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Figures, graphics, and photographs should be submitted as separate files (in TIFF or JPEG format)

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Case Report	1000	200	15	2	10 or total of 20 images
Technical Note	1500	No abstract	15	No tables	10 or total of 20 images
Letter to the Editor	500	No abstract	5	1	1

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

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

All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.

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

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

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

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

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

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

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

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

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

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

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CYTOTOXIC LESIONS OF THE CORPUS CALLOSUM: MAGNETIC RESONANCE IMAGING FINDINGS AND ETIOLOGIC FACTORS

KORPUS KALLOZUMUN SİTOTOKSİK LEZYONLARI: MANYETİK REZONANS GÖRÜNTÜLEME BULGULARI VE ETİYOLOJİK FAKTÖRLER

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ABSTRACT

Objective: Cytotoxic lesions of the corpus callosum (CLOCCs) are usually detected as a diffusion restriction in the splenium of the corpus callosum. They are rare secondary radiological findings associated with various clinical entities. The aim of this study is to evaluate the magnetic resonance imaging (MRI) findings and underlying clinical factors of cases with CLOCCs.

Materials and Methods: The MRI images of 850 patients who were admitted to the emergency services between January 2017 and March 2021 with encephalopathy and epilepsy-like neurological complaints were scanned retrospectively. Twenty nine patients (20 men, 9 women) with CLOCCs were included in the study. Their radiological and clinical findings were evaluated.

Results: The mean age was calculated as 26.4 years (5-72 years). The patients had neurological symptoms such as dysarthria, confusion, ataxia, syncope, epileptic seizure, and headache. Lesions were developed secondary to various infections in 20 (68.9%) patients. Diabetic decompensation was found in three patients and uremic decompensation in one patient. In the remaining patients, subarachnoid hemorrhage, asthma attack, trauma, high-dose lithium-levetiracetam intake and anti-epileptic drug withdrawal were responsible. Twenty patients had MRI control. In 16 (80%) patients, MRI findings returned to normal between 6 days and 8 months (median 30, Mean 53.8 days). One of the

ÖZET

Amaç: Korpus kallozumun sitotoksik lezyonları (KKSL) genellikle korpus kallozum spleniumunda diffüzyon kısıtlılığı olarak saptanan farklı birçok klinik durum ile ilişkilendirilmiş nadir bir radyolojik bulgudur. Çalışmamızın amacı kliniğimizde manyetik rezonans görüntülemesinde (MRG) KKSL saptanan olguların radyolojik ve klinik bulgularını incelemek ve literatür eşliğinde değerlendirmektir.

Gereç ve Yöntem: Ocak 2017 – Mart 2021 tarihleri arasında erişkin ve pediatri acil servislerine ensefalopati ve epilepsi benzeri nörolojik şikayetler ile başvuran, diffüzyon veya kraniyal MRG çekilmiş yaklaşık 850 hastanın görüntülemeleri retrospektif olarak tarandı. KKSL saptanan 29 hasta (20 erkek, 9 kadın) çalışmaya dahil edilerek radyolojik ve klinik bulguları değerlendirildi.

Bulgular: Yaş ortalaması 26,4 yıl (5-72 yıl) olarak hesaplandı. Hastalarda dizartri, bilinç bulanıklığı, ataksi, senkop, epileptik nöbet, baş ağrısı gibi nörolojik semptomlar mevcuttu. Hastalardan 20 (%68,9) tanesinde çeşitli enfeksiyonların merkezi sinir sistemine direkt veya indirekt etkisi ile lezyonların geliştiği görüldü. Üç hastada diyabetik, bir hastada üremiye bağlı metabolik dekompansasyon saptanmıştır. Diğer 5 hastada; subaraknoid hemoraji, astım atağı, travma, yüksek doz lityum - levetirasetam alımı ve antiepileptik ilaç çekilmesi gibi etyolojik faktörlere bağlı geliştiği değerlendirildi. Yirmi hastada MR kontrolü bulunmaktaydı. 16

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other 4 patients had partial regression, and 3 patients recovered with sequelae gliosis.

Conclusion: CLOCCs are nonspecific MRI findings associated with a broad underlying clinical spectrum. They are usually reversible. Determination of the underlying clinical etiology, avoidance of an ischemic stroke and tumor-like misdiagnoses are important for appropriate patient management.

Keywords: Cytotoxic callosal lesions, reversible splenial lesion, splenium, corpus callosum

(%80) hastada bulgular 6 gün ile 8 ay arasında normale dönmüştür (Median 30, Ortalama 53,8 gün). Diğer 4 hastadan birinde parsiyel regresyon olduğu, 3 hastada sekel gliozis ile iyileştiği izlendi.

Sonuç: Kallozal sitotoksik lezyonlar altta yatan geniş bir klinik spektrum ile ilişkili nonspesifik kranial MRG bulgularıdır. Genellikle geri dönüşümlüdür. Radyolojik olarak tanınmaları, altta yatan klinik etiyolojinin saptanması, iskemik inme ve tümör benzeri yanlış tanılardan kaçınılması, uygun hasta yönetimi için önemlidir.

Anahtar Kelimeler: Sitotoksik kallozal lezyonlar, reversibl splenial lezyon, splenium, korpus kallozum

INTRODUCTION

The corpus callosum is the largest white matter pathway that provides interhemispheric communication and coordination. Splenium is the part of the corpus callosum that remains in the posterior part and consists of thick fibers that connect both the temporal, posterior parietal and occipital cortices (1,2). In recent years, cytotoxic lesions observed in the splenium of the corpus callosum have been identified by Magnetic Resonance imaging (MRI). These lesions which are called cytotoxic lesions of the corpus callosum (CLOCCs), are rare radiological findings that can be seen secondary to a wide range of diseases (3,4). These lesions are usually ovoid, homogeneous, non-hemorrhagic lesions detected in the middle part of the splenium. Extracallosal involvement and irregular lateral extension have been described in some patients (4,5) . In MRI examinations, the signal features are usually mildly hyperintense on T2-weighted and FLAIR sequences, slightly hypointense on T1-weighted images, hyperintense in diffusion sequences and do not show contrast enhancement (3,4).

These lesions are usually detected incidentally when imaging for encephalopathy, encephalitis or epileptic seizures. Although the clinical prognosis is generally good in these patients, the clinical situation varies depending on the underlying etiology (5,6). The underlying pathogenesis of these lesions is still controversial. Currently, the most accepted hypothesis explains these lesions with cytotoxic edema and demyelination secondary to inflammatory cytokinopathy in the brain. Differential diagnoses include a wide range of etiological factors with varying clinical importance (7,8). Radiological identification of these lesions and knowing the associated clinical conditions are important for avoiding a misdiagnosis like an acute ischmeia or tumor -like lesions and to apply appropriate treatment to patients. In this study, 29 cases with CLOCCs detected on cranial MRI were included and these cases were evaluated in terms of MRI findings, clinical symptoms and underlying etiological factors. The similar features and differences of these cases were examined with the literature examples.

MATERIAL AND METHODS

After applying to the appropriate ethics committee, the MRI images of 850 patients who were admitted to the adult and pediatric emergency services between January 2017 and March 2021 with encephalopathy and epilepsy-like neurological complaints were scanned retrospectively. The review of our database records revealed 29 patients with CLOCCs who were included in the study. Their radiological and clinical findings were evaluated retrospectively through the image archive system and the hospital database records. MR images were evaluated by a neuroradiologist with 6 years of experience in neuroimaging. Patients with arterial territorial diffusion restriction due to acute ischemic stroke were not included in the study. Patients with a known diagnosis of demyelinating disease and presenting with callosal lesion due to an acute attack were excluded from the study.

Patients' age, initial complaints, clinical signs and symptoms, laboratory findings, chronic/previous disease parameters, initial and control MRI findings and final diagnosis were evaluated. MRI examinations of the patients were performed with 1.5 Tesla (T) or 3 Tesla devices, and the initial MRI examinations were made with or without contrast or just diffusion and T2, FLAIR sequences. The shape, localization, MR signal intensity, diffusion restriction, apparent diffusion coefficient (ADC) values, enhancement patterns, affected areas and extra callosal lesions were evaluated in MRI examinations. In addition, medical records of patients such as clinical symptoms, underlying diseases, clinical course, recurrence and post-treatment response were evaluated. All statistical analyses were calculated using Statistical Package for the Social Sciences (SPSS) for Windows version 20.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Demographic characteristics of the patients

In the study, 29 patients who were reported to have cytotoxic lesions of the corpus callosum according to the findings obtained in MRI examinations were included.

There were 20 female (68.9%) and 9 male patients, with a mean age of 26.4±19.6years (range, 5 -72 years). Eleven patients (37.9%) were aged 18 years or younger. Eighteen patients (62.1%) were older than 18 years. Patient gender, age, MRI findings and clinical characteristics are detailed in Table 1.

MRI findings

Corpus callosum splenium was involved in all cases. Splenial involvement was localized in the middle part in 20 patients (68.9%), in the middle and paramedian part in three patients (10.3%), in the middle and left lateral part in three patients (10.3%) and only in the lateral parts and paramedian parts of the splenium in three patients (10.3%). The lesions which were localized to the middle part of the splenium were oval, crescent-shaped and band-like morphological structures (Figure 1). All lesions restricted diffusion and ADC responses were monitored. In the additional T2 and FLAIR sequences taken in the diffusion examination, the lesions were evaluated as slightly hyperintense. It was observed that the lesions did not enhance contrast in 18 patients with contrast-enhanced examination.

As extracallosal involvement, one patient had acute diffusion restriction in the bilateral centrum semiovale simultaneously with the splenial lesion (Patient 19) due to disseminated intravascular coagulation (DIC), septicemia and acute respiratory distress syndrome (Figure 2). In a second patient, involvement of the corpus callosum genu and left centrum semiovale was observed as well as splenial lesion involvement (patient 20) due to meningitis and septicemia. One patient who presented with diabetic ketoacidosis had bilateral thalamic and left globus pallidus involvement concomitant in the splenial lesion (patient 26). In one patient with trauma, focal diffusion restriction was observed in the corpus callosum corpus and bilateral cerebral hemispheres other than the splenium (patient 28). One patient diagnosed with meningitis had concomitant leptomeningeal enhancement in post contrast MRI. In one of the patients, cerebellar meningitis findings were added in the MRI examination during the follow-up, and the findings were observed to regress after appropriate treatment (patient 8).

Control MRIs of 20 patients could be accessed. In 16 (80%) patients with MRI control, the imaging findings returned to normal between 6 days and 8 months (median 30, Mean 53.8 days). In the 2nd month follow-up MRI of one patient with meningitis, it was observed that the findings had partially regressed. In two patients who developed cytotoxic lesion due to diabetic ketoacidosis and one patient with post-traumatic lesion, the MRI findings healed with sequelae gliosis.

Clinical symptoms and findings

Patients often had neurological symptoms such as confusion, dysarthria, ataxia, dizziness, syncope, epileptic seizures, headache, meningeal irritation findings and visual complaints. Seven (24.1%) patients presented with upper respiratory tract infection (URTI) symptoms at the time of admission. Six patients (20.6%) had complaints related to the gastrointestinal system such as nausea, vomiting, abdominal pain and diarrhea. Two patients presented with epilepsy attacks. Other patients had complaints such as fatigue, tremor, hypertension at the time of admission.

In the follow-up and examinations, it was evaluated that callosal cytotoxic lesions developed in 20 (68.9%) patients due to the direct or indirect effects of various systemic or local infections. Influenza infection was diagnosed in three patients (one influenza pneumonia, two URTI), plasmodium falciparum malaria in one patient, Listeria monocytogenes meningitis in one patient, pneumonia in one patient, viral and bacterial serology negative cerebellitis in one patient, adenovirus enteritis in one patient, mastitis in one patient, otitis media in one patient, URTI in two patients, covid 19 in one patient, acinetobacter septicemia in one patient with a known diagnosis of methyl malonic acidemia, serology and culture negative meningitis in two patients and septicemia due to URTI in one patient. It was diagnosed after meningitis and septicemia in a patient who had been operated for subdural hematoma two months before the cytotoxic lesion. It was found that a patient diagnosed with familial Mediterranean fever (FMF) had delayed his treatment for the last seven months and a cytotoxic lesion developed after a mild respiratory infection. In addition, it developed secondary to metabolic decompensation after acute tonsillitis in a patient with a known diagnosis of Maple Syrup Disease.

Except for infection-related lesions, metabolic decompensation due to diabetes mellitus was found as an etiological factor in three patients (10.3%). Metabolic decompensation (osmotic demyelination) due to uremia was detected in one patient. Subarachnoid hemorrhage was determined as etiological factor in one patient and post-traumatic injury in one patient. Asthma attack, high-dose lithium-levetiracetam intake and discontinuation of anti-epileptic drug (drug withdrawal) were responsible for cytotoxic lesions in the remaining 3 patients.

DISCUSSION

Transient splenial lesions were first described by Chason et al. in 1996 in epilepsy patients (3). The actual mechanism in the formation of these lesions has not been fully elucidated. Some researchers have thought that it develops due to the involvement of crossing white matter fibers from the temporal lobes in the splenium. They suggested that this mechanism is caused by focal edema that develops due to the temporary deterioration of the blood-brain barrier in the splenium in the postictal period after epileptic seizures (3,9). Kim et al. attributed these lesions to transient demyelination induced by an-

Table 1: MRI findings, demographic and clinical characteristics of the patients

Patient No	Age	Gender	Pre-existing disease	Prodromal manifestations	Etiological factor-Diagnosis	Neurological manifestations	Splenial involvment	CSF examination	Extracallosal lesion, findings	Therapy for neurological symptoms	MRI normalization
1	22	M	No	Fever/URTI	Infection-associated (İnfluenza)	Dizziness	Middle	NE	No	Symptomatic treatment	1 month
2	5	M	MSUD	Fatigue	Infection-associated (Metabolic decompation due to tonsillitis)	Ataxia, increased deep tendon reflexes	Left paramedian	NE	No	Sodium phenylbutyrate, insülin, ceftriaxone	No control
3	63	M	No	URTI	Infection-associated (NBC, NVT)	syncope	Middle	NE	No	Oseltamivir, azithromycin	10 days later
4	25	M	No	Fever, vomiting	Infection-associated (Listeria Monocytogenes meningitis)	Headache, neck stiffness	Middle	Protein: 53.7 mg/dL, lymphocyte	leptomeningeal enhancement	Ampicillin-sulbactam	1 month
5	21	F	No	Sore throat, fever	Infection-associated (İnfluenza pneumonia)	Syncope	Middle	NE	No	Oseltamivir	No control
6	13	M	No	Fever, vomiting	Infection-associated (pneumonia)	Horizontal nystagmus	Middle	NE	No	Ceftriaxone	No control
7	20	M	No	Fever, fatigue	Infection-associated (malaria plasmodium falciparum)	Disturbance of consciousness	Middle	NE	No	Artemeter, lumefantrine	No control
8	21	F	No	Chills and shivering, fever	Infection-associated (NC, NVT cerebellitis)	Involuntary contraction of the body	Middle	Protein and lymphocyte	Cerebellitis (cerebellar enhancement, expansion)	Doxycycline, acyclovir, dexamethasone	6 days
9	19	F	No	URTI	Infection-associated (NBC,NVT)	Bilateral temporary loss of vision	Middle	NE	No	Symptomatic treatment	No control
10	9	M	No	Diarrhea, fever	Infection-associated (enteric adenovirus)	Hand spasms, meaningless speech, walking disorder	Middle	Normal	No	Ceftriaxone, acyclovir	8 days

Table 1: MRI findings, demographic and clinical characteristics of the patients (Continued)

Patient No	Age	Gender	Pre-existing disease	Prodromal manifestations	Etiological factor-Diagnosis	Neurological manifestations	Splenial involvment	CSF examination	Extracallosal lesion, findings	Therapy for neurological symptoms	MRI normalization
11	32	M	Hyperaldosteronism	Hypertension	Bilateral renal artery stenosis Chronic renal failure (metabolic decompensation due to uremia)	Dizziness, dysarthria	Right lateral	NE	No	Antihypertensive therapy, hemodialysis	No control
12	21	M	Methylmalonic acidemia, Chronic renal failure, epilepsy	Nausea, fever, fatigue	Infection-associated (acinetobacter septicemia)	headache	Middle	NE	No	Piperasiline, tazobactam	7 months
13	15	M	FMF	Stomach ache, fever	Infection-associated (oropharyngeal hyperemia)	Dizziness	Middle, left lateral	Normal	No	Ceftriaxone, acyclovir	1 month
14	11	M	No	Headache,	Infection-associated (otitis media)	Headache, otalgia	Middle, left lateral	NE	No	Ampicillin, sulbactam	1 month
15	18	M	Epilepsy	Epileptic seizure	Epileptic drug withdrawal (1 week after discontinuation of oxcarbazepine)	epileptic seizure	Middle	NE	No	Levesiteram, lacosamide	4 months
16	12	M	No	Sore throat, fever	Infection-associated (Covid 19 +)	Headache,	Middle	NE	No	Piperacillin, tazobactam	No control
17	18	M	Recurrent craniopharyngioma	Fever, vomiting	Infection-associated (NC,N-VT meningitis)	disturbance of consciousness, temporary loss of vision	Middle	Normal	Stable recurrent craniopharyngioma	Vancomycine, acyclovir	No control
18	15	M	Asthma	Stomach ache	Asthma attack	Headache,	Middle	NE	No	No	1 month
19	34	F	No	URTI	Infection-associated (DIC,ARDS, sepsis)	disturbance of consciousness	Middle, paramedian	NE	Diffusion restriction in bilateral sentrum semiovale	Intensive care hospitalization	20 days

Table 1: MRI findings, demographic and clinical characteristics of the patients (Continued)

Patient No	Age	Gender	Pre-existing disease	Prodromal manifestations	Etiological factor-Diagnosis	Neurological manifestations	Splenial involvment	CSF examination	Extracallosal lesion, findings	Therapy for neurological symptoms	MRI normalization
20	72	F	Operated for subdural hematoma 2 months ago	Epileptic seizure, confusion	Infection-associated (Sepsis, menengitis)	Epileptic seizure, confusion	Middle	Protein (164 mg/dl)	CC Genu involvement, left sentrum semiovale involvement	Vancomycine amphotericin b, meropenem	2 months later partial involution
21	65	F	No	SAH	Aneurysmal SAH	Headache, vomitting	Middle	NE	No	Endovascular embolization	8 months
22	22	F	Bipolar disorder		High-dose lithium and levetiracetam intake	Headache	Middle	Normal	No	Gastric lavage	1 month
23	60	M	Diabetes mellitus	Dizziness	Uncontrolled diabetes	Dizziness	Right and left lateral involvment	NE	No	Diabetes mellitus regulation	2 months control gliotic sequelae
24	68	F	Diabetes mellitus, hypertension	Disturbance of consciousness	Uncontrolled diabetes	Disturbance of consciousness	Middle	NE	No	Diabetes mellitus regulation	1 month
25	32	F	No	Headache, dizziness	Infection-associated (mastitis)	Headache, dizziness	Middle	Normal	No	Ampicillin-sulbactam for mastitis	No control
26	7	M	No	Abdominal pain, vomiting, confusion	Diabetic ketoacidosis	confusion	Middle, left paramedian	NE	Bilateral thalamic, left globus pallidus involvement	Diabetes mellitus regulation	9 months later control gliotic sequelae
27	22	M	No	Fever, vomiting	Infection-associated (NC, NVT meningitis)	Headache, Disturbance of consciousness	Middle	Protein:86 mg/dl, lymphocyte, (culture negative)	Bilateral globus pallidus calcification on CT	Ceftriaxone, acyclovir	Bilateral splenial paramedian extension on first week control MRI, 1 month control normal
28	20	M	No	Posttraumatic confusion	Posttraumatic	Confusion	Middle, left paramedian	NE	CC corpus involvement, bilateral DAI	Symptomatic treatment	1 month control gliotic sequelae
29	5	M	No	Sore throat, fever	Infection-associated (İnfluenza)	Headache	Middle	NE	No	Symptomatic treatment	8 days

CSF: Cerebrospinal fluid, MRI: Magnetic Resonance Imaging, M: Male, F: Female, URTI: Upper respiratory tract infection, NE: not examined, MSUD: Maple syrup urine disease, NBC: Negative blood cultere, NVT: Negative viral tests, mg: milligrams, dl: deciliter, NC: Negative culture, , FMF: Familial Mediterranean Fever, DIC: Disseminated Intravascular Coagulation, ARDS: Acute respiratory distress syndrome, CC: Corpus Callosum, SAH: Subarachnoid Haemorage, CT: Computed Tomography, DAI: Diffuse Axonal Injury

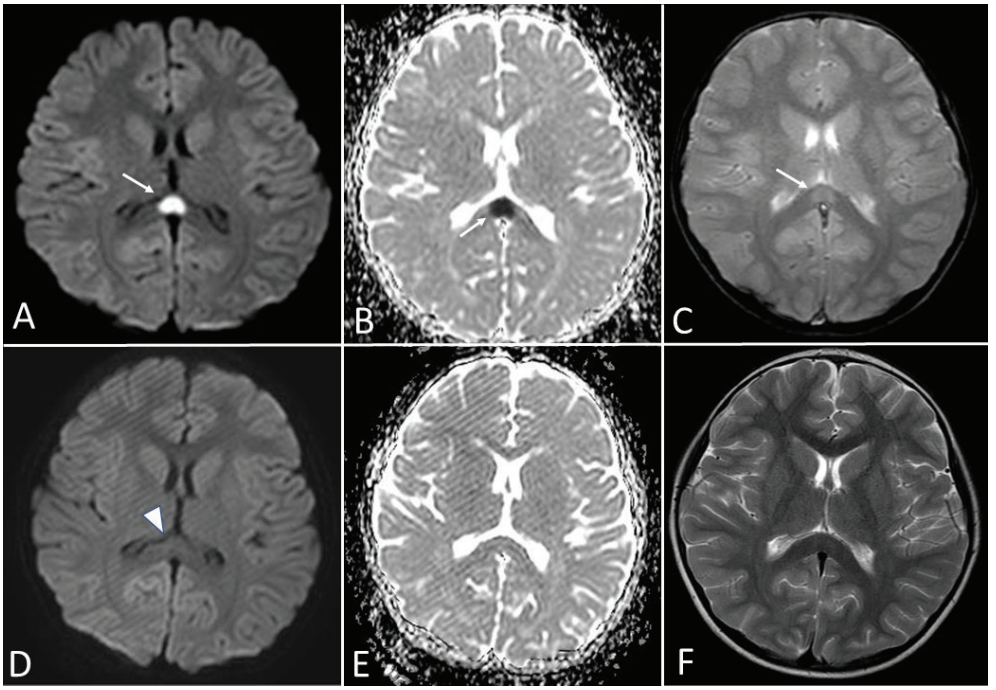

Figure 1: Cytotoxic splenial lesion. A) Increased signal intensity on axial plane Diffusion Weighted Image in the middle part of the splenium (arrow) B) ADC map shows low signal intensity on the same localization (arrow) C) T2 axial image show mildly hyperintensity (arrow) eight days control show normalization on DWI (arrow head) (D), ADC (E) and T2 (F) axial images

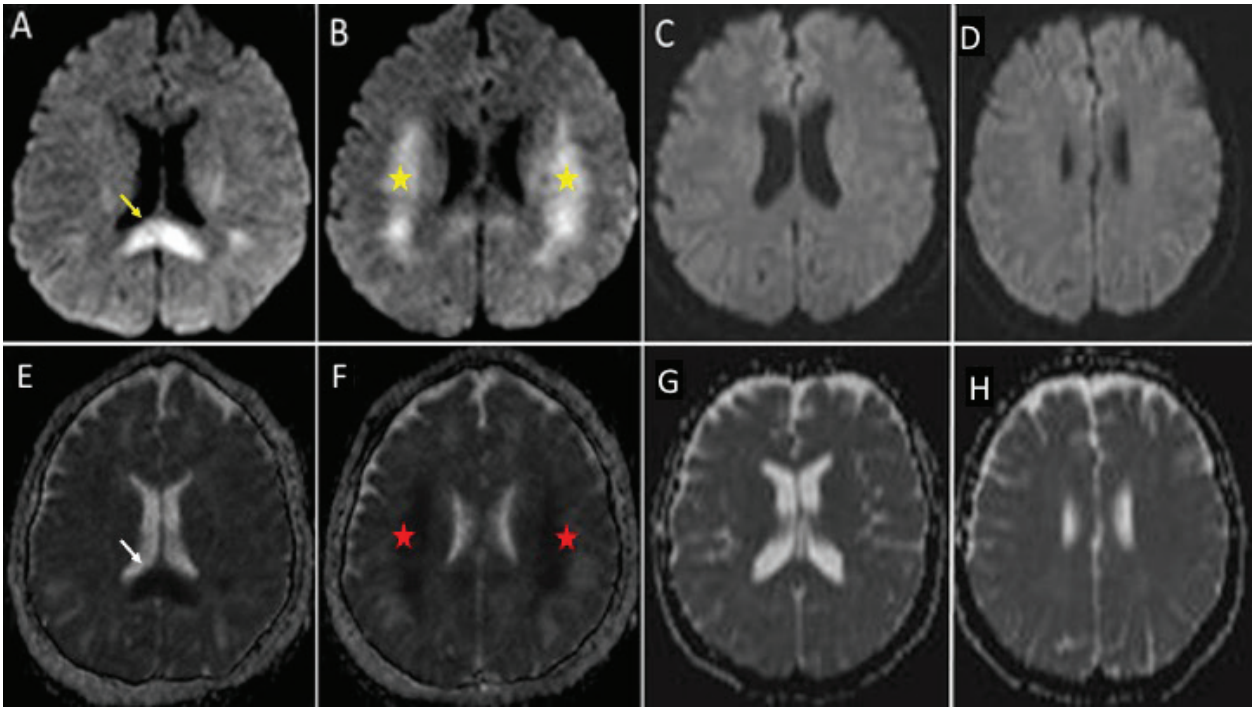

Figure 2: 34-year-old female images A) Acute diffusion restriction in the middle part of the splenium (yellow arrow), B) diffusion restriction in the bilateral centrum semiovale (yellow aterisks) C,D) Normalization is seen in the control diffusion images after 20 days, E) ADC map in the splenium (white arrow) indicates low signal intensity on her first MRI and F) indicates low signal intensity in bilateral centrum semiovale (red asterisks), G,H) after 20 days control ADC maps show normalization in splenium and bilateral centrum semiovale

ti-epileptic drug toxicity (4). As another possible putative mechanism, transient extrapontine osmotic myelinolysis caused by sodium and glucose imbalance due to toxicity, hypersensitivity or drug withdrawal of anti-epileptic drugs has been reported (10,11). Mild encephalopathy with transient splenial lesion (mild encephalitis/encephalopathy with reversible isolated splenial corpus callosum lesion (MERS)) is a clinical and neuroradiological syndrome with a good prognosis that has been used to describe a similar entity in the literature (5,7).

Nowadays, instead of the term transient splenial lesion, these lesions have started to be named cytotoxic lesions of the corpus callosum, which is a more inclusive term. Today, it is known that the splenium region of the corpus callosum has a higher density of cytokine, glutamate, drug and toxin receptors compared to other regions of the brain. For this reason, it has been reported in many studies that the splenium region of the corpus collosum is more sensitive to cytokineopathy (12). The widely accepted theory for CLOCC is that cytokine-mediated immunological reactions cause microvascular endothelial damage, causing perivascular-intramyelinic edema, inflammatory cell migration and associated cytotoxic edema (6). CLOCCs are nonspecific lesions that are usually centrally located in the splenium of the corpus callosum, oval, homogeneous, non-hemorrhagic, non-contrast, hyperintense on FLAIR and T2W images, slightly hypointense on T1W images, and with marked focal diffusion restriction on diffusion-weighted examination (8).

It is usually detected as an incidental finding in cranial MR imaging performed in patients presenting with encephalopathy, encephalitis or seizure complaints. Its etiology has been associated with a wide spectrum of diseases such as drug withdrawal of antiepileptic drug therapy, infection (influenza, EBV, adenovirus, varicella zoster, legionella pneumonia, rotavirus, HIV, tuberculous meningitis), malaria, malignancy, trauma, subaracnoid hemorrage, metabolic disorders (hypoglycemia, electrolyte imbalance) and hemolytic uremic syndrome (3-6).

Callosal cytotoxic lesions due to various infections developed in 20 (68.9%) of our patients. Meningitis was found as the etiological cause in four of our cases. Three of them were serology and culture negative meningitis and one was Listeria monocytogenes meningitis. One patient was diagnosed with cerebellitis of unknown etiology. Except these 5 patients, we did not detect direct central nervous system involvement in the other 15 patients which was associated with infection. Diabetic decompensation was found in three patients and uremic decompensation in one patient. Subarachnoid hemorrhage, asthma attack, trauma, high-dose lithium-levetiracetam intake and antiepileptic drug withdrawal were responsible for the remaining patients.

All patients included in the study showed clinical improvement without any deficits after appropriate treatments for the etiological factors. The clinic of the patients whose etiological factors were related to secondary infections returned to normal in a short time and no permanent deficits developed. In addition, radiological findings returned to normal in all patients except two who presented with diabetic ketoacidosis and one who presented with trauma. In these three patients, the splenial lesions healed with sequelae gliosis without any clinical deficit. We observed that many etiological factors played a role in our cases, as stated in different literatures. Trying to reveal the etiological cause with clinical and laboratory findings is important in terms of applying the appropriate treatment.

MR imaging findings of callosal cytotoxic lesions are from demyelinating diseases such as acute disseminated encephalomyelitis (ADEM) or multiple sclerosis (MS); it can be easily distinguished by the fact that generally it is a single lesion on the middle part of the splenium, and the absence of a prominent inflammatory margin. It has been described in the literature that in some cases, splenial lesions may extend to the lateral parts of the corpus callosum and lesions may occur in both frontoparietal regions (13). In this case, demyelinating diseases such as ADEM and pathologies such as progressive reversible encephalopathy should be excluded with clinical and laboratory findings in the differential diagnosis.

Involvement was observed only in the midline of the splenium in 16 patients (55.1%). Three patients had splenial involvement outside the splenium midline (left paramedian, right lateral part and bilateral lateral part involvement). Six patients had midline splenium and accompanying paramedian and/or lateral segment involvement. As extracallosal involvement, leptomeningeal enhancement in a patient with meningitis, cerebellum involvement in one patient in the following few days, bilateral centrum semiovale involvement in one patient, corpus callosum genu and centrum semiovale involvement in one patient, bilateral thalamus and globus pallidus involvement in one patient, corpus callosum corpus involvement and subcortical white matter involvement-compatible with axonal damage in one patient. Acute ischemic stroke should not be considered primarily in the differential diagnosis because of its incompatibility with the lesion localization, features, and vascularization territory. Recognition of the lesion radiologically and clinically is important for differential diagnosis. In the literature, it has been reported that MR findings quickly return to normal, but it is found in cases that last up to three months. In our patient group, it was observed that the lesions returned to normal between 6 days and 8 months in 16 of 20 patients who were under control. In three patients, the splenial lesions healed with sequelae

gliosis. In one patient, partial involution was observed in the 2nd month follow-up MRI.

Since it is a retrospective study, the inaccessibility of control MRI scans of all patients and the fact that the existing control images were not performed at regular intervals make it difficult to follow the true evolution of the lesions over time and make it difficult to predict the actual recovery time. This is one of the major weaknesses of the study. Prospective studies are needed to fully reveal the etiology of the indicated callosal cytotoxic lesions and to monitor the imaging findings in real time.

In conclusion, cytotoxic lesions of the corpus callosum are nonspecific MR imaging findings and they are associated with many underlying entities. The prognosis is generally good. It is important to be aware of the MRI findings of these lesions and to know that they are secondary lesions. The broad clinical spectrum associated with the lesion should be known for the identification and elimination of the underlying true etiology and for appropriate clinical management.

Ethics Committee Approval: This study was approved by Istanbul University Istanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 21.05.2021, No: 204262).

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THE ROLE OF DIFFUSION-WEIGHTED MR IMAGING IN EVALUATING THE RESPONSE TO STEROID THERAPY IN IDIOPATHIC GRANULOMATOUS MASTITIS LESIONS

İDİYOPATİK GRANÜLOMATÖZ MASTİT LEZYONLARINDA STEROİD TEDAVİSİNE YANITIN DEĞERLENDİRİLMESİNDE DİFÜZYON AĞIRLIKLI MR GÖRÜNTÜLEMENİN ROLÜ

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ABSTRACT

Objective: The aim of this study was to investigate the role of diffusion-weighted magnetic resonance imaging (DW MRI) with an apparent diffusion coefficient (ADC) map in evaluating the response to treatment of steroid-treated idiopathic granulomatous mastitis (IGM) lesions.

Materials and Methods: This retrospective study included 99 lesions of 58 female patients (average age: 32.91 years; range: 22–55 years) with biopsy-proven IGM. Patients were treated with oral and topical steroids. All pre-treatment and post-treatment MR examinations were evaluated. The maximal size of the masses and non-mass enhancement (NME) lesions were measured. Patients were classified as complete response (CR), partial response (PR), and non-response (NR) according to the dynamic contrast-enhanced (DCE) MR findings after treatment.

Results: ADC values of areas occupied by IGM (0.933±0.317x10^{-3} mm^2/sec) were lower than contralateral normal parenchyma (1.259±0.423x10^{-3} mm^2/sec). Twenty-two (22.22%) of the lesions were in the NR group, 30 (30.30%) in the PR group, and 47 (47.47%) in the CR group. There was no significant difference between the pre-treatment ADC values in NR, PR, and CR groups (p=0.228). There was a significant difference between the pre-treatment and post-treatment ADC values in the PR groups (p=0.001).

Conclusion: DW MR imaging in IGM is a useful method to monitor the response to treatment. However, it is not successful in predicting response to treatment.

Keywords: Granulomatous Mastitis, Magnetic Resonance Imaging, Diffusion-Weighted MRI, Treatment Response, Steroid Therapy

ÖZET

Amaç: Bu çalışmanın amacı, idiyopatik granülomatöz mastit (İGM) lezyonlarının steroit tedavisine yanıtını değerlendirmede, görünür bir difüzyon katsayısı (ADC) haritası ile difüzyon ağırlıklı manyetik rezonans görüntülemenin (DW MRG) rolünü araştırmaktı.

Gereç ve Yöntem: Bu retrospektif çalışma, biyopsi ile kanıtlanmış İGM'li 58 kadın (ortalama yaş 32,91 yıl, aralık=22-55 yıl) hastanın 99 lezyonunu içeriyordu. Hastalar oral ve topikal steroidlerle tedavi edildi. Tüm tedavi öncesi ve tedavi sonrası MR incelemeleri değerlendirildi. Kitlelerin ve kitlesel olmayan kontrastlanmaların maksimum boyutu ölçüldü. Tedavi sonrası dinamik kontrastlı (DK) MR bulgularına göre hastalar tam yanıt, kısmi yanıt ve yanıtsız olarak sınıflandırıldı.

Bulgular: İGM (0,933±0,317x10^{-3} mm^2/sn) tarafından işgal edilen alanların ADC değerleri, kontralateral normal parankimden (1,259±0,423x10^{-3} mm^2/sn) daha düşüktü. Lezyonların 22'si (%22,22) yanıtsız, 30'u (%30,30) kısmi yanıt ve 47'si tam yanıt (%47,47) grubundaydı. Üç tedavi grubunda tedavi öncesi ADC değerleri arasında anlamlı bir fark yoktu (p=0,228). Kısmi yanıt grubunda tedavi öncesi ve tedavi sonrası ADC değerleri arasında anlamlı fark vardı (p=0,001).

Sonuç: İGM'de DW MR görüntüleme, tedaviye yanıtı izlemek için yararlı bir yöntemdir. Ancak tedaviye yanıtı tahmin etmede başarılı değildir.

Anahtar Kelimeler: Granülomatöz mastitis, Manyetik rezonans görüntüleme, Difüzyon ağırlıklı MRG, Tedavi yanıtı, Steroid tedavisi

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INTRODUCTION

Idiopathic granulomatous mastitis (IGM) is a benign, recurrent, and rare inflammatory disease of the breast. It is most often diagnosed in women of childbearing age with a breastfeeding history. The most common clinical finding is a painful, unilateral palpable mass (1,2). Among imaging methods, mammography and ultrasound (US) are the preferred modalities. Imaging findings are nonspecific and vary according to the stage of the disease, as well as the degree of inflammation (3,4). Focal asymmetry is the most common finding in mammography. On US, IGM most often presents as a large, irregularly shaped, hypoechoic mass that is parallel to the skin (5,6). The sensitivity of magnetic resonance imaging (MRI) is high, and the most frequently reported findings are heterogeneous or a rim-enhancing mass and non-mass enhancement (NME) (3-8). IGM is an exclusion diagnosis and is histologically confirmed to distinguish it from malignancies and other inflammatory diseases of the breast. Histological examination reveals lobulocentric noncaseating granulomas with inflammatory cell infiltration. While abscess formations are frequently detected, necrosis and fibrosis are less noticeable features (9,10). Recently, medical treatment has been preferred in the first stage, with surgical treatment reserved for resistant cases. Steroids, methotrexate, and bromocriptine are medical treatment options and have been found useful (11-14). More than one treatment protocol may be required for complete regression (15). Spontaneous resolution can be seen after 6-12 months without any treatment (4,16). Recurrence occurs in both conservative and surgical treatment (15). Imaging is crucial in evaluating the response to treatment and detecting recurrence. Defining the extent of the disease, the response of lesions to treatment, and the presence of new lesions are important in evaluating the success of treatment (3,4). DW MRI is an examination technique based on measuring the mobility of water molecules in vivo to provide numerical data with ADC values without using contrast material. It analyzes the microscopic structure of tissues such as cellularity, membrane integrity, viscosity, organelles, and macromolecules (17).

The aim of this study is to investigate the role of DW MR imaging in evaluating treatment response in IGM lesions.

MATERIALS AND METHODS

This study is retrospective and was approved by Kartal Doktor Lutfi Kırdar Training and Research Hospital Ethics Committee (Date: 25.11.2020, No: 514/190/3). The requirement to obtain informed consent from patients was waived.

Patient population

Between June 2015 and November 2021, the results of MR examinations performed both before and after treatment on 65 patients with a diagnosis of IGM were evaluated. IGM diagnosis was proven by core biopsy in all patients. Microbiological testing (Gram, periodic acid-Schiff and Ziehl-Neelsen staining, mycobacterial cultures, fungal analysis with Grocott–Gomori methenamine silver staining) was performed to exclude other types of mastitis. Purified protein derivative skin test (PPD) and QuantiFERON test were also done to rule out tuberculous mastitis. Five patients were excluded from the study because new lesions developed in the breast after the treatment, and two patients' scans were not of optimal quality due to artifacts in the MR examinations. The remaining 58 patients and 99 lesions of these patients were included in the study. Patients were treated randomly with two methods. Thirty-one (53.44%) patients were treated with 0.4 mg/kg methylprednisolone once a day and 0.125% prednisolone pomad on weekdays. Twenty-seven (46.55%) patients were treated with 0.8 mg/kg of oral methylprednisolone once a day. Superficial abscesses with fluctuation were drained either on admittance or during the treatment.

MR imaging technique

MR examinations of all patients before and after treatment were performed on a 1.5 T system (Philips Ingenia, Philips Healthcare, Best, The Netherlands). Three-dimensional fat-saturated ultrafast spoiled gradient echo DCE sequences (FOV:342x342, matrix:342x340, FA:10, TR:5 TE:3, section thickness:2, section gap:1) were acquired. DCE sequences consisted of a total of five series, one of which was pre-contrast (90, 142, 194, 246, and 298 seconds after injection). Image parameters were FOV:342x342, matrix:342x340, FA:10, TR:5, TE:3, section thickness:2, section gap:1. Diffusion-weighted images were obtained with b-values of 0 and 1000 s/mm^2 (FOV:364x364, matrix:151x146, FA:90, TR:9400, TE:71, section thickness:3, section gap:3).

Examinations were performed with a dedicated phase array 16 channel breast coil in the prone position, and 0.1 mmol per kilogram of body weight gadoteric acid was used with an automated injector.

MR image analysis

MR evaluations were performed with consensus by two radiologists with six and nine years of experience in breast imaging (G.R, M.A.). All MR images were reviewed on the picture archiving and communication system of an EIZO GS520 workstation. Pre- and post-treatment MR examinations were evaluated.

Rim-enhancing lesions and mass lesions were considered masses. Masses and non-mass enhancements (NME) were noted. The characteristic features, maximal sizes, and average ADC values of the lesions detected in pre-treatment MR examinations were noted. These measurements were repeated by finding the corresponding

target lesions on MRI after treatment. Maximal lesion size was measured on the first DCE images, which were obtained after 90 seconds.

For ADC value measurements, the section with the longest diameter of the lesion was selected from the DCE MR images of the lesions. It was made manually by drawing the entire lesion by finding its equivalent from the ADC map. (Figure 1). The ADC measurement was not performed for lesions less than 1 cm in diameter in the MR examination before or after treatment. The ADC value was measured from the contralateral normal breast parenchyma using a 100 mm^2 ROI. Pre-treatment and post-treatment MR examinations were correlated, and ADC measurements of lesions showing complete regression with an ROI of 100 mm^2 from the area corresponding to the localization of the lesions were performed. The areas occupied by these lesions were found with the help of the residual architectural distortion. The localization of lesions that do not cause structural distortion was found by referring to the distance to the nipple, adjacent fat lobules, and vascular structures.

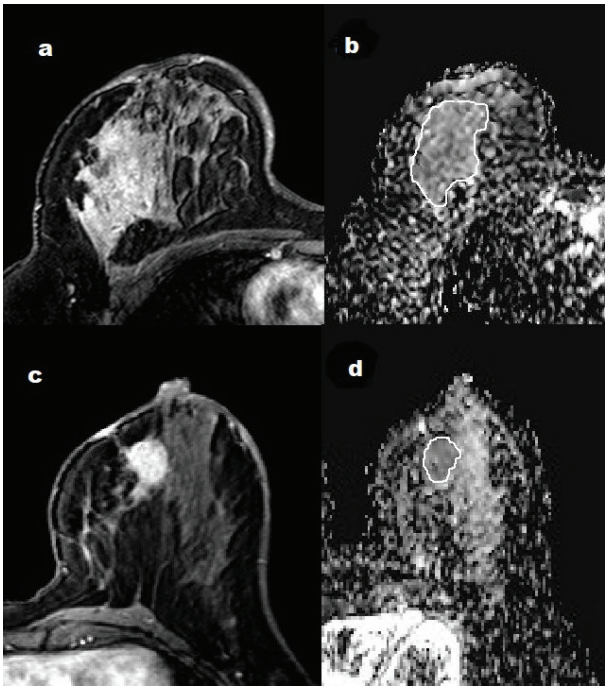

Figure 1: A 27-year-old female patient with IGM diagnosis has NME in the middle outer quadrant of the right breast in fat-suppressed T1W MR images (a). ADC values were calculated manually by drawing around the entire lesion in the section where the lesion was best seen (b). Contrast-enhanced fat-suppressed T1W MR images of a 28-year-old female patient with a diagnosis of IGM with a mass in the middle inner quadrant of the left breast (c). The ADC value was measured manually by drawing the entire lesion from the ADC map value (d).

Treatment response

Patients were divided into three groups according to the DCE MRI findings after treatment: complete response (CR), partial response (PR), and non-response (NR). The disappearance of all lesions in post-treatment MR imaging was accepted as CR. A ≥30% reduction in the maximal size of the targeted lesions was accepted as PR.

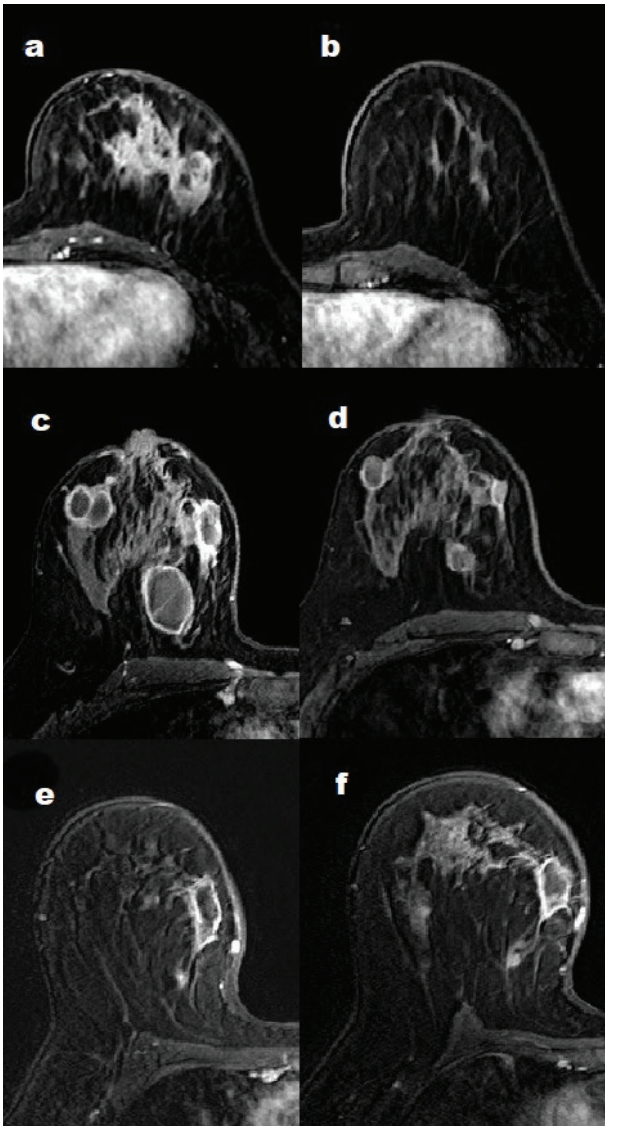

Figure 2: DCE MR images of patients in groups CR, PR, and NR according to treatment response. In the NR group, the heterogeneously enhanced mass in the left breast in a 30-year-old patient disappeared on MRI after 83 days (a,b) In the PR group, the size of 3 abscesses in the right breast of a 41-year-old female patient significantly regressed on MRI 54 days later (c,d). In the NR group, there was no significant change in the size of the abscess in the right breast of the 38-year-old female patient on MRI performed 146 days later (e,f).

Masses that showed resolution after treatment but had NME in the space they occupied were also included in the PR group. Targeted lesions that showed a <30% reduction, stability, and increased maximal size were evaluated as NR (18) (Figure 2). Since there was no lesion for comparison on MRI before treatment, patients who developed new lesions after treatment were excluded from the study.

Statistical analysis

Percentage, mean, and standard deviation were the statistics used to evaluate the descriptive findings. The one-sample Kolmogorov–Smirnov test was performed to determine whether the groups conformed to a normal distribution. Normally distributed results were evaluated using a paired t-test. Results not conforming to a normal distribution were evaluated using the Mann-Whitney U test to compare groups that did not show normal distribution. Wilcoxon signed-rank test was used to compare dependent groups and Kruskal Wallis test was used to compare three independent groups. One-way ANOVA was used to evaluate independent triple groups. Pearson's correlation test was used to evaluate within-group agreement. P-values <0.05 were considered statistically significant.

RESULTS

This study included 58 patients (mean age: 32.91 years; range: 22–55 years) with a total of 99 lesions. Post-treatment MR examinations of these patients were made after an average of 128 days (range: 27–233). In pre-treatment MR examinations, 72 (72.72%) of the lesions were masses, while 27 (27.27%) were NME.

Of the 58 patients, 25 (43.10%) were in the CR, 20 (34.48%) were in the PR, and 13 (22.41%) were in the NR group.

After treatment, 47 (47.47%) of the lesions were in the CR group, 30 (30.30%) in the PR group, and 22 (22.22%) in the NR group (Table 1). Nine (12.5 %) mass lesions included in the PR group disappeared in post-treatment MR imaging with residual NME in the same location. The mean maximal size of the lesions was 35.65±18.27 mm before treatment and 14.20±17.09 mm after treatment, and it decreased significantly after treatment (p=0.000).

Pre-treatment ADC values of areas occupied by IGM (0.933±0.317x10^{-3} mm^2/sec) were lower than those of contralateral normal parenchyma (1.259±0.423x10^{-3} mm^2/sec). There was a significant increase in ADC values of the PR group after treatment (p=0.001) (Table 2).

There was no significant difference between the pre-treatment ADC values in the CR, PR, and NR groups (p = 0.228). There was a significant difference in ADC values between PR and CR (p=0.017), PR and NR (p=0.041), and CR and NR (p=0.000) groups after treatment. There was no significant difference between the post-treatment ADC values of the CR group and the ADC values of the contralateral normal parenchyma (p=0.60).

Table 1: Mean size and response groups of lesions

Groups	Pretreatment mean size (mm±SD)	Posttreatment mean size (mm±SD)	CR	PR	NR
All lesions (n=99)	35.65±18.27	14.20±17.09*	47 (47.47%)	30 (30.30%)	22 (22.22%)
Mass (n=72)	33.00±16.52	13.08±15.51*	34 (47.20%)	22 (30.55%)	16 (22.22%)
NME (n=27)	42.74±20.99	16.22±20.30*	13 (48.14%)	7 (25.92%)	7 (25.92%)

*: p=0.000, NR: Non-response, PR: Partial response, CR: Complete response, NME: Non-mass enhancement

Table 2: Mean ADC and p values of groups

Lesion groups	Pre-treatment ADC (x10^{-3} mm^2/sec)	Post-treatment ADC (x10^{-3} mm^2/sec)	p value
Contralateral normal parenchyma (n=58)	1.259±0.423	1.367±0.567	0.18
All lesions (n=99)	823.85±299.51	1028.3±559.55	0.000
NR (n=22)	0.882±0.228	0.852±0.406	0.277
PR (n=30)	0.861±0.178	1.140±505	0.001
CR (n=47)	1.002±0.401	1.305±0.446	0.000
Mass (n=38)	0.918±0.357	1.102±0.443	0.001
NME (n=14)	0.973±0.169	1.294±0.571	0.008

NR: Non-response, PR: Partial response, CR: Complete response

DISCUSSION

IGM is a persistent, recurrent inflammatory disease often seen in women of childbearing age.

Response time to treatment is long and problematic, and recurrence is common (1,2). The duration of the complete disappearance of the lesions with conservative treatment ranges from 2 to 24 months (4). MR examination has a very high sensitivity in IGM imaging and can successfully show the extent of the disease. It is also successful in evaluating response to treatment and detecting recurrence (3,4,8).

In our study, there was a similar response to treatment in both NME and mass. This shows that with steroid therapy, mass and NME respond similarly to treatment; thus, characteristic features of lesions have no prognostic benefit in determining response to treatment. A study conducted by Altunkeser et al. showed that the characteristic features of IGM lesions, including MR imaging findings, do not provide any benefit for predicting treatment success (19).

We found that the ADC values of IGM lesions were lower than normal parenchyma and consistent with previous studies (3,20,21). Intense inflammatory cell infiltration without necrosis and abscess formation in areas occupied by IGM may be the cause of diffusion restriction.

According to the results of our study, the lack of significant difference between the pre-treatment ADC values in the CR, PR, and NR groups shows that the ADC values are not useful in predicting the response to treatment.

According to our results, DW-MR examination appears to be a good biomarker as a method of detecting changes in the microenvironment of lesions in the response of IGM to steroid treatment. The advantage of this method is that it does not require contrast material and does not contain radiation.

The limitations of our study are the small number of patients and the lack of agreement between observers. In addition, lesion responses after treatment were not evaluated histopathologically.

In conclusion, based on the findings of our study, ADC values are useful in monitoring response to treatment. ADC values and characteristic features of the lesions are also not useful in predicting treatment success. However, the findings in our study need to be supported by further studies.

Ethics Committee Approval: This study was approved by Kartal Doktor Lütfi Kırdar Training and Research Hospital Ethics Committee (Date: 25.11.2020, No: 514/190/3).

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IS THE AMOUNT OF WEIGHT LOSS AFTER BARIATRIC SURGERY A FACTOR DETERMINING CARDIOMETABOLIC RISK REDUCTION?

BARİYATRİK CERRAHİ SONRASI KİLO KAYBI MİKTARI KARDİYOMETABOLİK RİSK AZALMASINI BELİRLEYEN BİR FAKTÖR MÜDÜR?

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ABSTRACT

Objective: This study aimed to investigate whether differential weight loss amount appropriately reflects improvements in cardiometabolic health in patients undergoing bariatric surgery.

Materials and Methods: Patients who underwent bariatric surgery (BS) and were followed up for six months were divided into three groups according to their weight loss (Group-1: Low weight loss; Group-2: Moderate weight loss; Group-3: High weight loss). Before and after surgery, patients were evaluated for metabolic syndrome (MetS) using waist circumference, blood pressure, glucose, HDL-C, and triglyceride data, and a clustered cardiometabolic risk (CMR) score was obtained for each patient using a calculator available in the literature online. Changes in each MetS criterion and CMR score in the groups before and after the operation, and their relations with each other were compared.

Results: Sixty-six patients were included in the study. It was observed that the prevalence of MetS, which was 74.2% before the operation, decreased to 25.8%. A significant difference was observed between the groups in the decrease in weight, waist circumference, fat mass, fasting blood sugar, and HOMA-IR values. However, no significant difference was found between the groups in the change in MetS parameters and CMR scores. While a significant negative correlation was found between the amount of weight loss and the difference in the CMR score, no relation was observed between each MetS parameter and the amount of weight loss. It was also found that each 1% increase in weight loss was associated with a 57% decrease in the CMR score.

Conclusion: Although there is a significant decrease in cardiometabolic risk parameters after bariatric surgery, the amount

ÖZET

Amaç: Bu çalışma, bariyatrik cerrahi geçiren hastalarda farklı miktarda kilo kaybının kardiyometabolik sağlıktaki gelişmeleri uygun şekilde yansıtıp yansıtmadığını araştırmayı amaçlamıştır.

Gereç ve Yöntem: Bariyatrik cerrahi (BC) geçiren ve altı ay takip edilen hastalar geriye dönük olarak kilo kayıplarına göre üç gruba ayrıldı (Grup-1:Düsük kilo kaybı; Grup-2:Orta kilo kaybı; Grup-3: Yüksek kilo kaybı). Cerrahi öncesinde ve sonrasında hastalar, bel çevresi, kan basıncı, glukoz, HDL-K ve trigliserid verileri kullanılarak hem metabolik sendrom (MetS) varlığı açısından değerlendirildi hem de literatürde mevcut bir hesaplayıcı kullanılarak, her hasta için kümelenmiş bir kardiyometabolik risk (KMR) skoru elde edildi. Her bir MetS kriteri ve KMR skorunun, operasyondan önce ve sonra gruplardaki değişimleri ve birbirleriyle ilişkileri karşılaştırıldı.

Bulgular: Çalışmaya 66 hasta dahil edildi. Operasyon öncesi %74,2 olan MetS sıklığının operasyondan sonra %25,8'e düştüğü görüldü. Gruplar arasında kilo, bel çevresi, yağ kütlesi, açlık kan şekeri ve HOMA-IR değerlerindeki düşüşte anlamlı farklılık gözlendi. Ancak hem MetS parametrelerindeki hem de KMR skorundaki değişimde gruplar arası anlamlı bir farklılık bulunamadı. Kilo kaybı miktarıyla KMR skorundaki değişim arasında anlamlı negatif korelasyon saptanırken, ayrı ayrı MetS parametreleriyle kilo kaybı miktarı arasında herhangi bir ilişki gözlenmedi. Ayrıca kilo kaybındaki her %1'lik artışın, KMR skorunda %57'lik bir azalma ile ilişkili olduğu tespit edildi.

Sonuç: Bariyatrik cerrahi sonrası kardiyometabolik risk parametrelerinde anlamlı azalma olmasına rağmen hastaların kilo kayıp miktarı, ayrı ayrı parametreleri etkilememektedir. Ancak hastaların kardiyometabolik küme risklerini değerlendirmek ve izlemek

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of weight loss of the patients does not affect the individual parameters. However, using a scoring system to evaluate and monitor patients' cardiometabolic cluster risks will make it possible to follow the gradual changes in patients after surgery, making interventions by physicians and nutritionists more targeted and efficient.

Keywords: Metabolic syndrome, bariatric surgery, cardiometabolic risk score, amount of weight loss, obesity

için bir skorlama sistemi kullanmak, hastalarda ameliyat sonrasındaki kademeli değişiklikleri takip etmeyi mümkün kılacak, hekim ve beslenme uzmanlarının müdahalelerini daha hedefe yönelik ve verimli hale getirecektir.

Anahtar Kelimeler: Metabolik sendrom, bariyatrik cerrahi, kardiyometabolik risk skoru, kilo kaybı miktarı, obezite

INTRODUCTION

The probability of developing cardiovascular disease (CVD) and type-2 diabetes (T2D) in an individual is known as the "cardiometabolic risk" (CMR) and many risk factors coexist. Metabolic syndrome (MetS) elevates CMR because the presence of MetS causes a five-fold increase in the risk of developing T2D and a two-fold increase in the risk of developing CVD over the next five to ten years (1,2).

Conventional diagnosis of metabolic syndrome does not make it possible to follow the gradual changes after treatment in the diagnosed patients. This limitation seems to have been overcome with the continuous cardiometabolic risk (cCMR) index, which was used to estimate CMRs of patients diagnosed with MetS, especially in the pediatric age group, in previous studies (3). This index shows the continuous risk that the individual is exposed to and gives information about the severity of the risk (1,2). An article published in Diabetes Care in 2006 argued that CVR is a progressive function of various MetS risk factors and that separating variables into "metabolic risk" and "cardiovascular risk" would reduce statistical power; therefore, separate evaluation was suggested as not being necessary. It has been suggested that the continuous MetS risk score developed using the MetS risk factors of the International Diabetes Federation (IDF) is a more appropriate and valid alternative for epidemiological analyses (4). Similar indices have recently been developed for continuous cardiometabolic risk (cCMR) measurement. Although they contain the same components of the MetS, they were created using different methodologies (1,2).

In observational and randomized controlled studies with long-term follow-up of patients such as Surgical Treatment and Medications Potentially Eradicate Diabetes Efficiently (STAMPEDE) and Gastric Bypass to Treat Obese Patients With Steady Hypertension (GATEWAY) and with shorter follow-up periods, it has been shown that bariatric surgical interventions not only reduce weight, but also reduce glycemic and cardiometabolic risk, and are superior to intensive medical and lifestyle change treatments alone (5,6).

The most important indicator of treatment success and superiority of bariatric surgery is considered to be the amount of weight lost after surgery (7). However, in addition to patients who could not reach the target weight or regain weight after surgery, there are also studies showing improvements in MetS and CMR factors independent of weight loss with lifestyle changes such as diet and/or exercise without surgery (8,9). The findings of these studies highlight the necessity of considering other markers in managing obesity apart from weight loss alone. At this point, it comes to mind whether the amount of weight loss provided by bariatric surgery can be a treatment success on its own. This study evaluated the relationship between the amount of weight loss in obese individuals who underwent bariatric surgery and decreased CMR.

MATERIALS AND METHODS

In the study, the data of the patients who underwent bariatric surgery at Istanbul University, Istanbul Faculty of Medicine between the dates 2013-2017 and followed up afterward were used by scanning their files retrospectively. The study was approved by the local ethics committee (Date: 24.06.2022, No:12), and written informed consent was obtained from all patients.

Study population

The characteristics of the patients who were pre-evaluated for the study were as follows: Men and women aged between 18-60 years who had Roux-en-Y gastric bypass (RYGB) surgery by the same surgeon at Istanbul University, Istanbul Faculty of Medicine hospital, whose blood tests were performed at Istanbul Faculty of Medicine Clinical Biochemistry Laboratory, and who followed up in Istanbul Faculty of Medicine Endocrinology and Metabolic Diseases department.

Patients with the following characteristics were not included in the evaluation: Those diagnosed with cancer, kidney and liver failure, stroke, coronary artery disease, myocardial infarction or angina, coronary bypass surgery, and Percutaneous Coronary Angioplasty and stent placement.

Evaluated data: height (cm), weight (kg), fat mass (kg and %), fat-free mass (kg), waist and hip circumference (cm), systolic and diastolic blood pressure (mmHg), fasting blood sugar (FBG, mg/dl), fasting insulin (μU/mL), gly-

cated hemoglobin (HbA1c, %), low-density lipoprotein cholesterol (LDL-C, mg/dl), high-density lipoprotein cholesterol (HDL-C, mg/dl), triglycerides (TG, mg/dl), and C -reactive protein (CRP) measurements.

Patients were divided into tertiles according to the magnitude of percent weight loss; 1st tertile, Group-1: "mild weight loss; 2nd tertile, Group-2: "moderate weight loss"; 3rd tertile, Group-3: "high weight loss".

Metabolic syndrome criteria and cardiometabolic risk assessment

The following were accepted as diagnostic criteria for metabolic syndrome (MetS): Presence of at least one of these: insulin resistance (IR), diabetes mellitus (DM), or impaired glucose tolerance (IGT); Presence of any two of these: Hypertension (SBP≥130, DBP≥85 mmHg or antihypertensive use), Dyslipidemia (TG≥150 mg/dl or HDL-C <40 mg/dl in men, <50 mg/dl in women), presence of abdominal obesity (BMI)≥30 kg/m^2 or waist circumference ≥96 cm in men, ≥90 cm in women (based on TURDEP-II data) (10,11).

The Homeostasis Model Assessment (HOMA) formula, the most commonly used method in clinical practice, was used to detect the presence of insulin resistance. It has been reported that the HOMA value is lower than 2.7 in normal individuals, and a value above 2.7 reflects varying degrees of insulin resistance [HOMA: Fasting insulin (μU/mL) x Fasting plasma glucose (mg/dl)/405)] (11).

The definition of mean arterial pressure (MAP) is the average arterial pressure throughout one cardiac cycle; systole, and diastole. To perfuse vital organs requires 70-100 mmHg (minimum 60 mmHg) MAP. If the MAP drops below this point for an extended period, end-organ manifestations such as ischemia and infarction can occur. A standard method to estimate the MAP is the following formula: "MAP=DP+1/3(SP–DP)". This method is often more conducive to measuring MAP in most clinical settings as it offers a quick means of calculation if the blood pressure is known (14).

The metabolic syndrome z-score (MetS z-score)

To date, approximately 90% of the indices used in many studies for calculating cardiometabolic risk have been calculated using the sum or average of the statistical z-scores of the 5 MetS components. This analysis produces a score based on the individual's measurements of these components. This score behaves like a z-score in that it has a normal distribution with a mean of "0" and a standard deviation of "1". Technically, a z-score is the number of standard deviations from the mean of a given value. A z-score=0 for a given individual indicates that the value of that individual is equal to the population mean. Z-score=2 implies that the subject's population mean is two standard deviations above the mean, that is, "well above the mean", indicating MetS diagnosis. The components most frequently included were waist circumference (52%), triglycerides (87%), high-density lipoprotein cholesterol (67%), glucose (43%), and systolic blood pressure (52%) (12).

It cannot be excluded that there are different genetic and environmental controls on the expression of cardiometabolic risk factors, both in different racial/ethnic communities and in different genders. From this point of view, Gurka MJ et al. developed a gender and race/ethnic-specific equation to calculate the severity of MetS: MetS z-score (13). This calculator uses gender, ethnicity, height, weight, waist circumference, SBP, HDL-C, TG, and FBG parameters and is available online at https://metscalc.org/metscalc/. The calculator score is calculated in 2 different ways based on BMI (MetS z-score BMI) and waist circumference (MetS z-score waist). Both were calculated for each patient and used for this study.

This calculator, which uses IDF criteria, does not have a cut-offs value for the MetS z-score, and the CMR increases as the score value increases. Receiver operating characteristic (ROC) analysis has been used in studies to evaluate the differences of this score from the traditional definition of MetS, and cut-offs values were determined for the evaluated populations and interpreted (13).

In this study, the MetS z-score was evaluated by plotting receiver operating characteristic-(ROC) curves from which sensitivity and specificity were obtained to accurately classify patients at high and low cardiometabolic risk (CMR). The optimal thresholds for the MetS z-score to distinguish between low or high CMR were determined by calculating the Youden index.

Statistical analysis

Statistical analyses were performed in the SPSS 21 package program with an odds ratio (OR) and 95% confidence intervals (CI) and a significance level of $p<0.050$. Normality control was done by drawing Shapiro Wilk and single sample Kolmogorov Smirnov tests, box plot, Q-Q, and histogram graphs. Data were presented as mean and standard deviation (SD), frequency, and percentage. Normally distributed variables in the comparison of tertiles were compared using one-way ANOVA and t-test in independent groups and the others with the Mann-Whitney U test. Nominal variables were compared with Yates corrected chi-square and Fisher exact probability tests. Variables with a "p" value below 0.30 were included in the Binary Logistic Regression analysis.

The association of weight loss percentages with MetS z-score and cardiometabolic risk parameters: age, gender, HbA1c, FBG, HOMA, waist circumference, HDL-C, TG, and CRP were evaluated by Pearson's and Spearman's correlation. Statistical significance was accepted at $p<0.050$.

RESULTS

Sixty-six patients were included in the study. According to the conventional MetS criteria (10.11), the number of patients diagnosed with MetS was n=49 (74.2%) in the preoperative period and n=17 (25.8%) in the 6th postoperative month. The characteristics of the patients in the preoperative period are presented in Table 1.

In general, bariatric surgery provided a large and significant reduction in all parameters of the patients, regardless of the amount of weight loss (Table 2).

ΔWeight (kg) (p=0.000), ΔWeight (%) (p=0.000), ΔFM% (p=0.000), ΔEWL% (p=0.000), ΔWaist circumference (p=0.031), ΔFBG (p=0.038) and ΔHOMA (p=0.039) values were significantly different between groups with mild (Group-1), moderate (Group-2) and high (Group-3) weight loss.

ΔFBG and ΔHOMA values were found to be significantly lower at sixth months in the "High Weight Loss, Group-3", which had the lowest FBG and HOMA values before the operation. Changes in other CMR factors at 0-6 months did not differ significantly between groups (ΔMAP (p=0.752), ΔHbA1c (p=0.446), ΔTG (p=0.886), ΔHDL-C (p= 0.893), and ΔCRP (p=0.572), ΔMetS z-BMI (p=0.527) and ΔMetS z-bel (p=0.638)). The changes in Group-1, Group-2, and Group-3 over the period of 0-6 months are presented in Table 2 and Figure 1.

MetS z-score receiver-operating characteristic curve (ROC) was used to reflect Cardiometabolic risk assessed by conventional MetS criteria. The most appropriate thresholds for MetS z-score to distinguish the presence or absence of CMR were: Baseline: "1.282, p=0.003" and sixth months: "0.262, p=0.005" for MetS z-score BMI. Baseline: "1.006, p=0.000" and sixth months "0.203,

Table 1: Characteristics of the patients pre-operative and 6 months after surgery for weight loss tertiles

Variables	Mild weight loss Group-1 (n=22)		Moderate weight loss Group-2 (n=22)		High weight loss Group-3 (n=22)		p values	
	Pre-op	Post-op 6th months	Pre-op	Post-op 6th months	Pre-op	Post-op 6th months	Pre-op	Post-op 6th months
Age, year	40.29±12.36		38.00±11.77		36.82±8.45		0.648	
Weight, kg	141.42±25.51	108.02±18.61*	133.02±22.42	95.54±15.85*	142.99±39.70	92.62±26.98	0.594	0.087
Weight loss, %	23.30±2.97		28.12±1.40		35.28±3.32		**0.000**	
Fat mass, %	49.47±7.24	39.80±7.43*	47.19±6.68	34.20±8.59	49.77±7.20	31.22±8.04*	0.512	**0.011**
BMI, kg/m^2	51.08±7.49	38.97±5.64*	48.53±7.78	34.80±5.88*	49.75±8.66	32.32±7.43*	0.700	**0.013**
Waist, cm	136.88±19.71	115.73±15.31	134.52±13.95	107.00±10.61	135.58±23.41	108.46±19.15	0.256	0.309
MetS z-score BMI	2.06±1.10*	0.56±0.55	1.83±1.37	0.17±0.61	1.28±0.53*	-0.08±0.43	0.100	**0.004**
MetS z-score waist	1.80±1.05*	0.32±0.29	1.64±1.35	0.11±0.50	1.11±0.44*	-0.15±0.49	0.134	**0.034**
Fasting glucose, mg/dl	125.70±50.02	92.41±20.89	125.00±53.15*	80.92±11.64	93.72±11.08*	85.14±15.78	0.053	0.056
HbA1c, %	6.48±1.07*	5.45±0.74	6.36±1.58	5.10±0.43	5.73±0.74*	5.02±0.32	0.210	0.082
HOMA	8.60±5.97	2.25±1.10*	12.50±10.07*	1.92±1.18	5.97±2.94*	1.48±0.73*	0.060	0.104
Systolic blood pressure, mmHg	140.88±16.60*	126.91±24.14	130.00±12.99*	115.50±10.12	130.64±11.27*	124.00±8.43	0.398	0.273
Diastolic blood pressure, mmHg	84.70±13.04	78.00±13.71	81.76±11.71	73.50±4.11	80.88±12.77	74.50±5.98	0.880	0.499
MAP, mmHg	103.43±12.78	94.30±16.61	97.84±10.60	87.50±5.04	97.47±11.12	91.00±5.56	0.210	0.082
C-reactive protein, mg/L	9.19±6.51	4.66±3.18	11.95±8.22	1.92±1.18	8.79±5.88	1.48±0.73	0.590	0.479
HDL-C, mg/dl	45.70±9.98	47.43±8.67	43.58±10.77	45.96±7.45	43.76±8.67	46.00±7.42	0.611	0.834
LDL-C, mg/dl	125.58±20.28	102.43±27.55	122.52±32.25	107.87±36.79	125.17±24.84	103.87±29.72	0.934	0.881
Triglycerides, mg/dl	161.58±75.10	109.62±47.78	163.82±69.96	92.93±22.11	153.64±131.07	90.62±30.39	0.950	0.265

BMI: Body mass index, MetS: Metabolic syndrome, HbA1c: Glycated haemoglobin, HOMA: Homeostatic Model Assessment of Insulin Resistance, MAP: Mean Arterial Pressure, HDL-C: High-density lipoprotein-cholesterol, LDL-C: Low-density lipoprotein-cholesterol. "p" values that are significant by the ANOVA test are written in bold. *: p<0.050; t Test

Table 2: Changes in all cardiometabolic parameters of the patients 6 months after the bariatric surgery

Variables	Difference values (n=66)	Max-Min	Mild weight loss Group-1 (n=22)	Moderate weight loss Group-2 (n=22)	High weight loss Group-3 (n=22)	p values (intergroup difference)	p value (intragroup difference)
ΔWeight, kg	40.41±12.43	-7.00-149.00	33.40±8.52	37.47±6.94	50.36±13.98	*0.000*	**0.000**
ΔWeight, %	28.90±5.63	-13.98-41.30	23.30±2.97	28.12±1.40	35.28±3.32	*0.000*	**0.000**
ΔFat mass (%)	13.73±6.69	-0.40-34.10	9.67±5.67	12.99±4.49	18.54±6.68	*0.000*	**0.000**
ΔBMI, kg/m^2	14.42±3.58	7.00-23.60	12.11±2.24	13.73±2.20	17.42±3.79	*0.000*	**0.000**
ΔWaist, cm	30.41±9.11	8.00-50.00	26.35±7.20	30.41±10.06	34.47±8.44	*0.000*	**0.031**
ΔMetS z-score BMI	1.50±0.74	0.09-4.49	1.49±0.74	1.65±0.94	1.36±0.48	*0.000*	0.527
ΔMetS z-score waist	1.42±0.82	-0.44-4.78	1.47±0.75	1.53±1.12	1.27±0.49	*0.000*	0.638
ΔFasting glucose, mg/dl	28.82±33.89	-7.00-149.00	33.29±33.78	36.85±37.78	13.01±8.65	*0.000*	**0.038**
ΔHbA1c, %	1.08±1.09	-0.10-4.80	1.06±0.96	1.38±1.48	0.80±0.74	*0.000*	0.323
ΔHOMA	7.13±7.17	-1.54-39.82	6.34±5.76	10.53±9.99	4.51±2.70	*0.000*	**0.039**
ΔSBP, mmHg	10.74±13.61	-20.00-50.00	10.16±25.64	13.50±12.48	14.50±16.23	*0.000*	0.860
ΔDBP, mmHg	7.33±13.37	-30.00-50.00	5.75±20.25	6.50±10.01	9.50±16.90	*0.000*	0.861
ΔMAP, mmHg	8.47±11.40	-16.67-48.33	10.19±15.22	7.45±7.33	7.76±10.75	*0.000*	0.752
ΔC-reactive protein, mg/L	6.01±5.77	-4.55-21.46	5,17±6.16	7,20±5.99	5.66±5.27	*0.000*	0.572
ΔHDL-C, mg/dl	-1.58±7.96	-23.00-19.00	-1.47±8.33	-1.41±9.02	-1.88±6.85	0.160	0.983
ΔLDL-C, mg/dl	19.91±33.20	-97.00-81.00	22.75±34.45	14.68±40.19	22.31±24.67	*0.000*	0.750
ΔTriglycerides, mg/dl	60.54±85.16	-50.00-527.00	52.64±54.89	67.11±53.19	61.88±129.24	*0.000*	0.886

BMI: Body mass index, MetS: Metabolic syndrome, HbA1c: Glycated haemoglobin, HOMA: Homeostatic Model Assessment of Insulin Resistance, SBP: systolic blood pressure, DBP: Diastolic blood pressure, MAP: Mean Arterial Pressure, HDL: High-density lipoprotein-cholesterol, LDL: Low-density lipoprotein-cholesterol, *: p<0.050; t Test

p=0.003" for MetS z-score waist (Figure 2 and 3). Patients with higher scores than these cut-offs were considered to have higher CMRs. Accordingly, it was observed that the CMR of patients who lost less weight was higher than those who lost more (Table 3).

In the 6th month, the mean BMI of all patients in the groups was 35.36±6.83 kg/m^2, and the patients in all three groups were still in the "obese" class according to the BMI category (BMI values Group-1: 38.97±5.64 kg/m^2; Group-2: 34.80±5.88 kg/m^2; Group-3: 32.32±7.43 kg/m^2).

The high cardiometabolic risk, determined by preoperative MetS prevalence, was promoted in the low cardiometabolic risk class in 8% of patients in Group-2 who lost moderate weight and 11% in Group-3 who lost high weight at sixth months postoperatively. However, it was observed that "mild weight loss, Group-1" had a high cardiometabolic risk ratio of 60%.

At sixth months postoperatively, % weight loss showed a significant negative correlation with both MetS z-score BMI and MetS z-score waist, as well as glycemic parameters (FBP, HbA1c, HOMA) (Table 4). There was no relationship between the amount of weight loss and waist circumference, TG, HDL-C, LDL-C, MAP, SBP, DBP, and CRP values, each of which is a MetS component (Table 4).

Patients were classified as having high or low cardiometabolic risks according to the MetS z-score cut-offs values calculated six months after surgery, and Binary logistic regression was performed. It was determined that the probability of entering the low-risk group from the high-risk group in the cardiometabolic risk classification increased 0.57 times for each 1% weight loss of the patients (p=0.002). On the other hand, it was observed that the probability of remaining in the high cardiometabolic risk category of patients despite having undergone bariatric

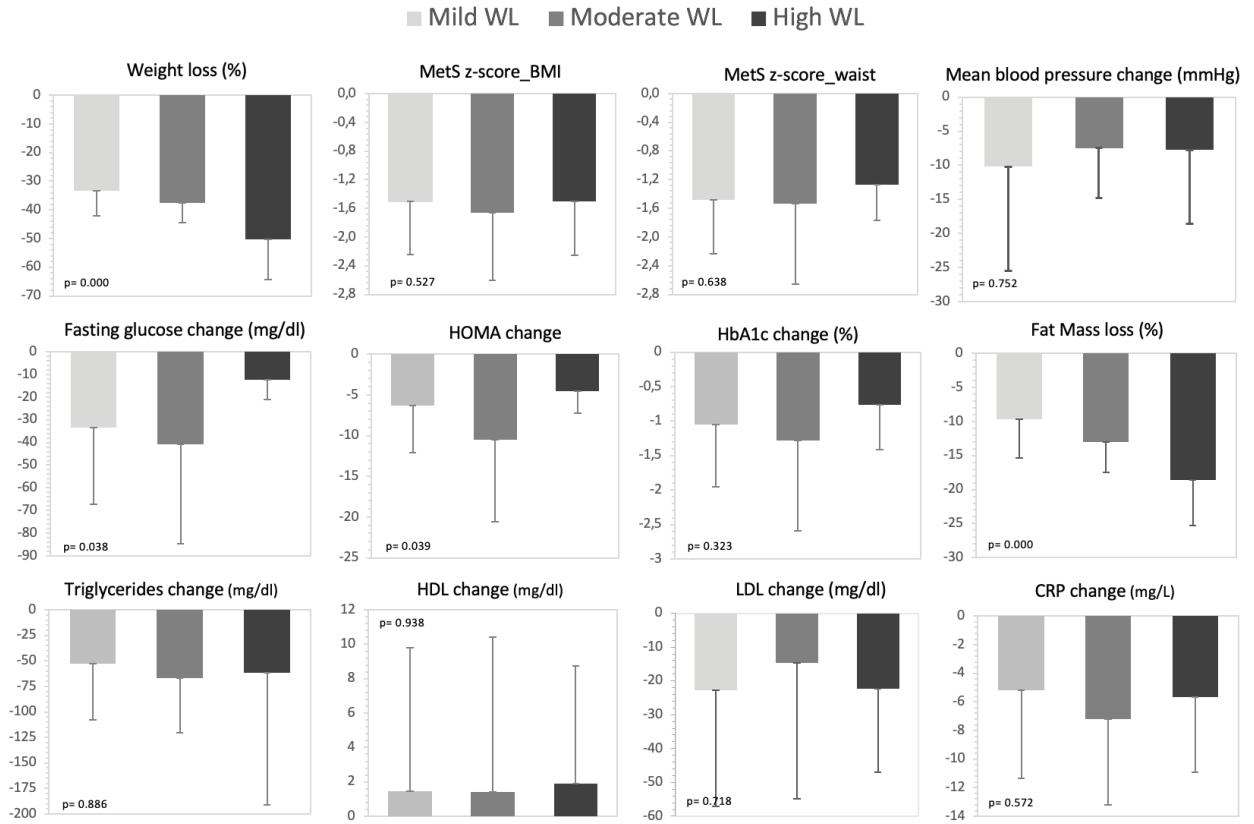

Figure 1: Comparison of changes in cardiometabolic risk parameters six months after surgery in high, moderate, and mild weight loss groups

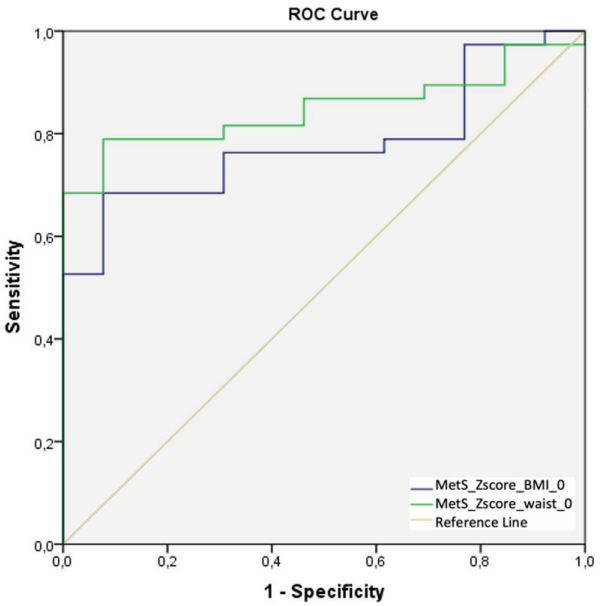

Figure 2: cMetS z-score ROC curve at pre-op for BMI and waist circumference

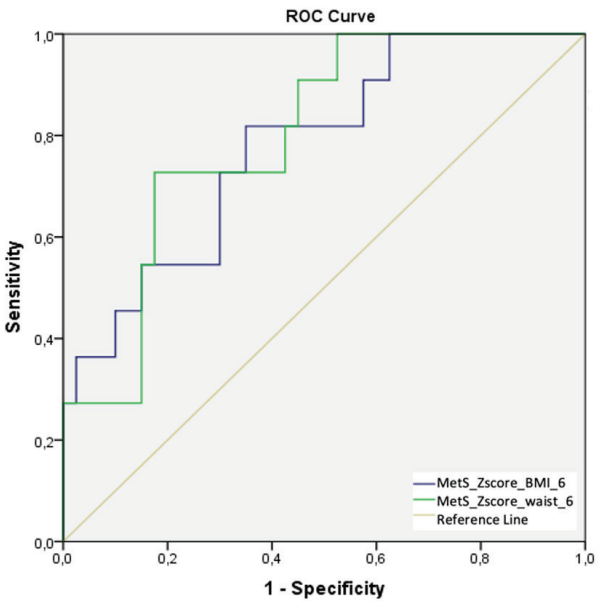

Figure 3: cMetS z-score ROC curve at 6 months for BMI and waist circumference

Table 3: Distribution of patients with high CMR in groups formed according to weight loss percentages

Variables	Mild weight loss (n%)	Moderate weight loss (n%)	High weight loss (n%)	p value
MetS z-scores BMI				
Baseline	40.0	33.3	26.7	>0.050
6th month	60.0	25.0	15.0	*0.004*
MetS z-scores waist				
Baseline	36.4	30.3	33.3	>0.050
6th month	47.4	31.6	21.1	>0.050

CMR: Cardiometabolic risk, MetS: Metabolic syndrome, BMI: Body mass index

Table 4: The relationship between weight loss (%) after bariatric surgery and values of cardiometabolic parameters at sixth months and changes at 0-6 months

Values	Weight loss, %			
	The values at 6th months		Change values between 0-6 months	
	p	r	p	r
MetS z-scores BMI	*0.000*	-0.566	0.569	-0.082
MetS z-scores waist	*0.001*	-0.470	0.272	-0.157
Waist, cm	0.119	-0.221	0.053	0.409
SBP, mmHg	0.986	-0.002	0.763	-0.043
Fasting blood glucose, mg/dl	*0.050*	-0.276	*0.042*	-0.285
HOMA	*0.016*	-0.337	0.624	-0.070
HbA1c, %	*0.010*	-0.357	0.426	-0.131
Triglyceride, mg/dl	0.061	-0.264	0.342	-0.136
HDL-C, mg/dl	0.643	-0.066	0.568	-0.082
C-reactive protein, mg/L	0.581	-0.079	0.365	0.130

MetS: Metabolic syndrome, BMI: Body mass index, SBP: systolic blood pressure, HOMA: Homeostatic Model Assessment of Insulin Resistance, HbA1c: Glycated haemoglobin, HDL-C: High-density lipoprotein-cholesterol

surgery increased 25.8 times (p=0.005) in the presence of TD2 and increased 0.20 times (p=0.013) if they were still in the obese class.

DISCUSSION

The main finding of our study is that although there was a significant decrease in all cardiometabolic risk parameters of the patients six months after bariatric surgery, no correlation was found between the amount of weight loss and any parameter except fasting blood glucose and HOMA. However, the statistically significant negative correlation between the change in the calculated MetS z-scores compared to the preoperative period, and the amount of weight loss suggested that the use of risk scores would be a more objective approach to evaluate the reduction in cardiometabolic risks of patients after bariatric surgery.

In the literature, the prevalence of MetS in morbidly obese patients before surgery varies (52% to 87%) (17, 18). In our study, the prevalence of MetS in the preoperative period was found to be approximately 74%, which is consistent with the rates reported in the literature. This rate, which decreased to 26% in the postoperative sixth months, is similar to the decrease rates in the literature (17-20).

It is accepted that the most important indicator of the success of treatment and superiority of bariatric surgery over other obesity treatments is the amount of weight lost after surgery (7). It is known that only a six-month period after bariatric surgery is sufficient for body weight to decrease in a way that positively affects primary cardiometabolic risk factors such as DM, hypertension, and dyslipidemia (21). In studies evaluating the change in car-

diometabolic risk during this period, there is a significant improvement after surgery in each risk parameter; however, no relationship has been shown between this improvement and the amount of weight loss (19,20). In their study, Gil S. et al. investigated the effects of the amount of fat mass lost instead of the total amount of weight loss. They showed that even if obesity remission is achieved after surgery, patients with higher fat mass loss have less insulin sensitivity and higher triglyceride levels and that, contrary to their previous findings, there is a significant relationship between the amount of fat mass loss and continued cardiometabolic risk score (16).

After RYGB, weight loss is very rapid because both food intake is reduced and malabsorption occurs, and it is essential to ensure the loss of fat mass by preserving lean body mass. For this reason, patients should be closely followed up on nutrition, and adequate protein intake should be ensured. In our study, when we made a separate evaluation of patients with a fat mass loss instead of weight loss, we did not obtain a different result from the previous one. We think this is because the total weight loss of the patients and the loss of fat mass are parallel to each other, and the lean body mass of the patients is preserved. In Goday A et al.'s study evaluating the factors affecting the decrease in cardiometabolic risk parameters, healthy obese patients with normal cardiometabolic risk parameters were compared to pathological ones in the pre-op period. Although the patients lost a similar amount of weight in the post-op period, it was observed that the values decreased more in the healthy obese group and reached healthier values than in the pathological group. In other words, in this study, it was emphasized that cardiometabolic risk reduction was associated with the pre-op health status of the patients, independent of weight loss (22). When we evaluated our study from this perspective, we also observed no significant difference between the weight and fat mass losses of those with pre-op MetS parameters within healthy limits and those with pathological parameters.

In the study by Honk YR et al. using the National Health and Nutrition Examination Survey (NHANES) 2015-2018, 6274 patients were divided into three groups: those who have undergone bariatric surgery but are still obese, obese patients who are candidates for surgery, and adults with normal weight (23). In this study, the selection bias of bariatric surgery was minimized using the propensity score weighting technique. Thus, only the effect of patients' current weight on cardiometabolic risk factors was evaluated. Although there was no significant difference between the parameters of patients who are still obese despite bariatric surgery and adults with normal weight, the cardiometabolic risk parameters of the obese who are surgical candidates were found to be different from the other two groups (23). This picture indicates that cardiometabolic risks are normalized mainly, even if a BMI within the normal range cannot be achieved after bariatric surgery, as we have seen in our study.

Studies show that insulin sensitivity in patients improves by 25% at one week after RYGB, without weight loss yet, and this is attributed to the post-op low-calorie diet. As a matter of fact, similar improvements in insulin sensitivity were observed in obese patients who followed the liquid diet prescribed after bariatric surgery for only four days without surgery. In the longer term, the effect on diabetes remission cannot, of course, be attributed to calorie restriction alone because the increase in GLP-1 secretion due to the mechanism of the operation was not observed in nonsurgical and hypocalorically fed obese subjects (24). On the other hand, in another study, three different weight maintenance diets with low, medium, and high carbohydrate contents were given to patients diagnosed with MetS for four weeks. At the end of the study, when patients were fed with low carbohydrates, it was observed that there was a significant improvement in TG and LDL-C levels without weight loss (25). In other words, lipid control as well, like glycemic control, can be modified by dietary content without weight loss. When we evaluate all these studies, it is not surprising that a low-calorie and low-carbohydrate nutrition program after surgery improves both glycemic and lipid parameters, independently of weight loss in the short term and with the effect of weight loss in the long term. The degree of improvement in the long term is closely related to the amount of weight lost by the patients. The frequency of care and follow-up visits of patients after the operation contributes to the success of the surgery. Studies show that 60% of patients who do not reach their target weight after surgery have no nutritional follow-up (26). For this reason, the importance of close follow-up of patients and nutritional guidance to get the most efficient results from surgery is indisputable.

The strongest aspect of this study is that although there are many studies evaluating the effect of bariatric surgery on metabolic syndrome parameters, it is one of the few studies evaluating its effect on cumulative cardiovascular risk using the MetS score. The weaknesses of the study are the small number of patients and the short follow-up period. Therefore, there is a need for studies in which more patients who were operated on with different types of bariatric operations (malabsorptive and restrictive) were followed for a more extended period.

In conclusion, although there is a significant decrease in cardiometabolic risk parameters after bariatric surgery, the amount of weight loss of the patients does not affect this. However, using a scoring system to evaluate and monitor the cardiometabolic cluster risks of patients will make it possible to follow the gradual changes in patients

after surgery, making the interventions of physicians and nutritionists more targeted and efficient.

Ethics Committee Approval: This study was approved by Istanbul University Istanbul Faculty of Medicine Clinical Research Ethics Committee (Date:24.06.2022; No:12).

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Author Contributions: Conception/Design of Study- B.F.Ç., U.B.; Data Acquisition- B.F.Ç., U.B.; Data Analysis/Interpretation- B.F.Ç., G.Y.Y., U.B.; Drafting Manuscript- B.F.Ç., G.Y.Y., U.B.; Critical Revision of Manuscript- B.F.Ç., G.Y.Y., U.B.; Final Approval and Accountability- B.F.Ç., G.Y.Y., U.B.; Material or Technical Support- B.F.Ç., U.B.; Supervision- B.F.Ç., G.Y.Y., U.B.

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OUR EXPERIENCE OF ENDOSCOPIC TRANSSPHENOIDAL SURGERY FOR CUSHING'S DISEASE: OUTCOMES AND COMPLICATION RATES IN 48 PATIENTS

CUSHİNG HASTALIĞINDA ENDOSKOPİK TRANSSFENOİDAL CERRAHİ DENEYİMLERİMİZ: 48 HASTADA SONUÇLARIMIZ VE KOMPLİKASYON ORANLARIMIZ

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ABSTRACT

Objective: This study aimed to analyze the results of patients who underwent the transsphenoidal endoscopic approach for Cushing's disease in our department and to determine the surgical outcomes, recurrence and complication rates.

Materials and Methods: A single-center retrospective study was performed on 48 patients who underwent endoscopic transsphenoidal surgery for Cushing's disease in our department between January 2005 and January 2019. Patients who underwent endoscopic transsphenoidal surgery received perioperative supraphysiological glucocorticoid therapy. Patients were evaluated for clinical features and basal cortisol levels without medication use in the last 24 hours when glucocorticoid therapy was reduced to a physiological dose. Patients were also evaluated with steroid replacement durations; the 3rd month, the first year and the last examination blood cortisol levels, 1mg dexametazon suppression test; MRI imaging post-operative in the first 24 hours, the 3rd month and the first year.

Results: A total of 48 patients underwent transsphenoidal endoscopic approach. Moreover, 38 patients (79.1%) had biochemical remission 1 year postoperatively. The mean follow-up of duration was 72 months. An additional recurrence of Cushing's disease was detected in 11 patients (22.9%). Consequent-

ÖZET

Amaç: Çalışmamızın amacı kliniğimizde endoskopik transsfenoidal cerrahi (ETC) yöntemle opere edilen Cushing hastalarının cerrahi sonuçları, nüks ve remisyon oranları ile komplikasyon oranlarını değerlendirmektir.

Gereç ve Yöntemler: Kliniğimizde Ocak 2005 – Ocak 2019 tarihleri arasında, ETC yöntemle opere edilen ve histopatolojik olarak ACTH salgılayan hipofiz adenomu tespit edilen olgular retrospektif olarak incelendi ve 48 hasta çalışmamıza dahil edildi. Steroid şemsiyesi altında opere edilen hastaların glukokortikoid replasmanı fizyolojik doza inildiğinde 24 saat ilaçsız iken bazal kortizol seviyesi ve klinik bulguları değerlendirildi. Hastalar steroid replasman süreleri, ilk 3. ay, 1. yıl ve son kontroldeki serum sabah kortizol ölçümleri ve 1 mg deksametazon supresyon testleri; ilk 24 saat içinde, 3. ay, 1. yıl kontrollerinde kontrastlı Manyetik Rezonans görüntülemeleri ile değerlendirildi.

Bulgular: Toplamda 48 hasta kliniğimizde ETC ile opere edilmiş olup bu hastaların 38'inde (%79,1) post-operatif ilk 12 aylık dönemde remisyon sağlandığı görüldü. Ortalama olarak 72 ay süreyle takip ettiğimiz bu hastalardan 11 (%22,9) tanesinde Cushing hastalığının nüks ettiği saptandı. Sonuç olarak uzun dönem takiplerimizde remisyonda kalan hasta sayısının 27 (%56,3) olduğu gözlenmiştir.

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ly, in our long-term results 27 patients (56.3%) remained in remission.

Conclusions: The mortality and morbidity rates of Cushing's disease are significantly decreased with treatment. Surgery is the first line treatment method for Cushing's disease. Transsphenoidal endoscopic surgery is a safe and effective treatment method for Cushing's disease with benefits such as better visualization, providing the opportunity to access parasellar regions, and lower complication rates.

Keywords: Cushing, surgery, endoscopic, transsphenoidal

Sonuç: Cushing hastalığı tedavi edildiği takdirde morbidite ve mortalite riski belirgin azalmaktadır. Cushing hastalığının seçkin tedavisi cerrahidir. Daha iyi bir görüş açısı ve parasellar bölgelere ulaşabilme imkânı sağlaması, daha az komplikasyon oranlarına sahip olması gibi nedenlerden dolayı ETC Cushing hastalığı tedavisinde etkin ve güvenli bir yöntemdir.

Anahtar Kelimeler: Cushing, cerrahi, endoskopik, transsfenoidal

INTRODUCTION

Cushing's disease was first described by Harvey Cushing in 1932, as a clinical condition caused by oversecretion of glucocorticoids due to an adrenocorticotropic hormone (ACTH)-secreting pituitary adenoma (1). The annual incidence rates are between 0.7 and 2.4 per million population, and it has a poor prognosis when not treated (2). In Harvey Cushing's case series, the average survival of patients is 4.6 years and cardiovascular diseases are the most common cause of increased mortality rates (1). Moreover, high blood cortisol levels lead to the developement of morbidities such as sarcopenia, osteopenia, central obesity, metabolic disease (dyslipidemia, diabetes mellitus etc.) and arterial hypertension. Previous studies have shown that these risks can be reduced if biochemical treatment is provided (3).

Endoscopic transsphenoidal surgery (ETS) is preferred as the first line treatment of Cushing's disease (4–8). However, survey results of approximately 30 centers within the borders of the USA has shown, that remission rates of these centers are highly variable and are between 10% and 90-100% (9). The major reasons for this variability are the centers' experience with ETS and surgical techniques.

This study aimed to analyze the results of patients who underwent ETS for Cushing's disease in our department and determine the surgical outcomes, recurrence and complication rates.

MATERIALS AND METHOD

The authors present a single-center (a third level reference hospital Istanbul University Faculty of Medicine, Department of Neurosurgery) retrospective review of 48 patients who underwent ETS for Cushing's syndrome. In our clinic, a council of specialists (neurosurgeon-neuroradiologist-endocrinologist) decide on the surgical treatment of pituitary adenomas. If a patient is diagnosed with Cushing's syndrome, but still have some inconsistencies regarding the clinical and radiological features; inferior petrosal sinus sampling (IPSS) is performed. Patients undergoing ETS received perioperative supraphysiological glucocorticoid therapy. In our clinic 69 patients underwent ETS between January 2005 and January 2019. Of these patients 48 who underwent preoperative detailed biochemical evaluations, pre- and postoperative magnetic resonance imaging (MRI), had histopathological diagnosis of ACTH-secreting pituitary adenoma, and were followed-up for at least 12 months postoperatively included our study. This study was approved by Istanbul University Istanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 31.05.2022, No: 09).

Biochemical diagnosis and remission criteria

In the initial testing for Cushing syndrome, the 1-mg overnight dexamethasone suppression test (DST) was performed, and 24-h urine free cortisol (UFC) levels, late-night salivary cortisol levels and 23:00 serum cortisol levels were obtained. After the 1-mg overnight DST after an 08:00 serum cortisol level >1.8 µg/dL (50 nmol/L), 24-h UFC level more than the normal range, late-night salivary cortisol level>145 ng/dL (4 nmol/L) and 23:00 serum cortisol level>7.5 µg/dL (207 nmol/L) were accepted as indicators of endogenous cause (10,11). As a verification test, we used the 48h 2mg/day DST. After the DST, if the following 08:00 serum cortisol level was >1.8 µg/dL (50 nmol/L), Cushing's syndrome was confirmed. After the verification of Cushing's syndrome, morning plasma ACTH level was determined. If the plasma ACTH level was >20 pg/mL, the patient was diagnosed with ACTH-dependent Cushing's syndrome. For differential diagnosis, pituitary imaging and an 8 mg DST were determined. After the 8 mg DST, if the serum cortisol levels reduced by >50% of the baseline level, the patient was evaluated for pituitary Cushing's disease. In cases without an adenoma larger than 6 mm in pituitary MRI, inferior petrosal sinus sampling (IPSS) performed (12). IPSS was accompanied by corticotrophin-releasing hormone level determination (100 µg), if the ratio of the third-minute central and peripheral ACTH level is >3, the condition is diagnosed as Cushing's disease, while if it is <2, it is diagnosed as Cushing's syndrome.

Patients were evaluated for clinical features and basal cortisol levels without medication in the last 24h when

glucocorticoid therapy was reduced to the physiological dose. Patients were also evaluated with steroid replacement durations; the 3rd month, the first year and the last examination blood cortisol levels, and 1 mg DST. Basal cortisol levels without medication in the last 24h<1.8 µg/dL (50 nmol/L); the third month, the first year and the last examination of blood basal cortisol levels after the 1-mg DST<1.8 µg/dl (50 nmol/L); the clinical regression of Cushing's disease symptoms and the patient's continuing need for steroid replacement are accepted as surgical cure criteria.

Radiologic evaluation

All patients underwent sellar magnetic resonance imaging (MRI) preoperatively, which was performed at 1.5 T in the sagittal and coronal planes with a 2.5-mm slice thickness, without an interslice gap. T1-weighted spin-echo images were obtained with a TR of 611 ms and TE of 8.9 ms, both before and after the administration of 10 mL of gadolinium contrast. T1-weighted images with gadolinium contrast were used to classify the tumors as microadenoma (<10 mm) and macroadenoma (≥10 mm). Follow-up MRI scans were performed on the first post-operative day and then at three months and one year post-operatively.

Surgical procedure

All patients were surgically treated using the pure endoscopic endonasal transsphenoidal approach. Most patients underwent surgery with uninostril technique. Although some patients had larger adenomas, a binostril and extended approach was preferred according to the surgeon's preference. Under general anesthesia, the patient was placed in the supine position, with routine rigid fixation of the head for fixed neuronavigational reference. Xylometazoline spray was administered on the nasal mucosa preoperatively. After adequate sterilization, cotton pieces soaked with adrenalin were applied. All surgeries were performed with a 0° rigid endoscope (Storz, Germany). The middle turbinate was lateralized to increase the surgical corridor, and the sphenoid sinus ostium was recognized. After gentle mobilization of the posterior wall of the nasal septum, the anterior wall of the sphenoid sinus was removed with a high-speed drill and rongeurs. The septum in the sphenoid sinus was removed cautiously if necessary, and then the sella was exposed. The anterior wall of the sella was removed using drill and rongeurs. The opening of the superior part of the wall was usually avoided to prevent an unnecessary risk of intraoperative cerebrospinal fluid (CSF) fistula.

The dura was opened with a horizontal incision, and the tumor was resected in fragments using forceps, ring curettes and an aspirator. For macroadenomas growing in the parasellar region, angled scopes (30° and 45°) were used additionally for gross-total resection. After the resection, the autologous grafts, fascia and fat tissue that were harvested from the abdominal wall and a fibrin sealant were used for duraplasty in each patient. A lumbar drain was placed postoperatively if the arachnoid membrane was opened during resection to prevent a CSF leak.

RESULTS

There were 12 male (25%) and 36 female patients (75%), and the mean age was 37.7±1.8 years (range, 17-68 years). Patients were followed for an average of 72 months (interquartile range 82.5; range 12-264 months) postoperatively. Twenty eight (58.3%) patients presented with weight gain; seven (15%) presented with partial vision loss; seven (15%) patients presented with irregular or absent menstrual periods and six (12.5%) presented with headaches. Of these patients 29 (60.1%) had a Cushingoid appearance (weight gain and fatty tissue deposits, particularly around the midsection and upper back, in the face (moon face), and between the shoulders (buffalo hump). A total of 15 (31%) macroadenomas (>10mm) and 27 (56.2%) microadenomas (<10mm) were detected in MRI. The MRI of 6 patients (12.5%) did not show any adenoma. Cavernous sinus invasion was detected in 32 (43.83%) of 73 patients. Twelve (24%) of these patients who had inconsistency between clinical and radiological features underwent IPSS preoperatively.

Twenty-two (45.8%) of 48 patients attained biochemical remission at the early postoperative period, and 13 additional patients (27%) were in remission based on their 12-month postoperative hormone levels. In the early postoperative MRI findings, 3 (6.2%) patients had residue adenoma. In their follow-up, 7 of the 13 patients who were not in remission after postoperatively, underwent a second surgery. Subsequently, two of these seven patients underwent remission. One patient who was not in remission after the first surgery, developed pituitary insufficiency and had remission while planning for a second surgery. Eight (16.7%) patients who were not in remission, received radiotherapy post-operatively and two of them were then in remission. Two patients who were not in remission postoperatively, underwent a bilateral adrenalectomy. One of these patients developed Nelson's syndrome, and underwent a second ETS, and the pituitary adenoma was totally resected. One patient who was not in remission underwent surgery for left adrenal adenoma two years after ETS, and had remission.

A total of 38 patients (79.1%) attained biochemical remission in the first 12-months post-operatively. There were 11 (22.9%) cases of reccurrence in the long-term follow-up. Consequently, in our long-term follow-up 27 patients (56.3%) had remission after surgery. The remission rates of patients are summarized in Table 1.

Table 1: Patient characteristics and remission-recurrence rates

Age at surgery, years	
Mean (range)	37.7 (17-68)
Gender, n (%)	
Female	36 (75%)
Male	12 (25%)
Follow up, (months)	
Mean (range)	72 (12-264)
Tumor type, n (%)	
Macroadenoma (>10mm)	15 (31%)
Microadenoma (<10mm)	33 (69%)
Remission-recurrence rates, n (%)	
Short-term (<12 months) remission	38 (79.2%)
Recurrence	11 (22.2%)
Long-term (>12 months) remission	27 (56.3%)

Of 48 patients who were included our study, 10 (20.8%) had arachnoid membrane lacerated. A lumbar drainage system was placed to these patients postoperatively and followed as immobile to prevent a CSF leak. Of the 10 patients with a lumbar drainage system, one developed rhinorrhea. This patient underwent endoscopic repair surgery for a CSF leak. In the follow-up of the same patient, meningitis and hydrocephalus developed. After appropriate antibiotic therapy, a ventriculoperitoneal shunt placed in this patient. Thirteen (27%) patients had transient diabetes insipidus post-operative. One (2%) patient had hyponatremia, one (2%) patient had hypopituitarism and appropriate replacement therapy was administered. One (2%) patient developed a postoperative hemorrhage in the operative area and was treated conservatively (Table 2).

Table 2: Complication rates in our study

Complication rates	**n (%)**
CSF fistula	10 (20.8)
Diabetes Insipidus	13 (27.1)
Hyponatremia	1 (2.1)
Hypopituitarism	1 (2.1)
Hemorrhage	1 (2.1)

CSF: Cerebrospinal fluid

DISCUSSION

Since being initially described by Harvey Cushing, Cushing's disease is more common in female patients, and previous studies showed the male/female ratio as between 1:2 and 1:15 (2,3). Although the exact cause of Cushing's disease predominance in female patients is unknown, some studies suggest that corticotropic adenoma tissue is more sensitive to estrogen (11,12). In our study, in line with previous studies Cushing disease is more common in female patients and the female/male patient ratio is 2.7.

In our study, the short-term (≦12 months) remission rate of patients was 79.1% , and the recurrence rate of patients was 22.9%. Additionally, our long-term (>12 months) remission rate was 56.3%. The remission and recurrence rates in our study were found to be similar to those of previous studies (9).

When meta-analyses are examined, microscopic transsphenoidal surgery and ETS have similar remission rates, approximately 80%. Both surgical techniques have similar recurrence rates (10%) and short-term mortality rates (<0.5%). Patients who underwent ETS have higher CSF fistula rate (12.9% vs 4.0%) and lower rates of transient diabetes insipidus (11.3% vs 21.7%) post-operatively. Especially in macroadenomas, ETS has better remission rates (76.3% vs 59.9%) and a lower recurrence rate (1.5% vs 17%) compared to microscopic transsphenoidal surgery (13). In our clinic, we are using ETS for Cushing's disease, because of its better remission rate (especially for patients with macroadenoma), better and wider surgical visualization, the opportunity to access the parasellar regions, a lower risk of nasal trauma peroperatively, greater comfort for patients and lower complication rates.

The most common complications of ETS in our study are transient diabetes insipidus (27%) and arachnoid membrane laceration (20.8%). If arachnoid membrane laceration occurs during surgery, a lumbar drainage system was placed postoperatively, and patients are followed as immobile to prevent a CSF leak. With this procedure, a CSF leak was prevented in nine of ten with an intraoperative arachnoid membrane rupture. One (2%) patient had hyponatremia, one (2%) had hypopituitarism and appropriate replacement therapy was administered.

The limitations of this retrospective study, are the inability to measure the early postoperative basal cortisol level because of perioperative supraphysiological glucocorticoid therapy, and the absence of the 24h UFC test of most patients because of not being routinely checked in our clinic. For this reason, remission in patients who underwent surgery was evaluated in the early postoperative period with clinical features and basal cortisol levels without medication use in the last 24 hour when glucocorticoid therapy was reduced to a physiological dose. It is also reported that there is no correlation between the short and long term remission rates in the literature (13).

CONCLUSION

Surgery is the first-line treatment in Cushing's disease. Nowadays ETS is increasingly used as the first surgical option in the treatment of Cushing's disease due to its effectiveness and safety.

Ethics Committee Approval: This study was approved by Istanbul University Istanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 31.05.2022, No: 09).

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EUTHYROID SICK SYNDROME: PREVALENCE AND PROGNOSIS IN ELDERLY PATIENTS WITH SEPSIS

ÖTİROİD HASTA SENDROMU: SEPSİSLİ YAŞLI HASTALARDA SIKLIĞI VE PROGNOZU

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ABSTRACT

Objective: Euthyroid sick syndrome (ESS) manifests by the decreased level of serum free thyroid hormones and with the normal or decreased secretion of thyroid-stimulating hormone. The study aims to identify the prevalence of ESS in elderly patients with sepsis and evaluate its possible effect on prognosis and mortality.

Material and Methods: Two hundred and three patients diagnosed with sepsis were prospectively evaluated. They were divided into two groups, a geriatric group (≥65 years), and a control group (<65 years). Patients with low free T3 (fT3) and/or free T4 (fT4) were considered to have euthyroid-sick syndrome. The demographic characteristics, comorbidities, laboratory results, hospitalization data, intensive care unit (ICU) duration, treatment outcomes, and mortality rates of the patients were recorded and compared.

Results: The incidence of ESS was significantly higher in the geriatric group (88.5% vs. 77.8%) (p=0.04). There was no difference between the groups in regard to mortality rate and ICU stay. However, 91.3% of deceased patients in the entire group and all deceased patients in the geriatric group were ESS (+) patients. The mean fT3 was significantly decreased in the deceased patients, and ICU patients (p=0.017). Additionally, the decreased levels of fT4 in both the entire group and the geriatric group were significantly associated with mortality and ICU stay (p-value: 0.020 and 0.019, respectively).

ÖZET

Amaç: Ötiroid hasta sendromu (ÖHS), serum serbest tiroid hormonlarının azalması ve tiroid stimülan hormonun normal veya azalmış sekresyonu ile kendini gösterir. Bu çalışmada, sepsisli yaşlı hastalarda ÖHS prevalansının belirlenmesi ve prognoz ve mortalite üzerindeki olası etkisinin değerlendirilmesi amaçlandı.

Gereç ve Yöntem: Sepsis tanısı alan 203 hasta prospektif olarak değerlendirildi. Hastalar geriatrik grup (≥65 yaş) ve kontrol grubu (<65 yaş) olarak iki gruba ayrıldı. Serbest T3 (sT3) ve/veya serbest T4 (sT4)'ü düşük olan hastalar ötiroid hasta sendromu olduğu kabul edildi. Hastaların demografik özellikleri, komorbiditeleri, laboratuvar sonuçları, hastaneye yatış verileri, yoğun bakım ünitesi (YBÜ) ihtiyacı ve kalış süreleri, tedavi sonuçları ve mortalite oranları kaydedildi ve karşılaştırıldı.

Bulgular: Geriatrik grupta ÖHS insidansı anlamlı olarak daha yüksekti (%88,5 vs %77,8) (p=0,04). Gruplar arasında mortalite ve YBÜ ihtiyaçları açısından anlamlı fark saptanmadı. Bununla birlikte, tüm hastalarda ölen hastaların oranı %91,3 ve geriatrik gruptaki ölen hastaların ise tamamı ÖHS (+) hastalardı. Ölen hastalarda ve YBÜ hastalarında ortalama sT3 düzeyleri anlamlı olarak azalmıştı (p=0,017). Ek olarak, hem tüm grupta hem de geriatrik grupta azalmış sT4 seviyeleri mortalite ve yoğun bakım ihtiyaçları ile anlamlı olarak ilişkiliydi (p-değeri: sırasıyla 0,020 ve 0,019).

Sonuç: Sepsisli yaşlı hastalarda ÖHS prevalansının (%88,5) daha yüksek olduğu açıkça gösterilmiştir. Ek olarak, ÖHS (+) grubunda

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Conclusions: The higher prevalence of ESS (88.5%) in elderly patients with sepsis was clearly demonstrated. Additionally, approximately two-fold higher mortality and ICU stay risk was documented in the ESS (+) group. Thus, simply screening of ESS in septic elderly patients will certainly contribute to treatment decisions and early prediction of complicated clinical course and poor prognosis.

Keywords: Sepsis, euthyroid sick syndrome, thyroid hormones

yaklaşık iki kat daha yüksek mortalite ve YBÜ kalış riski belgelenmiştir. Bu nedenle, sepsisi olan yaşlı hastalarda ÖHS'nin basitçe taranması, tedavi kararlarına ve karmaşık klinik seyrin erken tahminine ve kötü prognozun belirlenmesine kesinlikle katkıda bulunacaktır.

Anahtar Kelimeler: Sepsis, ötiroid hasta sendromu, tiroid hormonları

INTRODUCTION

Sepsis is defined as a life-threatening acute organ dysfunction, secondary to infection. Sepsis is the result of an uncontrolled systemic inflammatory response which leads to high morbidity and mortality. Over than 19 million people are estimated to have developed sepsis, which is responsible for over 6 million deaths annually worldwide (1). While more than half of sepsis cases are documented in the elderly population due to the numerous risk factors such as comorbidities, the incidence of sepsis-related mortality dramatically increases with advanced age (2). There are several scoring systems to predict mortality in patients with sepsis. Currently, quick Sequential Organ Failure Assessment (SOFA) is the most commonly used scoring system for organ dysfunction (3).

Additionally, metabolic changes and hormonal alterations may occur during sepsis. Thyroid hormones are often more affected in this process. Euthyroid sick syndrome (ESS), also known as nonthyroidal illness syndrome (NTIS), manifests by the decreased levels of serum thyroid hormones [T3, free T3, and/or thyroxine (T4)], normal or decreased secretion of thyroid-stimulating hormone (TSH) with higher reverse T3 levels, and normal thyroid hormone function (4).

The prevalence of ESS has been documented as a prognostic risk factor particularly in patients with severe/critical diseases. ESS is also associated with prolonged ICU stay, end stage organ dysfunction, poor prognosis and mortality (5). The prevalence of ESS is significantly higher in geriatric patients particularly with comorbid chronic diseases or malignancies (6). However, the predictive value of thyroid hormone levels as a prognostic marker has yet been debated to date. Furthermore, the number of published data evaluating ESS in elderly patients with sepsis is limited. This study aims to identify the prevalence of ESS in elderly patients with sepsis and evaluate the possible effect on prognosis and mortality.

MATERIALS AND METHODS

The present study was performed between March 2014 and February 2015 in the Department of Internal Medicine in Istanbul Faculty of Medicine with the approval of Clinical Research Ethics Committee (Date 21.06.2013, No: 12). Two hundred and three patients diagnosed with sepsis, according to the Surviving Sepsis Campaign Guidelines 2012 criteria, were prospectively evaluated. Written informed consent was obtained from the patients in the study. Patients with malignancies, chronic kidney failure, congestive heart failure, or thyroid dysfunction and patients using drugs that may affect thyroid hormone levels were excluded from the study. Patients were divided into two groups, the geriatric group age over 65 years and the control group aged between 18 to 65 years. The quick Sequential Organ Failure Assessment (qSOFA) scoring system was utilized in the evaluation of organ dysfunction. Patients with low free T3 and/or free T4 were considered to have ESS.

In addition, the demographic characteristics, detailed anamnesis, presence of comorbidities, physical examination findings, laboratory findings, hospitalization data, intensive care unit (ICU) stay, ICU duration, treatment follow-up outcomes, and mortality rate were recorded and compared.

The blood cell count analysis was performed from peripheral blood samples. Hematological parameters were analyzed using a hematology analyzer (Cell-Dyne 3700, Abbott, Abbott Park, IL, USA). Biochemical analysis was performed from serum samples by an electro-chemiluminescence immunoassay analyzer (Beckman Coulter Unicel DXI 800, Brea, CA, USA). The analysis of serum hormone levels was performed using an immunodiagnostic system (Siemens, Advia Centaur xp, Germany).

Statistical analysis

Data were analyzed with SPSS for Windows (v21.0; IBM, Armonk, NY, USA) and presented with descriptive statistics. The normality of data distribution was identified with the Kolmogorov-Smirnov test. A comparison of the variables with normal distribution was made with a Student t test. Mann Whitney and Kruskal Wallis tests were used to compare non-normally distributed variables. The categorical variables were assessed using the Chi-Square test. The presence of correlation was analyzed

with Spearman's Rho or Pearson tests. Multivariable analysis was performed by logistic regression method. P-Values of <0.05 were considered statistically significant.

RESULTS

Two hundred and three patients with sepsis were included in the study, and of these, 93 were female (45.8%). The mean age was 63.49±18.03 years (ranged=18-94 years) in our sample group. Of the patients, 122 (60.1%) were in the geriatric group and 81 (39.9%) were in the control group. The most common infection source was pneumonia with a rate of 61.6% (n=125) in both groups (geriatric 68% vs. control 51.9%) and followed by urosepsis (12.8%, n=26), cholangitis (4.4%, n=9), necrotic pancreatitis (3%, n=6), empyema (2%, n=4), febrile neutropenia (2%, n=4), cellulite (2%, n=4), abscess (2%, n=4), and pyelonephritis (1.5%, n=3).

The comparison of the clinical and laboratory characteristics of the study participants is presented in Table 1. The mean values of neutrophile, hemoglobin, hematocrit, fasting blood glucose (FBG), and blood urea nitrogen (BUN) were found statistically higher in the geriatric group compared to the control group. Similarly, the mean values of TSH and erythrocyte sedimentation rate (ESR) were significantly lower in the geriatric group than the control group. Additionally, no statistically significant difference was observed regarding fever, respiratory rate

Table 1: Comparison of the baseline clinical and laboratory characteristics of the groups

	Control group	Geriatric group	p-value*
Body temperature (°C)	38.31±0.90	38.37±1.03	0.219
Respiratory rate	24.74±3.95	25.40±4.29	0.200
Heart rate	111.27±14.57	108.30±12.50	0.092
FB Glucose (mg/dL)	122.50±54.74	146.27±75.27	**0.017***
Hemoglobin (g/L)	9.71±2.58	10.34±2.15	**0.017***
Hematocrit (%)	29.34±2.58	31.56±6.64	**0.029***
Platelet (x10^9/L)	207.6±190.0	222.9±121.2	0.051
WBC (10^6/uL)	12.80±12.89	13.81±7.14	0.082
Neutrophile (x10^9/L)	10.53±11.61	11.55±6.91	**0.044***
Lymphocyte (x10^9/L)	1.208±1.272	1.319±1.501	0.117
BUN (mg/dL)	28.06±24.56	34.86±25.81	**0.003***
Creatinine (mg/dL)	1.98±2.09	1.75±1.51	0.194
AST (U/L)	39.37±73.21	39.05±69.56	0.095
ALT (U/L)	36.13±86.63	35.82±80.91	0.657
GGT (IU/L)	84.08±90.69	82.88±119.5	0.117
ALP (IU/L)	140.59±137.8	125.2±119.5	0.121
CRP (mg/dl)	244.2±141.1	208.5±127.8	0.094
Procalcitonin (ng/mL)	13.07±31.39	9.10±18.56	0.522
ESR (mm/h)	88.81±34.05	73.63±34.04	**0.002***
Albumin (g/dL)	2.94±0.67	3.01±0.58	0.388
Total bilirubin (mg/L)	1.79±4.61	1.23±3.59	0.523
Direct bilirubin (mg/L)	2.02±7.72	0.81±2.70	0.666
TSH (uIU/mL)	1.90±1.52	1.51±1.74	**0.005***
fT4 (ng/dL)	15.01±4.31	14.53±4.14	0.432
fT3 (ng/dL)	2.38±0.98	2.28±0.79	0.449

*: p<0.05 statistically significant, FB: Fasting blood, WBC: White blood cell, BUN: Blood urea nitrogen, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma glutamyl transferase, ALP: Alkaline phosphatase, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, TSH: Thyroid-stimulating hormone, fT4: Free thyroxin, fT3: Free triiodotyronine

(min.), heart rate, leukocyte, lymphocyte, platelet count, creatinine, liver function test aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transferase (GGT) and total/direct bilirubin levels, C-reactive protein (CRP), and procalcitonin (PCT) as well as fT3 and fT4 levels between the groups (Table 1).

The rate of septic shock development was more frequent in geriatric patients in comparison to the control group (32.1% vs. 17.2% p=0.014). ESS developed in a total of 171 (84.2%) patients, 108 of which (88.5%) were in the geriatric group and 63 (77.8%) were in the control group. The incidence of ESS was significantly higher in geriatric group (p=0.04) than controls. There was no statistically significant difference between the control and geriatric groups in terms of mortality (12.3% vs. 10.7%) and ICU stay (24.7% vs. 18.0%) (p=0.435). There was also no statistically significant difference between the groups regarding qSOFA score (p=0.310) (Figure 1) (Table 2). The rate of septic shock was significantly higher (33.8% vs. 18.1%) in patients who developed mortality (p=0.013). Age and gender did not differ with mortality (p=0.180 and p=0.331, respectively).

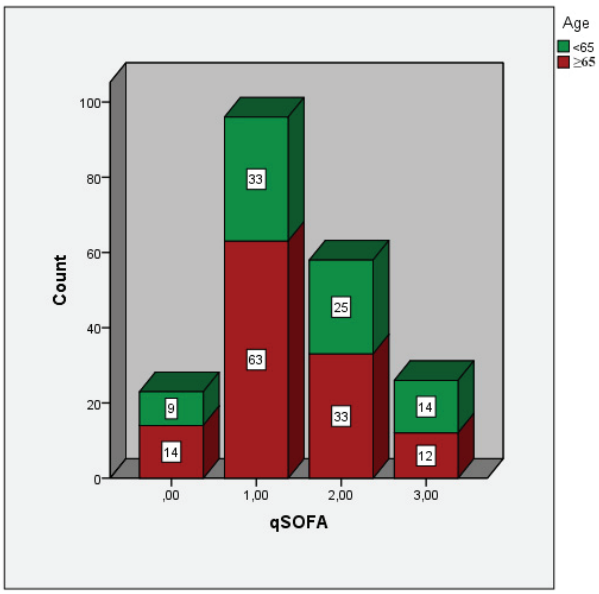

Figure 1: Distribution of the qSOFA score between the groups

Table 2: Comparison of the clinical characteristics between the groups

	Control group n (%)	Geriatric group n (%)	p-value
Septic shock	26 (32.1%)	21 (17.2%)	**0.014***
ICU	20 (24.7%)	22 (18.0%)	0.251
Mortality	10 (12.3%)	13 (10.7%)	0.435
ESS	63 (77.8%)	108 (88.5%)	**0.004***
qSOFA score			
0	9 (11.1%)	14 (11.5%)	
1	33 (40.7%)	63 (51.6%)	0.310
2	25 (30.9%)	33 (27.0%)	
3	14 (17.3%)	12 (9.8%)	

*: p<0.05 statistically significant, ICU: Intensive Care Unit, ICU: Intensive Care Unit, ESS: Euthyroid Sick Syndrome, qSOFA: quick Sequential Organ Failure Assessment

In the present study, the mean creatinine and blood urea nitrogen (BUN) values were significantly higher in ESS (+) group than the mean values in ESS (-) group (p=0.010 and p=0.011, respectively). In addition, the mean albumin, fT3, and fT4 values were significantly lower in ESS (+) group than the mean values in the ESS (-) group (p<0.001, p<0.000 and p<0.000, respectively) (Table 3). There was no statistically significant difference according to the septic shock rates, mortality, malignancy, ICU stay, gender, and qSOFA score between the ESS (+) and (-) groups (Table 4). There was also no statistically significant difference according to the mortality (p=0.187), ICU stay (p=0.172), gender (p=0.302), and qSOFA score (p=0.132) between the ESS (+) and (-) groups within only the elderly patients. Both 91.3% of all patients (n=23/21) who died in the entire sample group and all patients (n=13) who died in the geriatric group (≥65 years) were ESS (+) patients. The mean fT3 levels were significantly decreased in non-survived and ICU patients (2.42 vs. 2.11 ng/dL) (p=0.017) (Figure 2). Additionally, decreased levels of fT4 in both the entire group (15.19 vs. 13.72 ng/dL) and the geriatric group (15.09 vs. 13.15 ng/dL) were significantly associated with mortality and ICU stay (p=0.020 and p=0.019, respectively) (Figure 3). TSH levels were not significantly associated with mortality or ICU stay (p=0.255). In addition, the increased levels of procalcitonin in both the entire group (8.93 vs. 24.41 ng/mL) and the geriatric group (7.04 vs. 26.31 ng/mL) were significantly associated with mortality (p<0.001 and p=0.006, respectively). In addition, the increased levels of PCT were significantly associated with mortality in ESS (+) patients both in the control group (8.56 vs. 28.69 ng/mL) and geriatric group (12.48 vs. 16.50 ng/mL) (p=0.001 and p=0.01, respectively).

In multivariable analysis, higher qSOFA scores (B:3.08, p<0.001, odds ratio [OR]: 21.8, 95% confidence interval [CI]: 9-52), higher PCT levels (B: 0.03, p=0.004, OR: 1.03, 95 % CI: 1.01-1.06), the presence of hypotension (B: 3.4, p<0.001, OR: 30.8, 95% CI: 6.6-143) in entire group, higher qSOFA scores (B:2.04, p<0.001, OR: 7.7, 95% CI: 3.5-17), and lower fT4 levels (B:-0.13, p=0.034, OR: 0.87, 95% CI: 0.77-0.99) were independently associated with mortality in patients with geriatric population.

Table 3: Comparison of the baseline clinical and laboratory characteristics between euthyroid-sick syndrome (ESS) (-) and (+) groups

	ESS (-)	ESS (+)	p-value*
Age	59.25±19.89	64.28±17.61	0.185
Body temperature (°C)	38.37±1.08	38.34±0.96	0.360
Respiratory rate	25.28±3.83	25.11±4.23	0.914
Heart rate	109.46±12.18	109.49±13.66	0.817
FB glucose (mg/dL)	126.37±40.58	138.74±72.66	0.727
Hemoglobin (g/L)	10.67±3.20	9.98±2.14	0.378
Hematocrit (%)	32.58±9.37	30.31±6.58	0.199
Platelet (x10^9/L)	193.9±124.9	221.0±156.7	0.564
WBC (10^6/uL)	13.21±11.40	13.45±9.54	0.675
Neutrophile (x10^9/L)	10.56±9.735	11.25±8.970	0.538
Lymphocyte (x10^9/L)	1.248±0.924	1.280±1.488	0.826
BUN (mg/dL)	23.65±20.82	33.74±26.00	**0.011***
Creatinine (mg/dL)	1.29±1.35	1.95±1.81	**0.010***
AST (U/L)	31.37±37.83	40.64±75.44	0.747
ALT (U/L)	30.40±35.84	36.98±89.16	0.883
GGT (IU/L)	83.31±122.1	83.37±106.4	0.868
ALP (IU/L)	115.2±76.57	134.40±134.3	0.933
CRP (mg/dl)	199.0±117.8	227.2±136.7	0.315
Procalcitonin (ng/mL)	7.74±13.2	11.23±26.0	0.324
ESR (mm/h)	71.43±39.60	81.23±33.69	0.260
Albumin (g/dL)	3.32±0.68	2.92±0.58	**0.001***
Total bilirubin (mg/L)	1.14±2.04	1.51±4.30	0.612
Direct bilirubin (mg/L)	0.78±1.82	1.39±5.75	0.283
TSH (uIU/mL)	1.28±1.09	1.73±1.74	0.300
fT4 (ng/dL)	18.12±3.47	14.08±4.03	**<0.001**
fT3 (ng/dL)	3.59±0.56	2.08±0.70	**<0.001**

*: p<0.05 statistically significant, FB: Fasting blood, WBC: White blood cell, BUN: Blood urea nitrogen, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, GGT: Gamma glutamyl transferase, ALP: Alkaline phosphatase, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, TSH: Thyroid-stimulating hormone, fT4: Free thyroxin, fT3: Free triiodotyronine

Table 4: Comparison of the clinical characteristics between euthyroid-sick syndrome (ESS) groups

	ESS (-) n (%)	ESS (+) n (%)	p-value
Age			
<65 years	18 (56.3%)	63 (36.8%)	**0.004***
≥65 years	14 (43.8%)	108 (63.2%)	
Septic shock	6 (18.8%)	41 (24.0%)	0.520
ICU	4 (12.5%)	38 (22.2%)	0.188
Mortality	2 (6.3%)	21 (12.3%)	0.214
Gender			
Female	17 (53.1%)	76 (44.4%)	0.366
Male	15 (46.9%)	95 (55.65)	
qSOFA Score			
0	5 (15.6%)	18 (10.55)	
1	17 (53.1%)	79 (46.2%)	0.520
2	6 (18.8%)	52 (30.4%)	
3	4 (12.5%)	22 (12.9%)	

*: p<0.05 statistically significant, ICU: Intensive Care Unit, ICU: Intensive Care Unit, ESS: Euthyroid Sick Syndrome, qSOFA: quick Sequential Organ Failure Assessment

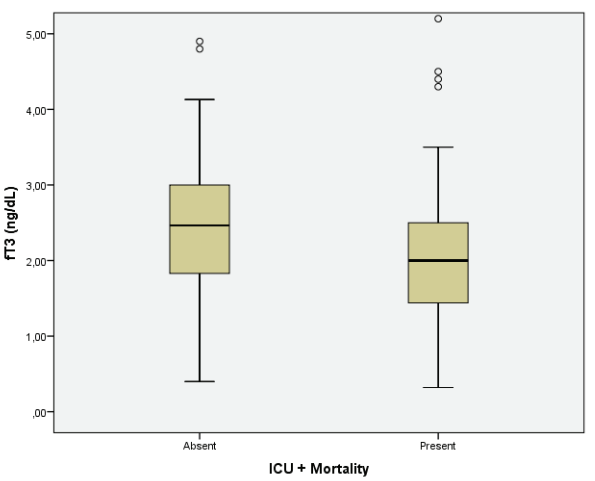

Figure 2: Alteration of the mean fT3 levels in accordance with mortality and ICU stay

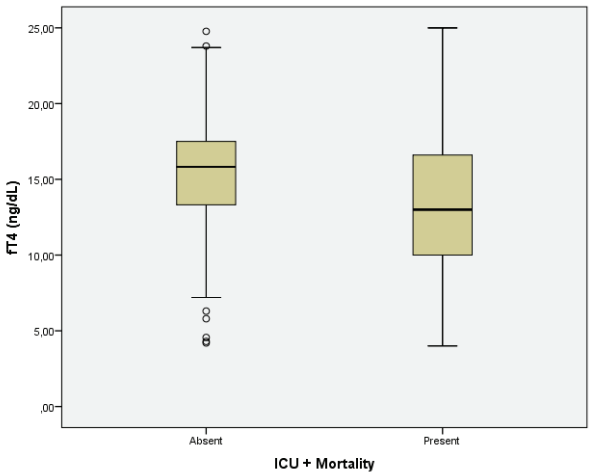

Figure 3: Alteration of the mean fT4 levels in accordance with mortality and ICU stay

DISCUSSION

In our study, the incidence of ESS was significantly higher in the geriatric group, but there was no statistically significant difference between the control and geriatric groups in terms of mortality and ICU stay. Mean fT3 levels, decreased levels of fT4, and increased levels of PCT were significantly associated with mortality and ICU stay. Elderly patients are known to be more prone to develop sepsis than the younger population due to several risk factors, such as comorbidities, invasive interventions, malnutrition, and immune system disorders. Moreover, sepsis typically occurs with more severe clinical course and potentially increased mortality risk in older patients (7). Respiratory tract infections are documented to be the most common origins of sepsis in the published data (8). Cheng et al. reported respiratory tract infections as the most common source of sepsis in their both sample groups (35.5% and 55.0%, respectively) which involves elderly (n=4414) and non-elderly (n=2673) patients (9). Supporting this, the most frequent infection source was pneumonia, with a rate of 61.6% in both groups (geriatric 68% vs. control 51.9%), in the present study.

It has been documented that blood glucose levels increase even in non-diabetic patients diagnosed with sepsis. Furthermore, blood glucose levels and glucose variability were significantly associated with sepsis severity (10). Sim et al. reported significantly increased glucose levels in highly elderly ICU patients and increased glucose levels associated with higher mortality rate (11). Researchers noted that high glucose levels have a predictive value for mortality in elderly patients (11). In addition, increased platelet, neutrophile, and BUN levels have been associated with poor prognosis and mortality in elderly patients with sepsis and septic shock in various studies (12, 13). Arihan et al. reported higher baseline mean BUN levels in older patients (70±12 years vs 62±14 years), and the researchers associated higher BUN levels with multi-organ failure and long-term mortality in their study, which included 4176 critically ill patients involved 544 sepsis patients (13). Consistent with this, the mean neutrophile, fasting blood glucose, and BUN values were found statistically higher in the geriatric group than the mean values in the control group in our study.

Euthyroid sick syndrome prevalence has been reported to have increased by aging particularly in critically ill elderly patients. Moreover, ESS has been associated with poor prognosis, the development of complications, severity of disease, and mortality in older subjects, particularly in critically ill geriatric patients (14). Poliniet al. reported an ESS prevalence of 31.9% in 808 critically ill geriatric patients. Researchers also stated ESS as a significant independent risk factor for mortality ($p<0.0001$) (6). Similarly, Zhu et al. reported a 37.3% ESS prevalence in 83 patients aged over 60 years, and researchers concluded that the prevalence of ESS increases with aging (15). Furthermore, it has been documented that the increase of ESS prevalence through sepsis ranges between 60% to 70% in the published data. Padhi et al. reported an ESS prevalence of 67% in 360 ICU patients with sepsis (16). Similarly, Neamtu et al. reported a 63% ESS prevalence in 65 children with sepsis (17). Supporting this in our study, the total ESS prevalence was 84.2%, 108 of which (88.5%) were in the geriatric group and 63 (77.8%) were in the control group. The incidence of ESS was significantly higher in the geriatric group, but there was also no statistically significant difference according to the mortality, ICU stay, gender, and qSOFA score between the ESS (+) and (-) groups within only the elderly patients.

Despite the prognostic value of ESS being well-documented in the published data, there is a limited amount of data with controversial results which evaluated ESS in sepsis-patients. Moreover, to our knowledge, there is no study available in the published data which evaluated ESS in elderly patients diagnosed only with sepsis. Ergan et al. significantly associated ESS with increased non-invasive ventilation failure rate ($p=0.04$) and mortality ($p=0.02$) in a study conducted with 44 elderly patients (≥65 years) with chronic obstructive pulmonary disease (18). In the present study, qSOFA scores, the presence of hypotension, and PCT levels were all significantly associated with mortality in both entire group and geriatric population. Similarly, these variables are also shown as important predictors of mortality in patients with septic shock in published data (5).

While the decreased level of thyroid hormones reported to have a predictive value for poor prognosis and

mortality in patients with sepsis or septic shock in some studies, other studies have not associated lower thyroid hormones with poor prognosis (19). Padhi et al. reported the overall mortality of 30%; non-ESS patients was reported as 13.4%, group ESS with low total T3 was 50.1%, and group ESS with low T3 with low thyroxine (T4) was 69.1% (p<0.001) in their study included 360 ICU patients with sepsis. Researchers concluded that low T3 and free T3 levels are significant prognostic factors for mortality (16). Brinker et al. documented ESS in 69 children with meningococcal sepsis. They reported that the TT3/rT3 ratio decreased with no increase in TSH level, and TT4 levels were negatively correlated with the severity of the disease. Researchers also concluded that both the TT3/rT3 ratio and TT4 levels had a predictive value for mortality (20). Similarly, Hagag et al. documented significantly lower free TT3 and free TT4 in 40 neonates with neonatal sepsis (21). In the present study, the increased prevalence of mortality (6.3% vs. 12.3%) and ICU stay (12.5% vs. 22.2%) was not found statistically significant between the ESS (+) and (-) groups. There was also no statistically significant difference found according to the mortality, ICU stay, and qSOFA score between the ESS (+) and (-) groups within only elderly patients. However, it is noteworthy that both 91.3% of all patients (n=23/21) who died in the entire sample group and all patients (n: 13) who died in the geriatric group were ESS (+) patients. Moreover, mean fT3 levels were significantly decreased in deceased and ICU patients (2.42 vs. 2.11 ng/dL) in the entire group. Additionally, the decreased fT4 levels in both the entire group (15.19 vs. 13.72 ng/dL) and the geriatric group (15.09 vs. 13.15 ng/dL) were independently associated with mortality and ICU stay.

The mean creatinine and BUN values were significantly higher in ESS (+) group. In addition, the mean values of albumin, fT3, and fT4 were significantly lower in ESS (+) group than the mean values in ESS (-) group in our sample group. Furthermore, higher levels of procalcitonin, increasing levels of which have been well-documented as a prognostic factor for mortality, in both the entire group (8.93 vs. 24.41 ng/mL) and the geriatric group (7.04 vs. 26.31 ng/mL) were significantly associated with mortality. In addition, increased levels of PCT were also significantly associated with mortality in ESS (+) patients both in the control group (8.56 vs. 28.69 ng/mL) and geriatric group (12.48 vs. 16.50 ng/mL). On the other hand, Arnau-Barrés et al. significantly associated lower albumin levels with mortality (Survivors:3.1 vs. Non-survivors:2.6 g/dl) (p<0.0001) in 235 patients diagnosed with sepsis or septic shock with a median age of 75 years. Researchers highlighted lower albumin levels (<2.6 g/dL) as a prognostic factor for mortality in elderly patients (22). In another study, Guo et al. evaluated the clinical characteristics of euthyroid sick syndrome in their sample group (n=305), which involved 118 (38.7%) ESS patients. Researchers reported that the albumin level was significantly lower in the ESS group than the levels in the non-ESS group (26.63±6.51 vs 30.13±7.13 g/L) (p<0.001). Additionally, the creatinine level was significantly higher in the ESS group than the creatinine level in the non-ESS group (120.3±165.8 vs 80.6±85.8 umol/L) (p=0.007).

There are a few limitations in our study. Since we included patients from different clinics in our hospital, the initial evaluation and the physical examination were performed by different clinicians, and this might have led to heterogenous results; however, we used a predefined protocol to minimize these disadvantages and missing data. Caution is needed when interpreting the final results because of the different groups of patients included in our study.

In conclusion, significantly higher ESS prevalence (88.5%) was clearly demonstrated in elderly patients diagnosed with sepsis in the present study. Additionally, approximately a two-fold higher mortality (6.3% vs. 12.3%) and ICU administration (12.5% vs. 22.2%) risk was documented in the ESS (+) group. Moreover, well-documented prognostic markers such as increased BUN, creatinine levels, and decreased albumin, fT3, fT4 levels were found in the ESS (+) group. Thus, simply screening for ESS in elderly patients with sepsis certainly will contribute to treatment decisions and early prediction of complex clinical course, poor prognosis, and mortality.

Ethics Committee Approval: This study was approved by Istanbul University Istanbul Faculty of Medicine Clinical Research Ethics Committee (Date 21.06.2013, No: 12).

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Author Contributions: Conception/Design of Study- R.Ç., M.K., T.S.A., T.T.; Data Acquisition- R.Ç., O.K.B., G.Y.; Data Analysis/Interpretation- R.Ç., M.B., A.A.; Drafting Manuscript- R.Ç., M.K., O.K.B., T.S.A.; Critical Revision of Manuscript- R.Ç., T.T., M.B., G.Y., A.A.; Final Approval and Accountability- R.Ç., M.K., T.S.A., T.T., O.K.B.; Material or Technical Support- R.Ç., M.K., O.K.B., M.B., G.Y.; Supervision- R.Ç., T.S.A., A.A., T.T.

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DIABETIC PATIENTS' COMPLIANCE WITH TREATMENT DURING COVID-19 PANDEMIC PERIOD

COVID-19 PANDEMİ DÖNEMİNDE DİYABET HASTASININ TEDAVİYE UYUM DURUMUNUN DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: Diabetes mellitus is a chronic, complex disease with many components that must be managed. Treatment success depends on excellent treatment compliance. In this study, we aimed to evaluate the treatment adherence of diabetic patients during the COVID-19 (Coronavirus Disease) pandemic and the factors affecting this condition.

Material and Methods: The study was carried out on 474 diabetic patients with a questionnaire consisting of questions based on The Medication Compliance Questionnaire (MCQ) and the World Health Organization (WHO) 2003 compliance guideline.

Results: The rate of non-compliance with the treatment based on the MCQ scale was 82.3%. Non-compliance with treatment was significantly associated with oral antidiabetic (OAD) drug use, smoking status, glycosylated hemoglobin (HbA1c) <7%, and patient comments of "I don't have regular doctor follow-up," "I can't communicate well with my doctor," "My blood glucose is not at the target value," and "My medications are not comfortable enough for use"(p=0.011; 0.010; 0.014; 0.011; 0.002; 0.019; 0.001). Patients under insulin treatment or with an HbA1c value of ≥7% were found to be more compliant with the treatment.

Conclusion: Unlike the classical results, the incompatibility of diabetic patients with HbA1c <7% and under OADs with the treatment was emphasized. Patients using insulin and with advanced duration of diabetes were more compliant with the treatment in the stressful period of the COVID-19 pandemic. Lack of follow-up by the doctor and low patient effort to communicate with the doctor have been decisive factors in the non-compliance.

Keywords: Diabetes mellitus, compliance, HbA1c, oral antiglycemic agent

ÖZET

Amaç: Diyabet, yönetimi çok bileşenli olan kronik, kompleks bir hastalıktır. Tedavi başarısı ancak iyi bir tedavi uyumuyla sağlanabilmektedir. Bu çalışmada, diyabet hastalarının COVID-19 (Koronavirüs hastalığı) pandemisi döneminde tedaviye uyum durumu ve bu durumu etkileyen faktörlerin değerlendirilmesi amaçlandı.

Gereç ve Yöntem: Çalışma, The Medication Compliance Questionnaire (MCQ) ve Dünya Sağlık Örgütü (DSÖ) uyum klavuzu 2003'e göre belirlenmiş sorularından oluşan bir anket ile 474 diyabetik hasta ile gerçekleştirilmiştir.

Bulgular: MCQ ölçeğine göre tedaviye uyumsuzluk oranı %82,3 idi. Tedaviye uyumsuzluk, oral antidiyabetik (OAD) ilaç kullanımı, sigara içme durumu, glukolize hemoglobinin (HbA1c) <%7 olması ve hasta yorum sorularından; ''düzenli doktor takibim yok'',"doktorumla iyi iletişim kuramıyorum", ''şekerim belirlenen hedef değerde değil'',"ilaçlarım kullanım yönünden yeterince konforlu değil" ifadeleriyle ilişkili bulunmuştur (p=0,011; 0,010; 0,014; 0,011; 0,002; 0,019; 0,000). İnsülin kullanan, HbA1c değeri ≥%7 olan hastalar ise tedaviye daha uyumlu olarak tespit edilmiştir.

Sonuç: Bu çalışmada klasik sonuçlardan farklı olarak HbA1c <%7 ve OAD kullanmakta olan diyabetik hastaların uyumsuzluğu gündeme getirilmiştir. COVID-19 pandemisi döneminde insülin kullanan ve hastalık süresi uzun olan hastalar tedaviye daha bağlı kalmışlardır. Doktor takibinin olmaması ve doktorla iyi iletişim kuramama uyumsuzluk açısından belirleyici olmuştur.

Anahtar Kelimeler: Diyabetes mellitus, tedavi uyumu, HbA1c, oral antidiyabetik ilaç

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INTRODUCTION

Coronavirus Disease 2019 (COVID-19), caused by Severe Acute Respiratory Syndrome-2 (SARS-CoV-2), was determined to be a new coronavirus agent. The virus was defined in Wuhan, China and declared a pandemic by the World Health Organization (WHO) in early March 2020 (1).

Diabetes is known to be another global epidemic, and the incidence of the disease is increasing very fast. According to 2019 data, diabetes mellitus (DM) is a chronic disease affecting approximately 9% of adults, causing an important health problem (2, 3). Although it is known that diabetic patients are susceptible to COVID-19 infection, only 12-20% of diabetic patients were infected with COVID-19 (3). Nevertheless, the risk and severity of infectious diseases and the rate of complications can be reduced by providing good glycemic control in diabetic patients (2).

In chronic diseases such as diabetes, treatment compliance is a decisive factor that ensures the success of treatment (4, 5). Poor compliance with the treatment recommendations results in individual, social, and economic costs (6). According to the WHO 2003 compliance guideline, compliance is a multidimensional situation and occurs with the interaction of five factors; 1-patient, 2-social and economic, 3-related to the disease state, 4-related to the health system, 5-related to treatment (6).

Metabolic control in diabetic patients is closely related to patients' attitudes (7). In addition, many other factors also affect drug treatment compliance, including the complexity of treatments, lack of knowledge, presence of co-morbidities, side effects of drugs, and patient's well-being (8). Diabetic patients may be affected by disorders in some conditions such as medication delivery, blood glucose monitoring, regular outdoor sports, and diet compliance due to the sudden COVID-19 pandemic. The information concerning the effects of COVID-19 pandemic quarantine on glycemic control is conflicting (3,9). Home quarantine causes fear in many patients and prevent these patients from going to the clinic. Throughout the COVID-19 pandemic, to reduce the spread, many hospitals have reduced their capacity for outpatients. Diabetic patients could not go to their routine check-ups during quarantine and treatment could not be rescheduled under these stressed conditions. Therefore, after the pandemic, the health system may have to deal with the burden of acute and chronic complications due to poor glycemic control. To prevent this, self-management of diabetic patients during home quarantine is very important (3).

The aim of this study was to evaluate treatment compliance status and the factors affecting this situation in diabetic patients during the COVID-19 pandemic.

MATERIAL AND METHODS

According to our Power analysis using the G*Power program, when we took the difference between the rates of non-compliance with treatment according to age as D=20.6%, the minimum sample number determined for Power: 0.80 and alpha: 0.05 was determined to be 198.

This was a cross-sectional study. It was conducted with 474 diabetic patients admitted to the secondary level state hospital between 05.11.2020 and 05.11.2021.

Written consent was obtained after all patients were informed. The data was collected with a questionnaire consisting of two parts. One of them was the Medication Compliance Questionnaire (MCQ) scale and the other part was interpretation questions based on the WHO 2003 compliance guideline. In outpatient clinics, face-to-face interviews were performed with the patients by the internal medicine doctors, and the answers were recorded. An average of 15-20 minutes was allocated for each patient for the questionnaire. All type 1 and type 2 diabetic patients, diagnosed according to the American Diabetes Society (ADA) 2021 guideline, who were over 18 years old and under antidiabetic medications were included in the study (10). The data were recorded without changing the treatments used. Patients with incomplete records, who had been diagnosed with diabetes for less than 6 months, who were having acute complications, or who were unable to answer the questions due to mental problems were excluded from the study. Patients' socio-demographic and laboratory data, clinical features, comorbidities, diabetes-related complications, and medications were recorded.

The MCQ scale, which is an approved questionnaire for drug compliance, was used. Permission was obtained from the corresponding author for the use of the scale (8, 11). The adaptation of the questionnaire into Turkish was carried out by this paper's study team. The Cronbach's alpha coefficient showing the internal consistency of the scale is 92.1%.

In our study, the MCQ scale developed from different studies was used (12-14). The MCQ scale consists of seven questions in total that aim to evaluate the non-compliance of patients with their drug treatments. A four-point Likert scale was used for every question. Accordingly: Never, 4 points; Sometimes (1-4 times in a month), 3 points; Most of the time (≥5 per month or ≥2 per week), 2 points; Always, 1 point. Total scores were 7 to 28 for a patient. ≥27 rated as compliant, <27 incompatible (8).

According to the WHO 2003 compliance guideline, the factors affecting compliance were examined under five headings, and comment questions for the pandemic period were added to these subheadings. This section was

specified as the second section of the questionnaire. Under these subheadings the following factors were evaluated: 1) patient factors: gender, body mass index (BMI), age, diet compliance status, ability to do sports (regular outdoor sports), and compliance with personal hygiene and hygiene rules during the pandemic; 2) social and economic factors: education, marital status, occupation, monthly income, family support, good communication with the doctor, "my blood glucose is at the determined target value," and "my medications are comfortable in terms of use"; 3) factors related to the disease status: smoking, comorbidities, complications, HbA1c value, and COVID-19 status; 4) factors related to the health care team and system: the condition of regular doctor follow-ups during the pandemic; 5) factors related to treatment: duration of diabetes (treatment period) and drugs used (6).

Ethics committee approval was optained from Dr. Cemil Taşcıoğlu City Hospital for the study (Date: 03.11.2020, No: 386). Our study was carried out following the Declaration of Helsinki.

Statistical analyses

IBM SPSS Statistics version 22 software (IBM Corp., Armonk, NY, USA) program was used for statistical evaluation. With the Kolmogorov-Smirnov Test, the conformity of the parameters to the normal distribution was evaluated. Data were evaluated with Continuity (Yates) Correction and Chi-Square Test. Logistic regression analysis was applied for multivariate analysis. $p<0.05$ value was considered as statistically significant.

RESULTS

The ages of the 474 patients evaluated were between 25 and 93 years (mean age=59.25±11.81). The mean glycosylated hemoglobin (HbA1c) level of the patients was 8.55±2.55% (median=7.6%). Mean duration of diabetes of the patients was 9.02±7.72 years (median=7) (Table 1).

Table 1: Demographic and clinical features

Socio-demographic features			
Age interval (year), mean±SD		25-93	59.25±11.81
BMI interval, mean±SD		16.7-57.5	31.03±5.72
Gender n	Female	285	60.1
	Male	189	39.9
Education n	Illiterate	43	9.1
	Primary school	347	73.2
	High school	69	14.6
	University and higher	15	3.2
Marital status n	Single	116	24.5
	Married	358	75.5
Working status n	Not working in the pandemic	414	87.3
	Working	60	12.7
Monthly income n	Below minimum wage	236	49.8
	Equal or more	238	50.2
Smoking n	No	399	84.2
	Yes	75	15.8
Clinical features			
HbA1c interval (%), mean±SD (median)			8.55±2.55 (7.6)
FBG interval (mg/dL), mean±SD (median)			165.95±75.19 (143)
PPBG interval (mg/dL), Mean±SD (median)			228.78±102.52 (200)
DM period (treatment duration) year, Mean±SD (median)			9.02±7.72 (7)

SD: standard deviation, BMI: body mass index, HbA1c: glycosylated hemoglobin 1c, FPG: fasting plasma glucose, PPBG: postprandial plasma glucose, DM: diabetes mellitus

As for the forms of treatment, 64.3% of the cases were under oral antidiabetic (OAD) drug treatment, 21.9% were using OAD and insulin, and 13.5% were using insulin alone.

The mean MCQ score was 21.94±5.76 (median=24). According to the MCQ scale classification, 82.3% (n=390) of the cases were non-compliant with the treatment (Table 2).

The non-compliance of the patients who used the expressions ''My blood glucose is not at the specified target value'' and/or "my medications are not comfortable enough in terms of use" was statistically significant (p=0.019; 0.001). Comorbidities, complications, and COVID-19 states could not be associated with non-compliance with treatment (p=0.813; 0.274; 0.295). Also, smoking was found to be one of the determining factors for non-compliance (p=0.01) (Table 3).

Non-compliance was higher in patients with HbA1c levels <7% (p=0.014) and patients with HbA1c values ≥7% were more compliant with the treatment. The cases who stated that they were not under regular doctor follow-up during the pandemic were more non-compliant with the treatment (p=0.011). When patients were grouped according to the drugs used, statistically significant for non-compliance (p=0.011). Patients using insulin were more compliant with the treatment (Table 3).

When we evaluated the effects of the parameters on non-compliance with treatment; smoking, HbA1c value, drugs used, and the comments "I don't have regular doctor follow-up in the pandemic, I can't communicate well with my doctor, my blood glucose is not at the specified target value, my medications are not comfortable enough for use" with Backward stepwise logistic regression analysis, the model was important (p=0.001). The Negelkerke R square value of 0.162 was detected, and descriptive of the model (82.3%) was found to be at a good level. The effects of all these parameters, except drugs used, were important on the model (p<0.05) (Table 4).

Table 2: The Medication Compliance Questionnaire (MCQ) score*

	MCQ	
Questions	**Mean±SD**	**Median**
Q1 (How often do you forget to take your medicine?)	3.11±0.9	3
Q2 (How often do you decide not to take your medicine?)	3.07±1.11	4
Q3 (How often do you miss taking your medicine because you feel better?)	3.23±0.98	4
Q4 (How often do you decide to take less of your medicine?)	2.86±1.08	3
Q5 (How often do you stop taking your medicine because you feel sick due to effects of the medicine?)	3.22±1	4
Q6 (How often do you forget to bring along your medicine when you travel away from home?)	3.18±0.93	3
Q7 (How often do you not take your medicine because you run out of it at home?)	3.34±0.98	4
Total MCQ score	21.94±5.76	24
MCQ Score	**n**	**%**
Incompatible	390	82.3
Compatible	84	17.7

*: There are 7 MCQ questions. A score of 27 and above is compatible. Below 27 is incompatible (11).

DISCUSSION

In our study, using the MCQ scale, we found that 82.3% of the diabetic patients were non-compliant with treatment. Before the COVID-19 pandemic, compliance was a problem in diabetic patients and the compliance rate was usually 30-70% (15). Non-compliance rates were reported to be 27.1% in Southern Brazil, 28% in New York, 28.9% in Uganda, 36% in Mexico, and around 59% in Nigeria (5). In a study conducted with diabetic patients in Turkiye in 2015, the rate of non-compliance was found to be 44.7% (16). In other studies conducted in Turkiye, the rate of treatment compliance was found to be moderate or just above moderate (5, 7, 8).

As a result of good compliance with treatment, clinical outcomes improve, and quality of life is positively affected. The costs of disability and death decrease, and the number of hospital admissions and emergency admissions decrease. On the other hand, non-compliance is called the "invisible epidemic". It is a frequent occurrence with chronic diseases and an important public health issue (5).

In order to prevent the spread of the disease, curfews were ordered in many countries during the COVID-19 pandemic. Millions of people had to stay at home for this reason (17). In a study conducted during the COVID-19 pandemic on hypertensive and diabetic cases in Epitopia, non-compliance with treatment was found to be 72% (19). In another study conducted during the COVID-19 pandemic period, 74.46% of type 2 and 64.89% of type 1 diabetics were regarded as having poor glycemic control (3). In a study conducted on diabetic patients in Saudi Arabia, compliance was 18.5% before the COVID-19 quarantine and 17.4% after quarantine (18).

Table 3: Evaluation of study parameters in terms of the Medication Compliance Questionnaire (MCQ) compliance

		MCQ		p
		Non-compliance (n=390)	Compliance (n=84)	
Demographic features		**n (%)**	**n (%)**	
1-Patient related factors				
Age	< 65 years	263 (82.4)	56 (17.6)	[1]0.892
	≥ 65 years	127 (81.9)	28 (18.1)	
Gender	Female	236 (82.8)	49 (17.2)	[1]0.711
	Male	154 (81.5)	35 (18.5)	
BMI	Non-obese	181 (81.5)	41 (18.5)	[1]0.689
	Obese	209 (82.9)	43 (17.1)	
Diet compliance in pandemic	No	242 (83.2)	49 (16.8)	[1]0.526
	Yes	148 (80.9)	35 (19.1)	
Sports in pandemic (regular outdoor sports)	No	298 (80.5)	72 (19.5)	[2]0.085
	Yes	92 (88.5)	12 (11.5)	
Personal hygiene in the pandemic	No	27 (79.4)	7 (20.6)	[2]0.825
	Yes	363 (82.5)	77 (17.5)	
2-Social and economic factors				
Education	Illiterate	34 (79.1)	9 (20.9)	[1]0.278
	Primary school	286 (82.4)	61 (17.6)	
	High school	60 (87)	9 (13)	
	University and higher	10 (66.7)	5 (33.3)	
Marital status	Single	89 (76.7)	27 (23.3)	[1]0.071
	Married	301 (841)	57 (15.9)	
Working status	Not working in the pandemic	344 (83.1)	70 (16.9)	[2]0.300
	Working	46 (76.7)	14 (23.3)	
Monthly income	Below minimum wage	194 (82.2)	42 (17.8)	[1]0.966
	Equal or more	196 (82.4)	42 (17.6)	
Family support	Absent	115 (87.8)	16 (12.2)	[2]0.071
	Present	275 (80.2)	68 (19.8)	
Good communication with the doctor	Absent	160 (89.4)	19 (10.6)	**[2]0.002***
	Present	230 (78)	65 (22)	
My blood glucose is at the determined target values	No	199 (86.5)	31 (13.5)	**[1]0.019***
	Yes	191 (78.3)	53 (21.7)	
Medicines are comfortable to use	No	93 (95.9)	4 (4.1)	**[2]0.000***
	Yes	297 (78.8)	80 (21.2)	

Table 3: Continue

		MCQ		p
		Non-compliance (n=390)	Compliance (n=84)	
		n (%)	n (%)	
3-Disease associated factors				
Smoking	No	320 (80.2)	79 (19.8)	[2]**0.010***
	Yes	70 (93.3)	5 (6.7)	
Comorbidities	No	222 (81.9)	49 (18.1)	[1]0.813
	Yes	168 (82.8)	35 (17.2)	
Complications	No	301 (81.1)	70 (18.9)	[2]0.274
	Yes	89 (86.4)	14 (13.6)	
HbA1c (%)	<7	146 (88.5)	19 (11.5)	[2]**0.014***
	≥7	244 (79)	65 (21)	
COVID-19 history	No	331 (81.5)	75 (18.5)	[2]0.295
	Yes	59 (86.8)	9 (13.2)	
4-Factors related to the health system				
Regular doctor follow-up in the pandemic	Absent	302 (84.8)	54 (15.2)	[1]**0.011***
	Present	88 (74.6)	30 (25.4)	
5-Treatment related factors				
DM period (Treatment duration)	under 10 years	222 (80.1)	55 (19.9)	[1]0.295
	10-20 years	120 (86.3)	19 (13.7)	
	20 years and over	48 (82.8)	10 (17.2)	
Medications	OAD	263 (85.9)	43 (14.1)	[1]**0.011***
	insulin	46 (71.9)	18 (28.1)	
	OAD-Insulin use	81 (77.9)	23 (22.1)	

[1]: Chi-square test, [2]: Continuity (Yates) correction, *: a value of p<0.05 is significant, MCQ: Medication Compliance Questionnaire, BMI: body mass index, HbA1c: glycosylated hemoglobin, COVID-19: Coronavirus disease, DM: diabetes mellitus, OAD: oral antidiabetic

Table 4: Evaluation of the effects of the parameters that cause non-compliance with treatment by logistic regression

		95% CI		
Step 2	**OR**	**Lower Bound**	**Upper Bound**	**p**
Smoking	2.823	1.076	7.403	**0.035***
HbA1c < 7%	2.115	1.165	3.84	**0.014***
Lack of regular doctor follow-up in the pandemic	1.716	0.995	2.958	**0.048***
Poor communication with doctor	1.938	1.085	3.462	**0.025***
Absence of blood glucose in expected target values	1.849	1.081	3.163	**0.025***
The inconvenience of drugs used	5.356	1.862	15.405	**0.002***

*: a value of p<0.05 is significant. Parameters included in the model: smoking, HbA1c: glycosylated hemoglobin, doctor follow-up during the pandemic, medications used, doctor support, blood glucose at target values, comfortable use of drugs.

We found that non-compliance was higher in patients using OAD. Similarly, in previous studies, patients using insulin were more compliant with their treatments (5, 20). However, in another study, as the frequency of daily insulin use increased, treatment compliance decreased (16). There is also a study stating that there is no significant association of the number of medications taken, or OAD or insulin use with compliance with treatment (8). Compliance with oral hypoglycemic agents was attributed to some factors. It has been shown that poor communication negatively affects compliance with OAD therapy and glucose monitoring in type 2 diabetic patients (6). In a previous study, problems associated with drug therapy were detected in 42.3% of diabetic patients (15). Some of the obstacles to the use of OADs were stress, forgetfulness, not being sufficiently aware of the course of diabetes, belief in OADs, the high number of drugs used, poor communication with health care practitioners, the perception that the effect of OAD is weak, the presence of concomitant diseases, and old age (21).

In this study, patients with HbA1c <7% were more discordant to the treatment. In a study examining the compliance of diabetic patients with treatment, HbA1c values were not different between groups (22). Conversely, in some studies, it was emphasized that the higher the treatment compliance, the lower the HbA1c levels (2). In addition, in a study conducted during the COVID-19 quarantine, a statistically insignificant increase in HbA1c, fasting, and postprandial blood glucose levels was observed (23). In a different study, the treatment compliance score was found to be higher in those with an HbA1c value below 7.5%, however there was no statistical difference (22). The higher discordance defined in patients with HbA1c <7% in our study may be associated with a short period having passed after the diagnosis, or discontinuation of drugs due to the absence of complications and comorbid conditions. In addition, HbA1c may not be a suitable parameter to be used in stressful situations such as a pandemic. Self-confidence and uncontrolled continuation of treatment may be another factor in diabetic patients who were under insulin treatment for a long time. When the patients' symptoms related to the disease disappear, the patients cease to use their medications or become non-compliant by reducing them. On the contrary, as the patients' diseases worsen, the patients adapt more to their treatment. It is stated that patients with high blood glucose levels are more likely to remain compliant with treatment than those with regular blood glucose levels. In a previous study, as in our study, patients with HbA1c >7 were more likely to comply with treatment (5). Moreover, it has been stated that patients who were minimally affected by the course of diabetes have less compliance. No complications in cases with early diabetes were another factor for non-compliance (5).

In a study that was similar to ours, age, gender, education, income, diabetes duration, type of treatment, complications, and comorbidities were not associated with treatment compliance (5). In addition, it was emphasized in another study that factors such as gender, duration of diabetes, training status, BMI, and the number of drugs used were not the determinants of treatment compliance (8).

In this study, smokers were more non-adherent to the treatment. It has also been stated that smoking increases the patient's stress level and impairs drug intake (24). The absence of regular doctor follow-up, inability to communicate well with the doctor, blood glucose level not being at the determined target value, insufficient comfort with the use of drugs, and absence of doctor follow-up in the pandemic were associated with non-compliance with the treatment. It was stated in a study that if the patient-physician relationship is good, treatment compliance increases 2.26 times (5). It has been shown that directorial factors such as continuous follow-up and time spent with the doctor, and the doctor's communication style outweigh characteristics such as weight, height, and education (6). It is stated that if patients' knowledge about their diseases and the drugs increases, their compliance with treatment increases. This can only be achieved as a result of patients receiving more counseling and interacting with healthcare professionals (11). It is emphasized that stressful situations such as war affect treatment compliance and the effects of this situation continue even after the war is over. The reasons for this are shown as economic distress and interruption of medical follow-up (6).

The quality of life, physical, social, and mental conditions of diabetic patients have deteriorated under extraordinary conditions such as the COVID-19 pandemic. It is emphasized that in the presence of disasters that negatively affect life with chronic diseases, the quality of life deteriorates and vulnerable groups should be monitored more closely (23). Person-to-person transmission was prevented during the COVID-19 pandemic by taking strict public health measures. However, people's lifestyles, mental states, and behaviors are negatively affected by this situation (25).

However, there is a need for innovative practices with independent physician studies instead of structured traditional systems (6). Services supporting diabetic patients via telephone and e-mail during the COVID-19 quarantine have been more accessible for patients who have difficulty going to the clinic (9). The continuing COVID-19 outbreak emphasizes the significance of electronic health records and the need for electronic health records to be remote. Thus, it could provide valuable input for strengthening general health service delivery in the coming years (26).

There are some limitations in our study. Firstly, it does not reflect the whole society because it is a single center.

Interpretation questions such as, "I cannot communicate well with the doctor," and "I think my medications are not comfortable enough" were evaluated according to the patients' responses. The patient's perception at that moment and the state of being affected by the hospital environment may have affected the response. The HbA1c value is the value when the patient applied to the hospital and does not reflect the current values. Although there were adaptation studies conducted before the pandemic, the comparison could not be fully achieved due to the lack of a study using the same place and scale. Also, in OAD drug use, drugs could not be specified in detail and it has not been determined which type of OAD was affecting incompatibility. Another limitation of our study is that patients could not be evaluated in terms of hypoglycemia.

CONCLUSION

The expected results in classical adaptation studies focus on the incompatibility of patients with HbA1c >7% and patients using insulin. Our study contributed to the literature by providing a different approach, because we emphasized the non-compliance with treatment of diabetic patients who use OAD drugs and have HbA1c <7%. HbA1c <7% is a desired condition in which blood glucose is under control. However, this may not indicate that all is well. This patient group, which decides that doctor follow-up is insufficient, may be using many medications containing combinational medications. Also, patients may be exposed to hypoglycemia. In the long term, this will cause complications and increased health expenditures. Further studies are warranted explaining acute complications such as hypoglycemia, analyzing sub-groups of OAD agents, and exploring the behaviors of insulin-using diabetic patients who are more treatment-compatible in the COVID-19 pandemic. It would also be useful to analyze in detail patients with HbA1c <7%.

Ethics Committee Approval: This study was approved by Prof. Dr. Cemil Tascioglu Education and Research Hospital Ethics Committee (Date: 03.11.2020, No: 386).

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HIDDEN DANGER OF SARS-COV-2; MULTISYSTEM INFLAMMATORY SYNDROME IN ADULTS (MIS-A): FIRST CASE SERIES IN A SINGLE CENTER FROM TURKIYE

SARS-COV-2'NİN GİZLİ TEHLİKESİ; ERİŞKİNLERDE MULTİSİSTEM İNFLAMATUAR SENDROM (MIS-A): TÜRKİYE'DE TEK MERKEZDEN İLK OLGU SERİSİ

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ABSTRACT

Objective: Multisystem Inflammatory Syndrome (MIS) is a condition seen in the early post-COVID-19 period and thought to develop with an impaired immune response. It has been usually reported in children but rarely in adults. Here we report the first adult MIS (MIS-A) case series from Turkiye.

Material and Methods: Six patients who met the Centers for Disease Control and Prevention's MIS-A diagnostic criteria were included in the study. The demographic, clinical, laboratory, radiological characteristics and therapy regimes and outcomes of the patients were recorded.

Results: All of our cases had a history of mild COVID-19. They presented with fever, severe fatigue and hypotension. Abnormal echocardiography findings were detected in five patients. Only one patient had multiple mucocutaneous findings. Common laboratory features were lymphopenia, markedly increased C-Reaktive Protein, procalcitonin, pro-brain natriuretic peptide (pro-BNP), D-dimer, and ferritin. All patients had positive SARS-CoV-2 antibody result. Corticosteroids and/or anakinra were used in five, and intravenous immunoglobulin was used in two patients. Low-molecular-weight heparin (LMWH) was used for all cases. Empirically initiated antibiotic treatments were discontinued after cultures were negative. After anti-inflammatory treatment, the hypotension of the patients resolved, they did not need intensive care follow-up and no mortality was seen in our cases.

ÖZET

Amaç: Multisistem İnflamatuar Sendrom (MIS), COVID-19 sonrası erken dönemde görülen ve bağışıklık yanıtının bozulmasıyla geliştiği düşünülen sıklıkla çocuklarda görülen bir durumdur. Erişkinlerde nadiren bildirilmiştir. Bu makalede Türkiye'de takip edilen ilk yetişkin MIS (MIS-A) olgu serisi sunulmaktadır.

Gereç ve Yöntem: Centers for Disease Control and Prevention'in MIS-A tanı kriterlerini karşılayan altı hasta çalışmaya dahil edildi. Hastaların demografik, klinik, laboratuvar, radyolojik özellikleri ile tedavi uygulamaları ve sonuçları kaydedildi.

Bulgular: Tüm olgular COVID-19'u hafif şiddette geçirmişti. Hastaların hepsi ateş, şiddetli yorgunluk ve hipotansiyon ile başvurdular. Beş hastada anormal ekokardiyografi bulguları saptandı. Sadece bir hastada çoklu mukokutanöz bulgular mevcuttu. Yaygın laboratuvar özellikleri arasında lenfopeni, C-Reaktif Protein, prokalsitonin, pro-brain natriüretik peptid (pro-BNP), D-dimer ve ferritin artışı vardı. Tüm hastaların SARS-CoV-2 antikoru pozitifti. Beş hastada kortikosteroid ve/veya anakinra, iki hastada intravenöz immunoglobulin tedavisi ve hepsinde düşük moleküler ağırlıklı heparin tedavisi kullanıldı. Ampirik olarak başlanan antibiyotik tedavileri alınan kültürler negatif sonuçlanınca kesildi. Antiinflamatuar tedavi sonrası hastaların hipotansiyonu düzeldi, hiçbirinde yoğun bakım takibi ihtiyacı olmadı ve olgularımızda mortalite görülmedi.

Sonuç: MIS-A, çeşitli klinik tablolara neden olan ve sepsis ile

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Conclusions: MIS-A is a severe and mortal condition that causes various clinical pictures and can be confused with sepsis. Anakinra, a recombinant IL-1 receptor antagonist, is a significant agent that can be used in the treatment of MIS-A since it blocks the cytokine cascade at an early stage. The satisfactory responses will be obtained with early diagnosis and anti-inflammatory treatment. In this period when the pandemic is not over yet, it is necessary to increase the awareness of clinicians about MIS-A, which can be fatal.

Keywords: SARS-CoV-2, COVID-19, multisystem inflammatory syndrome, adult, anakinra, steroid therapy

karıştırılabilen ciddi ve ölümcül bir durumdur. Rekombinant IL-1 reseptör antagonisti olan anakinra, sitokin kaskadını erken aşamada bloke ettiği için MIS-A tedavisinde kullanılabilecek önemli bir ajandır. MIS-A'da erken teşhis ve antiinflamatuar tedavi ile olumlu sonuçlar alınacaktır. Pandeminin hız kestiği ancak henüz bitmediği bu dönemde klinisyenlerin ölümcül olabilen MIS-A hakkında farkındalığının artırılması gerekmektedir.

Anahtar Kelimeler: SARS-CoV-2, COVID-19, multisystem inflamatuar sendrom, erişkin, anakinra, steroid tedavisi

INTRODUCTION

In the course of Coronavirus disease 2019 (COVID-19), severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) may affect all tissues that express angiotensin-converting enzyme 2 (ACE-2) receptors. Increased cytokine release from infected cells causes local inflammation (1). As it is known, inflammation is a self-limited process through the balance between inflammatory and anti-inflammatory systems of the organism. Somehow, the triggered and activated immune cells may cause a vicious inflammatory circle and some patients develop a cytokine-mediated hyperinflammatory state on the basis of some probable immunogenetic factors. We have learned during the SARS-CoV-2 pandemic that SARS-CoV-2 infection may cause hyperinflammation not only under the title of acute COVID-19 but also in post-COVID-19 period.

Multisystem inflammatory syndrome (MIS) is defined as an immune-mediated complication of COVID-19 in the early stage of post-illness period (2). It has predominantly been reported among children and the first adult case was reported on June 2020 (3). After this time, adult patients with Kawasaki-like illness have been reported. Adult patients who are infected with SARS-CoV-2 can develop MIS days to weeks after the initiation of COVID-19 (4). Clinical features have varied but predominantly included persistent fever, abdominal pain, mucocutaneous signs, edema, cardiac dysfunction, shock, markedly elevated inflammatory markers, hematological involvement and serologically positive COVID-19 (2, 4).

Here we report the first MIS in adults (MIS-A) case series in Turkey, examine the clinical course of the disease and possible pathogenesis, and also present our treatment experiences.

MATERIALS AND METHODS

The hospital files and electronic records of MIS-A cases were retrospectively examined. The administered treatments with the demographic, clinical, laboratory and radiological characteristics of the patients were recorded on the previously prepared forms. Six patients who met the Centers for Disease Control and Prevention (CDC)'s MIS-A diagnostic criteria (5) were included in the study. CDC's MIS-A case definition is: a patient aged ≥21 years with fever hospitalized for ≥24 hours, or with an illness resulting in death, who meets the clinical criteria (three criteria, but at least one primary clinical criteria) and laboratory criteria (laboratory evidence of SARS-CoV-2 infection and elevation of at least two inflammatory markers like C-Reaktive Protein (CRP), ferritin, IL-6, erythrocyte sedimentation rate, procalcitonin). The patient should not have a more likely alternative diagnosis for the illness (e.g., bacterial sepsis, exacerbation of a chronic medical condition). The primary clinical criteria are severe cardiac illness or rash and non-purulent conjunctivitis. The secondary clinical criteria are new onset neurologic signs and symptoms, shock or hypotension not attributable to medical therapy, abdominal pain/vomiting/diarrhea and thrombocytopenia. The transthoracic echocardiography was performed by the cardiologist to the patients. SARS-CoV-2 IgG antibody against Spike protein were tested for all patients, and the informed consent was obtained from the patients on hospital admission. Patients were treated with 40 mg methylprednisolone daily or 6 mg dexamethasone daily as steroid therapy. Intravenous immunoglobulin (IVIG) treatment was administered at a total of 2 g/kg dose divided into five days. In patients with high inflammatory markers, anakinra was administered as an anti-cytokine therapy at a dose of 100-200 mg two or three times a day, subcutaneous (SC)/intravenous (IV), after taking the opinion of rheumatologists according to the follow-up of these inflammatory markers. Prophylactic low-molecular-weight heparin (LMWH) was administered as 40 mg/day SC, LMWH was administered as 1 mg/kg SC twice a day as the treatment dose.

The study was approved by the local ethics committee of Istanbul University, Istanbul Faculty of Medicine (Date: 14.01.2022, No: 2021/2183) and the Turkish Ministry of Health.

RESULTS

Characteristics of the cases

Four of the six cases were men. Mean age of the patients was 43.3 years old (minimum age: 23, maximum

age: 56). All of our cases were middle-aged with a history of mild COVID-19 in the last 2-8 weeks. All had asymptomatic period after COVID-19 until the initiation of the MIS-A symptoms. They were followed after the second COVID-19 wave in Turkiye in November and December 2020. All the patients were unvaccinated against to SARS-CoV-2. The patients presented to emergency room with the symptoms of fever, and severe fatigue. None of the patients had respiratory problems. Five out of six patients developed severe hypotension. Abnormal echocardiography findings were detected in five patients. One patient had no echocardiographic evaluation. Only one patient had multiple mucocutaneous findings, although these findings except livedo reticularis occurred after the diagnosis of MIS-A (Figure 1). Common laboratory features were lymphopenia, markedly increased CRP, procalcitonin, pro-BNP, D-dimer, and ferritin. Mild thrombocytopenia developed in one patient, and moderate thrombocytopenia in another patient. All patients' anti-SARS-CoV-2 IgG antibodies were qualitatively positive (Table 1).

Treatment strategies

Corticosteroids were used in five out of six patients, and we preferred IVIG treatment in two cases who had severe, and IV fluid resistant hypotension (Case 1, and 5). LMWH was used for all cases, interleukin 1 receptor antagonist (Anakinra) was used in five patients (Table 2). Hypotension resolved after a few days with the initiation of anti-inflammatory therapy in MIS-A cases. As a result; none of our patients needed intensive care and none received.

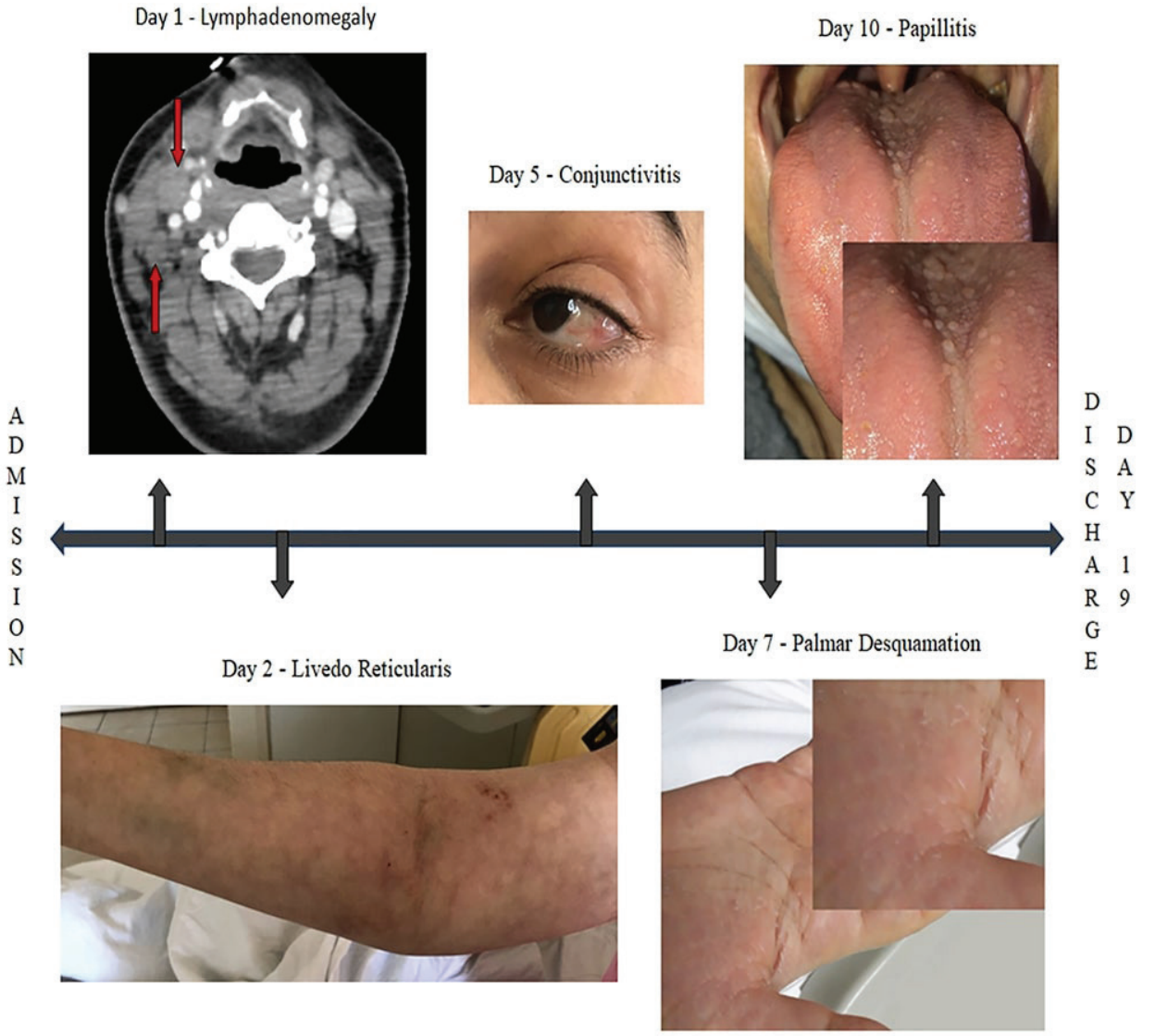

Figure 1: The mucocutaneous and radiological findings of Case 1

Table 1: Clinical, laboratory and radiologic characteristics of MIS-A cases

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Age	37	48	45	51	23	56
Gender	Female	Male	Male	Male	Male	Female
Coexisting diseases	Allergic rhinitis	None	Nephrolithiasis	Inactive Hepatitis B	None	Type 2 DM, Hypertension
Severity of COVID-19	Mild	Mild	Mild	Mild	Mild	Mild
Duration between COVID-19 diagnosis and initiation of MIS-A symptoms	29 days	10 days	24 days	29 days	50 days	12 days
Duration of the asymptomatic period	23 days	8 days	20 days	23 days	41 days	9 days
SARS-CoV-2 PCR - on admission for MIS-A	Negative	Positive	Negative	Negative	Negative	Negative
Anti-SARS-CoV-2 IgG antibody	Positive	Positive	Positive	Positive	Positive	Positive
Lymphocyte (absolute count/µl)	300	700	900	800	300	800
Neutrophil (absolute count/µl)	10,000	2,350	25,600	19,100	4,100	24,700
Platelet (absolute count/µl)	75,000	170,000	179,000	127,000	179,000	622,000
C-reactive protein (mg/L)	323.85	280	311.49	205.14	412	111
Ferritin (ng/mL)	426.3	780	3,714	2,748	1,595	998
Procalcitonin (ng/mL)	3.43	19	11.41	0.73	5.31	11.62
D-dimer (µg/L)	12.760	2800	1.400	2090	2900	730
Pro-BNP (pg/mL)	6.831	3600	23.077	>35.000	1006	443
Troponin-T (pg/mL)	3.54	14	54.89	540.5	30.16	6.24
Fever	7 days	6 days	12 days	4 days	4 days	4 days
Fatigue	+	+	+	None	+	+
Lymphadenomegaly	Unilateral cervical	None	None	Unilateral cervical	None	None
Cutaneous and mucocutaneous findings	Livedo Reticularis Conjunctivitis Papillitis Palmar desquamation	Macular rash on legs	Tonsillar hyperemia	None	None	None
Blood pressure (mm Hg)	75/45	85/40	88/60	120/80	70/40	85/60
Edema	Severe-Anasarca	Severe-Anasarca	Slightly	Slightly	Slightly	Slightly

Table 1: Clinical, laboratory and radiologic characteristics of MIS-A cases *(Continued)*

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Echocardiographic evaluation	EF: 68% PAP: 43mmHg Pericardial effusion	Not performed	EF: 50% PAP: 32 mmHg Right ventricular dysfunction Septal hypokinesia	EF: 50% Anteroseptal hypokinesia, pericardial effusion	EF: 60% PAP: 27 mm Hg Pericardial effusion	EF: 76% PAP: 27 mm Hg Left ventricular hypertrophy and diastolic dysfunction
Gastrointestinal involvement	Vomiting	None	None	None	Diarrhea	Vomiting/ Diarrhea
Respiratory findings	Mild coughing	Mild coughing	None	None	None	None
Duration of hospitalization	19 days	18 days	15 days	15 days	13 days	12 days

Laboratory results demonstrate the lowest absolute lymphocyte and platelet counts; and highest levels for other parameters.
MIS-A: Multisystem inflammatory syndrome in adults, COVID-19: Coronavirus disease 2019, SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2, DM: Diabetes mellitus, BNP: Brain natiruretic peptide, EF: Ejection fraction, PAP: Pulmonary artery pressure, PCR: Polymerase chain reaction

Table 2: Medical therapies of MIS-A cases

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Methylprednisolone	-	-	40 mg/day IV	40 mg/day IV	-	40 mg/day IV
Dexamethasone	-	6 mg qd - 10 days IV	-	-	6 mg qd - 12 days IV	-
Anakinra	100 mg q8h - SC	200 mg q8h - IV	200 mg q8h - IV	100 mg bid - SC	100 mg q8h - SC	-
Tocilizumab	-	-	-	-	-	-

IV: intravenous, SC: subcutaneous

DISCUSSION

Several hypotheses have been suggested to clarify the immunopathogenesis of MIS in children (MIS-C) and MIS-A. Inefficient and reduced neutralizing antibody activity against SARS–CoV-2, hyperinflammation triggered by spike protein which has a superantigen-like motif similar to *Staphylococcal* enterotoxin B, autoantibody mediated cell damage or inflammation and the composition of high viral load, slow viral clearance and delayed interferon response are among the current hypotheses (6-9). Another pathogenesis of MIS in children is the continuation of viral replication in the gastrointestinal system, leading to this strong systemic response in the post-infectious period. Mesenteric lymphadenitis was detected in laparotomy performed in MIS-C patients, and it was shown that enterocytes were infected with SARS-CoV-2 earlier (10, 11). In addition, one study which compared COVID-19, Kawasaki Disease and MIS-C in children; demonstrated that children diagnosed with MIS-C were significantly older, presented with higher CRP and ferritin, lower platelet count and interleukin (IL) 17A, also with different T-cell subsets. Outcomes of the study suggested that these three entity contained differences in immunological and antibody profiles (12).

The characteristics of our cases, and previously reported cases in the literature suggest that this clinical entity has a wide spectrum. Common features of the cases are; high fever, hypotension, systemic findings, lymphopenia, thrombocytopenia, high D-dimer levels, especially significantly higher procalcitonin and increased inflammation markers that mimic severe sepsis. Also, we observed that although patients had mild COVID-19, they had high SARS-CoV-2 antibody levels.

Abnormal echocardiography findings in MIS-C patients are seen in 50-60%, while in the CDC's MIS-A case series, this rate increases to 80% (4,13-15). Similar to this finding, five out of six patients in our study also had abnormal echocardiography findings. Echocardiography findings in patients were evaluated as secondary reactive-hyperdynamic changes to the advanced reduction of peripheral resistance. Pericardial-pleural effusion and peripheral edema were also suggested to develop due to fluid escape associated with reduced resistance, and endothelial damage.

As reported in children, previously reported adult MIS-A patients were mostly African-American and Hispanic, while a small number of patients of Asian descent were reported (2, 4, 15, 16). In the present study all patients were Caucasian.

The aforementioned study examining the COVID-19, Kawasaki, MIS-C and healthy children has shown that SARS-CoV-2 antibodies of several MIS-C patients target the endothelial glycoprotein named endoglin (12). Cellular damage caused by this cross reaction may be perceived by pattern recognition receptors of innate immunity as endogenous damage-associated molecular patterns. It is inevitable that this possible interaction would trigger many signal cascades known from the pathogenesis of sepsis, causing the production of cytokines such as tumor necrosis factor alfa, IL-1β, IL-18 via activation of nuclear factor kappa B and inflammasomes (17). In the light of the data, we have learned about innate immunity over the last decade, there is a possibility of that innate immunity might be trained and triggered with acute COVID-19, and the trained immunity may empower the severity of inflammation that occurs during the post-COVID-19 inflammatory disease (18).

During sepsis, IL-1β levels were shown to be higher in patients who have died compared to the levels of the survivors (19). The IL-1β is known to promote the amplification cascade, and induces the synthesis of various inflammatory genes such as IL-6, IL-8, monocyte chemoattractant protein-1, cyclooxygenase-2, nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor alpha, IL-1α, IL-1β, and mitogen-activated protein kinase (17). Accordingly, we hypothesized that the use of a human recombinant interleukin 1 antagonist would be more effective (Anakinra) to block the cytokine cascade at an earlier stage.

In conclusion MIS-A is a severe and mortal condition that causes various clinical pictures and can be confused with sepsis. Anakinra, a recombinant IL-1 receptor antagonist, is an agent that can be used in the treatment of MIS-A since it blocks the cytokine cascade at an early stage. Since this clinical picture can be fatal, our clinical strategy was an earlier diagnosis and rapid anti-cytokine treatment. We suggest that there was a demonstrative response to anakinra and corticosteroid therapy in our case series and it is worthwhile. We assume that there is a need to increase the awareness of clinicians about MIS-A.

Ethics Committee Approval: The study was approved by the local ethics committee of Istanbul University, Istanbul Faculty of Medicine (Date: 14.01.2022, No: 2021/2183).

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CLINICAL AND MOLECULAR RESULTS OF SIX CASES WITH ROBERTS SYNDROME: REVIEW OF CASES FROM TURKIYE

ROBERTS SENDROMLU ALTI OLGUNUN KLİNİK VE MOLEKÜLER SONUÇLARI İLE TÜRKİYE'DEN BİLDİRİLEN OLGULARIN GÖZDEN GEÇİRİLMESİ

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ABSTRACT

Objective: Roberts syndrome is a rare autosomal recessive disease characterized by limb defects, prenatal onset growth retardation, and craniofacial anomalies. We aimed to compare the clinical and molecular findings of six cases with Roberts syndrome with the previously reported patients from Turkiye and to emphasize that a definitive diagnosis can be made in the intrauterine period with cytogenetic tests in the early period without the need to wait for molecular test results.

Materials and Methods: Six cases, diagnosed with Roberts syndrome, in our outpatient clinic of Istanbul University, Istanbul Faculty of Medicine, Medical Genetics Department between 2015-2021, were included in the study. The family history, clinical information, and cytogenetic and molecular findings of the patients were retrospectively reviewed and compared with the cases reported from Turkiye in the literature. G and C-banding techniques and Sanger sequencing of the *ESCO2* gene were performed.

Results: Pathogenic variants in homozygous in four and compound heterozygous in two patients in the *ESCO2* gene were identified. Compound heterozygous c.[417dup];[1131+1G>A] (p.[(Pro140Thrfs*8)];[(?)]) in case 1, and c.[1111dup];[760del] (p.[(Thr371Asnfs*32)];[(Thr254Leufs*13)]) in case 6, homozygous c.1131+1G>A (p.(?)) in case 2, case 3 and case 5, and homozygous c.1111dup (p.(Thr371Asnfs*32)) in case 4 were detected. The variants reported in our case series were previously asso-

ÖZET

Amaç: Roberts sendromu; ekstremite anomalileri, prenatal başlangıçlı büyüme gelişme geriliği ve kraniyofasiyal anomaliler ile karakterize nadir görülen otozomal resesif kalıtılan bir hastalıktır. Roberts sendromlu altı olgunun klinik ve moleküler bulgularını Türkiye'den daha önce bildirilen olgularla karşılaştırmayı ve moleküler test sonuçlarını beklemeye gerek kalmadan erken dönemde sitogenetik testlerle kesin tanının intrauterin dönemde yapılabileceğini vurgulamayı amaçladık.

Gereç ve Yöntem: 2015-2021 yılları arasında İstanbul Üniversitesi İstanbul Tıp Fakültesi Tıbbi Genetik Anabilim Dalı polikliniğimizde Roberts sendromu tanısı alan altı olgu çalışmaya dahil edildi. Olguların aile öyküsü, klinik bilgileri, sitogenetik ve moleküler bulguları retrospektif olarak incelendi ve literatürde Türkiye'den bildirilen olgularla karşılaştırıldı. G ve C-bantlama teknikleri ve *ESCO2* geninin Sanger dizilimi gerçekleştirildi.

Bulgular: *ESCO2* geninde dört olguda homozigot ve iki olguda bileşik heterozigot patojenik varyant tespit edildi. Olgu 1'de birleşik heterozigot c.[417dup];[1131+1G>A] (p.[(Pro140Thrfs*8)];[(?)]) ve olgu 6'da c.[1111dup];[760del](p.[(Thr371Asnfs*32)]; [(Thr254Leufs*13)]) ile olgu 2, olgu 3 ve olgu 5'te homozigot c.1131+1G>A (p.(?)), olgu 4'te homozigot c.1111dup (p.(Thr371Asnfs*32)) saptandı. Olgu serimizde bildirilen varyantlar daha önce hastalıkla ilişkilendirilmiştir. c.760del değişimi ilk kez Türkiye'den bir olguda gösterilmesi bu hastalığa neden olan toplumuzdaki genotip bilgisine katkısı olmuştur. Ayrıca li-

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ciated with the disease. The first demonstration of the c.760del in a Turkish case contributed to the genetic association of this pathogenic variants with Roberts syndrome Although all the previously reported patients were homozygous, we have detected two patients with compound heterozygous pathogenic alterations from Turkiye indicating that the disease should also be considered in families with no consanguinity.

Keywords: Roberts syndrome, *ESCO2*, tetraphocomelia

teratürde Türkiye'den daha önce bildirilmiş olgulardaki patolojik varyantların homozigot olduğu rapor edilmiştir. Bizim olgu serimizde ise ebeveynler arasında akrabalık ilişkisi olmadığı halde iki olguda birleşik heterozigotluk gösterilmesi bu sendromun akrabalık ilişkisi olmayan olgularda da rastlanılabileceğini göstermektedir.

Anahtar Kelimeler: *ESCO2* geni, Roberts sendromu, tetrafokomeli

INTRODUCTION

Roberts syndrome (RBS) (OMIM #268300) is an autosomal recessive disorder, characterized by prenatal or postnatal growth retardation, extremity anomalies with bilateral symmetric tetraphocomelia/mesomelia, craniofacial anomalies with cleft palate/lip, and dysmorphic facial findings, intellectual disability, cardiac and renal anomalies. RBS is caused by biallelic mutations in the *ESCO2* (Establishment of sister chromatid cohesion N-acetyltransferase 2) gene, which encodes a protein necessary for the establishment of sister chromatid cohesion during the S phase of mitosis (1). It was first reported by Roberts in 1919 in three affected siblings with phocomelia and bilateral cleft palate/lip from a consanguineous Italian family (2). SC phocomelia (OMIM #269000) syndrome has a milder phenotype, which is allelic with RBS, described by Herrman et al. in 1969 and named this syndrome SC syndrome by using the initials of the surnames of these families (3). SC cases are also called pseudothalidomide syndrome because they resemble those exposed to thalidomide during pregnancy. RBS is the most severe form of the spectrum in which severely affected infants may be stillborn or die in the postnatal period, while individuals with SC phocomelia often represent the milder form of the spectrum (4,5). Premature centromere separation (PCS) and heterochromatin repulsion (HR) in heterochromatin regions are observed in chromosome analysis, and a diagnosis can be made by cytogenetic investigation before molecular analysis (6). The *ESCO2* gene encodes 601 amino acids and consists of 11 exons located at 8p21.1. So far, 34 variants associated with SC as reported in the HGMD (Human Genome Mutation Database) database (Professional Edition 2021.3 December 2021) composed of four missense, three nonsense, six splicing, 13 small deletion, and eight small insertion type alterations. Currently, no studies have been conducted to determine the mutation profile in patients from Turkiye. In this study, the clinical, radiological, cytogenetic, and molecular findings of six unrelated patients affected by RBS disease are presented and compared with the previously reported cases from Turkiye.

MATERIAL AND METHODS

Six cases, diagnosed with Roberts syndrome, in our outpatient clinic of Istanbul University, Istanbul Faculty of Medicine, Department of Medical Genetics between 2015-2021 were included in the study. The family history, clinical information, cytogenetic and molecular findings of the patients were retrospectively reviewed. This study was approved by Istanbul University Istanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 29.07.2021, No: 367380). Written informed consent was obtained from all parents of the patients included in the study.

Conventional cytogenetics

For cytogenetic analysis, 72-hour phyto-haemagglutinin-induced cell cultures were performed on cells obtained from peripheral blood in cases 2-4 and 6. Long term cultures were performed on cells obtained from chorionic villus biopsy in case 1 and amniocentesis in case 5. Chromosomes were harvested according to standard techniques and staining procedures were carried out using Giemsa-Pancreatin-Leishman (GPL) and C banding (7). A minimum of 20 metaphases for each banding method were analyzed at the 500-600 band level. The metaphases were also evaluated for PCS.

Molecular genetics

Genomic DNA was extracted from peripheral blood (case 2-4), chorionic villus (case 1) and cultured amniocytes (case 5) samples of the patients and the peripheral blood of the parents by using commercial kits according to the manufacturer's instructions (High Pure PCR Template Preparation Kit, Roche). Primers were designed to cover all the coding exons and exon intron boundaries ± 20 bp. *ESCO2* gene (NM_001017420. 2) was sequenced by Sanger methods (ABI 3500). Electropherograms were analysed using SeqScape software (SeqScape Version 3.0; Applied Biosystems). The variants were analyzed in the open source data bases [dbSNP (https://www.ncbi.nlm.nih.gov/snp/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and HGMD (http://www.hgmd.cf.ac.uk/ac/)]. The American College of Medical Genetics and Genomics' (ACMG 2015) standards were used for variant classification (8).

RESULTS

Clinical findings

The clinical, radiological, and molecular results of the cases are summarized in Table 1 and characteristic facial findings and limb malformation are shown in Figure 1.

Table 1: Clinical, radiological and molecular findings of RBS cases

Case	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6
Gender	♀	♀	♀	♂	♀	♂
Consanguinity **Affected siblings**	(-) (+)	1° cousin (-)	1.5° cousin (+)	1° cousin (-)	3° cousin (+)	(-) (-)
Age of diagnosed	16th GW	13 months	22 months	1.5 months	24th GW	19 months
Growth parameters Weight (at birth) Weight* Height*	MTP	1800 g (-4.56 SD) 6900 g (-2.40 SD) 52 cm (-7.7 SD)	1500 g (-4.69 SD) 4450 g (-7.1 SD) 55 cm (-8.82 SD)	2050 g (-3.48 SD) 3300 g (-2.36 SD) 48 cm (-3.02 SD)	MTP	1850 g (-4.24 SD) 7000 g (-4.28 SD) 65 cm (-5.21 SD)
Microcephaly*	nd	42 cm (-2.61 SD)	39 cm (-6.24 SD)	34 cm (-3.56 SD)	nd	45 cm (-2.37 SD)
Cleft palate/lip	-	-	Cleft lip/palate (unilateral)	-	-	Cleft lip/palate (unilateral)
Upper extremity findings	bilateral radial aplasia and ulnar hypoplasia bilateral hypoplastic thumbs	bilateral radial aplasia and ulnar hypoplasia bilateral thumbs aplasia	bilateral radial aplasia and ulnar hypoplasia bilateral thumbs aplasia	bilateral radial aplasia and ulnar hypoplasia bilateral hypoplastic thumbs	bilateral radial aplasia and ulnar hypoplasia bilateral hypoplastic thumbs	bilateral radial aplasia and ulnar hypoplasia bilateral hypoplastic thumbs
***ESCO2* gene variants in nucleotide**	c.[417dup]; [1131+1G>A]	homozygous c.1131+1G>A	homozygous c.1131+1G>A	homozygous c.1111dup	homozygous c.1131+1G>A	c.[1111dup]; [760del]

MTP: Medical Termination of Pregnancy, nd: not determined, GW: Gestation Week RBS: Roberts syndrome, *: the growth parameters at the age of diagnosed, SD: Standard Deviation, *ESCO2*: Establishment of sister chromatid cohesion N-acetyltransferase 2

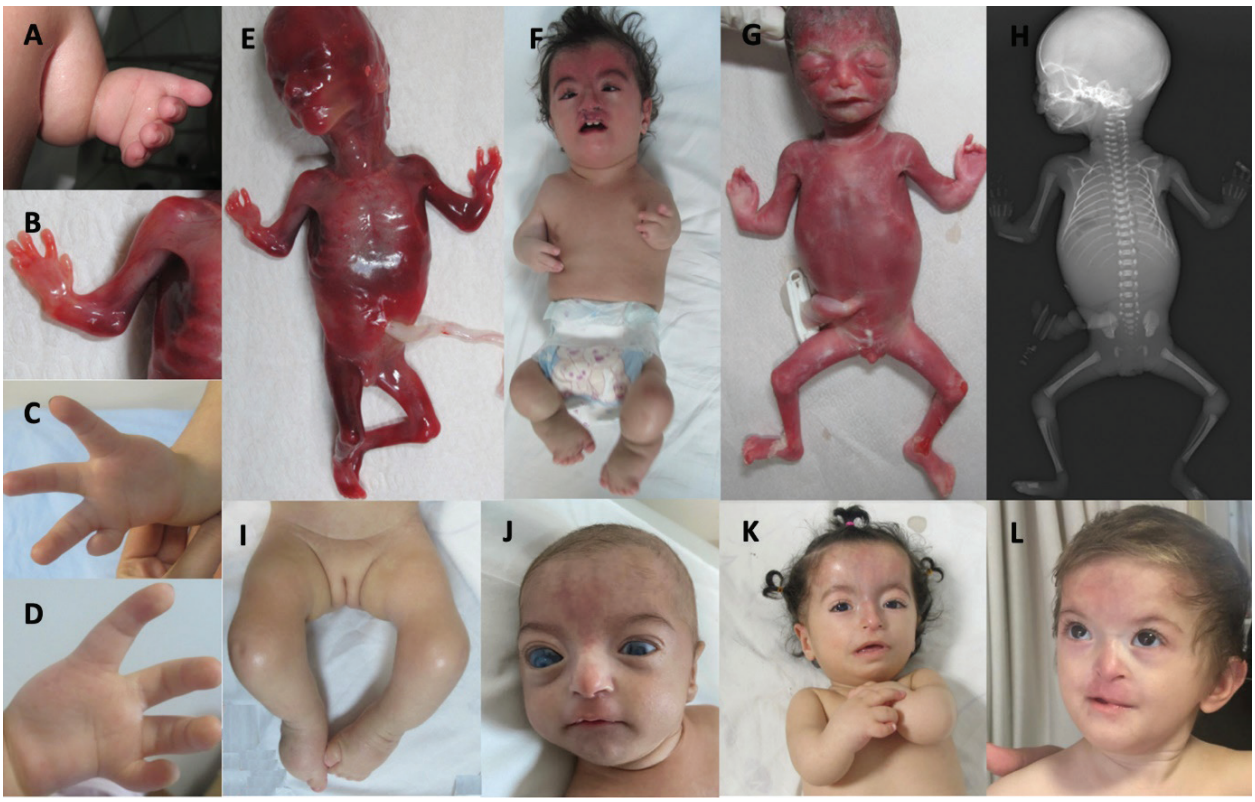

Figure 1: Clinical features of RBS cases. **A-D:** Severe mesomelic shortness and thumb a/hypoplasia in Case 3, Case 1, and Case 2. **E-G**: Bilateral cubiteal pterigium in Case 1, Case 3, and Case 5. **H:** Bilateral radial aplasia and ulnar hypoplasia in Case 5. **I:** Knee contractures in Case 3. **J-L:** Capillar malformation on the face of Case 4, Case 2, and Case 6.

Case 1, a female fetus, is the second pregnancy of a non-consanguineous couple. The family history revealed that the first pregnancy (G1) was medically terminated due to a hypoplasia of the bilateral tubular bones (humerus/radius/ulna) and a flexion deformity of the bilateral knees. The case was terminated due to a bilateral radial hypoplasia, flexion contractures in the elbows, and a ventricular septal defect (VSD) in the 15th week of the case [G2 Medical Termination of Pregnancy (MTP) 2]. In her 16th Gestation Week (GW) postmortem fetal examination; malar hypoplasia, micrognathia, bilateral low set ears, cubital pterygium, flexion contractures at the elbows, radially deviated hands, hypoplastic and proximally located thumbs, and bilateral clinodactyly were found. A Fetal radiological examination revealed bilateral radial aplasia, ulnar hypoplasia, and bilateral metacarpal and a hypoplastic phalanx structure.

Case 2, a 13-month-old female is the first child (G1P1) of a consanguineous marriage. She was born by Cesarean Section (CS) due to a breech presentation at the 40th GW, with a birth weight of 1800 g (-4.56 SD). The birth height and head circumference (HC) is unknown. The case, which was followed up in the Neonatal Intensive Care Unit (NICU) for one month, was evaluated in our outpatient clinic at 13 months old because of a small gestational age (SGA), dysmorphic findings, and extremity anomalies. Her hair was thin and sparse. She was found to have a growth deficiency and microbrachycephaly. Capillary malformation was observed on the forehead, which became more evident when crying. She had bitemporal narrowing, shallow orbit with downslanting palpebral fissures, malar hypoplasia, hypoplastic ala nasi, narrow/high palate, and micrognathia. Respiratory, cardiovascular (CVS), and gastrointestinal system (GIS) examinations were unremarkable. The patient had bilateral flexion contracture at the elbows and knees, and a marked shortening of the forearms (mesomelia). He gained head control at three months, the ability to unsupported sitting at nine months, and spelling at 13 months. Radiological examination revealed bilateral ulnar/radial hypoplasia and thumb aplasia.

Case 3, a 22-month-old girl, is the second child of a consanguineous marriage from their fourth pregnancy. She has a healthy 7-year-old brother. The couple's second and third pregnancies, which were not consulted with us, were medically terminated due to extremity anomalies (G4P2MTP2). Severe shortness was detected in all extremities in the antenatal ultrasound examinations prenatally. She was born with spontaneous vaginal delivery (SVD) at the 38th GW with a weight of 1500 g (-4.69 SD). The birth height and head circumference were unknown. She was followed up in the NICU for 90 days due to extremity anomalies. She was operated on for a unilateral cleft lip when she was 20 months old. At her 22-month-old physical examination, she had severe growth and developmental retardation and severe microbrachycephaly She had extensive capillary malformation covering the entire forehead, extending to the midline of the face, sparse hair, highly arched eyebrow, prominent eyelashes, long and downslanting palpebral fissures, hypertelorism, shallow orbits, short philtrum, hypoplastic ala nasi, malar hypoplasia, and a high palate. There was tetraphocomelia in all four extremities, in which the anterior segments were more severely affected, especially in the upper part. A single transverse line on small/hypoplastic palms and bilateral thumb aplasia were observed. She also had bilateral 40-degree knee flexion contractures and brachydactylic toes with partial syndactyly between the 4th and 5th toes. Respiratory, GIS, and CVS examinations were unremarkable. In her genital examination, labia major and minors were hypoplastic. A neurological examination revealed hypotonicity and neuromotor retardation. She has gained head control at nine months, but not yet achieved unsupported sitting.

Case 4; A 1.5-month-old male case is the first child from a consanguineous marriage. He was found to have short limbs at the 28th GW, but no further genetic counseling or invasive procedure was recommended. He was born with CS with a weight of 2050 g (-3.48 SD), a height of 47 cm (-1.28 SD), a HC of 33 cm (-1.27 SD) at the 38th GW. He was hospitalized in the NICU for one month due to postnatal respiratory distress, bilateral pes equinovarus (PEV) deformity, corneal clouding, and extremity anomalies. An eye examination revealed bilateral corneal clouding. At his 1.5-month-old physical examination, he had a growth deficiency and microcephaly. Diffuse cutis marmoratus and capillary malformation on the forehead were observed. Mild hypertelorism, proptosis, bilateral buphthalmos, which is more prominent on the right, corneal clouding, short-small nose, hypoplastic ala nasi, high/narrow palate, and micro- and retrognathia were observed. Significant mesomelic shortness in the upper extremity and bilateral hypoplastic thumbs were observed. A bilateral 90-degree elbow and 30-degree knee flexion contractures and popliteal pterygium were observed. There was bilateral clubfoot in the feet. His tonus was normal. A radiological examination revealed bilateral radius and ulna hypoplasia, aplasia of the right fibula, and hypoplasia of the left fibula.

Case 5, the second child from the second pregnancy of the consanguineous couple. The female fetus was medically terminated at the 24th GW (G2P1MTP1). He has a healthy 8-year-old sister. Nasal bone hypoplasia, absence of radius and ulna in the left upper extremity, single bone structure in the anterior segment of the right upper extremity, and a radial deviation of bilateral thumbs were detected at 19th GW. In postmortem 24th GW physical examination, the height was measured as 31 cm (25-50p),

620 g (25-50p), 22 cm (50p). There were hypertelorism, proptosis, hypoplastic ala nasi, malar hypoplasia, micrognathia, and low-set ears. Severe shortening of the forearms and radial deviation of the hands were observed bilaterally, more prominently on the left side. There was clinodactyly in the 5th fingers of the bilateral hands, and 20-degrees knee flexion contractures. A fetal X-ray examination revealed, severe mesomelic shortness in the upper extremity, radial aplasia and ulnar hypoplasia which is more prominent on the right.

Case 6; A 19-month-old male case was the second child of a non-consanguineous marriage. He has a healthy 3-year-old brother (G2P2). In the antenatal period, upper extremity shortness and PEV were observed at the 28th GW. However, invasive procedure and genetic evaluation could not be performed because the family was against medical termination. The case was born at the 38th GW by CS due to intrauterine growth retardation (IUGR), with a weight of 1850 g (-4.24 SD) and a height of 42 cm (-3.41 SD). The birth head circumference is unknown. The case was followed up in the NICU for 2 weeks due to SGA birth and congenital anomalies. He had a history of a unilateral cleft palate/lip operation at the age of 13 months. He was first evaluated by us at the age of 19 months. He had microbrachycephaly and capillary malformation extending to the forehead, glabella, nose, and philtrum. His hair was sparse and thin. He had hypertelorism, bilateral proptosis, arched brow structure, malar hypoplasia, short-small nose with hypoplastic ala nasi, cleft lip operation scar on the right part of the philtrum, micrognathia, and low-set ears. Severe mesomelia in the bilateral upper extremities and 90-degree elbow flexion contractures were observed. There were hypoplastic thumbs and bilateral PEV. Bilateral radius aplasia and ulna hypoplasia were observed in the X-Ray.

Genetic results

Cytogenetic analysis was performed on the cells obtained from the chorionic villus biopsy in case 1, amniocentesis in case 5, and the peripheral blood in cases 2-4, and 6. The karyotype were all normal [46,XX (Case 1-3 and 5) and 46,XY (Case 4, 6)]. PCS was found in all cases (Figure 2). A Sanger sequence analysis of the *ESCO2* gene revealed homozygous c.1131+1G>A variant in case 2, case 3, and case 5, and a homozygous c.1111dup variant in case 4. Two cases were compound heterozygous for the c.417dup with c.1131+1G>A, and c.1111dup with c.760del, in case 1 and case 6, respectively (Figure 3). The parents of the cases with homozygous variants were heterozygous, as expected. The mother of case 1 was heterozygous for the c.417dup, while the father was heterozygous for the c.1131+1G>A variant. The mother of case 6 was heterozygous for the c.417dup, while the father was heterozygous for the c.760del.

Figure 2: View of the C-banding of Case 3. Black arrows show characteristics premature centromere separation

DISCUSSION

RBS/SC phocomelia syndrome is currently also called a spectrum associated with the *ESCO2* gene. The diagnosis is established in a case with extremity anomalies and craniofacial findings by identification of the premature centromere separation via cytogenetic testing and/or biallelic pathogenic variants in the *ESCO2* gene by molecular testing.

The clinical diagnosis of RBS is considered when typical extremity and craniofacial malformations are accompanied by prenatal-onset growth deficiency and developmental retardation. Differential diagnosis includes preaxial reduction defects such as Holt-Oram and Fanconi syndrome if extremity findings are mild. Thrombocytopenia-Aplastic Radius (TAR) Syndrome is more likely if severe involvement is accompanied by thrombocytopenia. Cornelia de Lange syndrome (CdLs) should be considered, especially in the presence of SGA, if there are arched eyebrows with synophrys.

Clinical diagnosis can be distinguished according to four criteria observed in all patients; [1] symmetrical reduction defects in the extremities in which the upper extremities are affected more frequently and more severely than the lower extremities, [2] limb defect causing mesomelic shortness, which is always accompanied by a/hypoplasia of the thumb. The most frequently, and severely affected limb is the upper limb. The radius, ulna, and humerus are affected, respectively, in the upper extremity, while the fibula, tibia, and femur are affected, respectively, in the lower extremities. [3] Characteristic facial findings accompanied by microcephaly (facial capillary malformation, exophthalmos, hypertelorism, downward slanting palpebral fissures, malar hypoplasia, hypoplastic space,

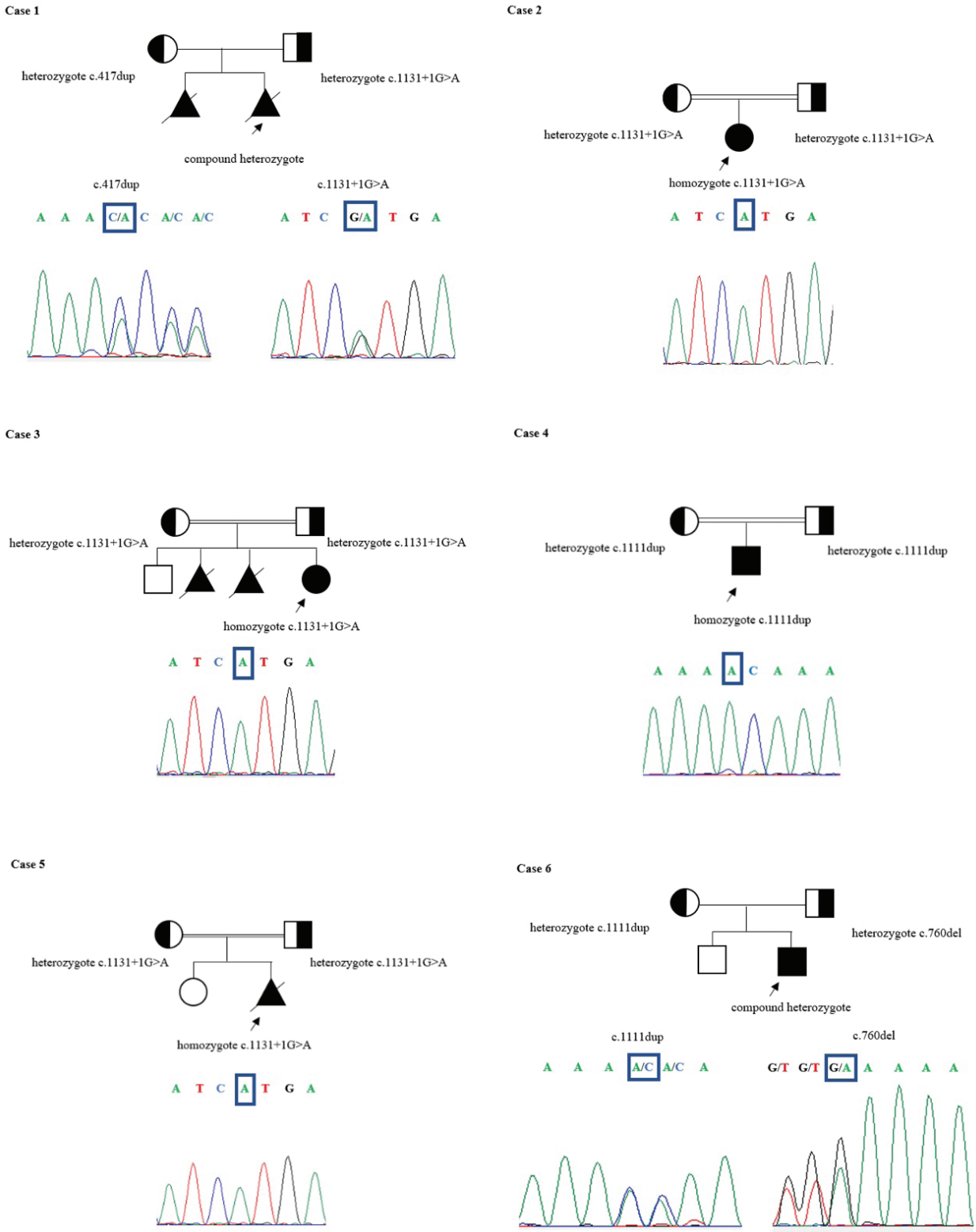

Figure 3: Molecular results of RBS cases. Sanger sequence analysis of the *ESCO2* gene revealed homozygous c.1131+1G>A mutation in case 2, case 3, and case 5, homozygous c.1111dup mutation in case 4. Two cases were compound heterozygous for the c.417dup with c.1131+1G>A, and c.1111dup with c.760del, in case 1 and case 6, respectively

cleft palate-lip, and micrognathia) [4] are consistent with the correlation between extremity malformations and craniofacial findings, and facial involvement is more pronounced in those with severe extremity defects (9).

In the study of Vega et al. in 2010, in which the clinical findings of 49 cases were compiled according to this algorithm, phocomelia with growth retardation and radial hypoplasia/aplasia and aplasia or hypoplasia of the hand and thumb in 100% of the cases, hypoplasia of the thumb in 98.7%, microcephaly in 95%, cleft lip/palate in 54.5%, corneal opacity in 36.1%, and cardiac anomalies in 25.8% (10).

Growth deficiency starts prenatally and continues in the postnatal period. All postnatally evaluated cases in our series [4/4] had SGA and the mean birth weight was -4.24 SD (minimum: -4.69 SD and maximum: -3.48 SD). In accordance with the literature, cases 2, 3, 4, and 6 diagnosed in the postnatal period had growth deficiency and microcephaly [4/4]. At the age of diagnosis, their mean weight is -4.03 SD (minimum: -7.1 SD and maximum: -2.36 SD), average height is -6.18 SD (minimum: -8.82 SD and maximum: 3.02 SD), and mean head circumferences were measured as -3.69 SD (minimum: -6.24 SD and maximum: -2.37 SD). Another finding observed in all our prenatal and postnatal cases is phocomelia [6/6]. Phocomelia is the most important feature of this syndrome that can be recognized in the prenatal period. It is a characteristic feature of shortness in all tubular bones in all four extremities where symmetrical and especially upper extremity anterior segments (mesomelia) are more severely affected. In severe cases, the upper and lower extremities are affected together. As in case 1 and case 5, diagnosed in the fetal period, the presence of especially symmetrical upper extremity forearm shortness should bring the diagnosis to mind, as in our cases in the prenatal period in the absence of craniofacial findings.

Another most common extremity finding is thumb aplasia or hypoplasia. In our series, symmetrical severe upper extremity mesomelic shortness [6/6] (bilateral radial aplasia and ulnar hypoplasia) accompanying thumb aplasia [2/6] and hypoplasia [4/6] were observed in all our cases. The relative preservation of the other fingers despite the involvement of the thumbs can be used as a clinical clue to exclude CdLs, which ranks first on the differential diagnosis list if SGA exists. Although the finding of a/hypoplasia in the thumb and radius was reported in all cases, Goh et al. reported a mildly affected adult male case in 2009 with a length of 152 cm with short tubular bones (11). Later, bilateral humeroradial synostosis without a shortening of the tubular bones was reported in a Thai and Indian case in 2020. It has been understood that phocomelia may not be present in mild cases of the spectrum associated with the *ESCO2* gene (12,13).

Cranial anomalies seen in the cases are microcephaly, brachycephaly, and craniosynostosis. Minor anomalies that cause a dysmorphic facial appearance are shallow orbits and proptosis, hypertelorism, facial capillary malformation, a wide nasal root, malar hypoplasia, and hypoplastic ala nasi. The typical dysmorphic facial appearance was observed in all [4/4] cases that were examined postnatally.

Cleft lip and/or cleft palate anomaly as a major craniofacial malformation is observed in half of the patients with RBS. The fact that a cleft palate anomaly alone was observed in less than 5% of the cases suggested that the presence of a cleft palate accompanying cleft lip anomaly is more typical for patients with RBS. It has been reported that there may be a high/narrow palate in cases where cleft lip and/or cleft palate anomalies are not observed (10). In our case series, a cleft lip anomaly was observed in case 3, a cleft palate accompanying a cleft lip anomaly in case 6, and a high/narrow palate was observed in cases 2 and 4.

To date, no correlation between genotype and phenotype associated with *ESCO2* variants has been established. It has been shown that there may be clinical differences even in different families carrying the same mutation or even within the same family. The difference in the severity of clinical findings in case 2, case 3 and case 5 with the same homozygous alterations in our case series is consistent with this observation.

RBS is an autosomal recessive disease. Although it is rare, it has been reported in many countries around the world of different racial and ethnic origins, although it is more common in Mediterranean countries such as Egypt and Turkiye, as well as in Germany and the American continent.

CdLs is on the differential diagnosis list for RBS. Both are known as, syndromes associated with mutations in the Cohesin complex and its regulators, a multiple subunit protein complexes known as cohesinopathies. The ESCO2 protein, responsible for RBS, acetylates the cohesion complex, contributing to the stability of the binding of the cohesion complex with DNA and holding the newly replicated sister chromatids together. Cohesin is a protein complex that mediates sister chromatid cohesion, homologous recombination, and DNA cycling. Mutations in the genes encoding the core proteins of the Cohesin complex, SMC1A, SMC3, and RAD21, and NIPBL, HDAC8, and BRD4 proteins, which are involved in the regulation and stabilization of the Cohesin complex, are responsible for CdLs (9).

In which the *ESCO2* gene was first associated with the disease, association with the 8p12-21.2 chromosome locus was established using linkage analyses in seven Co-

Table 2: *ESCO2* pathogenic variants in cases from Turkiye

Nucleotide change*	Amino acid change**	exon/intron	dbSNP	ClinVar	ACMG Classification	References of cases from Turkiye
c.417dup[1]	p.Pro140Thrfs*8	exon 3	rs80359848	VCV000021245.3	pathogenic	Vega et al. 2005, in this study
c.760del[2]	p.Thr254Leufs*13	exon 3	rs80359852	VCV000021247.10	pathogenic	in this study
c.879_880del[3]	p.Arg293Serfs*7	exon 4	rs80359857	VCV000021251.9	pathogenic	Vega et al. 2005, Gordillo et al. 2008, Vega et al. 2010
c.877dup	p.Arg293Lysfs*8	exon 4	-	-	likely pathogenic	Mengen et al. 2018
c.1111dup[4]	p.Thr371Asnfs*32	exon 6	rs80359859	VCV000021232.6	pathogenic	Vega et al. 2005, Avci et al. 2018, Sezer et al. 2019, in this study
c. 1131+1G>A	p.?	intron 6	rs80359861	VCV000021233.5	pathogenic	Gordillo et al. 2008, Doğan et al. 2014, Colombo et al. 2019, in this study
c.1461_1462del[5]	p.Arg487Serfs*19	exon 9	rs80359866	VCV000021237.2	pathogenic	Vega et al. 2005

*: DNA sequence based on GenBank number NM_001017420.2, **: Protein sequence based on GenBank number NP_001017420, c.417dup [1] [c.411_412insA veya 417_418insA], c.760del[2] [c.752del], c.879_880del[3] [877_878del], c.1111dup[4] [1104_1105insA], c.1461_1462del[5] [1457_1458del] these variants have been corrected from the original publications to conform to HGSV (*Human Genome Structural Variation*) nomenclature

lombian families and genome-wide homozygous mapping for RBS. It has been understood that it is caused by biallelic pathological variants of the gene (1).

We identified four known pathogenic variants; predicted truncating c.417dup, c. 760del, c.1111dup, and evolutionally conserved splice donor site alteration c.1131+1G>A in our study. According to ACMG guidelines, all variants are classified as pathogenic. The reported pathogenic variants detected in cases from Turkiye with RBS in the literature so far are summarized in Table 2 (14-18). The pathogenic variant c.760del, which has been reported in two cases of German and American origin in the literature, was reported in a Turkish case for the first time in our case series, which expanded the knowledge of the Turkish mutation profile (5,19). In addition, homozygous mutations were found in all previously reported Turkish cases. The determination of compound heterozygous mutations in two cases in our series shows that this disease can also occur without close consanguinity.

With the widespread use of clinical or whole-exome analyses in recent years, it is predicted that a definitive diagnosis can be made in more cases with RBS in the postnatal period. However, as seen in the family history of prenatal cases in our case series, it may be possible to medically terminate them without a diagnosis. In these cases, failure to diagnose the terminated fetus leads to the inability to provide appropriate genetic counseling to the family. It should also be kept in mind that with the increasing clinical recognition of RBS, cytogenetic diagnosis can be made without the need for further molecular analysis.

Ethics Committee Approval: This study was approved by Istanbul University Istanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 29.07.2021, No: 367380).

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INTER- AND INTRA-OBSERVER RELIABILITY OF THE MIRELS' SCORING SYSTEM FOR THE DETERMINATION OF PATHOLOGICAL FRACTURE RISK IN METASTATIC BONE LESIONS AMONG ORTHOPEDIC SURGEONS WITH DIFFERENT LEVELS OF EXPERTISE

METASTATİK KEMİK LEZYONLARINDA PATOLOJİK KIRIK RİSKİNİN BELİRLENMESİNDE KULLANILAN MİRELS SKORLAMASININ FARKLI KIDEMLERDEKİ ORTOPEDİK CERRAHLAR ARASINDAKİ GÜVENİLİRLİĞİ

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ABSTRACT

Objective: To assess inter- and intra-observer reliability of Mirels scoring for the determination of pathological fracture risk in metastatic bone lesions of 30 patients among six different levels of experienced orthopedic surgeons who were trained in the same university clinic.

Material and Methods: Thirty patients were randomly selected from oncology unit consultations. Six observers were selected in accordance to their orthopedic experience. Mirels parameters except pain were evaluated by observers on two different times without the observers being aware of each other.

Results: The Fleiss' Kappa values were detected as κ=0.21, p<0.0001, and κ=0.15, p<0.0001 by inter-observers at the first and second observational points, respectively. The Kappa values were in perfect (κ=0.95), fair (κ=0.27) and fair (κ=0.10) agreements for the region, size and type of the metastatic involvement by inter-observers at the first observational point. The same parameters had similiar scores as κ=0.83, κ=0.13 and κ=0.28 for region, size and type for the second observation. Fair (κ=0.333), moderate (κ=0.413), fair (κ=0.225), slight (κ=0.035), fair (κ=0.369

ÖZET

Amaç: Bu çalışmanın amacı, metastatik kemik lezyonları nedeniyle oluşan patolojik kırık riskinin tespiti için geliştirilmiş Mirels skorlamasının, metastatik lezyon tanısı alan 30 hastada aynı üniversite kliniğinde eğitim almış altı farklı tecrübedeki ortopedist arasındaki güvenilirliğini değerlendirmekti.

Gereç ve Yöntem: Şiddetli veya en az orta derecede ekstremite ağrısı nedeniyle onkoloji birimi tarafından konsülte edilen rastgele 30 hasta seçildi. Ağrı dışındaki parametreler gözlemciler tarafından bir ay arayla birbirlerinden habersiz olarak değerlendirildi.

Bulgular: Gözlemciler arasındak Fleiss' Kappa değerleri, sırasıyla birinci ve ikinci gözlem noktalarında κ=0,21, p<0,0001 ve κ=0,15, p<0,0001 olarak tespit edildi. İlk değerlendirmede gözlemciler arası metastatik tutulumun lokalizasyonu, boyutu ve tipi için Kappa değerleri sırasıyla mükemmel (κ=0,95), zayıf (κ=0,27) ve zayıf (κ=0,10) olarak bulundu. Aynı parametreler bir ay arayla ikinci kere değerlendirildiğinde ise lezyonun lokalizasyonu, boyutu ve tipi için κ=0.83, κ=0.13 ve κ=0.28 olarak benzer puanlara sahipti. Gözlemcilerin kendi içlerinde, iki farklı zaman diliminde

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and poor (κ=0.030) evidence of agreements were detected in the comparison of the first and second observations for total scores in seniority order.

Conclusions: Kappa analysis showed perfect agreement for region, but slight to fair for size and type. There was a significant difference in overall scores across experience levels for the most and least experienced observers. A new rating system with revised parameters may be required to predict impending fractures.

Keywords: Pathological fractures, mirels, bone metastasis

ağrı olmadan diğer parametrelere verdikleri cevapların toplamdaki skorları kıdem sırasına göre zayıf (κ=0,333), orta (κ=0,413), zayıf (κ=0,225), önemsiz düzeyde (κ=0,035), zayıf (κ=0,369) ve kötü (κ= -0,030) olarak görüldü.

Sonuç: Kappa analizine göre gözlemciler arasında lezyonun lokasyonu için mükemmel uyum görülürken, lezyonun tipi ve boyutu içinse önemsiz düzeyde ve zayıf bir uyum görülmüştür. Total skorlarda en deneyimli ve en az deneyimli gözlemciler arasında önemli bir fark tespit edilmiştir. Bu sebeple yaklaşan (impending) kırıkları öngörmek ve hastaların prognozunu dikkate almak için parametrelerinin revize edildiği yeni bir derecelendirme sistemi gereklidir.

Anahtar Kelimeler: Patolojik kırıklar, mirels, kemik metastazı

INTRODUCTION

More than 75% of diagnosed carcinoma patients exhibit an evidence of skeletal metastases during their treatment (1). The most prevalent etiology of skeletal system metastases are lung and breast cancers (2). Once cancer spreads to the skeleton, cure can rarely be attained (3). The most common regions of metastases are the spine and the pelvis, followed by the femur (4). The diagnosis is based on signs, symptoms, clinical findings and radiological imaging. The mobility of these patients usually decreases due to severe pain. Spinal cord compression due to pathologic vertebral fracture and pathologic fractures of a lower extremity are two major reasons of morbidity which may have devastating consequences for their ongoing oncological treatment (5).

With the increase in the global life expectancy of patients with bone metastasis, it is crucial to determine the appropriate protocols aiming to improve the quality of patients life. This has to be done even before the occurrence of a pathologic fracture, and to preserve such stabilization for the rest of their life (6).

Prediction of bone fracture risk due to metastasis, a scoring system including four parameters including region, pain, type (blastic, lytic or mixed) and the lesion size was described by Mirels et al (7). The rating system is based on 4 parameters, each scored from 1 to 3 (Table 1). The individual scores are added for a final total score. Treatment modalities are based on the risk of fracture according to the final score (Table 2).

Table 1: Mirels' scoring system (7)

Parameter	Score		
	1	2	3
Site	Upper limb	Lower limb	Peritrochanter
Pain	Mild	Moderate	Severe
Lesion	Blastic	Mixed	Lytic
Size	<1/3	1/3-2/3	>2/3

Table 2: Mirels' definitions and treatment recommendations (7)

Risk of pathological fracture	Total score	Treatment recommendations
Impending	≥9	Prophylactic stabilization
Borderline	8	Consider stabilization
Not impending	≤7	Nonoperative care

In this study, we aimed to investigate the intra- and inter-observer reliabilty of Mirels' scoring for the determination of pathological fracture risk in metastatic bone lesions of 30 patients among six different levels of experienced orthopedic surgeons who were trained in the same orthopedics clinic.

MATERIAL AND METHODS

This retrospective study was performed in accordance with the principles of the Declaration of Helsinki. The review included 30 patients radiologic demonstrations who were under oncologic treatment for a primary cancer diagnosed with bone metastasis at a single tertiary referral center. This study was approved by the ethics committee of the Istanbul Faculty of Medicine Clinical Research Ethics Committee, Istanbul University (Date: 11.02.2022, No: 03).

The thirty patients included in this study were randomly selected from oncology unit consultations. Patients with a pathological fracture at the time of initial presentation, patients without a standard radiograph of the painful extremity and patients with previous adjuvant radiotherapy (RT) to metastatic lesion were excluded from study. Plain radiographs of the 30 patients were obtained from the archives of our hospital server.

Six observers were selected in accordance to their orthopedic experience as following; one senior orthopedic oncologist; one senior orthopaedic surgeon of shoulder and elbow, one senior orthopedic surgeon, one junior orthopaedic surgeon, one senior resident of orthopedics and also one junior resident of orthopedics.

All observers were blinded to patients' data. Scoring was done to the region (upper extremity, lower extremity and trochanteric region), size (<1/3, 1/3-2/3, >2/3) with electronic ruler and type (blastic, mixed, lytic) of metastatic lesions by evaulating plain radiographs individually according to the Mirels' scoring system. To remove possible biases, pain criteria was not evaluated and other parameters of the Mirels Classification were based on by observers (8, 9). Therefore, scores were recorded from nine instead of twelve. Parametres were evaulated on two different times without the observers being aware of each other.

Statistical evaluation

The Fleiss' Kappa statistic was used for inter-observer agreement in each two time points (one month interval) individually. The intraclass correlation coefficient (ICC), shows how strongly units in the same group resemble each other and indicates the reliability of inter-observer agreement in each time point. The Kappa statistic (Cohen's Kappa coefficient) was used for intra-observer agreement (Table 3) (10). SPSS 24.0 was used for statistical analyses to evaluate intra observer variability and ICC value, but Stata 13.0 was performed for determining Fleiss Kappa coefficient.

Table 3: The Kappa statistic agreement scores (10)

Value of κ	Agreement
<0	Poor agreement
0.00-0.20	Slight agreement
0.21-0.40	Fair agreement
0.41-0.60	Moderate agreement
0.61-0.80	Substantial agreement
0.81-1.00	Almost perfect agreement

RESULTS

Region scores for the first and second observational points were shown in Table 4, respectively. The Fleiss' Kappa value was detected as 0.95, z=27.47, p<0.0001, according to the region of metastatic bone involvement by inter-observers (Table 5). The Kappa coefficient was in perfect agreement for the region by inter-observers. The intraclass correlation coefficient (ICC) was calculated as 0.93, and it indicates a good reliability of ratings by inter-observers (high similarity between ratings from the same group). Similar results were detected at the second observational time between scores by inter-observers as the Fleiss' Kappa=0.83, z=24.09, p<0.0001 and ICC=0.99.

The second criteria that was evaluated by observers was the size (<1/3, 1/3-2/3, >2/3) of the metastatic lesions. Scores given for the size of the lesions for the first and second observational time, are shown in Table 4. The Fleiss' Kappa value was detected as 0.27, z=7.32, p<0.0001, according to the size of metastatic bone involvement by inter-observers respectively (Table 5). The Kappa coefficient was detected as fair agreement for the size of lesion by inter-observers. Intraclass correlation coefficient (ICC) was calculated as 0.83, and it indicates a good reliability of ratings by inter-observers. Similar

Table 4: Inter-observer scores of Mirels criteria

	Region			Size			Type		
	UE	LE	PT	<1/3	1/3-2/3	>2/3	Blastic	Mixed	Lytic
	F/S	F/S	F/S	F/S	F/S	F/S	F/S	F/S	F/S
Orth. Onco	15/15	6/7	9/8	11/1	11/5	8/24	1/8	2/12	27/10
Sho&Elb Orth	15/15	5/7	10/8	3/15	8/7	19/8	2/1	11/11	17/18
Senior Orth	15/15	6/7	9/8	1/2	6/13	23/15	1/0	13/9	16/21
Junior Orth.	15/15	5/4	10/11	2/4	6/4	22/22	6/5	11/4	13/21
Sen. Orth Res	15/15	5/4	10/10	2/2	16/12	12/16	1/1	5/14	24/15
Jun Ort. Res.	15/15	7/8	8/9	2/0	9/8	19/22	3/4	8/9	19/7
					Total: 30				

F/S: First observational point/Second observational point, UE: Upper Extremity, LE: Lower extremity, PT: Pertrochanteric, Ort. Onco: Orthopedic Oncologist, Sho&Elb Orth: Senior Shoulder and Elbow Surgeon, Senior Orth: Senior Orthopedic Surgeon, Junior Orth: Junior Orthopedic Surgeon, Sen Orth Res: Senior Orthopedic Resident, Jun Ort. Res.: Junior Ort hopedic Resident

results (slight agreement) were detected at the second observational time between scores by inter-observers as the Fleiss' Kappa=0.13, z=3.34, p<0.0001 and ICC=0.76.

The third criteria evaluated by the observers was type (blastic, mixed, lytic) of the metastatic lesion. Scores given for type of the lesions for the first and second observational times are shown in Table 4. The Fleiss' Kappa value was detected as 0.10, z=2.72 p=0.0032, according to the type of lesions by inter-observers (Table 5). The Kappa coefficient was detected as slight agreement for the type by inter-observers. The intraclass correlation coefficient (ICC) was calculated as 0.50, and it indicates a moderate reliability of ratings by inter-observers. Similar but a little better results (fair agreement) were found at the second observational time between scores by inter-observers as the Fleiss' Kappa=0.28, z=7.57, p<0.0001 and ICC=0.78.

Lastly, total scores for the first and second observational times are shown in Table 6. The Fleiss' Kappa value was detected as 0.2, z=8.05, p<0.0001 by inter-observers (Table 5). The Kappa value was detected as fair agreement by inter-observers, and decreased due to decreasing seniority. The intraclass correlation coefficient (ICC) was calculated as 0.87, and it indicates a good reliability of ratings by inter-observers. Similar but a little lower results (slight agreement) were detected at the second time (two months) between scores by inter-observers as the Fleiss' Kappa=0.15, z=6.14, p<0.0001 and ICC=0.86.

For the orthopedic oncologist, there was a fair evidence of agreement in the comparison of the first and second observational times for total scores (κ=0.333, p<0.001), region (κ=0.947, p<0.0001), size (κ=0.019, p=0.821), and type (κ=0.097, p=0.160) (Table 7).

For the senior orthopedic surgeon of shoulder and elbow, there was a moderate evidence of agreement in the comparison of the first and second observational times for total scores (κ=0.413, p<0.0001), region (κ=0.947, p<0.0001), size (κ=0.209, p=0.134), and type (κ=0.427, p<0.007) (Table7).

For the senior orthopedic surgeon, a fair evidence of agreement was detected in the comparison of the first and second observational time-points for total scores (κ=0.225, p<0.012), region (κ=0.893, p<0.0001), size (κ=-0.074, p=0.588), and type (κ=0.195, p=0.237) (Table 7).

For the junior orthopedic surgeon, a slight evidence of agreement was detected in the comparison of the first I and second observational times for total scores (κ=0.035, p<0.012), region (κ=0.945, p<0.0001), size (κ=-0.172, p=0.210), and type (κ=0.024, p=0.840) (Table 7).

Table 5: Inter-observer analysis in each of the observational point for all observers

Subsets	First observational point				Second observational point			
	κ-statistic	z-score	ICC	P value	κ-statistic	z-score	ICC	p value
Overall	0.21	0.05	0.87	p<0.0001	0.15	6.14	0.86	p<0.0001
Site	0.95	27.47	0.93	p<0.0001	0.83	24.09	0.99	p<0.0001
Size	0.27	7.32	0.83	p<0.0001	0.13	3.34	0.76	p=0.0004
Type	**0.10**	**2.72**	**0.50**	**p=0.0032**	**0.28**	**7.57**	**0.78**	**p<0.0001**

Table 6: Results of total scores for intra-observer analysis

Total Scores	Orth. Onco	Sho&Elb Orth	Senior Orth	Junior Orth.	Sen. Orth Res	Jun Ort. Res.
	F/S	F/S	F/S	F/S	F/S	F/S
Score 3	0/0	0/0	0/0	1 /0	0/0	0/0
Score 4	0/1	0/0	1 /0	2 /1	0/0	1 /0
Score 5	4 /5	3 /3	0/1	3 /5	2 /3	1 /2
Score 6	10 /7	6 /7	6/7	4/4	6/8	8 /6
Score 7	11 /10	16/14	15/14	12/9	16/13	14 /13
Score 8	4 /6	2/6	6/6	4 /5	4/4	3 /7
Score 9	1/1	3 /2	2 /2	4 /6	0/2	3 /2
				Total: 30		

F/S: First observational point/Second observational point, Ort. Onco: Orthopedic Oncologist, Sho&Elb Orth: Senior Shoulder and Elbow Surgeon, Senior Orth: Senior Orthopedic Surgeon, Junior Orth: Junior Orthopedic Surgeon, Sen Orth Res: Senior Orthopedic Resident, Jun Ort. Res.: Junior Orthopedic Resident

Table 7: Intra-observer analyses for the comparison of two observational points

Observers	Subset	κ-statistic	p value
Orthopedic Oncologist	Overall	0.333	p<0.0001
	Site	0.947	p<0.0001
	Size	0.019	p=0.821
	Type	0.097	p=0.160
Senior orthopedic surgeon of shoulder and elbow	Overall	0.413	p<0.0001
	Site	0.947	p<0.0001
	Size	0.209	p=0.134
	Type	0.427	p<0.007
Senior orthopedic surgeon	Overall	0.225	p< 0.012
	Site	0.893	p<0.0001
	Size	-0.074	p=0.588
	Type	0.195	p=0.237
Junior orthopedic Surgeon	Overall	0.035	p<0.012
	Site	0.945	p<0.0001
	Size	-0.172	p=0.210
	Type	0.024	p=0.840
Senior resident of orthopedics	Overall	0.369	p<0.0001
	Site	0.945	p<0.0001
	Size	0.180	p=0.231
	Type	0.104	p=0.424
Junior resident of orthopedics	Overall	-0.030	p=0.769
	Site	0.740	p<0.0001
	Size	-0.049	p=0.761
	Type	0.026	p=0.851

For the senior resident of orthopedics, a fair evidence of agreement was detected in the comparison of the first and second observational times for total scores (κ=0.369, p<0.0001), for region (κ=0.945, p<0.0001), size (κ=0.180, p=0.231), and type (κ=0.104, p=0.424) (Table 7).

For the junior resident of orthopedics, a poor evidence of agreement was detected in the comparison of the first and second observational times for total scores (κ=-0.030, p=0.769) as well as comparing the scores for region (κ=0.740, p<0.0001), size (κ=-0.049, p=0.761), and type (κ=0.026, p=0.851) (Table 7).

DISCUSSION

Metastatic bone disease may cause a pathologic fracture with severe pain, hospitalization, and inevitably a surgery with high risks. Moreover, perioperative morbidity may be increased because of an established fracture. Prophylactic procedures such as surgical fixation become a necessity for patients with high pathological fracture risk. The main objective is to identify impending pathologic fractures that require surgical fixation prior to irradiation in clinical settings (8). Mirels created a scoring system to predict the metastatic bone fracture risk in 1988. He suggested as the score increased above a score of seven, the percentage of pathological fractures increased, but a score of nine is the most diagnostic value threshold to predict pathological fractures (7). However, Howard et al. found that a Mirels score of nine has a specificity of 35%, and they concluded that if the Mirels score is used as an indicator for surgery, two thirds of patients would have unnecessary surgery (9, 11). An impending pathologic fracture has some characteristics such as having a proximal femur lesion of ≥2.5 cm, and to occupy 50% or more of the bone diameter, and there is an adjacent lesser trochanteric fracture nearby (12). In our study, kappa and the Fleiss' kappa coefficient with intraclass correlation coefficient were used to score 30 patients with lesions according to the Mirels' rating system. We excluded the scoring of pain severity to remove subjectivity (8,15).

In the literature review, there are a few studies related to intra- and inter-observer variability scores in the prediction of metastatic lesions. Howard et al. conducted a study with four participants and 62 patients (9). Same as in our study, objective parameters of the Mirels classification were assessed two weeks apart, inter- and intra-observer reliability scores were calculated using the Fleiss' kappa statistic. Kappa values of scores for the inter-observer were detected as k=0.554 for region, k=0.342 for size, k=0.443 for radiographic view, and k=0.294 for the total score, similar to our results. The authors concluded that there was a fair to moderate agreement between observers at the first observational time, and moderate to substantial agreement after two weeks. They mentioned that the Mirels' score system is not objective and does not have reproducibility for the risk prediction of pathological fractures (9). Similarly in our study, the most experienced observers had the highest and the least experienced observer had the least agreement but the other observers had different agreement levels that do not belong to their experience level. In another study by Damron et al., they evaluated the intra- and inter-observer scores of 53 orthopedic surgeons or oncologists for 12 patients (12). They reported that the kappa values for the inter observer variability indicated a high agreement for region (k=0.752), moderate agreement for radiographic view, and fair agreement for size, similar to our results except for pain. Their overall sensitivity and specificity were 91% and 35%, respectively.

Furthermore, four orthopedic surgeons and four radiol ogists scored radiographs of 47 patients having bone metastases at the time of admission and after 12 weeks in the study of El-Husseiny and Coleman (14). Evans et al. suggested that the Mirels' ratings system may give false positive results leading patients to undergo an unnecessary prophylactic surgery (15). In the study by Mac Niocaill et al., radiographs with 35 lesions of 28 patients were scored twice by three orthopedic oncologists (8). In

their study, inter-observer agreement of the lesions were found for size (κ=0.27–0.60), region (κ=0.77–1.0) and type of the lesion (κ=0.55–0.81). Unlike us, Damron et al. and Macniocaill et al. stated that the Mirels scoring system is a reliable and reproduceable clinical tool (8, 12). However, their kappa values indicated slight and moderate to high agreement for the size and radiographic view, and perfect agreement for the region.

Borderline scores, such as score of 8, is problematic in the Mirels' classification system, and makes treatment options uncertain for the selection of prophylactic fixation or surgery. Therefore, the clinically use of Mirels' recommendation may cause unnecessary fixation in approximately 2/3 of the patients (16). Damron et al. reported that oncologists had scored very inconstantly, and they were advised to have an additional education (12). This is also true for our group but we had only one orthopedic oncologist. Today's golden standard, the Mirels' score, may lead to over treatment. Therefore, new methods such as finite element analysis and computed tomography-based structural rigidity analysis could be useful in the prediction of impending pathological fractures. Computed tomography-based structural rigidity analysis calculates the reduction of 35% or more in the affected femur rigidity with 100% sensitivity and 61% specificity for the prediction of pathological fractures (17). Finite element analysis measures the bone mineral density (11). These analyses are complex and difficult to use in clinical settings. One of the conflicting results of this study was that of not obtaining scores from pain. Our aim was to evaluate objective parameters of the classification, as no objective definitions were clarified related to this integral part of the original classification. So, it might be considered a highly subjective variable, and excluded from assessment.

CONCLUSION

In conclusion, the difference between clinicians with different backgrounds and experience may partly influence the scores. There was a significant difference in overall scores across experience levels for the most and least experienced observers but not for other observers. We believe that the Mirels' scoring system doesn't consider important factors such as comorbidities, radiotherapy, underlying diseases and expected survival. Moreover, there is a need for more specific guidelines for selectively fracture risk in metastatic lesions of long bones. New and more specific parameters and a satisfactorily revised rating system is required to predict impending fractures.

Ethics Committee Approval: This study was approved by the ethics committee of the Istanbul Faculty of Medicine Clinical Research Ethics Committee, Istanbul University (Date: 11.02.2022, No: 03).

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EVALUATING THE ROLE OF SCAPULA MORPHOLOGY IN ROTATOR CUFF TEARS: WHICH IS THE MOST USEFUL PREDICTOR?

ROTATOR MANŞET YIRTIKLARINDA SKAPULA MORFOLOJİSİNİN ROLÜNÜN İNCELENMESİ: EN KULLANIŞLI PREDİKTÖR HANGİSİ?

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ABSTRACT

Objective: The aim of this study was to determine the relationships between glenoid inclination (GI), acromial index (AI), critical shoulder angle (CSA), superior inclination (SI), and symptomatic degenerative full-thickness supraspinatus tears (SSTs).

Materials and Methods: Patients who were diagnosed with SSTs (n=39) between 2015 and 2017 were assessed retrospectively. Controls were matched to age, gender, and side. Measured GI, AI, CSA, and SI values were compared between the SSTs and control groups (n=39). The mean age for the SSTs group was 52.74±5.49 years, and the mean age for the control group was 51.15±5.22 years.

Results: The mean GI for the SSTs group was 19.97°±5.62°, and it was 13.72°±6.55° for the control group (p<0.001). The mean AI was 0.7±0.08 and 0.67±0.07 in the SSTs and control groups, respectively (p=0.035). The mean CSA for the SSTs group was 35.05°±4.09° and it was 33.06°±3.42° for the control group (p=0.022). The mean SI was 25.13°±5.71° and 25.91°±5.81° in the SSTs and control groups, respectively (p=0.552). For a cut-off value of GI ≥17.35°, sensitivity was 79.54%, and specificity was 79.51% (p=0.001). For a cut-off value of AI ≥0.67, sensitivity was 61.54% and specificity was 56.4% (p=0.031). For a cut-off value of CSA ≥33.45°, sensitivity was 64.12%, and specificity was 64.54% (p=0.014).

Conclusion: Higher measurement values of glenoid inclination, acromial index, and critical shoulder angle were associated with symptomatic degenerative full-thickness supraspinatus tears, and no correlation was found with superior inclination measurement. The glenoid inclination measurement had the highest

ÖZET

Amaç: Bu çalışmada glenoid inklinasyon (Gİ), akromial indeks (Aİ), kritik omuz açısı (CSA) ve superior inklinasyon (Sİ) ölçümlerinin semptomatik dejeneratif tam kat supraspinatus tendon yırtıkları (SSY) ile ilişkisinin incelenmesi amaçlanmıştır.

Gereç ve Yöntem: 2015 ve 2017 yılları arasında SSY tanılı hastaların verileri retrospektif olarak incelendi. SSY tespit edilen 39 hasta ile yaş, cinsiyet ve taraf yönünden eşleştirilmiş SSY olmadığı tespit edilen 39 hasta çalışmaya dahil edildi. Ölçülen Gİ, Aİ, CSA ve Sİ değerleri SSY grubu (n=39) ve kontrol grubu (n=39) arasında karşılaştırıldı. SSY grubunun yaş ortalaması 52,74±5,49 yıl, kontrol grubunun yaş ortalaması 51,15±5,22 yıl idi.

Bulgular: Gİ ortalaması yırtık grubunda 19,97°±5,62° iken kontrol grubunda 13,72°±6,55° idi (p<0,001). Aİ ortalaması yırtık grubunda 0,7±0,08, kontrol grubunda ise 0,67±0,07 idi (p=0,035). CSA ortalaması yırtık grubunda 35,05°±4,09°, kontrol grubunda 33,06°±3,42° idi (p=0,022). Sİ ortalaması yırtık grubunda 25,13°±5,71°, kontrol grubunda ise 25,91°±5,81° idi (p=0,552). Gruplar arasında istatistiksel anlamlı farklılık bulunan Gİ, Aİ ve CSA ölçümlerinin duyarlılık, özgünlük ve cut-off değerleri belirlendi. Buna göre Gİ'nin ≥17,35° cut-off değeri için duyarlılığı %79,54 iken özgünlüğü %79,51 idi (p=0,001). Aİ'nin ≥0,67 cut-off değeri icin duyarlılığı %61,54, özgünlüğü %56,4 idi (p=0,031). CSA'nın ≥33,45° cut-off değeri icin duyarlılığı %64,12, özgünlüğü ise %64,54 idi (p=0,014)..

Sonuç: Yüksek glenoid inklinasyon, akromial indeks, kritik omuz açısı ölçüm değerleri semptomatik dejeneratif tam kat supraspinatus tendon yırtıkları ile ilişkili iken superior inklinasyon ölçümü ile ilişki saptanmadı. Glenoid inklinasyon ölçümü semptomatik

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sensitivity and specificity in predicting symptomatic degenerative full-thickness supraspinatus tears.

Keywords: Acromial index, critical shoulder angle, glenoid inclination, rotator cuff tear, superior inclination

dejeneratif tam kat supraspinatus tendon yırtıklarının öngörülmesinde en yüksek duyarlılık ve özgünlüğe sahipti.

Anahtar Kelimeler: Akromiyal indeks, glenoid inklinasyon, kritik omuz açısı, rotator manşet yırtığı, superior inklinasyon

INTRODUCTION

Although various factors such as age, gender, overuse, and scapula morphology are held responsible for rotator cuff tears (RCTs), there are still many points waiting to be clarified in its etiology (1-3). With a better understanding of the importance of scapula morphology as a risk factor, various radiological parameters have been described consecutively to evaluate this relationship (4). Historically, subacromial impingement owing to scapular morphology as described by Neer had been recognized as a risk factor for RCTs (1). For a long time, the hook acromion in the sagittal plane described by Bigliani et al., as well as the thickening and shortening of coracoacromial ligament, have been considered to be correlated with RCTs (5-6). Attention was then turned to the orientation of the glenoid in the coronal plane (4). While some studies stated that glenoid inclination (GI) is associated with RCTs, no correlation could be demonstrated in a study by Moor et al. (3, 4, 7). This situation will lead to the need to describe the superior inclination (SI), which takes into account the position of the acromioclavicular joint in the coronal plane in the measurement of GI later on (8). The acromial index (AI), which was described by Nyfeller et al. and takes into account the amount of coverage of the humeral head by the acromial extension, found widespread support in revealing the aforementioned relationship (9, 10). On the other hand, the most widely accepted predictor for RCTs has been the critical shoulder angle (CSA) defined by Moor et al., which combines acromial extension with GI (11). However, the evidence in the recent literature is conflicting about the strength of the association of all the aforementioned radiological parameters, including CSA, with RCTs, which is a heterogeneous group (12, 13). Therefore, the aim of this study was to simultaneously evaluate the relationship between CSA, AI, GI, and SI with only full-thickness supraspinatus tears (SSTs). The study also aimed to determine the clinical usability of the parameters that could be correlated with SSTs.

MATERIAL AND METHODS

The data of 218 patients who were admitted with complaints of shoulder pain and underwent magnetic resonance imaging (MRI) at a single center (Acıbadem Maslak Hospital) between 2015 and 2017 were analyzed retrospectively. Exclusion criteria were:

1. having a true shoulder AP radiograph with >5 mm overlapping between the anterior and posterior border of the glenoid,
2. being younger than 40 years of age,
3. having arthrosis,
4. having had a glenoid or humeral fracture or deformity,
5. having an isolated subscapularis and infraspinatus tear or a partial SS tear,
6. having previous shoulder surgery.

Thirty-nine patients diagnosed with SSTs (19 female/20 male, 15 left/24 right) and 39 patients (21 female/18 male, 16 left/23 right) who had intact rotator cuff and were matched for age, gender, and sides were included in the study (Table 1). The control group was formed by matching method from patients who were admitted to the clinic with the complaint of shoulder pain and did not have a rotator cuff tear on MRI within the specified time. While it was determined that in the specified control group 34 patients had at least one of the diagnoses subacromial bursitis (n=21), supraspinatus tendinosis (n=13), or subacromial impingement syndromes (n=11), the shoulder MRI scan evaluation of 5 patients was found to be within normal limits. The mean age of the SSTs group was 52.74±5.49 years, and the mean age of the control group was 51.15±5.22 years. Measurements were carried out on true shoulder AP radiographs using the techniques originally described for GI, AI, CSA, and SI (Figure 1-4) (3, 8, 9, 14).

Table 1: Patient demographics

	SST group (n=39)	Control group (n=39)	*p*
Variables	**Mean±SD**	**Mean±SD**	
Age (years)	52.74±5.49	51.15±5.22	0.194*
Gender, n			0.651**
Female	19	21	
Male	20	18	
Side, n			0.817**
Left	15	16	
Right	24	23	

*: Independent samples t test, **: Pearson Chi-square test, Max: maximum, Min: minimum, SST: supraspinatus tear, SD: standard deviation

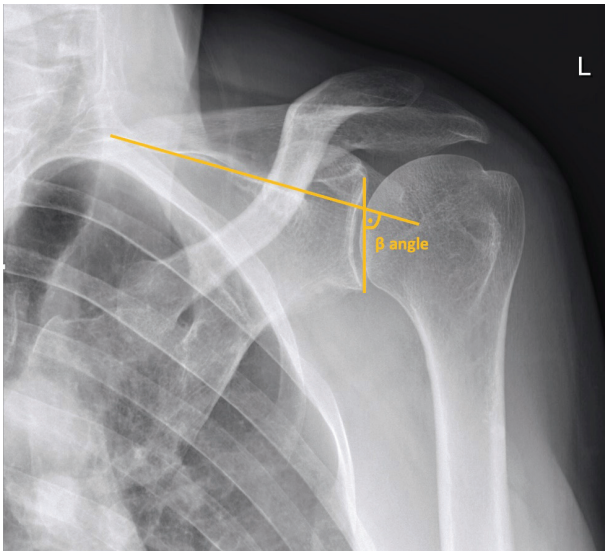

Figure 1: The ß-angle measurement. Glenoid inclination (GI) was obtained as 90° subtracted from the ß-angle

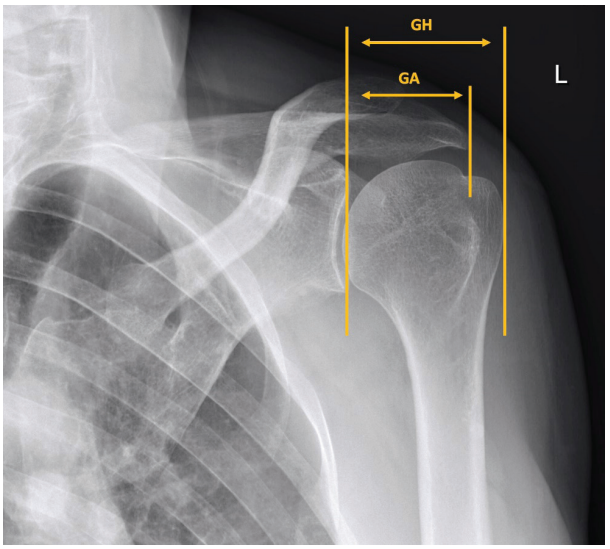

Figure 2: The acromial index (AI) measurement. AI is obtained by dividing the length from the glenoid joint face to the acromion (GA) by the length from the glenoid joint face to the lateral border of the humeral head (GH)

Statistical analysis

A Pearson Chi-Square test was used to compare categorical variables. An independent sample t-test was used to compare measurements between groups. Diagnostic screening tests (sensitivity, specificity) and ROC Curve analysis were used to determine cut-off for variables. Significance was evaluated at the p <0.05 level at least (SPSS 26.0.0.0 MacOS).

This study was approved by the Clinical Research Ethical Committee of the Acıbadem Mehmet Ali Aydınlar University (ATADEK) (Date: 24.06.2022, No: 2022-11/29).

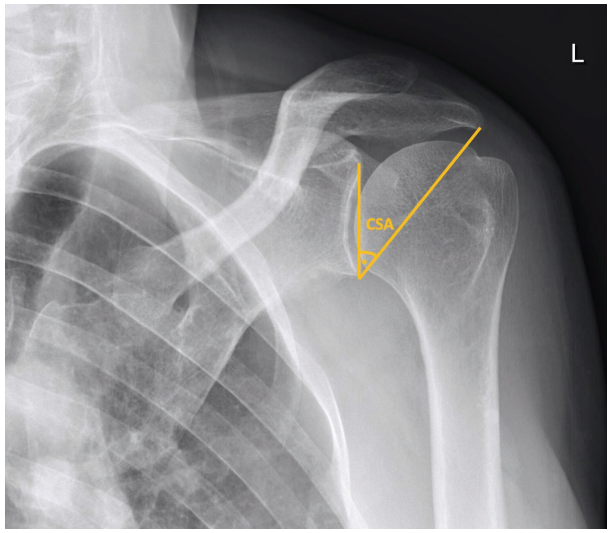

Figure 3: The critical shoulder angle (CSA) measurement

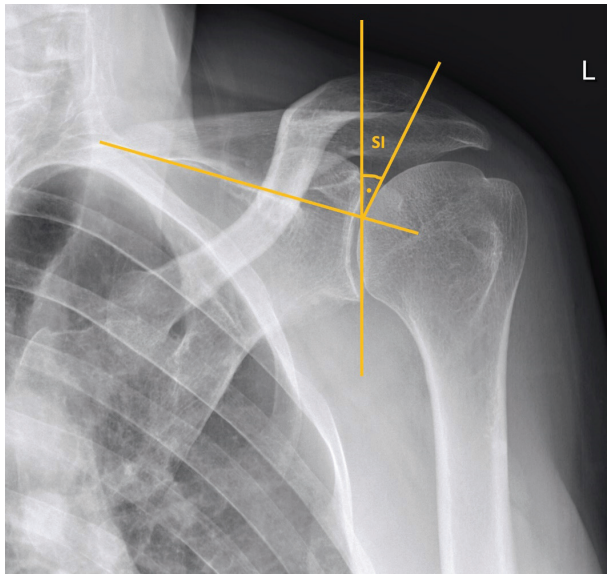

Figure 4: The superior inclination (SI) measurement

RESULTS

A statistically significant difference was found between the groups in all parameters evaluated except SI. The mean GI for the SSTs group was 19.97°±5.62° and it was 13.72°±6.55° for the control group (p<0.001). The mean AI for the SSTs group was 0.7±0.08 and it was 0.67±0.07 for the control group (p=0.035). The mean CSA for the SSTs group was 35.05°±4.09° and it was 33.06°±3.42° for the control group (p=0.022). The mean SI for the SSTs group was 25.13°±5.71° and it was 25.91°±5.81° for the control group (p=0.552) (Table 2). Based on detected significance, the cut-off point, sensitivity and specificity were calculated for GI, AI, and CSA. For a cut-off value

of GI ≥17.35°, sensitivity was 79.54%, while specificity was 79.51% (p=0.001). For a cut-off value of AI ≥0.67, sensitivity was 61.54%, while specificity was 56.4% (p=0.031). For a cut-off value of CSA ≥33.45°, sensitivity was 64.12%, while specificity was 64.54% (p=0.014). Table 3 summarizes the values calculated by diagnostic scan tests and ROC Curve analysis for GI, AI, and CSA.

Table 2: The evaluation of measurements between groups

	SST group (n=39)	Control group (n=39)	*p*
Variables	**Mean±SD**	**Mean±SD**	
CSA	35.05°±4.09°	33.06°±3.42°	0.022*
AI	0.7±0.08	0.67±0.07	0.035*
GI	19.97°±5.62°	13.72°±6.55°	0.000*
SI	25.13°±5.71°	25.91°±5.81°	0.552*

*: Independent samples t test, AI: acromial index, CSA: critical shoulder angle, GI: glenoid inclination, Max: maximum, Min: minimum, SD: standard deviation, SI: superior inclination; SST: supraspinatus tear

The SI measurement technique, which was first described by Chalmers et al., unfortunately does not express that classical glenoid inclination is excessive in the superior direction, which is the connotation of the phrase itself (8). In many studies, the line expressing the scapular spine (1st line) and the line connecting the top and bottom points of the glenoid articular surface (2nd line) are used for measuring the conventional glenoid inclination (14). However, a third line extending from the intersection of the two lines to the acromioclavicular (AC) joint is also needed in this technique. The angle the authors refer to with SI is the angle between this third line and the second line. However, this angle is affected by the position of the AC joint in the coronal plane and lacks an actual expression of the glenoid inclination. In their study comparing patients with full-thickness rotator cuff tears and patients without tears, the authors reported higher SI values in the tear group but stated that this difference was not clinically significant (8). No other study using the technique specified in the current literature could be found. Also, considering the findings of our study, it was concluded that the use of the SI technique has no place in predicting RCT.

Table 3: Diagnostic scan tests for CSA, AI, GI and ROC Curve outcomes

Variables	Diagnostic scan			ROC curve		*p*
	Cut off	Sensitivity	Specificity	AUC	95% CI	
CSA	*≥33.45°*	64.12	66.73	0.655	0.532-0.779	*0.014**
AI	*≥0.67*	61.54	56.4	0.635	0.512-0.758	*0.031**
GI	*≥17.35°*	79.54	79.51	0.778	0.669-0.888	*0.001**

*: Receiver operating characteristic, AI: acromial index, AUC: area under the curve, CI: confidence interval, CSA: critical shoulder angle, GI: glenoid inclination, ROC: receiver operating characteristic

DISCUSSION

Although there are studies in the current literature reporting that scapula morphology may not be a risk factor in the development of rotator cuff tears, our study's findings revealed that scapula morphology may be associated with SSTs (12, 13). Only the superior inclination parameter was not found to be related to SSTs among the parameters evaluated in this study. Otherwise, GI, AI, and CSA have been shown to be associated with SSTs. Based on this relationship, the sensitivity, specificity, and cut-off values of these parameters were determined in a patient who presented with shoulder pain and underwent direct radiography. All three parameters have been found to be clinically usable in making predictions for SSTs. In addition, for a GI ≥17.35° cut-off value, it stood out with 79.54% of sensitivity and 79.51% of specificity (p=0.001).

One of the interesting findings in our study is the conclusion that the glenoid inclination, which was examined in earlier periods, is more useful, with its higher sensitivity and specificity than the more popular predictors, than AI and CSA today. Before interpreting this data, it should be noted that various techniques have been described for GI measurement, and the ß angle is more useful in terms of reproducibility and reliability, as indicated by Maurer et al. (14). Based on this finding, the ß angle was used in GI measurements in our study, and GI was calculated according to the 90-ß definition as stated by Garcia et al. (15). Measuring GI with a standardized, reliable, and reproducible method may have allowed the existing relationship to be clearly demonstrated.

The acromial index refers to the degree of coverage of the humeral head by the acromion. This radiological predictor has continued to attract the attention of clinicians since it was first described. It is widely supported

in the literature that it is associated with RCTs (3, 9-11). However, the main reason for the interest is that it is a radiological parameter that can be corrected with lateral acromioplasty during rotator cuff repair (10, 16). Moreover, it has been reported that the risk of re-tear following repair of RCTs is higher in patients with high values of AI (17). Therefore, acromioplasty is usually recommended in patients with high values of AI (10, 16, 17). On the other hand, in a study by Chalmers et al. in which 110 patients with full-thickness RCTs were evaluated, the width of the acromion was evaluated instead of the acromial index (12). Acromion width was not found to be a statistically significant risk factor for retear and lateral acromioplasty was not recommended in that study. Contrary to Chalmers et al., the findings of our study support that lateral acromioplasty can be performed in patients with high values of AI in parallel with other studies reported in the literature (10, 12, 16, 17).

The critical shoulder angle defined by Moor et al. is one of the most popular predictors (3). CSA can be somehow defined as a geometric combination of acromial index and glenoid inclination (3). It has been reported that the high value of CSA is associated with both RCTs and retear after repair (15, 18, 19). However, some recent studies reporting that high values of CSA are not associated with RCTs (12, 13, 20). It was thought that the reason for this might be that CSA measurement can be affected by the anteversion of the scapula (21). Therefore, measurements should be made only on well-standardized true shoulder AP radiographs. In our study, the mean CSA of the patients in the SSTs group was higher than the control group (p=0.022). However, the sensitivity and specificity of CSA were lower than that of the GI predictor (64.12% and 66.73% for CSA, 79.54% and 79.51% for GI; respectively). This may be due to the confounding effect of scapula anteversion. Re-comparison of these two predictors using 3-dimensionally corrected MRI slices may be the subject of future studies.

This current investigation has several limitations. Firstly, it was a retrospective study and may have selection bias by its nature. The fact that the measurements were carried out once by a single observer is another limited aspect of the study. However, the strengths of the study are having detailed exclusion criteria, the fact that measurements were carried out by a senior surgeon trained in shoulder and elbow surgery, that fact that low-quality true anteroposterior shoulder radiographs that do not provide the strictly necessary adequacy were not included, and having a homogeneous control group in terms of age, gender, and side. Also, not including subscapularis tears, infraspinatus tears, or partial thickness supraspinatus tears allowed a homogeneous comparison to be carried out.

CONCLUSION

The findings of this study support the statement that high measurement values of GI, AI, and CSA are associated with SSTs, while high measurement values of SI are not. GI, AI, and CSA can be used in clinical practice to predict SSTs in patients with shoulder pain by taking the cut-off values determined in our study as a reference. Additionally, GI measurement stands out with its high sensitivity and specificity among the parameters examined in the prediction of SSTs.

Ethics Committee Approval: This study was approved by ATADEK (Clinical Research Ethical Committee of Acıbadem Mehmet Ali Aydınlar University) (Date: 24.06.2022, No: 2022-11/29).

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ARE JOINT POSITION SENSE, TWO-POINT DISCRIMINATION, FINE MOTOR CONTROL, GRIP STRENGTH, LIGHT TOUCH SENSATION, PAIN, AND FUNCTIONALITY AFFECTED BILATERALLY IN UNILATERAL CARPAL TUNNEL SYNDROME?

TEK TARAFLI KARPAL TÜNEL SENDROMUNDA EKLEM POZİSYON HİSSİ, İKİ NOKTA AYRIMI, İNCE MOTOR KONTROL, KAVRAMA KUVVETİ, HAFİF DOKUNMA DUYUSU, AĞRI VE İŞLEVSELLİK BİLATERAL OLARAK ETKİLENİR Mİ?

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ABSTRACT

Objective: This study aimed to evaluate joint position sense, two-point discrimination, fine motor control, grip strength, light touch sensation, pain, and functionality in patients with unilateral carpal tunnel syndrome and to determine whether these variables change in the unaffected hands of patients with unilateral carpal tunnel syndrome.

Materials and Methods: The study was planned as a single-blind, cross-sectional case-control study. Individuals between the ages of 20-65 who were diagnosed with carpal tunnel syndrome (n=22) and healthy (n=22) were included. The device was designed for the study of measurements; discriminator; 9-hole peg test, dynamometer, and pinch meter, Semmens Weinstein monofilament test; The visual analog scale was evaluated bilaterally with the Boston questionnaire.

Results: There was a significant difference between all measurements in the affected and unaffected hands between the CTS group and the control group ($p<0.05$). There was no significant difference between the joint position sense, two-point discrimination, fine motor control, grip strength, light touch sense, and functionality between the affected and unaffected hand in the CTS group ($p>0.05$).

Conclusion: This is the first study evaluating joint position sense in the wrist, metacarpophalangeal joints, and interphalangeal joints in unilateral CTS syndrome. It was noteworthy that patients

ÖZET

Amaç: Bu çalışmanın amacı, tek taraflı karpal tünel sendromlu hastalarda eklem pozisyon hissi, iki nokta ayırt etme, ince motor kontrol, kavrama kuvveti, hafif dokunma hissi, ağrı ve fonksiyonelliği değerlendirmek ve hastaların etkilenmeyen ellerinde de bu değişkenlerin değişip değişmediğini belirlemektir.

Gereç ve Yöntem: Çalışma tek kör, kesitsel vaka-kontrol çalışması olarak planlandı. 20-65 yaş aralığında karpal tünel sendromu tanısı alan (n=22) ve sağlıklı (n=22) bireyler dahil edildi. Ölçümler çalışma için tasarlanan cihaz; diskriminatör; 9 delikli peg test, dinamometre ve pinchmetre, semmens weinstein monoflament test; visuel analog scale, Boston anketi ile bilateral değerlendirildi.

Bulgular: Karpal tünel sendromu grubu ile kontrol grubu arasında etkilenen ve etkilenmeyen ellerinde tüm ölçümler arasında anlamlı farklılık vardı ($p<0.05$). KTS grubunda etkilenen el ve etkilenmeyen el arasında eklem pozisyon hissi, iki nokta diskriminasyonu, ince motor kontrol, kavrama kuvveti, hafif dokunma duyusu, fonksiyonellik arasında anlamlı fark bulunmadı ($p>0.05$).

Sonuç: Bu çalışma tek taraflı KTS sendromunda el bileği, metakarpofalangeal eklemler ve interfalangeal eklemlerde eklem pozisyon hissini değerlendiren bildiğimiz kadarıyla ilk çalışmadır. Tek taraflı karpal tünel sendromlu hastaların eklem pozisyon hissi, ince motor kontrol, iki nokta diskriminasyonu, hafif dokunma duyusu ve kavrama kuvveti sağlıklı kontrol grubuna göre eksik

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with unilateral carpal tunnel syndrome had deficiencies in joint position sense, fine motor control, two-point discrimination, light touch sense, and grip strength compared to the healthy control group, as well as deficiencies in the unaffected sides.

Keywords: Carpal tunnel syndrome, proprioception, motor skills, grip strength

bulunmasının yanı sıra etkilenmeyen taraflarında da eksikliklikler olması dikkat çekici bulundu.

Anahtar Kelimeler: Karpal tünel sendromu, propriosepsion, motor beceri, kavrama kuvveti

INTRODUCTION

Carpal tunnel syndrome (CTS) is one of the most common peripheral neuropathies caused by compression of the median nerve within the carpal tunnel. Its prevalence is about 2.7% to 8%. It is more common in women than men, between the ages of 40-46 and 70-85, in the general population (1). The median nerve affects the tendons, muscles, and skin receptors that provide hand proprioception. In this syndrome, which is seen due to frequent use, patients misposition the wrist, afferent impulses are carried to the upper centers and cause cortical changes. It is thought that these neuroplastic changes in the somatosensory cortex may affect the proprioceptive mechanism and functional skills (2). Localized sensory, motor and autonomic disorders occur because of the compression of the median nerve in the tunnel. In the clinic, patients mostly complain of pain, numbness, and gripping difficulties in the palm and first three fingers (3). Symptoms usually increase at night and may even wake the patient from sleep. There is also a tactile sensory weakness, two-point discrimination, and a loss of functional motor skills such as grasping small objects. Studies evaluating grip strength, fine motor control, and pain in patients with CTS have also been found before (4-6). However, the cause of the symptoms and the cause of bilateral deficiencies are not fully known.

Since there are many factors affecting proprioception, which has a multisensory structure, it is difficult to evaluate specifically, but two easy-to-apply methods have been determined, namely joint position sense and passive joint movement detection threshold, to provide ease of application in the clinic and to give an idea about the proprioceptive effect. Proprioception is considered a subcomponent of the sensorimotor system. It contains afferent information originating from receptors that contribute to postural control, joint stability, and motor control (7). The body diagram is created by the information coming from the afferent path. An increasing number of studies show that proprioceptive knowledge and thus body schema are impaired in chronic painful conditions (8,9). One of the ways to evaluate the body chart is with the two-point discrimination test. Two-point discrimination has been reported as a clinical measure of body schema in the primary somatosensory cortex. (10). In individuals with hand-wrist pathology, it was observed that the sense of two-point discrimination carried by the same spinal route as proprioception was decreased, but no study investigating whether there was a pathological change in the sense of joint position sense was found in the literature (11). Studies examining joint position sense changes in the unaffected hand in patients with unilateral CTS are one of the missing points in the literature. Since spinal motor neurons receive afferent information from both the ipsilateral and contralateral extremities, we think that the joint position sense should also be evaluated in the contralateral extremity in patients with CTS (12). Therefore, we aimed to evaluate the joint position sense, two-point discrimination, fine motor control, grip strength, light touch sensation, pain, and functionality in patients with unilateral CTS and to determine whether these variables change in the unaffected hands of unilateral CTS patients.

MATERIALS AND METHODS

This study was conducted with a cross-sectional case-control research model. The study was reviewed and approved by the local Clinical Research Ethics Committee (Date:13.03.2018, No: 2018/6-27), and the study was conducted by the principles of the Declaration of Helsinki. The individuals participating in the study were informed about the purpose, duration, and scope of the study, and informed consent was obtained from the individuals. Data were collected between March 2018 and June 2018.

The population of the study consisted of individuals between the ages of 25-65. Individuals diagnosed with unilateral carpal tunnel syndrome who were willing to participate in the study were included in the carpal tunnel syndrome group (CTSG), and healthy individuals were included in the control group (CG). Individuals with a history of any surgical operation, including CTS surgery on the wrist, with any condition that may affect the wrist, and individuals who refused to participate in the study were excluded from both groups. Patients who participated in the study and met the inclusion criteria were selected from the relevant population using the improbable random sampling method. Demographic variables of all participants were recorded, including age, gender, weight, height, and dominant hand.

Assuming that the contralateral healthy hand is affected by 10% in the power analysis performed before the start of the study, with α=0.05 and 1-β (power)=0.80, it was calculated that 22 subjects should be included in each

group in the study (13, 14). Power analysis was performed using the publicly available statistical software OpenEpi, version 3 (http://www.openepi.com) to calculate the sample size. A total of 44 individuals were evaluated within the scope of the study.

Measurement

The joint position sense was measured with the device designed for the study (Figure 1) by asking participants to actively move their wrists to recreate the predetermined target positions (wrist flexion and extension (WF-WE) 30°, radial deviation (RD) 10°, ulnar deviation (UD) 15°, metacarpophalangeal joint flexion (MCPF) 45°, metacarpophalangeal joint extension (MCPE) 10°, and proximal interphalangeal joint flexion (PİPF) 50°) (15) (Figure 2). Three replications were made for the target angles in all motion axes, and the arithmetic average was recorded. Throughout the measurement, no feedback on their performance was given to eliminate the possibility of participants rearranging their responses.

A two point discrimination evaluation was measured with a 1-millimeter precision discriminator. While the eyes are open, the individual's finger is warned about two points far from each other, "these are the two points in question", then a single point warning is given, and preliminary information is given as "this is the only point" (11). All the 1st, 2nd, and 3rd fingertips are touched from the widest to the narrowest distance for 1-1.5 seconds, and the individual is asked "single or even?" questions. For CTSG, the application was repeated for the affected and intact hand. The narrowest double point distance that the individual could feel was recorded.

Fine motor control, the assessment was measured with the Nine Hole Peg Test. It consists of a wooden surface with 9 holes on it and 9 cylinders attached to these holes. Individuals were asked to insert the rods into the holes as quickly as possible and then collect them back. This time was measured with a stopwatch (16). Measurements were repeated 3 times and averaged.

Evaluation of grip strength, hand grip strength according to the standard position recommended by the American Association of Hand Therapists; The patient was measured in an upright position, knees in 90° flexion, shoulder in adduction and a neutral position, elbow in 90° flexion, forearm in mid-rotation, and wrist in neutral, using a Baseline brand dynamometer, and 3 measurements were made with a one-minute break between each measurement, and the average was recorded (17). A baseline brand pinch meter was used for finger grip strength. Subjects were asked to sit with support, shoulder in adduction and neutral position, elbow in 90˚ flexion, forearm in a neutral position, wrist in 0-30˚ extension, and 0-15˚ ulnar deviation. For lateral grip (key grip) strength, the grip strength between the thumb and the radial side of the index finger was evaluated. While assessing the grip, the patient was told to press the pinch meter from the top with the middle of the distal phalanx of the thumb and support the second phalanx of the index finger from the lower part of the pinch meter. During the measurements, the patients were asked to squeeze at maximum force. Each measurement was made three times and the averages were recorded (18).

Semmes-Weinstein monofilaments were used for light touch assessment. The filaments (from thick to thin) were

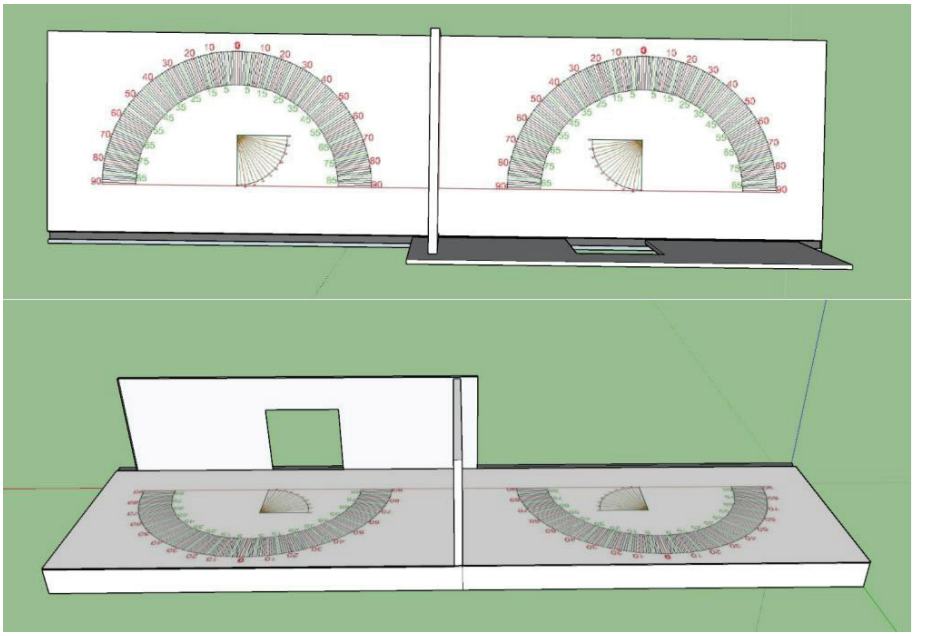

Figure 1: Device designed for the study

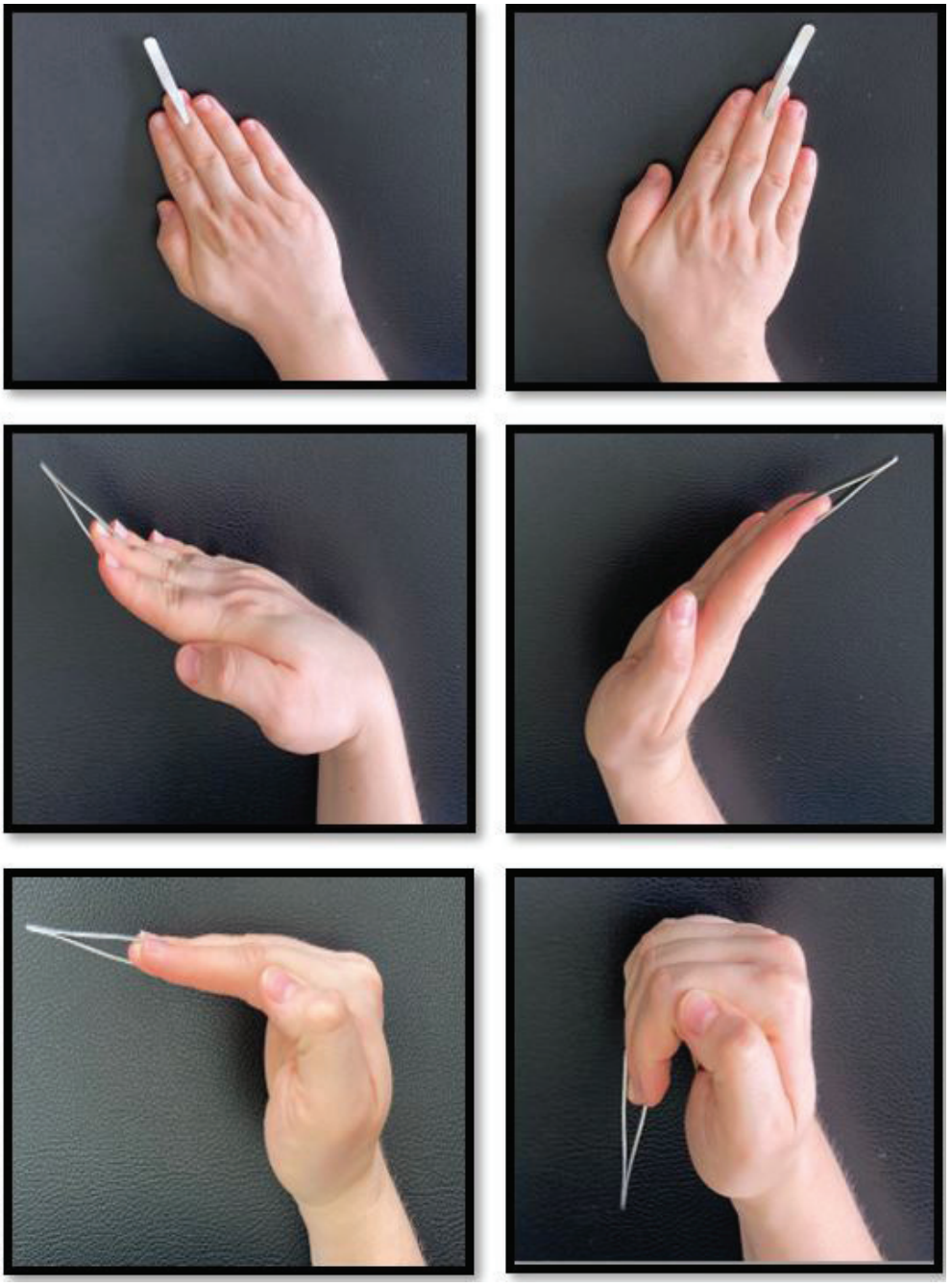

Figure 2: Assessment of joint position sense

touched for 1-1.5 seconds with the eyes closed so that a slight elastic deformation would occur, and the individual was asked if he felt the touch. The thinnest filament coefficient felt during the application was recorded (19).

Pain assessment, Visual Analogue Scale (VAS) was used. On a 10 cm horizontal line, the beginning was marked as 0 (no pain) and the ending (extreme pain), and the subjects were asked to mark on this horizontal line according to the degree of pain they felt. The point marked on the line was then measured with the help of a ruler and recorded as the VAS value in cm. In addition, the degree of pain they felt at rest, during activity, and at night was questioned and recorded in three different ways (20).

The Boston Carpal Tunnel Syndrome Questionnaire (BCTSQ), which consists of two subsections, the Boston Symptom Severity Scale (BSSS) and the Boston Functional Capacity Scale (BFCS), was used in the disease-specific assessment scale. There are 11 questions about the pa-

tient's symptoms in the BSSS and eight questions about the functional capacity in the BSSS. Questions are scored between 1 and 5. A high score indicates severe symptoms and reduced functional capacity (21). Results for BSSS and BFCS were recorded separately.

Statistical analysis

The data of the study were uploaded to the computer with the program "SPSS (Statistical Package for Social Sciences) for Windows 20.0 (SPSS Inc, Chicago, IL)" and evaluated. Descriptive statistics were presented as mean±standard deviation, percentage, and median (25%-75%). In the power analysis, it was calculated that 22 individuals should be included in each group, assuming that α=0.05 and 1-β (strength)=0.80, and the rate of involvement of the contralateral healthy hand is 10% (13). The Public OpenEpi program was used to calculate the sample size. The Pearson chi Square test was used to evaluate categorical variables. The conformity of numerical variables to the normal distribution was calculated using the Shapiro Wilk test. Statistically significant data were analyzed with the One Way ANOVA test for data with normal distribution. The Tukey test was used in case of significant difference and homogeneity of variances; the Tamhane test was used when homogeneity of variances was not achieved. The Kruskal Wallis Test was used for data that did not fit the normal distribution. The Mann-Whitney U test was used in the dual evaluation of the groups that differed in the Kruskal Wallis test.

RESULTS

A total of 44 individuals were evaluated within the scope of the study (Figure 3). All participants were female and right-handed dominant. According to the affected hand evaluation of individuals with CTS, the tinel test was negative in five individuals and positive in 17 individuals; The Phalen test was found to be negative in four individuals

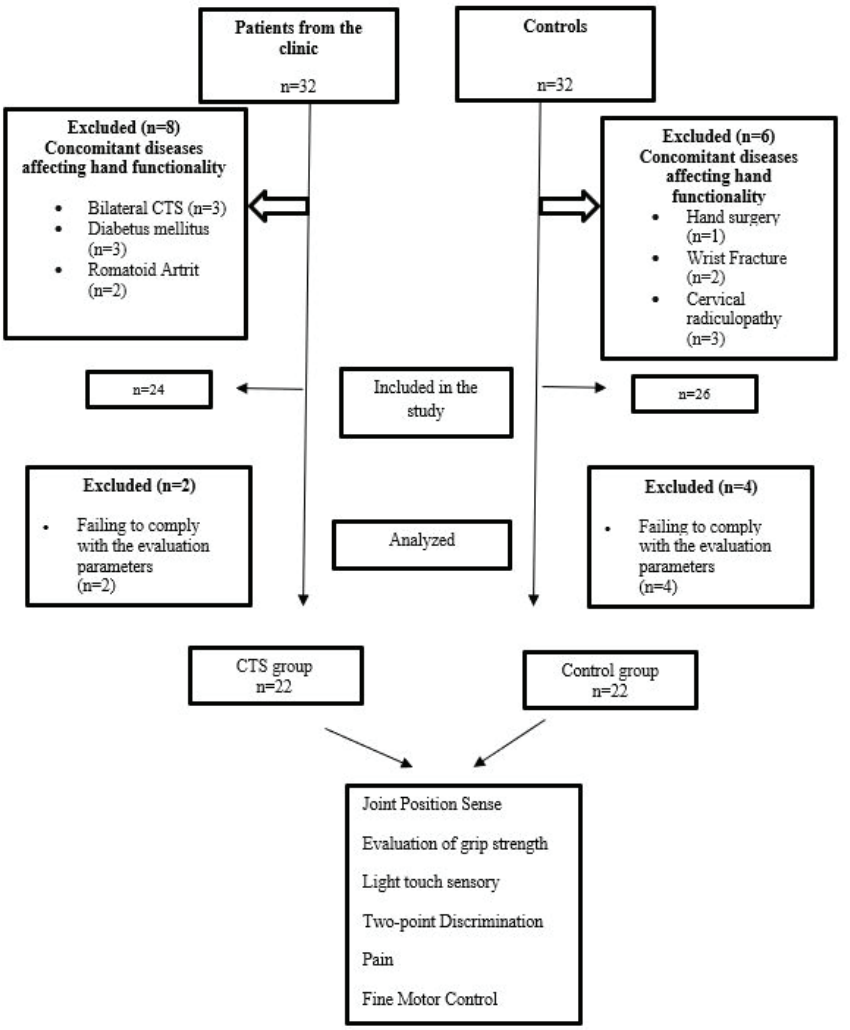

Figure 3: Flow diagram of the study population
CTS: Carpal tunnel syndrome

Table 1: Demographic data

	CTS (n=22) Mean±SD	Kontrol (n=22) Mean±SD	p[a]
Age (years)	50.4545±9.02	49.0±11.57	0.577
Height (m)	1.59±0.68	1.61±0.06	0.140
Weight (kg)	74.77±9.78	71.50±9.21	0.190
BMI (kg/m^2)	29.58±4.33	27.23±2.92	0.012

a: Independent Sample t Test, BMI: Body musk index

and positive in 18 individuals. Both tests were negative in the entire control group. The demographic characteristics of the individuals included in the study are shown in Table 1 (Table 1).

While there was a significant difference between CTS (affected side) and the control group in joint position sense, two-point discrimination, hand and finger grip strength, light touch sense, and functional skill tests (p>0.05); There was no significant difference between the affected and unaffected side in individuals with CTS (p>0.05) (Table 2).

Table 2. Intergroup joint position sense, hand and finger grip strength, light touch sense, two-point discrimination, functional skill results

	Patients (n=22)		Control (n=22)	P	Affected-non-affected	Affected-control	Unaffected-control
	Affected side	Unaffected side			p	p	p
JPS-RD	8.54±7.32	6.81±3.74	1.77±1.60	0.000[a]	0.467[b]	0.000[b]	0.003[b]
JPS-UD	10.63±6.82	7.36±6.31	2.31±2.12	0.000[a]	0.128[b]	0.000[b]	0.010[b]
JPS-WF	19.81±8.40	15.54±8.93	2.13±2.73	0.000[a]	0.133[b]	0.000[b]	0.000[b]
JPS- WE	12.45±8.41	10.22±6.95	2.72±2.76	0.000[a]	0.495[b]	0.000[b]	0.001[b]
JPS- MCPF	10.13±7.80	7.09±4.27	2.5±2.11	0.000[a]	0.143[b]	0.000[b]	0.015[b]
JPS-MCPE	8.09±4.86	5.0±4.01	2.18±2.46	0.000[a]	0.029[b]	0.000[b]	0.051[b]
JPS-PİPF	10.00±5.34	6.54±5.22	1.09±1.60	0.000[a]	0.031[b]	0.000[b]	0.000[b]
Two-point discrimination 1st Finger	3.82±2.13	2.86±1.98	1.68±0.71	0.001[a]	0.345[f]	0.000[f]	0.042[f]
Two-point discrimination 2nd Finger	3.18±2.28	2.5±1.84	1.45±0.59	0.006[a]	0.630[f]	0.006[f]	0.053[f]
Two-point discrimination 3rd finger	3.45±2.22	2.73±1.88	1.59±0.59	0.002[a]	0.575[f]	0.003[f]	0.036[f]
Hand grip strength[c]	12.50(3-21)	13.36(8-21)	18.00(14-31)	0.000[d]	0.364[e]	0.000[e]	0.000[e]
Finger grip strength[c]	3.64(1-7)	4.00(1-6)	5.00(3-8)	0.001[d]	0.836[e]	0.002[e]	0.001[e]
Light touch sensation 1st finger[c]	3.84(2.36-4.93)	3.84(2.83-5.07)	2.83(0.22-3.61)	0.000[d]	0.850[e]	0.000[e]	0.000[e]
Light touch sensation 2nd finger[c]	3.72(2.36-5.07)	3.61(2.83-5.07)	2.83(2.36-3.61)	0.000[d]	0.739[e]	0.000[e]	0.000[e]
Light touch sensation 3rd finger[c]	3.96(2.83-5.07)	3.61(2.83-4.93)	2.83(2.36-3.61)	0.000[d]	0.259[e]	0.000[e]	0.000[e]
Nini hole peg test[c]	26.50(16-47)[c]	23.50(17-44)	18.50(15-27)	0.000[d]	0.204[e]	0.000[e]	0.001[e]
Boston carpal tunnel severity scale	30.14±5.41	30.14±5.41	11±00	0.000	1.00[b]	0.000[b]	0.000[b]
Boston carpal tunnel status scale	25.82±5.78	25.82±5.78	8±00	0.000	1.00[b]	0.000[b]	0.000[b]

a: One Way ANOVA, b: Tukey, c: Median(min-max), d: Kruskall Wallis, e: Mann Whitney U, f: Tamhane
JPS: joint position sense, RD: radial deviation, UD: ulnar deviation, WF: wrist flexion, WE: wrist extension, MCPF: metacarpophalangeal flexion, MCPE: metacarpophalangeal extension, PİPF: proximal interphalangeal flexion

No significant relationship was found between pain level and motor functions in individuals with CTS.

While there was a significant difference in functional capacity between individuals with CTS and the healthy control group ($p<0.05$), there was no significant difference between the affected and unaffected sides of the patients ($p>0.05$) (Table 2).

DISCUSSION

In this study, it was observed that the joint position sense, two-point discrimination, fine motor control, grip strength, light touch sensation, pain, and functional level of patients with CTS were significantly changed when compared with the healthy control group. In addition, the most striking result is the loss of joint position sense, two-point discrimination, fine motor control, grip strength, light touch sense, and functional level in the unaffected hands, although the patients present with unilateral CTS.

So far, many studies have shown that joint position sense is impaired in different musculoskeletal problems (22-24). There has been no previous study that we found in the literature on joint position sense in patients with CTS. In our study, a significant difference was found in the sense of joint position between individuals with CTS and the healthy control group; There was no significant difference in any joints of the individuals with CTS on the affected and unaffected sides. Our study is the first to evaluate joint position sense in different joints in the affected and unaffected hand. In studies examining the loss of proprioception on the affected and unaffected side in different extremities such as the knee and shoulder, it was revealed that while the results of the affected and unaffected extremities were similar, there was a difference between the results of the healthy group (12-25). Robert et al. concluded that patients with anterior cruciate ligament injury also have a proprioceptive loss in the unaffected knee. They explained this situation by arguing that faulty afferent information from the periarticular receptors in the injured knee alters the functioning of muscle spindles on the contralateral side. This deficiency on the unaffected side is the result of processing feedback from the subcortical level. Spinal motor neurons receive afferent information from both the ipsilateral and contralateral limbs. Cross connections between contralateral limbs contribute to simultaneous learned responses in the cerebral cortex (12). While acquiring skills with one extremity by repeating a task, skill acquisition takes place in the other extremity in the same way. Similarly, functional loss in one-sided extremity may cause disorders on the other side. Two theories have been proposed to explain this situation. First, task-oriented training leaves a mark on the motor cortex of the extremity; task performance in the untrained extremity, in which the motor effects developed in the dominant hemisphere of the brain are accessible to the opposite hemisphere through the corpus callosum (callosum access hypothesis); the second is based on the observation that the execution of many unilateral tasks produces cortical activity (i.e., cross-activation) on both the opposite and the same side of the trained limb. According to the 'cross-activation' hypothesis, bilateral cortical activity produced by unilateral training leads to adaptations in both hemispheres (26). Thus, unilateral training causes changes in motor organization associated with task-specific activation of the same muscles on the contralateral side. Afferent nerves responsible for proprioception receive information from muscles, tendons, and cutaneous receptors at the wrist level and are involved in the perception of joint movement, the fluent maintenance of grip, and other functional movements (27). While contralateral activation is gained with unilateral training, the same functions can be lost on the contralateral side with unilateral involvement.

In a study conducted with individuals with CTS, a significant difference was observed in the sense of two-point discrimination and kinesthesia compared to the healthy control group (28). Caseiro et al. examined the integrity of the body chart in individuals with chronic non-traumatic unilateral shoulder pain using two-point discrimination and right/left discrimination test. In conclusion, there was no difference between the symptomatic and asymptomatic shoulders (9). A recent systematic review also found no difference in right-left discrimination between painful and healthy limbs in individuals with unilateral shoulder pain (29). Hearkens et al. evaluated tactile acuity in patients with chronic arm, neck, and shoulder pain with a two-point discrimination test, and no difference was observed in the symptomatic and asymptomatic regions (30). In a study evaluating grip strength and fine motor control in individuals with unilateral CTS, it was suggested that there was bilateral involvement in individuals with CTS compared to the healthy control group and that this was due to central sensitization (31). In our study, there was no difference in the two-point discrimination test between symptomatic and asymptomatic hands in individuals with CTS, but there was a significant difference between them and the healthy group. In a study conducted on women with CTS, two-point discrimination, grip strength, and kinesthetic differentiation (joint range of motion) of the radiocarpal joint were evaluated. A significant difference was found according to the group (32, 33). It has been observed that patients with unilateral CTS develop symptoms on the asymptomatic side as well as decreased fine motor control and grip strength in the affected hand (34). Similar to the literature, in our study, when individuals with CTS were compared with healthy controls, significant differences were observed in terms of fine motor control, two-point discrimination, light touch, and grip strength. There was no significant difference between the affected and unaffected sides. The underlying

cause of the reduction in grip strength has not been fully determined. It may cause loss of flexion in the metacarpophalangeal joint due to muscle tension in the forearm; Another reason is that there is tension in the lumbrical muscles in patients with CTS, which causes drag towards increased resistance/extension, preventing the DIP joints and PIP joints from achieving strong flexion. An additional cause of strength reduction may include sensory impairment common in CTS, which can reduce the ability to properly regulate force production (33).

Out of the scope of our work; previous studies investigating the relationship between motor functions and pain in CTS have reported conflicting results. In the Wiebusch et al. study, it was observed that patients with unilateral lateral elbow tendinopathy had a bilateral lack of fine motor control and joint position sense, and unilateral pain syndrome was found to cause bilateral deficits (35). Another study suggested that bilateral deficits in fine motor control and grip strength were negatively correlated with pain intensity in individuals with CTS (31). It has been suggested that central sensitization plays a role in the formation of CTS. However, since the device (Purdue pegboard) used in the study could not evaluate median nerve damage only, it was insufficient to explain contralateral deficits. Rincon et al. found that unilateral and bilateral deficits of fine motor control and grip strength were not associated with pain in individuals with CTS. In our study, the presence of bilateral symptoms was not found to be associated with pain. Another hypothesis explaining the bilateral findings of unilateral musculoskeletal problems is supraspinal mechanisms. The second somatosensory cortex and posterior parietal areas are responsible for areas of sensory and proprioceptive integration (37). Regions representing the hand in the primate motor cortex have callosal connections. Motor cortex activation is transferred to both hemispheres during ipsilateral hand use (15).

Study limitations

Our study has some limitations in that it used a small sample size. In addition, the fact that all the participants in the study were women is one of the limitations of the study. Future studies with larger samples will be needed.

CONCLUSION

This is the first study to evaluate joint position sense in the wrist, metacarpophalangeal joints, and interphalangeal joints in unilateral CTS syndrome. Patients who have bilateral deficits in joint position sense, fine motor control, two-point discrimination, light touch sense, grip strength, and the affected and unaffected sides were missing from the healthy control group. In addition, the detected bilateral deficiencies were not associated with pain.

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MACHINE LEARNING-BASED CLASSIFICATION OF HBV AND HCV-RELATED HEPATOCELLULAR CARCINOMA USING GENOMIC BIOMARKERS

GENOMİK BİYOBELİRTEÇLER KULLANILARAK HBV VE HCV İLE İLİŞKİLİ HEPATOSELLÜLER KARSİNOMUN MAKİNE ÖĞRENİMİ TABANLI SINIFLANDIRILMASI

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ABSTRACT

Objective: It is crucial to know the underlying causes of hepatocellular carcinoma (HCC) for optimal management. This study aims to classify open access gene expression data of HCC patients who have an HBV or HCV infection using the XGboost method.

Material and Methods: This case-control study considered the open-access gene expression data of patients with HBV-related HCC and HCV-related HCC. For this purpose, data from 17 patients with HBV+HCC and 17 patients with HCV+HCC were included. XGboost was constructed for the classification via ten-fold cross-validation. Accuracy, balanced accuracy, sensitivity, specificity, the positive predictive value, the negative predictive value, and F1 score performance metrics were evaluated for a model performance.

Results: With the feature selection approach, 17 genes were chosen, and modeling was done using these input variables. Accuracy, balanced accuracy, sensitivity, specificity, positive predictive value, negative predictive value, and the F1 score obtained from the XGboost model were 97.1%, 97.1%, 94.1%, 100%, 100%, 94.4%, and 97%, respectively. Based on the variable importance findings from the XGboost, the *ALDOC, GLUD2, TRAPPC10, FLJ12998, RPL39, KDELR2,* and *KIAA0446* genes can be employed as potential biomarkers for HBV-related HCC.

Conclusion: As a result of the study, two different etiological factors (HBV and HCV) causing HCC were classified using a machine learning-based prediction approach, and genes that could be biomarkers for HBV-related HCC were identified. After the

ÖZET

Amaç: Hepatoselüler karsinomun (HCC) optimal yönetimi için altında yatan nedenleri bilmek çok önemlidir. Bu çalışma, HBV veya HCV enfeksiyonu olan HCC hastalarının açık erişim gen ekspresyon verilerini XGboost yöntemini kullanarak sınıflandırmayı amaçlamaktadır.

Gereç ve Yöntem: Bu vaka-kontrol çalışmasında, HBV ve HCV ile ilişkili HCC'li hastaların açık erişimli gen ekspresyonu verileri dikkate alınmıştır. Bu amaçla, 17 HBV+HCC ve 17 HCV+HCC hastadan elde edilen veriler çalışmaya dahil edildi. Sınıflandırma için on katlı çapraz geçerlilik kullanılarak XGboost modeli oluşturuldu. Model performansı için doğruluk, dengeli doğruluk, duyarlılık, özgüllük, pozitif tahmin değeri ve negatif tahmin değeri ve F1 skor performans metrikleri değerlendirildi.

Bulgular: Özellik seçimi yaklaşımı ile 17 gen seçilmiş ve bu girdi değişkenleri kullanılarak modelleme yapılmıştır. XGboost modelinden elde edilen doğruluk, dengeli doğruluk, duyarlılık, özgüllük, pozitif tahmin değeri, negatif tahmin değeri ve F1 skor sırasıyla %97,1, %97,1, %94,1, %100, %100, %94,4 ve %97 idi. XGboost'tan elde edilen değişken önemliliği bulgularına dayanarak, *ALDOC, GLUD2, TRAPPC10, FLJ12998, RPL39, KDELR2* ve *KIAA0446* genleri, HBV ile ilişkili HCC için potansiyel biyobelirteçler olarak kullanılabilir.

Sonuç: Çalışma sonucunda, HCC'ye neden olan iki farklı etiyolojik faktör (HBV ve HCV), makine öğrenimi tabanlı bir tahmin yaklaşımı kullanılarak sınıflandırıldı ve HBV ile ilişkili HCC için biyobelirteç olabilecek genler tanımlandı. Ortaya çıkan genler sonraki araştırmalarda klinik olarak doğrulandıktan sonra, bu

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resulting genes have been clinically validated in subsequent research, therapeutic procedures based on these genes can be established and their utility in clinical practice documented.

Keywords: Hepatocellular carcinoma, Hepatitis B infection, Hepatitis C infection, machine learning, classification

genlere dayalı terapötik prosedürler oluşturulabilir ve klinik uygulamada kullanımları belgelenebilir.

Anahtar Kelimeler: Hepatosellüler kanser, Hepatit B enfeksiyonu, Hepatit C enfeksiyonu, makine öğrenimi, sınıflandırma

INTRODUCTION

Primary liver cancer ranks as the sixth most prevalent kind of cancer that is diagnosed and the fourth most common cause of death from cancer globally (1). The great majority of instances of primary liver cancer, which account for roughly 75-85 percent of all cases, are caused by hepatocellular carcinoma (HCC) (1). The most important risk factors associated with HCC are Hepatitis B virus (HBV), Hepatitis C virus (HCV), alcohol abuse, non-alcoholic steatohepatitis (NASH), and non-alcoholic fatty liver disease (NAFLD) (1-3).

Hepatitis B virus contributes to the development of HCC via both direct and indirect mechanisms (4). Recent estimates demonstrate HBV is responsible for more than half of all HCC cases globally, ranking it second only to cigarettes as the most frequent carcinogen (5, 6). Chronic HBV carriers are 10- to 25-fold more likely to develop HCC throughout their lifetime than people without HBV (7). Alcohol consumption has a synergistic effect, increasing the carcinogenic risk of HBV by more than twofold. Tobacco use is also linked to an increased risk of HCC in patients with HBV-related cirrhosis, indicating a quantitative link between smoking and a cancer risk. In some subtropical areas of Asia and Africa, aflatoxin B1 exposure combined with HBV infection results in an exceptionally high HCC frequency (5, 8). Additionally, HBV replication, genotype, and HBV genomic mutations contribute to an increased likelihood of developing HCC. In the clinical environment, elevated levels of HBV DNA in the serum are linked to liver damage, the progression to cirrhosis, and the development of HCC (9, 10).

Hepatitis C virus is a hepatotropic RNA virus that only infects the liver and is spread through the bloodstream. HCV infects around 71 million individuals worldwide, yet only 20–30% of those infected develop liver cirrhosis, and only 1–4% of cirrhotic patients develop HCC each year (11, 12). The HCC risk is raised 15 to 20-fold in HCV-infected individuals, with the yearly incidence of HCC in cirrhotics estimated to be 1% to 4% over a 30-year period (13, 14). Over the last decade, mortality from HCV-related HCC has increased by 21.1%, whereas deaths from HCC caused by sources other than HCV and alcohol remained unchanged (14).

The role of many demographic, socioeconomic and clinical variables in the development of HCC has been studied in detail. However, the underlying molecular pathogenesis of HCC development such as genetic mutations and expression of gene products, has not been sufficiently clarified (15). The most important reasons for this are the popularity of genetic analyses in recent years, the lack of access to genetic tests, and the economic burden of these analyses. It is known that the genes or gene products play a vital role in the development of HCC. However, the comprehensive understanding molecular mechanism of HCC carcinogenesis and tumor prognosis remains unclear (15).

In recent years, in parallel with the development of next-generation sequencing (NGS) technology, important developments have been made regarding the molecular pathogenesis of HCC. In this context, the molecular mechanisms that play a role in the pathogenesis of HCC are roughly genomic, transcriptomic and, epigenetic alteration viral integration, tumor microenvironment, cancer stem cell, and cancer metabolism (16). Thanks to NGS, large-scale mutation screening and gene expression detection in HCCs has paved the way. However, instead of classical statistical analysis methods, it has become necessary to use artificial intelligence (AI) technology for their analysis and interpretation.

Machine learning (ML) is a subfield of AI that aims to make predictions about new data by performing data-driven learning when exposed to new data. AI/ML methods are one of the most commonly utilized technologies in illness detection and clinical decision support systems in recent years, with a wide range of applications. In the last decade, with the availability of large datasets and greater computing power, ML methods have achieved high performance in various situations (17, 18). Today, it is crucial to diagnose HCC and determine/predict the genes that cause the presence of HCC as biomarkers and use them concerning the HCC stage. For this reason, many studies have used ML methods to identify genes that may be biomarkers related to HCC (19). A study studied Non-Coding RNAs for HCV-associated HCC (20). Another study used ML to diagnose HCC with HCV (21). One study used gene expression profiling and supervised ML to predict HBV-related metastatic HCC (22). This study aims to classify open-access gene expression data of patients with HBV-related HCC and HCV-related HCC using the XGboost method and reveal important genes that may cause HCC.

MATERIAL AND METHODS

Data collection and variables

The present research originated from a case-control study published by Ueda et al. (23). The XGboost approach, one of the ML methods, was used to open-access gene expression data of HBV-related HCC and HCV-related HCC in the current investigation. For this purpose, data from 17 patients with HBV+HCC and 17 patients with HCV+HCC were included in the study. In the dataset, complementary DNA (cDNA) microarrays obtained from liver samples were used (23). cDNA is the double-stranded DNA version of the mRNA molecule. Since introns are cut out, researchers prefer to work with cDNA rather than mRNA. RNA is inherently more unstable than DNA. In addition, no amplification and purification technique can be applied to the RNA molecule (24). The primary output of the study is to classify HBV and HCV-associated HCC using machine learning methods and identify genes that may be biomarkers for HBV-related HCC.

Feature selection

Variable selection is an essential step in predictive modeling processes, and one of the most critical steps in developing a statistical model is deciding which data to include in the modeling. Feature selection identifies the most prominent features affecting a data set's dependent variable. Too many explanatory variables can lead to long computation times and the risk of over-learning the data and obtaining biased results (25). Most ML and data mining methods can produce ineffective results when working with extensive data. Therefore, these methods give more effective results when the dimensionality is reduced (26).

Gene expression data sets are pretty large. Modeling analyses take a long time because gene expression datasets are large, and these datasets can cause computational inefficiency in the analysis. LASSO, one of the feature selection methods, was used to solve these problems in this study. The LASSO method requires that the sum of the model parameters' absolute values be less than a fixed value (upper limit). The method achieves this by penalizing the coefficients of the regression variables, causing some of them to drop to zero. It is beneficial when the data set has a lot of variables and few observations. Furthermore, by removing irrelevant variables unrelated to the response variable, LASSO improves model interpretability and eliminates the problem of over-learning (27).

XGBoost

Gradient Boost is a powerful ML technique used for regression and classification problems where weak predictive models often produce ensemble forms of decision trees. Gradient Boost is based on boosting techniques (28, 29).

XGBoost, the abbreviation for Extreme Gradient Boosting, is one of the applications of gradient boosting machines (GBM), one of the most effective supervised learning algorithms. Its basic structure is based on gradient boosting and decision tree algorithms. Compared to other algorithms, it is in a very advantageous position regarding speed and performance. Gradient boosting is an ensemble method combining weak classifiers with boosting to create a strong classifier. The strong learner is trained iteratively, starting with a basic learner (29, 30).

Bioinformatics analysis

For the samples of HBV-related HCC and HCV-related HCC patients whose gene expression profiles were examined, differential expression analyses were performed using the limma package in the R programming language (31). Differential expression analysis is the statistical analysis of normalized read count data to find quantitative differences in expression activities between treatment arms. A pipeline is designed for the relevant analyses via the R software environment. The achieved results are presented from a table of genes in order of importance and a graph to visualize differentially expressed genes. The result table contains adjusted P and log2-fold change (log2FC) values, with genes with the smallest p values will be the most reliable. Log2FC>1 was used to identify up-regulated genes, and log2FC<-1 was used to identify down-regulated genes (32). A volcano plot was graphed to highlight quickly large values regarding the relevant genes.

Study protocol and ethics committee approval

This study, which was prepared using the National Center for Biotechnology Information Gene Expression Omnibus open-access dataset involving human participants, followed the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Ethical approval was obtained from the Inonu University Institutional Review Board (IRB) for Non-Interventional Clinical Research (Date: 07.06.2022, No: 2022/3648). The STROBE (Strengthening the reporting of observational studies in epidemiology) guideline was utilized to assess the likelihood of bias and overall quality of this study (33).

Biostatistical analysis

The Shapiro Wilk test of normality was used to determine whether the variables had a normal distribution. Data were given as median (minimum-maximum) or mean± standard deviation. The Mann-Whitney U test was employed to compare non-normally distributed data, and an independent-sample t-test was utilized to compare non-normally distributed data where appropriate. Logistic regression analysis was performed to estimate each gene's odds ratio (a measure of effect size). Hosmer & Lemeshow's test for the goodness of fit and an omnibus

test of model coefficients were calculated for logistic regression. A P-value <0.05 was considered statistically significant. The IBM SPSS Statistics 25.0 program was used in the analysis.

Modeling process

The XGBoost, one of the ML methods, was used in the modeling. Analyses were carried out using the n-fold cross-validation method. In the n-fold cross-validation method, the data is first divided into n parts, and the model is applied to n parts. One of the n parts is used for testing, while the other n-1 parts are used for training the model. The mean of the obtained values is evaluated for the cross-validation method. In this study, 10-fold cross-validation was employed for the modeling process. Accuracy, balanced accuracy, sensitivity, selectivity, a positive predictive value, a negative predictive value, and an F1-score were used as performance evaluation criteria. In addition, variable importances were calculated, which gives information about how much the input variables explain to the output variable.

RESULTS

In the study, 34 HCC patients were used, of which 28 were male and six were female. The mean age of the patients was 61.7±9.4 years. While 15 of the HBV+HCC patients were male and two were female, 13 of the HCV+HCC patients were male, and four were female. The mean age of HBV+HCC patients is 60.5±9.0 years, and the mean age of HCV+HCC patients is 62.9±9.9 years. The dataset used contains 8516 expressions. According to the bioinformatics analysis, the first ten results are summarized concerning minimum adjusted-p values in Table 1. Based on the statistics from Table 1, two genes (ID: 7109 and 9136) were down-regulation, one gene (ID: 6412) was up-regulation, and the other seven genes were unregulated. According to Table 1, Log2FC values for the ID=7179, *ALDOC, RPL39, IFITM3, FLJ12998, KIAA0446, GLUD1, TNIP1, FLJ30092,* and *MRPS21* genes were -1,6096623, -0,8756088, -1,1163435, -0,8729706, -0,7040085, -0,9362293, 1,0475908, -0,7960824, -0,9509129, and -0,7807535, respectively. The volcano plot used to visualize differentially expressed genes is given in Figure 1. On the y- and x-axes, the volcano graph plots significance versus fold-change in log 2 base to observe differentially expressed genes quickly.

Seventeen expression results were obtained by applying the LASSO feature selection method to 8516 expression results. Table 2 presents descriptive statistics for the selected genes concerning the groups. The explanations of the data set with the selected expressions, the examined target variable, and the odds ratio per gene for the target variable are presented in Table 2. Based on the statistics in Table 2, significant differences were detected between groups in all genes (p<0.05). The findings of the performance metrics from the XGboost model are given in Ta-

Table 1: The results of the bioinformatics analysis

Gene ID	Gene	Gene product	Adj P value	p value	t	B	Log2FC	diffexpressed
7109	*GML*	glycosylphosphatidylinositol anchored molecule like	0.0000215	3.60E-09	-7.57217	10.3385	-1.60966	Down
2765	*ALDOC*	Aldolase C, fructose-bisphosphate	0.0006396	2.59E-07	-6.21883	6.59	-0.87561	No
9136	*RPL39*	Ribosomal protein L39	0.0006396	3.22E-07	-6.15147	6.3996	-1.11634	Down
4853	*IFITM3*	Interferon-induced transmembrane protein 3 (1-8U)	0.0021807	2.06E-06	-5.56952	4.7487	-0.87297	No
9176	*FLJ12998*	Hypothetical protein FLJ12998	0.0021807	2.13E-06	-5.55998	4.7216	-0.70401	No
7556	*KIAA0446*	KIAA0446 gene product	0.0021807	2.19E-06	-5.55042	4.6945	-0.93623	No
6412	*GLUD1*	Glutamate dehydrogenase 1	0.0022021	2.58E-06	5.498999	4.5485	1.047591	Up
3752	*TNIP1*	TNFAIP3 interacting protein 1	0.0040048	5.37E-06	-5.26902	3.8962	-0.79608	No
5909	*FLJ30092*	AF-1 specific protein phosphatase	0.0070976	1.07E-05	-5.05112	3.2804	-0.95091	No
7010	*MRPS21*	Mitochondrial ribosomal protein S21	0.0124601	2.09E-05	-4.83884	2.6838	-0.78075	No

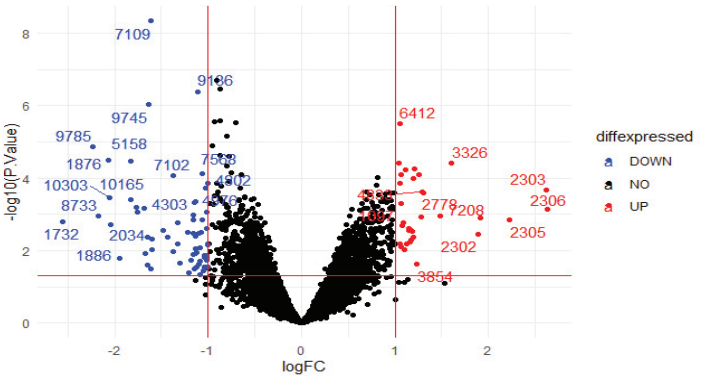

Figure 1: The volcano plot

Table 2: Descriptive statistics for Input variables

Gene	Prop number	Grups				OR	p
		HCV+HCC		HBV+HCC			
		Mean±SD	Median (min-max)	Mean±SD	Median (min-max)		
GLUD2	2747	0.87±0.31	0.89 (0.23-1.38)	-0.01±0.43	-0.02 (-0.64-0.72)	0.003	<0.001*
ALDOC	2765	0.53±0.39	0.52 (-0.33-1.17)	-0.34±0.35	-0.35 (-1.01-0.34)	0.003	<0.001*
TNIP1	3752	0.40±0.36	0.49 (-0.48-0.83)	-0.40±0.46	-0.46 (-1.24-0.61)	0.019	<0.001*
MX1	4303	0.75±1.07	0.43 (-1.32-2.98)	-0.39±0.72	-0.40 (-1.72-0.59)	0.187	0.001*
IFITM3	4853	0.55±0.40	0.56 (-0.17-1.14)	-0.32±0.46	-0.37 (-0.94-0.44)	0.011	<0.001*
C7orf30	4904	0.63±0.50	0.57 (-0.10-1.66)	-0.04±0.53	-0.01 (-1.56-0.72)	0.029	0.001*
RPL41	6171	-1.91±0.69	-1.83 (-3.47--0.77)	-0.98±0.82	-0.58 (-2.51-0.15)	4.779	0.004**
TRAPPC10	7109	1.74±0.69	1.69 (0.40-3.01)	0.13±0.57	0.01 (-0.78-1.94)	-	<0.001**
KIAA0446	7556	0.69±0.48	0.69 (-0.23-1.78)	-0.25±0.47	-0.11 (-1.72-0.35)	0.002	<0.001**
KDELR2	7919	0.33±0.50	0.22 (-0.57-1.38)	-0.34±0.49	-0.34 (-1.14-0.66)	0.050	<0.001*
OS-9	7949	0.17±0.38	0.22 (-0.45-1.10)	-0.36±0.38	-0.34 (-1.09-0.21)	0.014	<0.001*
ACP1	8178	0.16±0.25	0.12 (-0.24-0.69)	-0.23±0.28	-0.20 (-0.83-0.16)	0.001	<0.001*
RPL39	9136	0.99±0.52	0.90 (0.27-2.23)	-0.13±0.53	-0.26 (-1.02-0.59)	0.003	<0.001*
FLJ12998	9176	0.70±0.31	0.77 (0.09-1.13)	-0.01±0.32	-0.11 (-0.41-0.67)	0.001	<0.001*
WTAP	9589	0.55±0.33	0.58 (-0.31-1.05)	0.07±0.40	0.00 (-0.58-0.64)	0.028	0.001*
LMNA	9744	0.88±0.53	0.90 (-0.18-1.94)	0.09±0.76	0.27 (-2.30-1.60)	0.067	<0.001**
FKBP1A	10014	0.76±0.46	0.66 (0.13-1.84)	0.27±0.33	0.23 (-0.37-1.07)	0.022	0.001*

*: Independent sample t-test; **: Mann Whitney U test; OR. Odds ratio; SD: Standard deviation

Table 3: Performance metrics of the XGboost model

Metric	Value (%) (95% CI)
Accuracy	97.1 (91.4-100)
Balanced accuracy	97.1 (91.4-100)
Sensitivity	94.1 (71.3-99.9)
Specificity	100 (80.5-100)
Positive predictive value	100 (79.4-100)
Negative predictive value	94.4 (72.7-99.9)
F1 score	97 (91.2-100)

ble 3. Accuracy, balanced accuracy, sensitivity, specificity, the positive predictive value, the negative predictive value, and the F1 score obtained from the XGboost model were 97.1%, 97.1%, 94.1%, 100%, 100%, 94.4%, and 97%, respectively. The performance criteria values are plotted for the XGboost model in Figure 2. Figure 3 shows the importance levels of expressions for the selected genes in explaining the output variable. The *ALDOC* gene had the highest predictor importance of 100%, followed by *GLUD2* at 77.2 %, *TRAPPC10* at 59.2%, *FLJ12998* at 51.0%, *RPL39* at 33.2%, *KDELR2* at 24.8%, and *KIAA0446* at 23.8%.

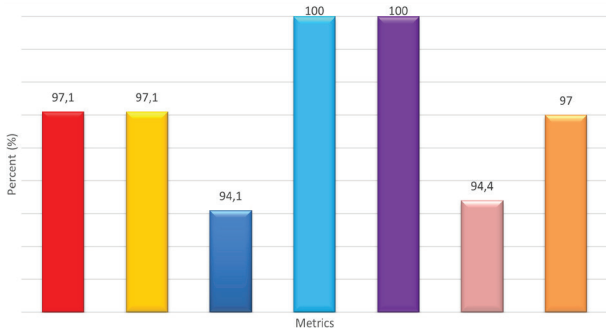

Figure 2: Graph of values for performance criteria obtained from XGboost models

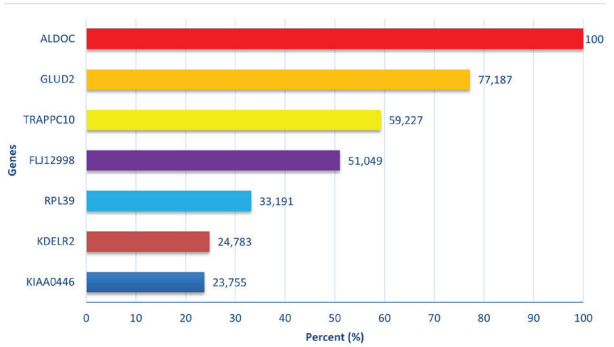

Figure 3: Graph of values for performance criteria obtained from XGboost models

DISCUSSION

Although the structure of gene expression profiling in HCC and the background liver tissue structure has been extensively studied, ML-based prediction of HBV-related HCC and HCV-related HCC and the detection of critical candidate biomarkers using an AI approach have not been clarified (23). The present study uses the XGboost method to classify HBV-related HCC and HCV-related HCC and identify important genes that may cause HBV-related HCC.

HCC is an aggressive type of cancer with well-defined epidemiological features. HCC continues to be an important public health problem worldwide, as it causes a significant economic and disease burden (1, 3, 34). The incidence and fatality rates of HCC vary significantly throughout the world. Discrepancies in the timing and quantity of exposure to environmental and infectious risk factors, the availability of healthcare resources, and the capacity to identify HCC at an earlier stage and administer possibly curative therapy are all variables that contribute to these differences (13, 35). HCC develops due to prolonged chronic hepatitis. In this case, patients have developed liver cirrhosis due to HBV or HCV infection. In patients with cirrhosis owing to chronic HBV or HCV infection, the annual incidence of HCC ranges from 2 to 5 percent overall. Chronic HBV and HCV infection are the major causes of HCC globally, accounting for 80% of all cases (34).

Except in northern Africa, where HCV incidence is most significant, chronic HBV infection is the primary cause of HCC throughout Eastern Asia and most African nations (36, 37). It is estimated that 257 million people worldwide have a chronic HBV infection. This situation leads to the high prevalence of chronic viral liver disease and HCC. It is also estimated that 20 million deaths can be attributed to acute hepatitis, chronic hepatitis, cirrhosis, and HCC caused by HBV between 2015 and 2030, with 5 million deaths from HCC alone (34).

HCV infection is still one of the most frequent bloodborne viral diseases and the leading cause of global infectious disease mortality (38, 39). HCV infection affects an estimated 71 million individuals worldwide, representing 1% of the population (40). Although direct-acting antiviral treatments have a high cure rate, 1.75 million new HCV infections and 400,000 HCV-related deaths occur yearly (41). HCV infection is a firmly established risk factor for HCC, increasing risk by 10- to 20-fold. Fatalities from HCV-related HCC grew by 21.1 percent during the last decade, but deaths from HCC caused by sources other than HCV and alcohol remained unchanged (14).

The overall survival of patients affected by HCC is low, and management of HCC risk factors needs to be rationally expanded to reduce the burden of HCC worldwide. There is a growing interest in genomics and molecular biology studies to identify early diagnosis and prognostic markers and new therapeutic targets to uncover the mechanisms of liver carcinogenesis and thus improve the clinical management of HCC patients (34, 42).

In the dataset investigated in this study, genomic data of samples obtained from liver tissues of 17 HBV-related HCC and 17 HCV-related HCC patients were used for the relevant analyses. cDNA microarrays were obtained from the samples, and the dataset used contained 8516 expressions. According to the Log2FC values used to determine the expression fold changes between the two groups from the bioinformatics analyses (detailed in Table 2), the GML gene has three-fold lower gene expression in HBV-related HCC patients than HCV-related HCC. Similarly, the RPL39 gene had a 2.15-fold lower gene expression. The *GLUD1* gene had two-fold upper gene expression in HBV-related HCC patients than in HCV-related HCC patients. Finally, the *ALDOC* gene, *IFITM3* gene, *FLJ12998* gene, *KIAA0446* gene, TNIP1 gene, *FLJ30092* gene, and *MRPS21* gene had the same expression between the two groups. In this instance, gene expression data are so large that modeling with these datasets can result in long analysis times and computational inefficiency in the analysis due to the size. Therefore, before mod-

eling with the existing data set, the most important genes associated with the output variable were selected with the Lasso variable selection method. Seventeen genes selected by the Lasso method were used in building Xgboost modeling. The accuracy, balanced accuracy, sensitivity, specificity, positive and negative predictive value, and F1 score metrics obtained with the XGboost model were 97.1%, 97.1%, 94.1%, 100%, 100%, 94.4%, and 97%, respectively. The performance metrics indicated that the proposed XGboost could correctly classify two groups of patients based on the AI approach. According to the variable importance obtained from the XGboost method, *ALDOC, GLUD2, TRAPPC10, FLJ12998, RPL39, KDELR2*, and *KIAA0446* genes can be used as candidates for predictive biomarkers for HBV-related HCC. According to the statistical analysis, 17 genes obtained by variable selection showed statistically significant differences for the two patient groups. Of the genes whose odds values were calculated, all genes, except *RPL41*, were downregulated in HBV-related HCC patients at significantly higher folds than in HCV-related HCC patients. The *RPL41* gene, on the other hand, was upregulated 4.779 fold in HBV-related HCC patients compared to HCV-related HCC patients. The OR values that were determined throughout the study and the Log2FC values support each other and support the values that were identified in the genes according to the variable significance. Additionally, the proposed pipeline produced a volcano plot, representing the up-and-down-regulation of the genes in this research. These plots are becoming more common in omics experiments such as genomics, proteomics, and metabolomics, where thousands of replicate data points between two conditions are often present.

One study reported that the *ALDOC* gene is associated with HBV-related HCC and is up-regulated by the MLX protein (43). In another study, it was reported that *ALDOC* was up-regulated in patients with HBV (44). In a study using matched tumor and adjacent liver tissues from 159 patients with HBV-related HCC, *GLUD2* showed high expression (45). Another study showed *GLUD2 d*own-regulation for the same condition (46). Another study found *GLUD2*, a potentially relevant gene for HCC (47). In one study, overexpression of RPL39 was reported to be associated with HCC (48). In one study, *KDELR2* was identified as a potential gene associated with HBV (49).

As it is known, all diseases that cause chronic liver damage are risk factors for the development of HCC. Therefore, international guidelines' follow-up of such patients is crucial for detecting possible HCC or its detection at an early stage (50). The most authoritative guidelines on monitoring chronic liver patients are published periodically by EASL, APASL, and AASLD (50). The above guidelines suggest that patients with chronic liver disease without suspected HCC should be followed up with ultrasonography and AFP at six-month intervals (50). Patients with suspected HCC should be followed up with ultrasound and AFP at three or six-month intervals. Patients with a strong suspicion of HCC should be followed up with ultrasound and AFP.

However, these approaches may not always give the expected results because it is not always easy for patients to reach healthcare providers in underdeveloped or developing countries. False-negative results may be higher than expected, especially since ultrasonography is an operator-dependent examination. It is a known fact that there is a correlation between the duration of chronic liver disease and the probability of developing HCC. In addition, as in all other cancer types, gene mutation and mutation-related mRNA expression changes are expected in HCC. Therefore, in the follow-up of patients with chronic liver disease, fundamental genetic analysis can be performed after a certain period to determine whether there is a genetic mutation. As seen in the results of this study, if changes in the expression of genes strongly associated with HCC are detected, and ideas are formed about the genetic mechanism underlying the different etiologies that cause HCC, patients can be followed more closely, and preventive treatments can be started when necessary. However, there is no evidence-based data on when genetic analysis should be performed on chronic liver disease. Therefore, a prospective multicenter study is needed on the timing of genetic analysis for patients with chronic liver disease. With this vital finding, increasing the number of patients may further increase the scope of genetic information and the power of the study.

CONCLUSION

In conclusion, this study identified potential genomic biomarkers for HBV-associated HCC using gene expression data from patients with HBV-associated HCC and HCV-associated HCC. The reliability of the genes discovered in the future, more thorough analyses may be evaluated, therapy techniques can be devised based on these genes, and their clinical utility can be detailed.

Ethics Committee Approval: This study was approved by Inonu University Institutional Review Board (IRB) for Non-Interventional Clinical Research (Date: 07.06.2022, No: 2022/3648).

Peer Review: Externally peer-reviewed.

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THE IMPORTANCE OF GLOMERULAR C3 ACCUMULATION IN ELDERLY PATIENTS WITH PRIMARY MEMBRANOUS NEPHROPATHY

İLERİ YAŞLI PRİMER MEMBRANÖZ NEFROPATİLİ HASTALARDA GLOMERÜLER C3 BİRİKİMİNİN ÖNEMİ

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ABSTRACT

Objective: The purpose of this study was to investigate the impact of glomerular C3 accumulation density on clinical, histopathological parameters and outcomes in elderly (>60 years) individuals with primary membranous nephropathy (PMN).

Material and Methods: In this study, we examined the patients (n=105) in two groups according to the C3 staining density in kidney biopsy samples as low intensity (C3: 1+; LI group) and high intensity (C3: 2+ or C3: 3+; HI group). The primary endpoint of our study was the end-stage renal disease, and the secondary endpoints were the development of partial remission (PR) or complete remission (CR).

Results: At the end of the follow-up (mean 30.6 months), more patients achieved the primary endpoint, and fewer patients achieved the secondary endpoints in the HI group compared to the LI group. (p=0.015 and p=0.016, respectively). Moreover, the glomerular filtration rate (eGFR) was lower (p<0.001), and proteinuria was higher in the HI group (p=0.018). Kaplan-Meier survival analysis revealed that renal survival (p=0.031) was lower in the HI group compared to the LI group. In the multivariate logistic regression analyses, no predictive parameters could be detected for the endpoints.

ÖZET

Amaç: Bu çalışmada, primer membranöz nefropatili (PMN) yaşlı (>60 yaş) hastalarda glomerüler C3 birikim yoğunluğunun klinik, histopatolojik özellikler ve hastalığın seyri üzerindeki etkilerini araştırmayı amaçladık.

Gereç ve Yöntem: Bu retrospektif gözlemsel çalışmaya dahil ettiğimiz PMN'li 105 hastayı böbrek biyopsi örneklerinde C3 birikiminin yoğunluğuna göre düşük yoğunluklu (C3 1+; LI) ve yüksek yoğunluklu (C3 2+ veya C3 3+; HI) olmak üzere iki grupta inceledik. Birincil sonlanım noktası son evre böbrek hastalığı, ikincil sonlanım noktaları ise tam (CR) veya kısmi remisyon (PR) idi.

Bulgular: İzlem sonunda (ortanca 30,6 ay), HI grubunda LI grubuna kıyasla daha fazla hasta birincil noktaya ulaşırken daha az hasta ikincil son noktalara erişti (sırasıyla p=0,015 ve p=0,016). Ayrıca HI grubunda LI grubuna göre glomerüler filtrasyon hızı (eGFR) daha düşük (p<0,001), proteinüri ise daha fazlaydı (p=0,018). Kaplan-Meier analizlerinde böbrek sağ kalımının (p=0,031) HI grubunda LI grubundan daha düşük olduğu saptandı. Çok değişkenli lojistik regresyon analizlerinde, son noktalar öngördürücü bir parametre saptanamadı.

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Conclusion: Intense glomerular C3 deposition in elderly (>60 years) patients with PMN may be related to poor clinical outcomes.

Keywords: Complementary system, C3, older age, membranous nephropathy

Sonuç: Yoğun glomerüler C3 birikimi, PMN'li yaşlı hastalarda olumsuz klinik sonuçlarla ilişkilidir.

Anahtar Kelimeler: Kompleman sistemi, C3, ileri yaş, membranöz nefropati

INTRODUCTION

Primary membranous nephropathy (PMN) is one of the most important etiologies of nephrotic syndrome in the non-diabetic adult population. Although it was previously known as an idiopathic disease, this approach has changed in the last decade. Today, the dominant role of autoreactive antibodies in the pathogenesis of the disease has been proven (1). It occurs in 25% of patients due to infections, drugs, systemic diseases such as systemic lupus erythematosus, and malignancies (secondary membranous nephropathy-MN) (2-4). The most common autoantibodies in PMN are M-type phospholipase A2 receptor (PLA2R) and thrombospondin type-1 domain-containing 7A (THSD7A); the number of responsible autoantibodies is increasing day by day (5-7). Although various risk factors such as older age, decreased GFR at diagnosis, male gender, and persistent heavy proteinuria have been identified, the clinical course of PMN is still quite interesting (8-10). Spontaneous complete remission develops in approximately 33% of the patients, and in 33% of the patients, proteinuria persists, albeit at varying levels. The remaining develop end-stage renal disease within ten years despite all treatments (11).

The immune deposits in PMN are rich in essential parts of the human complement system, such as C3 and C5b-9, indicating that the complement system has a vital role in PMN (12, 13). In PMN, immunofluorescent staining (IF) is characteristically detected for C3 and C4d, while C1q is negative (13). The process that begins with autoantibodies to cause glomerular damage results in building of a membrane attack complex (MAC) (13). It is accepted that the complement system activation in PMN is not via the classical pathway (CP). Anti-PLA2R IgG, which has an essential role in pathogenesis, predominantly activates mannose-binding lectin (MBL) or alternative complement (AP) pathways (14). On the other hand, glomerular MBL and C4b accumulation are also present in PMN (14). The accumulation of C1q, C3, C4, complement factor B (CFB), MBL, and C5b-9 accompanying the deposition of IgG in secondary MN supports the role of AP and MBL in the pathogenesis (14). Despite these data on the interaction between MN and the complement system, data on glomerular C3 accumulation, disease course, and prognosis are limited. However, in one study, the intensity of glomerular C3 accumulation was predictive of the development of kidney failure (15).

It is generally accepted that ageing activates the complement system (16). There is a strong relationship between the complement system activation and physiological ageing, as well as ageing diseases such as Alzheimer's and age-related macular degeneration. On the other hand, the complement system modulates many soluble and circulating factors responsible for renal ageing (17, 18).

Therefore, in this retrospective single-center study, our purpose was to examine the impact of C3 density on clinical, pathological parameters and endpoints in elderly (>60 years) patients with PMN.

MATERIAL AND METHODS

Study design

Patients over 60 years of age with biopsy-confirmed PMN, followed for at least six months between 1996 and 2019, were included after obtaining written informed consent. Patient information was gathered from hospital medical records. Patients with rheumatic diseases, hepatitis B or hepatitis C virus infections, cancers, or other secondary MN-related systemic diseases, and with an eGFR <15 ml/min/1.73 m^2 were excluded. In order to exclude malignancies in the elderly patient group, endoscopy, colonoscopy, thorax and abdominal tomography scans were performed and prostate-specific antigen levels were measured.

Standard laboratory methods were used for hemogram and biochemical parameters. The blood pressure (BP) measurements of the patients were measured twice with a manual sphygmomanometer and the higher value was recorded. The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) calculation was used for eGFR (19). Proteinuria was detected by urine protein-creatinine ratio (uPCR, g/g) in the first urine in the morning.

We examined the patients in two groups according to the density of C3 accumulation glomerular C3 immunofluorescence staining: Low-intensity (C3: 1+; LI group) and high density (C3: 2+ or C3: 3+; HI group).

All patients with no contraindications received a renin-angiotensin aldosterone system blocker (angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs). The low/intermediate-risk patients received only supportive treatments for six months. Immunosuppressive therapies (cyclophosphamide or calcineurin inhibitors and corticosteroids) were given to patients un-

responsive to these treatments (19). Patients diagnosed after 2012 were treated based on the treatment recommendations in the Renal Disease Improvement Global Outcomes (KDIGO) Glomerulonephritis Clinical Practice Guidelines (20). The study was performed in accordance with the Declaration of Helsinki and approved by Istanbul University Istanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 04.01.2013, No: 2013/11).

Study outcomes

The primary endpoint of the study was end-stage kidney disease. The secondary endpoints were complete remission (CR) or partial remission (PR). Proteinuria <0.3 g/g with eGFR of ≥60 mL/min/1.73 m2 (or a recovery of ±15% in those patient with eGFR <60 mL/min/1.73 m2) was defined as CR. PR was described as follows. 1- Proteinuria drop of >50% with proteinuria level of <3.5 g/g in those patients with nephrotic proteinuria at diagnosis 2- Recovery or stabilization (±25%) in eGFR.

The period between the histological diagnosis and the last clinical visit or the development of end-stage renal disease was considered the follow-up period. Demographic characteristics, clinical and histopathological findings (interstitial fibrosis, tubular atrophy, and immunofluorescent staining intensity pattern for IgG and C3) were analyzed.

Histopathological evaluation

A semiquantitative scale was used to define the fluorescence intensity of IgM, IgA, IgG, C3, C1q, lambda and kappa from 0 to 3. According to this scale, 0 is negative; 1 is weak; 2 is medium; 3 is strong. These lesions were grouped into four grades according to the Ehrenreich and Churg's criteria (21). Similarly, tubular atrophy and interstitial fibrosis were classified using a semiquantitative scale as follows: 1- mild, <25% of interstitium, 2- moderate, 25–50%, 3- severe >50%.

Statistical analyses

Quantitative parameters were depicted using standard deviations or medians with interquartile range (IQR, 25-75). Categorical parameters were expressed by percentages and numbers. Chi-square test was used for qualitative parameters. The Mann-Whitney U test was used for quantitative variables that did not show parametric distribution. Renal survival was evaluated by Kaplan-Meier analysis. Logistic regression analyzes were used to figure out risks associated with study endpoints. SPSS statistical software (SPSS version 26.0, IBM Corp., USA) and MedCalc were used for statistical analysis. A $p<0.05$ was accepted as a statistically significant value.

RESULTS

In total, 105 patients with PMN (36.1% female, median age 57.0 (IQR 45.0-66.0) were followed for a median of 30.6 (IQR 13.8-63.8) months. There were 49 patients in the LI group and 56 patients in the HI group. The mean age was higher in the HI group (71.0±6.1) than in the LI group (67.8±5.0 years) (p=0.003). Systolic and diastolic BPs and follow-up time were similar between groups. Higher serum albumin levels (2.9±0.8 versus 2.5±0.7 g/dL, p<0.001) and hemoglobin (13.1±1.9 versus 12.1±1.8, p=0.001) levels were determined in the LI group. Other demographic, clinical, and laboratory parameters of the study groups are shown in Table 1.

Therapeutic and histopathological features

There was no difference between the groups according to histopathological (Ehrenreich and Churg's) stage (p=0.751) and tubular atrophy/interstitial fibrosis density (p=0.414). However, the IgG density was greater in

Table 1: Demographic, clinical and laboratory characteristics of patients according to C3 accumulation

	LI group (n=49)	HI group (n=56)	p
Age mean±SD, years	67.8±5.0	71.0±6.1	0.003
Gender n (%)			
Male	30 (61.2)	37 (66.1)	0.606
Female	19 (38.8)	19 (33.9)	
Blood pressure mean±SD, mmHg			
Systolic	129.6±19.0	129.8±17.6	0.938
Diastolic	81.1±11.6	81.4±11.2	
Baseline proteinuria level mean±SD, g/g	5613.0±3395.6	6848.6±4099.2	0.199
Baseline serum albumin level mean±SD, g/dL	2.9±0.8	2.5±0.7	0.004
Baseline hemoglobin mean±SD, g/dL	13.1±1.9	12.1±1.8	0.001
Baseline eGFR mean±SD, mL/min/1.73 m^2	78.9±23.8	68.7±28.4	0.052

eGFR: estimated glomerular filtration rate, HI: high intensity, IQR: interquartile range, LI: low intensity, SD: standard deviation
Note: p-values compared low intensity and high intensity, obtained from the Chi-Square test, Fisher's exact test, or Mann–Whitney U test

Table 2: Histopathological characteristics of patients according to C3 accumulation density

	LI group (n=49)	HI group (n=56)	p
Histological stage n (%)			
Stage I			
Stage II	12 (24.5)	11 (19.6)	0.751
Stage III	28 (57.1)	32 (57.7)	
	9 (18.4)	13 (23.2)	
IgG intensity n (%)			
II +	20 (40.8)	7 (12.5)	0.001
III +	29 (59.2)	49 (87.5)	
IFTA intensity n (%)			
Mild	19 (38.8)	15 (26.8)	0.414
Moderate	1 (38.8)	1 (1.8)	

HI: high intensity, IFTA: interstitial fibrosis tubular atrophy, LI: low intensity
Note: p-values compared low intensity and high intensity, obtained from the Chi-Square test, Fisher's exact test, or Mann–Whitney U test

the HI group compared to the LI group (p<0.001). The histopathological features of the patients are shown in Table 2. There was no difference between therapeutic regimens (antiproliferative drugs, CNIs, and rituximab) (Table 3).

Study outcomes

After a follow-up of 30.6 (IQR 13.8-63.8) months, the primary endpoint developed in nine (16.1%) patients in the HI group, and in one patient (2.0%) in the LI group (p=0.015). The Kaplan-Meier survival analysis revealed that renal survival (p=0.031) was lower in the HI group than in the LI group (Figure 1). The number of patients who achieved the composite secondary endpoint was lower in the HI group (p= 0.016). However, CR [12 (24.5%) vs. 7 (12.5%)] and PR [23 (46.9%) vs. 20 (35.7%)] rates did not achieve statistical significance (p=0.111 and p=0.243, respectively).

The last eGFR was lower [52 (IQR 40-88) vs. 71 (IQR 69-117) mL/min/1.73 m^2, p<0.001)], and the last proteinuria

Table 3: Outcomes and treatment modalities according the groups

	LI group (n=49)	HI group (n=56)	p
Follow-up time months, median, (IQR 25-75)	49.7 (15-72)	47 (19-69)	0.716
Last eGFR level mL/min/1.73 m^2, median, (IQR 25-75)	71 (69-117)	52 (40-88)	<0.001
Last proteinuria level g/g, median (IQR 25-75)	2.1 (0.6-3.5)	3.8 (1.3-4.7)	0.018
Medication n (%)			
Calcineurin inhibitors	17 (34.7)	18 (32.1)	0.782
Cyclophosphamide	3 (6.1)	1 (1.8)	0.247
Antiproliferative agent	8 (16.3)	10 (17.9)	0.836
Rituximab	2 (4.1)	4 (7.1)	0.500
No immunosuppression	29 (59.2)	31 (55.4)	0.693
Primary endpoint n (%)	1 (2.0)	9 (16.1)	0.015
Secondary endpoint n (%)	35 (71.4)	27 (48.2)	0.016
Complete remission	12 (24.5)	7 (12.5)	0.111
Partial remission	23 (46.9)	20 (35.7)	0.243

eGFR: estimated glomerular filtration rate, HI: high intensity, IQR: interquartile range, LI: low intensity, SD: standard deviation
Note: P-values compared low intensity and high intensity, obtained from the Chi-Square test, Fisher's exact test, or Mann–Whitney U test

Table 4: The logistic regression analyses with factors that may predict primary outcome

	Univariate analysis		Multivariate analysis	
	OR (%95 CI)	P value	OR %95 CI	P value
Patient age	0.147 (0.977- 1.173)	0.147		
Initial eGFR	0.968 (0.941- 0.996	0.024	0.981 (0.952- 1.011)	0.219
Initial proteinuria	1.000 (1.000- 1.001)	0.833		
HI group	9.191 (1.120- 75.418)	0.039	5.856 (0.666- 51.459)	0.111
Serum albumin level	0.454 (0.169- 1.218)	0.117		
Histological stage	1.025 (0.379- 2.773)	0.961		
Baseline hemoglobin level	0.631 (0.423- 0.941)	0.024	0.800 (0.517- 1.239)	0.318

eGFR: estimated glomerular filtration rate, HI: high intensity, CI: Confidence interval

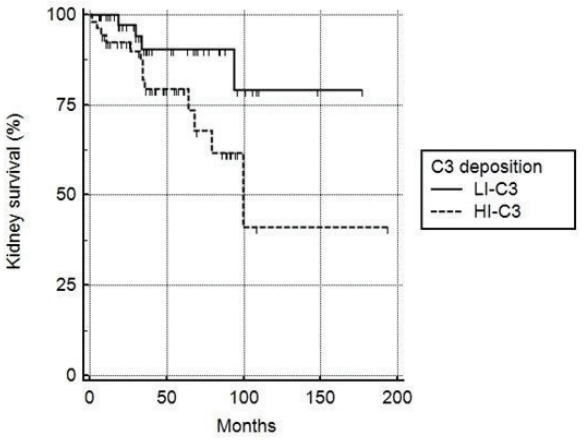

Figure 1: Kaplan-Meier survival analysis revealed that renal survival was lower in the HI group compared to in the LI group. (p= 0.031 with log-rank test)

DISCUSSION

In this single-center retrospective study examining the effect of glomerular C3 staining on disease outcomes in elderly (>60 years) patients with PMN, patients with strong C3 accumulation had lower final eGFR and higher last proteinuria. We also found that patients with strong C3 deposition were found to have lower baseline hemoglobin and serum albumin levels compared to patients with mild C3 deposition. Moreover, patients in this group (extensive C3 accumulation) had a high incidence of end-stage renal disease.

Studies show that ageing is associated with an increased immunoreactivity associated with alternative and classical pathway dysregulation of the complement system (22). This chronic inflammatory environment appears to be a contributing factor to many essential diseases of ageing (23-25). The kidney is susceptible to complement-mediated injury, mainly due to its high ultrafiltration capacity, the local increase in the production of complement compounds, and partially low renal expression of complement regulatory factors (26). This explains why the complement system is an essential pathogenic mediator in developing various kidney diseases such as lupus nephritis, antineutrophil cytoplasmic antibody-associated vasculitis, IgA nephropathy, C3 glomerulopathy, and atypical hemolytic uremic syndrome (aHUS) (27-29). There is evidence to support the idea that the complement system activation is one of the initiating factors that lead to tissue damage and subsequent proteinuria resulting from these immune reactions (30-32). Although it is unclear which pathway is more active in PMN, previous studies show that C4b, Bb, and MBL residues are related to the lectin pathway activation. In addition, accumulation of MAC, C3b, and renal excretion of C3dg suggest that the AP pathway is also effective in the pathogenesis of PMN (33-35).

Table 5: Logistic regression analysis with factors that may predict secondary outcomes

	Univariate analysis		Multivariate analysis	
	OR (%95 CI)	P value	OR %95 CI	p value
Patient age	0.947 (0.892-1.007)	0.082		
Initial eGFR	0.991 (0.976-1.006)	0.224		
Initial proteinuria	1.000 (1.000-1.000)	0.848		
HI group	2.685 (1.192-6.046)	0.017	2.196 (0.946-5.101)	0.067
Serum albumin level	2.179 (1.166-4.071)	0.015	1.911 (1.000-3.653)	0.050
Histological stage	0.729 (0.400-1.330)	0.303		
Baseline hemoglobin level	1.138 (0.919-1.408)	0.235		

eGFR: estimated glomerular filtration rate, HI: high intensity, CI: Confidence interval

levels were significantly higher (3.8 IQR 1.3-4.7) g7g vs. 2.1 (IQR 0.6-3.5) g/g, p=0.018) in the HI group compared to the LI group. Further details are shown in Table 3.

In univariate logistic regression analyses, baseline eGFR (OR 0.968, 95%CI 0.941-0.996, p=0.024), baseline hemoglobin (OR 0.631, 95%CI 0.423-0.941, p=0.024), and the presence of HI (OR 9.191, 95%CI 1.120-75.418, p=0.039) were associated with the primary endpoint. However, none of these parameters predicted the primary endpoint development in multivariate logistic regression analyses.

In univariate logistic regression analysis, the presence of HI (OR 2.685, 95%CI 1.192-6.046, p=0.017) and baseline serum albumin (OR 2.179, 95%CI 1.166-4.071, p=0.015) predicted the secondary outcome. However, none of these predicted secondary endpoint development in multivariate logistic regression analyses (Table 5).

Results of the studies investigating the prognostic importance of pathological parameters in PMN show significant differences (36, 37). Moreover, there are conflicting data about the intensity of complement deposition and clinicopathological findings. Zhang et al. reported higher serum anti-PLA2R antibody levels, more severe proteinuria, higher serum creatinine, and lower serum albumin levels in patients with strong complement accumulation. On the other hand, they found that C3 density was not predictive of adverse outcomes (38). Similarly, Horvatic et al. did not find any relationship between C3 density and negative results. Although a study reports that quantitative complement accumulation and disease progression are strongly associated, it is challenging to reach decisive conclusions due to the semiquantitative and unconfirmed grading system and differences in the specificity of the reagents used to predict complement accumulation (37). Our previous studies found that intense C3 accumulation was predictive of renal survival in patients with PMN (15). In this study, we showed that patients with extensive C3 deposition had worse kidney outcomes than patients with mild C3 accumulation. However, the C3 deposition amount was not predictive of end-stage renal disease development and complete or partial remission. The fact that the HI group was older than the LI group might have affected the results of the logistic regression analyses. Other reasons for the differences between these two outcomes may be related to the number of patients, the duration of the follow-up, differences in the scaling of C3 accumulation, variability in treatment regimens, and changes in the disease course in different populations.

Our study suffers from some limitations because of its retrospective nature. Serum anti-PLA2R was not detected in all patients at the time of diagnosis, and changes in the disease course could not be recorded. Hence, we were not able to obtain information about the relationship between C3 accumulation and autoantibody levels. In addition, due to technical limitations, electron microscopic evaluation, distribution of C3 residues, and an IgG subgroup determination could not be performed.

In conclusion, elderly patients with PMN with extensive glomerular C3 deposition have worse clinical outcomes than those with mild C3 deposition; therefore, it would be beneficial to determine and apply individualized treatment protocols for this patient group.

Ethics Committee Approval: This study was approved by Istanbul University Istanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 04.01.2013, No: 2013/11).

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A CROSS-SECTIONAL STUDY ON MARBLE WORKERS: OCCUPATIONAL EYE HEALTH PROBLEMS AND RISK FACTORS

MERMER İŞÇİLERİ ÜZERİNE KESİTSEL BİR ARAŞTIRMA: MESLEKİ GÖZ SAĞLIĞI SORUNLARI VE RİSK FAKTÖRLERİ

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ABSTRACT

Objective: Occupational health and safety is one of the most important aspects of the workplace. Working in a healthy and safe environment is one of our constitutional rights. In terms of occupational health, which is of the utmost importance, assessing the occupational eye health of marble quarry workers is also critical. The aim of this study was to determine the prevalence of corneal nebula, refraction error, pterygium, and similar ocular surface diseases and the factors affecting these diseases in marble quarry workers.

Material and Methods: A cross-sectional study in a marble quarry was performed. All the marble quarry workers who wished to participate in this cross-sectional study were included. Examinations of 126 workers were done in a large, appropriately lit room using a portable Snellen chart and a portable biomicroscope, and Volk 90D for fundus examination.

Results: Of the total 126 workers, 83 (65.9%) were working in an environment of high-risk in respect to ocular health, and eye disease was determined in 32 (25.4%). Refraction error was determined in 19 (15.1%), pterygium in 11 (8.7%), amblyopia in 2 (1.6%), and corneal nebula in 1 (1.1%).

Conclusions: Pterygium and refraction error were determined at a significantly high rate in marble quarry workers. It is important for workplace doctors to be trained in the early diagnosis of pathologies affecting the ocular surface, such as pterygium, for these workers to be referred early to an ophthalmology specialist. With the measurement and recording of the visual acuity of workers at certain intervals, workplace accidents can be reduced by taking precautions such as the referral to ophthalmology specialists of those determined to have a decrease in visual acuity.

Keywords: Occupational health, pterygium, wounds, and injuries

ÖZET

Amaç: İş sağlığı ve güvenliği çalışma yaşamının en önemli unsurlarından olup, sağlıklı ve güvenli bir ortamda çalışmak anayasal haklarımızdan birisidir. Mermer ocağında çalışan işçilerin mesleki göz sağlığının değerlendirilmesi de önemlidir. Bu çalışmanın amacı, mermer ocağı işçilerinde kornea nebulası, kırılma kusuru, pterjiyum ve benzeri oküler yüzey hastalıklarının prevalansını ve bu hastalıkları etkileyen faktörleri belirlemektir.

Gereç ve Yöntemler: Mermer ocağında yapılan bu kesitsel çalışmaya katılmak isteyen tüm mermer ocağı çalışanları dahil edilmiştir. Yüz yirmi altı işçinin muayeneleri, portatif bir Snellen eşeli, portatif bir biyomikroskop ve fundus muayenesi için Volk 90D kullanılarak geniş, uygun şekilde aydınlatılmış bir odada yapılmıştır.

Bulgular: Toplam 126 işçinin 83'ü (%65,9) göz sağlığı açısından yüksek riskli bir ortamda çalışmakta olup, 32'sinde (%25,4) göz hastalığı tespit edilmiştir. On dokuzunda (%15,1) kırılma kusuru, 11'inde (%8,7) pterjium, 2'sinde (%1,6) ambliyopi ve 1'inde (%1,1) kornea nebulası tespit edilmiştir.

Sonuç: Mermer ocağı işçilerinde önemli oranda pterjiyum ve kırılma kusurları tespit edildi. İşyeri hekimlerinin pterjium gibi oküler yüzeyi etkileyen patolojilerin erken teşhisi konusunda eğitim almaları, bu çalışanların bir göz hastalıkları uzmanına erken sevk edilmesi için önemlidir. Çalışanların görme keskinliklerinin belirli aralıklarla ölçülmesi ve kayıt altına alınması ile görme keskinliğinde azalma tespit edilenlerin göz hastalıkları uzmanlarına sevk edilmesi gibi önlemler alınarak ileride oluşabilecek iş kazaları azaltılabilir.

Anahtar Kelimeler: İş sağlığı, pterjiyum, yaralar ve yaralanmalar

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INTRODUCTION

Turkiye has an important position in the world as a country with high-quality marble quarries (1). There are natural stone reserves in Turkiye of various colors and designs, and marble quarrying has been ongoing since ancient times (2). With increasing exports of marble and stone to China, India, and the United States of America (USA), Turkiye has become the largest exporter of marble in the world (a total of approximately 8 million tons, at a total value of approximately 2 billion USD) (3). The marble industry and stone quarries affect the health of workers in the industry and have a negative effect on those living in the area of the industry, the whole ecosystem, and public health as a whole (4).

As there are many types of procedures in the production process and because of the dangers that these entail, marble quarries were defined as very dangerous according to the workplace danger classifications published in 2017 (5). The possibility of industrial accidents is increased during the process of making large marble blocks portable (cutting, loading, transport, etc.), and this leads to serious outcomes, such as permanent disability (meaning the inability to work) or death (6). Industrial accidents occurring in marble quarries are still a great source of concern for many countries in respect to occupational health and safety (7). In several studies, mining and quarrying have been shown to be one of the most dangerous sectors because of the inherent characteristics, such as exposure to damp, dust, gas, smoke, noise, and mechanical vibration (8-10). In marble quarries, the workers may be working above or below ground. Marble workers above ground are more exposed to climactic conditions, solar radiation, and the reflection of sunlight from marble walls and floors. Consequently, temporary blindness can be seen as the most important occupational risk in these workers, and photokeratitis, dermatitis, and potentially sunstroke in the summer months (11).

The aim of this study was to determine the prevalence of corneal nebula, refraction error, pterygium, and similar ocular surface diseases and the factors affecting these diseases in marble quarry workers.

MATERIAL AND METHODS

The Afyonkarahisar marble quarry, which is one of the most important in Turkiye, has been operational since 1991 (12).

Study subjects

This cross-sectional study was conducted with 156 workers employed at the marble quarry in the town of Şuhut in the province of Afyonkarahisar between 3.9.2021 and 31.12.2021. There was no calculation of sample size for the study and the researcher attempted to reach all the workers at the marble quarry. All the workers who agreed to participate were included for evaluation, with the only criteria for exclusion being unwillingness to participate. The study was completed with 126 (80.7%) workers who agreed to participate. Marble quarries have workplace features that make them a particularly dangerous type of workplace, necessitating the presence of a regular workplace doctor and nurse. It has been reported by employers that dust measurements in the workplace are done every two years. Nonetheless, there are no records of how many work accidents occurred in the previous year; instead, employers have reported the absence of work accidents verbally.

Data collection

A questionnaire was administered to the workers in face-to-face interviews. The items in the questionnaire included sociodemographic and health-related characteristics (6 items), job description and risks of the working environment (11 items), information about ocular problems originating from the work environment, a form of resolution, and time of most recent presentation to an ophthalmologist.

Examinations lasting 15-20 mins were performed by an ophthalmology specialist (a Member of the Royal College of Surgeons of Edinburgh (UK)), in a large, appropriately lit room using a portable visual acuity chart, a portable biomicroscope (Reichert Inc., NY, USA), and Volk 90D (Volk Optical Inc., USA) for fundus imaging. First, the visual acuity was examined separately in both eyes from a distance of 6 meters. Then, intraocular eye pressure was measured using a portable rebound tonometer (MSLYZ06, Guangdong, China). In cases with visual acuity (VA) <20/20, the pupils were dilated, and examinations were made of the anterior segment with a manual biomicroscope (Portable Slit Lamp, Reichert Inc, NY, USA) and the posterior segment with 90D Volk. Cases who wore spectacles and had VA <20/20, with no pathology determined, were accepted as a refraction error. When any pathology was determined, primarily refraction error, amblyopia, and pterygium in the anterior segment, the findings were recorded.

Permission for the study was granted by the local Ethics Committee (Date: 03.09.2021, No: 2021-KAEK-2).

Statistical analysis

Data obtained in the study were analyzed statistically using the Statistical Package for Statistical Sciences software (SPSS, Version 23.0). In the presentation of descriptive data, continuous variables were stated as mean ± standard deviation (SD) values, and categorical variables as number (n) and percentage (%). To evaluate the relationships between categorical variables, the Chi-square test and Fisher's Exact test were applied. A value of $p<0.05$ was accepted as statistically significant.

RESULTS

Of the total 126 subjects participating in the study, 2 (1.6%) were illiterate, 7 (5.6%) had basic literacy, 34 (27%) had an educational level of primary school, 42 (33.3%) had an educational level of middle school, 33 (26.2%) had an educational level of high school, and 8 (6.3%) were university graduates. Diabetes mellitus was present in 3 (2.4%) subjects, hypertension in 3 (2.4%), hyperlipidemia in 2 (1.6%), and asthma in 1 (0.8%), and all of these cases were taking medication for these diseases. No employee had a history of cancer diagnosis and associated drug use. Work experience was determined as mean of 8.7±7.7 years, median of 6.5 years (range, 1-32 years). The working hours per week were determined as mean 56.7±9.1 hours, median 56 hours (range, 40-96 hours).

As a result of the examinations made, the macular disease was determined in 32/126 (25.4%) subjects, recorded as refraction error in 19 (15.1%), pterygium in 11 (8.7%), and amblyopia in 2 (1.6%). Of the total subjects, 72 (57.1%) had never consulted an ophthalmologist, 27 had not had an ocular examination in the last year, and 27 (21.4%) had been examined by an ophthalmology specialist within the last year.

Forty-three (34.1%) workers stated that there was no ocular health risk in the environment in which they worked, and 83 (65.9%) stated that they worked in an environment which posed a risk in terms of ocular health. Of these, 63 (50%) stated that they worked in a dusty environment, 12 (9.5%) that they worked in an environment where metal and similar fragments could be dispersed, and 8 (6.3%) that they were exposed to extremely high temperatures. Exposure to flying metal slivers, glass fragments, wood shavings, or soil particles while working was reported by 30 (20.8%) workers. In the work processes, 57 (45.2%) workers reported that they used machines/tools with colored signals/warning lights. Welding machines were used by 13 (10.3%) workers, of which 9 (7.2%) stated that they used protective equipment, and 4 (3.2%) that they did not use protective equipment while using welding machinery. Protective goggles or visors were reported to be used by 77 (61.1%) workers.

A problem in the eyes related to the working environment (heat, light, dust, or foreign body) was reported to have been experienced by 37 (29.4%) workers. Of the 7 who were treated because of the problem, 5 (4%) went to their family doctor, 1 (0.8%) to the workplace doctor, and 1 (0.8%) used eyedrops on his own initiative.

For 31 (24.6%) of the study participants the place of work was in the offices and for 95 (75.4%) it was in the field. Of those working in the offices, no ocular disease was determined in 19 (61.3%), refraction error was present in 6 (19.4%), pterygium was present in 4 (12.9%), and amblyopia was present in 2 (6.5%). No ocular disease was observed in 75 (78.9%) of those working in the field, refraction error was present in 13 (13.7%), and pterygium was present in 7 (7.4%). In 1 (1%) of the workers with refraction error, multiple nebulae were determined associated with previous foreign body trauma to the cornea. This was photographed and recorded (Figure 1). Amblyopia was not determined in any of the workers in the field.

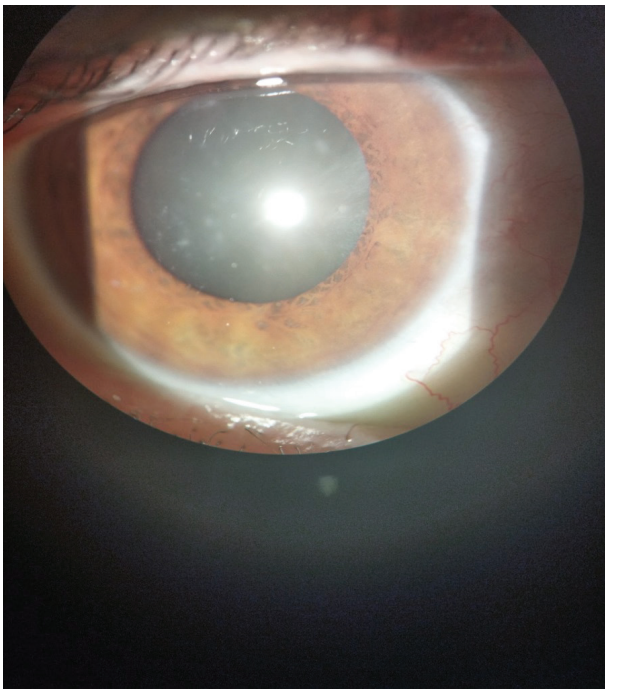

Figure 1: Multiple nebulae formed because of a stone fragment striking the cornea

No correlation was determined between the ocular diseases determined and the risk status in terms of eye health of the environment in which they were working (p=0.202) (Table 1).

No visit to an ophthalmologist was reported by 15 (48.4%) of the office workers and by 58 (61.7%) of those working in the field. No statistically significant difference was determined between the areas of work in respect of having consulted an ophthalmologist (p=0.257). Of the 58 (61.7%) workers who had not been to an ophthalmologist, refraction error was determined in 7 (7.4%), pterygium in 6 (6.3%), and amblyopia in 1 (1%). In all these cases, the disorder was diagnosed for the first time by a specialist in the examinations made for this study.

No significant difference was found between the determination of ocular disease and the use of protective goggles and educational level (p=0.535, p=0.124, respectively). There was determined to be a statistically significantly

Table 1: Eye diseases determined in the marble quarry workers, the units where they worked, the environmental conditions, and visits to an ophthalmologist

	None (n=94)	Pterygium (n=11)	Refraction error (n=19)	Amblyopia (n=2)	p value
Unit where employed n (%)					
Office	19 (61.3)	4 (12.9)	6 (19.4)	2 (6.5)	0.044
In the field	75 (78.9)	7 (7.4)	13 (13.7)	0 (0)	
Risk status n (%)					
None	27 (62.8)	4 (9.3)	10 (23.3)	2 (4.7)	0.202
Dust	51 (81.0)	5 (7.9)	7 (11.1)	0 (0)	
Hot environment	5 (62.5)	2 (25.0)	1 (12.5)	0 (0)	
Dispersion of particles (metal, soil, splinters, etc.)	11 (91.7)	0 (0)	1 (8.3)	0 (0)	
Exposure to particles in the air of the workplace, such as metal shavings, glass fragments, wood chippings, or soil dust n (%)					
No	71 (74.0)	9 (9.4)	14 (14.6)	2 (2.1)	1.000
Yes	23 (76.7)	11 (8.7)	19 (15.1)	2 (1.6)	
Use of welding machinery n (%)					
No	85 (75.2)	10 (8.8)	16 (14.2)	2 (1.8)	0.747
Yes	9 (69.2)	1 (7.7)	3 (23.1)	0 (0)	
Wearing protective goggles when using welding machinery n (%)					
No	2 (50.0)	0 (0)	2 (50.0)	-	0.471
Yes	7 (77.7)	1 (11.1)	1 (11.1)	-	
Use of protective goggles or face visor n (%)					
No	35 (71.4)	3 (6.1)	10 (20.4)	1 (2.0)	0.490
Yes	59 (76.6)	8 (10.3)	9 (11.6)	1 (1.2)	
Problems experienced related to the working environment (heat, light, dust) n (%)					
No	63 (70.8)	8 (9.0)	16 (18.0)	2 (2.2)	0.458
Yes	31 (83.7)	3 (8.1)	3 (8.1)	0 (0)	
Eye injury due to foreign body in the workplace n (%)					
No	90 (75.6)	9 (7.6)	18 (15.1)	2 (1.7)	0.274
Yes	4 (57.1)	2 (28.5)	1 (14.2)	0 (0)	
The treatment method for problems experienced related to the working environment (heat, light, dust) n (%)					
My eye was not injured.	90 (75.6)	9 (7.6)	18 (15.1)	2 (1.7)	N/A
I used eye drops myself.	1 (100)	0 (0)	0 (0)	0 (0)	
I went to the workplace doctor.	0 (0)	1 (100)	0 (0)	0 (0)	
I went to my family doctor.	3 (60)	1 (20)	1 (20)	0 (0)	
Previous visit to an ophthalmologist (apart from this examination)					
I have not seen an ophthalmologist.	58 (80.6)	6 (8.3)	7 (9.7)	1 (1.4)	0.207
I have seen an ophthalmologist.	36 (66.7)	5 (9.3)	12 (22.2)	1 (1.9)	

higher rate of the use of protective goggles in the group working in an environment of high risk to eye health compared to the group at no risk (p<0.001). Protective goggles were used at a statistically significantly higher rate by workers who had previously experienced eye problems related to the working environment compared to those who had not (p<0.001) and by workers who had suffered an eye injury because of a foreign body compared to those who had not (p=0.042) (Table 2).

Among the office workers, there was determined to be a statistically significantly higher rate of the use of protective goggles by those who had previously experienced eye problems related to the working environment compared to those who had not (p=0.010^{adj}). Protective goggles were used by 28 (87.5%) of the 32 workers in the field who had experienced eye problems because of the working environment, and by 37 (58.7%) of the 63 who stated they had not experienced such problems. This difference was determined to be statistically significant (p=0.010^{adj}) (Table 3).

Table 2: Associations between the education level of the workers, eye health, and environmental characteristics and the use of protective eyewear

	Protective goggles		
	No, n (%)	**Yes, n (%)**	**p value**
Eye disease			
Absent	35 (37.2)	59 (62.8)	0.535
Present	14 (43.8)	18 (56.3)	
Education			
Middle school or below	37 (43.5)	48 (56.5)	0.124
High school or above	12 (29.3)	29 (70.7)	
Risk status			
None	27 (62.8)	16 (37.2)	**<0.001**
Present	22 (26.5)	61 (73.5)	
Problems experienced related to the working environment (heat, light, dust)			
No	45 (50.6)	44 (49.4)	**<0.001**
Yes	4 (10.8)	33 (89.2)	
Eye injury due to foreign body in the workplace			
No	49 (41.2)	70 (58.8)	**0.042**
Yes	0 (0)	7 (100)	
Previous visit to an ophthalmologist (apart from this examination)			
I have not seen an ophthalmologist.	32 (44.4)	40 (55.6)	0.140
I have seen an ophthalmologist.	17 (31.5)	37 (68.5)	

Table 3: Associations of problems experienced related to the working environment and the use of protective eyewear

Unit	**Problems experienced related to the working environment (heat, light, dust)**	**Protective goggles**		
		No, n (%)	**Yes, n (%)**	**p**
Office	No	19 (73.1)	7 (26.9)	**0.010**^{adj}
	Yes	0 (0)	5 (100)	
In the field	No	26 (41.3)	37 (58.7)	**0.010**^{adj}
	Yes	4 (12.5)	28 (87.5)	

adj: Bonferroni correction

DISCUSSION

Eye injuries occurring in stone quarries and the marble industry are usually to the ocular surface and anterior segment, with a general lifetime prevalence of 4.4%, and age-specific prevalence varying between 2% and 6% (13). After injuries to the hands and feet, eye injuries are the third most common injuries in marble quarries, and therefore it has been reported that ocular health is negatively affected (14). Ocular injuries are a significant preventable cause of blindness, and it has been reported that 55 million ocular injuries per year cause blindness in 1.6 million individuals (15). The most common ocular symptoms range from simple eye fatigue to cornea, lens, and ocular surface degeneration, exposure to foreign bodies, and serious perforating injuries causing blindness (16,17). In the prevention of eye injuries, it is thought that the appropriate identification of workplace risk factors, evaluation of the use of personal protective equipment and training of employees on this subject can provide a cost-effective solution (18).

All the 126 workers in the current study were male, which is understandable when the difficulties of working in stone quarries are considered. In the evaluation of the educational level of the workers, there was seen to be a higher rate of those with primary school, middle school, and high school education compared to university graduates, which was consistent with the distribution seen in previous similar studies (18,19). Although this is associated with the socioeconomic status of the country and education policies, widespread difficult conditions in Turkiye and relatively low-paid work are associated with education level (20).

More than half (57.1%) of the workers in the current study, and the vast majority of workers in a previous study in Ghana, did not visit an ophthalmologist for routine check-ups (19). Moreover, the fact that no difference was determined in the status of visiting an ophthalmologist according to the unit of work (office or in the field) is an important sign of the workers' perception and awareness of health and safety at work. In a study in Nigeria, it was reported that only 52% of the workers in a stone quarry were aware of the ocular dangers in the workplace that could potentially have a negative effect on ocular health (21). In the current study, 43 (34.1%) workers stated that there was no risk to eye health in the environment where they worked and 83 (65.9%) stated that they worked in an environment that constituted a risk to eye health.

While a significant proportion of eye injuries occur in the workplace, ocular trauma continues to be a global preventable cause of morbidity of eye diseases (22). Eye injuries that occur in the workplace are related to the profession and the nature of the work conducted, and therefore some individuals are at a higher risk of eye injuries because of their occupation (23). Corneal foreign body, globe rupture, lamellar laceration of the cornea and hyphema have been reported in literature as the most frequent forms of ocular injuries (24).

In the current study, 20.8% of the workers were exposed to sudden flying particles of metal, glass, wood, or soil, and 5.5% experienced an eye injury of foreign body origin. However, there were no findings in this study of injuries similar to globe rupture, lamellar laceration, or hyphema in the anterior segment. Khorshed et al. reported that 47.6% of the workers in their study had experienced an ocular foreign body, and 10.8% had suffered an eye injury (25). In a study by Ezisi al., the general prevalence of work-related eye injuries was determined to be 32.0%, and of those working in stone quarries, there was a higher rate in stone processing workers (26). Koffuor et al. determined the prevalence of eye injuries to be 59.2% in workers in stone and sand quarries, and 43.7% in workers in areas other than mining and quarrying (19). The differences in eye injuries between published studies can be attributed to the laws and investments of countries, the extent of the use of personal protective equipment, training of the workers in health and safety at work, and differences in data collection methods (26).

The use of personal protective equipment is valuable with the aim of being able to prevent injuries in the workplace. In marble and stone quarries, protective goggles with which there can be full and clear vision in the workplace should be worn. It is known that the use of protective eyewear can prevent more than 90% of serious eye injuries (27). However, studies have shown that the use of protective equipment has still not reached high levels (22,28). In a study by Prasa et al., it was reported that 81.0% of workers did not use protective equipment, and in a study examining eye injuries in industrial accidents, Ngo et al. determined that protective eyewear was not used by 78.6% of workers (16,29). In the current study, it was determined that a relatively higher rate of workers used protective goggles or a visor, and the rate of protective goggle use was reported to be higher in those who had experienced ocular health problems and eye injuries because of the working environment ($p<0.001$, $p=0.042$). Similar to these findings, there are studies in the literature reporting that the use of protective goggles was associated with having experienced ocular trauma in the past and it had then become a personal habit (21,30). There may be various reasons that the use of protective equipment is not at an adequate level. The reasons most commonly focused on are that the employer does not always provide appropriate protective equipment, unsuitable protective goggles frequently steam up (reducing vision quality), users experience discomfort, and the risk of eye injury can be easily ignored by workers (21,31).

Of the 126 workers included in the current study, ocular disease was determined in 32 (25.4%), refraction error was recorded in 19 (15.1%), pterygium was recorded in 11 (8.7%), and amblyopia was recorded in 2 (1.6%) as a result of the examinations. In a recent study in Egypt, conjunctival hyperemia was seen most in the eye examinations in 59.6% of workers, and in another study that investigated ocular problems in stone quarry workers, pterygium was determined at the high rate of 22.0% (25,32). Similarly, in another study, pterygium, cataract, refraction error, pinguecula, and conjunctival hyperemia were predominant (19,26). That the ocular examination findings were at a lower level in this study compared to previous studies can be said to be related to the sample size. The development of pterygium can be caused by exposure to dust, smoke, and ultraviolet light while working outside (33). There are studies in literature that have reported that long-term exposure to marble dust in the process of producing marble in quarries causes ocular irritation and subsequent conjunctival hyperemia and conjunctivitis (25,34).

To the best of our knowledge, this is the first study which has been conducted by an ophthalmology specialist in stone quarries in Turkiye. In the light of the objective data obtained, reducing the factors threatening ocular health by educating workers in the use of protective equipment, and the workers becoming accustomed to using the protective equipment is important for the protection of the health of workers. Since it is a quarry, the workplace is classified as extremely hazardous, and there is a workplace doctor on site on a regular basis. Despite the fact that the workplace doctor conducts annual general routine checks on employees, no additional ocular examinations have been performed. As a specific specialty, ophthalmology attracts attention, and given that marble workers may have difficulty reaching an ophthalmologist, it is possible that these workers are unaware of their eye health. Our study is significant in this context because it emphasizes the importance of screening marble quarry workers for eye diseases. In addition, along with other studies, it will be beneficial to investigate whether there is a correlation between the ambient dust measurements to be taken in this occupational group and eye diseases. Mines and quarries employ 192,793 people in the Republic of Turkiye, according to data published by the Ministry of Labor and Social Security in 2020 (35). Only three occupational accidents were reported during the inspections of the mines and quarries, which employed 13,063 people. It is unknown if any of them were injured in the eyes. We cannot make any comparisons because we lack reliable data on whether or not an occupational accident occurred in the quarry under consideration.

In addition, workplace doctors can examine the visual acuity of workers at certain intervals with a portable chart, and by recording the results, workers determined to have a decrease in visual acuity can be referred to an ophthalmology specialist. Taking the time to examine workers with reduced visual acuity is of great importance in respect to preventing potential workplace accidents.

The correct diagnosis by a workplace doctor of eye diseases, which involve the ocular surface, such as pterygium, and which can be determined with light examination, can ensure the referral of workers to an ophthalmology specialist before progression of the disease. Therefore, workplace doctors may need training in this area. Together with the results of future similar studies, the neglect of the ocular health of workers, which is a potential public health problem, can be eliminated.

In this study, cases with visual acuity determined to be lower than normal and with no pathology determined in the examination were accepted as low vision because of refraction error. In future similar studies, it would be appropriate to use a portable autorefractometer. This study was conducted in two randomly selected marble quarries in a specific region. Further multicenter studies in marble quarries in various regions of Turkiye with a greater number of participants could determine more accurate results.

CONCLUSION

It is clear that the working conditions of marble quarries have an undeniable effect on ocular surface diseases. The current study showed a highly significant rate of pterygium and refraction error in the marble quarry workers, and a significant proportion of these were diagnosed for the first time in examinations by the ophthalmologist in the study. Although the use of personal protective equipment was seen to be relatively more than in other studies, the level of use should be independent of personal accident history and should be brought up to an adequate level. Workers and workplace doctors should be given training about eye health. In addition to being able to identify high risks in the workplace in terms of eye health, routine examinations by an ophthalmologist should be provided by the employer. With certain training, workplace doctors can measure visual acuity with a Snellen chart and screen for ocular disorders, primarily pterygium, and refer workers to specialists when necessary. Thus, by minimizing the exposure to working environments that have an effect on eye health, it will be possible to provide early diagnosis and treatment.

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THE EFFECTS OF ALPHA LIPOIC ACID ON LENS INJURY IN RATS ADMINISTERED WITH VALPROIC ACID

VALPROİK ASİT UYGULANAN SIÇANLARDA ALFA LİPOİK ASİDİN LENS HASARI ÜZERİNE ETKİLERİ

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ABSTRACT

Objective: Valproic acid (2-propyl valeric acid; VPA) is an effective short-chained fatty acid which is used for the treatment of migraine and schizophrenia. Though it provides effective treatment, its side effects are associated with free radicals and in this way it affects many organs and tissues. Alpha lipoic acid (ALA) is known to be a powerful antioxidant.

Material and Methods: The aim of this current study was to investigate the protection of ALA on VPA induced lens injury. Female rats were split into four groups as follows: 1st group, control animals (corn oil per day for 15 days); 2nd group, ALA administered group (50 mg per kg each day for 15 days); 3rd group, VPA administered group (500 mg per kg each day for 15 days) and 4th group, VPA and ALA administered group to which the same dose was given at the same time each day. On the 16th day, lens tissues were taken.

Results: Lens glutathione levels and glutathione-S-transferase activities were decreased while lipid peroxidation and protein carbonyl levels, superoxide dismutase, glutathione peroxidase and reductase, aldose reductase and sorbitol dehydrogenase activities were elevated after VPA administration. ALA reversed these levels and activities in the VPA group.

Conclusion: We can conclude that ALA used its antioxidant property and ameliorated VPA induced lens injury.

Keywords: Valproic acid, alpha lipoic acid, lens tissue, oxidative stress

ÖZET

Amaç: Valproik asit (VPA) kısa zincirli bir yağ asididir ve migren ile şizofreni tedavisinde kullanılır. Tedavi edici etkilerinin olmasına rağmen, bu ilacın yan etkileri serbest radikaller ile ilişkilidir ve bu yol ile birçok organ ve dokuyu etkilemektedir. Alfa lipoik asit (ALA), güçlü bir antioksidandır.

Gereç ve Yöntem: Bu çalışmada, ALA'in VPA ile oluşturulan lens hasarı üzerine koruyucu etkileri araştırıldı. Dişi sıçanlar dört gruba ayrıldı: 1. grup, kontrol hayvanları (15 gün boyunca her gün mısır özü yağı verildi); 2. grup, ALA verilen grup (15 gün boyunca her gün 50 mg/kg verildi); 3. grup, VPA uygulanan grup (15 gün boyunca her gün 500 mg/kg uygulandı), 4. grup; Her gün aynı saatte ve dozda VPA ile ALA uygulanan grup, 16'ıncı günde, lens dokuları alındı.

Bulgular: VPA uygulanmasından sonra, lens glutatyon düzeyleri ve glutatyon-S-transferaz aktiviteleri azalırken lipid peroksidasyonu ve protein karbonil seviyeleri, süperoksit dismutaz, glutatyon peroksidaz ve redüktaz, aldoz redüktaz ve sorbitol dehidrojenaz aktiviteleri artış gösterdi. ALA, VPA grubundaki bu seviyeleri ve artışları tersine çevirdi.

Sonuç: ALA'in antioksidan özelliğini kullandığı ve VPA ile oluşturulan lens hasarını iyileştirdiği sonucuna varılmıştır.

Anahtar Kelimeler: Valproik asit, alfa lipoik asit, lens dokusu, oksidatif stres

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INTRODUCTION

Valproic acid (VPA) is a short-chained and branched fatty acid having the chemical formula of "2-propyl valeric acid". It is derived from valeric acid which exists in *Valeriana officinalis* and was synthesized in the late 19th century by Burton (1). Nowadays, this molecule is used and widely preferred due to its anticonvulsant effect in the treatment of many seizures such as complex partial/tonic-clonic, depression, strong headaches like migraine, and schizophrenia (2-4). Despite its protective and preventive effects, VPA has been declared to have many serious side effects and this drug has taken the lead in the formation and construction of free radical species which damage many tissues and organs like heart, lung, liver, brain, testis, etc. (5-8).

Researchers have been trying to eliminate the harmful effect of VPA by using different protective agents. One of the solutions was put forward by Sokmen et al. and this involved using a sulphur containing compound like S-methyl methionine sulfonium chloride (MMSC), also known as Vitamin U and a powerful antioxidant. This was then proven to be effective by Gezginci-Oktayoglu et al. and Oztay et al. (9-11).

Based on this approach, we used alpha lipoic acid (ALA) in our study. ALA occurs in many types of nutrition such as meat and green vegetables (12). This sulphur containing compound has a unique structure in that it carries lipid while at the same time having water-soluble properties (13). ALA has a stimulant effect on insulin-sensitive cells, thereby facilitating glucose uptake. It has a stabilizing effect on cell redox system, proteins and some molecules such as glutathione (14). In addition, ALA has been described as being involved in the most important energy production pathway, the Krebs cycle, where ALA plays a vital role as a coenzyme assisting in the transfer of acyl groups (15). Its protective effects on different systems of VPA and α-cypermethrin have been reported by many researchers, respectively (7, 16, 17).

The lens has a special closed system and is well designed for transmitting light. It is made up of proteins at a much higher level when compared with other organs (18). In the crystalline structure of proteins, many cysteine residues are found (19). The biological composition of these crystalline structures are sometimes affected by free radical attacks, and as a result become damaged by processes such as oxidation (20). Moreover, VPA affects the lens by increasing free radical levels. It may lead to cell death since it affects glucose levels and forms hypoxia in human epithelial cells (21-23).

In the light of this, the aim of our study was to investigate the protection provided by ALA in VPA induced lens injury.

MATERIAL AND METHODS

Animals

The rats were supplied by the Experimental Animal Implementation and Research Centre, DEHAMER, Marmara University. Ethical approval was obtained from the Marmara University Animal Care and Use Committee (Date: 23.03.2015, No: 34.mar). Female Sprague-Dawley rats (6 months old) were used. The animals received standard food as pellets. They had access to tap water *ad libitum*.

Experimental procedures

The Sprague-Dawley rats were split into four groups. The 1st group consisted of control animals which received corn oil; the 2nd group consisted of animals which received ALA (50 mg per kg); the 3rd group included animals to which VPA (0.5 g per kg) was administered; and the 4th group incluced animals which received ALA (50 mg per kg) 60 minutes prior to the VPA administration (0.5g per kg). Corn oil and ALA were applied by gavage technique while VPA was applied intraperitoneally for 15 days. On the last day of experiment, all animals fasted overnight after which blood was taken from their hearts using a sterile injector. Their lens tissues were collected. The lens tissues were homogenized in physiological saline (0.9% NaCl) for the preparation of 10% (w/v) homogenate. The homogenates were centrifuged at 10000 x g at +4°C for 10 minutes and supernatants were used for the analysis.

Biochemical analyses

In clear supernatants, reduced glutathione (GSH), lipid peroxidation (LPO) and protein carbonyl (PC) levels were taken according to Beutler, Ledwozyw et al. and Levine et al. (24-26). Glutathione-S-transferase (GST), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione reductase (GR) activities were performed using the methods of Habig and Jakoby, Mylroie et al., Wendel, and Beutler (27-30). The lens marker enzymes aldose reductase (AR) and sorbitol dehydrogenase (SDH) were determined as per the methods of Hayman and Kinoshita, and Barretto and Beutler (31, 32). All the levels and enzyme activities were recorded according to the protein levels of the lens with reference to Lowry et al. (33).

Statistical analyses

Statistical analysis of biochemical results was performed via GraphPad Prism 6.0 (GraphPad Software, San Diego, California, USA). The values were expressed as means ± standard deviation (SD). The results were evaluated using an unpaired t-test and analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. The value of $p<0.05$ was considered statistically significant.

RESULTS

The lens GSH levels are shown in Figure 1. VPA administration decreased GSH levels in the control group in a

significant manner (p<0.01). ALA significantly reversed this level in the VPA group (p<0.01) (Figure 1).

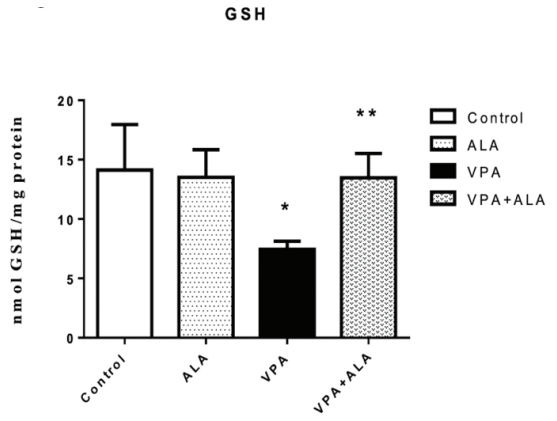

Figure 1: The lens GSH levels of control and experimental groups

GSH: reduced glutathione, ALA: alpha lipoic acid, VPA: valproic acid, Control group: received corn oil, ALA group: received alpha lipoic acid (50 mg per kg), VPA group: administered VPA (0.5 g per kg), VPA+ALA group: received ALA (50 mg per kg) 60 minutes prior to the VPA administration (0.5 g per kg). Each column represents the mean±SD. *p<0.01 vs control group; **p<0.01 vs VPA group.

The lens LPO levels are presented in Figure 2. According to the results, LPO levels of the VPA group were found to be increased significantly as compared to the control group (p<0.0001). Administration of ALA significantly decreased this level in the VPA group, (p<0.0001) (Figure 2).

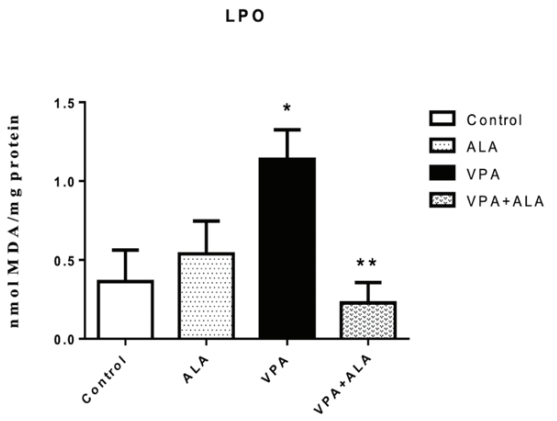

Figure 2: The lens LPO levels of the control and experimental groups

LPO: lipid peroxidation, ALA: alpha lipoic acid, VPA: valproic acid, Control group: received corn oil, ALA group: received alpha lipoic acid (50 mg per kg), VPA group: administered VPA (0.5 g per kg), VPA+ALA group: received ALA (50 mg per kg) 60 minutes prior to the VPA administration (0.5 g per kg). Each column represents the mean±SD. *p<0.0001 vs control group; **p<0.0001 vs VPA group.

The lens PC levels are given in Figure 3. VPA significantly increased the PC levels of the control groups (p<0.01). In the VPA+ALA group, this level was reversed in a significant manner as compared to the VPA group (p<0.0001) (Figure 3).

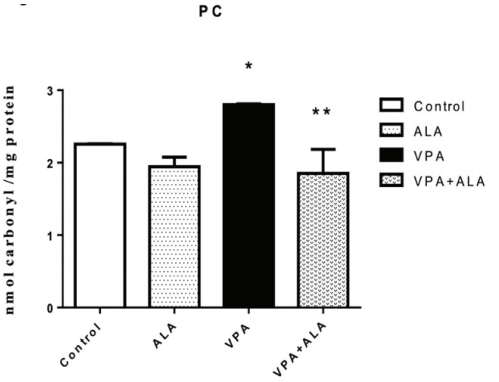

Figure 3: The PC levels of the control and experimental groups

PC: protein carbonyl, ALA: alpha lipoic acid, VPA: valproic acid, Control group: received corn oil, ALA group: received alpha lipoic acid (50 mg per kg), VPA group: administered VPA (0.5 g per kg), VPA+ALA group: received ALA (50 mg per kg) 60 minutes prior to the VPA administration (0.5 g per kg). Each column represents the mean±SD. *p<0.01 vs control group, **p<0.0001 vs VPA group.

The lens GST activities can be seen in Figure 4. ALA significantly decreased GST activities of the control group (p<0.001). Administration of VPA resulted in a diminishment in GST activities in the control group which was statistically significant (p<0.0001). In the VPA+ALA group, this activity was reversed as compared to the control group (p<0.0001) (Figure 4).

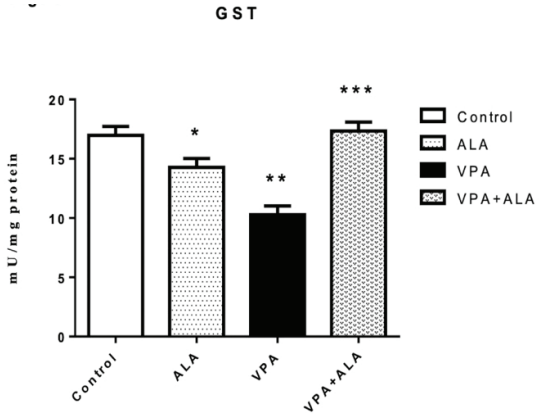

Figure 4: The GST activities of the control and experimental groups

GST: glutathione-S-transferase, ALA: alpha lipoic acid, VPA: valproic acid; Control group: received corn oil, ALA group: received alpha lipoic acid (50 mg per kg), VPA group: administered VPA (0.5 g per kg), VPA+ALA group: received ALA (50 mg per kg) 60 minutes prior to the VPA administration (0.5 g per kg). Each column represents the mean±SD. *p<0.001 vs control group; **p<0.0001 vs control group; ***p<0.0001 vs VPA group.

The lens SOD activities are shown in Figure 5. An elevation in SOD activities was observed in the VPA group as compared to the control group ($p<0.01$). Administration of ALA reversed the activities of the VPA group which was statistically significant ($p<0.001$) (Figure 5).

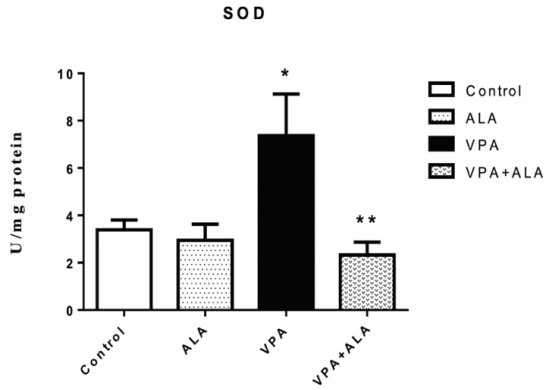

Figure 5: The SOD activities of the control and experimental groups
SOD: superoxide dismutase, ALA: alpha lipoic acid, VPA: valproic acid, Control group: received corn oil, ALA group: received alpha lipoic acid (50 mg per kg), VPA group: administered VPA (0.5 g per kg), VPA+ALA group: received ALA (50 mg per kg) 60 minutes prior to the VPA administration (0.5 g per kg). Each column represents the mean±SD. *p<0.01 vs control group; **p<0.001 vs VPA group.

The lens GPx activities are shown in Figure 6. According to the results, a significant elevation was observed after ALA and VPA administration to the control group ($p<0.01$, $p<0.001$) respectively. These activities were significantly reversed in the VPA+ALA group as compared to the VPA group ($p<0.0001$) (Figure 6).

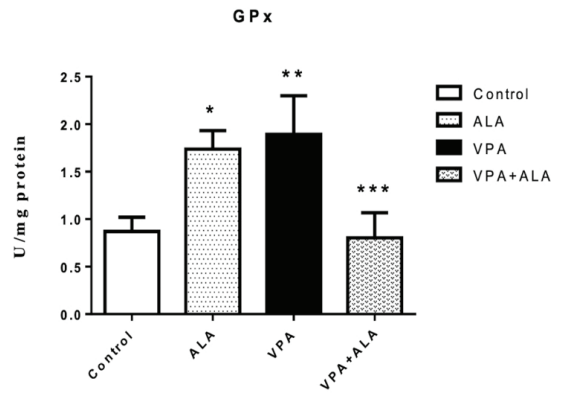

Figure 6: The GPx activities of the control and experimental groups
GPx: glutathione peroxidase, ALA: alpha lipoic acid, VPA: valproic acid, Control group: received corn oil, ALA group: received alpha lipoic acid (50 mg per kg), VPA group: administered VPA (0.5 g per kg), VPA+ALA group: received ALA (50 mg per kg) 60 minutes prior to the VPA administration (0.5 g per kg). Each column represents the mean±SD. *p<0.01 vs control group; **p<0.001 vs control group; ***p<0.0001 vs VPA group.

The lens GR activities are seen in Figure 7. The GR activities of the VPA group were recorded as being elevated as compared to the control group ($p<0.05$). These activities were found to be significantly decreased after ALA administration to the VPA group ($p<0.05$) (Figure 7).

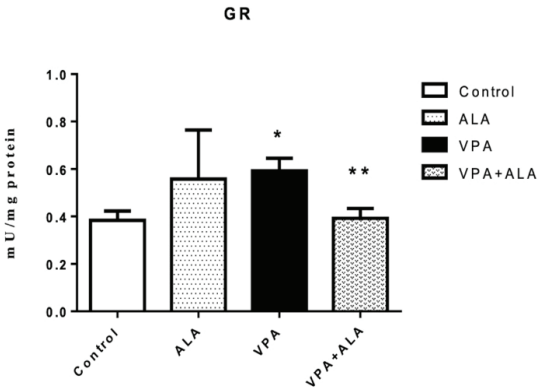

Figure 7: The GR activities of the control and experimental groups
GR: glutathione reductase, ALA: alpha lipoic acid, VPA: valproic acid, Control group: received corn oil, ALA group: received alpha lipoic acid (50 mg per kg), VPA group: administered VPA (0.5 g per kg), VPA+ALA group: received ALA (50 mg per kg) 60 minutes prior to the VPA administration (0.5 g per kg). Each column represents the mean±SD. *p<0.05 vs control group; **p<0.05 vs VPA group.

The lens AR activities are presented in Figure 8. AR activities were increased after VPA administration to the control group which was statistically significant ($p<0.0001$). ALA significantly reversed these activities in the VPA group ($p<0.05$) (Figure 8).

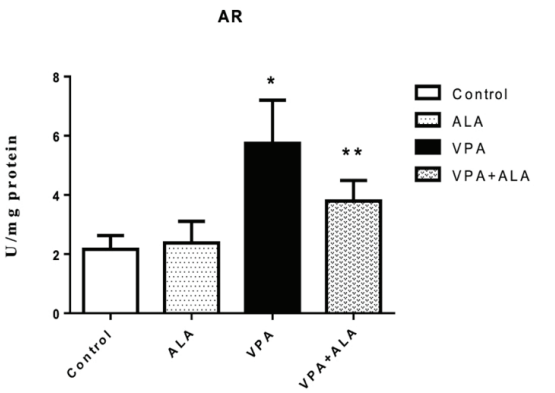

Figure 8: The AR activities of the control and experimental groups
AR: aldose reductase, ALA: alpha lipoic acid, VPA: valproic acid; Control group: received corn oil, ALA group: received alpha lipoic acid (50 mg per kg), VPA group: administered VPA (0.5 g per kg), VPA+ALA group: received ALA (50 mg per kg) 60 minutes prior to the VPA administration (0.5 g per kg). Each column represents the mean±SD. *p<0.0001 vs control group; **p<0.05 vs VPA group.

The lens SDH activities are given in Figure 9. After VPA administration, SDH increased in the control group which was statistically significant ($p<0.01$). ALA insignificantly decreased these activities of the VPA group.

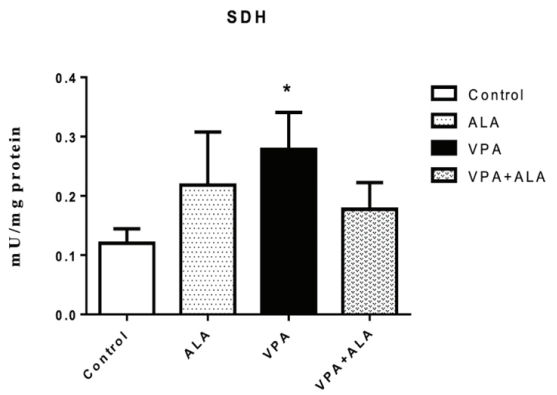

Figure 9: The SDH activities of the control and experimental groups
SDH: sorbitol dehydrogenase, ALA: alpha lipoic acid, VPA: valproic acid, Control group: received corn oil, ALA group: received alpha lipoic acid (50 mg per kg), VPA group: administered VPA (0.5 g per kg), VPA+ALA group: received ALA (50 mg per kg) 60 minutes prior to the VPA administration (0.5 g per kg). Each column represents the mean±SD. *$p<0.01$ vs control group.

DISCUSSION

The main function of the lens is the transmission and focusing of light onto the retina. This high-speed transmission requires the highest protein levels (34). Due to the function of the eye being related to light, all layers of an eye, including the lens, are open to being affected by the presence of oxygen (35). This means that the lens layer is susceptible to damage induced by reactive oxygen species (ROS) . In addition, VPA has been widely described as having a triggering effect on free radicals in many tissues including lens tissue (5, 22, 36).

The lens has been documented as evolving an anaerobic biological system by consisting of glutathione at mM levels (37). The protective effect of GSH is essential for GPx activity, and in general for protection of protein structure against oxidant molecules (38). When GSH is oxidized by the presence of free radicals, it will transform into oxidized glutathione (GSSG). Their transformation should be kept under strict regulation because lens membrane is capable of permitting GSSG while it is impermeable to GSH (38). Besides, lens crystallin consists of many cysteine residues and Lou reported that their connection with GSH might be initially protective (39). In this study, GSH levels decreased in the lenses of VPA treated rats, probably due to the free radical triggering effect of VPA. Reduced form of ALA (dihydrolipoic acid) helps convert cystine to cysteine. The decreased GSH level of the VPA group may have been increased in the VPA+ALA group owing to effects of ALA on the conversion of cystine to cysteine (a substrate for GSH synthesis) (40, 41).

Moreover, a diminishing tendency of GSH levels via VPA affected the membrane stability and protein structure by increasing LPO and PC levels. This is because amino acids like cysteine and tyrosine and other proteins are responsible for the prevention of excess oxygen derived metabolites (38). In addition, VPA has been referred to as increasing LPO and PC levels in the lens by affecting membrane and protein composite (22). ALA reversed these levels in VPA administered rats. In addition to the radical scavenging activity of ALA, the ameliorative property of ALA has been described by Neal et al. as having an elevator effect on cystine levels which can be related to protein stabilization (23).

GSH-based redox systems in the lens carry a great importance due to high protein levels and a necessity of their thiol group stabilization (42). Likewise, GST, GPx, GR and SOD are important antioxidant enzymes in metabolism and, according to research , lens tissue hosts GPx and SOD at a very high level (43). VPA has been reported as having a lowering effect on mitochondrial membrane potential and oxygen levels and an elevating effect on cell death and depletion of ATP levels which in turn has resulted in an increase in ROS levels (44). In our study, we found elevated SOD, GPx and GR activities in VPA treated rat lenses, but GST activities were diminished. Increased SOD and GPx reveals the increased levels of superoxide anion and hydrogen peroxide levels and increased GR activity means the regeneration of GPx. Decreased GST activity is associated with a decrease in GSH concentration. This is usually due to increased levels of free radicals.The positive effect of ALA may have occurred due to the ability of a reduced form of ALA (dihydrolipoic acid, DHLA) to scavenge free radicals such as superoxide and hydroxyl. More so, DHLA participates in the recycling of GSH from GSSG (45). The present findings support these approaches.

The NADPH dependent enzymes, AR and SDH, are important markers of the polyol pathway. In this case, glucose gains importance by being reduced to sorbitol by AR, and in turn, sorbitol is catalyzed to fructose by SDH. Aldose reductase (AR) has a low affinity for glucose compared to hexokinase. However, AR activity does increase in lens tissue in cases where blood glucose levels rise (such as in diabetes) (46, 47). Chateauvieux et al. mentioned in their review publications that VPA had the effect of changing fasting glucose levels in young children/teenagers (48). In the light of this , we determined that VPA increased AR and SDH activities in the lens tissues of rats. ALA decreased these activities. ALA is known as having an ability

to regulate energy metabolism and glucose regulation through its cofactor property on pyruvate / α-ketoglutarate dehydrogenase in the Krebs cycle (49). ALA may be preferred due to this positive and regulative effect in diabetic conditions. In addition, ALA is reported to increase glucose uptake by insulin-sensitive/resistant muscles, which is related to it triggering glucose transporters activities of plasma membranes in the presence of glucose (50).

CONCLUSION

Based on these results, we can assume that ALA protects lens tissue by using it's antioxidant and ameliorative the effect which decreases oxidative stress on lens tissues which were affected by the VPA administration.

Ethics Committee Approval: This study was approved by Marmara University Animal Experiments Local Ethics Committe (Date: 23.03.2015, No: 34.mar).

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- R.Y., İ.B.T., Y.Ö.; Data Acquisition- Y.Ö., İ.B.T.; Data Analysis/Interpretation- R.Y., İ.B.T., Y.Ö.; Drafting Manuscript- R.Y. İ.B.T.; Critical Revision of Manuscript- R.Y.; Final Approval and Accountability- R.Y., İ.B.T.; Material or Technical Support- R.Y., İ.B.T., Y.Ö.; Supervision- R.Y.

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

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RESEARCH / ARAŞTIRMA
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ASSOCIATION BETWEEN VITAMIN D AND URIC ACID AMONG NORTH CYPRUS ADULTS: FIRST PRELIMINARY REPORT

KUZEY KIBRIS YETİŞKİNLERİNDE D VİTAMİNİ VE ÜRİK ASİT ARASINDAKİ İLİŞKİ: BİRİNCİ ÖN HAZIRLIK RAPORU

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ABSTRACT

Objective: The aim of the study is to evaluate the association between serum uric acid (SUA) and 25-OH Vitamin D (25-OH-VIT D). There is no data about the association between hyperuricemia and 25-OH-VIT D deficiency for North Cyprus.

Material and Methods: A cross-sectional study was performed on 356 adults aged between 18-75 years in the North Cyprus. Patients socio-demographic information, clinical and biochemical characteristics, eating habits, and lifestyle choices were recorded. Biochemical parameters were evaluated in all patients. Data were analysed by Chi-square test, Student's t test, and ANOVA, as appropriate. Also, the Gamma correlation coefficient was calculated for the relationship between 25-OH Vitamin D groups and the other variables.

Results: The prevalence of 25-OH-VIT D deficiency was 55.9% among the patients (12.86±4.63 ng/mL), the prevalence of 25-OH-VIT D insufficiency (23.44±2.38 ng/mL) was 27%, and 25-OH-VIT D levels were sufficient in only 17.1% of the patients (36.01±5.83 ng/mL). The ANOVA results indicated statistically significant differences SUA for both male and female patients between the 25-OH-VIT D groups. According to the Gamma correlation coefficient values, 25-OH Vitamin D levels were significantly negatively correlated with SUA.

Conclusion: In our study indicated a high prevalence of 25-OH-VIT D deficiency and insufficiency in North Cyprus adults and we have found a significant association between SUA levels and 25-OH-VIT D. As a result, both vitamin D supplementation and uric acid-lowering therapies are important in protecting patients against future atherosclerotic diseases.

Keywords: North Cyprus, Vitamin D, uric acid, prevalence, atherosclerosis

ÖZET

Amaç: Kuzey Kıbrıs için hiperürisemi ile 25-OH-VIT D eksikliği arasındaki ilişki hakkında veri yoktur. Bu çalışmanın amacı serum ürik asit (SUA) ile 25-OH-VIT D arasındaki ilişkiyi değerlendirmektir.

Gereç ve Yöntem: Kuzey Kıbrıs'ta yaşları 18-75 arasında değişen 356 yetişkin üzerinde kesitsel bir çalışma yapılmıştır. Hastaların sosyodemografik bilgileri, klinik ve biyokimyasal özellikleri, beslenme alışkanlıkları ve yaşam tarzı tercihleri kaydedildi. Tüm hastalarda biyokimyasal parametreler değerlendirildi. Veriler Ki-kare testi, Student t testi ve uygun şekilde ANOVA ile analiz edildi. Ayrıca 25-OH Vitamin D grupları ile diğer değişkenler arasındaki ilişki için Gama korelasyon katsayısı hesaplandı.

Bulgular: Hastalar arasında 25-OH-VIT D eksikliği prevalansı %55,9 (12,86±4,63 ng/mL), 25-OH-VIT D yetmezliği prevalansı (23,44±2,38 ng/mL) %27 idi ve 25-OH-VIT D düzeyleri hastaların sadece %17,1'inde yeterliydi (36,01±5,83 ng/mL). ANOVA sonuçları, 25-OH-VIT D grupları arasında hem erkek hem de kadın hastalar için istatistiksel olarak anlamlı SUA farklılıkları gösterdi. Gama korelasyon katsayısı değerlerine göre, 25-OH Vitamin D düzeyleri SUA ile anlamlı olarak negatif korelasyon gösterdi.

Sonuç: Çalışmamızda Kuzey Kıbrıs erişkinlerinde 25-OH-VIT D eksikliği ve yetersizliği prevalansının yüksek olduğu gösterildi ve SUA düzeyleri ile 25-OH-VIT D arasında anlamlı bir ilişki bulundu. Sonuç olarak, hem D vitamini takviyesi hem de ürikasit düşürücü tedaviler, hastaları gelecekteki aterosklerotik hastalıklara karşı korumada önemlidir.

Anahtar Kelimeler: Kuzey Kıbrıs, Vitamin D, ürik asit, prevalans, ateroskleroz

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INTRODUCTION

Hyperuricemia is an important marker increasing the risk of cardiovascular disease (CVD) and is a common health problem in all societies. As it is known, uric acid is the end product of purine metabolism, and produced in the intestines, liver, muscles and is excreted by the kidney. Elevated serum uric acid (SUA) level was found associated with diabetes mellitus (DM), hypertension (HT), low-grade inflammation, metabolic syndrome (MS), insulin resistance, stroke and significant cardiovascular events (1, 2). Additionally, hyperuricemia can lead to gouty arthritis, nephrolithiasis, and chronic renal disease if it is constant (3).

Vitamin D (VIT D) deficiency is now recognized as a worldwide epidemic and an increasingly important public health issue. It has many significant functions in extraskeletal tissues as well as the musculoskeletal system (4). Although VIT D has been defined a fat soluble vitamin in bone mineralization, it is rather a hormone since it can be synthesized in the body. It is a secosteroid which is widely known as the sun hormone (5, 6). It is estimated that more than one billion people in the world have VIT D deficiency (7). There are many studies supporting the relationship between hyperuricemia and VIT D deficiency, as well as unclear literature data (7-12). It is thought that SUA can reduce the conversion of 25-hydroxyvitamin D (25-OHD) to 1,25-di hydroxyvitamin D (1,25-$(OH)_2$D) active VIT D form by suppressing the 1-alpha-hydroxylase enzyme (3, 13).

Therefore, the study aims to determine the possible relationship between 25-OH-VIT D insufficiency-deficienc and hyperuricemia, and to evaluate clinical-biochemical characteristics in adults aged 18-75 years inNorth Cyprus.

MATERIAL AND METHODS

Subjects and design

This research was a cross-sectional study that was conducted in the Near East University Hospital. The records of 356 patients who presented to the Near University Hospital Internal Medicine Outpatient Clinic between the dates of June 2020 to June 2021 were retrospectively reviewed after obtaining the approval of the Near East University Hospital Ethics Committee (Date: 29.04.2021, No: YDU/2021/90-1327). Exclusion criteria included being under 18 years of age, pregnant, breastfeeding, having psychiatric disease, receiving calcium and/or VIT D supplements, use of uric acid-lowering medication, skeletal musculoskeletal disease, gastrointestinal system disease, chronic renal disease, and individuals using drugs that interact with the VIT D metabolism (such as anticonvulsants, antibiotics, glucocorticoids, and bile acid binding).

In this study, we used the Turkey Endocrinology and Metabolism Society for Metabolic Bone Diseases Diagnosis and Treatment 2020 guidelines as reference values for 25-OH-VIT D levels: >30 ng/mL sufficient, 20-30 ng/mL VIT D insufficiency, <20 ng/mL VIT D deficiency (14). According to the 25-OH vitamin D levels, patients were divided into three groups as the deficient vitamin D group D1 (25-OH vitamin D<=20 ng/mL), insufficient vitamin D group D2 "20 ng/mL<25-OH vitamin D<30 ng/mL" (D2) and sufficient vitamin D group D3 "25-OH vitamin D>=30 ng/mL".

Laboratory studies and calculation

Venous blood samples were collected in the morning under fasting conditions at the time of admission. Waist circumference (WC), Systolic blood pressure, Diastolic blood pressure, body mass index (BMI), fasting blood sugar, total cholesterol level (TCL), High density lipoprotein (HDL)-cholesterol, Low density lipoprotein (LDL)-cholesterol, triglycerides, uric acid, hs-CRP (high sensitivity C-reactive protein), homeostatic model assessment–insulin resistance (HOMA-IR) and 25-OH-VIT D vitamin levels of the patients were recorded. Also, the individuals participating in our study were asked whether they used VIT D, their age, gender, height, weight measurements, smoking and alcohol use, skin color, sunscreen use, sun exposure, nutritional status, supplemental vitamins or minerals use, and fish oil use.

Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) values of 2.5 and above were considered as "insulin resistance exists". Low density lipoprotein (LDL) values of 130 mg/dL and above were considered as "high LDL". Patients with total cholesterol (TC) levels of 200 mg/dL and above were considered as "high cholesterol". Fasting SUA levels were measured by uricase-based commercial kit (Architect i2000, Abbott, USA). In addition, individuals with SUA levels of 6 mg/dL and above in women and SUA 7mg/dL and above in men were considered as "high uric acid". High-sensitivity C-reactive protein (hs-CRP) values of 0.5 mg/dL and above were accepted as "hs-CRP high". In addition, individuals with a body mass index (BMI) of 30 kg/cm^2 and above were classified as obese.

Statistical analysis

The IBM Statistical Package for Social Sciences (SPSS) for Windows version 23.0 was used to perform statistical analysis. Continuous variables obtained were presented with mean±standard deviation and median (min, max) values. In addition, the chi-square (test was used to determine statistical significance for the categorical variables. The degree and direction of the relationship between 25-OH-VIT D groups and other variables were evaluated with the Gamma correlation coefficient (G). The One-way ANOVA test was applied to investigate whether significant differences between the mean levels of clinical and biochemical characteristics existed with-

in Vitamin D groups in cases where the homogeneity of variances was met according to the Levene test results. For the post hoc test, the Scheffe test was used if the variances were equal. According to the Levene test results, in cases where the homogeneity of variances was not met, Welch's Anova test was applied to investigate whether there were significant differences between the mean levels of clinical and biochemical characteristics within Vitamin D groups. For the post hoc test, the Games-Howell test was used because the variances were not equal. Also, the Student's t test was used to assess the significance of differences between the mean value of 25-OH-VIT D levels within two groups. A level of 5% was set as the level for statistical significance for all two tailed statistical tests.

RESULTS

A total of 356 patients, comprising 265 women (74.4%) and 91 men (25.6%), were included in the study. The mean age of the patients was 50.89±18.45 years. The mean age of the male patients (54.03±18.19 years) was statistically higher than the female patients (49.83±18.45 years) (p=0.000). In addition, 40.7% of the patients in the research group had obesity, while 59.3% of them did not have obesity.

The mean serum 25-OH-VIT D level of the study group was found to be 19.68±9.73 ng/mL. The serum 25-OH-VIT D levels of the female patients were significantly lower than the male patients (18.96±9.08 ng/mL; 21.79±11.23 ng/mL; p=0.017). The prevalence of VIT D deficiency was 55.9% (12.86±4.63), the prevalence of VIT D insufficiency (23.44±2.38) was 27%, and VIT D levels were sufficient in only 17.1% of the patients (36.01±5.83).

Table 1 presents the descriptive statistics of the clinical parameters and biochemical characteristics; age, BMI, WC, Systolic blood pressure, Diastolic blood pressure, fasting glucose, TCL, HDL-cholesterol, LDL-cholesterol, triglycerides, uric acid, hs-CRP, HOMA-IR and 25-OH Vitamin D levels of the patients according to the VIT D groups (Table 1). Table 2 and Table 3 present the ANOVA results of the patients' biochemical and clinical characteristics levels according to the 25-OH-VIT D groups. The results of the Welch's ANOVA test showed that there was a statistically significant difference for the mean value of SUA between the 25-OH-VIT D groups for both male and female patients, respectively.

The Games-Howell post hoc test for significance revealed that the mean value of SUA for the male patients with 25-OH-VIT D deficiency (9.144.45) was significantly greater than the mean value of SUA for the male patients with 25-OH-VIT D insufficiency (6.02 and with sufficient 25-OH-VIT D (4.611.68)), respectively. The mean value of SUA for the 25-OH-VIT D insufficient group was also

Table 1: Descriptive statistics of clinical and biochemical characteristics

		Mean	Median
Age (years)	D1	47.58	46.00
	D2	54.10	56.50
	D3	56.40	56.00
BMI (kg/m²)	D1	30.12	30.11
	D2	27.85	27.00
	D3	25.17	25.00
WC (cm)	D1	105.67 (male) 94.48 (female)	106.00 93.55
	D2	99.81 (male) 90.94 female)	98 92
	D3	90.65 (male) 81.75 (female)	90 80
Systolic blood pressure (mmHg)	D1	121.99	120.00
	D2	122.48	120.00
	D3	122.36	120.00
Diastolic blood pressure (mmHg)	D1	72.70	70.00
	D2	71.60	70.00
	D3	72.10	70.00
Fasting blood sugar	D1	110,17	98.00
	D2	104,15	97.00
	D3	104.27	95.00
HOMA-IR	D1	2.91	2.15
	D2	2.61	2.07
	D3	2.74	1.94
hs-CRP (mg/dL)	D1	1.04	0.65
	D2	0.50	0.20
	D3	0.30	0.10
Uric acid (mg/dL)	D1	9.14 (male) 6.17 (female)	7.8 5.5
	D2	6.02 (male) 4.61 (female)	5.85 4.3
	D3	4.61 (male) 4.02 (female)	3.70 3.8
Total Cholesterol (mg/dL)	D1	208.89	192.30
	D2	204.48	203.40
	D3	194.93	196.60
HDL	D1	38.83 (male) 45.65 (female)	37.00 46
(mg/dL)	D2	40.86 (male) 50.91 (female)	40.50 51
	D3	47.35 (male) 53.04 (female)	47 54

Table 1: Continue

		Mean	Median
LDL (mg/dL)	D1	128.90	126.00
	D2	130.81	126.50
	D3	120.66	117.00
Triglycerides (mg/dL)	D1	144.67	136.00
	D2	127.07	112.00
	D3	110.63	102
25-OH Vitamin-D (ng/mL)	D1	12.86	13.10
	D2	23.44	23.40
	D3	36.01	34.00

BMI: Body Mass Index, WC: Waist circumference, HOMA-IR: Homeostatic model assessment for insülin resistance, hs-CRP: High-sensitive C-reactive protein, HDL: high density lipoprotein, LDL: Low density lipoprotein

Table 2: Welch's ANOVA results according to vitamin D groups

	Statistic	df1	df2	Sig.	Significant difference
Uric acid (male)	11.769	2	55.994	**0.000**	D1-D2 D1-D3 D2-D3
Uric acid (female)	13.708	2	146.707	**0.000**	D1-D2 D1-D3
BMI	34.068	2	186.344	**0.000**	D1-D2 D1-D3 D2-D3
hs-CRP	12.802	2	216.497	**0.000**	D1-D2 D1-D3
Triglyceride	11.168	2	184.955	**0.000**	D1-D3
WC (male)	21.201	2	49.804	**0.000**	D1-D3 D2-D3
WC (female)	24.842	2	126.630	**0.000**	D1-D3 D2-D3

BMI: Body Mass Index, WC: Waist circumference, hs-CRP; High-sensitive C-reactive protein

significantly greater than the 25-OH-VIT D -sufficient group. For female patients, the mean value of SUA for the 25-OH-VIT D -deficient group (6.17 4.36) was significantly greater than the mean value of SUA for the male patients with insufficient 25-OH-VIT D (4.61 and with sufficient 25-OHVIT D (4.021.19)), respectively.

25-OH-VIT D levels were not significantly related with systolic blood pressure, diastolic blood pressure, fasting blood sugar, LDL, total cholesterol level, HOMA-IR, gender, smoking, alcohol use, use of sun cream and use of milk products. VIT D levels were significantly negatively correlated with BMI, WC, hs-CRP, USA and triglycerides.

25-OH-VIT D levels were significantly positively correlated with HDL, use of fish oil, use of vitamin and mineral supplements, use of salmon, use of tuna fish, use of egg yolk and sun benefit status. There was a significant relationship between 25-OH-VIT D deficiency or insufficiency accompanying high SUA levels. Patients with a SUA value of 6 mg/dL and above in women, SUA value 7 mg/dL and above in men and also 25-OH-VIT D deficiency or insufficiency comprised 25% (89/356) of all patients. A total of 69 people with high SUA levels along with 25-OH-VIT D deficiency were observed.

DISCUSSION

The strength of our current study is that it was planned to include all of the patients' metabolic status in a well characterized cohort of patients whose 25-OH-VIT D levels were measured. This is the first reported study to determine the 25-OH-VIT D status in the adult population of North Cyprus.

The relationship between 25-OH-VIT D and SUA levels has been extensively investigated in the literature. Important results have been reported showing that 25-OH-VIT D deficiency can increase the cardiovascular mortality (14-16). Also, according to previous estimates, the frequency of metabolic syndrome is significantly high in North Cyprus due to sedentary lifestyles and eating habits. In a study which was done among the adult health check-up subjects in the Near East University in North Cyprus, considering the NCEP ATP-III criteria for determining metabolic syndrome, the prevalence of Met S was found at 37.0 % generally, 22.4 % for women and 51.5 % for men respectively (17,18). The prevalence of 25-OH-VIT D deficiency was found to be 55.9%, the prevalence of 25-OH-VIT D insufficiency was 27%, and 25-OH-VIT D levels were sufficient in only 17.1% of the patients in our study. In addition, we have observed that 25-OH-VIT D deficiency was quite common in North Cyprus due to possible inaccuracies in lifestyle perceptions in the society. We found low 25-OH-VIT D levels in both genders; however 25-OH-VIT D levels were lower in females than males. Patients with a SUA value of 6 mg/dL and above in women, SUA 7 mg/dL and above in men and also 25-OH-VIT D deficiency or insufficiency comprised 25% (89/356) of all patients. A total of 69 people with high SUA levels along with 25-OH-VIT D deficiency were observed. There was a significant relationship between 25-OH-VIT D D deficiency or insufficiency accompanying high SUA levels in this study.

Low circulating 25-OH-VIT D levels have been associated with a wide variety of disease states and physiological

Table 3: Chi-square analysis results between VIT D groups and clinical-biochemical characteristics, skin color, sunscreen use, sun exposure, nutritional status and habits

n		D1		D2		D3		p
		%	n	%	n	%		
Gender	**Female**	150	75.4%	74	77.1%	41	67.2%	0.347
	Male	49	24.6%	22	22.9%	20	32.80%	
BMI	**<30 (kg/m²)**	95	47.7%	59	61.5%	57	93.4%	**0.000**
	(kg/m²)	104	52.3%	37	38.5%	4	6.6%	
WC	**<88 cm (female) <102 cm (male)**	80	40.2%	40	41.7%	49	80.3%	**0.000**
	88 cm (female) 102 cm (male)	119	59.8%	56	58.3%	12	19.7%	
Systolic blood pressure	**<130 mmHg**	114	57.9%	56	58.3%	36	59.0%	0.987
	mmHg	83	42.1%	40	41.7%	25	41.0%	
Diastolic blood pressure	**<85 mmHg**	164	82.4%	87	90.6%	48	78.7%	0.091
	mmHg	35	17.6%	9	9.4%	13	21.3%%	
Fasting blood sugar	**<100 mg/dL**	108	54.3%	57	59.4%	30	49.2%	0.447
	mg/dL	91	45.7%	39	40.6%	31	50.8%	
HOMA-IR	**<2.5**	130	65.3%	67	69.8%	40	65.6%	0.736
	2.5	69	34.7%	29	30.2%	21	34.4%	
hs-CRP	**<0.5 mg/dL**	113	56.8%	74	77.1%	51	83.6%	**0.000**
	0.5mg/dL	86	43.2%	22	22.9%	10	16.4%	
Uric acid	**<7 mg/dL (male) <6 mg/dL (female)**	130	65.3%	76	79.2%	55	90.2%	**0.000**
	7 mg/dL (male) 6 mg/dL (female)	69	34.7%	20	20.8%	6	9.8%	
Total cholesterol	**<200 mg/dL**	117	58.8%	43	44.8%	34	55.7%	0.076
	200 mg/dL	82	41.2%	53	55.2%	27	44.3%	
LDL-C	**<130 mg/dL**	130	65.3%	54	56.3%	36	59.0%	0.286
	130 mg/dL	69	34.7%	42	43.8%	25	41.0%	
HDL-C	**<40 mg/dL (male) <50 mg/dL (female)**	125	62.8%	38	39.6%	19	31.1%	**0.000**
	40 mg/dL (male) 50 mg/dL (female)	74	37.2%	58	60.4%	42	68.9%	
Triglycerides	**<150 mg/dL**	103	51.8%	63	65.6%	48	78.7%	**0.000**
	150 mg/dL	96	48.2%	33	34.4%	13	21.3%	
Metabolic syndrome	**No**	94	47.2%	68	70.8%	57	93.4%	**0.000**
	Yes	105	52.8%	28	29.2%	4	6.6%	
Smoking	**No**	148	74.4%	72	75.0%	49	80.3%	0.448
	Yes	39	19.6%	14	14.6%	9	14.8%	
	Previously used	12	6.0%	10	10.4%	3	7.0%	
Alcohol	**No**	172	86.4%	87	90.6%	49	80.3%	0.183
	Yes	27	13.6%	9	9.4%	12	19.7%	

Table 3: Continue

n		D1		D2		D3		p
		%	n	%	n	%		
Skin colour	**Light**	110	55.3%	63	65.6%	53	86.9%	**0.000**
	Dark	89	44.7%	33	34.4%	8	13.1%	
Use of sun-cream	**No**	137	68.8%	66	68.8%	48	78.7%	0.306
	Yes	62	31.2%	30	31.3%	13	21.3%	
Use of fish oil	**No**	196	98.5%	87	90.6%	52	85.2%	**0.000**
	Yes	3	1.5%	9	9.4%	9	14.8%	
Use of vitamin and mineral supplements	**No**	180	90.5%	72	75.0%	41	67.2%	**0.000**
	Yes	19	9.5%	24	25.0%	20	32.8%	
Use of milk products	**No**	11	5.5%	7	7.3%	5	8.2%	0.704
	Yes	188	94.5%	89	92.7%	56	91.9%	
Use of salmon	**No**	192	96.5%	86	89.6%	52	85.2%	**0.001**
	Yes	7	3.5%	10	10.4%	9	14.8%	
Use of tuna fish	**No**	136	68.3%	63	65.6%	11	18.0%	**0.000**
	Yes	63	31.7%	33	34.4%	50	82.0%	
Use of egg yolk	**No**	28	14.1%	13	13.7%	1	1.6%	**0.025**
	Yes	171	21.1%	82	20.0%	60	14.8%	
Sun benefit status	**Not direct exposure to the sun**	170	14.1%	78	13.7%	38	1.6%	**0.000**
	Direct exposure to the sun	29	21.1%	18	20.0%	23	14.8%	

BMI: Body Mass Index; WC: Waist circumference, HOMA-IR: Homeostatic model assessment for insülin resistance, hs-CRP: High-sensitive C-reactive protein, HDL-C: high density lipoprotein chlosterol, LDL-C: Low density lipoprotein chlosterol

disorders. In observational studies, lower 25-OH-VIT D levels have been associated with higher blood pressure levels, type 2 diabetes, stroke, myocardial infarction, and heart failure (19,20). When relationship between 25-OH-VIT D and the metabolic syndrome components were examined, a statistically significant difference was found between high TG, WC and BMI, and between low HDL and low 25-OH-VIT D levels. It has been suggested that SUA levels are associated with metabolic syndrome risk factors. Our previous study indicated that the mean concentration of SUA in patients with Met S was higher than that in non-affected people (19). Additionally, Abbasian et al. showed that SUA levels were significantly higher in Met S patients (20). In our study, SUA levels were found to be high in people with low 25-OH-VIT D. Also, a statistically significant difference was found between the groups with both deficiency and insufficiency of 25-OH-VIT D and hyperuricemia. Although the relationship between low 25-OH-VIT D D and hyperuricemia can be explained indirectly by the presence of metabolic syndrome, larger case-control studies with more patients are needed to explain the direct relationship in the pathogenesis. Due to the fact that hyperuricemia is a factor that increases the risk of metabolic syndrome, it is recommended that it should be included in routine tests (21).

Observational data suggest that low 25-OH-VIT D levels are associated with an increased risk of hypertension, but randomized controlled trials have shown minimal support for the beneficial effect of 25-OH-VIT D supplementation on blood pressure (22, 23). In other words, conflicting results were found in various meta-analyses examining observational and randomized controlled studies. However, another meta-analysis that included individual data suggested that 25-OH-VIT D supplementation can be considered in the blood, and even individuals with low baseline 25-OH-VIT D levels or high baseline blood pressure reported no significant effect on their blood pressure. Similarly, limited effects of VIT D supplementation on glycaemic control were observed in participants with type 2 diabetes (23). No significant relationship was found between patients with low 25-OH-VIT D and HT, DM, impaired fasting glucose or insulin resistance in our study. There was also no significant relationship between low 25-OH-VIT D and

LDL cholesterol. However, a statistically significant relationship was observed between patients with low 25-OH-VIT D levels and low HDL and high TG in our study.

Many studies have shown that poor 25-OH-VIT D status is associated with obesity (24). Although the reason for this relationship is not known exactly, it seems that extra body fat may decrease its bioavailability with 25-OH-VIT D sequestration (25). In our study, individuals with low 25-OH-VIT D levels had a BMI >30, and we found a statistically significant relationship between increased waist circumference and those with a 25-OH-VIT D level of <20 ng/mL, which is consistent with the literature.

The amount of exposure to sunlight through the skin, the type and style of clothing, the use of sunscreen, latitude, institutionalization (pollution), condition and skin colour can all alter the levels of 25-OH-VIT D in the blood. Therefore, diet, lifestyle, working environment, cultural habits, and demographics all play a role and these should be taken into account when planning food supplement programs and public health policies (26). Despite the fact that the sun generally shines for 11 months of the year in North Cyprus, people avoid sun exposure due to the scorching heat. This may explain some of the reasons for VIT D deficiency due to the lack of exposure.

In our study, 25-OH-VIT D levels of those who regularly consumed fish oil, salmon, tuna fish and egg yolks were better than of those who did not consume these sources in their diet. A statistically significant negative relationship was found between those with 25-OH-VIT D deficiency and fish oil, fish and egg consumption. In other combined vitamin-mineral supplement areas, vitamin D levels were within normal limits (>30). Valer-Martinez et al. reported that 25-OH-VIT D status showed a strong inverse association with subcutaneous adipose tissue and visceral adiposity, measured in different ways with other body measurements (i.e., body mass index) (27). Studies have shown that hypovitaminosis D has a potential inverse association with insulin resistance and cardiovascular risk factors (28).

The main limitations of this study were the limited number of participants and lifestyle characteristics. The population of the study was not comprised of randomly selected individuals, but patients who were admitted to the hospital. Genetic analysis could not be performed to determine possible genetic risk factors for VIT D deficiency due to the high cost. Another limitation of this study is that it is not known whether SUA levels will change positively with VIT D supplementation. Therefore, further research is needed with a larger cohort.

CONCLUSION

Our study results are the principal data for North Cyprus and our study showed that there is a significant inverse relationship between 25-OH-VIT D deficiency and SUA in North Cyprus. The significant relationship between 25-OH-VIT D and the metabolic status components such as HDL, triglyceride levels, SUA and WC should also be emphasized. Attention should be paid to the importance of 25-OH-VIT D in providing metabolic control in primary care. As a result, it is possible to find a solution to the problem with treatment protocols in accordance with the recommended guidelines. Improvement of both 25-OH-VIT D levels and hyperuricemia may reduce the risk of possible DM, coronary artery disease, and HT in the future. For this purpose, VIT D supplementation is as important as changing the lifestyle and eating habits.

Ethics Committee Approval: This study was approved by Near East University Scientific Research Ethics Evaluation Board (Date: 29.04.2021, No: YDU/2021/90-1327).

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THE IMPACT OF AGE, SEX, AND SOCIO-ECONOMIC STATUS ON SERUM VITAMIN B12, FOLATE CONCENTRATION AND IRON STATUS IN TURKISH CHILDREN: A CROSS-SECTIONAL STUDY

TÜRK ÇOCUKLARINDA YAŞ, CİNSİYET VE SOSYO-EKONOMİK DURUMUNUN SERUM DEMİR, VİTAMİN B-12 VE FOLİK ASİT KONSANTRASYONLARI ÜZERİNDEKİ ETKİLERİ: KESİTSEL BİR ÇALIŞMA

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ABSTRACT

Objective: The aim of this study is to evaluate the effects of age, sex, and socio-economic status (SES) on concentrations of vitamin B12 (vit-B12), folate, iron, and ferritin through the pediatric age span, and to show the relationship of these nutrients with blood count parameters such as hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), and red cell distribution (RDW).

Material and Method: The study comprised of 30,504 venous blood samples (54.5% of girls and 45.5% of boys). The study group was stratified; group I: 1-6 years (n=3,870), group II: 7-12 years (n=11,019), and group III: 13-18 years (n=15,615). Serum vit-B12, folate, ferritin, iron and total iron-binding capacity (TIBC) were measured using a Beckman Coulter DXI 800, and the blood count was analyzed using a Sysmex XE 2100 analyzer.

Results: The Hb, Hct, MCV, and RDW levels were significantly different between the age groups of boys and girls ($p<0.001$, for all). Hb and Hct were the highest in boys aged 13-18 years, and higher than those of girls in the same age group ($p<0.001$). The MCV and RDW were also significantly different across the age groups ($p<0.001$). The iron deficiency prevalences were 12.3% and 4.2% for the girls and boys respectively. Serum folate and vit-B12 showed decrement with age across the age groups. The prevalences of vit-B12 deficiency were 27.1% and 28.3% and 6.0% and 6.8% for folate deficiency for girls and boys. Iron, TIBC,

ÖZET

Amaç: Bu çalışmanın amacı, pediyatrik yaş gruplarında; yaş, cinsiyet ve sosyo-ekonomik durumun (SED) B12 vitamini, folik asit, demir ve ferritin konsantrasyonlarına etkisi, bu parametrelerin hemoglobin (Hb), hematokrit (Hct), ortalama eritrosit hacmi (MCV) , RDW gibi kan sayımı parametreleri arasındaki ilişkisinin saptanmasıdır.

Gereç ve Yöntem: Çalışma 30504 venöz kan örneğinden oluştu (%54,5 kız, %45,5 erkek). Çalışma grupları; grup I: 1-6 yaş (n=3.870), grup II: 7-12 yaş (n=11.019), grup III: 13-18 yaş (n=15.615). Serum B12 vitamini, folik asit, ferritin, demir, demir bağlama kapasitesi (TDBK), Beckman Coulter'ın DXI 800 analizörü kullanılarak, tam kan sayımı Sysmex XE 2100 hematoloji cihazında çalışıldı.

Bulgular: Kız ve erkek çocuklarının yaş grupları arasında Hb, Hct, MCV, RDW düzeyleri anlamlı olarak farklı bulundu (tüm parametreler için, $p<0.001$). Hb ve Hct düzeyleri 13-18 yaş grubu erkek çocuklarında en yüksek seviyede olup, aynı yaş grubundaki kız çocuklarına göre anlamlı olarak daha yüksekti ($p<0.001$). Demir eksikliği prevalansı, kız çocuklarda %12,3, erkek çocuklarda %4,2 idi. Serum folik asit ve B12 vitamin konsantrasyonlarında yaş grupları içerisinde yaşla azalma görüldü (sırasıyla $r=-0,480$, $p<0,001$; $r=-0,377$, $p<0,001$). Kız ve erkek çocuklarında, B12 vitamin eksikliği prevalansı sırasıyla %27,1 ve %28,3 iken, folik asit eksikliği prevalansı %6,0 ve %6,8 olarak bulundu. Demir, TDBK

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and ferritin levels were significantly different between the high and medium SES.

Conclusion: The results of this study are important in that the monitorization of ferritin, vit-B12, and folate levels greatly contribute to clinical practice because of the roles of vit-B12, folate, and iron in mental, emotional, and metabolic development. However, there is a need for larger and multicenter studies that can represent the nutrients of Turkish children and adolescents nationwide.

Keywords: Serum vitamin B-12, folic acid, iron status, iron deficiency prevalence, B-12 and folate deficiency prevalence

ve ferritin düzeylerinde yüksek ve orta SED arasında anlamlı farklılıklar görüldü (p<0,001, tüm parametreler için).

Sonuç: Bu çalışmanın sonuçları, ferritin, B12 vitamini, folik asit düzeylerinin mental, emosyonal ve metabolik gelişimlerindeki rolleri nedeniyle çocukluk çağı ve ergenlikte izlenmesinin klinik uygulamaya katkıları açısından önem taşımaktadır. Ancak, bu nutrientleri ülke genelinde Türk çocuk ve ergenlerindeki temsil edebilecek daha geniş ve çok merkezli çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Serum B12 vitamini, folik asit, demir eksikliği prevalansı, B12 vitamin eksikliği prevalansı, folik asit eksikliği prevalansı

INTRODUCTION

Adequate levels of micronutrients are essential for any stage of life, especially in childhood and adolescence. Folate and vitamin B12 (vit-B12) are crucial micronutrients for growth and development. They have indisputable roles in DNA synthesis, optimal hematopoiesis, and neuronal function. Studies have shown the association between inadequate levels of these vitamins with developmental problems and neurologic deficits (1, 2).

Micronutrient deficiency, especially iron, vit-B12, and folate, are the main causes of anemia. Vit-B12 participates in the methylation reaction of homocysteine (Hcy) and the conversion of methylmalonyl-CoA to succinyl-CoA. With inadequate levels of Vit-B12, the methylation reaction and the conversion of methyl-tetrafolate to its active form (tetrahydrofolate) are disrupted, resulting in functional folate insufficiency and macrocytic anemia. The intracellular and circulating levels of Hcy, which is an independent risk factor for atherosclerosis, are increased with a lack of vit-B12 and folate, and several potentially harmful effects of hyperHcy on the vascular endothelium and bone metabolism have been demonstrated (3, 4).

Vitamin B12 deficiency is common in developed countries, especially in vegetarians, alcoholics, and the geriatric population (1). Although some researchers have demonstrated the relationship between low folate and Vit-B12 levels and some chronic diseases in childhood, studies about the effects of low vit-B12 and folate on general health status in children and adolescents, and reference values are not certain, unlike in adults (5). National surveys investigating the prevalence of vit-B12 and folate deficiency in childhood and adolescence have shown different results in different parts of the world (6, 7). Thus, it should be noted that cultural and nutritional attitudes of populations in different parts of the world are influential on vit-B12 and folate concentrations.

Iron is an essential micronutritient for almost all living organisms with participating oxygen transport, deoxyribonucleic acid (DNA) synthesis, electron transport, and hematopoiesis, and is the most frequently seen nutritional deficiency in the world according to the World Health Organization (WHO), and its effects on the central nervous system, such as the metabolic function of the brain and roles in myelin synthesis, enhance its importance in general health (8). Cognitive, social, and emotional disturbances have been reported in adolescence due to iron deficiency (9). According to the recommendations of American Academy of Pediatrics, anemia screening in children is recommended to be performed with hemoglobin (Hb) measurement at the age of 1 year, because of the low sensitivity and specificity of Hb, the WHO recommends to use ferritin with Hb in the determination of iron deficiency (10).

In this cross-sectional study, our purpose was to evaluate the effects of age, sex, and socioeconomic status on concentrations of vit-B12, folate, iron, and ferritin through the pediatric age span, and to show the relationship of these nutrients with blood count parameters such as Hb, hematocrit (Hct), mean corpuscular volume (MCV), and red cell distribution (RDW).

MATERIALS AND METHOD

Study population

Thirty thousand five hundred and ninety-four venous blood samples from residual material of the Istanbul Public Health Laboratory workflow comprised our study group (54.5% of girls and 45.5% of boys; mean age: 12.1±4.3 years). The study group was stratified according to age groups; group I: 1-6 years (n=3,870), group II: 7-12 years (n=11,019), and group III: 13-18 years (n=15,615). Blood samples were collected into serum separator tubes (SST, Becton Dickinson, Plymouth, UK), centrifuged for 10 minutes at 2000 g and the supernatants were used for serum vit-B12, folate, ferritin, iron and total iron-binding capacity (TIBC) measurements. For the complete blood count (CBC), venous blood samples collected into K2–EDTA anti-coagulated tubes (Becton Dickinson, Plymouth, UK) were used, and Hb, red blood cells (RBC), MCV, RDW levels were measured. All the parameters were studied on the same day within three hours. Fasting was not required for these analyses because the majority of our study group was children.

Our study included healthy children and adolescents between 1 year to 18 years of age. Children with a history of acute or chronic illnesses or type 1 diabetes, celiac disease, renal or liver disease and those using any medications were excluded.

The current thresholds recommended by the WHO were used for serum ferritin, folate, and vit-B12, which are lower than 12 ng/mL, 4 ng/mL, and 148 pg/mL, respectively (10). Socioeconomic status was defined according to the classification of the Human Developmental Index, which includes the seven main indices (Governance, Social Inclusion, Economic Status, Education, Health, Social Life, Municipal Environmental Performance and Transportation) in 39 districts of Istanbul (11). The study group was stratified in three groups according to SES classification; group A; high human development, group B; medium development group C; low development group.

The study protocol was approved by the Ethics Committees of the Istanbul University, Istanbul Faculty of Medicine (Date: 25.2.2020, No: 332). Informed consent was received from the families of the participants.

Methodology

The CBC was analyzed using a Sysmex XE 2100 analyzer (Sysmex Corp. Kobe, Japan). Serum ferritin, folate, and vit-B12 levels were measured using a Beckman Coulter DXI 800 (Beckman Coulter, USA) with a sandwich immunoenzymatic method, and serum iron and TIBC were determined using an AU5800 analyzer with colorimetric method. The precision results for vit-B12, folate, and ferritin were 2.3%, 5.0%, and 4.5%, respectively. The coefficient of variation (CVs) values of the hematologic parameters were lower than 2.0%.

Statistical analysis

The data were analyzed using the SPSS 21 software (SPSS, Chicago, IL, USA). The results are expressed as mean ± standard deviation (SD). The normality of data distribution was evaluated using the Kolmogorov-Smirnov test. The Chi-square (χ^2), Kruskal–Wallis, and a post-hoc analysis using the Mann–Whitney U-test was used for the variables distribution that was not normally distributed, and a student-t test was used for the variables distribution that was normally distributed . Correlation analyses were performed using the Spearman test. Binary logistic regression was used to evaluate the variables affecting the risk of iron, vit-B12, and folate deficiencies. Statistical significance was defined as $p<0.05$.

RESULTS

Table 1 presents the descriptive characteristics of the study group. When the CBC parameters were compared in all age groups of the girls and boys, the Hb, Hct, MCV, and RDW levels were significantly different between the age groups; the Hb and Hct concentrations were the highest in boys aged 13-18 years ($p<0.001$). The Hb and Hct levels of boys aged 13-18 years were also significantly higher than those of girls in the same age group (14.5±1.2 vs. 12.6±1.2 g/dL and 43.0±3.1 vs. 38.4±2.9%, $p<0.001$ for both). The results of other CBC parameters, MCV, and RDW in 1-6 years, 7-12 years and 13-18 years age groups were significantly different in both sexes across the age groups of both sexes ($p<0.001$ for all).

The prevalence of iron deficiency was 12.3% and 4.2% for the girls and boys, respectively. When we investigated through the age groups, the prevalence was 17.3% and 4.6% for the 13-18 years' age group, 4.0% and 1.5% for the 7-12 years' age group, and 4.9% and 7.3% for the 1-6 years' age group, respectively. The prevalence of iron deficiency anemia in the 7-12 years' and 13-18 years' age groups were found significantly different between the boys and girls ($p=0.009$, $p<0.001$, respectively).

When the serum vit-B12, folate, iron, and ferritin levels were evaluated, the serum B12, folate, iron, and ferritin concentrations were significantly different through the age groups of girls and boys ($p<0.001$, for all). Serum iron, TIBC, and ferritin levels were also different between girls and boys in the 7-12 years' age group ($p=0.033$, $p=0.011$, $p<0.001$, respectively), and in the 13-18 years' age group ($p<0.001$, for all). However, the serum folate and vit-B12 concentrations showed a decrement across the age groups. Vit-B12 and folate levels were significantly lower in boys and girls aged 13-18 years compared with the other age groups ($p<0.001$, in all age groups)

The prevalence of serum vit-B12 deficiency in all study groups was 27.1% and 28.3% for the girls and boys. When we investigated through the age groups, the prevalence of B12 deficiency was 36.1% and 43.1% for the 13-18 years'; 15.3% and 16.5% for the 7-12 years' age and 7.2% and 9.7% for the 1-6 years' age group for girls and boys respectively, and the differences were significant between the age groups ($p<0.001$, for all). The prevalence of serum folate deficiency in all study groups was 6.0% and 6.8% for the girls and boys, respectively. Across the age groups; the prevalence of folate deficiency was 8.7% and 12.7% for the 13-18 years' age group, 0.7% and 1.3% for the 7-12 years' age group, and 0.9% and 0.4% for the 1-6 years' age group, and significant differences were obtained between the age groups ($p<0.001$, for all).

Ten thousand nine hundred six subjects (35.8) were in high SES, 16,641 (61,1%) was in medium SES, and 937 (3.1%) was low SES. When we compared the metabolite levels according to SES, in 1-6 years of age; the Vitamin B12 and MCV levels were different between high and medium SES ($p=0.004$, $p=0.019$, respectively), RDW was different between low and high SES ($p=0.034$). In 7-12 years of age, ferritin, Hgb, RDW and Vitamin B12 levels

Table 1. Complete blood counts (Hb, Hct, MCV, RDW), serum folate, vitamin B12 levels and the iron status parameters (iron, iron binding capacity, ferritin) in children and adolescence. Each value represents mean±SD

	Girls					Boys				
	Age range (years)	**Lower-Upper Limit**	**Mean±SD**	**Median (IQR)**	**Lower-Higher 95% CI**	**Age range (years)**	**Lower-Upper Limit**	**Mean±SD**	**Median (IQR)**	**Lower-Higher 95% CI**
Hemoglobin (g/dL)	1-6 n=751	32-213	12.3±0.9	12.3 (1.2)	12.2-12.3	1-6 n=759	7.7-15.2	12.2±1.0	12.3 (1.2)	12.2-12.3
	7-12 n=873	17-356	12.8±0.9	12.8 (1.1)	12.7-12.8	7-12 n=923	8.1-15.5	12.9±0.9	12.9 (1.2)	12.8-12.9
	13-18 n=2229	26-393	12.6±1.2*	12.7 (1.4)	12.6-12.7	13-18 n=1483	8.2-18.3	14.5±1.2	14.6 (1.5)	14.5-14.6
Hematocrit (%)	1-6 n=751	80-270	36.7±2.6	36.7 (3.2)	36.5-36.9	1-6 n=759	25-47.4	36.4±2.7	36.6 (3.6)	36.2-366
	7-12 n=873	85-306	38.4±2.4	38.4 (3.1)	38.2-38.5	7-12 n=923	27.9-45.8	38.4±2.4	38.4 (3.3)	38.2-38.5
	13-18 n=2229	81-330	38.4±2.9*	38.6 (3.5)	38.3-38.5	13-18 n=1483	29.2-52.4	43.0±3.1	43.3 (4.1)	42.9-43.2
MCV (fL)	1-6 n=751	36.6-193	77.4±4.9*	77.8 (5.7)	77-77.7	1-6 n=759	51.9-95.8	76.1±5.0	76.9 (5.5)	75.7-76.4
	7-12 n=873	24.4-233.6	79.6±4.6*	80 (5.4)	79.3-79.9	7-12 n=923	53.9-94.2	78.8±4.9	78.8 (5.2)	78.3-78.9
	13-18 n=2229	12.8-246	83.3±5.9*	84.1 (6.3)	83.1-83.6	13-18 n=1483	55.8-95.4	82.7±5.4	83.4 (5.9)	82.4-83
RDW (%)	1-6 n=751	31-100	13.8±1.1*	13.6 (1.1)	13.7-13.8	1-6 n=759	11.8-22.1	14.0±1.1	13.8 (1.0)	13.9-14
	7-12 n=873	27-98	13.6±0.9*	13.3 (0.9)	13.4-13.5	7-12 n=923	11.8-19.5	13.6±0.9	13.5 (1.0)	13.5-13.6
	13-18 n=2229	25-108	13.7±1.5*	13.4 (1.2)	13.7-13.1	13-18 n=1483	11.4-26.2	13.5±1.0	13.3 (0.9)	13.4-13.5
Iron (ng/mL)	1-6 n=891	4-200	67.7±33.6	65 (46)	65.5-69.9	1-6 n=112	4-257	68.1±34.2	65 (44)	65.9-70
	7-12 n=1317	1-218	73.7±33.4**	72 (44)	71.9-75.5	7-12 n=1057	3-215	71.7±33.8	67 (41)	69.8-73.5
	13-18 n=3394	1-455	70.5±40.3*	65 (54)	69.1-71.8	13-18 n=1329	7-302	90.2±40.6	87 (54)	88.4-92

Table 1. Continue

	Girls					Boys				
	Age range (years)	**Lower-Upper Limit**	**Mean±SD**	**Median (IQR)**	**Lower-Higher 95% CI**	**Age range (years)**	**Lower-Upper Limit**	**Mean±SD**	**Median (IQR)**	**Lower-Higher 95% CI**
Ferritin (ng/dL)	1-6 n=914	2.4-117.1	23.3±15.4	19.7 (17.1)	22.3-24.3	1-6 n=112	2.8-140.7	22.7±16.0	18.6 (15.2)	21.6-23.6
	7-12 n=1358	1.2-169.2	22.6±15.1*	19.1 (14.8)	21.8-23.4	7-12 n=1057	1.7-392.9	25.8±18.5	21.8 (16.7)	24.8-26.8
	13-18 n=3608	0.8-860.9	17.6±20.9*	13.4 (14.6)	16.9-18.3	13-18 n=1329	1.7-528	34.4±25.8	28.5 (25.9)	33.3-35.5
Iron Binding Capacity (µg/dL)	1-6 n=743	140-912	305.1±62.8	300 (70.5)	300.6-309.8	1-6 n=112	51-661	306.2±57.9	304 (73)	302.2-310.2
	7-12 n=1165	133-678	309.5±58.7**	307 (72)	306.1-312.8	7-12 n=1057	121-550	301.8±51.5	302 (68)	298.8-304.8
	13-18 n=3001	48-716	321.7±73.9*	316 (97)	319.1-324.3	13-18 n=1329	18-571	286.8±63.5	285 (81)	283.8-289.8
Folate (ng/mL)	1-6 n=234	3.9-23.5	11.2±4.1	10.5 (4.4)	10.6-11.7	1-6 n=112	3.8-23.8	11.5±4.4	11 (5.8)	11-12
	7-12 n=441	3.3-23.3	9.4±3.2	8.6 (4.1)	9.1-9.7	7-12 n=1057	2.4-23.3	9.5±3.4	8.9 (4.8)	9.2-9.8
	13-18 n=1313	1.9-21.8	6.9±2.6*	6.5 (3)	6.7-7	13-18 n=1329	1.3-19.9	6.4±2.5	5.9 (3.2)	6.3-6.6
Vitamine B12 (pg/mL)	1-6 n=948	67-948	291.5±126.9**	269 (150)	283.5-299.6	1-6 n=112	64-1038	279.5±130.0	250 (158)	271.6-287.5
	7-12 n=1493	53-844	237.9±101.8	218 (116)	232.8-243.1	7-12 n=1057	57-1449	246.3±116.6	226 (129)	240.4-252.2
	13-18 n=4081	51-1420	192.5±103.9*	170 (89)	189.3-195.7	13-18 n=1329	54-993	178.3±84.8	158.5 (84.3)	174.9-181.6

*: $p<0.001$, **: $p<0.05$

altered significantly, (p=0.029, p=0.001, p=0.002, p<001) between high and medium SES, and however the differences in Hgb and Vitamin B12 levels were also different between medium and low SES (p=0.020, p<0.001) together with Hct. However, beside Hgb, Vitamin B12 and ferritin (p=0.006, p=0.001 for Vit-B12 and ferritin), serum iron, TIBC levels showed a significant difference between high and medium SES (p<0.001 for both). The variables affecting the risk of iron and vit-B12 deficiencies were evaluated using binary logistic regression analysis and are presented in Table 2. Girls had a 2.8-fold increased higher risk compared with boys, and increased age was associated with a 2.0-fold higher risk associated with iron deficiency. For vit-B12 deficiency, boys had a 1.2-fold higher risk, and increased age was associated with a 1.8-fold higher risk in the 7-12 years' age group; however, the risk increased 5.9-fold in the 13-18 years' age group. For folate deficiency, boys had a 1.5-fold higher risk, and increased age was associated with a 20.7-fold higher risk in 13-18 years' age group; however, the risk was 1.8-fold for the 7-12 years' age group. No associations were obtained regarding SES and iron and folate deficiencies. Only lower SES was associated with a 1.4-1.8 times higher risk for vit-B12 deficiency.

Table 2: Evaluation of factors associated with the risk of iron deficiency anemia (IIA) and serum vit-B12 (IIB)

Table 2A: Predict independent variables for the iron deficiency anemia

						95% CI	
	B	**Wald**	**df**	**P**	**Odds ratio**	**Lower**	**Upper**
Age groups							
1-6 years (reference)							
7-12 years	-0.796	40.451	1	<0.001	0.451	0.353	0.577
13-18 years	0.742	78.341	1	<0.001	2.099	1.781	2.474
Gender							
Male (reference)							
Female	1.015	67.100	1	<0.001	2.759	2.164	3.518
Socioeconomic status							
A (reference)							
B	0.034	0.075	1	0.7840	1.035	0.81	1.32
C	0.091	0.157	1	0.6918	1.095	0.698	1.719

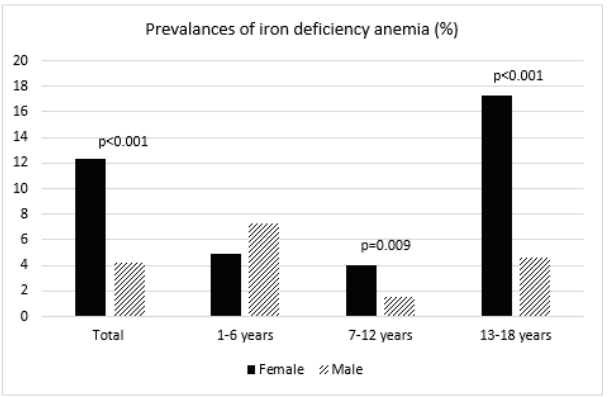
Table 2B: Predict independent variables for the vitamin B12 deficiency

						%95 CI	
	B	**Wald**	**df**	**P**	**Odds ratio**	**Lower**	**Upper**
Age groups							
1-6 years (reference)							
7-12 years	0.576	84.633	1	<0.001	1.779	1.574	2.012
13-18 years	1.774	950.833	1	<0.001	5.896	5.267	6.600
Gender							
Female (reference)							
Male	0.144	12.469	1	<0.001	1.154	1.06	1.25
Socioeconomic status							
A (reference)							
B	0.300	50.385	1	<0.001	1.350	1.243	1.467
C	0.561	19.371	1	<0.001	1.753	1.365	2.250

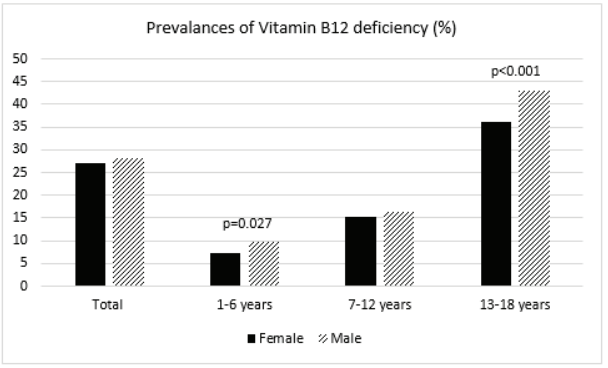
Hosmer&Lemeshow Model χ^2 (6)=36.822, p<0.001 for iron deficiency and χ^2 (6)=13.633, p=0.034 for vit-B12.
Variable(s) entered on step 1: Age groups, gender and socio-economic status.

Correlation analyses were conducted in the children and significant correlations between age and the following parameters: Hb (r=0.535, p<0.001), Hct (r=0.409, p<0.001), MCV (r=0.535, p<0.001), RDW (r=-0.142, p<0.001). Additionally, age was correlated with folate (r=-0.480, p<0.001), vit-B12 (r=-0.377, p<0.001). Significant correlations also existed between Hb and MCV (r=-0.393, p<0.001), Hb and RDW (r=-0.374, p<0.001).

(A)



(B)



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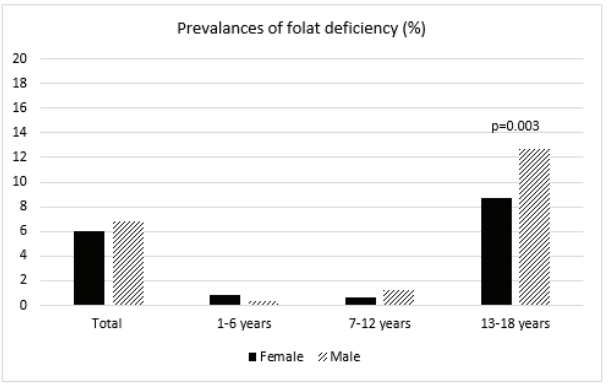

Figure 1: Prevalence of (A) iron deficiency (B) Vit-12 deficiency (C) folate deficiency in Turkish children aged 1-18 years. Mann Whitney U test was used for significance; p<0.05.

DISCUSSION

Folate and vit-B12 are essential throughout life, especially in childhood and adolescence because many metabolic, hormonal, and developmental changes take place physiologically during these periods. The reference values of childhood and adolescence due to hormonal changes, healthy growth, and metabolic roles in those periods are of great importance.

In this study, blood concentrations of serum vit-B12, folate, iron, and ferritin were measured to establish reference intervals in children and adolescents aged 1-18 years. Although ferritin concentrations are recommended as the single best indicator to evaluate the body's total iron status by the WHO, interfering factors such as inflammation that cause falsely increased ferritin results should not be neglected (12). In the present study, the Hb, Hct, MCV, and RDW levels were found significantly different across the age groups in girls and boys. The iron, TIBC, and ferritin levels were also lower in girls aged in 7-12 and 13-18 years' age groups compared with the same age groups of boys. Consequently, boys aged 13-18 years have higher Hb and Hct levels than girls, and the prevalence of iron deficiency in girls was higher than in boys in this age group (17.3% vs. 4.6%), However, the girls aged 7-12 years also had higher iron deficiency rates than the same age group of boys depending on the beginning of menarche. The ferritin levels of girls showed an approximately 22% decrease in the 13-18 years' age group compared with the 7-12 years' age group, and an approximately 30% increase was seen in boys between the same age groups. In Spanish and South-East Asian adolescents, a higher prevalence of iron deficiency was reported compared with our results (13, 14). Studies of Turkish children reported that the iron deficiency rate was between 15.7% and 28%, higher in girls than in boys; however, the rate was higher in boys compared with girls early in life (15, 16). According to our findings, the lower ferritin levels were obtained compared to the results of the National Health and Nutrition Examination Survey (NHANES), and Korean NHANES (2010-2012) (17, 18). In the Korean NHANES study, the higher ferritin results were reported in boys than girls in all age groups, in accordance with our findings (18). In general, adolescents have a greater risk of iron deficiency due to an increased requirement for iron, a poor dietary intake of iron, a high rate of infection, and malabsorption due to parasitosis of the gastrointestinal tract (19). The low ferritin levels in adolescent girls can be attributed to the onset of menarche, and dietary habits such as low food and/or energy intake compared with boys. When we evaluated the iron and ferritin levels depending on the SES levels, the iron and ferritin levels of children from the highly developed region were found significantly higher compared with those of medium developed regions.

According to our study results, we found significant age-related decreases in serum vit-B12 and folic acid levels, and these alterations in both vitamin levels may be associated with the higher requirements of vitamins due to the increased metabolic demands of growing and developing children (20). There are previous studies supporting the relation of accelerated metabolism with lower vit-B12 and folate levels and less intake of folate-rich foods in the adolescence period (5). Therefore, similar age and sex-related declines in vit-B12 and folate levels were also reported by a previous study (21). In our study, the serum vit-B12 levels of girls were higher than boys in the 1-6 years' and 13-18 years' age groups (5), however, the serum folate levels of girls were only higher in the 13-18 years' age group compared with their peer group. But the results of the other age groups were similar (5). Although the folate levels obtained in our study were in agreement with the results of previous studies, we obtained lower vit-B12 levels in Turkish children than their peers; higher serum vit-B12 levels have been reported, especially from European-based population studies (5, 22-24). However, similar serum vit-B12 results were reported in a study from Turkey (25).

Studies reported that folate deficiency due to a decreased intake was seen more frequently in developing and socioeconomically distressed countries (26). When we investigated the effect of SES on vit-B12 and folate concentrations, it was found that the risk of vit-B12 deficiency increased 1.8 times with decreased SES. However, no significant effects on folate levels were observed. Similarly, the serum vit-B12 levels of children from highly developed regions were significantly higher than those of middle and poorly developed regions.

According to our results, the prevalence of vit-B12 deficiency was 27.1% and 28.3%, and 6.0% and 6.8% for folate, girls and boys, respectively. The highest deficiency rate for vit-B12 was obtained in the 13-18 years' age group with 36.1% and 43.1% in girls and boys, and the highest rate for folate deficiency was 12.7% in boys during adolescence. Studies reported that the folate deficiency rate was 15% at cut-off levels of 4.5 ng/mL for adolescents (22, 23). Also, studies documented similar or higher deficiency rates for vit-B12 compared with our findings (24-27). In a cross-sectional study performed in urban and rural parts of Turkey in adults, the prevalence of vit-B12 and folate deficiency were reported as 27.4% and 4.6%, the highest being in the southern part and the lowest in western parts of Turkey (28). These changes were associated with socioeconomic factors and regional dietary habits in different regions of Turkey (28).

In some countries, mandatory folate and vit-B12 fortification is recommended to support the metabolic effects of these vitamins. Vit-12, Hcy and folate status are considered to be important for the follow-up of cardiovascular disease development and Hcy is an independent risk factor. In our previous study, strong associations were reported between vit-B12 and folate deficiencies and hypertension (28). In North America, studies showed that the fortification of flour with folic acid made a significant contribution to public health, and decreased mortality rates were reported due to complications of CVD, especially in subjects with preexisting CVD or renal disease (29-31).

This study has some limitations and strengths. The strengths of our study are its large sample size with almost equal numbers of both sexes in all age groups. Nevertheless, the lack of detailed information about subjects including the weight, height, and nutritional habits are the most important limitations of the study.

CONCLUSION

The results of this study are important in that the monitorization of serum ferritin, vit-B12, and folate levels greatly contribute to clinical practice because of the roles of vit-B12, folate, and iron in mental, emotional, and metabolic development. It is also of great value to monitor whether dietary intakes of vit-B12 and folate are at the required level to protect future generations from CVDs. In conclusion, mandatory fortification of cereals with iron, folic acid, and/or vit-B12 might be beneficial for public health in preventing the deficiencies of these nutrients and intercepting their adverse effects on general health.

Ethics Committee Approval: This study was approved by Istanbul University Istanbul Faculty of Medicine Clinical Research Ethics Committee (Date: 21.02.2020, No: 2020/311).

Peer Review: Externally peer-reviewed.

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

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

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CASE REPORT / OLGU SUNUMU
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TWO PATIENTS WITH TRANSIENT GLOBAL AMNESIA IN WHICH REPEATED DIFFUSION-WEIGHTED IMAGING REVEALED HIPPOCAMPAL HYPERINTENSITY: CASE REPORTS

TEKRARLANAN DİFÜZYON AĞIRLIKLI GÖRÜNTÜLEMEDE HİPOKAMPAL HİPERİNTENSİTE SAPTANAN İKİ GEÇİCİ GLOBAL AMNEZİ OLGUSU: OLGU SUNUMLARI

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ABSTRACT

An episode of temporary memory loss which is recovered within 24 hours is called transient global amnesia. The etiology and triggering factors are unclear but hippocampal lesions may contribute to this condition. Diffusion-weighted magnetic resonance imaging is used to detect these lesions. In this case report, two cases with transient global amnesia who had hippocampal lesions are presented.

Keywords: Transient global amnesia, hippocampus, diffusion weighted magnetic resonance image

ÖZET

Geçici global amnezi, 24 saat içinde iyileşen geçici hafıza kaybı ataklarıdır. Etyolojisi ve tetikleyici faktörleri aydınlanmış değildir ancak hipokampal lezyonlar geçici global amneziye katkıda bulunabilir. Bu lezyonları saptamak için difüzyon ağırlıklı manyetik rezonans görüntüleme kullanılır. Bu olgu sunumunda hipokampal lezyonu olan geçici global amnezili iki olgu sunuldu.

Anahtar Kelimeler: Geçici global amnezi, hipokampus, difüzyon ağırlıklı manyetik rezonans görüntüleme

INTRODUCTION

Transient global amnesia (TGA) is a condition that is characterized by sudden onset anterograde amnesia including partially retrograde features and episodes of memory loss that resolve within 24 hours. It is not accompanied by other neurological deficits such as weakness or numbness. Cranial imaging is used to support the diagnosis of TGA. Diffusion-weighted magnetic resonance imaging (DW-MRI) can usually help to detect the lesions in the brain. In early stages of TGA, a DW-MRI may not generate any findings so the MRI may need to be repeated (1). We present two patients in which DW-MRI revealed hippocampal lesions because the detection of these lesions changes the treatment strategies.

CASE PRESENTATIONS

CASE 1

A 50-year-old female patient was admitted complaining of the inability to remember the last 3 hours. The patient was cooperative. She followed all instructions given by examiner, but she was disorientated. She could not remember how or when she came to the hospital. She was constantly asking where she was. Aside from these issues, neurological examination was normal. There

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was no limb weakness or numbness**.** She was on medical treatment for asthma and hypothyroidism and had a history of one abortion. There was no pathology on the brain computerized tomography (CT) and a DW-MRI was performed within 3 hours after symptom onset. The patient was hospitalized with TGA as preliminary diagnosis. She completely recovered 7 hours after symptom onset. Electroencephalogram (EEG) was normal. A second DW-MRI repeated 48 hours after symptom onset revealed diffusion restriction in both hippocampi. Hypointensities were detected on the apparent diffusion coefficient (ADC) map consistent with areas of the DW-MRI hyperintensities (Figure 1). The appearance of suspected signal increase in the right mesencephalon on the DW-MRI was interpreted in favor of artifact because although the second DW-MRI in Figure 1 was performed at 48 hours after the symptom onset, when cytotoxic edema is greatest during this time period in ischemic brain tissue, there was no marked hypointensity on the ADC map. The patient also had no symptoms associated with brainstem involvement. Acetylsalicylic acid (ASA) and low molecular weight heparin were added to treatment. Electrocardiogram (ECG) and echocardiogram (ECHO) for the etiology of ischemic stroke was normal. No abnormality was detected in 24-hour Holter monitoring. No significant stenosis was detected on head and neck CT angiography. In the blood test, the low-density lipoprotein cholesterol (LDL-C) level was 84.5 mg/dl. Atorvastatin was added to treatment. For detailed investigation of a young patient with cerebrovascular disease, anti-beta 2 glycoprotein 1 (anti-B2GPI) and anti-cardiolipin (ACA) IgMs that had been tested twice with an interval of 12 weeks were detected as high titration. Anti-B2GPI was positive with the values of 63.37 and 30.75 U/ml (normal laboratory value: <20 U/ml) and ACA IgM was positive with the values of 10.24 and 19 U/ml, in the first and second tests, respectively. No abnormality was found in other hemogram and biochemistry parameters. No mutation was detected in thrombophilia genetic panel (TGP). We continued with antiaggregant therapy until the autoimmune tests and TGP were completed. We planned treatment with warfarin for secondary prophylaxis against risk of embolism recurrence after positive antibodies were detected but the patient did not come to the 3rd month follow up. There was no new neurological symptom within the 3 months.

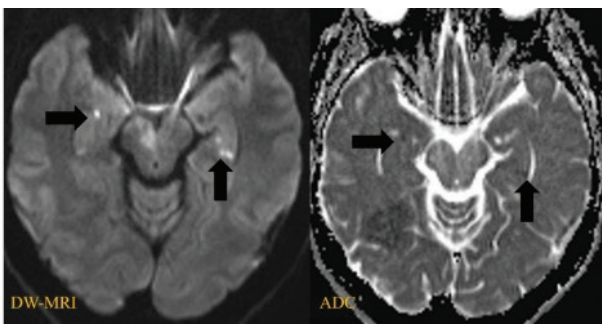

Figure 1: DW-MRI shows areas with restricted diffusion in both hippocampi (arrows). There are hypointensities in the corresponding areas on ADC (arrows)

CASE 2

A 56-year-old male patient was admitted with complaints of feeling unwell and not being able to remember the last few hours. When his relatives tried to tell him what had happened during the day, he could not remember. The patient was cooperative and had no lateralized motor impairment. Other neurological examination was normal. He did not have any history of chronic diseases. Brain CT and DW-MRI which were performed soon after symptom onset were normal. No abnormality was detected in EEG recording. In the left hippocampal gyrus, a DW-MRI repeated 12 hours after symptom onset showed millimetric area of restricted diffusion, with a corresponding low signal on ADC images (Figure 2). ASA was started. The patient completely recovered at 6 hours but did not recall this time period. In the evaluation of ECHO in terms of cardioembolism, mild mitral regurgitation was observed and no thrombus was detected. ECG was normal. No abnormality was detected in the 24-hour Holter monitoring. No stenosis was found on head and neck CT angiography. Atorvastatin was added to the treatment because the (LDL-C) level was 91 mg/dl. He had no new episodes at the 3rd month follow-up.

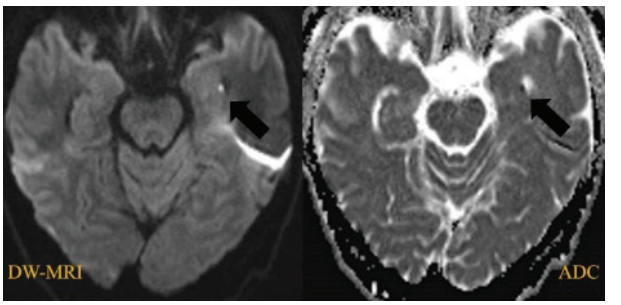

Figure 2: There is hippocampal restricted diffusion in the left hippocampus on DW-MRI and hypointensity is seen in the same area on the ADC map (arrows)

DISCUSSION

Transient global amnesia is a confusional clinical syndrome that resolves within 24 hours with anterograde amnesia and sometimes retrograde amnesia (2). Generally, patients tend to be disorientated and ask repetitive questions. The patient is awake during the attack and has no other neurological symptoms such as weakness or numbness. The patient can perform daily activities such as driving but is confused. Its incidence is higher in the 50-80 age range. Annual incidence of TGA is 3.4–

10.4/100,000 and in the over 50 population, this increases to 23.5/100.000 (4). Although clinical and imaging findings suggest hippocampal (especially cornu ammonis 1) dysfunction, the etiology of TGA is not clear. The diagnosis is made clinically according to the Hodges and Warlow criteria (3). It is suggested that a triggering situation including physical or psychological stress is usually detected before the disease onset, but the triggering factors are obscure. Migraine, focal ischemia, venous flow abnormalities and epileptic seizures have been suggested as factors that play a role in pathophysiology and differential diagnosis of TGA (5). A right hippocampal infarct was detected on DW-MRI in a case report with TGA (6) and a different study examining the retrospective data of 56 patients also suggested that TGA has a cerebrovascular background (7). It was stated in the same study that no relationship was found between migraine, epilepsy and TGA but another recent meta-analysis argued that there was a significant relationship between migraine and TGA (8). In our patients, epileptic amnesia was excluded because of the normal EEG recording and the absence of recurrent events in the follow-up. In our first patient with TGA, the hippocampal infarction was detected on the DW-MRI, and we detected APS in an investigation of etiologies of ischemic stroke. In 1998, the Sapporo criteria were established for APS (9). In 2006, the APS criteria were revised in the 11th International Antiphospholipid Symposium (10). Accordingly, while the clinical criteria remained the same, the laboratory criteria changed. Anti-B2GPI IgM and IgG were added to laboratory criteria. In our first patient, this diagnosis was considered due to the presence of a history of abortion, newly developed cerebrovascular ischemia, and positive test results for two different antiphospholipid antibodies (Anti-B2GPI and ACA IgMs). For secondary prophylaxis of APL, standard dose warfarin (INR: 2-3), low-dose ASA plus warfarin and high-intensity warfarin (INR: >3) have been recommended (11). We continued with antiaggregant therapy with ASA for the first patient until the autoimmune tests and TGP were concluded. We planned to start a standard dose of warfarin, but she did not come to her 3 months follow-up. We also investigated our second patient in terms of stroke etiology, but we could not find a cause and we continued his treatment with ASA. In one study, hippocampal hyperintensity was detected on the DW-MRI applied 24 and 36 hours later in 5 patients with TGA and it has been suggested that a DW-MRI performed at least 24 hours after symptom onset is important in supporting the diagnosis of TGA (1). In a meta-analysis, it was stated that the diagnostic yield of DW-MRI in TGA patients was 39% and a DWI performed between 24 and 96 hours after symptom onset showed higher diagnostic yield (12). In our patients, while the DW-MRI (3T MRI) within a short time (approximately 3 hours) after symptom onset was normal, a DW-MRI performed 48 hours later for the first patient and 12 hours later for the second patient showed hippocampal hyperintensity.

CONCLUSION

In this case report, two patients with TGA that had hippocampal hyperintensity on DW-MRI were presented. We think that repeating a DW-MRI in TGA cases increases the sensitivity for detecting lesions. Hippocampal lesions contribute to TGA. Detecting the lesion on the DW-MRI changes the treatment approach.

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LETTER TO THE EDITOR / EDİTÖRE MEKTUP
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THE EXPECTED SEROPROTECTION RATE AFTER THE SIXTH DOSE OF THE COVID-19 VACCINE: A NOTE FROM A CLINICAL MODEL ON KIDNEY TRANSPLANT RECIPIENTS

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There is still no cure for COVID-19, making it a global emergency (1). The best, greatest choice for management is vaccination (2). Traditionally, a complete vaccination requires two doses of the vaccine. Several experts advise taking an additional booster dose of the COVID-19 vaccine when there is a novel variant and a probable drop in antibodies after routine immunization (3, 4). The Australian Technical Advisory Group on Immunization (ATAGI) recommends a single booster dose for adults aged 16 and older, three months after the initial course (https://www.health.gov.au/initiatives-and-programs/covid-19-vaccines/advice-for-providers/clinical-guidance/clinical-recommendations). A second booster dose is advised four months following the first for patients who are at high risk of developing severe COVID-19.

For instance, the additional third and fourth doses of COVID-19 are already in use in Southeast Asia, where the background two dosage immunization has shown to be ineffective for immunocompromised groups (5, 6). The fifth dosage of the COVID vaccination is currently being administered with good results (7, 8). The local public health plan in some places, like Thailand, is to immunize people with a sixth dose that serves as a booster shot. The general indication for the sixth dose is usually for the immunocompromised cases such as renal transplant recipient.

The objective of the current study is to estimate the immunoprotection rate among a renal transplant recipient group following the sixth dose of a COVID-19 vaccination. Basic data on the protective effectiveness rates of various vaccine kinds are referred to as "primary data" (9). To create the model, the previously reported data on immunoprotection following five vaccinations is employed (9).

The basic concept is that different vaccinations have distinct immunogenicity mechanisms. The highest level of protective efficacy or effective immunity will be obtained once routine immunization is completed. The extra sixth dose will be employed to improve the performance of the immune system. The modeling approach employed in this research is the same as that used in a previous study to investigate the effect of a booster dose (10). After the sixth dose, the protective efficacy is likely to be treated as background protective efficacy for modeling. If delivered as a boosting dose, the sixth dose can increase the protected efficacy rate. However, it will not exceed the baseline protective effectiveness rate. The predicted protective efficacy rate after the sixth dose will be calculated as "background protective effect after the fifth dose + extra protection from the sixth dose."

Table 1 illustrates the estimated efficiency of the fifth vaccination dosage. The expected protective efficacy rates

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Table 1: Expected immunoprotection for renal transplant recipients after the sixth dose of the COVID-19 vaccine

The sixth dose vaccine		Protective efficacy rate (%)	
Type	Specific boosting* activity (%)	Background protective effect before the new additional dose** (%)	Expected protective efficacy rate after the sixth dose (%)
Inactivated	27	88.7	88.7
Viral vector	37	88.7	89
mRNA	24	88.7	94

*: Specific boosting activity refers to the ability to raise the protective efficacy rate of the first dose of vaccination when given as the second dose. **: Background protective effect prior to the new additional dose refers to the documented immunoprotection for renal transplant recipients after completing the fifth dose vaccination of that vaccine, and the statistics are based on publicly accessible data (8).

for the sixth dose vaccination using inactivated, viral vector, and mRNA type COVID-19 vaccine are 88.7%, 89.9%, and 94%, respectively, compared to the baseline protective level of 88.7%.

According to this study, the sixth dosage of vaccination still has a function in improving immunity, and all types of vaccines play a role, but an mRNA vaccine is the best vaccine for enhancing immunity. As a result, the sixth dosage of boosting immunization may be recommended for transplant recipients.

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