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ARAŞTIRMA MAKALELERİ

Iturin A'nın 6-OHDA ile indüklenmiş SH-SY5Y Parkinson Hastalığı hücre modeli üzerindeki anti-inflamatuar etkileri Pinar Altın Celik Muazzez Derya Andeden Hamiyet Donmez Altuntas	193
Depremle ilişkili periferik sinir yaranalmalarında nöropatik ağrı ile elektrodyagnostik testlerin korelasyonu Metin Balduz Halit Fidancı	201
Denetimli serbestlik tedbirli bulunan metamfetamin kullanıcılarının saldırganlık düzeylerinin incelenmesi Fatma Dilek Şeker Dilek Öker Keleş	209
Thiostrepton ile foxM1 inhibisyonu, DNA hasar yanıt genlerini baskılayarak meme kanseri hücrelerinin tedaviye duyarlılığını artırır Funda Demirtas Korkmaz Zekeriya Duzgun Fadime Mutlu İcduygu	223
GBA1 varyantları ve Parkinson Hastalığı: Klinik veriler ve literatür incelemesini birleştiren çift yaklaşım Sezin Canbek Goncagul Mert M. Fatih Gulseven M. Guney Senol	231
Yetişkin bireylerin hipertansiyondan korunmaya yönelik tutumları ve etkileyen faktörlerin incelenmesi Güler Kayabaşı Kadriye Sayın Kasar	243
PSA dansitesinin robotik radikal prostatektomi sonuçlarına etkisinin incelenmesi Alp Akyol Kasım Emre Ergun Serdar Kalemci Adnan Şimsir Fuat Kızılay	256
Kalp yetersizliği ve ileri yaş: Komorbiditeler, tedavi uygulamaları ve sağkalım analizi Emre Demir	262
Kronik karaciğer hastalıklarında FIB-4 ve APRI skorlarının klinik tanı ile uyumu Pinar Peker Ali İbrahim Hatemi	274
Ekstremita sinovyal sarkomlarının manyetik rezonans görüntüleme bulguları İpek Tamsel İlhan Hekimsoy	283
Anksiyete ve obsesif kompulsif bozuklukta siberkondri ve yaşam kalitesi: Bir üniversite kliniğinden kesitsel bir değerlendirme Selin Tanyeri Kayahan Özlem Kuman Tunçel Damla Işman Haznedaroglu Şebnem Pırıldar	289
Çoklu beyin metastazlarının stereotaktik radyoterapisi için tek izomerizli dinamik konformal ark tedavi (DCAT) ve volumetrik ayarlı ark tedavi (VMAT) planlama tekniklerinin dozimetrik karşılaştırılması Nezahat Olacak Sinan Hoca Mert Delikaya Yusuf Ziya Hazeral Deniz Yalman	300
Gebelik kayıplarının sık kullanılan hematolojik parametrelerle ilişkisi Atakan Turgutkaya Ayşe Hilal Eroğlu Küçükçiler Niyazi Alper Seyhan Ali Zahit Bolaman Zehra Manav Yiğit Gökay Bozkurt İrfan Yavaşoğlu	310
Diyabetik ayak enfeksiyonu ile takipli hastalarda HBV seroprevalansı Nazlıhan Yalçın Gökçen Ünal Kocabaş Tansu Yamazhan Hüsnü Pullukçu Anıl Murat Öztürk Meltem Işıkgöz Taşbakan	319
Preeklampsiyi öngörmeye biyokimyasal belirteçlerin rolü Ufuk Atılhan Begüm Ertan Mehmet Emre Peker Can Ata Onur Yavuz Hüseyin Ayтуğ Avcı Alper İleri Tefik Berk Bildacı Selçuk Erkinç	324
40 yaş üstü kadınlarda gebelik sonuçları ve riskler; Türkiye'nin en yüksek doğum oranına sahip bölgesinin retrospektif analizi Elif Ucar Melih Bestel	330
Multipl sklerozda huzursuz bacak sendromu: huzursuz bacak sendromu ile yorgunluk, yaşam kalitesi, gündüz uyku kalitesi, uyku kalitesi ve anksiyete-depresyon arasındaki ilişki Asiye Tuba Özdoğan Enes Aldemir Pervin Yeşiloğlu Vedat Çilingir	337
Beyin ve omurga cerrahisi geçiren hastalarda perioperatif koagülasyonun değerlendirilmesi Alev Atalay Abdurrahim Derbent Mustafa Nuri Deniz Sezgin Ulukaya Sacittin Taner Balcıoğlu	346
Spinal disrafizmi yeni doğanlarda cerrahi zamanlama ve gelişen hidrosefalide tedavi modalitesi Hakan Korkmaz Ali Özcan Binatlı	356
JZL184 iki farklı ARDS modelinde okludin ve TNF-α yoluyla akciğer hasarını modüle etmektedir Yusuf Elma Emine Yılmaz Can Meryem Akpolat Ferah Mete Keleş	363
Nefrolitiazis tanısında ve böbrek taşı boyut ölçümünde ultrasonografinin etkinliği ve doğruluğu İlhan Hekimsoy	374
Maternal trifenil fosfit maruziyetinin nörodavranışsal etkileri: valproik asit modeli ile karşılaştırmalı deneysel bir çalışma Petek Bilim Kübra Çelik Gurur Garip Ahmet Giray Yardımcı Buket Şura Yardımcı Meral Baka	381

OLGU SUNUMU

İntraoral lipom: 2 olgu sunumu Mehmet Ali Memişoğlu Mert Zeytinoğlu Ümran İncoğlu Ekin Çelikten	390
Epileptik nöbetler, beyin sapı tutulumu ve alt vücudun kas sertliği ile seyreden atipik Stiff Person Sendromu - bir olgu sunumu Göker Gören Abdullah Arı Hüseyin Nezi Özdemir Figen Gökçay	394
Sezaryen sonrası vezikouterin fistül olgusu: Olgu sunumu Mehmet Emre Peker Ufuk Atılhan Duygu Uçar Kartal	398

DERLEMELER

Periferik sinir sistemi yenilenmesinde kök hücre ve biyomateryallerin rolü Dila Hatun SAL Zeynep Simge YILMAZ Büşra HORUZ Aybike Berrak ÖZDEMİR Kubilay Doğan KILIÇ	402
Malign melanomda kanser kök hücreleri ve SHH yolağı: Terapötik çıkarımlar Berrin Özdiil Hüseyin Aktug	415



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İÇİNDEKİLER
CONTENTS

ARAŞTIRMA MAKALELERİ
RESEARCH ARTICLES

- Anti-inflammatory effects of iturin A on the 6-OHDA-induced SH-SY5Y cell model of Parkinson's Disease** 193
Iturin A'nın 6-OHDA ile indüklenmiş SH-SY5Y parkinson hastalığı hücre modeli üzerindeki anti-inflamatuar etkileri
Pinar Altın Celik Muazzez Derya Andeden Hamiyet Donmez Altuntas
- Corelation of the neuropathic pain and electrodiagnostic tests in earthquake-related peripheral nerve injuries** 201
Depremle ilişkili periferik sinir yaralanmalarında nöropatik ağrı ile elektrodyagnostik testlerin korelasyonu
Metin Balduz Halit Fidancı
- Investigation of aggression levels of methamphetamine users in probation** 209
Denetimli serbestlik tedbiri bulunan metamfetamin kullanıcılarının saldırganlık düzeylerinin incelenmesi
Fatma Dilek Şeker Dilek Öker Keleş
- FoxM1 inhibition by Thiostrepton downregulates DNA damage response genes, enhancing sensitivity of breast cancer cells to therapy** 223
Thiostrepton ile FoxM1 inhibisyonu, DNA hasar yanıt genlerini baskılayarak meme kanseri hücrelerinin tedaviye duyarlılığını artırır
Funda Demirtas Korkmaz Zekeriya Duzgun Fadime Mutlu Icduygu
- GBA1 variants and Parkinson's Disease: A dual approach combining clinical data and literature review** 231
GBA1 varyantları ve Parkinson Hastalığı: Klinik veriler ve literatür incelemesini birleştiren çift yaklaşım
Sezin Canbek Goncagul Mert M. Fatih Gulseven M. Guney Senol
- Yetişkin bireylerin hipertansiyondan korunmaya yönelik tutumları ve etkileyen faktörlerin incelenmesi** 243
Investigation of adults' attitudes towards protection from hypertension and affecting factors
Güler Kayabaşı Kadriye Sayın Kasar
- PSA dansitesinin robotik radikal prostatektomi sonuçlarına etkisinin incelenmesi** 256
Evaluation of the effect of PSA density on robotic radical prostatectomy outcomes
Alp Akyol Kasım Emre Ergun Serdar Kalemci Adnan Şimsir Fuat Kızılay

Kalp yetersizliđi ve ileri yař: Komorbiditeler, tedavi uygulamaları ve sađkalım analizi	262
<i>Heart failure in advanced age: Comorbidities, treatment approaches, and survival outcomes</i>	
Emre Demir	
Kronik karaciđer hastalıklarında FIB-4 ve APRI skorlarının klinik tanı ile uyumu	274
<i>Relationship with FIB-4, APRI scores and the clinical diagnosis in chronic liver diseases</i>	
Pınar Peker Ali İbrahim Hatemi	
Ekstremiteler sinovyal sarkomlarının manyetik rezonans görüntüleme bulguları	283
<i>Magnetic resonance imaging findings of extremity synovial sarcomas</i>	
İpek Tamsel İlhan Hekimsoy	
Cyberchondria and quality of life in anxiety and obsessive-compulsive disorder: A cross-sectional assessment from a tertiary center	289
<i>Anksiyete ve obsesif kompulsif bozuklukta siberkondri ve yaşam kalitesi: Bir üniversite kliniđinden kesitsel bir deđerlendirme</i>	
Selin Tanyeri Kayahan Özlem Kuman Tunçel Damla İşman Haznedaroğlu	
Şebnem Pırıldar	
Çoklu beyin metastazlarının stereotaktik radyoterapisi için tek izomerkezli dinamik konformal ark tedavi (DCAT) ve volumetrik ayarlı ark tedavi (VMAT) planlama tekniklerinin dozimetrik karşılaştırılması	300
<i>Dosimetric comparison of single isocenter dynamic conformal arc therapy (DCAT) and volumetric modulated arc therapy (VMAT) planning techniques for stereotactic radiotherapy of multiple brain metastases</i>	
Nezahat Olacak Sinan Hoca Mert Delikaya Yusuf Ziya Hazeral	
Deniz Yalman	
The relationship of pregnancy losses with frequently-used hematologic parameters	310
<i>Gebelik kayıplarının sık kullanılan hematolojik parametrelerle ilişkisi</i>	
Atakan Turgutkaya Ayşe Hilal Erođlu Küçük diler Niyazi Alper Seyhan	
Ali Zahit Bolaman Zehra Manav Yiđit Gökay Bozkurt İrfan Yavařođlu	
Diyabetik ayak enfeksiyonu ile takipli hastalarda HBV seroprevalansı	319
<i>HBV seroprevalence in patients with diabetic foot infection</i>	
Nazlıhan Yalçın Gökçen Ünal Kocabaş Tansu Yamazhan Hüsnü Pullukçu	
Anıl Murat Öztürk Meltem İşıkgöz Taşbakan	

The role of biochemical markers in predicting pre-eclampsia	324
<i>Preeklampsiyi öngörmeye biyokimyasal belirteçlerin rolü</i>	
Ufuk Atılhan	Begüm Ertan Mehmet Emre Peker Can Ata
Onur Yavuz	Hüseyin Aytağ Avşar Alper İleri Tevfik Berk Bildacı Selçuk Erkılınc
Pregnancy outcomes and risks in over 40; a retrospective analysis from Turkey's highest birth rate region	330
<i>40 yaş üstü kadınlarda gebelik sonuçları ve riskler; Türkiye'nin en yüksek doğum oranına sahip bölgesinin retrospektif analizi</i>	
Elif Ucar	Melih Bestel
Restless leg syndrome in multiple sclerosis: relationship with restless legs syndrome and fatigue, quality of life, daytime sleepiness, sleep quality and anxiety-depression	337
<i>Multipl sklerozda huzursuz bacak sendromu: huzursuz bacak sendromu ile yorgunluk, yaşam kalitesi, gündüz uyukluluğu, uyku kalitesi ve anksiyete-depresyon arasındaki ilişki</i>	
Asiye Tuba Özdoğar	Enes Aldemir Pervin Yeşiloğlu Vedat Çilingir
Evaluation of perioperative coagulation in patients undergoing brain and spine surgery	346
<i>Beyin ve omurga cerrahisi geçiren hastalarda perioperatif koagülasyonun değerlendirilmesi</i>	
Alev Atalay	Abdurrahim Derbent Mustafa Nuri Deniz Sezgin Ulukaya
Sacittin Taner Balcıoğlu	
Spinal disrafizmli yenidoğanlarda cerrahi zamanlama ve gelişen hidrosefalide tedavi modalitesi	356
<i>Surgical timing and treatment modalities for developing hydrocephalus in newborns with spinal dysraphism</i>	
Hakan Korkmaz	Ali Özcan Binatlı
JZL184 modulates lung injury via occludin and TNF-α in two distinct ARDS models	363
<i>JZL184 iki farklı ARDS modelinde okludin ve TNF-α yoluyla akciğer hasarını modüle etmektedir</i>	
Yusuf Elma	Emine Yılmaz Can Meryem Akpolat Ferah Mete Keçeci
Nefrolitiazis tanısında ve böbrek taşı boyut ölçümünde ultrasonografinin etkinliği ve doğruluğu	374
<i>The effectiveness and accuracy of ultrasound in the diagnosis of nephrolithiasis and measurement of kidney stone size</i>	
İlhan Hekimsoy	
Neurobehavioral effects of maternal triphenyl phosphite exposure: A comparative experimental study using the valproic acid model	381
<i>Maternal trifenil fosfit maruziyetinin nörodavranışsal etkileri: Valproik asit modeli ile karşılaştırmalı deneysel bir çalışma</i>	
Petek Bilim	Kübra Çelik Gurur Garip Ahmet Giray Yardımcı
Buket Şura Yardımcı	Meral Baka

OLGU SUNUMLARI

CASE REPORTS

İntraoral lipom: 2 olgu sunumu

390

Intraoral lipoma: 2 case report

Mehmet Ali Memişoğlu

Mert Zeytinoğlu

Ümran İnceoğlu

Ekin Çelikten

An atypical Stiff Person Syndrome patient presenting with epileptic seizures, brainstem involvement, and muscle stiffness of the lower body- a case report

394

Epileptik nöbetler, beyin sapı tutulumu ve alt vücutun kas sertliği ile seyreden atipik Stiff Person Sendromu - bir olgu sunumu

Göker Gören

Abdullah Arı

Hüseyin Nezih Özdemir

Figen Gökçay

Vesicouterine fistula after cesarean section: A case report

398

Sezaryen sonrası vezikouterin fistül olgusu: Olgu sunumu

Mehmet Emre Peker

Ufuk Atlıhan

Duygu Uçar Kartal

DERLEMELER

REVIEWS

The role of stem cells and biomaterials in peripheral nervous system regeneration

402

Periferik sinir sistemi yenilenmesinde kök hücre ve biyomateryallerin rolü

Dila Hatun SAL

Zeynep Simge YILMAZ

Büşra HORUZ

Aybike Berrak ÖZDEMİR

Kubilay Doğan KILIÇ

Cancer stem cells and the SHH pathway in malignant melanoma: Therapeutic implications

415

Malign melanomda kanser kök hücreleri ve SHH yolağı: Terapötik çıkarımlar

Berrin Ozdil

Huseyin Aktug

Anti-inflammatory effects of Iturin A on the 6-OHDA-induced SH-SY5Y cell model of Parkinson's Disease

Iturin A'nın 6-OHDA ile indüklenmiş SH-SY5Y parkinson hastalığı hücre modeli üzerindeki anti-inflamatuar etkileri

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ABSTRACT

Aim: Biosurfactants produced by bacteria are particularly important due to their antibacterial, antifungal, and antiviral properties, which offer therapeutic and biomedical potential. Nevertheless, no research has examined the impact of Bacillus-derived biosurfactant Iturin A on Parkinson's disease. This study examined the neuroprotection through cytokine modulation of Iturin A to formulate a novel therapeutic approach for Parkinson's disease.

Materials and Methods: The effect of Iturin A on cell viability in SH-SY5Y cells was evaluated by the [3-(4,5-Dimethyl thiazole-2) 2,5-diphenyltetrazolium bromide] method. As a result of this experiment, the non-toxic dose was determined by cell viability (%). To evaluate the neuroprotective effect of Iturin A, SH-SY5Y cells were treated with a non-toxic dose of Iturin A and then induced with 6-OHDA. After these treatments, cytokines (interleukin 6, transforming growth factor beta) and chemokine (interleukin 8) in SH-SY5Y cells were evaluated by ELISA kit. GraphPad Prism 9 was used to analyze all data.

Results: Using one-way ANOVA and Dunn's post hoc test, normally distributed data was tested for multiple comparisons. Treatment with Iturin A for 48 hours showed a neuroprotective effect on 6-hydroxy dopamine-induced SH-SY5Y cells by statistically significantly decreasing the expression levels of interleukin 6 ($P<0.001$), transforming growth factor beta ($P<0.001$), and interleukin 8 ($P<0.0001$).

Conclusion: Iturin A might offer new approaches to treating Parkinson's patients, and additional *in vivo* studies are necessary to confirm the *in vitro* results.

Keywords: Parkinson's Disease, Biosurfactants, Neuroinflammation, Cytokines

Öz

Amaç: Bakteriler tarafından üretilen biyosüpfaktanlar, terapötik ve biyomedikal potansiyel sunan antibakteriyel, antifungal ve antiviral özellikleri nedeniyle özellikle önemlidir. Bununla birlikte, hiçbir araştırma Bacillus türevi biyosüpfaktan İturin A'nın Parkinson hastalığı üzerindeki etkisini incelememiştir. Bu çalışma, Parkinson hastalığı için yeni bir terapötik yaklaşım formüle etmek amacıyla İturin A'nın sitokin aracılı nöroprotektif etkisini incelemiştir.

Gereç ve Yöntem: İturin A'nın SH-SY5Y hücrelerindeki hücre canlılığı üzerindeki etkisi MTT [3-(4,5-Dimetil tiyazol-2) 2,5-difeniltetrazolium bromür] yöntemi ile değerlendirilmiştir. MTT deneyinin sonucunda, toksik olmayan doz hücre canlılığı (%) ile belirlendi. İturin A'nın nöroprotektif etkisini değerlendirmek için, SH-SY5Y hücreleri toksik olmayan bir doz İturin A ile muamele edildi ve ardından 6-OHDA ile indüklendi. Bu muamelelerden sonra, SH-SY5Y hücrelerindeki sitokinler (IL-6, TGF- β) ve kemokin (IL-8) ELISA kiti ile değerlendirildi. Tüm verileri analiz etmek için GraphPad Prism 9 kullanıldı.

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Bulgular: İturin A ile 48 saatlik tedavi, sitokinlerin (IL-6, TGF- β) ve kemokin (IL-8) ifade seviyelerini değiştirerek 6-OHDA ile indüklenen SH-SY5Y hücrelerinde nöroprotektif bir etki gösterdi ($p<0,05$ - $p<0,0001$).

Sonuç: İturin A, Parkinson hastalarının tedavisinde yeni yaklaşımlar sunabilir ve *in vitro* sonuçları doğrulamak için ek *in vivo* çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: Sitokinler, İnflamasyon, İturin A, Nöroprotektif, SH-SY5Y

INTRODUCTION

Parkinson's disease (PD), characterized by motor and non-motor features, is a progressive neurological disease (1). Neuroinflammation mediated by microglia is a common feature in PD (2). Although the exact etiology of PD remains unknown, the loss of dopaminergic neurons in the substantia nigra (SN) is thought to be the underlying pathophysiology behind the gross motor symptoms of the disease. Recent studies have shown that PD is associated with a strong inflammatory response characterized by activation of microglia in the brain due to increased cytokines (3). PD leads to a significant increase in cytokines such as tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-2, IL-4, IL-6, transforming growth factor (TGF)- α , TGF- β 1 and TGF- β 2 (4). Although the factors underlying the neurodegenerative cascade, such as aging, environmental and genetic factors, are known the intricate processes responsible for the progression of neurodegeneration are still not fully understood (5). TGF- β , IL-6, and IL-8 are among the cytokines that may be used as PD biomarkers. These cytokines contribute significantly to neuroinflammatory characteristics. Their profiles could be useful for neurodegenerative disease prognosis, early detection, and treatment result documentation (6–10).

Excessive production of reactive oxygen species (ROS) and inadequate antioxidant defenses lead to mitochondrial damage and dysfunction, and ultimately to the pathogenesis of PD (11). Due to the high mortality and morbidity associated with this disease, the development of diagnostics and drugs to modify the disease is an urgent medical need for PD (12). Therefore, it is important to understand the pathophysiological mechanisms underlying PD (13). Biosurfactants are surface-active compounds produced by some biological agents such as yeast and bacteria (14). Scientific studies show that they are safer than chemical surfactants, making them a preferred alternative in terms of the environment (14). The anti-

inflammatory, antioxidant and antibacterial potential of biosurfactants has been demonstrated in numerous studies (15–17). Iturin A is a type of lipopeptide produced by *Bacillus* spp. (18). It has multiple activities inhibiting fungi, bacteria and viruses (19). Iturin A biosurfactant with a fatty acid chain and a cyclic peptide isolated from the fermentation of *Bacillus subtilis* exhibits a variety of biological activities and is of interest due to its anti-cancer properties (20,21). Iturin A also exhibits strong anti-inflammatory property on cells (22). Although the mechanisms that affect these anti-inflammatory processes are not fully elucidated, it is known to affect specific inflammatory pathways such as Nuclear Factor kappa B NF- κ B (23). This study examined the neurotherapeutic effect of Iturin A biosurfactant via some inflammatory cytokines in the 6-hydroxy dopamine (6-OHDA)-induced *in vitro* PD model.

MATERIALS and METHODS

Cell Culture

Dulbecco's Modified Eagle's Medium/Ham's Nutrient Mixture F12 (DMEM/F12) (Gibco, Thermo Fisher Scientific, UK), supplemented with 10% fetal bovine serum (FBS) (Biological Industries, Kibbutz Beit-Haemek, Israel) and 2% penicillin-streptomycin (Capricorn Scientific GmbH, Ebsdorfergrund, Germany) were used to culture human neuroblastoma cell lines SH-SY5Y (CRL-2266) purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). The culture grew at 37 °C with 5% CO₂.

Cell Proliferation and Cytotoxicity Assays

After the cultures of SH-SY5Y cells were 80–90% confluent, the cells were detached with 0.05% trypsin-EDTA (Capricorn Scientific GmbH, Ebsdorfergrund, Germany) for two minutes and then centrifuged for five minutes at 1200 rpm. Trypan blue staining was used to determine the total number of cells using an automatic cell counter (Luna II, Logos Biosystems, Gyeonggi-do, South Korea). To perform the 3-(4,5-dimethyl-2-

thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay (Sigma, Missouri, ABD), 96-well plates were seeded with 5×10^3 /well SH-SY5Y cells, which were then incubated for 24 hours. Subsequently, Iturin A (No: I1774, Sigma-Aldrich, Saint Louis, USA) was added to the SH-SY5Y cells at six different concentrations (30, 40, 50, 60, 70, and 80 μ M) for a 48-hour treatment. As a positive control, SH-SY5Y cells were treated with L-DOPA at doses ranging from 0.05 to 1.3 μ M for 48 hours. After a 48-hour incubation period, 10 μ L of MTT solution (5 mg/ml in DPBS) was added to each well and the cells were incubated for a further three hours. After discarding the medium, 100 μ L of DMSO was given into each well. A microplate reader (TECAN-sunrise, Männedorf, Switzerland) was used to determine the viability of the cells at 570 nm. Each experiment was done three times. Using a range of Iturin A concentrations, the 50% inhibitory concentration (IC_{50}) value (48.709 μ M) was calculated using GraphPad Prism version 9 software. Non-linear regressions of log (inhibitor) versus response with four parameters were selected for IC_{50} estimation. Only 0% and 100% could be the low- and high-end values, respectively.

Establishment of an *in vitro* Parkinson's Disease Model

Neurotoxicity was induced by 6-OHDA (Sigma, H4381), which can disrupt cellular signaling pathways related to mitochondrial damage and oxidative stress, in SH-SY5Y cells. The 6-OHDA stock concentration was freshly prepared and was 10 mM in 1 mL of solvent (0.01% (w/v) L-ascorbic acid (Sigma, A4403). Then, this stock was diluted in the medium to 200 μ M, 300 μ M, 400 μ M and 500 μ M concentrations (2X) to be used on the cells, and the appropriate concentration was determined in SH-SY5Y cells by MTT method. After the SH-SY5Y cells adhered to the 6-well plates, they were incubated for 48 hours to evaluate the protective effect of Iturin A. Subsequently, the cells were incubated with 6-OHDA at 250 μ M concentration for 24 hours to induce neurodegeneration after 48 hours. The group treated with Iturin A was compared to the 6-OHDA group and negative control by not treating them.

ELISA Assay

For the ELISA assay, SH-SY5Y cells (1×10^6 /mL) were seeded in 6-well plates and incubated for 24 hours. Iturin A was added at a concentration of 30 μ M, and the cells were incubated with 6-OHDA for

24 hours to induce neurodegeneration after 48 hours. At the end of the incubation, the supernatant of the cells was collected. Direct competitive chemiluminescence ELISA kits (Bioassay Technology Laboratory, Shanghai, China) were used to measure the levels of IL-6, IL-8, and TGF- β in the supernatant (extracellular) according to the manufacturer's instructions. The cell culture supernatant was collected in sterile Eppendorf tubes after centrifugation at 2000-3000 rpm for about 20 minutes to determine cytokines released extracellularly. 50 μ L of each diluted cell pellet (1:40 in 1 $\times$ PBS, pH 7.2-7.4) was lysed by multiple freeze-thaw cycles to remove internal components to determine the intracellular cytokines and chemokines. After centrifugation at 2000 rpm for 20 minutes, the supernatants were collected in sterile Eppendorf tubes. A microplate reader (TECAN-sunrise, Männedorf, Switzerland) was used to determine the absorbance of the samples at 450 nm.

Statistical Analysis

GraphPad Prism 9 (GraphPad Software Inc., San Diego, CA, USA) was used to analyze all of the data. In paired comparisons, the Mann-Whitney U test was used to evaluate non-normal data while the student's t-test was used to analyze regularly distributed data. Using one-way ANOVA and Dunn's post hoc test, normally distributed data was tested for multiple comparisons. Tukey's post hoc adjustment was applied to the Kruskal Wallis test when analyzing non-normally distributed data. $p < 0.05$ value was considered statistically significant (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.001$).

RESULTS

Evaluation of the Cytotoxicity of Iturin A in the SH-SY5Y Cells

First, SH-SY5Y cells were treated with Iturin A at different concentrations (30, 40, 50, 60, 70, and 80 μ M) for 48 hours. Cell viability decreased with increasing concentrations (Figure-1). The cell viability of SH-SY5Y cells remained almost unchanged when treated with Iturin A up to a concentration of 30 μ M, indicating the non-toxicity of Iturin A. Cell viability was 85.2% at a concentration of 30 μ M. The subsequent experiments employed Iturin A at a concentration of 30 μ M because it showed cytotoxic effects at higher concentrations than 30 μ M and the IC_{50} value was determined as 48.709 μ M by calculating the effect of increasing concentrations of Iturin A

on cell viability (%). For subsequent experiments, the non-toxic dose of Iturin A, 30 μ M, was used.

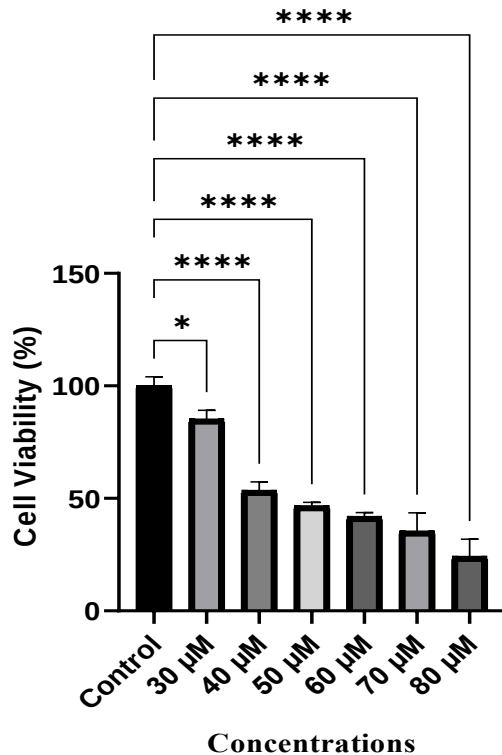


Figure-1. Iturin A decreases the viability of SH-SY5Y cells due to increasing concentration. SH-SY5Y cells were treated with six different Iturin A concentrations for 48 hours. The IC_{50} value was calculated as 48.709 μ M. The significant difference compared to the control group, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

The Effect of Iturin A on 6-OHDA-Induced Cytotoxicity in SH-SY5Y Cells

SH-SY5Y human neuroblastoma cells are commonly used for 6-OHDA-induced *in vitro* PD model (24). In agreement with previous studies, 6-OHDA showed a cytotoxic effect on the cells. After an application of a 250 μ M concentration, cell viability was expressed as a percentage and an IC_{50} was calculated. Iturin A at a concentration of 30 μ M dramatically improved cell viability (104.27%) similar to that in the L-DOPA group (125.08%), compared to the 6-OHDA-induced neurotoxicity group (Figure 2). Since the lowest

Figure 3.A). IL-8 level significantly increased in 6-OHDA-induced SH-SY5Y cells compared to the control group ($P<0.01$) and significantly decreased in Iturin A-treated cells compared to 6-OHDA-

concentration of Iturin A provided the greatest benefit, a concentration of 30 μ M Iturin A was used for further experiments.

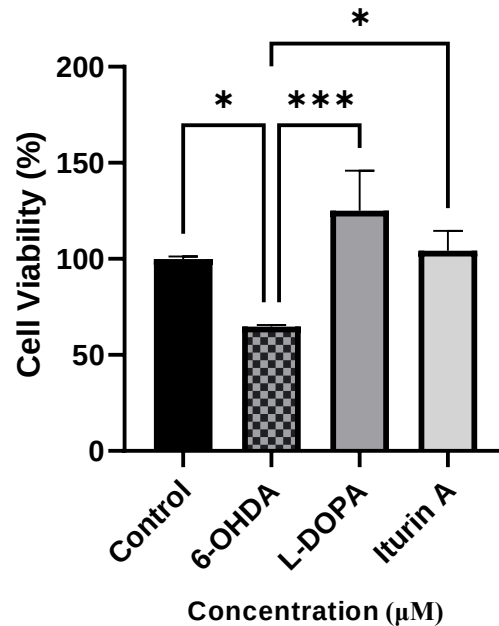


Figure-2. Iturin A reduces cytotoxicity in 6-OHDA-induced SH-SY5Y cells. Significant difference compared to control and 6-OHDA-induced neurotoxicity groups, * $p<0.05$, *** $p<0.001$.

Iturin A Reduces the Inflammatory Response Induced by 6-OHDA in SH-SY5Y Cells

TGF- β , IL-6, and IL-8 levels increased in the 6-OHDA-induced SH-SY5Y cells compared to the control group (Figure-3). The therapy with Iturin A in 6-OHDA-induced SH-SY5Y cells dramatically decreased the production of TGF- β , IL-6, and IL-8 compared to the 6-OHDA-induced neurotoxicity group (Figure-3).

While the IL-6 level increased statistically significantly in 6-OHDA-induced SH-SY5Y cells compared to the control group ($P<0.001$), it significantly decreased in these cells treated with Iturin A compared to 6-OHDA-induced SH-SY5Y cells ($P<0.001$). There was a decrease in IL-6 level in L-DOPA-treated cells (positive control), but it was not statistically significant ($P>0.05$) (

induced SH-SY5Y cells ($P<0.0001$). IL-8 levels significantly decreased in L-DOPA-treated cells (positive control) ($P<0.0001$) (Figure 3.B). Similarly, TGF- β level significantly increased in 6-

OHDA-induced SH-SY5Y cells compared to the control group ($P<0.05$) and significantly decreased in Iturin A-treated cells compared to the 6-OHDA-

induced cells ($P<0.01$). TGF- β level significantly decreased in L-DOPA-treated cells (positive control) ($P<0.01$) (Figure-3.C)

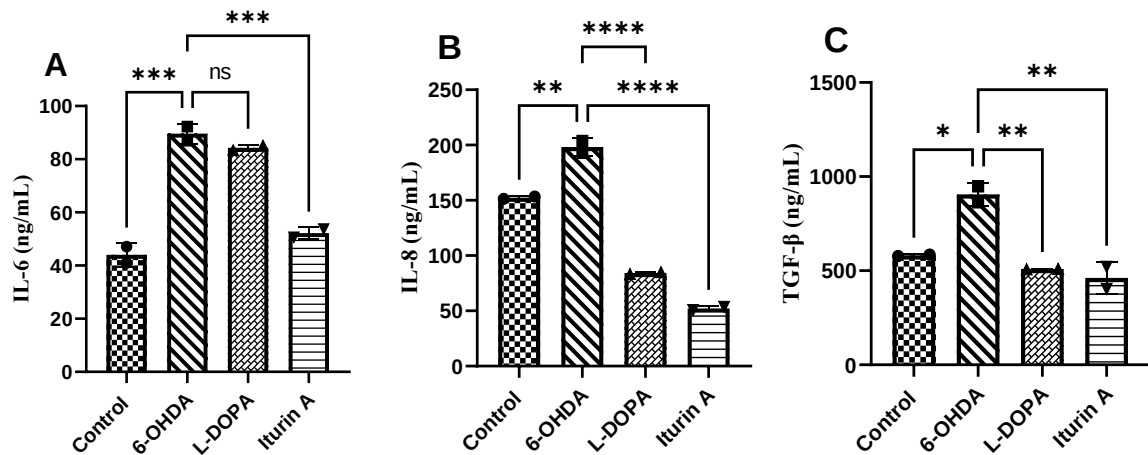


Figure-3. Iturin A reduces inflammation in 6-OHDA-induced SH-SY5Y cells. IL-6 (A), IL-8 (B), and TGF- β (C) protein expressions. Significant difference compared to control and 6-OHDA-induced neurotoxicity groups, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

DISCUSSION

The prevalence and incidence of PD are increasing worldwide as the population ages, and it is estimated that there will be approximately 30 million PD patients worldwide by 2030 (25). The disease is a major public health concern because it becomes more common with increasing age (26). There is currently no cure for PD, but clinical trials are investigating potential disease-modifying approaches (27,28).

Biosurfactants, particularly Iturin A, are attracting attention due to their anti-inflammatory effects (22). Although the importance of biosurfactants in developing strategies to prevent and treat the disease is well known, studies in this area are quite limited. Although there are no studies in the literature on the effect of Iturin A in the treatment of PD, it cannot be ignored due to its effects on neurodegeneration. (29–31). The cytotoxic, anti-/pro-inflammatory, and anti-tumor properties of the biosurfactant Iturin A were investigated in this work using ELISA and MTT assays on an *in vitro* PD cell model.

The source of Iturin A used in our study is a *Bacillus* from the soil. As far as we know, there is no information in the literature about the anti-tumor properties of Iturin A obtained from the same

source in SH-SY5Y cells. In this context, our study is of particular importance. In this study, we calculated the IC_{50} value for Iturin A in SH-SY5Y cells as 48.709 μ M for 48 hours. Moreover, Iturin A decreased the viability of SH-SY5Y human neuroblastoma cells to below 50% and lowered neurotoxicity.

The 6-OHDA-induced *in vitro* PD model is a useful model to investigate the molecular basis of cytotoxicity, to study the cellular processes activated by neuroinflammation and neuronal death, and to understand new treatment mechanisms, as it reproduces the cellular processes described in PD (32). The biology of PD and the efficacy of potential neuroprotective drugs are being investigated using SH-SY5Y cells as an experimental model (33).

There are a large number of activated microglial cells in the substantia nigra striatum of PD patients, which produce various cytokines (e.g. TNF- α , IL-6 and IL-1 β , etc.) and then induce an inflammatory response and promote neuronal lesions (34,35). Therefore, reducing microglial inflammation is a useful approach for the treatment of PD (36) Previous studies have shown that levels of various cytokines such as TNF- α , IL-1 β , IL-2, IL-4, IL-6, IL-8, epidermal growth factor (EGF), TGF- α , TGF- β are elevated in PD (10,37–39).

Interleukins are released by activated microglia and leukocytes. IL-6 is a multifunctional cytokine secreted by neurons and glial cells and IL-6 plays an important role in neuronal development and differentiation (38). They possess a number of receptors, one of which is IL-6, which promotes neuronal cells survival at normal concentrations but can cause neurodegeneration when overactivated (40). In our study, 6-OHDA-induced SH-SY5Y cells, which we created to mimic the PD model, had elevated IL-6 levels in line with neurodegeneration. Iturin A treatment reduced inflammation in 6-OHDA-induced SH-SY5Y cells by lowering IL-6 levels.

Interleukin-8 (IL-8/CXCL8), a major CXC chemokine, exerts profound effects on cell-cell activation and brain function, suggesting possible involvement in neuroinflammation (41). IL-8 is secreted by a variety of cell types and is released when an inflammatory trigger occurs (42,43). IL-8 levels are particularly high in Parkinson's

patients (10). In our study, 6-OHDA-induced SH-SY5Y cells had higher IL-8 levels than the control group. Similar to the L-DOPA treatment group (positive control), treatment with Iturin A reduced the levels of IL-8 in these neurodegenerated cells.

PD is associated with higher TGF- β levels, suggesting a possible modulatory involvement in neurodegeneration (44,45). Elevated TGF- β levels are even protective against neurotoxicity (46). In our study, we found that the 6-OHDA-induced neurotoxicity group had higher TGF- β levels. Similar to the positive control L-DOPA group, treatment with Iturin A dramatically decreased inflammation in these neurotoxic cells by lowering TGF- β levels.

In addition, when all these findings are evaluated together, these cytokines/chemokines seem to work synergistically and thus contribute to neurodegeneration and neuroprotection by supporting each other.

Credit authorship contribution statement

Pinar Altin Celik: Conceptualization, Investigation, Data curation, Methodology, Validation, Writing-original draft, Writing – review & editing. **Muazzez Derya Andeden:** Methodology, Investigation, Writing – review & editing. **Hamiyet Donmez Altuntas:** Validation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

Conflicts of interest: Authors declared no conflict of interest.

CONCLUSION

The expression of cytokines, which increases and/or decreases during biosurfactant treatment, could contribute to the development of PD. In the present study, the biosurfactant Iturin A was shown to decrease the increased levels of IL-6, IL-8 and TGF- β in 6-OHDA-induced SH-SY5Y cells, an *in vitro* PD model. High levels of these cytokines are known to be particularly common in PD. In conclusion, the Iturin A treatment has a protective effect against the toxicity of 6-OHDA in SH-SY5Y cells. However, these findings are preliminary and were studied *in vitro* based on a single cell line. In this context, it is supported *in vivo* with additional cytokines and chemokines to better understand its efficacy for clinical translation.

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Correlation of the neuropathic pain and electrodiagnostic tests in earthquake-related peripheral nerve injuries

Depremle ilişkili periferik sinir yaralanmalarında nöropatik ağrı ile elektrodyagnostik testlerin korelasyonu

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ABSTRACT

Aim: Peripheral nerve injury resulting from being buried under earthquake-related debris can lead to neuropathic pain. This study investigates the relationship between neuropathic pain and electrodiagnostic test findings in patients with peripheral nerve lesions caused by entrapment under debris.

Materials and Methods: This retrospective study included patients who developed peripheral nerve lesions due to entrapment under debris during the Kahramanmaraş-centered earthquakes in February 2023. Abnormalities in compound nerve action potentials (CNAPs) and compound muscle action potentials (CMAPs) were categorized as mild, moderate, or severe.

Results: 62 (34 female, 28 male) patients were included in this study. The mean (min-max) disease duration, time under debris, and Douleur Neuropathique 4 Questions (DN4) scores were 59.4 ± 37.1 (21-180) days, 22.6 ± 29.7 (0.5-130) hours, and 4.9 ± 1.7 (1-9), respectively.

A positive correlation was observed between the number of severe CNAP/CMAP abnormalities and DN4 scores ($p=0.024$, $r=0.287$ / $p=0.003$, $r=0.371$). Patients with severe CMAP abnormalities in at least one or at least two nerves had higher DN4 scores compared to those without ($p=0.039$ / $p=0.009$).

Conclusion: This study highlights a relationship between neuropathic pain and severe CNAP/CMAP abnormalities in patients with peripheral nerve lesions due to entrapment under debris.

Keywords: Earthquake, nerve conduction study, neuropathic pain, peripheral neuropathy,

ÖZ

Amaç: Depremle ilişkili enkaz altında kalmaya bağlı gelişen periferik sinir yaralanmaları nöropatik ağrıya yol açabilir. Bu çalışma, enkaz altında kalma sonucu periferik sinir hasarı olan hastalarda nöropatik ağrı ile elektrodyagnostik test bulguları arasındaki ilişkiyi araştırmaktadır.

Gereç ve Yöntem: Bu retrospektif çalışma, Şubat 2023'te Kahramanmaraş merkezli depremler sırasında enkaz altında kalma sonucu oluşan periferik sinir hasarı gelişen hastaları içermektedir. Bileşik sinir aksiyon potansiyelleri (CNAP) ve bileşik kas aksiyon potansiyellerindeki (CMAP) anormallikler hafif, orta veya şiddetli olarak kategorize edilmiştir.

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Bulgular: Bu çalışmaya 62 (34 kadın, 28 erkek) hasta dahil edilmiştir. Ortalama (min-maks) hastalık süresi, enkaz altında kalma süresi ve Douleur Neuropathique 4 soru (DN4) skorları sırasıyla $59,4 \pm 37,1$ (21-180) gün, $22,6 \pm 29,7$ (0,5-130) saat ve $4,9 \pm 1,7$ (1-9) olarak bulunmuştur.

Şiddetli CNAP/CMAP anormalliklerinin sayısı ile DN4 skorları arasında pozitif bir korelasyon gözlenmiştir ($p=0,024$, $r=0,287$ / $p=0,003$, $r=0,371$). En az bir veya en az iki sinirde şiddetli CMAP anormallikleri olan hastalar, anormallik bulunmayan hastalara göre daha yüksek DN4 skorlarına sahipti ($p=0,039$ / $p=0,009$).

Sonuç: Bu çalışma, enkaz altında kalmaya bağlı periferik sinir hasarı olan hastalarda nöropatik ağrı ile şiddetli CNAP/CMAP anormallikleri arasındaki ilişkiye dikkat çekmektedir.

Anahtar Sözcükler: Deprem, sinir ileti çalışması, nöropatik ağrı, periferik nöropati

INTRODUCTION

Abnormalities in the peripheral nervous system can lead to the development of weakness, sensory abnormalities, and neuropathic pain (1, 2). These issues can range from mild impairment to disability in affected patients. Traumas, alongside chronic systemic diseases, are known to cause disorders involving the peripheral nervous system, including peripheral nerve damage, plexopathy, or radiculopathy (1-4). Nerve conduction studies and needle electromyography are valuable tools not only for diagnosing peripheral nerve lesions but also for providing insights into differential diagnosis and prognosis (5, 6). Peripheral nerve lesions may result from traumas such as being trapped under debris caused by earthquakes (4, 7-9). Moreover, individuals affected by earthquakes may develop neuropathic pain (10). This study investigates the potential relationship between neuropathic pain and nerve conduction study findings in peripheral nerve lesions resulting from individuals being trapped under debris due to earthquakes centered in Kahramanmaraş.

MATERIALS AND METHODS

Subjects

This retrospective study included patients who suffered peripheral nerve lesions due to being trapped under debris in the Kahramanmaraş-centered earthquakes on February 6, 2023. The study was conducted at Adana City Training and Research Hospital's Neurology Clinic and Clinical Neurophysiology Laboratory between February 2023 and June 2023. Ethics committee approval for the study was obtained from Adana City Training and Research Hospital Clinical Research Ethics Committee (number: 129/2669, date: 2023). Patients exhibiting clinical features and electrodiagnostic findings consistent with single or

multiple nerve lesions, plexopathy, and/or radiculopathy were included. Exclusion criteria comprise individuals with conditions predisposed to polyneuropathy, neurodegenerative diseases, or a history of radiculopathy/plexopathy or nerve lesions. Douleur Neuropathique 4 Questions (DN4) pain scale was applied to the patients. The cut-off point for DN4 score was considered as 4 points (11, 12).

Electrodiagnostic tests

Nerve conduction study and needle electromyography were performed with the Cadwell Sierra Summit EMG unit (Cadwell Laboratories, Kennewick, Washington, USA). Recommended methods and reference values for nerve conduction study and needle electromyography were used. Considering the clinical findings of the patients', recommended methods were and reference values for nerve conduction study and needle electromyography were performed on the median, ulnar, radial, superficial radial, medial antebrachial cutaneous, lateral antebrachial cutaneous, posterior tibial, peroneal, and sural nerves. The normal values for these nerve conduction studies are as follows: Posterior tibial CMAP: 4.2 μ V, Median CMAP: 4.3 μ V, Median CNAP: 10.3 μ V, Ulnar CMAP: 6.4 μ V, Ulnar CNAP: 7.1 μ V, Peroneal CMAP: 3.7 μ V, Sural CNAP: 5.1 μ V, Superficial peroneal CNAP: 5.3 μ V, Superficial radial CNAP 10 μ V, Medial antebrachial cutaneous (MAC): 3 μ V, Lateral antebrachial cutaneous (LAC): 6 μ V (13-16).

For motor and sensory nerve conduction studies, upper and lower band filters were set as 20 Hz-10kHz and 20Hz-2KHz, respectively. In sensory and motor nerve conduction studies, the sweep speed was 1 ms/division and 5 ms/division, respectively. Sensitivity in sensory and motor nerve conduction studies was set as 10 μ V/division and 2 mV/division, respectively.

Compound muscle action potential (CMAP) and compound nerve action potential (CNAP) amplitudes were calculated from peak to peak. Considering clinical findings of the patients, nerve conduction studies of the median, ulnar, radial, superficial radial, medial antebrachial cutaneous, lateral antebrachial cutaneous, posterior tibial, peroneal, and sural nerves were performed on the patients. CMAP or CNAP were classified according to their amplitudes as follows (17): 1) Mild nerve injury: CNAP / CMAP amplitude is less than 50% of the normal value 2) Moderate nerve injury: CNAP / CMAP can be obtained but the amplitude is between 50-100% 3) Severe nerve injury: CMAP/CNAP cannot be obtained.

Concentric needle electrodes (length=50mm, 26 G, Natus, Galway, Ireland; length=50mm, diameter=0.46mm, Bionen Medical Devices, Florence, Italy) were used for needle electromyography (EMG). Band filter 10 Hz It was set to -10 kHz. Positive sharp waves and fibrillation potentials at rest were carefully examined. When the motor unit action potential (MUAP) amplitude was >3.5 mV and/or duration was >15 ms, this MUAP was considered neurogenic. Muscles with positive sharp wave/Fibrillation potentials were divided into two groups according to whether MUAP could be obtained or not. Muscle selection for needle EMG was made according to the clinical characteristics of the patient.

Statistical analysis

Categorical variables were expressed as frequency and percentage, while numerical data were presented as mean $\pm$ standard deviation (SD) and range. Statistical analysis employed the Mann-Whitney U test for numerical data comparison between groups and the Pearson-Chi Square test for categorical variables. Spearman correlation analysis assessed correlations. A p-value < 0.05 was deemed statistically significant. SPSS 22.0 was used for statistical analysis.

RESULTS

The findings of 97 patients were reviewed. There were 22, eight and five patients with a history of diabetes mellitus, polyneuropathy and radiculopathy, respectively. These patients were excluded from the study. Finally, 62 patients (34 female, 28 male) were included in the study. The mean age of the patients was 35.3 ± 14.3 years. The time interval between the time the lesion

occurred and the time the clinical/electrodiagnostic examination was performed was 59.4 ± 37.1 (min-max 21-180) days. The mean time spent under debris is 22.6 ± 29.7 (0.5-130) hours. The clinical features of the patients are given in Table-1. When patients were grouped according to lesion localization, there were 24 (38.7%), 18 (29.1%) and two (3.1%) patients with only peripheral nerve lesion, plexopathy and radiculopathy, respectively. Eighteen patients had at least two of these three types of injuries.

The mean DN4 score of the patients was 4.9 ± 1.7 (min-max 1-9). There were 40 (64.5%) patients with a DN4 score > 4. DN4 scores of individuals with one extremity affected and more than one extremity were 4.5 ± 1.8 (min-max 1-9) and 5.6 ± 1.3 (min-max 3-8), respectively (p=0.007). The numbers of mild, moderate, and severely affected CNAP/CMAPs in patients are shown in Table-2. Table-3 shows the correlation between the number of mildly, moderately, and severely affected nerves / disease duration / time under debris and DN4 scores. Figure 1 shows the correlation between DN4 scores and the number of nerves with severe CMAP abnormality in each patient. The comparison of disease duration/time in debris/DN4 scores between the groups formed by separating the patients according to the presence of MUAP and the number of severe CNAP/CMAP abnormalities is available in Table-4. Correlation between DN4 scores and the number of nerves with severe CMAP abnormality in patients with and without severe CMAP abnormality in at least two nerves shows in Figure 2.

Table-1. The clinical features of the patients.

Clinical feature	Number of patients (%)
Median / Ulnar nerve	7 (11.2) / 10 (16.1)
Radial / Axillary nerve	6 (9.6) / 1 (0.01)
Peroneal / Posterior tibial / Sural nerve	15 (24.1) / 3 (0.04) / 2 (0.03)
Superficial / Sciatic nerve	1 (0.01) / 15 (24.1)
Brachial / Lumbosacral / Sacral plexus	9 (14.5) / 4 (0.06) / 10 (16.1)
Cervical / Lumbosacral radiculopathy	1 (0.01) / 4 (0.06)
Affected extremity	
Only one extremity / >1 extremity	18(29.1)/ 24 (38.7)
Right / Left / Bilateral	23(37.1)/21(33.9)/18(29.0)
Upper / Lower / Both	20(32.3)/34(54.8)/8(12.9)

Table-2. The numbers of mild, moderate and severely affected CNAP/CMAPs in patients.

Electrodiagnostic abnormality	Number of patients				
CNAP	Abnormality in one nerve	Abnormality in two nerves	Abnormality in three nerves	Abnormality in four nerves	Abnormality in five nerves
Mild	13	4	2	-	-
Moderate	1	-	-	-	-
Severe	18	16	8	-	3
CMAP					
Mild	16	4	2	-	-
Moderate	14	2	-	-	-
Severe	15	15	9	10	2

Table-3. The correlation between the number of mild, moderate and severely affected nerves /disease duration/ time under debris and DN4 scores.

Clinical and electrodiagnostic features	DN4 scores
Disease duration (day)	p=0.044, r=0.257
Time under debris (hour)	p=0.865, r=0.022
Number of nerves with CNAP/CMAP abnormality in patients	
CNAP abnormality	
Mild	p=0.574, r=0.073
Moderate	p=0.125, r=-0.197
Severe	p=0.024, r=0.287
CMAP abnormality	
Mild	p=0.480, r=-0.091
Moderate	p=0.331, r=-0.126
Severe	p=0.003, r=0.371

CMAP: compound muscle action potential; CNAP: compound nerve action potential; DN4: Douleur Neuropathique 4 Questions.

Table-4. The comparison of clinical features / DN4 scores between the patients with and without the electrodiagnostic abnormalities.

Electrodiagnostic feature	Disease duration (day) mean±SD(IQR)	Time under debris (hour) mean±SD	DN4 scores mean±SD
Severe CNAP abnormality in at least one nerve +			
+ (n=45)	59.4±40.0(45)	25.7±29.9(19.5)	5.2±1.6(2)
- (n=17)	59.3±29.17(45)	14.0±28.2(9.5)	4.3±1.8(3)
P value	0.585	0.006	0.106
Severe CNAP abnormality in at least two nerves			
+ (n=27)	63.3±42.1(77)	25.4±26.6(14)	5.3±1.7(2)
- (n=35)	56.3±33.2(36)	20.3±32.1(9)	4.6±1.7(2)
P value	0.749	0.046	0.110
Severe CMAP abnormality in at least one nerve +			
+ (n=51)	60.2±38.7(55)	23.5±28.8(17)	5.1±1.6(2)
- (n=11)	55.3±29.8(34)	17.9±34.6(9)	4.0±1.9(3)
P value	0.861	0.068	0.039
Severe CMAP abnormality in at least two nerves			
+ (n=36)	62.9±40.3(54)	27.1±33.3(41.8)	5.4±1.5(2)
- (n=26)	54.4±32.3(36)	16.2±23.1(14.5)	4.2±1.7(2.2)
P value	0.488	0.387	0.009
Absence of MUAP in at least one muscle			
+ (n=35)	57.9±36.4(40)	30.7±36.3(53)	5.1±1.6(2)
- (n=24)	60.5±35.2(50)	12.1±12.4(8.8)	4.7±1.9(2.7)
P value	0.676	0.049	0.315

CMAP: compound muscle action potential; CNAP: compound nerve action potential; DN4: Douleur Neuropathique 4 Questions; MUAP: motor unit action potential; SD: standard deviation.

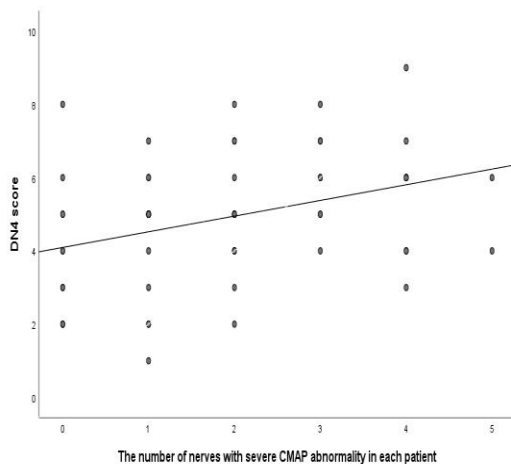


Figure-1. The correlation between DN4 scores and the number of nerves with severe CMAP abnormality in each patient
CMAP: compound muscle action potential; DN4: Douleur Neuropathique 4 Questions.

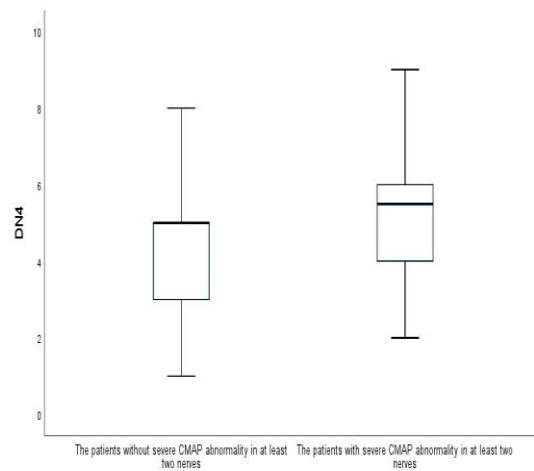


Figure-2. The comparison of DN4 scores between the patients with and without severe CMAP abnormality in at least two nerves
CMAP: compound muscle action potential; DN4: Douleur Neuropathique 4 Questions.

DISCUSSION

Neuropathic pain was prevalent in approximately 65% of the patients in this study, indicating its significant impact on individuals with peripheral nerve lesions resulting from being trapped under earthquake debris. Unlike acute nerve injuries seen in typical trauma, entrapment under debris causes prolonged compression and ischemic damage, leading to more severe axonal degeneration and delayed recovery. This mechanism distinguishes earthquake-related nerve injuries from standard traumatic neuropathies. A noteworthy finding was the positive correlation observed between DN4 scores, indicative of neuropathic pain severity, and the presence of severe CNAP abnormalities. This suggests that as the degree of axonal degeneration in sensory nerves increases, the severity of neuropathic pain may also escalate (18-21). Interestingly, a similar relationship was observed between severe CMAP abnormalities and DN4 scores, implying that in patients trapped under debris, not only the sensory branches of the peripheral nerves are affected, but also the motor and sensory branches more proximally. Another explanation for this situation may be in the pathophysiology of neuropathic pain. Both peripheral and central mechanisms are involved in the pathophysiology of neuropathic pain (18-20). Affecting Damaged to motor fibers may have affected the motor cortex, which is known to be related to the somatosensory cortex (22, 23), and thus may have led to the development of neuropathic pain in these patients. Entrapment duration (time under debris) did not correlate with DN4 scores but correlated with CNAP abnormalities in patients with one or two CNAP abnormalities. Neuropathic pain is a progressive condition, and its severity is influenced by the duration of nerve injury rather than the initial entrapment duration. While entrapment duration objectively correlates with the degree of nerve damage (reflected by CNAP abnormalities), subjective pain perception (DN4) is influenced by multiple factors, including central sensitization and the chronicity of the injury. In this study, neuropathic pain was present in more than half of the patients. It shows that neuropathic pain is an important problem in patients with disorders in the peripheral nervous system as a result of being trapped under earthquake-related debris (10). Similarly, patients without detectable MUAP, indicative of more severe nerve injury, tended to have longer exposure durations under debris

compared to those with detectable MUAP. As the time spent under debris increases, the severity of axonal degeneration in the peripheral nerve may gradually increase, or the peripheral nerve may be completely damaged, or an event such as compartment syndrome may damage the peripheral nerve (8, 24). These findings underscore the importance of timely rescue efforts to prevent or mitigate severe peripheral nerve damage in individuals trapped under debris following earthquakes.

Although some studies suggest that the lower extremities are more affected in those trapped under earthquake debris, some studies have reported the opposite (4, 7, 25, 26). In this current study, peripheral neuropathy was more common in the lower extremities. Although lower extremity neuropathy was more common in our study, with ulnar nerve damage prevalent in the upper extremity and sciatic and peroneal nerve damage in the lower extremity, the specific distribution of nerve injuries varied. This variability may be attributed to anatomical vulnerabilities. Peripheral nerves become more superficial or susceptible to neuropathy as they pass through some parts of the extremities. The ulnar nerve is more prone to injury across the elbow segment and the peroneal nerve at the head of the fibula (27-29). Contrary to this situation, the deeper located sciatic nerve is also affected. The most common causes of sciatic nerve neuropathy are hip fractures/dislocations and hip surgery (17). Similarly, there are reports that suggest that approximately 45% of the patients injured in the earthquake had tibia/fibula or femur fractures (4). Therefore, sciatic nerve neuropathy may have been observed more frequently in patients trapped under debris due to traumatic hip and knee injuries.

This study had some limitations. In addition to being a retrospective study, the disease durations of the patients were different. It is known that electrodiagnostic tests are affected by disease duration (6, 30). The low number of patients can also be considered a limitation, but the criteria for inclusion of patients in the study were strict. The nerve conduction studies examine myelinated nerve fibers rather than the A delta and C fibers associated with pain (6). This was also one of the limitations.

CONCLUSION

This study highlights the significant burden of neuropathic pain in individuals with peripheral

nerve lesions following earthquake-related entrapment under debris. The observed correlations between neuropathic pain severity and CNAP/CMAP abnormalities underscore the importance of early detection and intervention in mitigating the impact of such injuries. Further

research is warranted to elucidate optimal management strategies for neuropathic pain and peripheral nerve injuries in disaster settings.

Conflicts of interest: Authors declared no conflict of interest.

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Investigation of aggression levels of methamphetamine users in probation

Denetimli serbestlik tedbiri bulunan metamfetamin kullanıcılarının saldırganlık düzeylerinin incelenmesi

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ABSTRACT

Aim: The use of methamphetamine is a global health concern that profoundly impacts individual well-being, potentially resulting in severe health consequences and mortality. Probation, a prevalent legal substitute for imprisonment, is essential in the oversight and rehabilitation of methamphetamine-related probationers. This study aimed to identify the characteristics of methamphetamine users and assess their aggression levels.

Materials and Methods: The sample for this descriptive cross-sectional study comprises 200 adult probationers (27 females, 173 males). The study utilized personal and criminological information forms, the Addiction Profile Index (API), and the Buss-Perry Aggression Questionnaire (BPAQ).

Results: The mean age of the probationers was 32.32 ± 8.41 years (Female: 30.07 ± 7.14 ; Male: 32.67 ± 8.55). It was determined that 52.5% were unmarried, 36.5% had experienced traumatic events (including earthquakes, violence, or death), 39% had a history of incarceration, 18% had attempted suicide, and 39% exhibited self-injurious behaviors. Upon analyzing the addiction profiles of probationers, it was shown that 40% had engaged in drug use for a duration of 1 to 5 years, while 64.5% exhibited polydrug usage. The average overall aggressiveness score of probationers was 76.36 ± 20.47 , whereas the average total API score was 9.08 ± 3.99 . The correlation study indicated a somewhat positive and statistically significant association between probationers' levels of hostility and addiction ($p < 0.05$).

Conclusion: Acknowledging the traits of methamphetamine users and their aggression levels during probation may enhance the efficacy of probation practices and aid in diminishing drug consumption.

Keywords: Crime, Methamphetamine, Probation, Substance Use, Aggression

This research is an extended version of the oral presentation at the 33rd Congress of Psychology held in Prague between 21-26 July.

Öz

Amaç: Bu çalışmada madde kullanım davranışı ile ilgili takip ve tedavi sürecinde önemli bir yere sahip olan denetimli serbestlik müdürlüklerinde, metamfetamin kullanıcılarının özelliklerini ortaya koyarak saldırganlık düzeylerinin belirlenmesi ve metamfetamin kullanımı ile ilişkin faktörlerin ortaya konulması amaçlanmıştır.

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Gereç ve Yöntem: Tanımlayıcı kesitsel desende bu araştırmanın örneklemini 200 (27 kadın, 173 erkek) yetişkin yükümlü oluşturmaktadır. Çalışmada yükümlülerin kişisel ve kriminolojik bilgilerini içeren bilgi formu, Bağımlılık Profil İndeksi (BAPİ) ve Buss-Perry Saldırganlık Ölçeği (BPSÖ) kullanılmıştır.

Bulgular: Veriler değerlendirilirken tanımlayıcı istatistikler ve pearson korelasyon analizi kullanılmıştır. Metamfetamin kullanımı olan yükümlülerin yaş ortalaması $32,32 \pm 8,41$ (K:30,07±7,14; E:32,67±8,55) olup %52,5'i bekâr, %39,5'i ortaokul mezunu olduğu, %36,5'inin travmatik olay geçmişi (depresyon, şiddet, ölüm vb.) olduğu, %33'ünün birden fazla denetimli serbestlik tedbirinin bulunduğu, %39'unun ceza infaz kurumu öyküsünün olduğu, %68,5'inin ailesinin, %71'inin arkadaşının denetimli serbestlik tedbirinin bulunduğu, %18'inin intihar girişiminin olduğu ve %39'unun kendine zarar verme davranışının bulunduğu sonucuna ulaşılmıştır. Metamfetamin kullanımı olan yükümlülerin bağımlılık özellikleri incelendiğinde %40'ının 1-5 yıldır madde kullandığı, %64,5'inin ise çoklu madde kullanımı olduğu sonucuna ulaşılmıştır. Yükümlülerin toplam saldırganlık puan ortalamaları $76,36 \pm 20,47$; BAPİ toplam puan ortalamalarının ise $9,08 \pm 3,99$ olduğu ve %86,5'inin yüksek bağımlılık şiddetinde riskli kullanımının olduğu saptanmıştır. Araştırmada hipotezleri test etmek için pearson korelasyon analizi yapılmıştır. Yapılan analiz sonucuna göre yükümlülerin saldırganlık ve bağımlılık düzeyleri arasında orta düzeyde pozitif ve anlamlı bir ilişki bulunmuştur ($p < 0.05$).

Sonuç: Metamfetamin kullanıcıların özellikleri ve saldırganlık düzeylerinin denetimli serbestlik sürecinde yürütülen iyileştirme faaliyetlerinin odağında tutulması denetimli serbestlik uygulamalarının başarısının artırılmasında ve madde kullanımının azaltılmasında etkili olabileceği düşünülmektedir.

Anahtar sözcükler: Suç; Metamfetamin; Denetimli Serbestlik; Madde Kullanımı; Saldırganlık

INTRODUCTION

Substance use disorders, characterized as a neurological condition necessitating a biopsychosocial approach, represent a significant public health concern demanding urgent attention in numerous jurisdictions. In the context of combating substance use, diverse strategies are utilized, encompassing demand and production reduction, drug trafficking regulation, and rehabilitation initiatives, with addiction treatment varying based on the substance type, usage duration, and potential complications. In many communities, substance-use disorders are perceived as a deterioration, a threat, and a stigmatization of the individual, leading to a marginalized and often illegal existence (2). Concerns surrounding the accessibility and utilization of methamphetamine have been escalating for an extended period. The rising prevalence of methamphetamine misuse in recent years has led to a heightened workload for addiction clinics. Methamphetamine-related disorders present a substantial issue for people, their families, and society as a whole (3).

Methamphetamine, commonly known as meth, is a powerful and highly addictive stimulant that can have severe consequences for individuals and communities. According to research, short and long-term use of methamphetamine causes circulatory, respiratory, and

neurological problems, as well as mental health problems ranging from anxiety, aggression, and depression to acute paranoid psychosis (4). Also, methamphetamine is commonly associated with psychosis. This may be a factor in frequent criminal justice referrals and lengthy treatment required by meth users (5; 6). Despite the lack of precise epidemiological data, efforts to struggle with the rapidly increasing misuse of these substances in Turkey, as in other countries, have been implemented, with various sanctions outlined (7; 8; 9). Probation acts as an alternative to imprisonment for certain types of offenders, primarily those involved in less severe crimes. It is particularly significant and commonly utilized in countries with high incarceration rates, owing to a greater awareness of the costs of imprisonment and the increased cost-effectiveness of probation (10). With the establishment of supervised release in the Turkish Penal Code in 2004, a new era began in the field of addiction (11). A supervision plan is prepared for the probationer for whom a supervised release cautionary decision is issued, and it is ensured that he/she participates in the programs (individual interview, group work, seminars) planned to raise awareness on addiction within the scope of educational improvement studies (12;13). Methamphetamine abuse is increasingly

emerging as a major public health issue, with devastating effects on both individuals and their communities (14). Its abuse is linked to numerous mental health disorders and negatively impacts cognitive functioning, resulting in various detrimental behavioral changes and, ultimately, social isolation. (15). Methamphetamine is linked to a range of mental health disorders and adversely affects cognitive functioning, leading to numerous negative behavioral changes and, ultimately, social isolation (15). Mental disorder symptoms include depression, anxiety, anger, aggression, hallucinations, and delusions, while cognitive impairment encompasses deficiencies in learning, memory, attention, decision-making, social cognition, executive function, and working memory (16; 17). Also, methamphetamine is a drug closely associated with intense aggressive behavior, and the psychoactive nature of the substance leads to high rates of violence and violent crimes (18, 19; 20).

Violence is a significant social issue in numerous countries. Methamphetamine use disorder is a long-term, recurring condition increasingly linked to a range of harms, including mental and physical health issues, intimate partner violence, family disruption, homelessness, crime, and mortality (21). A review of the literature shows that while topics such as anxiety, self-efficacy, problem-solving skills, and stigma are explored among probationers (22; 23; 24), and there are studies on probation measures related to cannabis use (25), research specifically focusing on probationers under supervised release due to methamphetamine use is relatively limited. The research question of the study was "*Is there a relationship between aggression and methamphetamine use in the probation sample ?*" In this context, the study aims to identify the substance use characteristics of methamphetamine users in probation samples and to determine their levels of aggression. Thus, it also aims to contribute to treatment and rehabilitation by revealing the factors associated with methamphetamine use. Considering the original value of the research, it is believed that rehabilitative interventions targeting the concepts of aggression associated with substance use will contribute to a more effective effort to protect the health and well-being of both individuals and society.

MATERIALS AND METHODS

This study, designed in a cross-sectional, was conducted with persons over the age of 18 who were given probation measures within the scope of Article 191 of the Turkish Penal Code (2004) due to the offense of "buying, accepting or possessing drugs or stimulants for use or using drugs or stimulants."

Sample

The study sample, selected by random sampling, included 200 probationers with a history of methamphetamine use who were receiving treatment and probationary measures at the İzmir Probation Office from July 2023 to September 2023. The study's dependent variable is aggressiveness scores. The independent factors include the probationers' socio-demographic traits, substance use patterns, and criminal history.

Procedure

The study was conducted after obtaining permission for the research application from the Directorate of Probation of the Ministry of Justice. Face-to-face interviews were conducted with the probationers. An average of 30 to 50 minutes was spent on each" probationer's session.

Data Collection Tools

Personal Information Form: The form consists of two parts: socio-demographic questions, obtaining data such as age, occupation, education level, employment status, number of siblings, criminal history, and a clinical data form querying psychiatric history and substance use-related characteristics was used.

Buss-Perry Aggression Questionnaire (BPAQ): The BPAQ is a self-report instrument consisting of 29 items, answered on a 5-point Likert-type scale, ranging from 1 (*extremely uncharacteristic of me*) to 5 (*extremely characteristic of me*). The BPAQ assesses four dimensions of aggression: Physical Aggression (nine items), Verbal Aggression (five items); Anger (seven items), and Hostility (eight items) (26). The validity and reliability study of the Turkish version of the scale was tested by Madran (27). The score obtained for each scale sub-factor indicates the individual's aggressive attitudes towards that factor.

Addiction Profile Index (API): The scale consists of 37 questions and five subscales. The validity

and reliability study of the scale was conducted by Ögel and colleagues in 2012. Symptoms related to severe craving and cessation motivation are questioned for the past week, while other categories are assessed for the past year (28).

Data Analysis

The SPSS (Statistical Package for Social Sciences) for Windows 25.0 program was used for statistical analysis. Pearson Correlation Analysis, Independent t-test, and One-Way Analysis of Variance were used as hypothesis tests. The results were evaluated in the 95% confidence interval, and the significance was assessed as $p < 0.05$.

RESULTS

A total of 200 probationers in the study, consisting of 27 females and 173 males, aged between 18 and 65 years old, with a mean age of 32.32 ± 8.41 . 13.5% of the probationers were female (mean age: 30.07 ± 7.14), and 85.5% were male (mean age: 32.67 ± 8.55). Regarding education level, 39.5% had completed middle school, 52.5% were single, 33% were married, and 14.5% were divorced, 46% of the probationers were parents. 68% were currently employed, 36.5% had a history of traumatic events, 32% had a history of divorce within their families, 31.5% had a history of domestic violence, and 31.5% had experienced childhood neglect. 11% had a history of psychological treatment, 39% had engaged in self-harming behaviors, and 18% had attempted suicide. Furthermore, 39% had previously received a sentence for a crime, and 55.5% described themselves as "calm" (Table-1).

Results Regarding Probationers' Substance Use Characteristics

Probationers with methamphetamine use have a lifetime substance use rate that was determined to be 100%. The mean age at which probationers first tried a substance was found to be 20.65 ± 7.70 years old, with 65% trying marijuana for the first time and 22.5% trying methamphetamine. It was found that 68.5% of probationers had people in their family and environment who used substances, 33% were repeat offenders under probation, 71% had a friend under probation, and 64.5% engaged in multiple substance use. When the distribution of the probationers according to the duration of substance use is analyzed, It was observed that

40% had substance use between 1-5 years, 16% had substance use between 5-10 years, and 27% had substance use more than 10 years. The 'probationer' Addiction Profile Index (API) average score was 9.08 ± 3.99 . In this study, it was found that all participants had low addiction severity. Although the severity of addiction of the participants was low, the rate of high-risk substance use behaviors was found to be 86% (Table-2).

Results Related to Aggression Levels of Probationer

The total score obtained from the Buss-Perry Aggression Questionnaire (BPAQ) was found to be 76.36 ± 20.47 . The mean score of the physical aggression sub-dimension of the BPAQ was 22.28, the mean score of the verbal aggression sub-dimension was 14.09, the mean score of the anger sub-dimension was 18.10, the mean score of the hostility sub-dimension was 21.89. The highest mean score of the probationer was obtained from the physical aggression sub-scale. There was a statistically significant difference between the education levels of probationers and BPAQ verbal aggression sub-dimension scores ($F=3.067$; $p = 0.01 < 0.05$). According to the results of Post Hoc analysis, when the verbal aggression levels of probationers were analyzed according to their educational levels, it was found that the average of university graduates ($\bar{x}=15.42$) was higher than high school graduates ($\bar{x}=14.71$). Similarly, there was a significant difference between 'probationer's education levels and anger sub-dimension scores ($F=2.464$; $p<0.05$).

There was a statistically significant difference between the total score obtained from BPAQ and having a history of traumatic events ($t = 3.118$; $p<0.05$), domestic violence ($t = 6.113$; $p < 0.05$), childhood neglect ($t = 6.385$; $p<0.05$), self-harming behavior ($t=5.450$; $p<0.05$), suicide attempt ($t = 3.958$; $p<0.05$), presence of substance users in family and surroundings ($t = 3.819$; $p<0.05$) and Polysubstance use ($t=0.944$; $p<0.05$). There was a statistically significant difference in all sub-dimensions (physical aggression, verbal aggression, hostility, anger) of the BPAQ for the variables that have a history of traumatic events, domestic violence, childhood neglect, self-harming behavior, suicide attempt, presence of substance users in family and surroundings and Polysubstance use. There was a statistically significant difference between the total score

obtained from BPAQ and the number of polysubstance use ($t=.944$; $p<0.05$), as well as physical aggression ($t=4.942$; $p<0.05$), verbal aggression ($t=-2.903$; $p<0.05$), the hostility ($t=-3.879$; $p<0.05$) and anger ($t=-3.407$; $p<0.05$) sub-dimensions. In other words, it was found that the total aggression levels of multiple substance use of probationer ($\bar{x}=18.15$) were higher than single substance use of probationer ($\bar{x}=15.25$) (Table-3).

The relationship between the probationer' BPAQ total score ($\bar{x}=76.36$, $sd=20.47$) and API total score levels ($\bar{x}=9.08$, $sd=3.99$) was evaluated by Pearson Correlation. A moderate, positive and significant relationship was found between these

variables ($r_{(198)}=.552$, $p=.000$). It was concluded that there was a statistically moderate, positive and significant relationship between physical aggression, which is one of the sub-dimensions of BPAQ, and API total score ($r_{(198)}=.486$, $p=.000$), API- Substance Use Characteristics ($r_{(198)}=.498$, $p=.000$), API diagnosis ($r_{(198)}=.525$, $p=.000$), API effect on life ($r_{(198)}=.463$, $p=.000$) and API craving dimensions ($r_{(198)}=.548$, $p=.000$). In other words, as physical aggression increases, substance use total score, substance use characteristics, substance use diagnosis status, adverse effects of substance use on life and substance use desire increase (Table-4).

Table-1. Socio-demographic data of participants (n=200)

	Probationers with Methamphetamine Use (N=200)	
	Mean $\pm$ SD	
Age	32.32 $\pm$ 8.41	
	N	%
Sex		
Female	27	13.5
Male	173	86.5
Total	200	100
Education		
Literate	7	3.5
Primary School Graduate	37	18.5
Middle School Graduate	79	39.5
High School Graduate	70	35
University Graduate	7	3.5
Total	200	100
Marital Status		
Single	105	52.5
Married	66	33
Divorced	29	14.5
Total	200	100
Having Children		
Yes	92	46
No	108	54
Total	200	100
Employment Status		
Employed	136	68
Working irregularly	44	22
Unemployed	20	10
Total	200	100
History of Traumatic Events		
Yes	73	36.5
No	127	63.5
Total	200	100
Family Divorce History		
Yes	64	32
No	136	68
Total	200	100
Domestic Violence History		
Yes	63	31.5
No	137	68.5
Total	200	100

Childhood Neglect Experience		
Yes	63	31.5
No	137	68.5
Total	200	100
Psychological Treatment History		
Yes	22	11
No	178	89
Total	200	100
Self-Harming Behavior		
Yes	78	39
No	122	61
Total	200	100
Suicide Attempt		
Yes	36	18
No	164	82
Total	200	100
How do you describe yourself?		
Calm	111	55.5
Hyperactive	23	11.5
Irritable	17	8.5
Social	25	12.5
Introverted	24	12
Total	200	100
Previous Incarceration Status		
Yes	78	39
No	122	61
Total	200	100

Table-2. Substance use characteristics of offenders with methamphetamine use

Probationers with Methamphetamine Use (N=200)		
	Mean $\pm$ SD	
	N	%
Lifetime Substance Use		
Yes	200	100
Total	200	100
Presence of Substance Users in Family and Surroundings		
Yes	137	68.5
No	63	31.5
Total	200	100
Previous Probation Measure Status		
Yes	66	33
No	134	67
Total	200	100
Friend's Probation Measure Status		
Yes	142	71
No	58	29
Total	200	100

Lifetime Substance Use Duration

Less than 1 year	34	17
1-5 years	80	40
5-10 years	32	16
More than 10 years	54	27
Total	200	100

Substance Use Frequency

Almost every day	43	21.5
Most days of the week	39	19.5
Several days a week	67	33.5
Several days a month	44	22
Several days a year	7	3.5
Total	200	100

Marijuana Use Frequency

Never	40	20
At least one use	19	9.5
Three or more	141	70.5
Total	200	100

Polysubstance Use

Single Substance	71	35.5
Multiple Substances	129	64.5
Total	200	100

Addiction Profile Index (API) Severity of Addiction

Low Addiction Severity	7	3.5
Moderate Addiction Severity	20	10
High Addiction Severity	173	86.5
Total	200	100

Table-3. Comparison of probationers' aggression scores by some variables

Demographic Characteristics	n	BPAQ Total Score	Physical Aggression	Verbal Aggression	Hostility	Anger
Sex		Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD	Mean $\pm$ SD
		78.66 $\pm$ 22.69	22.14 $\pm$ 6.99	13.92 $\pm$ 4.49	22.85 $\pm$ 6.67	19.74 $\pm$ 7.61
Female	27	76 $\pm$ 20.15	22.30 $\pm$ 6.92	14.11 $\pm$ 3.57	21.75 $\pm$ 6.85	17.84 $\pm$ 5.56
Male	173		-.106	-.247		1.55
t		.62	.916	.805	.786	.121
p		.531			.433	

Education		Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
Literate	7	80.71±24.06	23.14±7.38	15.42±4.96	22.85±7.94	19.28±4.49
Elementary School	37	78.21±15.07	22.08±5.29	14.72±3.11	22.24±6.43	19.16±4.00
Gra.Middle School	79	71.78±20.12	21.62±6.95	13±3.47	20.63±6.88	1653±5.78
GraduateHigh School	70	79.44±22.32	22.85±7.46	14.71±3.96	22.65±6.87	19.21±6.62
Graduate University	7	83.14±21.82	24.14±8.97	15.42±4.96	25.57±5.44	18±6.40
F		1.757	0.458	3.067	1.473	2.464
p		0.139	0.767	0.01	0.21	0.04
PostHoc				4>3 (p<0.05)		4>3 (p<0.05)
History of Traumatic Events		Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
		82.19 ±20	23.72±6.96	15±3.77	23.67±6.77	19.79±5.92
Yes	73	73.01±20.05	21.44±6.78	13.56±3.57	20.86±6.66	17.13±5.67
No	127					
t		3.118	2.264	2.677	2.847	3.140
p		0.002	0.025	0.008	0.005	0.002
Domestic Violence		Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
Yes		88.36±17.58	25.61±6.57	16.04±3.52	25.34±5.60	21.34±5.19
No	63	70.84±19.36	20.74±6.54	13.18±3.43	20.29±6.76	16.61±5.60
t		6.113	4.886	5.422	5.163	5.675
p		0.000	0.000	0.000	0.000	0.000
Childhood Neglect Experience		Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
		88.80±18.33	25.76±7.37	16.04±3.29	25.23±5.76	21.76±5.18
Yes	63	70.64±18.85	20.67±6.08	13.18±3.53	20.35±6.74	16.42±5.43
No	137					
t		6.385	5.123	5.422	4.976	6.543
p		0.000	0.000	0.00	0.000	0.000
Psychological Treatment History		Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
		86±19.66	24.81±5.79	15.50±4.04	24.63±6.67	21.04±7.02
Yes	22	75.17±20.30	21.96±6.99	13.91±3.63	21.55±6.78	17.74±5.65
No	178					
t		2.367	1.835	1.906	2.015	2.512
p		0.019	0.068	0.058	0.045	0.013
Self-harming Behavior		Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
		85.58±18.46	25.97±6.15	15.29±3.37		
Yes	78	70.46±19.55	19.91±6.34	13.31±3.70	23.83±6.43	20.48±5.31
No	122	5.450	6.663	3.803	20.64±6.80	16.58±5.76
t		0.000	0.000	0.000	3.298	4.817
p					0.001	0.000

Suicide Attempt		Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
Yes	36	88.16±17.52	25.72±5.71	15.44±3.82	25.55±6.36	21.44±5.44
No	164	73.77±20.20	21.52±6.94	13.79±3.61	21.08±6.67	17.37±5.75
t		3.958	3.381	2.455	3.667	3.883
p		0.000	0.001	0.015	0.000	0.000
Presence of Substance Users in Family and Surroundings		Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
Yes	137	79.99±20.02	23.26±6.87	14.65±3.60	22.90±6.61	19.16±5.76
No	63	68.47±19.31	20.14±6.57	12.85±3.64	19.68±6.81	15.79±5.55
t		3.819	3.022	3.271	3.171	3.890
p		0.000	0.003	0.001	0.002	0.000
Lifetime Substance Use Duration		Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
Less than 1 year		61.32±17.17	16.70±5.92	12.73±3.51	17.64±6.80	14.23±4.94
1-5 years	34	74.58±18.72	21.47±6.15	13.73±3.70	21.57±6.06	17.80±5.90
5-10 years	80	87.50±17.32	26.03±6.18	15.75±3.16	24.81±5.87	20.90±5.54
More than 10 years	32	81.87±20.88	24.75±6.59	14.48±3.78	23.29±7.22	19.33±5.41
F	54	12.672	16.171	4.312	7.896	9.140
p		0.000	0.000	0.006	0.000	0.000
PostHoc		3>1, 4>1 (p<0.05)	2>1, 3>1, 4>1 (p<0.05)	3>1 (p<0.05)	3>1, 4>1 (p<0.05)	2>1, 3>1, 4>1 (p<0.05)
PolySubstance Use		Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
Single Substance	71	67.97±19.44	19.19±6.34	13.08±3.76	19.45±6.71	16.23±5.30
Multiple Substances	129	80.98±19.60	23.97±6.65	14.64±3.55	23.23±6.53	19.13±5.97
t		.944	4.942	-2.903	-3.879	-3.407
p		.000	0.000	.004	.000	0.001
Addiction Profile Index(API) Severity of Addiction		Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD	Mean ±SD
	7	63.28±18.21	20.14±6.06	11.42±1.90	17.57±7.97	14.14±4.48
Low Addiction Severity	20	63.65±15.10	17.80±5.88	12.70±3.61	18.45±5.06	14.70±4.49
Moderate Addiction Severity	173	78.36±20.45	22.88±6.88	14.35±3.70	22.46±6.81	18.65±5.91
High Addiction Severity		6.445	5.416	3.777	4.717	5.962
F		0.002	0.005	0.025	0.010	0.003
p		3>2 (p<0.05)	3>2 (p<0.05)	3>1 (p<0.05)	3>2 (p<0.05)	3>2 (p<0.05)
PostHoc						
Total	200					

F: Anova Test; t: Independent Samples T-Test; PostHoc:Tukey, LSD

Table-4. Correlation between Variables

	Mean	Sd	API- Total	API-Substance Use Characteristics	API- Diagnosis	API- Effects on Life	API- Craving	API- Motivation	BPAQ Total Score	Physical Aggression	Verbal Aggression	Hostility	Anger
API-Total	9.0892	3.99683	1										
API-Substance Use Characteristics	1.9068	1.59429	.708**	1									
API-Diagnosis	10.332	6.15325	.894**	.620**	1								
API-Effects on Life	21.27	11.46579	.886**	.559**	.804*	1							
API-Craving	5.14	4.54021	.829**	.591**	.738*	.680*	1						
API-Motivation	9.005	3.38114	.496**	.075	.262*	.312*	.150*	1					
BPAQ Total Score	76.365	20.47285	.552**	.540**	.525*	.463*	.548*	.075	1				
Physical Aggression	22.28	6.91961	.486**	.498**	.489*	.421*	.509*	-.016	.887*	1			
Verbal Aggression	14.09	3.70141	.441**	.424**	.385*	.368*	.396*	.139*	.790*	.580*	1		
Hostility	21.89	6.82722	.498**	.460**	.455*	.409*	.494*	.114	.888*	.666*	.676*	1	
Anger	18.105	5.89642	.493**	.490**	.481*	.410*	.483*	.059	.907*	.770*	.654*	.720*	1

**p<0.01; *p<0.05

**Correlation is significant at the 0.01 level (2-tailed).

DISCUSSION

The supervised probation program in Turkey, established in 2006, plays a crucial role in managing substance use disorders by focusing on socio-demographic factors, criminal records, and treatment outcomes (29). This program aims to correct criminal behavior, prevent recidivism, and rehabilitate substance abusers through regular urine analysis and psychosocial evaluations (30). Studies have shown that most individuals in these programs are male, young, with low education levels, and primarily use marijuana as their substance of choice (29, 31). Additionally, research highlights the importance of early education on substance abuse, social awareness, and the need for detailed psychological assessments for individuals with multiple substance use (29). Similar results were obtained in this study conducted to determine the aggression levels and substance use characteristics of methamphetamine users on probation.

The study found that 85.5% of the probationers were male. When reviewing research in the field of addiction, it's evident that substance use is

more prevalent among males compared to females (32; 33). Similarly, in some studies conducted with individuals under probation (34; 35), it's noteworthy that there is a higher proportion of male participants, with some samples consisting entirely of males. According to the 2021 data from TUBİM regarding methamphetamine use disorder in Turkey, it's shown that methamphetamine use is more common among males, with a rate of 92.9% (36). A study indicates that the proportion of women under probation increased from 31% in 2000 to 36% in 2010 (37). According to the 2023 profile research results of the General Directorate of Security, it's stated that the proportion of women among methamphetamine users is higher compared to other drug users. The same report also notes an increase in the proportion of women in the sample compared to the previous year in 2022. Therefore, there is a need for more research involving women in this context (36). When examining the rates of high severity of substance use obtained from API, it was found that 86.5% of the participants had high addiction severity. However, the total score averages from

API were found to be low, with a mean of 9.08 ± 3.99 . Considering this finding, it is observed that individuals using methamphetamine are aware of the addictive potential and harms of the substance, but they may not accurately reflect how they use it due to efforts to present themselves positively. Therefore, their levels of dependency may appear low. According to the research results, the average scores obtained from the API-effects of life subscale of methamphetamine users were higher at 21.27 ± 11.46 compared to other subscales. It is concluded that probationers have low insight into the harms of the substance and have low motivation for change. A study by Levin et al. (2006) examining the motivations of cocaine and marijuana users found that motivation varied depending on the type of substance used (38). The results obtained from the study show a significant difference between the total aggression scores with all subscales and traumatic event history, domestic violence, childhood neglect experiences, self-harm behaviors, suicide attempts, the presence of substance use in family and environment and the amount of substance use among individuals who are under probation due to methamphetamine use. In a study in 2009, it was found that children with a history of substance use in their parents are more likely to exhibit problematic behaviors (39). In a study examining the relationship between childhood trauma and aggression, it was reported that individuals exhibit aggressive behaviors to get rid of negative emotions (40). Gomez, in a study in 2011 examining the effect of childhood experiences on violent behavior in young adults, suggests that childhood traumatic experiences result in suicide attempts and involvement in violence-related crimes in adulthood (41).

The intricate and multifaceted connections between substance use and psychiatric or behavioral disorders have been a subject of significant interest for a long time and remain an active area of investigation, given the well-established impact of substances on user behavior. The connection between substance use and aggressive or impulsive behaviors is of particular significance. According to the results of the study, as the aggression scores of probationers increase, their levels of severity of addiction also increase. In a study conducted by Akan et al. (2019) for probationers, it is emphasized that practices that will reduce the

level of aggression and change the view of violence are very important rather than imposing sanctions on men who commit violence (42). In a study in 2016, examining the relationships between anger, depression, anxiety, and addiction severity in substance users, a positive correlation between BPAQ and API subscales was observed (43). It was found that as BPAQ scores increase, there is an increase in the subscale scores related to the impact of substance use on life, the intense desire for substance use, and motivation to quit substance use. Our findings are parallel with previous studies. It is observed that there is a significant difference between API high-risk substance use rates and total aggression scores, as well as scores for physical aggression, verbal aggression, hostility, and anger sub-dimensions among probationers. Additionally, it was determined that the total aggression and sub-dimension scores of probationers with high addiction severity were higher than those of probationers with moderate addiction severity. As the severity of addiction increases, health problems and problems in work and social life also increase. As the cost of substance use increases in the individual's life, acceptance of the problem may be more likely. However, it is thought that some of the probationers in the study may have experienced a decrease in the severity of addiction because some of them started probation measures within the first 3 days after the penal institution and some of them started probation measures after Alcohol and Substance Abuse Treatment Center (AMATEM) treatment. Therefore, it can be said that treatment or execution is effective in reducing the severity of addiction. Another explanation for the low addiction severity data obtained from our study could be that the participants were unable to respond to the questions impartially. It is considered possible that individuals under probation, whose judicial processes have not yet been finalized, may have provided biased answers out of fear that their probation might be terminated due to violations. Some limitations of our study should be acknowledged. Firstly, the assessment of aggression levels and substance use characteristics relied on self-report measures, which may be subject to recall bias or social desirability bias. Secondly, the sample size was limited, which may affect the generalizability of the findings. Thirdly, there was a smaller number of female participants compared to male

participants, which may limit the generalizability of the results to the broader population

CONCLUSION

In conclusion, methamphetamine is considered more dangerous due to its high potential for dependence, acute complications, and long-term neurotoxicity. Additionally, our findings suggest that methamphetamine increases aggression and leads to similar outcomes among individuals under probation. It is noteworthy that individuals who use methamphetamine may not disclose accurate information about their usage patterns, levels, and durations to avoid disclosure and exclusion, leading to lower reported dependence levels but higher risk usage. Therefore, incorporating forensic/analytical toxicological approaches alongside subjective reports could provide more reliable and concrete information about substance use. Furthermore, given the increased suicide risk among methamphetamine users with a history of past treatment, family and environmental substance use, and probation, comprehensive evaluations for addiction and suicide risk are essential to decrease these risks. Moreover, understanding

the risk factors associated with substance use and preventing substance dependence and promoting willingness to quit and seek treatment are crucial in probation sampling. While larger, longitudinal, multicenter studies are needed in this regard, it is believed that our research will also shed light on future studies.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Conflicts of interest: Authors declared no conflict of interest.

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FoxM1 inhibition by ThioStrepton downregulates DNA damage response genes, enhancing sensitivity of breast cancer cells to therapy

ThioStrepton ile FoxM1 inhibisyonu, DNA hasar yanıt genlerini baskılayarak meme kanseri hücrelerinin tedaviye duyarlılığını artırır

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ABSTRACT

Aim: Chemotherapy resistance, often linked to the development of resistance against genotoxic agents, is a major obstacle in cancer treatment. FoxM1, a transcription factor frequently overexpressed in malignancies such as breast cancer, is strongly associated with genotoxic therapy resistance. The aim of this study is to conduct a comparative analysis of the effects of thioStrepton (THIO), a FoxM1 inhibitor, on the DNA damage response in HUVEC cells (non-malignant) and MDA-MB-231 breast cancer cells (malignant)

Materials and Methods: THIO's impact on cell viability were evaluated in both cell lines using the MTT assay. Oxidative DNA damage levels were measured with the 8-OHdG kit, and apoptosis was assessed using the Caspase 3 ELISA kit. The expression levels of DNA damage response genes (BRCA-1, DNAPKC, FOXM1, RAD51, MRE11 and XRCC1) were analyzed by RT-PCR.

Results: MDA-MB-231 cells exhibited greater sensitivity to the cytotoxic effects of THIO than HUVEC cells. In HUVEC cells, THIO caused a significant increase in oxidative DNA damage, whereas no such effect was observed in MDA-MB-231 cell lines. Conversely, breast cancer cells showed a significant increase in Caspase 3 levels. RT-PCR results revealed a marked downregulation of DNA damage response genes, particularly BRCA-1, DNAPKC, MRE11, FOXM1, and XRCC1, in both cell types.

Conclusion: THIO has been shown to inhibit FoxM1 expression and downregulate DNA damage response genes in both malignant and non-malignant cells, demonstrating its potential to enhance the sensitivity of breast cancer cells to therapy by disrupting DNA repair pathways. However, its potential to induce oxidative damage in non-malignant cells underscores the need for further comprehensive studies to validate its therapeutic efficacy and assess its safety in normal tissues.

Keywords: FoxM1, resistance to chemotherapy, ThioStrepton, breast cancer, DNA damage response

This study was presented as an oral presentation at the 11th International Medicine and Health Sciences Research Congress (UTSAK), held on December 24-25, 2022, in Ankara.

ÖZ

Amaç: Kemoterapi direnci, genellikle genotoksik ajanlara karşı direnç gelişimiyle ilişkilendirilir ve kanser tedavisinde önemli bir engel teşkil eder. FoxM1, meme kanseri gibi malignitelerde sıkça aşırı eksprese edilen ve genotoksik tedavi direnci ile güçlü bir şekilde ilişkili olan bir transkripsiyon faktörüdür. Bu çalışmanın amacı, FoxM1 inhibitörü olan thioStrepton'un (THIO) malign olmayan HUVEC hücreleri ile malign MDA-MB-231 meme kanseri hücrelerinde, DNA hasar yanıtı üzerindeki etkilerini karşılaştırmalı olarak analiz etmektir.

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Gereç ve Yöntem: THIO'nun hücre canlılığı üzerine etkileri her iki hücre hattında MTT testi kullanılarak değerlendirildi. Oksidatif DNA hasar seviyeleri 8-OHdG kiti ile ölçülürken, apoptoz seviyeleri Caspase 3 ELISA kiti ile belirlendi. DNA hasar yanıt genlerinin (BRCA-1, DNAPKC, FOXM1, RAD5, MRE11, ve XRCC1) ekspresyon düzeyleri RT-PCR yöntemiyle analiz edildi.

Bulgular: THIO'nun, MDA-MB-231 hücrelerinde HUVEC hücrelerine kıyasla daha belirgin sitotoksik etki gösterdiği gözlemlendi. HUVEC hücrelerinde THIO, oksidatif DNA hasarında anlamlı bir artışa neden olurken, MDA-MB-231 hücrelerinde bu tür bir etki gözlenmedi. Buna karşın, meme kanseri hücrelerinde Caspase 3 seviyelerinde belirgin bir artış saptandı. RT-PCR analizleri, DNA hasar yanıt genlerinin, özellikle BRCA-1, DNAPKC, MRE11, FOXM1, ve XRCC1'in ekspresyonunda her iki hücre hattında anlamlı bir düşüş olduğunu gösterdi.

Sonuç: THIO, hem malign hem de malign olmayan hücrelerde FoxM1 ekspresyonunu inhibe ederek DNA hasar yanıt genlerini baskılamış ve DNA onarım yollarını bozarak meme kanseri hücrelerinin tedaviye duyarlılığını artırma potansiyeli sergilemiştir. Bununla birlikte, malign olmayan hücrelerde oksidatif hasar oluşturabilme potansiyeli, bu ajanının terapötik etkinliğinin doğrulanması ve normal dokular üzerindeki güvenliğinin değerlendirilmesi için daha kapsamlı araştırmaların gerekliliğine işaret etmektedir.

Anahtar sözcükler: FoxM1, kemoterapi direnci, Thiostrepton, meme kanseri, DNA hasar yanıtı

INTRODUCTION

Breast cancer ranks among the most prevalent cancers affecting women globally (1). The triple-negative breast cancer (TNBC) subtype is particularly challenging due to its poor prognosis, frequent recurrences, and limited treatment options (2). TNBC tumors are typically more sensitive to chemotherapy, but less responsive to targeted therapy than other breast cancer subtypes. Consequently, individuals diagnosed with TNBC face a greater likelihood of distant metastasis, shorter overall survival, and higher mortality rates (3). The diverse molecular characteristics and absence of specific therapeutic targets in TNBC present major obstacles for treatment development. Despite progress in understanding TNBC biology and discovering potential therapeutic targets, there remains a substantial need for effective therapies for TNBC patients.

Numerous studies have indicated that the FoxM1 transcription factor is critical in fostering resistance to chemotherapies that inflict DNA damage, and can facilitate the development of drug resistance if its activation or expression becomes aberrant (4,5). FoxM1 orchestrates DNA repair processes by regulating the transcription of various genes involved in detecting, mediating, signaling, and repairing DNA damage (6,7). FoxM1 has been shown to promote DNA repair by regulating the transcription of its direct target, EXO1, which helps protect ovarian cancer cells from apoptosis induced by cisplatin (8). Cells lacking FoxM1 show

reduced levels of XRCC1 and BRCA2 gene expression. Additionally, phosphorylation of FoxM1 by checkpoint kinase 2 (Chk2) occurs in response to DNA damage (6). Notably, studies have shown that FoxM1 indirectly stabilizes the MRN subunits, which are essential for the homologous recombination repair process (5). Given its pivotal role in the DNA damage response, FoxM1 is a key regulator of genotoxic resistance and presents a promising target for therapies designed to overcome resistance to genotoxic agents

Thiazole antibiotics like Thiostrepton (THIO) and Siomycin A are known to reduce mRNA and protein levels of FoxM1, leading to cell death by inhibiting its transcriptional activity (9,10). THIO is a natural thiopeptide antibiotic isolated from *Streptomyces* species and is used in veterinary applications to prevent bacterial infections (11,12). In addition to its antibacterial properties, THIO exhibits anticancer activity by inducing apoptosis, autophagy, and proteotoxic stress (13,14). THIO is also a specific inhibitor of the FoxM1 transcription factor, which is overexpressed in cancer cells. It has been shown that THIO directly or indirectly suppresses cancer cell proliferation through FoxM1 inhibition, reduces the metastatic ability of cancer cells by preventing epithelial-mesenchymal transition, and inhibits tumor growth (15–17). Although THIO has been reported to have important effects in cancer studies, its cytotoxic and genotoxic effects on non-malignant healthy cells are unknown.

The current study aimed to explore and contrast the cytotoxic and genotoxic responses of HUVECs and MDA-MB-231 cells when exposed to THIO. By evaluating cell viability, DNA damage responses, and apoptotic changes, we intended to clarify whether THIO exerts different effects on healthy endothelial cells and aggressive breast cancer cells. Comprehending these differential impacts may be paramount in assessing the therapeutic possibilities of using THIO to inhibit FoxM1 and overcome resistance to genotoxic agents.

MATERIALS AND METHODS

Cell culture and test chemicals

This study utilized MDA-MB-231 cells, which are a malignant breast cancer cell line, and HUVEC cells, representing a non-malignant cell line. Both cell types were grown in RPMI 1640 medium, which was enriched with 10% fetal bovine serum (FBS, sourced from the US), 100 units/mL of penicillin, and 100 µg/mL of streptomycin. The cultures were kept at 37°C in an atmosphere with 5% CO₂, ensuring their optimal growth and maintenance. THIO was procured from Enzo Life Sciences and dissolved in DMSO to prepare a 10 mg/ml stock solution, with the required dilutions made for all experiments. DMSO (Dimethyl sulfoxide) was obtained from Sigma-Aldrich (St. Louis, MO, USA).

Cell viability assay

The assessment of cell viability was carried out using the MTT assay. Cells were seeded at a concentration of 5x10³ cells in each well of 96-well plates, followed by exposure to different THIO concentrations (1.25µM-40 µM) at 37°C for durations of 24 and 48 hours. At the highest concentration of THIO applied to the cells, the DMSO percentage was 0.67%, which remains below 1% and is widely considered safe for most cell lines in terms of cytotoxicity. Notably, the highest concentration of DMSO was used in our control group. After completing the treatment, MTT solution (5 mg/ml) was added to each well and incubated for 3–4 hours. Following incubation, the medium was carefully removed, and 100 µL of DMSO was added to each well to dissolve the formazan crystals. The plates were subsequently placed on an orbital shaker for 5 minutes, and the absorbance was measured utilizing a spectrophotometer. The viability of control cells

(those not treated with THIO) was designated 100%, and the viability of the cells exposed to THIO was expressed as a percentage relative to this control.

Real-time PCR analysis

Following a 24-hour treatment with 5 µM THIO, total RNA was extracted by employing TRIzol reagent (Invitrogen), with slight modifications to the originally published protocol (Chomczynski and Sacchi, 1987). The purity and concentration of the RNA were assessed using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Complementary DNA (cDNA) was subsequently synthesized utilizing a cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Waltham, MA, USA). Real-time PCR amplification was executed using a light cycler instrument (Bio-Rad) with HOT FIREPol EvaGreen qPCR Mix Plus (no ROX) (SolisBioDyne, Inc.). Primers specific to BRCA1, DNAPKC, FoxM1, H2AX, MRE11, RAD51, and XRCC1 genes were designed with Oligo7 software, and their sequences were as follows:

BRCA1 forward primer - GACAGGCTGTGGGGTTTCTC; BRCA1 reverse primer - TCACACTTTGTGGAGACAGGT;
 DNAPKC forward primer - TAGGGGAAAGCCATTGCCAG; DNAPKC reverse primer - GGGATCACTCAGGTAAGCCG;
 FoxM1 forward primer - AGGGTCTCCACAATTGCCCG; FoxM1 reverse primer - CCCAGGGGGAGTTCGGTTTT;
 H2AX forward primer - TTCACCGGTCTACCTCGCTA; H2AX reverse primer - CGGGCCCTCTTAGTACTCCT;
 MRE11 forward primer - GGACGCCGTTCTCTCCC; MRE11 reverse primer - TGGGACCAGGTTCTTCCTCA;
 RAD51 forward primer - AGACCGAGCCCTAAGGAGAG; RAD51 reverse primer - AGTTGCAGTGGTGAAACCCA;
 XRCC1 forward primer - CCACCACTGCTCCAGGAAG; XRCC1 reverse primer - TGTCCGTGTTCTCATCCGTG.

GAPDH served as the internal reference gene. All experiments were performed in triplicate. Gene expression levels were normalized relative to GAPDH (Δ Ct values), and fold changes in mRNA expression were quantified employing the 2^{- $\Delta\Delta$ Ct} method.

Measurement of 8-OH-dG and Caspase-3 levels in cell lysates

The quantification of oxidative DNA damage was carried out utilizing the 8-OHdG ELISA kit (BT Lab

no.: EA0048Hu), whereas caspase 3 activity was assessed with the caspase ELISA kit (Sunred Bio Cat No:201-12-2249). For both measurements, cells were seeded into 6-well plates at a density of 5×10^5 cells per well. Subsequent to a 24-hour incubation with 5 μ M THIO, the cells were transferred into sterile tubes and centrifuged. The cell pellets were then rinsed with phosphate buffered saline, and cell lysis was achieved through three successive freeze-thaw cycles. After centrifugation (2000 rpm for 20 min), the lysate supernatants were collected and stored at -20°C . Finally, the analyses were performed based on the kit instructions, with spectrophotometric measurements at a wavelength of 450 nm.

Statistical Analysis The statistical differences observed between the experimental groups and the control group were evaluated using Student's t-test (GraphPad Software, Boston, Massachusetts USA). Statistical significance was defined as a p-value under 0.05.

RESULTS

Assessment of cell viability

The anti-proliferative effects of THIO on HUVEC and MDA-MB-231 cell lines were evaluated using the MTT assay (Figure 1a, 1b). After incubation with the specified concentrations of THIO, the viability of MDA-MB-231 cells dropped below 50% at distinct concentrations depending on the time of exposure. At 24 hours, cell viability decreased below 50% at a concentration of 40 μ M, reaching 50.97%. In contrast, after 48 hours of incubation, cell viability dropped below 50% at a much lower concentration of 2.5 μ M, further emphasizing the time-dependent inhibitory effects of THIO on cell viability. In contrast, HUVEC cells exhibited a dose- and time-dependent decrease in viability, but the concentrations required to reduce viability below 50% were higher compared to MDA-MB-231 cells. At 24 hours, cell viability remained above 50% at all tested concentrations, with the lowest recorded viability being 71.24% at 40 μ M. However, after 48 hours, cell viability dropped below 50% at a concentration of 5 μ M, reaching 30.62%. This highlights the relatively greater resistance of HUVEC cells to THIO compared to MDA-MB-231 cells, demonstrating that malignant cells are more sensitive to THIO.

Figures and Legends

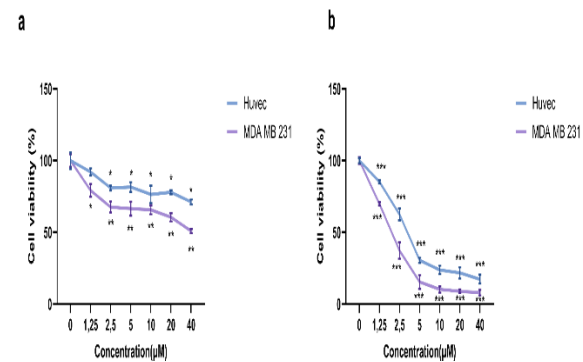


Figure-1. Effects of THIO on the cell viability of HUVEC and MDA-MB-231 cells after 24 hours (a) and 48 hours (b) of treatment. Data are shown as the mean $\pm$ SEM obtained from four separate experiments (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.0001$).

Evaluation of 8-OHdG and Caspase-3 level in cell lysates

Figure-2a illustrates the levels of 8-OHdG and Caspase-3 in lysates derived from HUVEC and MDA-MB-231 cells that were treated with 5 μ M THIO. The findings revealed that exposure to 5 μ M THIO resulted in a notable increase in oxidative DNA damage within HUVEC cells (p -value = 0.0496). Conversely, the breast cancer cells demonstrated a non-significant reduction in oxidative DNA damage compared to the control (p = 0.5972). As depicted in Figure 2b, treatment with 5 μ M THIO did not induce a significant change in Caspase-3 levels in HUVEC cells. However, a noticeable increase in Caspase-3 was observed within the breast cancer cells (p = 0.0072).

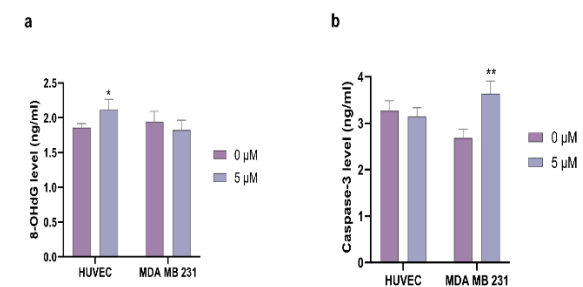


Figure-2. Levels of 8-OHdG (a) and Caspase-3 (b) in HUVEC and MDA-MB-231 cells treated with THIO for 24 hours. The values are reported as the mean $\pm$ SD (* $p < 0.05$, ** $p < 0.01$).

Assessment of DNA Damage Response Gene Expression

Figure-3 demonstrates the alterations in the expression patterns of DNA damage response genes and FoxM1 in both HUVEC and MDA-MB-231 cells after a 24-hour exposure to 5 μ M THIO. In line with expectations, THIO significantly reduced FoxM1 expression in both cell lines; the reduction was more marked in the malignant cell line, with a 10.10-fold decrease, compared to a 1.82-fold decrease in HUVEC. Notably, THIO's impact on DNA damage response genes varied between cell lines. In HUVEC cells, THIO induced suppression of BRCA1, DNAPKC, MRE11, and XRCC1 expression, with reductions of 3.37-, 2.38-, 2.01-, and 3.29-fold, respectively. Conversely, in MDA-MB-231 cells, all assessed DNA damage response genes (BRCA1, DNAPKC, H2AX, MRE11, Rad51, and XRCC1) experienced significant downregulation, with fold changes spanning from 1.52 to 3.04.

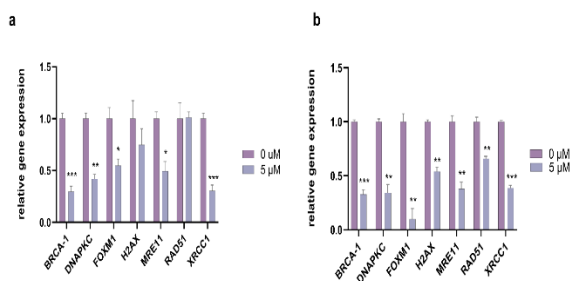


Figure-3. Expression levels of DNA damage response genes in (a) HUVEC and (b) MDA-MB-231 cells following treatment with THIO. The mean $\pm$ SD is used to present the data (* p <0.05, ** p <0.01, and *** p <0.001).

DISCUSSION

This investigation focused on contrasting the potential of FoxM1 inhibitor THIO in influencing DNA damage responses across both non-malignant and malignant cells. Cell viability was markedly decreased by THIO treatment in both cell lines, with a clear dependence on both dose and duration. In various studies evaluating the cytotoxic effects of THIO, the IC₅₀ value has been observed to range between 3-10 μ M in cancer cells (18–20). Consistent with the literature, we found that 2.5-5 μ M THIO significantly affected cell viability, especially after 48-hour treatment. However, THIO showed a higher cytotoxic effect on MDA-MB-231 cells than on HUVEC. These findings suggest that THIO's cytotoxic impact may

vary in malignant (MDA-MB-231) and non-malignant (HUVEC) cell lines, with MDA-MB-231 cells displaying higher sensitivity or susceptibility to THIO. The stronger effect observed in malignant cell lines such as MDA-MB-231 may support the idea that THIO could be an effective agent against cancer cells. However, observing cytotoxic effects at 48 h of treatment on HUVEC cells also indicates the need to consider potential side effects during treatment or the risk of affecting normal tissues.

Oxidative DNA damage may occur due to various factors. Free radicals, which are reactive molecules generated during metabolic processes or from external sources, such as radiation, certain chemicals, mitochondrial dysfunction, and pollutants, can attack DNA molecules and cause oxidative damage. (21). There are several cellular pathways responsible for repairing oxidative DNA damage, such as base excision repair (BER), single-strand break repair (SSBR), and nucleotide excision repair (NER)(22). Our findings revealed that oxidative DNA damage levels were higher in HUVEC cells compared to malignant MDA-MB-231 cells. Notably, the malignant cells exhibited no significant alterations in oxidative DNA damage, suggesting differential responses to THIO treatment between the two cell types. Previous studies have demonstrated that silencing FoxM1 can induce oxidative stress in cells(23,24). In our study, although THIO treatment significantly suppressed FoxM1 mRNA expression in both cell types, it is possible that THIO may have an indirect effect on oxidative damage and repair in these cells, independent of FoxM1. Additionally, cancer cells have been reported to exhibit high ROS-scavenging activity (23,25), which may contribute to their ability to mitigate oxidative stress. This may explain the lack of significant changes in oxidative DNA damage observed in our study in malignant MDA MB 231 cells. Furthermore, the differential metabolic activity and antioxidant defense mechanisms between malignant and non-malignant cells could also play a role, as cancer cells often adapt their redox homeostasis to support survival under stress. (26). Further investigations are essential to elucidate the comprehensive effects of THIO on oxidative DNA damage and repair pathways, as well as its implications for therapeutic applications in cancer and normal cells.

The difference in caspase 3 levels is also noteworthy. The increased level of caspase 3 in

breast cancer cells suggests that THIO may induce apoptosis in these cells. Studies have shown that THIO induces apoptosis in certain cancer cell lines (13, 19, 27). This suggests that THIO may promote apoptosis in these cells, leading to inhibition of uncontrolled cell growth. The change in caspase 3 levels in HUVEC cells was not statistically significant, indicating that THIO may not effectively activate apoptotic pathways in these non-malignant cells. This finding aligns with our cytotoxicity analyses, which demonstrate that breast cancer cells exhibit a markedly more sensitive response to THIO treatment. The exact mechanism by which THIO affects caspase 3 levels is not yet fully understood. However, THIO may interfere with specific pathways or signaling molecules involved in caspase activation and apoptosis (28).

Our real-time PCR results indicated that THIO significantly suppressed the expression of BRCA1, DNAPKC, MRE11, and XRCC1, which play a crucial role in the DNA damage response, in both cell lines. However, we found that H2AX and RAD51 damage-response genes were also suppressed in breast cancer cells. THIO, a FoxM1 inhibitor, decreased its expression in both cell types. Recent studies have revealed that the oncogenic transcription factor, FoxM1, plays a pivotal role in chemotherapy resistance associated with DNA damage. Aberrant activation or expression of FoxM1 has been shown to potentially promotes drug resistance (4,5). The ability of FoxM1 to induce DNA repair encompasses transcriptional control of a network of genes involved in detecting, mediating, signaling, and repairing DNA damage (6,7). Kuthetuher et al. revealed that targeting FoxM1 using miR-4521 mimics could potentially serve as a novel therapeutic approach in breast cancer by modulating the DNA damage response (29). Wang et al. demonstrated that inhibiting FoxM1, either through shRNA or FDI-6 inhibitor, sensitizes BRCA- expressing TNBC cells to the PARP inhibitor olaparib by reducing DNA damage repair (30). In another study evaluating the anticancer effects of niclosamide on prostate cancer, it was

found that inhibition of FoxM1 significantly reduced the expression of genes involved in DNA repair, particularly those associated with homologous recombination (31). These findings indicate that FoxM1 regulates the expression of genes critical for the cellular DNA damage response and that inhibition of FoxM1 may enhance the therapeutic sensitivity of cancer cells through suppression of DNA repair.

CONCLUSION

In conclusion, our study highlights the differential effects of THIO on DNA damage response and cellular mechanisms in malignant and non-malignant cell lines. While the findings provide valuable insights into the therapeutic potential of targeting FoxM1 via THIO in cancer treatment, the study is based on gene expression analyses. Protein-level validation of these findings is crucial to achieving a more in-depth understanding of the mechanisms underlying THIO's effects and its therapeutic potential in cancer treatment. Further research is needed to fully elucidate the complex interplay between THIO, FoxM1, and DNA damage response pathways.

Author contributions

FDK was involved with the conceptualization of the study, developing the methodology, and was responsible for the initial draft of the manuscript. ZD contributed to the conceptualization and methodology of the study, as well as the preparation of the manuscript. FMI was the supervisor of the project.

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Declarations

Conflict of interests

The authors declare that they have no conflicts of interest.

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GBA1 variants and Parkinson's Disease: A dual approach combining clinical data and literature review

GBA1 varyantları ve Parkinson Hastalığı: Klinik veriler ve literatür incelemesini birleştiren çift yaklaşım

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ABSTRACT

Aim: The *GBA1* (*ENSG00000177628*) gene, a key susceptibility gene in Parkinson's disease (PD) and closely linked to Gaucher disease, has garnered significant research attention over the past two decades. Mutations in *GBA1* are associated with an elevated risk of developing PD. This study aims to elucidate genetic mutations related to PD in a cohort of patients undergoing clinical exome analysis. We aimed to investigate the clinical and genetic profiles of patients with Parkinson's disease, focusing on *GBA1* gene mutations and additional neurodegenerative-related genetic alterations.

Materials and Methods: Fourteen patients with a preliminary diagnosis of Parkinson's disease underwent clinical exome analysis. The research included genetic testing to identify variants of uncertain significance, likely pathogenic mutations, and pathogenic mutations in the *GBA1* gene. Further genetic alterations were evaluated in patients with positive *GBA1* mutation results.

Results: 14 out of 177 diagnosed individuals had *GBA1* mutations. The group had 8 men and 6 women with a mean onset age of 53.7 years. Tremor, stiffness, bradykinesia, hypomimia, and postural instability predominated. Dopamine agonists were often administered for symptoms. Disorders like depression, anxiety, hypercholesterolemia, and diabetes were frequent. *GBA1* gene study found many missense variants, with the p.Asn409Ser (N370S) mutation being the most common. Four individuals also had mutations in *EIF4G1*, *APP*, and *SLC20A2*, demonstrating a complex genetic landscape affecting PD.

Conclusion: Our research highlights PD's genetic diversity, particularly *GBA1* mutations' role in disease onset and progression. Additional genetic variants may worsen PD symptoms. Further research is needed on these mutations' pathogenicity and consequences on patient care.

Keywords: Parkinson's disease, *GBA1* gene, glucocerebrosidase, clinical exome analysis, motor symptoms.

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ÖZ

Amaç: Parkinson hastalığında (PH) önemli bir duyarlılık geni olan ve Gaucher hastalığıyla yakından ilişkili olan GBA1 (ENSG00000177628) geni, son yirmi yılda önemli bir araştırma ilgisi topladı. GBA1'daki mutasyonlar, PH geliştirme riskinin artmasıyla ilişkilidir. Bu çalışma, klinik ekzom analizi geçiren bir hasta kohortunda PH ile ilişkili genetik mutasyonları açıklamayı amaçlamaktadır. GBA1 gen mutasyonlarına ve ek nörodejeneratif ilişkili genetik değişikliklere odaklanarak Parkinson hastalığı olan hastaların klinik ve genetik profillerini araştırmak amaçlanmıştır.

Gereç ve Yöntem: Parkinson hastalığı ön tanısı olan on dört hasta klinik ekzom analizinden geçti. Araştırma, belirsiz öneme sahip varyantları, muhtemel patojenik mutasyonları ve GBA1 genindeki patojenik mutasyonları belirlemek için genetik test içeriyordu. Pozitif GBA1 mutasyon sonuçları olan hastalarda daha fazla genetik değişiklik değerlendirildi.

Bulgular: Araştırmamız PH'nin genetik çeşitliliğini, özellikle GBA1 mutasyonlarının hastalığın başlangıcında ve ilerlemesindeki rolünü vurgulamaktadır. Ek genetik varyantlar PH semptomlarını kötüleştirebilir. Bu mutasyonların patojenitesi ve hasta bakımı üzerindeki sonuçları hakkında daha fazla araştırmaya ihtiyaç vardır.

Sonuç: Teşhis konulan 177 kişiden 14'ünde GBA1 mutasyonu vardı. Grupta ortalama başlangıç yaşı 53,7 yıl olan 8 erkek ve 6 kadın vardı. Titreme, sertlik, bradikinezi, hipomimi ve duruş bozukluğu baskındı. Semptomlar için genellikle dopamin agonistleri uygulandı. Depresyon, anksiyete, hiperkolesterolemi ve diyabet gibi bozukluklar sıklıkla görüldü. GBA1 gen çalışması, p.Asn409Ser (N370S) mutasyonunun en yaygın olduğu birçok anlamsız varyant buldu. Dört kişide ayrıca EIF4G1, APP ve SLC20A2 mutasyonları vardı ve bu da PH'yi etkileyen karmaşık bir genetik manzarayı göstermektedir.

Anahtar sözcükler: Parkinson hastalığı, GBA1 geni, glukoserebrosidaz, klinik ekzom analizi, motor semptomlar

INTRODUCTION

Parkinson's disease (PD) is the second-most common neurodegenerative disorder. Defining features of PD include bradykinesia with tremor, rigidity, or postural instability. On the basis of the pathology, dopaminergic neuron loss in the substantia nigra (SN), alpha synuclein accumulation, and Lewy body formation are evident (1). Although the majority of cases occur at random, about 15% of individuals carry a gene that causes the condition (OMIM; 168600). Familial and early-onset types are more likely to be genetic. Disease incidence ranges from 8 to 18 cases per 100,000 people-years. Less than one percent of those over the age of 60 have PD, and the prevalence rises with age (1, 2).

There are 33 current PD phenotypes (PS168600) in OMIM: one third is inherited as an autosomal recessive form, one form is X-linked, and the remainder, which constitutes the majority, is inherited as an autosomal dominant form. Inheritance associated with the GBA1 gene, identified as the late-onset PD susceptibility gene with PMIM number 168600, has been described as autosomal dominant.

Parkinson's disease and other comparable illnesses are often linked to glucocerebrosidase (GBA1) gene mutations, which encode the lysosomal enzyme defective in Gaucher disease. Rare cases of Gaucher disease and their obligatory carriers showed parkinsonism in the clinic, revealing this relationship. Findings from extensive studies indicate that individuals with PD and Lewy body-related disorders exhibit a higher prevalence of GBA1 mutations when compared to control subjects (3).

The incidence of the GBA1 mutation in PD varies between populations. The GBA1 mutation has been detected in approximately 10–15% of patients, followed by a diagnosis of PD. The penetrance of GBA1 mutations and the lifetime risk of developing PD vary with age. In one study, the frequency of GBA1 mutation in PD patients over the age of 80 was approximately 30%, and in another study, the frequency of mutation in the GBA1 gene in the PD population was around 3% (4-6).

The risk of PD is increased 20-fold in people with Gaucher disease. The risk of PD at age 65 is 1%, so 20% of people with Gaucher will have PD by age 65. However, 80% of people with Gaucher's

disease will not have Parkinson's disease at age 65. People with a *GBA1* mutation have a 5-8 times higher risk of PD. 5–8% of people with a *GBA1* mutation will have PD. However, 92–95% of patients will not have PD by age 65 (4, 7).

***GBA1* Gene**

A gene-dense area located on chromosome 1q21, measuring 7.6 kb, encompasses the *GBA1* gene, in addition to nine other genes and two pseudogenes within a 100-kb segment. Though the GGCGGG motif is absent from the *GBA1* promoter region, around 250 base pairs upstream of the ATG start site, there are potential TATA and CAAT-like boxes.

Within the intergenic space that separates the *GBA1* and *MTX1* genes are two pseudogenes, *GBA1P* and *MTX1P*. It seems that a duplication event, about 27–40 million years ago on average, gave rise to these pseudogenes.

Other animals do not possess these pseudogenes, with the exception of humans and primates.

Intronic Alu sequences are found in *GBA1*, yet they are absent from the *GBA1P* gene sequence. These sequences represent a type of transposon that has the potential to relocate within DNA during the course of evolutionary processes. *GBA1P* exhibits a 96% sequence similarity with *GBA1*; however, the lengths of these sequences are limited to just 5 kb.

Additionally, the pseudogene is delineated from the *GBA1* gene by a deletion of 55 base pairs, which is flanked by a brief inverted repeat within exon 9. GCase, a lysosomal hydrolase composed of 497 amino acids, is the functional protein synthesized by *GBA1* through the process of transcription.

The primary role of GCase involves the hydrolysis of glucosylceramide into ceramide and glucose, while also exhibiting the capability to cleave glucosylsphingosine and possibly other β -glucosides. (Human genome assembly hg38, chromosome 1, positions 155234452 to 155241249, UCSC Genome Browser version 462)

Effects of *GBA1* Gene Mutations on Glucocerebrosidase Structure and Function

In 2018, over 300 *GBA1* gene mutations and rearrangements were discovered. These mutations and rearrangements were classified into three types: type I, type II, and type III

Gaucher disease (GD). The term "severe *GBA1* mutations" refers to genetic abnormalities that, when inherited from both progenitors, result in profound signs of Gaucher disease, more precisely types II or III. Subtle genetic differences, on the other hand, lead to a manifestation of the disorder that is less severe when it is passed down in either a homozygous or compound heterozygous form, which ultimately results in Gaucher disease type I (8, 9).

Furthermore, certain variants exhibit ambiguous functions in GD while distinctly serving as risk factors for PD, with E326K being the most prominent example. The prevalent mutations associated with *GBA1*-related PD include N370S, L444P, D409H, R120W, V394L, 84insGG, IVS2+1 G→A, IVS10-4 C→T, IVS10-1 G>A, V460V, and R496H (8).

Different *GBA1* mutations affect GCase's enzymatic activity in their own unique ways. There are cases when the range of enzymatic activity seen in moderate and severe *GBA1* mutations overlaps, and there are other cases where the degree of enzymatic activity does not correlate with the severity of GD. Assessing GCase activity exclusively within the lysosomal environment could yield a more precise understanding of GCase's true biological role. Furthermore, factors related to genetics, biology, or the environment, aside from residual GCase activity, could influence the severity of the disease (7, 10).

***GBA1* Gene and Potential Mechanisms of Parkinson's Disease**

GBA1 causes neurodegeneration via many mechanisms. Mazzulli et al. found that glucosylceramide, a GCase substrate, may increase α -synuclein and decrease GCase activity. Regardless of whether they are carriers of the *GBA1* mutation or not, studies show that α -synuclein overexpression reduces GCase activity in cell models and animals. PD patients, on the other hand, have lower GCase levels in brain tissue, cerebrospinal fluid, and peripheral blood than controls (10).

GCase inhibitors promote α -synuclein aggregation in human iPSc neural models. Recent research suggests a neurotoxic cycle between α -synuclein and GCase, which may partly explain *GBA1* PD, although the sensitivity of particular neuron types remains unclear. Studies have not found elevated

glucosylceramide concentrations in *GBA1* heterozygotes (7, 10, 11).

Another mechanism by which *GBA1* mutations may lead to PD is through the disorder of ER-associated degradation and the cell death associated with ER stress. The buildup of α -synuclein can lead to endoplasmic reticulum stress, hinder the degradation of ER-associated degradation substrates, and disrupt the trafficking between the endoplasmic reticulum and Golgi apparatus. Certain genes associated with PD, including *PARK2*, are involved in the endoplasmic reticulum-associated degradation process, which corroborates this finding (12).

It is highly likely that ER stress and ERAD contribute significantly to the development of PD. In this context, the detection of ER involvement in experiments with certain mutated forms of GCase indicates that ER stress may contribute to the pathogenesis of PD in individuals carrying specific *GBA1* mutations. Mutated GCase has been observed to interact with parkin, facilitating the accumulation of GCase (12). The proposed processes are called into question by the fact that certain mutations, such as 84GG, IVS2+1, R359X, and others, increase the likelihood of developing PD yet do not produce a protein product. The ER stress noted in models exhibiting *GBA1* mutations may stem from the buildup of α -synuclein, rather than from GCase itself. *GBA1* mutations may enhance susceptibility to PD through multiple pathways, with each mechanism potentially playing a role in the progression of the disease (7, 10, 13). The precise mechanism underlying this relationship remains ambiguous. In conclusion, it is proposed that there are interactions involving α -synuclein, lysosomal dysfunction, endoplasmic reticulum stress, and ceramide metabolism.

Synucleinopathies and the *GBA1* Gene

GBA1-REM Sleep Disorder

The majority of individuals with REM Sleep Behavior Disorder (RBD) are predisposed to developing synucleinopathies, including PD, Dementia with Lewy Bodies (DLB), or Multiple System Atrophy (MSA). Numerous similarities exist between *GBA1*-associated PD and RBD-associated PD (14-16).

Patients with PD who possess *GBA1* mutations, as well as those with REM sleep behavior disorder (RBD), exhibit accelerated advancement

of nonmotor symptoms, including autonomic dysfunction, cognitive deterioration, and dementia. Research consistently corroborates the association between *GBA1* and RBD (14, 17).

GBA1-Lewy Body Dementia

Research indicates that the prevalence of *GBA1* mutation carriers is elevated in individuals with DLB, and there exists an inverse correlation between *GBA1* mutations and the severity of Alzheimer's pathology. While it indicates that *GBA1* mutations may play a role in the emergence of synucleinopathies, the underlying mechanism of this association is still not well understood. Nonetheless, it is probable that lysosomal dysfunction plays a significant role, given that multiple lysosomal genes such as *SCARB2*, *SMPD1*, and *MCOLN1* have been linked to DLB (14, 18, 19).

GBA1-Multiple System Atrophy

Most likely because MSA is so uncommon, the association between *GBA1* mutations and the disease is less solid. In six of the eight investigations done, no correlation was seen between *GBA1* variations and MSA. The most extensive worldwide MSA clinical investigation to date, undertaken at Columbia University with Ashkenazi Jews, identified a correlation between *GBA1* and MSA (8, 17); (20-23).

Methodology in *GBA1* Gene Mutations

In 2022, studies indicated around 500 mutations associated with Gaucher disease, with a predominant occurrence of missense variants arising from single nucleotide variants (SNVs) (24, 25). The identification of *GBA1* mutations occurs 6.9 kb upstream, exhibiting an overall homology of 96% to *GBA1*. The presence of a pseudogene, specifically *GBA1P1*, located downstream, presents a significant challenge. The percentage rises to 98% within the area extending from intron 8 to the 3'-UTR, characterized by five identical segments, each exceeding 200 base pairs. The presence of high homology facilitates non-allelic homologous recombination events between *GBA1* and *GBA1P1*, resulting in a diverse array of structural variants (SVs) (26, 27).

GBA1-MTX1 and its Pseudogenes

Gauchian, a novel *GBA1* browser for Illumina WGS data that can discover SVs and SNVs inside *GBA1*, performed differently from BWA-

GATK analysis utilizing ONT-targeted sequencing in 2022, proving its confirmatory validity (28). BWA-GATK: Burrows-Wheeler Aligner (BWA) read-mapper with Broad Institute Genome Analysis Toolkit (GATK). A revised ONT amplicon sequencing workflow can identify reciprocal recombinants, indels, and homopolymer alterations in coding exons. Illumina WGS data are now reliably analyzable, and *GBA1* analysis can make ONT-targeted sequencing cost-effective (28).

***GBA1* Meta Analysis**

A meta-analysis indicated that the *GBA1* variant frequency was markedly elevated in the DLB group compared to the control group, as were the frequencies of L444P (p. Leu483Pro), N370S (p. Asn409Ser), and E326K, but the variant frequency of T369M was Research indicates no significant difference between the groups; however, in patients with DLB, those with the *GBA1* variant exhibit an earlier onset age and a lower Montreal Cognitive Assessment score compared to the non-*GBA1* variant group, with no gender differences observed among *GBA1* variant-positive DLB patients (8, 19, 25).

Clinical Applications—Diagnosis, Counseling and Treatment

Despite the extensive research evidence available in recent years, *GBA1* PD has not significantly impacted patient care. Genetic testing is not now the normal procedure in PD clinical follow-up (4, 7, 29).

After the initiation of precision medicine clinical trials aimed at *GBA1* mutation carriers, patients want to ascertain their genotypes. Numerous studies are now being conducted for the treatment of *GBA1*-PD in patients (Clinicaltrials.gov).

At present, amroxol hydrochloride, a small molecular chaperone demonstrated to augment brain GCase activity in murine and primate models, is under evaluation as a potential therapeutic option (30, 31).

Ambroxol, first discovered via a library screening of FDA-approved chemicals, is now undergoing clinical studies for PD (clinicaltrials.gov identifier: NCT02941822-completed-April-2020) and PD with dementia (clinicaltrials.gov identifier: NCT02914366-Active-August 2022).

Despite indicating a potentially favorable future, the efficacy of these medicines in alleviating

symptoms has not yet been conclusively shown (32). Although *GBA1*'s limited penetrance makes it difficult to advise *GBA1* mutant carriers, the vast majority of these individuals will not develop synucleinopathy (33).

Although a definitive correlation exists between *GBA1* and PD, there are no established genetic counseling guidelines that specifically outline how to handle this matter with *GBA1* mutation carriers, particularly the relatives of those diagnosed with *GBA1* PD. Some individuals who carry *GBA1* mutations can determine their genetic status through prenatal testing or during the screening of their infants for Gaucher disease (34).

MATERIALS AND METHODS

Patient Selection

For this study, we recruited a cohort of fourteen patients who had received a preliminary diagnosis of PD. Neurologists evaluated the clinical features consistent with PD to select the patients. The Ümraniye Research and Training Hospital Ethics Committee (Approval No. 257254374) granted ethical approval for the study, and all patients provided informed consent for genetic testing.

Demographic, Clinical and Laboratory Profile of Patients

In the study, various demographic, clinical and laboratory parameters of Parkinson's patients with *GBA1* gene mutation were examined, including gender, age of disease onset, presence of mutation in another gene, presence of concomitant diseases such as diabetes-cholesterol, neurological findings and symptoms (tremor, hypomimia, bradykinesia, etc.). These parameters contributed to systematic evaluation to understand their relationship with outcomes.

Genetic Testing

We extracted DNA from peripheral blood samples using standard procedures (e.g., QIAamp DNA Blood Mini Kit, Qiagen).

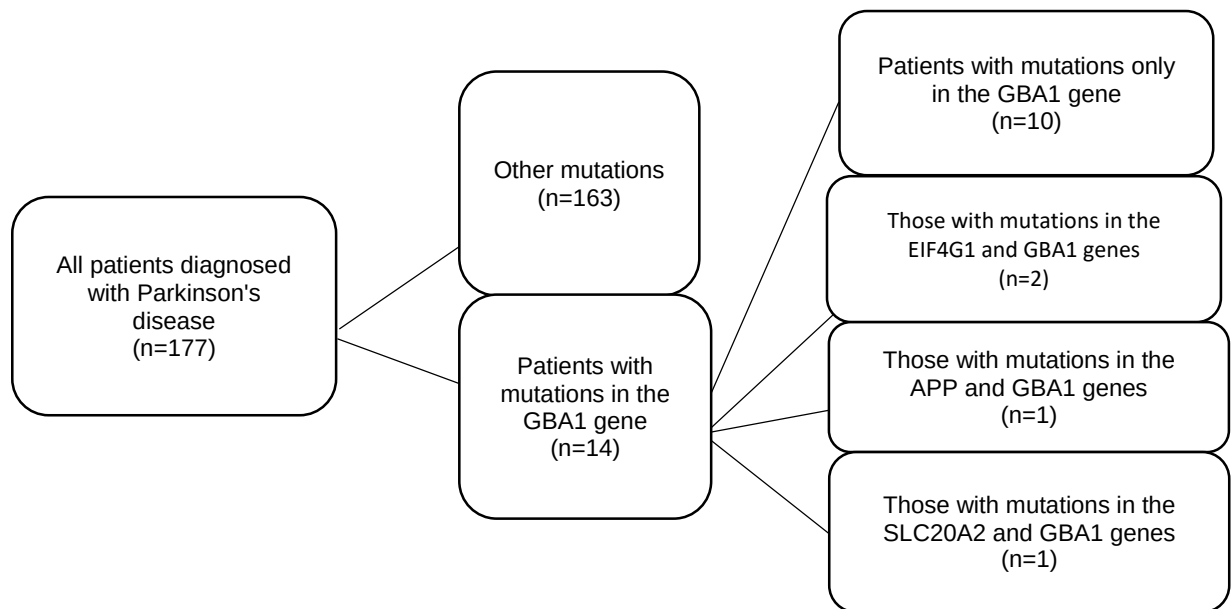


Figure-1: Categorization of Parkinson's disease patients. n: Number of patients.

We performed clinical exome sequencing (CES) to identify genetic variants associated with PD. We carried out CES using Illumina NextSeq, following the manufacturer's protocol. The Sophia DDM platform aligned the sequencing data to the human genome reference (GRCh38) for variant calling and annotation.

Next-generation sequencing was performed using the prepared DNA library and capture probes hybridized to a Celemics Target Enrichment Kit (Celemics, Korea) in a buffer to capture the retina panel of interest. After capture and washing, the captured library was amplified using post-PCR. Finally, the PCR products were sequenced using the NextSeq platform from Illumina Inc. The average depth of coverage of the panel was more than 200-fold.

Single nucleotide variations and small insertion-deletion variants were identified using the Genome Analysis Toolkit (GATK) 3.0 best practice and annotated using ANNOVAR. Variants with a variant allele fraction of less than 0.35 for heterozygous and 0.85 for homozygous were filtered. Copy number variation analysis was also performed for all target genes using the Celemics pipeline. We used several public databases including the Genome Assembly Database (gnomAD), the National Heart, Lung, and Blood Institute (NHLBI) Exome Sequencing

Project, and the Korean Reference Genome Database to identify common variants.

Variants in genes that cause disease in an autosomal dominant manner were filtered using an allele frequency filter threshold of <0.01%. The filter threshold for autosomal recessive variants was set at <0.05% in the East Asian population. In addition, variants previously reported to cause disease were included as disease-causing variants even if they had higher allele frequencies. Using the ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>) and HGMD (<https://www.hgmd.cf.ac.uk/ac/index.php>) databases, variants in genes reported to cause PD were analyzed.

Variants were excluded if there was insufficient clinical correlation with the identified genes. To classify the clinical significance of each variant, we followed the latest recommendations of the American College of Medical Genetics and Genomics (ACMG) standards for the interpretation and reporting of sequence variations: Variants were classified as pathogenic, likely pathogenic, variants of uncertain significance; benign or probably benign.

Cases with two causative variants in genes with recessive inheritance patterns, with more than one pathogenic or likely pathogenic variant, were included. Cases with pathogenic or likely pathogenic variants were included in genes with

dominant inheritance patterns. To assess whether a pair of variants in a gene occurred in cis (same copy of the gene) or in trans (different copies of the gene), we used variant co-occurrence information in the gnomAD browser (<https://gnomad.broadinstitute.org/variant-cooccurrence>).

GBA1 Gene Mutation Analysis

We focused particularly on identifying mutations in the *GBA1* gene, given its established role as a risk factor for PD. We analyzed both homozygous and heterozygous alterations in the *GBA1* gene. Patients carrying *GBA1* variants underwent further evaluation to assess potential additional genetic alterations. We used Sanger sequencing to confirm the presence of *GBA1* mutations identified by exome sequencing. To find out what the identified variants meant for patients, we used the Sophia program and databases like ClinVar, HGMD, and gnomAD to annotate and interpret the variants. We conducted a further assessment of potential pathogenicity using silico prediction tools such as PolyPhen-2 and SIFT.

RESULTS

As a result of the evaluation of 177 patients diagnosed with PD, 14 patients with *GBA1* mutation were examined. Alongside *GBA1* mutations, clinical exome sequencing identified mutations in other genes in 4 of the 14 patients. Two of the four patients had mutations in the *EIF4G1* gene, which are believed to contribute to autosomal dominant PD; nevertheless, the gene's function in neurodegeneration linked to PD remains inadequately understood (35). Furthermore, one patient exhibited an APP gene mutation linked to early-onset autosomal dominant Alzheimer's disease, while another patient presented an *SLC20A2* gene mutation responsible for familial brain calcification.

Within our group, Table-1 provides a comprehensive collection of information on the patients, including the mean age of onset (AO) of the illness, the gender ratio, Parkinson's symptoms, and other disorders. In accordance with what is shown in Table 1, the research concentrated mostly on the non-autonomic (motor) symptoms of the condition. The majority of the patients had classic Parkinson's symptoms, which include tremor (shaking), rigidity (muscle stiffness), bradykinesia (slowing down of movements), hypomimia (decreased facial expression), and postural instability

(balance issues). These symptoms were noticed in the majority of patients. In order to ease the motor symptoms that the patients were experiencing, dopamine agonists were often administered. Furthermore, depression and anxiety, which are commonly noted independently of motor symptoms and have a substantial impact on the quality of life of the patients, were detected in two individuals in addition to these characteristic symptoms. During the same time period, 66.6% of the patients were found to have excessive cholesterol levels as well as diabetes. On the other hand, this high prevalence might be attributed to the short cohort size or the high average age of patients.

Table-1. Demographic data of patients with mutations detected in the *GBA1* gene.

Patient Characteristics	Percentage
Sex	8 ♂, 6 ♀
Mean AOO + SD	53,7 ± 9,9
Tremor	33,30%
Postural Instability	16,60%
Hypomimia	50,00%
Bradykinesia	50,00%
Rigidity	33,30%
Psychiatric Symptoms	33,30%
Cholesterol	66,60%
Diabetes	66,60%
Benign Prostatic Hyperplasia	50%

(AOO: Age of onset of disease, SD: Standard deviation)

The results of the investigation and categorization of mutations on the *GBA1* gene in PD patients are shown in Table 2. It can be seen from the table that patients have a variety of mutations that are situated in different exons. The most frequent sort of mutation is known as a missense mutation, which means that it results in the production of an erroneous amino acid in the structure of the protein by the modification of a single nucleotide. Out of the fourteen patients, only one of them had a frameshift mutation. One such mutation that was found in a large number of patients was the p.Asn409Ser mutation. It is estimated that the majority of patients possess the mutation in a heterozygous (HET) form, which indicates that the mutation is only present on one chromosome rather than both. One

patient was classed as "LP" (Likely Pathological), and one patient was classified as "PAT" (Pathological). In terms of categorization, numerous mutations were rated as "VUS" (Variant of Unknown Clinical Significance), and one patient was described as "Pathological." The fact that this is the case suggests that there are

varying degrees of ambiguity about the possible pathogenic implications of the mutations. Additionally, it was shown that the mutation was inherited in an autosomal dominant (OD) manner in each of the fourteen individuals who were included in the cohort.

Table-2. Analysis and Classification of Mutations in the GBA1 Gene in PD Patients

Patient Number	Coordinate	Nucleotide Change	Exon	c.DNA	Protein	Mutation Type	HET/HOM	Classification	Heredity
P1	Chr1: 155205634	T>C	Exon 10	c.1226A>G	p.(Asn409Ser)	missens	-	-	OD
P2	Chr1: 155205634	T>C	Exon 9	c.1226A>G	p.(Asn409Ser)	missens	HET	VUS	OD
P3	Chr1: 155205634	T>C	Exon9	c.1226A>G	p.(Asn409Ser)	missens	HET	VUS	OD
P4	Chr1: 155205634	T>C	Exon 9	c.1226A>G	p.(Asn409Ser)	missens	HET	MP	OD
P5	Chr1: 155207203	T >C	Exon 7	c.928A>G	p.(Ser310Gly)	missens	HET	VUS	OD
P6	Chr1: 155205043	A>G	Exon 10	c.1448T>C	p.(Leu483Pro)	missens	HET	VUS	OD
P7	Chr1: 155207249	A>C	7	c.882T>G	p.(His294Gln)	missens	HET	VUS	OD
P8	Chr1: 155208388	-	exon 5	c.508C>T	p.(Arg170Cys)	missens	HET	PAT	OD
P9	Chr1: 155206193	AG>A	Exon8	c.1066del	p.(Leu356Trpfs*8)	frameshift	HET	-	OD
P10	-	-	-	c.1448T>C	p.(Leu483Pro)	missens	HET	VUS	OD
P11	Chr1: 155205518	C>G	Exon 9	c.1342G>C	p.(Asp448His)	missens	HET	VUS	OD
P12	Chr1: 155205634	T >C	Exon 9	c.1226A>G	p.(Asn409Ser)	missens	HET	VUS	OD
P13	Chr1: 155205634	T>C	Exon 9	c.1226A>G	p.(Asn409Ser)	missens	HET	VUS	OD
P14	Chr1: 155207925	-	-	c.761A>C	p.(Lys254Thr)	missense	HET	VUS	OD

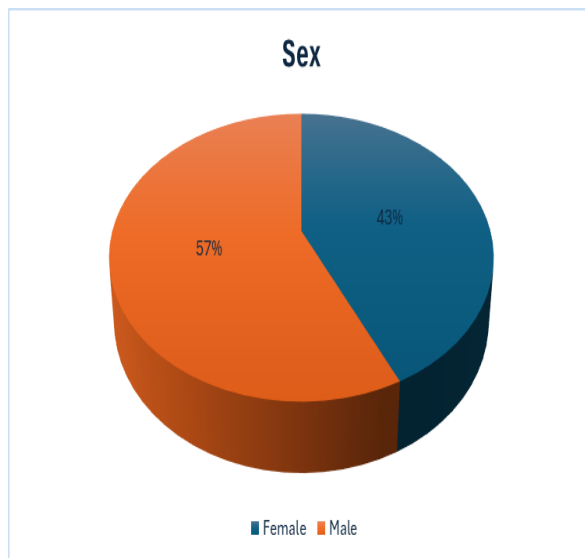


Figure-2. Percentage of women and men in the cohort

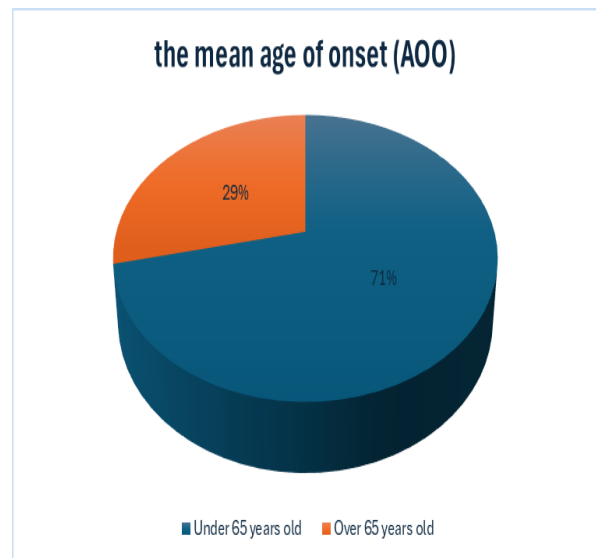


Figure-3. Percentage of age at onset of PD

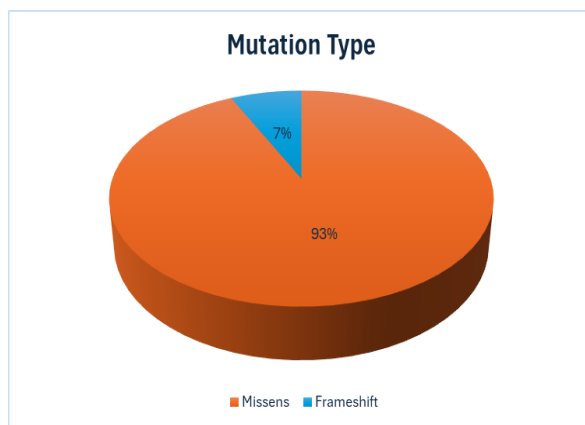


Figure-4. Mutation type percentages of PD patients in the cohort

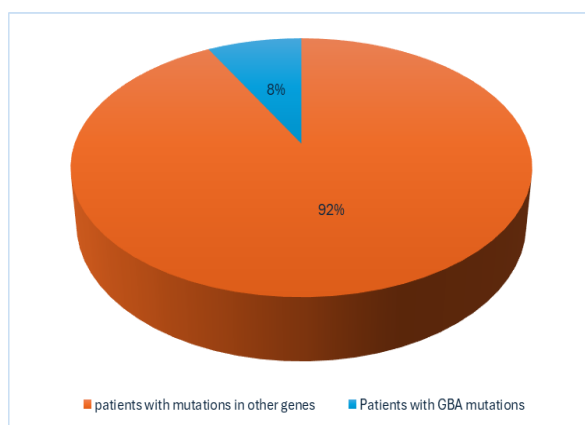


Figure-5. Percentage of PD patients with mutations in the *GBA1* gene and other genes

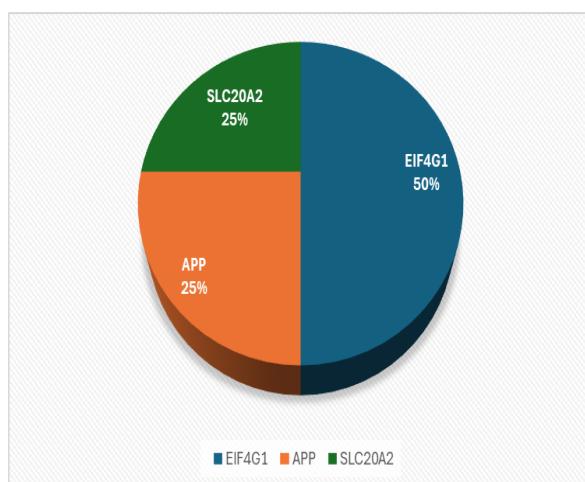


Figure- 6. Percentage of mutations in genes other than *GBA1* in PD patients.

DISCUSSION

Many studies on the correlation between *GBA1* gene mutations and PD have been conducted and are still being conducted worldwide. This study contributes to the existing evidence by identifying *GBA1* mutations including pathogenic, likely pathogenic and variants of uncertain significance (VUS) in 14 patients diagnosed with PD. The findings attempt to highlight the complex role of *GBA1* mutations in the pathogenesis of PD and demonstrate the clinical and genetic diversity observed in *GBA1*-associated PD.

The prevalence of the p.Asn409Ser (N370S) mutation in heterozygous form is a significant observation in our cohort, affecting the majority of patients. This variant is linked to a mild form of Gaucher disease (GD) and an elevated risk of PD, as noted in prior literature. The role of this factor in PD is ambiguous, despite its classification as pathogenic in Gaucher disease (GD), particularly since heterozygous carriers exhibit a relatively early age of onset for PD. The mean age of onset in our cohort was 53.7 years, partially aligned with prior studies indicating that *GBA1*-associated PD manifests earlier than idiopathic PD (36). The variability in age of onset and clinical presentation among *GBA1* mutation carriers indicates the impact of additional genetic and environmental factors on disease progression.

In our study, in addition to motor symptoms, psychiatric findings such as depression and anxiety, which affected approximately one-third of the patients, were also observed in *GBA1* mutation carriers. The findings align with earlier research indicating that non-motor symptoms, including cognitive decline and psychiatric issues, are prevalent among *GBA1*-PD patients (37,38). The prevalence of metabolic comorbidities, such as hypercholesterolemia and diabetes, in two-thirds of patients indicates that *GBA1* mutations exert a broader systemic impact. Prior research indicates that lysosomal dysfunction in *GBA1*-PD could influence metabolic processes; however, the precise mechanisms remain unclear (39).

Our investigation also discovered mutations in additional genes, including EIF4G1, APP, and SLC20A2, which are associated with various neurological illnesses. The existence of these mutations indicates a complex etiology in certain patients, wherein *GBA1* mutations may interact with other genetic variations to affect PD

progression (40). Importantly, APP, which is linked to Alzheimer's disease, and EIF4G1, which is linked to autosomal dominant PD, suggest possible shared mechanisms between PD and other neurodegenerative disorders (41).

The established association between *GBA1* mutations and PD indicates a need for further investigation into the underlying pathogenic mechanisms, which are not yet fully understood. A well-known hypothesis proposes that decreased glucocerebrosidase (GCase) activity leads to lysosomal dysfunction and the resulting accumulation of α -synuclein in vital organelles, a characteristic feature of PD. This accumulation may create a feedback loop, in which α -synuclein further inhibits GCase activity, worsening neurodegeneration (42). Further research is necessary to clarify these mechanisms and to investigate whether *GBA1*-PD constitutes a distinct subtype of the disease or exists on a continuum with idiopathic forms.

Clinically, finding PD-related *GBA1* mutations could pave the way for more effective precision treatment. Ambroxol hydrochloride and other small molecule chaperones show promise in improving GCase function and potentially reducing PD progression (43). Ongoing clinical trials are evaluating treatments targeting GCase activity. One obstacle, though, is that genetic counseling does not yet have established standards. Due to partial penetrance, not all carriers of *GBA1* mutations will get PD, which makes genetic counseling and risk assessments more challenging (44).

CONCLUSION

This work emphasizes the critical significance of *GBA1* mutations in the development of PD, underscoring the necessity for thorough clinical care techniques that encompass both motor and non-motor symptoms in afflicted patients. Our

findings indicate the extensive genetic complexity of *GBA1*-associated PD, highlighting the necessity for further study to elucidate the molecular processes underlying this association, especially the link between glucocerebrosidase malfunction and α -synuclein buildup.

Despite significant advancements in understanding the genetics of PD over the last twenty years, the precise mechanisms connecting *GBA1* mutations to neurodegeneration are still unclear. The interactions between *GBA1* mutations and other genetic and environmental modifiers require additional investigation. Further research is necessary to investigate the development of genotype-specific therapies targeting glucocerebrosidase activity, which may provide tailored treatments for the varied clinical manifestations of the disease.

The increasing availability of genetic testing necessitates the establishment of standardized guidelines for genetic counseling, particularly due to the incomplete penetrance associated with *GBA1* mutations. The integration of new precision medicine approaches, including GCase-enhancing therapies, into clinical practice will be essential. Future studies should clarify the relationship between *GBA1* mutations and other synucleinopathies, such as REM sleep behavior disorder (RBD), dementia with Lewy bodies (DLB), and multiple system atrophy (MSA), to enhance understanding of the full spectrum of *GBA1*-related neurodegenerative diseases.

Expanding our understanding of *GBA1*-associated PD and its broader clinical and genetic context is critical to developing promising targeted therapies for patients and their families.

Conflicts of interest: Authors declared no conflict of interest.

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Yetişkin bireylerin hipertansiyondan korunmaya yönelik tutumları ve etkileyen faktörlerin incelenmesi

Investigation of adults' attitudes towards protection from hypertension and affecting factors

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ÖZ

Amaç: Bu araştırma yetişkin bireylerin hipertansiyondan korunmaya yönelik tutumları ve etkileyen faktörlerin incelenmesi amacıyla yapılmıştır.

Gereç ve Yöntem: Tanımlayıcı ve kesitsel tipteki çalışma, Ocak- Haziran 2023 tarihleri arasında Aksaray Eğitim ve Araştırma Hastanesi dahiliye polikliniklerine (kardiyoloji, endokrinoloji, romatoloji, nefroloji, gastroenteroloji, göğüs vb.) çeşitli nedenlerle başvuran 18 yaş ve üzeri ve hipertansiyon tanısı olmayan 525 bireyle yürütülmüştür. Araştırmadaki verileri, "Birey Tanıtım Formu" ve "Hipertansiyondan Korunma Tutumları Ölçeği (HKTÖ)" ile yüz yüze görüşmeyle toplanmıştır. Verilerin değerlendirilmesinde, iki ortalama arasındaki farkın önemlilik testi, tek yönlü varyans analizi ve çoklu doğrusal regresyon modelinden yararlanılmıştır.

Bulgular: Çalışmaya katılan bireylerin %44,4'ünün yaşı 18-30 aralığında olup, %52,8'i kadın, %60'ı evli, %39,2'si üniversite mezunu ve %57,9'unun gelirlerinin giderlerine eşit olduğu bulunmuştur. Çalışmaya katılan bireylerin %66,3'ü kendisini hipertansiyon açısından riskli grupta görmediği, %60,4'ünün hipertansiyonu önlemek ile ilgili eğitim alma isteğinin olduğu ve %76,2'sinin sağlık kontrollerine zamanında gittiği bulunmuştur. Katılımcıların HKTÖ toplam puan ortalamasının 105,67±12,44 olduğu bulunmuştur. Katılımcıların HKTÖ toplam puanı ile değişkenler olarak kurulan çoklu doğrusal regresyon modeli üzerinde; medeni durum, eğitim durumu, gelir durumu, ruhsal durum, hipertansiyon açısından risk grubunda yer alma düşüncesi ve sağlık kontrollerine gitme durumunun istatistiksel açıdan anlamlı bir etkisinin olduğu bulunmuştur. Hipertansiyondan korunma tutum ölçeği puanları üzerinde istatistiksel olarak anlamlı etkiye sahip olan değişkenler arasında en yüksek etkiye sahip olan değişkenin eğitim düzeyi (standardize edilmiş β katsayısı=-0,201) olduğu bulunmuştur.

Sonuç: Katılımcıların hipertansiyondan korunma tutumları üzerinde bazı sosyodemografik ve sağlık/hastalık değişkenlerinin etkisinin olduğu belirlenmiştir. Hipertansiyondan korunmaya yönelik tutumların olumlu yönde gelişmesi için bireylere hipertansiyona yönelik bireysel eğitimlerin düzenlenmesi ve özellikle risk altındaki grupların farkındalığının sağlanmasının önemli olduğu düşünülmektedir.

Anahtar Sözcükler: Hipertansiyon, tutum, etkileyen faktörler

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ABSTRACT

Aim: This research was conducted to examine the attitudes of adults towards prevention of hypertension and the factors affecting it.

Materials and Methods: The descriptive and cross-sectional study was carried out with 525 individuals aged 18 years and above and without a diagnosis of hypertension who applied to the internal medicine outpatient clinics of Aksaray University Training and Research Hospital between January and June 2023 for various reasons (cardiology, endocrinology, rheumatology, nephrology, gastroenterology, chest, etc.). The data in the research were collected through face-to-face interviews with the "Individual Introduction Form" and the "Attitudes towards the Prevention of Hypertension (ASPH)". Significance test of the difference between two means, one-way analysis of variance and multiple linear regression model were used to evaluate the data.

Results: It was found that 44.4% of the individuals participating in the study were between the ages of 18-30, 52.8% were women, 60% were married, 39.2% were university graduates, and 57.9% had income equal to their expenses. It was found that 66.3% of the individuals participating in the study did not see themselves in the risk group for hypertension, 60.4% had a desire to receive training on preventing hypertension, and 76.2% went to health check-ups on time. It was found that the total mean score of ASPH of the participants was 105.67 ± 12.44 . It was found that marital status, education status, income status, mental status, the idea of being in the risk group in terms of hypertension and going to health checkups had a statistically significant effect on the multiple linear regression model established as variables with the scale total score of the participants. It was found that among the variables that had a statistically significant effect on the prevention of hypertension attitude scale scores, the variable with the highest effect was the level of education (standardized β coefficient=-0.201).

Conclusion: It was determined that some sociodemographic and health/disease variables had an effect on the participants' attitudes to prevent hypertension. It is thought that it is important to organize individual training on hypertension for individuals and to raise awareness of groups at risk in order to develop attitudes towards prevention of hypertension in a positive way.

Keywords: Hypertension, attitude, affecting factors.

GİRİŞ

Hipertansiyon (HT), sürekli yüksek kan basıncıyla kendini gösteren, toplumda yaygın görülen sistemik bir hastalık olup, aynı zamanda ciddi komplikasyonlara neden olabilen önemli bir halk sağlığı sorunudur (1,2). Dünya genelinde 30-79 yaşları arasındaki tahminen 1,28 milyar yetişkin hipertansiyona sahip olduğu belirtilmektedir (3). Ülkemizde hipertansiyon prevalansı ile ilgili ilk geniş kapsamlı çalışma olan "Türk Erişkinlerinde Kalp Hastalıkları ve Risk Faktörleri (TEKHARF)" çalışmasında hipertansiyon prevalansının %33,7 olduğu, yaş ilerledikçe prevalansın arttığı saptanmıştır (4). Türk Hipertansiyon Prevalans Çalışmasında (*Prevalence, awareness and treatment of hypertension in Turkey, PatenT*) ise, erişkin yaş grubunda hipertansiyon prevalansı %31,8 ve daha sonra yapılan PatenT2 çalışmasında %30,3 olarak bulunmuştur (5,6). Ayrıca, hipertansiyonlu hastaların sadece %40,7'sinin hastalıklarının farkında olduğu, %31,1'inin antihipertansif tedavi aldığı ve tedavi

alanların sadece %20,7'sinin kan basıncının kontrol altında olduğu saptanmıştır (5). Yapılan projeksiyonlara göre ise, 2030 yılında 23 milyon kardiyovasküler ölümün hipertansiyon ile ilişkili olacağı ve bu ölümlerin %85'inin düşük ve orta gelirli ülkelerde gerçekleşeceği bildirilmektedir (7).

Hipertansiyon sıklığı yaşla birlikte artmakla olup, etkileyen faktörler arasında cinsiyet, etnik köken, ailede hipertansiyon öyküsü, diyabet veya böbrek hastalığı gibi eşlik eden hastalık gibi faktörlerin yanında; fiziksel hareketsizlik, sağlıksız beslenme (düşük meyve ve sebze tüketimi, aşırı tuz alımı, doymuş yağ ve trans yağlardan zengin diyet), aşırı kilolu ya da obez olma, tütün ve alkol tüketimi gibi değiştirilebilir risk faktörleri yer almaktadır (2,3). Bununla birlikte, hipertansiyonu olan yetişkinlerin tahminen %46'sı hipertansiyon olduklarından habersiz olduğu bilinmektedir (3). Hipertansiyonun farkına varılmaması ya da etkili tedavi edilmemesi nedeniyle bireyler önlenebilir, ancak ölümcül olabilen koroner kalp hastalığı, iskemik ve hemorajik inmenin yanında kalp

yetmezliği, periferik damar hastalığı, böbrek yetmezliği ve görme bozuklukları açısından artmış riske sahiptirler (8).

Hipertansiyon bireylerin yaşam tarzına dikkat etmesi sonucunda kontrol edilebilen hastalıklar arasında yer almaktadır. Hipertansiyon riskini azaltmak için hastalıklar ve semptomları hakkında bilgi edinme, mevsime uygun sebze ve meyve tüketme, normal vücut ağırlığına sahip olma, stresi azaltma ve yönetme, kan basıncını düzenli olarak kontrol etme, fiziksel hareket etme, tuz tüketimini kısıtlama, doymamış yağ grubundan zengin sıvı yağları tercih etme, yüksek tansiyon tedavisi ve diğer tıbbi durumların yönetimi alkol ve tütün kullanmamadır (3,9,10). Hipertansiyon riskinin azaltılmasıyla bireyde gelişebilecek böbrek hasarı, inme ve kalp krizinin yanı sıra diğer sağlık sorunlarını da önleyebildiği bulunmuştur. Bu nedenle, bireyin sağlıklı yaşam biçiminde davranışlarını alışkanlık hâline dönüştürmesi, iyilik hâlini sürdürmesi ve sağlık durumunun iyi olmasıyla kronik hastalıklar azaltılabilir ya da engellenebilir olduğu görülmektedir (11). Bununla birlikte, ülkemizde yetişkin bireylerin hipertansiyondan korunmaya yönelik tutumlar ile etkileyen faktörlere ilişkin yeterince veri bulunmamakta olup, bu konuda herhangi bir çalışmaya rastlanmamıştır. Bu çalışmada yetişkin bireylerin hipertansiyondan korunmaya yönelik tutumları ve etkileyen faktörlerin incelenmesi amacıyla yapılmıştır.

GEREÇ ve YÖNTEM

Araştırmanın Amacı ve Tipi

Tanımlayıcı ve kesitsel tipteki bu araştırma, yetişkin bireylerin hipertansiyondan korunmaya yönelik tutumları ve etkileyen faktörlerin incelenmesi amacıyla yapılmıştır.

Araştırmanın Yeri ve Örneklemi

Çalışmanın örneklemi Ocak-Haziran 2023 tarihleri arasında Aksaray Eğitim ve Araştırma Hastanesi dahiliye polikliniklerine (kardiyoloji, endokrinoloji, romatoloji, nefroloji, gastroenteroloji, göğüs vb.) herhangi bir nedenlerle başvuran; 18 yaş ve üzeri, hipertansiyon tanısı olmayan, bilişsel ve fiziksel sağlık durumları toplama formları cevaplamaya uygun ve araştırmaya katılmaya gönüllü olan bireylerden oluşmaktadır. Örneklemi dâhil edilme kriterlerine sahip ve araştırmaya katılmaya gönüllü olan 525 yetişkin birey oluşturmuştur. Araştırmanın örneklem büyüklüğünü hesaplamak için,

Cochran'ın bilinmeyen örneklem büyüklüğü formülü kullanılarak minimum örneklem $p = ,50$ ve $q = 0,50$ için %5 hata ile 384 kişide olarak hesaplanmıştır. Değişkenler arasındaki ilişkinin daha iyi görülebilmesi amacıyla çalışma 525 birey ile tamamlanmıştır.

Veri Toplama Araçları

Araştırmadaki verileri “Birey Tanıtım Formu” ve “Hipertansiyondan Korunma Tutumları Ölçeği (HKTÖ)” yüz yüze görüşme tekniği kullanılarak toplanmıştır.

Birey Tanıtım Formu: Bu form, ilgili literatür (2,9,10) bilgileri ışığında araştırmacılar tarafından oluşturulmuştur. Form bireye yönelik; yaşı, cinsiyeti, medeni durumu, eğitim durumu, sosyal güvencesi, gelir durumu, kronik hastalık durumu, hipertansiyon hakkında bilgisi gibi hipertansiyon ve hipertansiyondan korunmaya yönelik sorulardan oluşmaktadır.

Hipertansiyondan Korunma Tutumları Ölçeği (HKTÖ): Hipertansiyondan Korunma Tutumları Ölçeği, Albayrak ve Şengezer tarafından 2022 yılında geliştirilmiştir (12). Hipertansiyondan Korunma Tutumları Ölçeği 26 madde ve 5 faktörden oluşmakta olup, ölçeğin 15. ve 20. soruları olumsuz olduğu için tersten puanlanarak değerlendirilmektedir. Ölçekte bulunan faktörler ve maddeleri: 1. Korunma ve kontrol (1, 4, 7, 10, 13, 18, 22, 25. Soru), 2. Alışkanlık ve yaşam şekli (6, 12, 17, 21, 24, 26. Soru), 3. Beslenme tutumu (5, 11, 16, 20. Soru), 4. Ruhsal durum ve fiziksel aktivite (3, 9, 15. Soru) ve 5. Hastalık ve risk bilgisi (2, 8, 14, 19, 23. soru) şeklinde yer almaktadır. Her madde “Hiç katılmıyorum-Kesinlikle katılıyorum” şeklinde 5’li Likert puanlanmıştır (12). Ölçekten 26-130 arası puan alınabilmekte olup, hipertansiyondan korunma tutumlarıyla ölçek skorları arasında pozitif bir ilişki olduğu söylenebilir. Ölçeğin Cronbach alfa değeri 0,91 bulunmuştur.

İstatistiksel Analiz

Verilerin değerlendirilmesi, SPSS (Statistical Package for Social Science for Windows 22.0, IBM Corp., Armonk, NY, USA) programı kullanılarak yapılmıştır. Sosyodemografik, sağlık ve hipertansiyon hastalığı ile ilgili değişkenlerin ölçek boyutları ve toplam puanlarına göre istatistiksel açıdan anlamlı bir farklılık gösterip göstermediğinin değerlendirilmesinde hipotez testlerinden faydalanılmıştır. Veri setinin normal dağılım göstermesi ve gözlem sayısının yüksek

olması sebebiyle parametrik test yöntemleri kullanılmıştır. Değişkenlerden iki kategorili olanların değerlendirilmesinde iki ortalama arasındaki farkın önemlilik testinden, ikiden fazla kategorisi olan değişkenlerin değerlendirilmesinde ise tek yönlü varyans analizinden yararlanılmıştır. Tek yönlü varyans analizi sonucunda istatistiksel açıdan anlamlı bulunan farklılığın hangi grup/gruplardan kaynaklandığının belirlenmesinde ise Tukey HSD post-hoc testi kullanılmıştır. Farklılığı yaratan grup/gruplar tablolar içerisinde koyu renkle belirtilmiştir. Parametrik testlerden faydalandığı için merkezi eğilim ve yaygınlık ölçüsü olarak ortalama ve standart sapma ile tablolarda bilgiler özetlenmiştir. Hipotez testleri sonucuna göre (toplam puan için) anlamlı çıkan değişkenler bağımsız değişkenler olarak, ölçeğin toplam puanı da bağımlı değişken olarak kurulan çoklu doğrusal regresyon modeline bu bölümde yer verilmiştir. Sonuçlar, %95 güven aralığında, $p < 0,05$ anlamlılık düzeyinde değerlendirilmiştir.

Araştırmanın Etik Yönü

Çalışma, Helsinki Bildirgesi'nin etik standartlarına uygun olarak yürütülmüştür. Araştırmanın yürütülebilmesi için Aksaray Üniversite'sinin İnsan Araştırmaları Etik Kurulu'ndan (Karar no:142-SBKA EK/2022/21-07, Tarih:22/12/2022) izin alınmıştır Aynı zamanda çalışmanın yapıldığı Aksaray Eğitim ve Araştırma Hastanesi'nden kurum izni (Karar sayı: E-74114513-929, Tarih:26/12/2022) ve ölçek sahibinden kullanım izni alınmıştır. Örnekleme dahil edilen bireylere çalışma öncesinde araştırmanın amacı açıklanmış ve onamları alınmıştır.

BULGULAR

Çalışmaya katılan bireylerin %44,4'ünün yaşı 18-30 aralığında olup, %52,8'i kadın, %60'ı evli, %39,2'si üniversite mezunu ve yarıdan fazlasının (%57,9) geliri giderine eşit bulunmuştur. Katılımcıların %62,1'inin sigara kullanmadığı ve %87,8'inin alkol kullanmadığı saptanmıştır. Çalışmaya katılan bireylerin %62,7'sinin ailesinde hipertansiyon hastasının olmadığı, %66,3'ünün kendisini hipertansiyon açısından riskli grupta görmediği, %60,4'ünün hipertansiyonu önlemek ile ilgili eğitim alma isteğinin olduğu ve %76,2'sinin sağlık kontrollerine zamanında gittiği bulunmuştur (Tablo-1).

Katılımcıların HKTÖ toplam puan ortalamasının $105,67 \pm 12,44$ olduğu bulunmuştur. Katılımcıların

HKTÖ alt boyutlarına göre puan ortalamaları karşılaştırıldığında ise; koruma ve kontrol alt boyutu puan ortalaması $33,46 \pm 4,15$, alışkanlık ve yaşam şekli alt boyutu puan ortalaması $24,28 \pm 3,23$, beslenme tutumu alt boyutu puan ortalaması $15,39 \pm 2,90$, ruhsal durum ve fiziksel aktivite alt boyutu puan ortalaması $12,66 \pm 2,03$ ve hastalık ve risk bilgisi alt boyutuna ilişkin puan ortalamasının $19,88 \pm 3,17$ olduğu tespit edilmiştir (Tablo-2).

Tablo-3'te hipertansiyon gelişiminin önlenmesine ilişkin tutum ölçeğinin alt boyutları ve toplam puanının sosyodemografik ve sağlık değişkenlere göre farklılık gösterip göstermediği incelenmiştir. Buna göre, ölçeğin toplam puanının cinsiyet, medeni durum, yaş, eğitim durumu, gelir durumu, alkol kullanımına, tuzdan kaçınma, sebze-meyve tüketimi, günlük egzersiz ve genel ruh hali değişkenlerine göre istatistiksel açıdan anlamlı bir farklılık gösterdiği bulunmuştur ($p < 0,05$). Buna göre, kadınların erkeklere, evlilerin bekarlara, üniversite mezunlarının diğer mezunlara, geliri giderine eşit olanların diğer gelir gruplarına ve alkol kullanmayanların alkol kullananlara göre daha yüksek puan aldığı görüldüğünde, 18-30 yaş grubundakilerin diğer yaş grubundakilere, lise mezunlarının diğer mezunlara ve geliri giderin fazla olanların diğer gelir gruplarına göre daha düşük puan aldığı görülmüştür. Aynı şekilde, tuzdan kaçınanların kaçınmayanlara, günde iki porsiyon sebze-meyve tüketenlerin diğerlerine, günlük egzersiz yapmayanların yapanlara ve genel ruh hali uyumlu olanların diğerlerine göre daha yüksek puan aldığı görüldüğünde, günlük üç ve daha fazla sebze-meyve tüketenlerin diğerlerine göre daha düşük puan aldığı görülmüştür (Tablo-3).

Tablo-4'te hipertansiyon gelişiminin önlenmesine ilişkin tutum ölçeğinin alt boyutları ve toplam puanının hipertansiyon hastalığı ile ilgili değişkenlere göre farklılık gösterip göstermediği incelenmiştir. Ölçeğin toplam puanının; ailede hipertansiyon tanısı alanların varlığı, hipertansiyon risk grubunda yer alma, hipertansiyon önleme konusunda eğitim alma isteği ve sağlık kontrollerine gitme değişkenlerine göre istatistiksel açıdan anlamlı bir farklılık gösterdiği bulunmuştur ($p < 0,05$). Buna göre, hipertansiyon tanısı almış yakının varlığının almayanlara, hipertansiyon risk grubunda yer alanların almayanlara, hipertansiyon önleme eğitim talebinde bulunanların bulunmayanlara ve

sağlık kontrolüne gidenlerin gitmeyenlere göre daha yüksek puan aldığı görülmüştür (Tablo-4).

Hipotez testleri sonucuna göre (toplam puan için) anlamlı çıkan değişkenler bağımsız değişkenler olarak, ölçeğin toplam puanı da bağımlı değişken olarak kurulan çoklu doğrusal regresyon modeli Tablo 5'te yer almaktadır. Katılımcıların HKTÖ puanları üzerinde medeni durum, eğitim durumu, gelir durumu, ruhsal durum, hipertansiyon açısından risk grubunda yer alma düşüncesi ve sağlık kontrollerine gitme durumunun istatistiksel açıdan anlamlı bir etkisinin olduğu bulunmuştur. Buna göre, bekar bireylerin hipertansiyondan korunma düzeylerinin evli bireylere kıyasla daha düşük olduğu görülmüştür. İlköğretim ve altı ile lise mezunu olan bireylerin üniversite mezunu olan bireylere göre hipertansiyondan korunma tutum puanlarının daha az olduğu tespit edilmiştir. Gelir düzeyi giderlerine göre daha fazla olan katılımcıların diğer katılımcılara kıyasla

hipertansiyondan korunma tutumlarının daha yüksek olduğu görülmüştür. Ruhsal durumu öfkeli ve stresli olan bireylerin ruhsal durumu uyumlu olan bireylere kıyasla hipertansiyondan korunma tutum düzeylerinin daha düşük olduğu saptanmıştır. Katılımcılar arasında kendisini hipertansiyon açısından riskli grupta görmeyen kişilerin hipertansiyondan korunma tutum ölçeğine ilişkin puanlarının riskli grupta görenlere kıyasla daha az olduğu bulunmuştur. Son olarak katılımcılar arasında tavsiye edilen sağlık kontrollerine gitmeyen bireylerin de hipertansiyondan korunma tutum düzeylerinin daha düşük olduğu sonucuna ulaşılmıştır. Hipertansiyondan korunma tutum ölçeği puanları üzerinde istatistiksel olarak anlamlı etkiye sahip olan değişkenler arasında en yüksek etkiye sahip olan değişkenin ise eğitim düzeyi (standardize edilmiş β katsayısı=-0,201) olduğu bulunmuştur (Tablo-5).

Tablo-1. Katılımcıların Bazı Sosyodemografik ve Hastalık/Sağlık Özellikleri (n=525)

Özellikler	n	%
Yaş		
18-30	233	44,4
31-40	109	20,8
41-50	121	23,0
51 yaş ve üzeri	62	11,8
Cinsiyet		
Kadın	277	52,8
Erkek	248	47,2
Kilo		
45-60 kg	109	20,8
61-75 kg	203	38,7
76 kg ve üzeri	213	40,6
Medeni durum		
Evli	315	60,0
Bekar	210	40,0
Eğitim durumu		
İlköğretim ve altı	142	27,0
Lise	177	33,7
Üniversite	206	39,2
Gelir durumu		
Geliriniz giderinizden fazla	118	22,5
Geliriniz giderinize eşit	304	57,9
Geliriniz giderinizden az	103	19,6
Sigara kullanımı		
Yok	326	62,1

Var	199	37,9
Alkol kullanımı		
Yok	461	87,8
Var	64	12,2
Sağlık güvencesi		
Evet	443	84,4
Hayır	82	15,6
Düzenli ilaç kullanma durumu		
Evet	109	20,8
Hayır	416	79,2
Kronik hastalık durumu		
Evet	94	17,9
Hayır	431	82,1
Günlük beslenmede tuzdan kaçınma durumu		
Evet	262	49,9
Hayır	263	50,1
Günlük en çok tüketilen yağ		
Sıvı yağ	467	89,0
Katı yağ	58	11,0
Günlük tüketilen sebze ve meyve porsiyonu		
Bir porsiyon	336	64,0
İki porsiyon	130	24,8
Üç ve fazlası	59	11,2
Günlük egzersiz yapma durumu		
Evet	150	28,6
Hayır	375	71,4
Genel ruh hali ifadesi		
Ben genelde çok öfkeliyim	91	17,3
Ben çok stres yaparım	198	37,7
Ben çok uyumluyum	236	45,0
Ailede hipertansiyon hastası olma durumu		
Evet	196	37,3
Hayır	329	62,7
Hipertansiyon tanısı olan akraba ile aynı evde yaşama durumu		
Evet	92	17,5
Hayır	433	82,5
Hipertansiyon risk grubunda yer aldığını düşünme		
Evet	177	33,7
Hayır	348	66,3
Hipertansiyonu önleme konusunda eğitim alma isteği		
Evet	317	60,4
Hayır	208	39,6
Tavsiye edilen sağlık kontrollerine gitme durumu		
Evet	400	76,2
Hayır	125	23,8

Tablo-2. Katılımcıların Hipertansiyondan Korunma Tutumları Ölçeğine (HKTÖ) İlişkin Puan Ortalamaları (n=525)

Hipertansiyondan Korunma Tutumları Ölçeği (HKTÖ)	Ort.	SS	Min.	Maks.
Koruma ve kontrol	33,46	4,15	8,00	40,00
Alışkanlık ve yaşam şekli	24,28	3,23	11,00	30,00
Beslenme tutumu	15,39	2,90	5,00	20,00
Ruhsal durum ve fiziksel aktivite	12,66	2,03	3,00	15,00
Hastalık ve risk bilgisi	19,88	3,17	6,00	25,00
HKTÖ Toplam	105,67	12,44	48,00	130,00

Tablo-3. Hipertansiyon Gelişiminin Önlenmesine İlişkin Tutum Ölçeğinin Sosyodemografik ve Sağlık Özelliklerine Göre Karşılaştırılması

Demografik Özellikler	Boyut 1	Boyut 2	Boyut 3	Boyut 4	Boyut 5	Toplam Puan
	X±SS	X±SS	X±SS	X±SS	X±SS	X±SS
Cinsiyet						
Kadın	33,86±3,61	24,84±2,87	15,82±2,67	12,90±1,82	20,08±3,05	107,51±11,14
Erkek	33,00±4,65 t=2,375 p=0,018*	23,64±3,49 t=-4,291 p<0,001*	14,92±3,07 t=3,601 p<0,001*	12,39±2,21 t=2,882 p=0,004*	19,65±3,30 t=1,581 p=0,114	103,60±13,52 t=3,631 p<0,001*
Medeni durum						
Evli	34,14±3,40	24,67±3,01	15,90±2,74	12,83±1,87	20,29±2,98	107,84±11,02
Bekar	32,43±4,91 t=4,703 p<0,001*	23,67±3,46 t=3,500 p=0,001*	14,62±2,98 t=5,061 p<0,001*	12,40±2,22 t=2,391 p=0,017*	19,24±3,35 t=3,753 p<0,001*	102,36±13,76 t=5,037 p<0,001*
Yaş						
18-30 Yaş	32,43±4,88	23,71±3,53	14,73±3,04	12,35±2,32	19,17±3,40	102,37±14,05
31-40 Yaş	34,18±3,28	25,02±2,98	16,07±2,69	13,07±1,64	20,40±2,85	108,75±10,53
41-50 Yaş	34,19±3,48	24,70±2,94	15,87±2,76	12,85±1,83	20,39±3,14	108,02±11,43
51 Yaş ve üzeri	34,60±2,74 F=9,036 p<0,001*	24,26±2,65 F=5,137 p=0,002*	15,71±2,47 F=7,655 p<0,001*	12,75±1,66 F=3,837 p=0,010*	20,58±2,28 F=7,186 p<0,001*	107,89±7,61 F=10,216 p<0,001*
Eğitim durumu						
İlköğretim ve altı	33,43±3,42	24,19±2,98	15,63±2,65	12,56±1,94	19,70±2,91	105,51±11,02
Lise	33,05±4,22	23,63±3,34	14,82±2,97	12,47±2,07	19,46±3,30	103,43±12,88
Üniversite	33,82±4,53 F=1,663 p=0,191	24,89±3,20 F=7,534 p=0,001*	15,72±2,94 F=5,301 p=0,005*	12,90±2,03 F=2,356 p=0,096	20,35±3,18 F=4,048 p=0,018*	107,68±12,76 F=5,632 p=0,004*
Gelir durumu						
Geliri giderinden fazla	32,77±4,61	23,63±3,68	14,97±3,64	12,28±2,38	19,46±3,34	103,10±14,15
Geliri giderine eşit	33,79±3,67	24,45±2,93	15,61±2,71	12,78±1,86	20,06±2,82	106,71±10,93
Geliri giderinden az	33,26±4,83 F=2,713 p=0,067	24,50±3,45 F=3,095 p=0,046*	15,22±2,84 F=2,345 p=0,097	12,76±2,03 F=2,740 p=0,066	19,79±3,85 F=1,607 p=0,202	105,54±14,21 F=3,586 p=0,028*
Kilo (Kg)						
45-60 kg	33,10±4,15	24,55±3,12	15,86±2,74	12,87±1,89	19,44±3,19	105,83±12,24
61-75 kg	33,40±3,93	24,22±3,29	15,18±2,75	12,50±2,21	19,83±2,97	105,13±12,32
76 kg ve üzeri	33,69±4,36 F=0,769 p=0,464	24,18±3,23 F=0,507 p=0,602	15,35±3,10 F=1,971 p=0,140	12,71±1,90 F=1,268 p=0,282	20,14±3,33 F=1,826 p=0,162	106,08±12,75 F=0,317 p=0,729
Sigara kullanımı						
Yok	33,56±4,10	24,58±3,06	15,56±2,91	12,66±1,98	19,89±3,17	106,28±12,21
Var	33,27±4,24 t=0,779 p=0,437	23,77±3,44 t=2,823 p=0,005*	15,10±2,86 t=1,768 p=0,078	12,65±2,10 t=0,040 p=0,968	19,84±3,18 t=0,180 p=0,857	104,65±12,84 t=1,454 p=0,147

<u>Alkol kullanımı</u>						
Yok	33,62±3,76	24,37±3,12	15,50±2,80	12,73±1,93	19,92±3,06	106,15±11,67
Var	32,25±6,19 t=2,493 p=0,013*	23,58±3,89 t=1,845 p=0,066	14,62±3,45 t=2,265 p=0,024*	12,17±2,57 t=2,073 p=0,039*	19,51±3,88 t=0,970 p=0,332	102,14±16,81 t=2,424 p=0,016*
<u>Sağlık güvencesi</u>						
Evet	33,44±4,04	24,24±3,25	15,47±2,94	12,60±2,05	19,85±3,20	105,61±12,39
Hayır	33,56±4,75 t=-0,246 p=0,806	24,46±3,11 t=-0,575 p=0,566	14,96±2,63 t=1,456 p=0,146	12,98±1,91 t=-1,595 p=0,113	19,98±3,06 t=-0,357 p=0,722	105,95±12,90 t=-0,221 p=0,825
<u>İlaç kullanımı</u>						
Evet	33,86±3,32	24,09±3,11	15,35±2,70	12,79±1,90	20,30±3,14	106,40±11,09
Hayır	33,35±4,34 t=1,337 p=0,183	24,32±3,26 t=-0,682 p=0,496	15,40±2,95 t=-0,181 p=0,857	12,63±2,06 t=0,821 p=0,413	19,76±3,17 t=1,588 p=0,114	105,47±12,81 t=0,757 p=0,450
<u>Kronik hastalık</u>						
Evet	33,33±3,99	24,05±3,33	15,51±2,69	12,58±1,94	20,02±3,25	105,50±12,15
Hayır	33,48±4,19 t=-0,338 p=0,736	24,32±3,21 t=-0,717 p=0,475	15,36±2,94 t=0,466 p=0,642	12,68±2,05 t=-0,424 p=0,672	19,84±3,16 t=0,480 p=0,632	105,70±12,55 t=-0,143 p=0,886
<u>Tuzdan kaçınma</u>						
Evet	33,89±3,73	24,61±3,02	15,88±2,58	12,65±1,98	20,02±3,02	107,06±11,54
Hayır	33,02±4,50 t=2,412 p=0,016*	23,94±3,40 t=2,362 p=0,019*	14,90±3,11 t=3,893 p<0,001*	12,67±2,07 t=-0,072 p=0,943	19,73±3,31 t=1,058 p=0,291	104,26±13,20 t=2,576 p=0,010*
<u>Sebze-meyve tüketimi</u>						
Bir porsiyon	33,36±3,99	24,43±3,13	15,50±2,83	12,74±1,93	19,93±3,10	105,97±12,01
İki porsiyon	34,14±3,63	24,24±3,31	15,36±3,01	12,58±2,17	20,24±2,97	106,57±11,97
Üç ve fazlası	32,47±5,67	23,46±3,52	14,84±3,05	12,35±2,22	18,74±3,75	101,88±15,28
	F=3,559 p=0,029*	F=2,306 p=0,101	F=1,274 p=0,280	F=1,062 p=0,347	F=4,748 p=0,009*	F=3,196 p=0,042*
<u>Tüketilen yağ</u>						
Sıvı yağ	33,47±4,21	24,36±3,24	15,52±2,88	12,74±1,97	19,90±3,09	106,01±12,49
Katı yağ	33,31±3,66 t=0,318 p=0,751	23,56±3,08 t=1,835 p=0,071	14,34±2,85 t=2,961 p=0,004*	12,00±2,32 t=2,346 p=0,022*	19,62±3,77 t=0,557 p=0,579	102,84±11,93 t=1,898 p=0,062
<u>Günlük egzersiz</u>						
Evet	32,99±4,42	23,75±3,30	15,08±3,08	12,42±2,20	19,41±3,47	103,65±13,21
Hayır	33,64±4,03 t=-1,557 p=0,121	24,49±3,18 t=-2,343 p=0,020*	15,51±2,81 t=-1,466 p=0,144	12,76±1,94 t=-1,739 p=0,083	20,06±3,02 t=-2,030 p=0,043*	106,47±12,07 t=-2,260 p=0,025*
<u>Genel ruh hali</u>						
Öfkeliyim	32,12±5,05	23,32±3,36	14,28±3,07	12,29±2,23	19,43±2,83	101,42±13,11
Stresliyim	32,88±4,23	23,83±3,45	15,19±2,84	12,52±2,10	19,42±3,28	103,83±12,96
Uyumluyum	34,46±3,42	25,01±2,81	15,99±2,74	12,92±1,86	20,43±3,12	108,83±10,96
	F=14,148 p<0,001*	F=12,476 p<0,001*	F=12,679 p<0,001*	F=3,970 p=0,019*	F=6,626 p=0,001*	F=15,767 p<0,001*

*: p<0,05 anlamlılık

Tablo-4. Hipertansiyon Hastalığı ile İlgili Değişkenlere Göre Hipertansiyon Gelişiminin Önlenmesine İlişkin Tutum Ölçeğinin Karşılaştırılması

Demografik Özellikler	Boyut 1	Boyut 2	Boyut 3	Boyut 4	Boyut 5	Toplam Puan
	X±SS	X±SS	X±SS	X±SS	X±SS	X±SS
<u>Ailede HT tanısı alma durumu</u>						
Evet	33,96±4,07	24,67±2,92	15,68±2,80	13,16±1,71	20,70±2,89	108,17±10,92
Hayır	33,16±4,18	24,04±3,38	15,22±2,95	12,37±2,14	19,38±3,23	104,16±13,09

	t=2,159 p=0,031*	t=2,273 p=0,023*	t=1,780 p=0,076	t=4,394 p<0,001*	t=4,840 p<0,001*	t=3,772 p<0,001*
<u>HT tanılı</u>						
<u>yakınla aynı</u>						
<u>evde yaşama</u>						
Evet	33,86±3,32	24,09±3,11	15,35±2,70	12,79±1,90	20,30±3,14	106,40±11,09
Hayır	33,35±4,34	24,32±3,26	15,40±2,95	12,63±2,06	19,76±3,17	105,47±12,81
	t=0,995 p=0,322	t=0,452 p=0,652	t=-0,041 p=0,968	t=1,732 p=0,086	t=1,845 p=0,067	t=1,199 p=0,233
<u>HT risk</u>						
<u>grubunda yer</u>						
<u>alma</u>						
Evet	34,26±4,10	24,80±2,68	15,85±2,63	13,12±1,85	20,81±2,64	108,83±10,71
Hayır	33,05±4,12	24,01±3,45	15,16±3,00	12,43±2,07	19,40±3,31	104,04±12,99
	t=3,190 p=0,002*	t=2,655 p=0,008*	t=2,699 p=0,007*	t=3,854 p<0,001*	t=4,935 p<0,001*	t=4,230 p<0,001*
<u>HT önleme</u>						
<u>eğitim isteği</u>						
Evet	34,06±3,42	24,53±2,96	15,45±2,69	12,77±1,86	20,15±2,92	106,96±10,73
Hayır	32,54±4,94	23,88±3,58	15,30±3,20	12,50±2,25	19,46±3,48	103,70±14,52
	t=4,146 p<0,001*	t=2,251 p=0,025*	t=0,565 p=0,572	t=1,448 p=0,148	t=2,437 p=0,015*	t=2,953 p=0,003*
<u>Sağlık</u>						
<u>kontrollerine</u>						
<u>gitme</u>						
Evet	34,01±3,55	24,46±3,07	15,51±2,82	12,89±1,83	20,11±3,01	106,99±11,32
Hayır	31,69±5,30	23,66±3,65	15,01±3,11	11,94±2,43	19,12±3,54	101,42±14,84
	t= 5,611 p<0,001*	t=2,433 p=0,015*	t=1,613 p=0,108	t=4,628 p<0,001*	t=2,831 p=0,005*	t=4,436 p<0,001*

*: p<0,05 anlamlılık; HT: Hipertansiyon

Tablo-5. Katılımcıların HKTÖ İlişkin Puanlarını Etkileyen Faktörlerin Çoklu Doğrusal Regresyon Analizi

Değişkenler	Standardize Edilmemiş B Katsayısı	Standardize Edilmiş Beta (β) Katsayısı	t	p	VIF
Sabit katsayı	147,401		10,387	0,000	
Yaş	0,070	0,073	1,303	0,193	2,036
Cinsiyet (referans grup: erkek)	-0,722	-0,029	-0,498	0,619	2,203
Medeni durum (referans grup: bekar)	-3,613	-0,142	-2,811	0,005	1,663
Eğitim durumu (referans grup: ilköğretim ve altı)	-5,628	-0,201	-4,001	0,000	1,637
Eğitim durumu (referans grup: lise)	-4,492	-0,171	-3,782	0,000	1,322
Gelir durumu (referans grup: gelirim giderimden fazla)	-3,481	-0,117	-2,171	0,030	1,878
Gelir durumu (referans grup: gelirim giderime eşit)	-0,120	-0,005	-0,091	0,928	1,784
Günlük beslenmede tuzdan kaçınma durumu (referans grup: hayır)	-0,851	-0,034	-0,802	0,423	1,179
Günlük sebze ve meyve tüketimi (referans grup: bir porsiyon)	2,542	0,098	1,566	0,118	2,548

Günlük sebze ve meyve tüketimi (referans grup: iki porsiyon)	2,247	0,078	1,250	0,212	2,523
Günlük egzersiz yapma durumu (referans grup: hayır)	1,771	0,064	1,506	0,133	1,183
Sizi en iyi anlatan ruhsal durum (referans grup: öfkeli)	-4,343	-0,132	-2,963	0,003	1,291
Sizi en iyi anlatan ruhsal durum (referans grup: stresli)	-4,811	-0,188	-4,353	0,000	1,204
Ailede hipertansiyon hastası olma durumu (referans grup: hayır)	-1,251	-0,049	-1,101	0,272	1,268
Hipertansiyon risk grubunda yer alma durumu (referans grup: hayır)	-2,664	-0,101	-2,289	0,022	1,269
Hipertansiyonu önleme konusunda eğitim alma isteği (referans grup: hayır)	-1,365	-0,054	-1,287	0,199	1,130
Tavsiye edilen sağlık kontrollerine gitme durumu (referans grup: hayır)	-3,407	-0,117	-2,771	0,006	1,150
F			7,902		
p			<0,001		
R ²			0,22		
Durbin-watson			1,709		

Tablo-5'te katılımcıların HKTÖ puanlarını etkileyen faktörleri belirlemeye yönelik yapılmış olan çok değişkenli doğrusal regresyon modeli sonuçları yer almaktadır. Söz konusu amaçla kurulmuş olan modelin istatistiksel olarak anlamlı olduğu ($F=7,902$; $p<0,001$) görülmüştür. Ayrıca modelde otokorelasyon (Durbin-watson= 1,709) ile çoklu bağlantı sorununun (VIF değer aralığı= 1,130-2,548) olmadığı da tespit edilmiştir

TARTIŞMA

Bu çalışma yetişkin bireylerin hipertansiyondan korunmaya yönelik tutumları ve etkileyen faktörlerin incelenmesi amacıyla yapılmıştır. Katılımcıların HKTÖ toplam puan ortalaması $105,67 \pm 12,44$ olduğu bulunmuştur. Ölçekten 26-130 arası puan alınabilmekte olup, hipertansiyondan korunma tutumuyla ölçek skorları arasında pozitif ilişki olduğu bilindiğinden, katılımcıların hipertansiyondan korunma tutumlarının iyi düzeyde olduğu söylenebilir. Bu çalışmada kullanılan ölçek ülkemizde yeni (2022) geliştirildiği için aynı ölçekle yapılmış bir çalışmaya ulaşılamamıştır. Ancak, Rahman ve ark. (2018), yetişkinlerde hipertansiyon hakkında bilgi, tutum ve uygulamaları değerlendirdiği çalışmasında katılanların yarısından fazlası hipertansiyon hakkında yeterli bilgiye sahip olduğu ve %85,68'inin hipertansiyona karşı olumlu tutuma sahip olduğu belirtilmektedir (13). Bu konuda, literatürde hem hipertansif hem de hipertansif olmayan hastalar arasında, özellikle kadınlar olmak üzere (14-17), benzer olarak hipertansiyon bilgisinin iyi düzeyde olduğu rapor edilmiştir (18). Bu konuda ülkemizde yapılan PatenT 2 çalışmasında da (2016) belirtildiği gibi, 2003'ten 2012'ye kadar hipertansiyonun

tanınmasında bir miktar ilerleme kaydedilmiş olmasına rağmen, Türkiye'de hala tedavi edilmemiş veya yetersiz tedavi edilmiş yüksek bir hipertansif popülasyon bulunmaktadır (6). Hipertansiyonun önlenmesi, erken teşhisi ve tedavisini iyileştirmek için toplum temelli çabaların güçlendirilmesi gerekmektedir.

Yetişkin bireylerin hipertansiyondan korunmaya yönelik tutumları ve etkileyen faktörleri değerlendirildiğinde, HKTÖ puanları üzerinde medeni durum, eğitim durumu, gelir durumu, ruhsal durumu, hipertansiyon açısından risk grubunda yer alma düşüncesinin ve sağlık kontrollerine gitme durumunun istatistiksel açıdan anlamlı bir etkisinin olduğu bulunmuştur. Bu çalışmada bekar bireylerin hipertansiyondan korunma düzeylerinin, evli bireylere kıyasla daha düşük olduğu görülmüştür. Paulose ve ark. (2022) tarafından yapılan bir çalışmada hipertansiyonu önlemeye yönelik olumlu bilgi ve tutumla ilişkili düzeyi ve faktörleri değerlendirmek amacıyla yapılan çalışmada, boşanmış/dul bireylerin, evlilere kıyasla daha az olumlu tutum sergiledikleri belirtilmektedir (19). Bu durumun evli olan bireylerde birlikte yaşadığı eş ve çocuğuna karşı sorumluluk hissederek, daha fazla sağlığı koruyucu davranışta bulunması ve

daha düzenli yaşam tarzının olmasına bağlı olabileceği düşünülmektedir.

Eğitim, hipertansif bireylerde küresel kardiyovasküler riskin en iyi belirleyicisi olarak kabul edildiğinden, hipertansiyon ve kardiyovasküler risk yönetimi stratejilerinde değerlendirilmesi önemlidir (20). Bu çalışmada, ilköğretim ve altı ile lise mezunu olan bireylerin üniversite mezunu olan bireylere göre hipertansiyondan korunma tutum puanlarının daha az olduğu tespit edilmiştir. Bu konuda Paulose ve ark. (2022) tarafından hipertansiyonu önlemeye yönelik olumlu bilgi ve tutumla ilişkili düzeyi ve faktörleri değerlendirmek amacıyla yapılan çalışmada, ilk, orta ve yüksek eğitim durumu olan katılımcıların okuma yazma bilmeyenlere göre daha olumlu tutumlarının olduğu belirlenmiştir (19). Hipertansiyon hastalarının, hastalıkları hakkında bilgi düzeyleri ve ilişkili faktörlerin ortaya konması amacıyla yapılan bir çalışmada da ilaç uyum bilgi düzeyinin ortaokul, lise, üniversite ve üzeri mezun grubun, ilkökul ve altı gruptan anlamlı olarak daha yüksek olduğu belirtilmektedir (21). Farklı olarak, Malezya'da Selangor kentinde konut sakinleri arasında hipertansiyona ilişkin bilgi, tutum ve uygulamaların incelendiği bir çalışmada; eğitim düzeyi ve hipertansiyona ilişkin bilgi, tutum ve uygulama arasında anlamlı ilişki saptanmamıştır (22). Bu çalışmada, hipertansiyondan korunma tutum ölçeği puanları üzerinde istatistiksel olarak anlamlı etkiye sahip olan değişkenler arasında en yüksek etkiye sahip olan değişkenin eğitim düzeyi olması da, dikkat çekici bir bulgu olarak karşımıza çıkmaktadır.

Geliri giderlerine göre daha fazla olan katılımcıların, diğer katılımcılara kıyasla hipertansiyondan korunma tutumlarının daha yüksek olduğu görülmüştür. Düşük gelirli ailelerden gelen bireylerin hipertansiyon tedavisine uyumunun düşük olduğunu belirten bir çalışmada da maddi zorluklar veya kısıtlamalar nedeniyle tedavi rejimine uyumun azaldığı, hipertansiyon ilaçlarının karşılanamaması bağlı kontrolsüz kan basıncına yol açtığı belirtilmektedir (23). Ek olarak, zayıf uyum, bilgi eksikliği ve ilaç temini ile ekonomik durum arasında ilişki belirtilmektedir (24). Farklı olarak, hipertansiyona ilişkin bilgi, tutum ve uygulamaların incelendiği bir çalışmada, gelir durumu ile hipertansiyona ilişkin bilgi, tutum ve uygulama ile anlamlı bir ilişkisi saptanmamıştır (22). Ayrıca bu çalışmada ruhsal durumu “*öfkeli*

ve *stresli*” olan bireylerin ruhsal durumu “*uyumlu*” olan bireylere kıyasla hipertansiyondan korunma tutum düzeylerinin daha düşük olduğu saptanmıştır. Kang ve ark. (2018) tarafından stres, uyum ve kan basıncı kontrolü arasındaki ilişkiyi incelenmiştir. Aynı stresin daha düşük yaş ve bekar ebeveyn olma ile ilişkili olduğunu bulmuştur. Stres ayrıca daha yüksek sistolik kan basıncı ve zayıf kan basıncı kontrolü ile ilişkilendirilmiştir (25). Bununla birlikte, stres ve anksiyetenin hem kardiyovasküler hastalıklar hem de hipertansiyon üzerinde etkili olduğu bilinmektedir.

Bu çalışmada, kendisini hipertansiyon açısında riskli grupta görmeyen kişilerin hipertansiyondan korunma tutum ölçeği puanlarının, riskli grupta görenlere kıyasla daha az olduğu bulunmuştur. Aynı zamanda, tavsiye edilen sağlık kontrollerine gitmeyen bireylerin de hipertansiyondan korunma tutum düzeylerinin daha düşük olduğu sonucuna ulaşılmıştır. Bununla birlikte, katılımcıların %66,3'ü kendini hipertansiyon açısından risk grubunda görmediği bulunmuştur. Benzer şekilde Albayrak ve Şengezer (2022) tarafından yapılan çalışmada katılımcıların %68,8'i hipertansiyon için risk grubu olmadığını ifade etmiştir (12). Malezya'da Selangor kentindeki konut sakinleri arasında hipertansiyona ilişkin bilgi, tutum ve uygulamaların incelendiği bir çalışmada, aile öyküsü ile hipertansiyona ilişkin bilgi, tutum ve uygulama ile anlamlı bir ilişkisi saptanmamıştır (22). Bu durumun çalışmamızda, ailede hipertansiyon hastası olan birey bulunan (%37,3) ve hipertansiyon tanısı olan akraba ile aynı evde yaşayan (%17,5) katılımcıların oranının düşük olmasıyla da ilişkili olabileceği düşünülmektedir.

SONUÇ

Katılımcıların hipertansiyondan korunma tutumları üzerinde bazı sosyodemografik ve sağlık/hastalık değişkenlerinin etkisinin olduğu bulunmuştur. Hipertansiyondan korunma tutum ölçeği puanları üzerinde istatistiksel olarak anlamlı etkiye sahip olan değişkenin ise eğitim düzeyi olduğu belirlenmiştir. Hipertansiyondan korunmaya yönelik tutumların gelişmesi için bireylerin yaşam kalitesinin artırılmasına olanak sağlayacağı düşünülmektedir. Hipertansiyondan korunmaya yönelik tutumların gelişmesi için bireylere hipertansiyona yönelik bireysel eğitimlerin düzenlenmesi ve özellikle risk altındaki grupların farkındalığının sağlanması önemlidir. Hipertansiyon riskini azaltmak için dergilerde ve sosyal alanlarda

destekleyici eğitimlerin ve sosyal faaliyetlerin yürütülmesi hastalığa karşı olumlu tutumun artmasına katkı sağlayabilir. Bu konuda daha fazla örneklem grubu ve nedenselliği araştıran derinlemesine yeni çalışmaların yapılması önerilmektedir.

Araştırmanın Sınırlılıkları

Bu çalışmanın bazı sınırlılıkları bulunmaktadır. Bu çalışmanın bulguları sadece çalışmanın yapıldığı kurumdaki yetişkin bireylerle ilgili olduğu

için, araştırma sonuçları genellenebilirliği açısından kısıtlıdır. Bununla birlikte, çalışmaya katılım gönüllük esasına dayalı olduğundan ve anket sorularının genel yapısı nedeniyle ölçeklere ilişkin soruları katılımcıların öznel olarak değerlendirildiği kabul edilmektedir.

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PSA dansitesinin robotik radikal prostatektomi sonuçlarına etkisinin incelenmesi

Evaluation of the effect of PSA density on robotic radical prostatectomy outcomes

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ÖZ

Amaç: Prostat spesifik antijen dansitesi (PSAD), prostat kanseri tanısında kullanılan serum prostat spesifik antijen (PSA) düzeyinin prostat volümüne oranlanması ile elde edilen tanısal bir belirteçtir. Prostat büyümesi ile PSA düzeylerindeki artışın korele olması nedeniyle tanısal değerlendirmede bu oran sıklıkla kullanılmaktadır ancak cerrahi sonuçların öngörülmesinde kullanılmamaktadır. Bu çalışmanın amacı, lokalize prostat kanseri için robotik radikal prostatektomi geçiren hastalarda PSA dansitesinin cerrahi sonuçlar ve postoperatif komplikasyonlar üzerindeki etkisini değerlendirmektir.

Gereç ve Yöntem: Bu retrospektif kohort çalışması, Temmuz 2020 ile Haziran 2022 arasında robotik RP uygulanan 154 hastanın verilerini analiz etmiştir. PSAD, preoperatif PSA düzeyinin (ng/mL) transrektal ultrason (TRUS) veya multiparametrik MRG ile elde edilen prostat hacmine (mL) oranı olarak hesaplanmıştır. Elektronik dosya arşivi taraması ile toplanan veriler demografik bilgiler, preoperatif PSA düzeyleri, prostat hacmi, cerrahi parametreler ve postoperatif sonuçları içermektedir.

Bulgular: Hastaların yaş ortalaması 65 (6,69) olup, yaş aralığı 46 ile 80 arasındadır. Preoperatif PSA düzeyleri 2.19 ile 35.4 ng/mL arasında değişmekte ve ortalama 7,75 (4,73) ng/mL olarak bulunmuştur. Prostat hacmi ortalama 47,73 (16,1) mL'dir. PSAD değerlerinin dağılımında hastaların %18,8'i düşük risk, %34,4'ü orta-düşük risk, %22,1'i orta-yüksek risk ve %24,7'si yüksek risk grubundadır. Daha yüksek PSAD ile pozitif cerrahi sınır oranları %18,75, %31,91, %44,12 ve %51,22 olarak saptanmıştır. PSAD'nin vezikouretral anastomoz stenozu (VUAS) ($p=0.8$) veya anastomoz kaçakları ($p=0.9$) üzerinde anlamlı bir etkisi bulunmamıştır. Lojistik regresyon analizi, PSAD (katsayı 3.02, $p=0.051$) ve T3a kanserin (katsayı 1.48, $p=0.018$) pozitif cerrahi sınırların anlamlı prediktörleri olduğunu göstermiştir. ROC eğrisi analizinde, PSAD'nin AUC değerinin (0.65), T3a kanserin AUC değerinden (0.55) daha yüksek olduğu saptanmış olup bu durum PSAD'nin T3a kanserden daha iyi bir pozitif cerrahi sınır belirteci olabileceğini göstermektedir.

Sonuç: Bu çalışma, robotik radikal prostatektomi geçiren hastalarda yüksek PSAD'nin pozitif cerrahi sınır riskini artırdığını ve cerrahi sonuçları öngörmeye önemli bir biyobelirteç olabileceğini göstermektedir. Ancak, PSAD'nin postoperatif komplikasyonlar üzerinde anlamlı bir etkisi yoktur, bu da daha fazla araştırmaya ihtiyaç olduğunu göstermektedir. PSAD, cerrahi sonuçların iyileştirilmesinde önemli bir rol oynayabilir.

Anahtar Sözcükler: Prostat Kanseri, Robotik Cerrahi, PSA Dansitesi, Cerrahi Sınırlar, Postoperatif Komplikasyonlar

ABSTRACT

Aim: Prostate-specific antigen density (PSAD) is a diagnostic marker derived by dividing the serum prostate-specific antigen (PSA) level by prostate volume, commonly used in the evaluation of prostate cancer.

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This ratio is frequently employed during diagnostic assessments due to the correlation between prostate enlargement and elevated PSA levels; however, its utility in predicting surgical outcomes remains unexplored. This study aimed to assess the impact of PSA density on surgical outcomes and postoperative complications in patients undergoing robotic radical prostatectomy for localized prostate cancer.

Materials and Methods: *This retrospective cohort study included data from 154 patients who underwent robotic radical prostatectomy between July 2020 and June 2022. PSAD was calculated as the ratio of the preoperative PSA level (ng/mL) to prostate volume (mL), determined using transrectal ultrasound (TRUS) or multiparametric MRI. Data collected from electronic records included demographic information, preoperative PSA levels, prostate volume, surgical parameters, and postoperative outcomes.*

Results: *The mean age of the patients was 65 years ($\pm 6,69$), with an age range of 46–80 years. Preoperative PSA levels ranged from 2,19 to 35,4 ng/mL, with a mean of 7,75 ng/mL ($\pm 4,73$). The mean prostate volume was 47,73 mL ($\pm 16,1$). Patients were stratified into risk groups based on PSAD: 18,8% were low risk, 34,4% intermediate-low risk, 22,1% intermediate-high risk, and 24,7% high risk. In terms of the risk groups, the positive surgical margin rates with higher PSAD were 18,75%, 31,91%, 44,12%, and 51,22%, respectively. PSAD had no significant association with vesicourethral anastomotic stenosis (VUAS) ($p=0.8$) or anastomotic leakage ($p=0.9$). Logistic regression analysis identified PSAD (coefficient 3,02, $p=0.051$) and T3a cancer (coefficient 1,48, $p=0,018$) as significant predictors of positive surgical margins. ROC curve analysis revealed that the AUC value of PSAD (0,65) was higher than that of T3a cancer (0,55), suggesting PSAD may be a superior predictor of positive surgical margins.*

Conclusion: *This study demonstrates that elevated PSAD is associated with an increased risk of positive surgical margins in patients undergoing robotic radical prostatectomy, highlighting its potential as an important biomarker for predicting surgical outcomes. However, PSAD showed no significant effect on postoperative complications, underscoring the need for further research. PSAD may play a crucial role in optimizing surgical outcomes.*

Keywords: *Prostate Cancer, Robotic Surgery, PSA Density, Surgical Margins, Postoperative Complications*

GİRİŞ

Prostat kanseri, erkeklerde en sık teşhis edilen ikinci kanser türü ve küresel olarak en yaygın beşinci ölüm nedenidir (1). Klinik olarak lokalize prostat kanseri olan hastaların büyük çoğunluğu için standart tedavi radikal prostatektomi operasyonudur (2). Radikal prostatektominin amacı, uygulama yöntemi ne olursa olsun, kanseri tamamen ortadan kaldırırken mümkün olduğunca pelvik organ fonksiyonlarını korumaktır. Robotik radikal prostatektomi (RP), lokalize prostat kanserinin tedavisinde yaygın olarak kullanılan minimal invaziv bir cerrahi prosedürdür (3). Avantajları arasında kan kaybının azalması, hastanede kalış süresinin kısalması ve geleneksel açık cerrahiye kıyasla daha hızlı iyileşme süreleri yer alır (4). Serum prostat spesifik antijen düzeyinin prostat hacmine oranı olarak hesaplanan PSAD, PSA'nın tek başına olduğundan daha spesifik bir prostat kanseri belirtici olabilir. Prostat boyutunun PSA düzeyleri üzerindeki etkisini hesaba katarak potansiyel olarak daha iyi tanı doğruluğu sağlar (5).

PSAD, prostat kanserinin tanısında önemli bir yere sahiptir (6–11). Ancak prediktif bir prognostik faktör olarak kullanılmasıyla ilgili farklı görüşler mevcuttur (12). Yashi ve ark.'larının 2017'de yaptıkları bir kohort çalışmasında PSAD özellikle yüksek risk grubundaki hastalarda olumsuz patolojik sonuçlar için bağımsız bir risk faktörü olarak bulunmuştur (13). Freedland ve ark.'nın çalışmasında PSAD ile kanser rekürrensi arasında anlamlı ilişki bulunduğu gösterilmiştir (11). PSAD'nin operasyonun onkolojik sonuçlarını öngörmedeki gücü Dilme ve ark.'nın çalışmasında incelenmiş ve pozitif cerrahi sınır, gleason 7 ve üzeri tümör ve biyokimyasal rekürrens için prediktif faktör olabileceği belirtilmiştir (12). Çalışmamızın birincil amacı, PSA yoğunluğunun robotik RP'nin sonuçları üzerindeki etkisini değerlendirmek, cerrahi başarıya ve postoperatif komplikasyonlara odaklanmaktır. İkincil amaç, PSA dansitesinin bu sonuçlar için öngörücü bir biyobelirteç olarak hizmet edip edemeyeceğini belirlemektir.

GEREÇ ve YÖNTEM

Bu retrospektif kohort çalışmasında kurumumuzda robotik RP uygulanan hastaların verileri analiz edilmiştir. Kohort, Temmuz 2020 ile Haziran 2022 arasında opere edilen 154 hastayı içermektedir.

Dahil etme kriterlerinde 40-80 yaş aralığında olmak, vücut kitle indeksinin 18,5-35 kg/m² arasında olması, operasyon öncesi metastatik hastalığın dışlanması için kemik sintigrafisi ve tüm vücut kontrastlı tomografide metastaz saptanmamış olması, preoperatif biyopside Gleason skorunun 6-8 arasında olması, lokalize prostat kanseri nedeniyle robotik RP operasyonu uygulanmış olması bulunmaktadır. Hariç tutma kriterleri PSA değerleri ya da prostat hacimleri bilgilerine ulaşılamayan hastalar, daha önce çeşitli nedenlerle prostat ameliyatı geçirmiş hastalar, laparoskopik prostat cerrahisi geçirmiş hastalar, lokal ileri prostat kanseri tanısı alan hastalar ve pelvik radyoterapi öyküsü olan hastalardır.

PSA dansitesi, preoperatif PSA düzeyinin (ng/mL), transrektal ultrason (TRUS) veya multiparametrik prostat MRG ile elde edilen prostat hacmine (mL) oranı olarak hesaplanmıştır. Tüm ameliyatlar en az 10 yıldır üroloji uzmanı olan ve en az 100 adet robotik RP operasyonu gerçekleştirmiş deneyimli ürologlar tarafından da Vinci Cerrahi Sistemi kullanılarak gerçekleştirilmiştir. Tüm prosedürler genel anestezi altında gerçekleştirildi ve hasta hazırlanıp örtüldükten sonra mesaneye 16 fr Foley kateter yerleştirildi. Palmer noktasına Veress iğnesi yerleştirilerek karbondioksit insuflasyonu ile 20 mm Hg basınçta pnömoperiton oluşturuldu, ardından kamera ve diğer portlar yerleştirildi. Trendelenburg pozisyonundaki hastaya da Vinci robotu yerleştirilerek pelvis ve prostata erişim sağlandı. Operasyonların tamamı intraperitoneal anteroposterior kombine yaklaşım kullanılarak gerçekleştirilmiştir. Tüm hastalarda bilateral sinir koruyucu cerrahi yapılmıştır. Vezikoüretal anastomoz 3/0 balık sırtı emilebilir sütürler kullanılarak kontinü şekilde gerçekleştirilmiştir. Tüm hastalara operasyon sonunda bir adet pelvik dren ve 20 fr foley sonda tatbik edilmiştir. Postoperatif dönemde hastalara derin ven trombozu için mekanik ve medikal profilaksi uygulanmıştır. Toplanan veriler demografik bilgiler, preoperatif PSA düzeyleri, prostat hacmi, PSA dansitesi, cerrahi parametreler (ameliyat süresi, tahmini kan kaybı) ve postoperatif sonuçları (pozitif cerrahi sınırlar, patolojik evre, komplikasyonlar) içermektedir. Tahmini kan kaybı hesaplanırken operasyon sırasında kullanılan aspiratördeki sıvı

seviyesi ölçülmüş ve cerrahi sırasında yıkama için kullanılan sıvı miktarı bu değerden çıkarılmıştır. PSA dansitesi risk grupları için, Avrupa Üroloji Derneği prostat kanseri kılavuzlarında da prostat kanseri tanısında riske dayalı biyopsi kararı verilmesi için kullanılan, 2021 yılında Schoots ve ark tarafından geliştirilen tablodaki PSA dansitesi değerleri kestirim değerleri olarak belirlenmiştir (14). Veriler SPSS 29.0 programında değerlendirildi. Populasyon dağılımı kolmogorov-smirnov testi ile değerlendirildikten sonra, normal dağılıma sahip parametrelerde pearson korelasyon testi uygulandı. Lojistik regresyon analizi ile anlamlı saptanan parametrelere etki eden başka faktörlerin olup olmadığı değerlendirildi. P<0.05 istatistiksel olarak anlamlı kabul edildi.

BULGULAR

Çalışma popülasyonunun demografik özellikleri incelendiğinde, hastaların yaş ortalaması 65 (6,69) olup, yaş aralığı 46 ile 80 arasında değişmektedir. Hastaların preoperatif PSA değerleri 2,19 ng/ml ile 35,4 ng/ml arasında değişmekte ve ortalama 7,75 (4,73) ng/ml olarak bulunmuştur. Preoperatif prostat volümleri ise ortalama 47,73 (16,1) ml olarak ölçülmüştür.

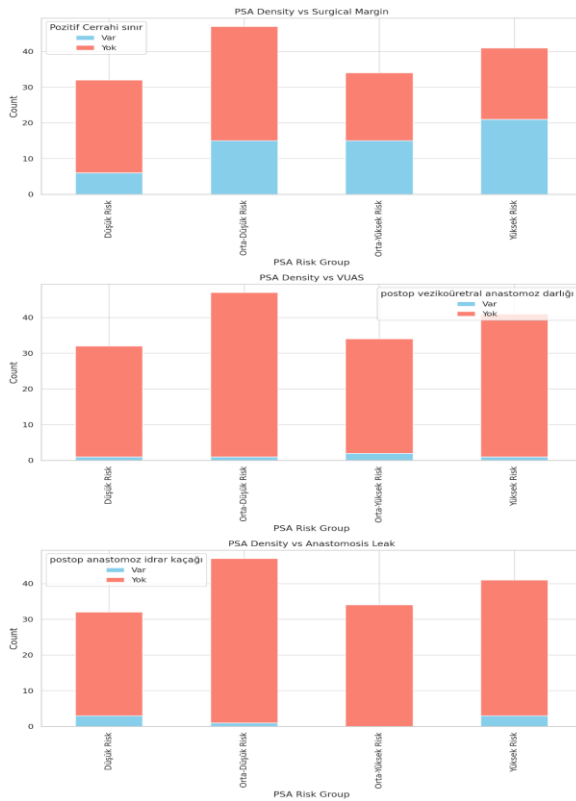
PSA dansitesi değerlerinin dağılımına bakıldığında, hastaların %18,8'inin düşük risk, %34,4'ünün orta-düşük risk, %22,1'inin orta-yüksek risk ve %24,7'sinin yüksek risk grubunda yer aldığı görülmüştür. Bu dağılım, PSA dansitesinin hastalar arasında geniş bir yelpazede değiştiğini göstermektedir.

Cerrahi parametrelerde tahmini kan kaybı ortalama 142,63 (84,30) ml olarak hesaplanmıştır. Cerrahi sınır pozitifliği oranı PSA dansitesine göre değişiklik göstermiştir. Düşük risk grubunda %18,75, orta-düşük risk grubunda %31,91, orta-yüksek risk grubunda %44,12 ve yüksek risk grubunda %51,22 oranında cerrahi sınır pozitifliği saptanmıştır.

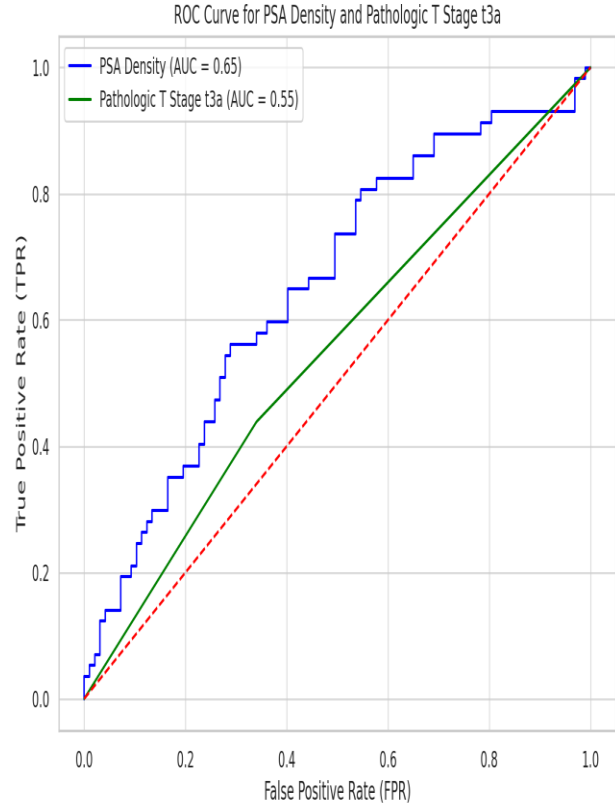
Postoperatif komplikasyonlar değerlendirildiğinde, PSA dansitesinin vezikoüretal anastomoz darlığı (VUAS) ve anastomoz kaçakları üzerindeki etkisi incelenmiştir. PSA dansitesi düşük olan hastalarda VUAS %3,12, orta-düşük risk grubunda %2,13, orta-yüksek risk grubunda %5,88 ve yüksek risk grubunda %2,44 olarak bulunmuştur. Anastomoz kaçakları ise düşük risk grubunda %9,38, orta-düşük risk grubunda %2,13, orta-yüksek risk grubunda %0 ve yüksek risk grubunda %7,32 olarak saptanmıştır. Lojistik regresyon analizi sonuçlarına göre PSA dansitesinin VUAS(p=0,940)

ve anastomoz kaçağı(p=0,991) üzerinde anlamlı etkisinin bulunmadığı saptanmıştır (Şekil-1). Patolojik bulguların lojistik regresyon analizine göre nihai patolojide saptanan T3a kanserin cerrahi sınır pozitifliği üzerinde anlamlı etkileri bulunmuştur. PSA dansitesinin cerrahi sınır pozitifliği üzerindeki katsayısı 3,02 (p=0,051), T3a kanserin katsayısı ise 1,48 (p=0,018) olarak hesaplanmıştır. PSA dansitesi ve T3a kanserin cerrahi sınır pozitifliğini öngörmedeki performansları ROC eğrisi

ve AUC hesaplanarak karşılaştırıldığında PSA dansitesinin AUC değeri (0,65), T3a kanserin AUC değerinden (0,55) daha yüksektir (Şekil-2). Bu, PSA dansitesinin cerrahi sınır pozitifliğini öngörmede T3a kanserden daha değerli olduğunu göstermektedir.



Şekil-1. PSAD risk gruplarına göre Cerrahi sınır, VUAS ve Anastomoz kaçağı parametrelerinin lojistik regresyon analizi grafiği.



Şekil-2. Cerrahi sınır pozitifliğini öngörmede PSA Dansitesi ve T3a patolojik evresinin ROC eğrileri.

TARTIŞMA

Çalışmamızda, PSA dansitesinin robotik RP ilişkili sonuçlar ve komplikasyonlar üzerindeki etkileri değerlendirilmiştir. Elde edilen sonuçlar, PSA dansitesinin cerrahi sınır pozitifliği ile anlamlı bir ilişkiye sahip olduğunu, ancak VUAS ve anastomoz kaçağı üzerinde anlamlı bir etkisinin olmadığını göstermektedir. Bu bulgu, daha yüksek PSA dansitesinin, cerrahi sınır pozitifliği riskini artırabileceğini ve bu nedenle, cerrahi sonuçların optimizasyonunda dikkate alınması gerektiğini vurgulamaktadır.

PSA dansitesinin onkolojik sonuçlar üzerindeki etkisi, literatürde sıkça araştırılmış bir konudur. Örneğin, S. Bishara ve arkadaşlarının çalışmasında, PSA dansitesinin biyokimyasal nüksü (BCR) öngörmede PSA'ya kıyasla daha üstün bir prediktör olduğu bulunmuştur (15). Çalışmamız, PSA dansitesinin sadece onkolojik sonuçlar üzerinde değil, aynı zamanda cerrahi sınır pozitifliği üzerinde de güçlü bir öngörücü olduğunu göstermektedir. Ayrıca, Roser Vives Dilme ve arkadaşlarının çalışmasında, PSA dansitesinin pozitif cerrahi sınırlar, Gleason skoru ≥ 7 ve PSA persistansı ile anlamlı bir ilişkiye sahip olduğu belirtilmiştir (12). Ancak, bu çalışmalarda PSA

dansitesinin cerrahi sonuçlar ve postoperatif komplikasyonlar üzerindeki spesifik etkileri incelenmemiştir.

PSA dansitesinin cerrahi sınır pozitifliği üzerindeki anlamlı etkisi, bu biyobelirtecin robotik RP geçiren hastalarda cerrahi sonuçların öngörülmesinde önemli bir rol oynayabileceğini göstermektedir. ROC eğrisi ve AUC analizi sonuçlarına göre, PSA dansitesinin AUC değerinin (0,65), T3a kanserin AUC değerinden (0,55) daha yüksek olması PSA dansitesinin cerrahi sınır pozitifliğini öngörmeye T3a kanserden daha değerli olduğunu ortaya koymaktadır. Bu sonuç analizleri literatürde daha önce saptanmamış bulgulardır.

PSA dansitesinin bu etkilerinin altında yatan potansiyel mekanizmalar tam olarak anlaşılamamakla birlikte, PSA dansitesinin daha yüksek olduğu durumlarda tümör yükünün ve agresifliğinin artabileceği düşünülmektedir (17). Bu da cerrahi sınırların daha sık pozitif olmasına neden olabilir. Ayrıca, prostat hacminin PSA düzeylerine olan etkisini dengeleyerek, PSA dansitesinin daha spesifik bir kanser belirtici olarak işlev görmesi de olasıdır (16–18).

Çalışmamızın bazı sınırlamaları bulunmaktadır. İlk olarak, retrospektif doğası nedeniyle veri toplama ve analiz süreçlerinde yanlılıklar oluşabilir. Ayrıca, hasta sayısının nispeten küçük olması, sonuçların genellenebilirliğini sınırlandırabilir. Gelecekteki araştırmalar, daha büyük ve prospektif kohortlarda PSA dansitesinin cerrahi sonuçlar ve postoperatif komplikasyonlar üzerindeki etkilerini daha ayrıntılı olarak inceleyebilir.

Sonuç olarak, bu çalışma, PSA dansitesinin robotik RP sonrası cerrahi sınır pozitifliği üzerinde önemli bir öngörücü olduğunu göstermektedir. Bu bulgu, PSA dansitesinin preoperatif değerlendirmelerde dikkate alınması gerektiğini ve cerrahi sonuçların iyileştirilmesinde önemli bir rol oynayabileceğini vurgulamaktadır. Ancak, PSA dansitesinin postoperatif komplikasyonlar üzerindeki etkisi belirgin değildir ve bu konudaki daha fazla araştırmaya ihtiyaç vardır.

SONUÇ

Çalışmamız, PSA dansitesinin robotik RP sonrası cerrahi sınır pozitifliği üzerinde anlamlı bir etkisi olduğunu ve daha yüksek PSA dansitesine sahip hastalarda cerrahi sınır pozitifliği riskinin arttığını göstermiştir. Bununla birlikte, PSA dansitesinin VUAS ve anastomoz kaçakları üzerinde anlamlı bir etkisinin olmadığı bulunmuştur. Bu bulgular, PSA dansitesinin robotik RP' de cerrahi sonuçların öngörülmesinde önemli bir biyobelirteç olarak hizmet edebileceğini ve preoperatif değerlendirmelerde dikkate alınması gerektiğini vurgulamaktadır. Ancak, postoperatif komplikasyonlar üzerindeki etkisi belirgin olmadığından, bu alanda daha fazla araştırmaya ihtiyaç duyulmaktadır. Sonuç olarak, PSA dansitesi, cerrahi sınır pozitifliği riskinin değerlendirilmesinde ve robotik RP sonrası cerrahi sonuçların iyileştirilmesinde klinik olarak önemli bir rol oynayabilir.

Çıkar Çatışması: Yazarlar aralarında çıkar çatışması olmadığını beyan etmişlerdir.

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Kalp yetersizliği ve ileri yaş: Komorbiditeler, tedavi uygulamaları ve sağkalım analizi

Heart failure in advanced age: comorbidities, treatment approaches, and survival outcomes

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ÖZ

Amaç: Bu çalışmanın amacı, Kalp Yetersizliği Polikliniğinde takip edilen ileri yaş grubundaki hastaların eşlik eden komorbiditelerini, kalp yetersizliğinde sağkalımı artıran tedavileri kullanma ve hedef dozlara ulaşma oranlarını belirlemek, primer ve sekonder sonlanım farklılıklarını erken yaş grubu ile karşılaştırmak ve ileri yaş grubunda medikal tedavi dozlarının primer sonlanım noktasına ulaşmadaki etkilerini araştırmaktır.

Gereç ve Yöntem: Çalışmaya kalp yetersizliği tanısı almış ve poliklinikte takip edilen 1624 hasta dahil edilmiştir. Hastaların klinik verileri hasta dosyaları, elektronik kayıtlar ve gerektiğinde telefon görüşmeleri ile elde edilmiştir. İleri yaş kalp yetersizliği grubu, 75. persentil ve üzeri yaşında tanı alan hastalar olarak tanımlanmıştır. Hastalar medikal tedavi kullanımı ve dozlarına göre incelenmiştir.

Bulgular: İleri yaş kalp yetersizliği hastalarının %39,2'si NYHA fonksiyonel sınıf III ve %9,2'si NYHA fonksiyonel sınıf IV olarak bulunmuştur. İleri yaş grubunda sistolik ve diyastolik kan basınçları, erken yaş grubuna göre daha düşük saptanmıştır. Komorbid hastalıkların (hipertansiyon, diabetes mellitus, hiperlipidemi, KOAH) ileri yaş grubunda daha yaygın olduğu tespit edilmiştir. Beta-bloker ve ACE-i/ARB kullanımında hedef dozlara ulaşma oranları ileri yaş grubunda daha düşüktür. İleri yaş grubunda primer sonlanım noktasına ulaşma oranı %35 iken, erken yaş grubunda bu oran %25,2 olarak bulunmuştur. Cox-regresyon analizleri, sol ventrikül ejeksiyon fraksiyonunun artışının sağkalıma olumlu etkisini göstermiştir.

Sonuç: İleri yaş kalp yetersizliği hastalarında komorbid hastalık yükü daha fazladır ve bu durum tedavi sürecini olumsuz etkilemektedir. Medikal tedavilerin hedef dozlara ulaşılması sağkalımı artırmakta ve tedaviye uyumun önemi vurgulanmaktadır.

Anahtar Sözcükler: Kalp yetersizliği, İleri yaş, Komorbidite, Medikal tedavi, Sağkalım

ABSTRACT

Aim: This study aims to identify the comorbidities, the usage rates of heart failure treatments that improve survival, and the achievement of target doses in elderly patients followed up at the Heart Failure Outpatient Clinic. Additionally, the study seeks to compare the differences in primary and secondary outcomes between the elderly and younger patient groups, and to investigate the impact of medical treatment doses on reaching the primary endpoint in the elderly group.

Materials and Methods: The study included 1624 patients diagnosed with heart failure and followed up in the outpatient clinic. Clinical data of the patients were obtained from patient files, electronic records, and, when necessary, through phone interviews.

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The elderly heart failure group was defined as patients diagnosed at or above the 75th percentile age. Patients were evaluated based on their use and doses of medical treatment.

Results: Among the elderly heart failure patients, 39.2% were classified as NYHA functional class III, and 9.2% as NYHA functional class IV. Systolic and diastolic blood pressures were found to be lower in the elderly group compared to the younger group. Comorbid conditions (hypertension, diabetes mellitus, hyperlipidemia, COPD) were more prevalent in the elderly group. The rates of achieving target doses for beta-blockers and ACE inhibitors/ARBs were lower in the elderly group. The rate of reaching the primary endpoint was 35% in the elderly group, compared to 25.2% in the younger group. Cox regression analyses showed that an increase in left ventricular ejection fraction had a positive impact on survival.

Conclusion: The burden of comorbid diseases is higher in elderly heart failure patients, which negatively affects the treatment process. Achieving target doses of medical treatments improves survival, emphasizing the importance of adherence to therapy.

Keywords: Heart failure, Elderly, Comorbidity, Medical treatment, Survival

GİRİŞ

Kalp yetersizliği prevalansı, kardiyovasküler koruyucu uygulamalar ve akut koroner sendromda erken reperfüzyon girişimleri ile artmaktadır. Kalp yetersizliğinin tüm dünyadaki prevalansı %1-2, Avrupa'daki prevalansı ise %2'nin üzerindedir. (1,2) Amerika Birleşik Devletleri'nde 2030 yılında kalp yetersizliği tanısı almış hasta sayısının sekiz milyonu aşması beklenmektedir. İleri yaş popülasyonunda bu artışın daha belirgin olacağı düşünülmektedir. (3)

Kalp yetersizliği hastalarında yaşın ilerlemesi ile sağkalım oranları azalmakta ve kalp yetersizliği nedenli hastaneye yatış sıklığı artmaktadır. Yaşlı kalp yetersizliği popülasyonunda komorbid (koroner arter hastalığı, hipertansiyon, diabetes mellitus, dislipidemi, kronik obstrüktif akciğer hastalığı) hastalık birlikteliği ve sıklığı artmaktadır. İleri yaş grubunda üçlü komorbidite oranı %90 ve beş komorbidite oranı ise %50'ye ulaşmaktadır. Komorbid hastalıkların birlikteliği ve sıklığının artması kalp yetersizliği gelişme riskini artırmaktadır ve yaşlı kalp yetersizliği hastalarında tedavi başlama zorluklarına, yan etki sıklığının artmasına neden olmaktadır. (4)

Kalp yetersizliği kılavuzları, mortalite ve morbiditeyi azaltmak amacıyla etkinliği kanıtlanmış çoklu farmakoterapileri önermektedir. Kalp yetersizliği kılavuzları medikal tedavide dört farklı tedavi noktasını hedeflemektedir: anjiyotensin konverting enzim inhibitörleri/anjiyotensin reseptör blokörleri /anjiyotensin reseptör-nepresilin inhibitörleri, beta-blokerler, mineralokortikoid reseptör antagonistleri ve sodyum glukoz transporter inhibitörlerinin kullanımı önerilmektedir. (5) Dört

farklı tedavi seçeneğinin kombinasyonunun kullanılması en fazla klinik fayda sağlamaktadır. Önerilen medikal tedavi seçeneklerinde hedef dozlara ulaşılmasının sağkalımı artırıcı ve kalp yetersizliği nedeniyle hastaneye yatış oranlarını azaltıcı etkisi, kalp yetersizliği hastalarının hayat kalitesini artırdığı gösterilmiştir. (6)

Bu çalışmada, kalp yetersizliği polikliniğinde takip edilen hastalar içerisinde belirlenen ileri yaş grubundaki hastaların eşlik eden komorbiditelerini, kalp yetersizliğinde sağkalımı artıran tedavileri kullanma ve hedef dozlara ulaşma oranlarını belirlemek, primer-sekonder sonlanım farklılıklarını erken yaş grubu ile karşılaştırmak ve ileri yaş grubunda medikal tedavi dozlarının primer sonlanım noktasına ulaşmadaki etkilerini araştırmak amaçlanmıştır.

GEREÇ ve YÖNTEM

Kalp yetersizliği tanısı almış ve kalp yetersizliği polikliniğinde takip edilen 1624 hasta çalışmaya dahil edilmiştir. Ege Üniversitesi Tıp Fakültesi Tıbbi Araştırmalar Etik Kurulundan çalışma onayı alınmıştır [Karar No: 24-8T/116, Tarih:22.8.2024]. Bilgilendirilmiş gönüllü olur formu hasta veya birinci derece yakınlarından alınmıştır.

Kalp yetersizliği hastalarının klinik verisi hasta dosyası, elektronik hasta dosyası, eksik veri durumunda ise hasta veya birinci derece yakınından telefon görüşmesi ile elde edilmiştir.

Kalp yetersizliği hastalarında, sol ana koroner arter veya sol ön inen arterde %50'den fazla darlık saptanmış ya da sağ koroner arter veya sirkumfleks arterin her ikisinde %50'den fazla

darlık saptanan hastalar iskemik nedenli kalp yetersizliği olarak kategorize edilmiştir. (7)

Hastaların kalp yetersizliği tanısı aldıkları yaş değerlerinin çeyreklikler arası değerleri belirlenerek, 75'inci persentil ve üzeri yaşında tanı alan hastalar ileri yaş kalp yetersizliği hasta grubu olarak tanımlanmıştır. Hastaların çoğunluğunu oluşturan 55 yaş altında tanı almış hastalar ise erken kalp yetersizliği grubu olarak tanımlanmıştır.

Hasta karakteristikleri ve komorbid hastalıkları, ekokardiyografi verileri, kardiyovasküler nedenli kullandığı tedavi ve dozları kayıt altına alınmıştır. Kalp yetersizliği tedavisinde kullanılan, sağkalımı artıran, kalp yetersizliği kılavuzu tarafından önerilen ve yüksek doza çıkılması önerilen tedavilerin doz aralıkları çeyrek doz, yarım doz ve tam doz olarak kategorize edilmiştir.

Primer ve Sekonder Sonlanım Noktası:

1. Primer Sonlanım Noktası: Tüm nedenli ölüm veya kalp replasman tedavisi (kalp nakil cerrahisi veya uzun dönem kalp mekanik destek cerrahisi) olarak belirlenmiştir.
2. Sekonder Sonlanım Noktaları:
 - a. Tüm nedenli ölüm
 - b. Kalp replasman tedavisi
 - c. Ventriküler aritmi, uygun ICD şoklaması veya ani kardiyak ölüm
 - d. Kardiyak toparlanma (recovery)

İstatistiksel Analiz:

İstatistiksel analizler, SPSS 28 (IBM Armon New York) ve Medcalc 19,0® istatistiksel veri analiz programları kullanılarak yapılmıştır. Sürekli değişkenler ortalama $\pm$ standart sapma, medyan-çeyrekler arası aralık, kategorik değişkenler ise sayı ve oran olarak belirtilmiştir. Gruplar arası karşılaştırmalar iki yönlü Student t testi, Wilcoxon rank testi, Mann Whitney U testi ve Ki-kare testi veya Fischer exact testi kullanılmıştır.

Log-rank (Mantel-Cox) testi, gruplar arası sağkalım farkını belirlemek amacıyla kullanılmıştır. Kaplan-Meier sağkalım grafikleri sağkalım özelliklerinin grafik çizimlerinde kullanılmıştır. Cox-regresyon risk modelleri, risk oranlarının (Hazard ratio) belirlenmesinde kullanılmıştır ve değerler ortalama ve %95 güven aralığında belirtilmiştir.

Yaş kategorilerine göre kalp yetersizliği medikal tedavileri ve sağkalımı artıran tedavilerin çeyrek, yarım ve tam dozlarına göre farklı Cox-regresyon modelleri oluşturulmuştur. Primer sonlanım noktasını belirlemede tekli değişken analizinde anlamlı olan kalp yetersizliği medikal tedavileri ve tedavi dozlarına göre yeni Cox-regresyon modeli oluşturulmuştur.

BULGULAR

Çalışmaya kalp yetersizliği polikliniğinde takip edilen 1624 hasta dahil edilmiştir. Yaş grupları belirlendiğinde, 75. persentil üzeri hasta grubu yaşı 55 yıl ve üstü olarak belirlenmiştir. Kalp yetersizliği hastalarının medyan takip süresi 58,4 (ÇAA: 30,2-88,3) ay olarak bulunmuştur. 427 hasta, tanı aldıkları tarihte ileri yaş kalp yetersizliği grubunda yer almıştır. İleri yaş kalp yetersizliği grubunun tanı anında yaş ortalaması 60,6 $\pm$ 4,4 yıldır ve %72,6'sı erkek cinsiyettedir.

İleri yaş kalp yetersizliği hastalarının %39,2'si ve erken yaş kalp yetersizliği hastalarının %29,4'ü NYHA fonksiyonel sınıfı III, ileri yaş kalp yetersizliği hastalarının %9,2'si ve erken yaş kalp yetersizliği hastalarının %5,9'u NYHA fonksiyonel sınıfı IV olarak bulunmuştur ve ileri yaş kalp yetersizliği hastalarında fonksiyonel sınıfının giderek kötüleştiği bulunmuştur (p değeri <0,0001).

İleri yaş kalp yetersizliği hastalarının sistolik kan basıncı ortalaması 112 $\pm$ 17 mmHg, diyastolik kan basıncı 67 $\pm$ 10 mmHg, erken yaş kalp yetersizliği hastalarının sistolik kan basıncı 114 $\pm$ 18 mmHg, diyastolik kan basıncı 70 $\pm$ 12 mmHg, (sırasıyla p değeri = 0,038; p < 0,0001) daha düşük bulunmuştur. İleri yaş grubu kalp yetersizliği hastalarında, kalp yetersizliği nedeni %67,7'sinde koroner arter hastalığı nedenlidir ve erken yaş kalp yetersizliği grubuna göre daha yüksek orandadır (p değeri <0,0001).

İleri yaş kalp yetersizliği hastalarında, komorbid hastalıklar, hipertansiyon %44,3, diabetes mellitus %33,5, hiperlipidemi %21,8 ve KOAH %9,3 ile erken yaş kalp yetersizliği hastalarına göre daha yüksek oranda bulunmuştur (p <0,0001). Sigara kullanım öyküsü ileri yaş grubunda %30,6 oranında ve erken kalp yetersizliği hasta grubuna göre daha düşük bulunmuştur (p = 0,002).

İleri yaş kalp yetersizliği hastalarında atriyal fibrilasyon %26,8, erken yaş grubu kalp yetersizliği hastalarında ise %11,4 bulunmuştur

(p değeri <0,0001) ve ileri yaş kalp yetersizliği hastalarında atriyal fibrilasyon daha yüksek oranda bulunmakla birlikte iskemik serebrovasküler olay oranı %6,4 ve erken yaş kalp yetersizliği hasta grubuna göre daha yüksek bulunmamıştır (p = 0,17). İleri yaş kalp yetersizliği hastalarında ICD implantasyonu %29,5, CRT-D implantasyonu %10,3, erken yaş kalp yetersizliği hastalarında ICD implantasyonu %35,2 ve CRT-D implantasyonu %8,4 oranında bulunmuştur (p = 0,002). (Tablo-1).

Ekokardiyografi değerlendirmesinde, sol ventrikül diyastol ve sistol çapı arasında her iki grup arasında fark bulunmazken, sol atriyum çapı ileri kalp yetersizliği hastalarında ortalama 47,5±16,9 mm bulunmuştur ve erken yaş kalp yetersizliği hastalarına göre sol atriyum çapı 46,0±7,9 daha yüksek bulunmuştur (p <0,0001). Mitral kapak yetersizliği, aort kapak yetersizliği ve triküspit kapak yetersizliği ciddiyetinin artması ile ileri yaş kalp yetersizliği hastalarında görülme oranı daha yüksektir.

İleri yaş kalp yetersizliği hastalarının sol ventrikül ejeksiyon fraksiyonu %27,0±9,5, erken yaş kalp yetersizliği hastalarının sol ventrikül ejeksiyon fraksiyonu %28,4±11,4 bulunmuştur ve ileri yaş kalp yetersizliği hastalarında sol ventrikül ejeksiyon fraksiyonu daha düşüktür (p değeri = 0,018). Sağ ventrikül fonksiyonunu değerlendirmek amaçlı kullanılan TAPSE ve RVsm-TDI ileri yaş kalp yetersizliği hastalarında sırasıyla 16,8±4 mm, 17,3±5,1 m/s bulunmuş ve erken yaş kalp yetersizliği hastaları ile arasında fark bulunmamıştır (sırasıyla p değerleri = 0,13 ve 0,36). İleri yaş kalp yetersizliği hastalarında, triküspit kapak regürjitasyon hızı 2,94±0,54 m/s, sistolik pulmoner arter basıncı 38,9±15,7 mmHg, erken yaş kalp yetersizliği hastalarına göre her iki değerinde daha yüksek bulunmuştur (p <0,0001). (Tablo-3).

Her iki grup arasında beta-bloker kullanımı, anjiyotensin reseptör-blokeri-neprisilin inhibitörü, antiplatelet, If kanal-blokeri ve antiaritmik kullanım oranları benzer bulunmuştur. ACE-i-ARB kullanım oranı erken yaş kalp yetersizliği hastalarında daha fazla oranda (%88,2, p değeri = 0,002), aldosteron antagonisti kullanım oranı erken yaş kalp yetersizliği hastalarında (%93,8, p değeri = 0,001) daha fazla bulunmuştur. SGLT-2i kullanım oranı (%18,3 p değeri = 0,014), kıvrım diüretik kullanım oranı (%82,2, p değeri = 0,003), antikoagülan kullanım oranı (%32,1, p değeri = 0,045), digoksin kullanım oranı (%21,3, p değeri

= 0,024) ve statin kullanım oranı (%45,7, p değeri <0,0001) ile ileri yaş kalp yetersizliği grubunda daha yüksek bulunmuştur.

ACE-i-ARB dozlarına göre değerlendirildiğinde, ileri yaş kalp yetersizliği grubunda ACE-i-ARB kullanan hasta oranı daha az olmasına rağmen ileri yaş kalp yetersizliği hasta grubunda yarım ve yüksek dozlara ulaşma oranlarının daha yüksek olduğu bulunmuştur. Beta-bloker kullanım oranında her iki grup arasında fark bulunmazken, beta-bloker doz artışları ileri yaş kalp yetersizliği hastalarında daha düşük oranlarda kalmıştır. Beta-bloker tam doza ulaşma oranı ileri kalp yetersizliği hastalarında %8,4, erken yaş kalp yetersizliği hastalarında ise %12,0'dir (p değeri <0,0001). Aldosteron antagonisti kullanım oranı ve ileri yaş kalp yetersizliği grubunda hedef yüksek dozlara ulaşma oranı daha düşük bulunmuştur (sırasıyla %69,3, %72, p değeri <0,0001). Ayrıca, ileri yaş grubu kalp yetersizliği hastalarında aldosteron antagonisti kullanmama oranı erken yaş kalp yetersizliği grubuna göre daha yüksektir. (Tablo-2).

Primer sonlanım noktası ileri yaş kalp yetersizliği grubunda 149 hastada %35 oranında, erken yaş kalp yetersizliği grubunda ise 300 hastada %25,2 oranında hastada primer sonlanım noktasına ulaşılmıştır ve ileri yaş kalp yetersizliği grubundaki hastalar daha fazla oranda primer sonlanım noktasına ulaşmıştır (p değeri <0,0001). Kardiyak toparlanma oranı ileri yaş kalp yetersizliği grubunda 40 hastada %9,4 oranında gelişmiş ve bu oran erken yaş kalp yetersizliği grubunda ise 119 hastada %10 oranında gerçekleşmiştir. Sekonder sonlanım noktalarından olan ventriküler aritmi, uygun ICD şoklanması 27 hastada %6,3, erken yaş grubunda ise 95 hastada %7,0 oranında gerçekleşmiştir ve anlamlı fark bulunmamıştır (p değeri = 0,62).

Sağ kalım analizlerinde Log-rank Mantel-Cox testi kullanılmış ve tüm hastaların bir yıllık sağkalım oranı %97, beş yıllık sağkalım oranı %81 ve on yıllık sağkalım oranı %55 bulunmuştur. İleri yaş kalp yetersizliği hastalarının sırasıyla 1-5-10 yıllık sağkalımı %98, %84, %60, erken yaş kalp yetersizliği hastalarının 1-5-10 yıllık sağkalımı %94, %72 ve %42 bulunmuştur. İleri ve erken yaş kalp yetersizliği hastaları karşılaştırıldığında, ileri yaş kalp yetersizliği hastalarının Hazard ratio'su 2,0; %95 GA (1,59-2,52); p değeri <0,0001 bulunmuştur. (Şekil-1).

Kullanılan ilaç tedavileri ve tedavi hedef dozlarına ulaşma oranının primer sonlanım noktasına ulaşmaya etkisinin incelenmesi amacıyla farklı Cox-regresyon modelleri oluşturulmuştur. Tedavi dozları ile yaş ve sol ventrikül ejeksiyon fraksiyonu modellemeye eklenmiştir. İleri yaş kalp yetersizliği grubunda kardiyovasküler medikal tedavi kullanmanın oluşturulduğu modelde, tekli değişken analizinde istatistiksel anlamlılık kazanan değerler çoklu değişken analizine dahil edilmiştir. Çoklu değişken analizinde sol ventrikül ejeksiyon fraksiyonu artışının sağkalıma anlamlı-olumlu etkisi bulunmuştur (HR: 0,94, %95 GA 0,90-0,97, p değeri <0,0001). Medikal tedavide ise ACE-i veya ARB kullanımı (HR: 0,51 %95 GA 0,36-0,73, p değeri <0,0001) ve beta-bloker kullanımı (HR: 0,28, %95 GA 0,14-0,54, p değeri <0,0001) ile dozdan bağımsız olarak sağkalıma anlamlı-olumlu katkısı gösterilmiştir.

Aldosteron antagonisti (HR: 0,70, %95 GA 0,45-1,07, p değeri = 0,1) kullanımının olumlu etkisi görülmemiştir. Loop diüretik kullanımının (HR: 3,24, %95 GA 1,32-7,96, p değeri = 0,01) sağkalıma olumsuz etkisi gösterilmiştir.

Kalp yetersizliği kılavuzu tarafından önerilen sağkalıma olumlu etkisi olduğu kanıtlanmış tedavi seçeneklerinden; anjiyotensin reseptör blokleri-

neprisinil inhibitörü, ivabradine ve sodyum glukoz transporter 2 inhibitörü kullanımının tekli değişken analizinde sağkalıma ileri yaş kalp yetersizliği hastalarında etkisinin olmadığı bulunmuştur. Kalp yetersizliğinde sağkalıma olumlu etkisi gösterilmiş ve yüksek dozlara ulaşmanın sağkalıma olumlu etkisinin gösterildiği tedavilerin ileri yaş kalp yetersizliği hastalarında Cox-regresyon modeli ile değerlendirilmiştir. ACE-i veya ARB kullanımında çeyrek doz kullanımının (HR: 1,09, %95 GA 0,69-1,72, p = 0,68) sağkalıma olumlu etkisi gösterilmemiştir.

ACE-i veya ARB kullanımında yarım doza veya tam doza ulaşmanın sağkalıma olumlu etkisi görülmüştür (sırasıyla HR: 0,59; %95 GA 0,37-0,93; p değeri = 0,024; HR: 0,32; %95 GA 0,17-0,58; p değeri <0,0001). ACE-i veya ARB kullanımında doz artışının yarım doz veya tam doza ulaşma ile sağkalıma artan olumlu etkisi gösterilmiştir. Beta-bloker kullanımı ise ileri yaş kalp yetersizliği hastalarında sağkalıma olumlu etkisi çeyrek doz ile başlamaktadır ve tam doza ulaşmak ile en düşük risk oranına ulaşmaktadır (sırasıyla HR: 0,45; %95 GA 0,23-0,89; p değeri = 0,021; HR: 0,20; %95 GA 0,09-0,42; p değeri <0,0001; HR: 0,15; %95 GA 0,05-0,40; p değeri <0,0001).

Tablo-1. Kalp yetersizliği hasta karakteristikleri ve yaş gruplarına göre karşılaştırılması

	Toplam	Yaş <55	Yaş ≥55	P değeri
Yaş yıl	46,0±12,8	40,7±20,6	60,6±4,4	<0,0001
Erkek cinsiyet %	72,8	72,9	72,6	0,89
BKİ kg/m ²	26,5±3,99	26,5±4,2	26,4±3,1	0,74
VYA m ²	1,91±0,96	1,92±0,19	1,89±0,15	0,016
NYHA FS (%)	-	-	-	<0,0001
I	12,8	14,7	7,9	-
II	48,1	50,0	43,2	-
III	32,2	29,4	39,7	-
IV	6,8	5,9	9,2	-
SKB mmHg	113,9±0,18	114,6±18,5	112,2±17,7	0,038
DKB mmHg	69,5±11,9	70,4±12,3	67,4±10,5	<0,0001
İskemik nedenli kalp yetersizliği (%)	56,3	52,2	67,7	<0,0001
Hipertansiyon (%)	32,2	27,8	44,3	<0,0001
Diabetes mellitus (%)	26,8	24,4	33,5	<0,0001
Dislipidemi (%)	15,7	13,5	21,8	<0,0001
Sigara kullanımı (%)	36,5	34,3	30,6	0,002
İskemik serebrovasküler olay (%)	5,1	4,7	6,4	0,17
KOAH (%)	5,7	4,4	9,3	<0,0001
Kronik böbrek hastalığı (%)	3,3	3,0	4,3	0,22
Kronik Karaciğer hastalığı (%)	0,7	0,8	0,6	0,74
Periferik arter hastalığı (%)	2,3	2,1	2,8	0,53
AF (%)	18,4	11,4	26,8	<0,0001

İntrakardiyak cihaz (%)	-	-	-	0,002
Pacemaker (%)	11,3	12,9	6,9	-
ICD (%)	31,0	29,5	35,2	-
CRT-D (%)	8,9	8,4	10,3	-
Kalp yetersizliği aile öyküsü (iskemik olmayan KM hastaları) (%)	12,4	15,2	4,7	<0,0001
Primer sonlanım noktası (%)	27,8	25,2	35,0	<0,0001
Kardiyak toparlanma (%)	9,8	10,0	9,4	0,73
Ventriküler aritmi veya Ani kardiyak ölüm (%)	6,8	7,0	6,3	0,62
Takip süresi ay (ÇAA)	58,4 (30,2-88,3)	61,3 (31,9-93,2)	52,8 (27,2-73,2)	0,68
NTpro-BNP pg/ml (ÇAA)	1487 (566-4086)	1320 (510-3985)	1769 (710-4268)	0,001

Tablo-2. Kalp yetersizliği hastalarının kullandıkları tedavi-dozları ve yaş gruplarına göre karşılaştırılması.

	Toplam	Yaş <55	Yaş ≥55	P değeri
ACE-i / ARB (%)	81,4	76,3	83,2	0,002
ACE-i / ARB dozu (%)	-	-	-	<0,001
Kullanmayan (%)	13,9	12,2	18,7	-
Çeyrek doz (%)	33,1	35,8	25,6	-
Yarım doz (%)	36	35,8	36,6	-
Tam doz (%)	17,0	16,2	19,2	-
Beta-bloker (%)	98,2	98,4	97,4	0,19
Beta-bloker dozu (%)	-	-	-	<0,0001
Kullanamayan (%)	1,9	1,7	2,7	-
Çeyrek doz (%)	32,6	29,5	41,2	-
Yarım doz (%)	36,0	37,1	32,8	-
Tam doz (%)	11,0	12,0	8,4	-
ARNI (%)	8,0	7,9	8,4	0,70
ARNI dozu (%)	-	-	-	0,68
Kullanamayan (%)	92,1	92,2	91,6	-
Çeyrek doz (%)	1,8	1,7	2,3	-
Yarım doz (%)	2,8	2,9	2,3	-
Tam doz (%)	3,3	3,2	1,0	-
Aldosteron antagonisti (%)	92,5	93,8	88,8	0,001
Aldosteron antagonist dozu (%)	-	-	-	<0,0001
Kullanamayan (%)	7,2	5,9	11,1	-
Çeyrek doz (%)	21,7	23,7	19,7	-
Yarım doz (%)	1,0	1,1	0,7	-
Tam doz (%)	70,0	69,3	72,0	-
SGLT-2i (%)	14,7	13,4	18,3	0,014
Loop diüretik (%)	77,0	75,1	82,2	0,003
Antikoagülan (%)	28,3	27,0	32,1	0,045
Antiplatelet (%)	53,7	52,3	57,6	0,059
If kanal blokeri (%)	30,7	30,1	32,6	0,34
Digoksin (%)	17,7	16,5	21,3	0,024
Antiaritmik (%)	9,7	9,7	9,5	0,90
Statin (%)	32,0	27,2	45,7	<0,0001

Değerler ortalama ± SS, medyan ÇAA ve frekans dağılımı % olarak belirtilmiştir, p değeri ≤0,05 istatistiksel olarak anlamlı olarak kabul edilmiştir,

ACE-i: Anjiyotensinojen konverting enzim inhibitörü; ARB: Anjiyotensin reseptör blokeri; ARNI: Anjiyotensin reseptör blokeri-neprilisin inhibitörü, SGLT-2i: Sodyum glukoz transporter 2 inhibitörü

Tablo-3. Kalp yetersizliği hastalarının ekokardiyografik verileri ve yaş gruplarına göre karşılaştırılması.

	Toplam	Yaş<55	Yaş≥55	P değeri
SVDSç mm	62,7±10,5	62,8±10,9	62,5±9,6	0,52
SVSSç mm	52,5±12,5	52,5±12,9	52,3±11,4	0,22
SAç mm	46,4±7,6	46,0±7,9	47,5±16,9	<0,0001
SVEF %	28,0±10,9	28,4±11,4	27,0±9,5	0,018
Mitral kapak yetersizliği (%)	-	-	-	<0,001
Yok	12,2	11,9	13,1	-
Hafif	27,5	30,3	19,7	-
Orta	40,6	38,3	46,8	-
Ciddi	19,7	19,5	20,4	-
Aort kapak yetersizliği (%)	-	-	-	<0,0001
Yok	76,2	79,4	67,2	-
Hafif	17,0	15,9	20,0	-
Orta	5,7	3,5	11,6	-
Ciddi	1,2	1,2	1,2	-
Triküspid kapak yetersizliği (%)	-	-	-	0,005
Yok	16,1	17,6	11,9	-
Hafif	37,0	37,9	34,4	-
Orta	32,0	30,3	36,6	-
Ciddi	15,0	14,2	17,1	-
TAPSE mm	17,1±5,0	17,3±5,1	16,8±4,73	0,13
RVsm (TDI) m/s	10,1±2,72	10,1±2,7	10,0±2,6	0,36
TRV m/s	2,84±0,54	2,81±0,54	2,94±0,54	<0,0001
SPAP mmHg	35,9±15,4	34,8±15,2	38,9±15,7	<0,0001

Değerler ortalama ± SS, medyan ÇAA ve frekans dağılımı % olarak belirtilmiştir, p değeri ≤0,05 istatistiksel olarak anlamlı olarak kabul edilmiştir,

ÇAA: Çeyreklikler arası aralık, SVDSç: Sol ventrikül diyastol sonu çapı, SVSç: Sol ventrikül sistol sonu çapı, SAç: Sol atriyum çapı, SVEF: Sol ventrikül ejeksiyon fraksiyonu, TAPSE: Triküspid anüler planar sistolik uzanımı, RVsm: Sağ ventrikül sistolik hareketi-doku Doppler görüntüleme, TRV: Triküspid kapak yetersizlik velositesi, SPAP: Sistolik pulmoner arter basıncı

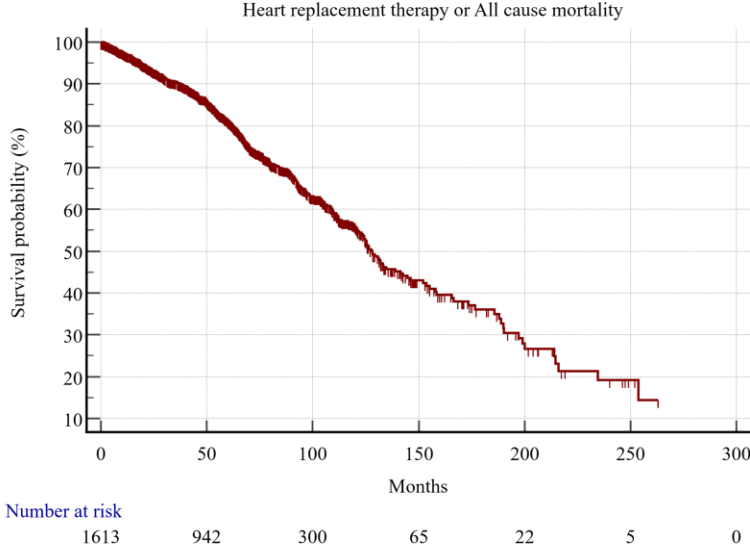
Tablo-4. İleri yaş ve üzeri kalp yetersizliği hastalarında medikal tedavi ve dozlarının Cox-regresyon analizi

	Tekli değişken		Çoklu değişken	
	HR 95% GA	P değeri	HR 95% GA	P değeri
Yaş yıl	1,02 (0,98-1,05)	0,19	-	-
SVEF%	0,92 (0,90-0,95)	<0,0001	0,94 (0,90-0,97)	<0,0001
ACE-i / ARB	0,43 (0,31-0,61)	<0,0001	0,51 (0,36-0,73)	<0,0001
ACE-i / ARB dozu	-	<0,0001	-	-
Kullanmayan	-	-	-	-
Çeyrek doz	0,19 (0,11-0,32)	<0,0001	1,09 (0,69-1,72)	0,68
Yarım doz	0,40 (0,26-0,61)	<0,0001	0,59 (0,37-0,93)	0,024
Tam doz	0,89 (0,58-1,36)	0,61	0,32 (0,17-0,58)	<0,0001
ARNI	1,52 (0,91-2,52)	0,10	-	-
Beta-bloker	0,21 (0,11-0,41)	<0,0001	0,28 (0,14-0,54)	<0,0001
Beta-bloker dozu	-	<0,0001	-	<0,0001
Kullanmayan	-	-	-	-
Çeyrek doz	0,40 (0,20-0,77)	<0,0001	0,45 (0,23-0,89)	0,021
Yarım doz	0,010 (0,05-0,21)	<0,0001	0,20 (0,09-0,42)	<0,0001
Tam doz	0,10 (0,039-0,27)	<0,0001	0,15 (0,05-0,40)	<0,0001
Aldosteron antagonist	0,45 (0,30-0,68)	<0,0001	0,70 (0,45-1,07)	0,10
SGLT-2i	0,88 (0,58-1,34)	0,57	-	-
İvabradine	0,92 (0,65-1,29)	0,63	-	-
Statin	0,84 (0,60-1,16)	0,29	-	-
Loop diüretik	5,38 (2,2-13,1)	<0,0001	3,24 (1,32-7,96)	0,01
Antiplatelet	0,84 (0,60-1,17)	0,31	-	-

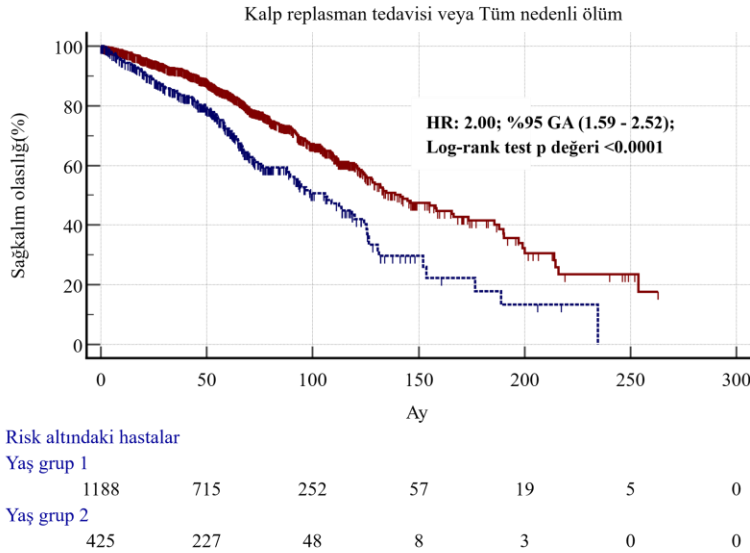
Antikaogulan	1,54 (1,10-2,16)	0,012	-	-
Antiaritmik	0,87 (0,53-1,42)	0,59	-	-
Digoksin	1,21 (0,85-1,73)	0,28	-	-

Değerler Hazard oranı ve 95 % güven aralığı olarak belirtilmiştir, P değeri $\leq 0,05$ istatistiksel olarak anlamlı olarak kabul edilmiştir,

ACE-i: Anjiyotensinojen konverting enzim inhibitörü; ARB: Anjiyotensin reseptör blokeri; ARNI: Anjiyotensin reseptör blokeri-neprilisin inhibitörü, SGLT-2i: Sodyum glukoz transporter 2 inhibitörü



Şekil-1. Tüm hastalarda primer sonlanım noktasına ulaşma oranlarına göre Kaplan Meier sağkalım eğrisi.



Şekil-2. Erken ve ileri yaş kalp yetersizliği hastalarında primer sonlanım noktasına ulaşma oranlarına göre Kaplan Meier sağ-kalım eğrisi.

TARTIŞMA

Bu çalışmada, kalp yetersizliği hastalarında yaş gruplarına göre farklı klinik ve demografik özelliklerin yanı sıra tedavi yaklaşımları ve sağkalım üzerindeki etkileri detaylı bir şekilde incelenmiştir. Sonuçlar, ileri yaş KY hastalarının klinik seyir, komorbidite profili ve tedaviye yanıt açısından erken yaş KY hastalarına kıyasla belirgin farklılıklar sergilediğini ortaya koymuştur. Tartışma kısmında, bu bulguların literatürdeki mevcut bilgilerle karşılaştırılması, klinik uygulamalar için çıkarımları ve çalışma kısıtlılıkları ele alınmıştır.

İleri Yaş Kalp Yetersizliği Grubunun Klinik ve Demografik Özellikleri

Çalışmada, ileri yaş KY hastalarının önemli bir bölümünde koroner arter hastalığı temel etiyolojik neden olarak bulunmuş ve bu durumun erken yaş KY hastalarına göre anlamlı şekilde daha yüksek oranda olduğu tespit edilmiştir. Bu bulgu, yaşla birlikte KAH prevalansının artması ve aterosklerotik süreçlerin KY gelişimindeki rolünün daha belirgin hale gelmesi ile uyumludur. (8) Bu bulgu, yaşla birlikte KAH prevalansının artması ve aterosklerotik süreçlerin KY gelişimindeki rolünün daha belirgin hale gelmesi ile uyumludur. Benzer şekilde, ileri yaş KY grubunda hipertansiyon, diabetes mellitus, hiperlipidemi ve KOAH gibi komorbiditelerin daha yaygın olduğu gözlenmiştir. Bu durum, yaşlanma sürecinin bir sonucu olarak vasküler ve metabolik bozuklukların artmasıyla açıklanabilir. Ancak, sigara kullanım öyküsünün erken yaş KY grubunda daha yüksek oranda olması, sigara içme alışkanlığının genç yaş gruplarında daha yaygın olabileceğini ve bu durumun erken dönemde KY gelişimine katkıda bulunabileceğini düşündürmektedir. (9)

Çalışmada ileri yaş KY grubunda atriyal fibrilasyon prevalansının belirgin şekilde yüksek olduğu tespit edilmiştir. Atriyal fibrilasyon prevalansının yaşla birlikte artış gösterdiği, özellikle yaşlı popülasyonda insidansın anlamlı şekilde yükseldiği mevcut bilgi ile uyumludur. (10) Bu bulgu, atriyal fibrilasyonun yaşla artan bir aritmi olduğu ve KY hastalarında hemodinamik yüklenme ile ilişkili olarak daha sık gözlemlendiği literatürle uyumludur. (10) Ancak, bu hasta grubunda iskemik serebrovasküler olay oranlarının anlamlı şekilde artmamış olması dikkat çekicidir. Atriyal fibrilasyonun serebrovasküler komplikasyon riskini artırdığı

bilinmekle birlikte, bu çalışmada kullanılan antikoagülan tedavi oranlarının etkili bir şekilde bu riski azaltmış olabileceği düşünülmektedir.

Tedavi ve Doz Hedeflerine Ulaşmanın Sağkalıma Etkisi:

KY tedavisinde rehberlerle önerilen farmakolojik ajanların kullanımı, sağkalımı iyileştirme potansiyeli açısından kritik öneme sahiptir. Çalışmada, ileri yaş KY hastalarında ACE-i/ARB ve beta-bloker kullanımı ile sağkalım arasında pozitif bir ilişki tespit edilmiştir. (11) Özellikle tam doz veya hedef dozlara ulaşmanın sağkalımı anlamlı şekilde iyileştirdiği gösterilmiştir. Bu bulgu, KY tedavisinde hedef dozların önemini vurgulayan önceki çalışmaları desteklemektedir. (12) Ancak, ileri yaş KY hastalarında hedef dozlara ulaşma oranlarının düşük olması, bu hasta grubunda ilaca toleransın azalabileceği veya yan etkiler nedeniyle doz artırmada sınırlamalar yaşanabileceğini düşündürmektedir. (13)

Ayrıca, çalışmada aldosteron antagonisti kullanımının ileri yaş KY hastalarında sağkalıma olumlu bir etkisinin bulunmaması dikkat çekicidir. Bu durum, aldosteron antagonistlerinin yaşlı popülasyonda etkinliğine dair literatürdeki görüş ayrılıkları ile uyumludur. Özellikle, bu ajanların yaşlı bireylerde renal fonksiyonlar ve hiperkalemi riskine etkisini ele alan yeni çalışmaların bu alandaki bilgi eksikliğini giderebileceği düşünülmektedir. (14) Bu bulgu, aldosteron antagonistlerinin yaşlı popülasyonda kullanımına dair sınırlı verilerin varlığına işaret edebilir. İleri yaş grubunda diüretiklerin yan etkilerine karşı daha dikkatli olunması gerektiği bilinmektedir ve bu durum, çalışma sonuçlarını etkileyen bir faktör olabilir. Bununla birlikte, kıvrım diüretik kullanımının sağkalımı olumsuz etkilediği gösterilmiş ve bu durum KY hastalarında diüretik kullanma ihtiyacının oluşması negatif sonuçlara yol açabileceğini düşündürmektedir. (15,16)

Fonksiyonel Kapasite ve Kardiyak Performans

İleri yaş grubunda kardiyak performansı etkileyen faktörlerle ilgili literatürde farklı görüşler bulunmaktadır. Yaşlanma ile birlikte miyokardiyal fibrozis ve vasküler sertliğin artışı, kardiyak fonksiyonlardaki gerilemeyi hızlandırabilir. (17) NYHA fonksiyonel sınıfındaki kötüleşme bu fizyolojik değişimlerle ilişkilendirilebilir. (18) NYHA fonksiyonel sınıfı değerlendirmesinde, ileri yaş KY hastalarının fonksiyonel kapasitesinin

erken yaş KY hastalarına göre belirgin şekilde daha kötü olduğu görülmüştür. Bu bulgu, yaşlanma ile birlikte kardiyovasküler rezervlerin azalması ve komorbid hastalık yükünün artması ile açıklanabilir. Ek olarak, sol ventrikül ejeksiyon fraksiyonunun ileri yaş KY hastalarında daha düşük bulunması, bu hasta grubunda miyokard disfonksiyonunun daha yaygın olduğunu düşündürmektedir.

Ekokardiyografik değerlendirmeler, sol ventrikül diyastol ve sistol çapı arasında fark olmamasına rağmen, sol atriyum çapının ileri yaş grubunda daha büyük olduğunu göstermiştir. Sol atriyum genişlemesi, kronik KY hastalarında artmış hemodinamik yükün bir göstergesi olarak değerlendirilebilir. Ayrıca, mitral, aort ve triküspit kapak yetersizliklerinin ileri yaş KY hastalarında daha sık ve şiddetli olması, bu hasta grubunda kapak disfonksiyonunun progresif yapısını ortaya koymaktadır. (8)

Sağkalım Analizleri ve Risk Faktörleri:

Sağkalım analizleri, yaşlı KY hastalarında progresif kardiyak disfonksiyon ve komorbidite yükünün kötü prognoz ile ilişkilendirildiğini vurgulamaktadır.

Çalışmada, ileri yaş KY hastalarının sağkalım oranlarının erken yaş KY hastalarına göre daha düşük olduğu gösterilmiştir. Özellikle, ileri yaş grubunda 10 yıllık sağkalım oranının %60 iken, erken yaş grubunda %42 olması dikkat çekicidir. Bu durum, ileri yaş KY hastalarında yüksek komorbidite yükü ve kötüleşen kardiyak fonksiyonlarla ilişkilendirilebilir. Ayrıca, Hazard oranlarının ileri yaş grubunda daha yüksek bulunması, bu hasta grubunun daha kırılgan olduğunu ve kötü prognoza sahip olduğunu göstermektedir.

Tedavi dozları ve sağkalım arasındaki ilişkiyi inceleyen Cox-regresyon analizleri, ACE-i/ARB ve beta-bloker kullanımının sağkalımı iyileştirdiğini ve doz artışının bu olumlu etkiyi güçlendirdiğini ortaya koymuştur. Bu durum, KY hastalarında tedavi hedeflerine ulaşmanın önemini vurgulamaktadır. Ancak, ileri yaş KY grubunda ilaç toleransının düşük olması ve hedef dozlara ulaşmada zorlukların varlığı, klinik pratiğe yönelik önemli bir engel olarak karşımıza çıkmaktadır. (11–13,19,20)

Çalışmanın Güçlü Yönleri ve Kısıtlılıkları

Bu çalışmanın güçlü yönleri arasında geniş hasta popülasyonunun yanı sıra detaylı analizlerin

yapılması yer alırken, literatürde de vurgulanan yaşlı bireylerde tedavi uyumunun değerlendirilmesindeki eksiklik, önemli bir kısıtlılık olarak öne çıkmaktadır.

Bu çalışmanın güçlü yönlerinden biri, geniş bir hasta popülasyonunda ileri yaş ve erken yaş KY gruplarının detaylı bir şekilde karşılaştırılmış olmasıdır. Ayrıca, tedavi dozları ve sağkalım arasındaki ilişkinin kapsamlı bir şekilde değerlendirilmesi, çalışmayı literatürdeki benzer çalışmalardan farklı bir konuma taşımaktadır. Bununla birlikte, çalışmanın bazı kısıtlılıkları da bulunmaktadır. İlk olarak, çalışma retrospektif bir tasarıma sahip olduğundan, elde edilen veriler arasındaki neden-sonuç ilişkisini kesin olarak belirlemek mümkün değildir. İkinci olarak, ileri yaş grubunda daha düşük tedaviye uyum veya yan etkilerin görülme sıklığı gibi faktörlerin değerlendirilmemesi, sonuçların yorumlanmasını sınırlandırabilir. Ayrıca, çalışmanın tek merkezli olması, elde edilen sonuçların genellenebilirliğini kısıtlayabilir.

Klinik Uygulamalar ve Geleceğe Yönelik Öneriler

Bu çalışma, KY hastalarında yaşa bağlı farklılıkların klinik ve tedavi yaklaşımlarını şekillendirmede önemli bir rol oynadığını göstermektedir. İleri yaş KY hastalarında tedavi hedeflerine ulaşmanın sağkalıma olumlu etkisi dikkate alındığında, bu hasta grubunda daha bireyselleştirilmiş tedavi yaklaşımlarına ihtiyaç duyulmaktadır. Özellikle, yaşlı hastalarda ilaç toleransını artırmaya yönelik stratejilerin geliştirilmesi ve yan etkilerin etkin yönetimi, tedaviye uyumu artırabilir.

Gelecekteki çalışmaların, ileri yaş KY hastalarında optimal tedavi dozlarını ve yan etki yönetimini daha iyi anlamak için prospektif ve çok merkezli tasarımlara odaklanması önerilmektedir. Ayrıca, bu hasta grubunda yeni farmakolojik ajanların etkinliğini değerlendiren randomize kontrollü çalışmaların gerçekleştirilmesi, klinik pratiği yönlendirmede faydalı olacaktır.

SONUÇ

Sonuç olarak, bu çalışma, ileri yaş KY hastalarının klinik ve tedavi profillerini detaylı bir şekilde analiz ederek, bu hasta grubunun özel ihtiyaçlarına dikkat çekmiştir. Bu bulgular, KY hastalarında yaşa bağlı farklılıkları ele alan güncel literatürle uyumludur. Bu alanda yapılan çalışmalar, yaşla birlikte kardiyovasküler

sistemde meydana gelen fizyolojik deęişikliklerin ve komorbidite profilindeki farklılıkların klinik yaklaşımları etkilediğini göstermiştir. Elde edilen bulgular, ileri yaş KY hastalarının tedavisinde daha bireyselleştirilmiş yaklaşımların gerekliliğini

vurgulamakta ve sağkalımı iyileştirme çabalarının önemini ortaya koymaktadır.

Çıkar Çatışması: Yazarlar aralarında çıkar çatışması olmadığını beyan etmişlerdir.

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Kronik karaciğer hastalıklarında FIB-4 ve APRI skorlarının klinik tanı ile uyumu *Relationship with FIB-4, APRI scores and the clinical diagnosis in chronic liver diseases*

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ÖZ

Amaç: Karaciğer biyopsisi, siroz tanısında altın standart yöntem olmasına rağmen invazif olması, nadir ancak yaşamı tehdit edici komplikasyonlara neden olması gibi bazı sınırlılıkları bulunmaktadır. Karaciğer biyopsisinin bu sınırlılıkları siroz tanısında kullanılabilecek invazif olmayan yöntemlerin geliştirilmesini gerektirmektedir. Bu invazif olmayan yöntemler arasında aspartat transaminazın platelete oranı (APRI) ve dört faktörü temel alan fibrozis indeksi (FIB-4) kronik hepatit B (HBV) ve C (HCV) hastalarında fibrozis ve sirozun tanımlanmasında sık tercih edilmektedir.

Gereç ve Yöntem: Bu çalışmanın amacı çeşitli etiyolojilerdeki kronik karaciğer hastalarında APRI ve FIB-4'ün sirozun tahmin edilmesindeki performansını değerlendirmektir. Çalışmamızda klinik pratikte kullanım için yüksek sensitivite ve spesifitede yeni eşik değerler oluşturmak için kronik karaciğer hastalarında APRI ve FIB-4'ün yeni eşik değerleri analiz ettik.

Bulgular: Çalışmamızda ROC analizlerinde, siroz tanısı için optimal eşik değerin APRI için 1,32, FIB-4 için 4,24 olduğu görüldü.

Sonuç: Bu invazif olmayan yöntemlerin daha sık kullanımı sirozun erken tanınmasını ve önemli komplikasyonları olan gereksiz biyopsilerin önlenmesini sağlayacaktır.

Anahtar Sözcükler: Siroz, Kronik Karaciğer Hastalığı, APRI, FIB-4

ABSTRACT

Aim: Liver biopsy is the gold standard for the diagnosis of cirrhosis, but has limitations such as invasive procedure, high cost, risk of rare but potentially life-threatening complications. These limitations of liver biopsy promote the development of noninvasive methods for assessments of cirrhosis. Among these models, aspartate transaminase-to-platelet ratio index (APRI) and fibrosis index based on four factors (FIB-4) are commonly used for identifying liver fibrosis and cirrhosis in patients with chronic hepatitis B (HBV) and C virus (HCV) infection.

Materials and Methods: The aim of this study was to evaluate the performance of APRI and FIB-4 to predict cirrhosis in chronic liver diseases of various etiologies. To propose a new set of cut-offs with high specificity and sensitivity for use in clinical practice, we evaluated new cut-offs of APRI and FIB-4 patients with chronic liver diseases.

Results: Based on ROC analysis, the optimal cut-offs were 1,32 for APRI, and 4,24 for FIB-4, for diagnosing cirrhosis in this study.

Conclusion: The more frequent use of these non-invasive methods in clinical practice will lead to the early identification of cirrhosis and prevent unnecessary biopsy with significant complications.

Keywords: Cirrhosis, Chronic Liver Disease, APRI, FIB-4

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GİRİŞ

Sıklıkla alkol kullanımı ve viral hepatitlerle ilişkili olan kronik karaciğer hastalığı (KKH) gelişmiş ve gelişmekte olan ülkelerde önemli bir morbidite ve mortalite nedeni olarak görülmektedir. KKH'nın ilk evreleri asemptomatik seyretme eğiliminde olan hepatit ve steatozla karakterize olmuştur. Bu nedenle, metabolik sendromda karaciğer hastalığının ana bileşeni olarak kabul edilmektedir. KKH'nın son dönemi olarak kabul edilen siroz histolojik olarak mikronodüler veya makronodüler formda, portal alanlarda fibröz septalarla karakterizedir. KKH tedavisi ve prognozu karaciğerde fibrosis varlığı ve derecesiyle oldukça yakından ilişki içerisinde (1). Ancak karaciğer biyopsisi komplikasyon riski taşıyan bir girişimsel işlemdir. Bu nedenle siroz tanısını değerlendirmek için güvenilir, girişimsel olmayan yöntemlerin geliştirilmesine ihtiyaç bulunmaktadır. Bu amaçla öne sürülen yöntemler içerisinde transient elastografi, C-aminopirin nefes testi, indosiyanin yeşili plazma klerensi gibi metabolik karaciğer fonksiyon testleri, aspartat aminotransferaz/platelet oranı (APRI), yaş, AST, platelet sayısı, ALT ile değerlendirilen fibrosis-4 skoru (FIB-4), yaş platelet (AP) indeksi yer almaktadır (2,3,4).

Koagülopati riskinin yüksek olması siroz tanısı için yapılacak olan biyopsiye bağlı kanama riskini arttırmaktadır. Bu nedenle siroz tanısı klinik pratikte sıklıkla laboratuvar analizlerine dayanmaktadır. Ancak tek başına laboratuvar analizlerinin tanı hassasiyeti düşüktür. Tam tersine, hastaları sosyo-demografik özellikleriyle birlikte değerlendirilen birden fazla laboratuvar parametresini temel alan yöntemler giderek daha sık kullanılmaya başlamıştır (5). Ancak bu yöntem ve indeksler hakkında, siroz ve/veya fibrosis tanısındaki hassasiyetlerinin gösterilmesi amacıyla daha çok çalışma yapılmasına ihtiyaç bulunmaktadır. Bu çalışmada, APRI ve FIB-4 indekslerinin klinik siroz tanısı ile uyumunun gösterilmesi, bu indekslerin siroz tanısındaki değerinin analiz edilmesi amaçlanmıştır.

GEREÇ ve YÖNTEM

Bu çalışma, retrospektif ve kontrollü bir tasarıma sahip olup, klinik bulguların yanı sıra radyolojik, laboratuvar ve endoskopik verilerin analizine dayanmaktadır. Veriler, Üniversite Hastanesi Gastroenteroloji polikliniğinden retrospektif olarak elde edilmiştir. Çalışmaya toplamda 67 hasta dahil edilmiştir. Bunların 29'u siroz grubunda, 38'i ise kontrol grubunda yer almaktadır. Dahil edilme

kriterleri ise, 18 yaş ve üzeri olmak, klinik bulgularla siroz veya kronik karaciğer hastalığı tanısı alınmış olmak, tüm klinik ve laboratuvar verilerine erişim sağlanabilen hastalardan oluşmaktadır. Dışlanma kriterleri ise eksik veri kaydı bulunan ve 18 yaş altı bireyler olarak belirlenmiştir. Bu retrospektif çalışmada herhangi bir müdahale uygulanmamış, tüm veriler hasta dosyalarından retrospektif olarak elde edilmiştir. Hastalardan aktif olarak bilgi toplanmamıştır. Birincil sonuç ölçütü, APRI ve FIB-4 skorlarının siroz tanısı ile uyumunu değerlendirmektir. Sensitivite, spesifite, pozitif ve negatif prediktif değerler hesaplanmış ve ROC analizi ile optimal eşik değerler belirlenmiştir. Bu analizler, noninvazif tanı yöntemlerinin etkinliğini göstermek için gerçekleştirilmiştir. Toplam 67 hasta, retrospektif veriler üzerinden örneklem büyüklüğünü oluşturmuştur. Çalışma için spesifik bir örneklem büyüklüğü hesaplama yapılmamıştır. Verilerin analizinde SPSS 20.0 istatistiksel yazılımı kullanılmıştır. Nicel değişkenler ortalama ve standart sapma olarak sunulmuş, nitel değişkenler ise frekans ve yüzde olarak ifade edilmiştir. Gruplar arası farkların değerlendirilmesi için Mann-Whitney U testi kullanılmış, kategorik veriler için Ki-kare testi uygulanmıştır. ROC analizi ile APRI ve FIB-4 skorlarının tanısal performansı ve eşik değerleri değerlendirilmiştir. Bu çalışma, Üniversite Klinik Araştırmalar Etik Kurulu tarafından onaylanmış ve Helsinki Bildirgesi'ne uygun olarak yürütülmüştür (Onay Numarası: 1068; Tarih: 01.09.2017).

Bu bağlamda çalışma verilerinin incelenmesi sırasında; radyolojik olarak karaciğerin konturunun lobule olması, splenomegali ve asit bulgularından üçünün birden olması splenomegali, asit ve özofagus varisi bulgularının üçünün birden mevcut olması ya da karaciğer konturlarında lobülasyon, asit ve varis birlikteliği klinik olarak karaciğer sirozu olarak kabul edilmiştir. Asit olmadan diğer özelliklerden (splenomegali, varis ve kontur düzensizliği) ikisinin olması durumunda; laboratuvar parametrelerine bakılmıştır. Laboratuvar değerlerindeki 3 parametreden (Total bilirubin 2mg/dL den fazla, INR 1,7'den fazla ya da albumin değeri 3,5g/dL un altında olması) en az birinin olması halinde klinik olarak siroz kabul edilmiştir. FIB-4 $\geq 3,25$ ve APRI skoru > 2 değerlerinin siroz tanısındaki sensitivite ve spesifitesi değerlendirilmiştir. Tam tersine FIB-4

<1.45 ve APRI $\leq$ 0,5 değerlerinin sirozun dışlanmasıdaki sensitivite ve spesifitesi olarak değerlendirilmiştir. FİB-4 skoru dört parametre ile hesaplanmaktadır: AST, ALT, yaş ve PLT. FİB-4 skoru Vallet-Pichard tarafından 2007 yılında yapılan çalışmada geliştirilmiştir (6). Siroz tanısıyla uyumlu eşik değerler 2,9-3,6 arasında bildirilirken, sirozun dışlanmasında bildirilen eşik değerler 1,45-1,62 arasında ifade edilmiştir. Çalışmada 1,45 ve 3,25 eşik değerleri kabul edilmiştir (7). Ancak ROC analizi ile kendi çalışmamızın en iyi sensitivite ve spesifiteye sahip eşik değeri de oluşturulmuştur. APRI skoru, AST/PLT oranını ifade eden APRI skoru karaciğer fibrozisi ve sirozun tanımlanmasında kullanılan non-invazif yöntemler arasında bulunmaktadır. Wai ve ark'ı tarafından yapılan çalışmada 192 kronik HCV enfeksiyonu olan hastada geliştirilmiştir (8). Çalışmada 0,5 ve 2,0 eşik değerleri kabul edilmiştir (9,10). Ancak çalışmamızın en iyi eşik değerleri ROC analizi ile oluşturulmuştur.

BULGULAR

Çalışmada siroz grubunda 29 hasta, kontrol grubunda 38 hasta olmak üzere 67 hasta yer almaktadır. Hastaların yaş ortalaması $55,8 \pm 12,4$ yıldır (medyan 57 yıl, 24-78). Siroz grubunun yaş ortalaması $58,7 \pm 11,9$ yıl (medyan 59 yıl, 24-78 yıl aralığında), kontrol grubunun yaş ortalaması $53,6 \pm 12,4$ yıldır (medyan 55,5 yıl, 26-76 yıl aralığında). Hastaların %65,7'si (n=44) erkek, %34,3'ü (n=23) kadın hastalardan oluşmaktadır. Siroz grubunda, hastaların %86,2'si (n=25) erkek, %13,8'i (n=4) kadın, kontrol grubunda hastaların %50'si (n=19) erkek, %50'si (n=19) kadın hastalardır. Siroz grubunda erkek cinsiyet kontrol grubundan daha sık görülmektedir (p=0.002). KKH etiyolojisi, hastaların %44,8'inde (n=30) HBV idi (Tablo-1). KKH etiyolojilerinden kriptojenik (p=0,129), NAYKH (p=0,226), AYKH (p=0,433), HCV (p=0,878), HBV + HCV (p=0,379), HBV + HDV (p=0,379) sıklığı gruplar arasında benzerken, kontrol grubunda HBV sıklığı (p=0,048) siroz grubundan daha fazladır.

Gruplar arasında AST ve ALT seviyesi açısından farklılık yoktur (p=0,111, p=0,349). ALP seviyesi daha yüksektir. Ancak gruplar arasındaki fark anlamlılığa ulaşamamıştır (p=0,064). Albümin seviyesi siroz grubunda kontrol grubundan daha düşüktür (p<0,001). Total ve direk bilirubin seviyesi siroz grubunda daha yüksektir

(p<0,001). PLT seviyesi siroz grubunda kontrol grubundan daha düşüktür (p<0,001). INR seviyesi siroz grubunda $1,2 \pm 0,1$, kontrol grubunda $1,0 \pm 0,1$ 'di. INR seviyesi siroz grubunda daha yüksektir (p<0,001). PT seviyesi siroz grubunda daha uzundur (p<0,001). Gruplarda laboratuvar verilerinin dağılımı Tablo-2'de belirtilmiştir.

Görüntüleme bulgularında siroz grubunda KC kontur düzensizliği (p<0,001) ve splenomegali (p<0,001) sıklığı kontrol grubundan daha fazladır. Hepatomegali (p=0,122) sıklığı gruplar arasında benzerdir. Endoskopik değerlendirmede Siroz grubunda hastaların %86,2'sinde (n=25) özofageal varis varken, kontrol grubunda hastaların %18,9'unda (n=7) vardır. Özofageal varis sıklığı siroz grubunda daha fazladır (p<0,001).

Hastaların ortalama APRI skoru $0,99 \pm 0,79$ 'dur (medyan 0,75, 0,13-3,69 aralığında), ortalama FİB-4 skoru $3,06 \pm 2,32$ 'dir (medyan 2,30, 0,29-10,41 aralığında). Siroz grubunda ortalama APRI skoru $1,30 \pm 0,74$, kontrol grubunda $0,75 \pm 0,76$ 'dır. Siroz grubunun APRI skoru kontrol grubundan daha yüksektir (p<0.001). Siroz grubunun ortalama FİB-4 skoru $4,67 \pm 2,54$, kontrol grubunun $1,83 \pm 1,05$ 'dir. Siroz grubunun FİB-4 skoru kontrol grubundan daha yüksektir (p<0,001). FİB-4 skoru 3,25 ve üzerinde olduğunda siroz hastalarını diğer kronik karaciğer hastalarından oluşan kontrol grubundan %58 sensitivite, %86 spesifite ile ayırmaktadır. APRI skoru 2'nin üzerinde olduğunda, siroz hastalarını kontrol grubundan %10 sensitivite, %92 spesifite ile ayırmaktadır (Tablo-3).

FİB-4 skoru 1,45 olduğunda kronik karaciğer hastalarını siroz hastalarından %39 sensitivite, %96 spesifite ile ayırmaktadır. APRI skoru 0,5 ve altında olduğunda kronik karaciğer hastalarını siroz hastalarından %50 sensitivite, %89 spesifite ile ayırmaktadır. ROC analizinde siroz tanısında en iyi APRI skorunun 1,32 olduğu görülmüştür. APRI için 1,32 eşik değer olarak kabul edildiğinde siroz tanısındaki sensitivitenin %48, spesifitenin %90 olduğu görülmüştür (AUC=0,777, %95 GA 0,665-0,888, p<0,001) (Şekil-1).

ROC analizinde siroz tanısında en iyi FİB-4 skorunun 4,24 olduğu görülmüştür. FİB-4 için 4,24 eşik değer olarak kabul edildiğinde siroz tanısındaki sensitivitenin %51, spesifitenin %97 olduğu görülmüştür (AUC=0,863, %95 GA 0,775-0,948, p<0,001) (Şekil-2).

Tablo-1. Gruplarda laboratuvar verilerinin dağılımı.

	Siroz		Kontrol		
	Medyan	Ortalama ± SS	Medyan	Ortalama ± SS	p
AST (U/l)	36	44,1 ± 29,9	24,5	36,7 ± 25,8	0,111
ALT (U/l)	26	33,5 ± 28,7	25,5	46,5 ± 48,4	0,349
ALB (g/dl)	3,4	3,1 ± 1,2	4,3	4,2 ± 0,7	<0,001
T.BİL(mg/dl)	1,1	1,5 ± 1,0	0,6	0,6 ± 0,3	<0,001
D.BİL(mg/dl)	0,5	0,7 ± 0,7	0,2	0,2 ± 0,1	<0,001
ALP (U/l)	109	118 ± 75	81	86 ± 45	0,064
PLT(10 ³ x µL)	103	138 ± 140	188	200 ± 104	<0,001
INR	1,2	1,2 ± 0,1	1,0	1,0 ± 0,1	<0,001
PT (sn)	14,7	13,8 ± 4,2	12,1	11,9 ± 3,0	<0,001

*Mann Whitney U testi

** AST; Aspartat aminotransferaz, ALT, Alanin aminotransferaz, ALB; Albumin, T.BİL; Total Bilirubin, D. BİL; Direkt bilirubin, ALP; Alkalen Fosfataz, PLT; Platelet, INR; International Normalized Ratio, PT; Protrombin Zamanı

Tablo-2. FIB-4 ve APRI skorlarının siroz tanısındaki yeri.

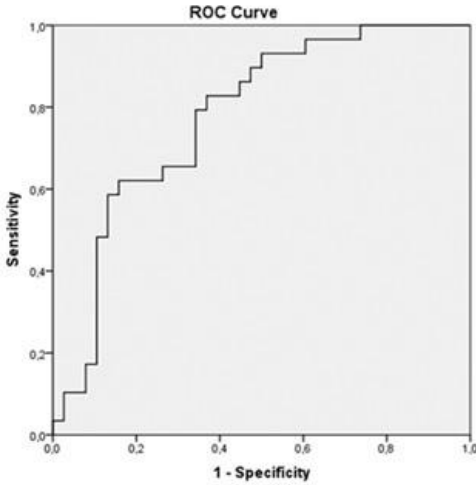
	FIB-4 < 1.45	APRI ≤0.5
Sensitivite	%58,62	%10,34
Spesifite	%86,84	%92,11
Pozitif Olabilirlik Oranı	4,46	11324
Negatif Olabilirlik Oranı	0,48	0,97
Pozitif Prediktif Değer	%77,27	0,5
Negatif Prediktif Değer	%73,33	%57,38
Doğruluk	%74,63	%56,72

*Ki-kare testi

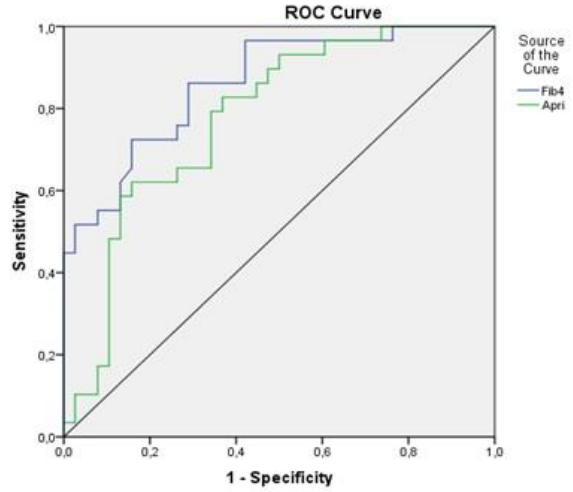
Tablo-3. FIB-4 ve APRI skorlarının siroz tanısını dışlamadaki yeri

	FIB-4 < 1.45	APRI ≤0.5
Sensitivite	%39,47	0,5
Spesifite	%96,55	%89,66
Pozitif Olabilirlik Oranı	11,45	4,83
Negatif Olabilirlik Oranı	0,63	0,56
Pozitif Prediktif Değer	%93,75	%86,36
Negatif Prediktif Değer	%54,90	%57,78
Doğruluk	%64,18	%67,16

*Ki-kare testi



Şekil-1. APRI değerlerinin ROC analizi



Şekil-2. FIB-4 skorunun ROC analizi

TARTIŞMA

Günümüzde karaciğer fibrozisi ve siroz tanısında altın standart yöntem hala histolojik incelemedir. Ancak invazif ve ağırlı bir işlem olmasının yanı sıra önemli komplikasyonlara sahiptir. Bu nedenle siroz tanısında invazif olmayan yöntemlere başvurulmaktadır. Bu yöntemler arasında olan APRI ve FIB-4 indeksleri giderek daha yaygın kullanılmaktadır. Karaciğer biyopsisi gerekliliğini azalttığı yönünde veriler olmasına rağmen, karaciğer fibrozisi ve siroz tanısında, her iki indeks için çok sayıda eşik değer, değişen oranlarda sensitivite ve spesifiteler bildirilmiştir (11). Bu yöntemlerin siroz tanısında kullanılabilirlikleri hakkında daha çok veriye ihtiyaç bulunmaktadır (12-13).

Çalışmada ilk dikkat çeken bulgu siroz grubunda, diğer kronik karaciğer hastalıklarından oluşan kontrol grubuna kıyasla APRI ve FIB-4 skorlarının daha yüksek olmasıdır. Bu sonuç farklı etiyolojik faktörlerle gelişen karaciğer yetersizliği vakalarında APRI ve FIB-4 testlerinin klinik tanıyı desteklediğini göstermektedir. Ancak henüz klinik olarak siroz tanısı almamış bir hastada bu testlerin siroz tanısını öngörmeye yararı kısıtlıdır. Çalışmamızda testlerin sensitivite ve spesifite analizlerinin bu yöntemlerin tek başına tanıda kullanılamayacağına işaret etmektedir. Yapılan çalışmaların çoğunda APRI ve FIB-4'ün siroz tanısında önemli olduğu bildirilmesine rağmen, çeşitli eşik değerler, değişen sensitivite ve spesifite değerleri bildirilmiştir. Bizim çalışmamızdaki eşik değerlerle uyumlu birçok

çalışma bulunmaktadır. Vallet-Pichard ve ark'ı (2007)⁶ tarafından FIB-4 hakkında yapılan öncül çalışmada çalışmamızla aynı eşik değerler tercih edilmiştir (1,45 ve 3,25). FIB-4'ün siroz için tanı gücü AUC= 0,91 (%95 GA 0,86-0,93) bildirilmiştir. FIB-4 <1,45 altında belirgin fibroziste negatif prediktif değeri %94,7, FIB-4>3,25 üzerinde belirgin fibrozis için pozitif prediktif değeri %82,1 ifade edilmiştir. Çalışma sonucunda FIB-4 için 1,45-3,25 değerleri dışında doğru ve güvenilir sonuçlar verdiği görülmüştür. FIB-4'ün çalışmamızda siroz tanısı için 3,25 üzerindeki pozitif prediktif değeri %77, sirozun dışlanmasında 1,45 altındaki negatif prediktif değeri %55 bulunmuştur. Çalışmalar arasındaki farkın en önemli nedeni Vallet-Pichard ve ark.'nın (6) çalışmasında belirgin fibrozis (F3-F4) incelenirken, çalışmamızda sadece klinik olarak siroz tanısı alan dolayısıyla (F4) kabul edilecek hastaların incelenmesi olabilir.

APRI ve FIB-4'ün için tanı değeri yüksek olan daha farklı eşik değerlerin tanımlandığı çalışmalar da bulunmaktadır. Li ve ark. (10) tarafından yapılan çalışmada 822 HBV hastasında FIB-4 ve APRI skorunun belirgin fibrozis ve siroz tanısındaki yeri değerlendirilmiştir. 30 yaş üzeri hastalarda APRI skorunun siroz tanısındaki AUC değeri 0,78 (%95 GA 0,75-0,82), FIB-4'ün 0,72 (%95 GA 0,68-0,76) hesaplanmıştır. Bu çalışmada ayrıca, siroz tanısında en az %90 spesifite sağlayan en yüksek FIB-4 değerinin 2,6 olduğu, siroz tanısını dışlamada en iyi değer 1,2 olduğu (sensitivite

%91, spesifitesi %32) ifade edilmiştir. Benzer şekilde en az %90 spesifitedeki siroz tanısındaki en iyi APRI skorunun 1,5 olduğu, siroz tanısını dışlamadaki en iyi eşik değerinin 0,4 olduğu (sensitivite %91, spesifite %36) ifade edilmiştir.

Lin ve ark'ı (2015) (14) tarafından yakın bir zamanda yapılan çalışmada 631 kronik HBV hastasında FIB-4 ve APRI skorlarının siroz tanısındaki yeri değerlendirilmiştir. Çalışmada FIB-4 indeksinin belirgin fibrozis ve siroz tanısında (AUC=0,869) APRI skorundan daha değerleri (AUC=0,706) olduğu ifade edilmiştir. FIB-4 indeksi için 0,87 ve 3,40 eşik değerlerinin belirgin fibrozis yokluğu ve siroz tanısı için doğruluğun sırasıyla %69,2 ve %84,4 olduğu bildirilmiştir. FIB-4<1,63'ün siroz tanısını dışlamadaki sensitivitesi %89,8, spesifitesi %64,2, FIB-4>3,40'ın siroz tanısı için sensitivitesi %55,1, spesifitesi %93,2'ydi. APRI < 1,10 skoru için sensitivite %71,4, spesifitesi %67,9, APRI > 4,03 için sensitivite %16,3, spesifite %95,1 bildirilmiştir.

Lin ve ark'ı (2011) (15) yaptıkları meta-analizde 40 çalışma ve 8739 HCV hastasını değerlendirmiştir. APRI skorunun siroz tanısında AUC değeri 0,83 bildirilmiştir. APRI için 1,0 eşik değerinin siroz tanısında %76 spesifitede, %72 spesifitede olduğu ifade edilmiştir. 2,0 eşik değerinin siroz tanısındaki spesifitesinin %91, sensitivitesinin ise %46 olduğu ifade edilmiştir.

Zhu ve ark'ı (2011) (16) yaptıkları prospektif çalışmada 175 kronik HBV hastası değerlendirilmiştir. Siroz tanısında APRI indeksinin AUC değeri 0,83 (%95 GA 0,77-0,90) ifade edilmiştir. FIB-4 için AUC değeri ise 0,77 (%95 GA 0,68-0,85) olarak ifade edilmiştir. Siroz tanısında APRI için 1,0 eşik değerinin %76 sensitivite, %70 spesifiteye, FIB-4 için 1,9 eşik değerinin %69 sensitivite ve %75 spesifiteye sahip olduğu belirtilmiştir. Belirgin fibrozis saptamadaki en iyi eşik değerin APRI için 0,5 (sensitivite %82, spesifite %83), FIB-4 için 1,7 olduğu (sensitivite %74, spesifite %84) bildirilmiştir.

Usluer ve ark (2012) (17) tarafından yapılan çalışmada kronik HCV enfeksiyonu olan hastalarda FIB-4 ve APRI skorlarının belirgin fibrozisi saptamadaki yeri değerlendirilmiş, daha kompleks yöntemler (Fibrotest, AP indeksi) ile karşılaştırılmıştır. FIB-4 için 1,45 eşik değerinin sensitivitesi %62, spesifitesi %67 bulunurken, APRI 0,5 eşik değeri için sensitivite %71,

spesifite %55 bildirilmiştir. Çalışmada APRI ve FIB-4 indekslerinin belirgin fibrozis tanısında daha kompleks yöntemlerle benzer sonuçlar verdiği ifade edilmiştir. Belirgin fibrozis siroz ile eş değer olmamasına rağmen, sirozun öncüsü olması nedeniyle bu çalışmanın sonuçları çalışmamızla karşılaştırılabilir.

Zhang ve ark. tarafından yapılan çalışmada karaciğer biyopsisi yapılan 212 kronik HBV hastası değerlendirilmiştir. Biyopsi ile fibrozis ve siroz tanısı evrelenmiş veya doğrulanmıştır. FIB-4'ün siroz tanısındaki AUC değeri 0,756 (%95 GA 0,687-0,825) bildirilmiştir (18). Çalışmanın sonucunda kronik hepatit hastalarında FIB-4'ün siroz tanısında kullanılabileceği, biyopsi gerekliliğini azaltabileceği ifade edilmiştir.

Siroz tanısında APRI ve FIB-4 indekslerinin başarılı olduğunu ifade eden çalışmaların aksine, siroz tanısında istenen düzeyde belirleyici olmadığını ifade eden çalışmalar da bulunmaktadır. Cheng ve ark. tarafından yapılan çalışmada kronik HBV hastalarında on non-invazif yöntemin siroz tanısı ve fibrozis tahminindeki rolü değerlendirilmiştir. APRI ve FIB-4 yönteminin siroz tanısında AUC değerleri sırasıyla 0,69 (%95 GA 0,64-0,74) ve 0,76 (%95 GA 0,72-0,80) bildirilmiştir. AUC değerlerine göre sıralandığında APRI indeksinin onuncu sırada, FIB-4 indeksi sekizinci sırada olduğu görülmüştür. Tanı için en yüksek AUC değerlerini HALF indeksi, FibroScan ve Zeng modeli sağlamıştır (19).

Siroz tanısında invazif olmayan yöntemlerin giderek yaygın kullanılmasına rağmen, sensitivite ve spesifite istenen düzeyde değildir. Bu nedenle tek başına kullanımları önerilmemektedir. Diğer taraftan özel durumlarda FIB-4 ve APRI modellerinin kullanımları sınırlanmaktadır. Xiao ve ark. tarafından yapılan çalışmada HCC'nin eşlik ettiği HBV hastaları değerlendirilmiştir. Bu hastalarda siroz tanısında APRI ve FIB-4 modellerinin AUC değeri sırasıyla 0,676 ve 0,652 hesaplanmıştır. Çalışmada APRI ve FIB-4 değerlerinin fibrozisle korele olmasına rağmen, HCC'nin eşlik ettiği hastalarda siroz için tanı değerinin azaldığı bildirilmiştir (20). Bu nedenle FIB-4 ve APRI sonuçlarının yorumlanmasına dikkat edilmeli, yardımcı yöntemler dikkate alınmalıdır. Ek olarak aminotransferazların normalin üst limiti tanımlaması çalışmalar arasında farklılık göstermektedir. Perazzo ve ark. yaptıkları çalışmada AST için normalin üst limiti

tanımlamalarının değişimin siroz tanısını nasıl etkilediğini değerlendirmiştir. Çalışmada APRI indeksiyle değerlendirilen siroz sıklığının %34,7 ile %68,5 arasında değişim gösterdiği, yalancı pozitiflik oranının %0-61 arasında değişim gösterdiği ifade edilmiştir (21). Diğer taraftan siroz ve fibrozis için APRI ve FIB-4'ün çalışmalar arasında farklı sensitivite, spesifite ve AUC değerlerinin bildirilmesinde, çalışmaların bir kısmında HBV, bir kısmında HCV, çalışmamızın da aralarında olduğu bir kısmında ise tüm nedenlerle siroz gelişen hastaların dahil edilmesi önemli bir neden olabilir.

APRI indeksi sıklıkla HBV ve HCV hastalarında incelenmiştir. APRI'nin diğer etyolojilerdeki kullanımı sınırlı sayıda çalışmaya konu olmuştur. Festi ve ark. (22) tarafından yapılan çalışmada NAYKH hastalarında fibrozisin ve siroz tanısında APRI indeksinin 0,54 olduğunda AUC=0,75, sensitivitesi %77,3, spesifitesi %70,9 olduğu ifade edilmiştir. Naveau ve ark. (23) tarafından yapılan çalışmada alkol ile ilişkili karaciğer hastalığında APRI skorunun siroz tanısında AUC değerinin 0,67 olduğu bildirilmiştir. Bu çalışmada APRI skorunun Fibrotest ve Hepaskor'dan daha düşük düzeyde belirleyici olduğu görülmüştür. Çalışmamızda ise APRI skorunun viral hepatit, alkol ile ilişkili karaciğer hastalığı, kriptojenik ve NAYKH'dan oluşan hasta grubunda iyi bir belirleyici olduğu gösterilmiştir. Benzer şekilde FIB-4'ün fibrozis ve siroz tanısındaki belirleyiciliği sıklıkla viral nedenlerde incelenmiştir (24). Ancak NAYKH hastalarında özellikle fibrozis tanısında AUC değerinin 0,80 olduğu, hastaların %60'ında biyopsi gerekliliğini azalttığı ifade edilmiştir (25-27). Viral hepatitlere bağlı siroz hastalarının yanında, alkolik karaciğer hastalığı, non-alkolik yağlı karaciğer hastalığı da yer almaktadır (28). Viral nedenler dışında da FIB-4 hakkındaki sonuçlar olumlu yönde olmasına rağmen kesin bir sonuca varmak için yeterli sayıda çalışma bulunmamaktadır (29, 30). Çalışmamızda tüm etiyolojiler değerlendirildiği için sonuçlarımız dikkatli yorumlanmalıdır.

Çalışmamızın bazı kısıtlılıkları bulunmaktadır. Birincisi, çalışmamız retrospektif çalışma dizaynında olduğu için retrospektif dizaynın sahip olduğu tüm kısıtlılıklara sahiptir. İkincisi, çalışmamıza dahil edilen hasta sayısı kısmen azdır. Diğer taraftan, APRI ve FIB-4 indekslerinin

sadece klinik olarak siroz tanısı olan hastalardaki yeri değerlendirilmiştir, bu hastalarda karaciğer biopsisi olmadığı için fibrozis derecesi konusundaki sensitivite ve spesifiteleri değerlendirilmemiştir. Ek olarak, çalışmamıza dahil edilen siroz hastalarında tek bir nedenden ziyade tüm nedenler dikkate alınmıştır (31). Çalışmamızın diğer bir kısıtlılığı siroz tanısında biyopsi haricinde hastaların klinik semptomları, görüntüleme bulguları ve laboratuvar yöntemleri de kullanılmıştır. Bu nedenle APRI ve FIB-4 indekslerinin siroz tanısındaki sensitivite ve spesifiteleri olduğundan daha fazla bulunmuş olabilir.

SONUÇ

Karaciğer biyopsisi siroz tanısında önemini korumasına rağmen, komplikasyonlar ve yüksek maliyetli oluşu dikkate alındığında invazif olmayan yöntemlerin popülaritesini arttırmaktadır. Siroz ve fibrozis tanısında, giderek artan sayıda non-invazif yöntem ileri sürülmektedir. Bu yöntemlerin içerisinde ön sıralarda yer alan APRI ve FIB-4'ün çalışmamızda siroz tanısını desteklediği gösterilmiştir. APRI indeksinde 1,32 eşik değerinde siroz tanısında AUC=0,777 (sensitivite %48, spesifite %90), FIB-4 indeksinde 4,24 eşik değerinde AUC=0,863 (sensitivite %51, spesifite %97) olarak bulunmuştur. Her iki testte de yüksek spesifite yanında yetersiz bir duyarlılık görülmektedir.

Siroz tanısında hem APRI hem de FIB-4 indeksleri önemli non-invazif yöntemler arasındadır. Bu yöntemlerin klinik pratikte daha sık kullanılması siroz gelişen hastaların önceden belirlenmesine, yüksek maliyetli anti-viral tedavilerin uygun hastalara yönlendirilmesine rehberlik edecektir. Diğer taraftan önemli komplikasyonlara neden olabilen karaciğer biyopsisi gerekliliğini azaltacaktır. Ancak APRI ve FIB-4 yöntemlerinin sensitivite ve spesifite analizleri dikkate alındığında tek başına siroz tanısında yeterli olmadıkları söylenebilir. Bu nedenle tanı ve takip yöntemleri içerisine entegre edilerek, tanı veya tanının dışlanmasında diğer laboratuvar ve görüntüleme yöntemleri ile birlikte dikkate alınması daha güvenli sonuçların alınmasını sağlayacaktır.

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Ekstremité sinoviyal sarkomlarının manyetik rezonans görüntüleme bulguları

Magnetic resonance imaging findings of extremity synovial sarcomas

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ÖZ

Amaç: Bu çalışma ekstremité sinoviyal sarkomlarının manyetik rezonans görüntüleme (MRG) bulgularının lezyon yerleşim yeri ile ilişkisini inceleyerek ayırıcı tanıda faydalı olabilecek görüntüleme bulgularını saptamayı amaçlamaktadır.

Gereç ve Yöntem: Ocak 2004- Aralık 2022 tarihleri arasında histopatolojik olarak sinoviyal sarkom tanısı almış ve bölümümüze cerrahi öncesi görüntüleme için yönlendirilen olguların MRG bulguları kas-iskelet sistemi radyolojisinde on yıldan fazla deneyimi olan radyoloji hekimi tarafından retrospektif olarak değerlendirildi.

Bulgular: Çalışmaya 29 lezyon (16 proksimal, 13 distal apendiküler) dahil edildi. Lezyonların 20 (%69) tanesinde T1 ağırlıklı (T1A) serilerde hiperintens sinyal izlendi. T2 ağırlıklı (T2A) görüntülerde sıvı sinyali lezyonların 24 (%82,8)'ünde saptandı. 'Triple sign' lezyonların 23 (%79,3) tanesinde mevcuttu. T1A sekansta lezyonların 25 (%86,2)'inde, T2A serilerde ve kontrastlı imajlarda ise 28 (%96,6)'inde inhomojenite izlendi. Lezyonların 18 (%62,1)'i solid ağırlıklı olarak sınıflandı. Perilezyonel ödem olguların 27 (%93,1)'sinde, fasyal ödem ise 25 (%86,2)'inde izlendi. Lezyonların 9 (%31)'unda komşu kemikte osteit lehine ödem veya infiltrasyon saptandı. Kalsifikasyon ise lezyonların 15 (%51,7) tanesinde tespit edildi. Proksimal ve distal apendiküler yerleşim gösteren lezyonların MRG bulguları arasında anlamlı farklılık izlenmedi ($p>0,05$).

Sonuç: T1A görüntülerde yüksek sinyal, 'triple sign' varlığı, heterojen kontrastlanan solid ağırlıklı lezyon, kalsifikasyon varlığı, perilezyonel ve fasyal ödem sinoviyal sarkomun çalışmamızda yaygın olarak tespit edilen MRG bulgularıdır. Ekstremité sinoviyal sarkomlarının görüntüleme bulguları apendiküler iskeletteki yerleşimine göre değişkenlik göstermemektedir.

Anahtar Sözcükler: Ekstremité, Manyetik rezonans görüntüleme, Sinoviyal sarkom, Yumuşak doku sarkomu

ABSTRACT

Aim: To identify imaging findings that could aid in differential diagnosis by assessing the relationship between magnetic resonance imaging (MRI) characteristics and lesion location of extremity synovial sarcomas.

Materials and Methods: MRI findings of patients diagnosed with synovial sarcoma histopathologically between 2004–2022 and referred to our department for preoperative imaging were retrospectively evaluated by a radiologist with more than ten years of experience in musculoskeletal radiology.

Results: The study included 29 lesions (16 proximal, 13 distal appendicular). In 20 (69%) lesions, hyperintense signals were observed in T1-weighted (T1W) series. In T2W images, fluid signal was

detected in 24 (82.8%) lesions. The 'triple sign' was present in 23 (79.3%) lesions. In T1W images, inhomogeneity was observed in 25 (86.2%) lesions, while in T2W series and contrast-enhanced images, it was detected in 28 (96.6%) lesions. 18 (62.1%) lesions were classified as predominantly solid. Perilesional edema was observed in 27 (93.1%) cases, while fascial edema was observed in 25 (86.2%) cases. In 9 (31%) lesions, edema favoring osteitis or infiltration was identified in the adjacent bone. Calcification was detected in 15 (51.7%) lesions. No significant difference was observed in the MRI findings of lesions with proximal and distal appendicular localization ($p>0.05$).

Conclusion: High signal on T1W images, presence of the 'triple sign', a predominantly solid lesion with heterogeneous enhancement, presence of calcification, perilesional and fascial edema are the MRI findings commonly detected in synovial sarcoma in this study. The imaging findings of extremity synovial sarcomas do not vary per their location.

Keywords: Extremity, Magnetic resonance imaging, Synovial sarcoma, soft tissue sarcoma

GİRİŞ

Primer yumuşak doku malignitelerinin %2,5 - 10'unu oluşturan sinoviyal sarkom, pleomorfik sarkom, liposarkom ve rabdomyosarkomdan sonra dördüncü en sık görülen yumuşak doku sarkomudur (1-3). Adölesan ve genç erişkin olgularda sıklıkla 15-40 yaş arasında saptanır (1,3,4). Olguların %80-95'inde ekstremitelerde yerleşim gösterir. Alt ekstremitte tutulumu daha sık olup, popliteal fossa en sık görüldüğü lokalizasyondur (5-7). İsmi aksine sinoviyumdan köken almaz ve intraartiküler yerleşim nadirdir (1). Olgular genellikle ele gelen kitle ve şişlik şikâyeti ile başvurur. Lezyonların yavaş büyüme gösterdikleri ve şikâyetlerin başlangıcından tanıya kadar geçen sürenin ortalama 2-4 yıl olduğu bildirilmiştir (8). Şikâyetlerin uzun süreli olması ve lezyonların yavaş büyümesi agresif olmayan iyi huylu yumuşak doku lezyonları ile karıştırılmalarına neden olmaktadır.

Radyolojik değerlendirme tümörün yayılımı, evreleme ve cerrahinin planlanmasında önemli olmakla birlikte, bulgular tanı için patognomonik değildir. Lezyonların %30'unda kalsifikasyon mevcut olup, eklem çevresinde kalsifikasyon barındıran yumuşak doku kitlesi saptanan genç hastalarda ayırıcı tanıda sinoviyal sarkom ön planda düşünülmelidir (1,3). Nadir olmayarak olguların %11-20'sinde komşu kemikte benign görünümlü düzgün sınırlı erozyon ve agresif olmayan periost reaksiyonu oluşturarak tanısız yanılgıya sebep olabilir (1). Manyetik rezonans görüntülemesinde (MRG) T2 ağırlıklı görüntülerde belirgin heterojen görünüme sahip olup literatürde bu görünüm 'triple sign' olarak tanımlanmıştır (9). Lezyon içerisindeki solid sellüler komponent, kanama/nekroz ve kalsifiye/fibrotik komponent nedeniyle oluşan ara, yüksek ve düşük sinyalli

alanları tanımlamaktadır (10). Ancak bu bulgu başka yumuşak doku sarkomlarında da görülebileceği için özgüllüğü düşüktür (1). Multilobüle görünüm, lezyon içerisinde septa varlığı ve sıvı seviyelenmeleri tanımlanan diğer MRG bulguları olup, üzüm kâsesi (bowl of grapes) görünümü olarak adlandırılmıştır (1,11). Bu bulguların aksine 5 cm'den küçük boyutlu lezyonlar homojen iç yapıya ve düzgün sınıra sahip olarak tanısız yanılgıya neden olabilmektedir (12).

Karakteristik klinik ve görüntüleme bulgularının olmaması nedeniyle sinoviyal sarkom en sık yanılgılıkla benign tanısı konulan yumuşak doku tümörüdür (13,14). Klinik bulguların iyi huylu lezyonlar ile karışabilecek şekilde uzun süreli olması ve lezyonların yavaş büyümesi, doğru tanı için görüntüleme bulgularının önemini arttırmaktadır. Erken ve doğru tanı ile hastaların uygun klinik yönetimi için ayırıcı tanıda sinoviyal sarkomu ön plana çıkaracak görüntüleme bulgularına farkındalık büyük önem taşımaktadır. Bu nedenle çalışmamızda ekstremitte sinoviyal sarkomlarının MRG bulgularının lezyon yerleşim yeri ile ilişkisini inceleyerek ayırıcı tanıda faydalı olabilecek görüntüleme bulgularını saptamayı amaçladık.

GEREÇ ve YÖNTEM

Etik kurul onayı alındıktan sonra Ocak 2004-Aralık 2022 tarihleri arasında histopatolojik olarak sinoviyal sarkom tanısı almış ve bölümümüze cerrahi öncesi görüntüleme için yönlendirilen olguların MRG tetkiklerinin analizi için hastanemizin Görüntü Arşivleme ve İletişim Sistemleri (PACS) veri tabanı sinoviyal sarkom anahtar kelimeleri kullanılarak retrospektif olarak tarandı. Bulunan olgulardan cerrahi öncesi MRG tetkiki veya histopatolojik tanısı olmayan olgular dışlandıktan sonra 29 hasta çalışmaya dahil edildi.

Olguların görüntüleri hastanemizde mevcut olan 1,5 Tesla veya 3 Tesla (Magnetom Symphony, Magnetom Amira veya Magnetom Verio, Siemens Healthineers, Erlangen, Germany) MRG cihazlarında multikanallı koiller kullanılarak elde olundu. Görüntüleme protokolü birbirine dik en az iki plan T1 ağırlıklı, üç plan sıvıya duyarlı T2 ağırlıklı veya "short tau inversion recovery" (STIR) ve kontrast öncesi ile kontrast sonrası yağ baskılı T1 ağırlıklı (dinamik) imajları içermekte olup, lezyon yeri ve boyutu göz önüne alınarak uygun uzaysal ve kontrast çözünürlüğü sağlayacak şekilde kas-iskelet radyolojisi sorumlu radyoloji hekimi tarafından belirlendi.

Tüm MRG tetkikleri kas-iskelet sistemi radyolojisinde on yıldan fazla deneyimi olan radyoloji hekimi tarafından retrospektif olarak değerlendirildi. Lezyon boyutları birbirine dik üç planda (anteroposterior, mediolateral ve kraniokaudal) ölçüldükten sonra, en uzun çapı kaydedildi.

Lezyon lokalizasyonu, sinyal karakteristikleri ve eşlik eden bulgular yönünden MRG tetkikleri incelendi. Lezyonların lokalizasyonu üst ve alt ekstremitelerde dirsek ve diz eklemleri referans alınarak proksimal ve distal yerleşim göstermelerine göre sınıflandırıldı. Lezyonların sinyal karakteristiklerinde T1 ağırlıklı imajlarda kas dokusunu göre yüksek sinyal varlığı, sıvıya duyarlı sekanslarda sıvı sinyalinin varlığı, tüm sekanslarda sıvı seviyelenmesi ve heterojenitenin varlığı, sıvıya duyarlı sekanslarda 'triple sign' varlığı yönünden incelendi. Triple sign, literatürde tanımlandığı gibi sıvıya duyarlı sekanslarda yağ dokusu referans alınarak yüksek, ara ve düşük sinyalli alanların her üçünün lezyondaki varlığının göstergesi olarak kabul edildi (9). Lezyonun %25'inden fazlası inhomojen ise lezyon heterojen olarak kabul edildi. Kontrastlı serilerde kontrastlanan solid komponent %75'ten fazla ise lezyon solid ağırlıklı, %25–75 arasında ise kısmi solid ve %25'ten az ise minimal solid olarak gruplandırıldı.

Eşlik eden bulgulardan lezyon çevresinde ödem veya fasyal ödem varlığı, komşu eklemden efüzyon veya infiltrasyon varlığı, komşu kemikte ödem veya infiltrasyon varlığı ve lezyon içerisinde kalsifikasyon varlığı araştırıldı. Kalsifikasyon varlığı olguların aynı dönemde çekilmiş bilgisayarlı tomografi (BT) veya direkt grafi tetkikleri ile de korele edildi.

İstatistik analiz 'Statistical Package for the Social Sciences (SPSS)' versiyon 25 kullanılarak yapıldı. Sürekli değişkenlerin normal dağılıma uyup

uymadığı Shapiro-Wilk testi ile değerlendirildi. Sürekli değişkenler ortalama ve standart sapma değerleri ile verildi. Kategorik veriler ise sayı ve yüzdeleri ile ifade edildi. Sürekli değişkenler Mann-Whitney U testi, kategorik değişkenler ise ki-kare testi veya Fisher exact test ile değerlendirildi. P değeri 0,05 in altında olan sonuçlar istatistiksel olarak anlamlı kabul edildi.

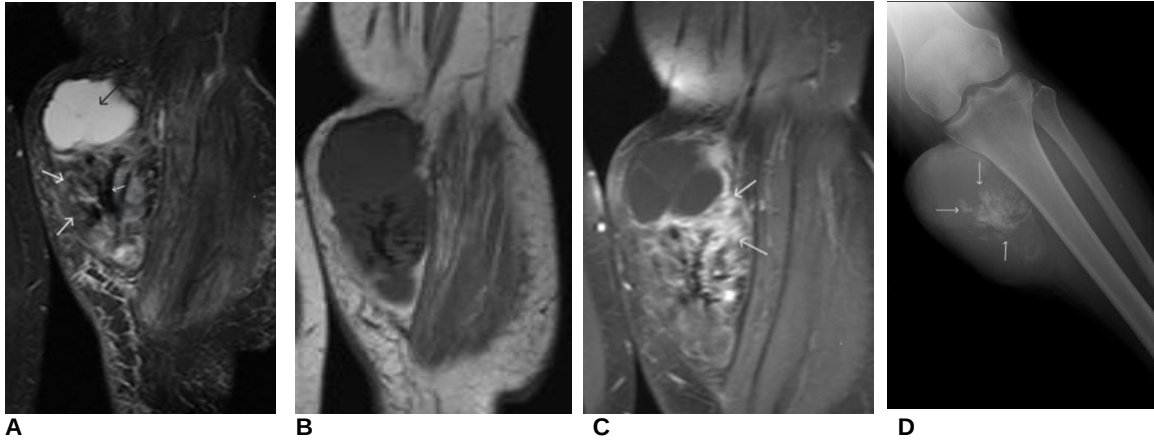
BULGULAR

Çalışmaya 14 (%48,3) erkek ve 15 (%51,7) kadın hasta dahil edildi. Tüm hasta grubunun yaş ortalaması 31,1±16,9 olup, erkek ve kadınların yaş ortalamaları arasında anlamlı fark izlenmedi ($p=0,71$). Lezyonların 16 (%55,2) tanesi proksimal, 13 (%44,8) tanesi distal apendiküler iskelette yerleşim göstermekteydi. Lezyonların ortalama boyutları 10,9±4,9 cm olarak ölçüldü. Lezyon yerleşim yeri ile lezyon boyutu arasında anlamlı ilişki saptanmadı ($p=0,135$).

Lezyonların 20 (%6) tanesinde kas dokusuna kıyasla T1 ağırlıklı serilerde hiperintens sinyal izlendi. T2 ağırlıklı görüntülerde sıvı sinyali lezyonların 24 (%82,8)'ünde saptandı. Herhangi bir sekansta sıvı seviyelenmesi lezyonların 5 (%17,2)'inde görüldü. 'Triple sign' lezyonların 23 (%79,3) tanesinde mevcuttu (Şekil-1). T1 ağırlıklı sekansta lezyonların 25 (%86,2)'inde, T2 ağırlıklı serilerde ve kontrastlı imajlarda ise 28 (%96,6)'inde inhomojenite izlendi. Lezyonların 18 (%62,1)'i solid ağırlıklı, 4 (%13,8)'ü kısmi solid, 7 (%24,1)'i ise minimal solid olarak sınıflandı.

Perilezyonel ödem olguların 27 (%93,1)'inde, fasyal ödem ise 25 (%86,2)'inde izlendi. Lezyona komşu eklemden efüzyon 12 (%41,4) olguda, komşu eklem invazyonu ise 17 (%58,6) olguda saptandı. Lezyonların 9 (%31)'unda komşu kemikte osteit lehine ödem veya infiltrasyon saptandı. Kalsifikasyon ise lezyonların 15 (%51,7) tanesinde tespit edildi.

Lezyonları 16 (%55,2) tanesi proksimal ve 13 (%44,8) tanesi distal apendiküler iskelette yerleşim göstermekteydi. T1 ağırlıklı serilerde hiperintens sinyal, T2 ağırlıklı görüntülerde sıvı sinyali, herhangi bir sekansta sıvı seviyelenmesi, 'triple sign' varlığı, T1, T2 ve kontrastlı sekanslarda inhomojenite, lezyonların iç yapısı, perilezyonel ve fasyal ödem varlığı, komşu eklemden efüzyon ve invazyon varlığı, komşu kemikte osteit lehine ödem veya infiltrasyon, kalsifikasyon varlığı yönünden lezyonlar yerleşimine göre karşılaştırıldı. Değerlendirilen görüntüleme bulgularından hiçbirinde lezyon yerleşimine göre anlamlı farklılık saptanmadı ($p>0,05$, Tablo-1).



Şekil-1. 31 yaşında kadın hasta, sol kruris postero-medial kompartmanda sinoviyal sarkoma ait kitle lezyonu. T2 ağırlıklı koronal planda görüntüde kitle içinde düşük (kalsifikasyon/ fibrotik komponent) (ince ok), ara (solid sellüler komponent) (kalın ok) ve yüksek (kanama/ nekroz) (siyah ok) sinyal içeren 'triple sign' görünümü mevcuttur (A). T1 ağırlıklı görüntüde kitle ara ve düşük sinyal özelliğinde izlenmektedir(B). Postkontrast T1 ağırlıklı yağ baskılı görüntüde kitlenin solid kesimlerinde belirgin kontrast parlaklaşması (ok) mevcuttur (C). Tibia ön-arka radyografide kitle içindeki düşük sinyalli alanların kalsifikasyona ait (ok) olduğu izlenmektedir (D).

Tablo -1. Sinoviyal Sarkomlu Olguların Yerleşimine Göre Manyetik Rezonans Görüntüleme Bulguları.

Görüntüleme bulguları	Tüm hastalar (n=29)	Proksimal Apendiküler (n=16)	Distal Apendiküler (n=13)	p
Lezyon boyutu (maksimum çap, cm)	10,9±4,9	12,2±5,5	9,4±3,5	0,13
Puls sekanslarında sıvı sıvı seviyesi	5 (17,2)	3 (18,8)	2 (15,4)	1,0
T1A hiperintensite	20 (69,0)	11 (68,8)	9 (69,2)	1,0
T2A sıvı sinyali	24 (82,8)	15 (93,8)	9 (69,2)	0,14
Triple sign	23 (79,3)	13 (81,3)	10 (76,9)	1,0
İnhomojen sinyal				
T1A imajlar	25 (86,2)	11 (84,6)	14 (87,5)	1,0
T2A imajlar	28 (96,6)	15 (93,8)	13 (100)	1,0
Kontrastlı imajlar	28 (96,6)	15 (93,8)	13 (100)	1,0
Lezyon içyapısı				0,42
Ağırlıklı solid	18 (62,1)	8 (50)	10 (76,9)	
Kısmi solid	4 (13,8)	3 (18,8)	1 (7,7)	
Minimal solid	7 (24,1)	5 (31,3)	2 (15,4)	
Perilezyonel ödem	27 (93,1)	15 (93,8)	12 (93,2)	1,0
Fasyal ödem	25 (86,2)	14 (87,5)	11 (84,6)	1,0
Komşu eklemdede efüzyon	12 (41,4)	7 (43,8)	5 (38,5)	0,77
Komşu eklemdede invazyon	12 (41,4)	6 (37,5)	6 (46,2)	0,64
Komşu kemikte osteit veya invazyon	9 (31,0)	3 (18,8)	6 (46,2)	0,23
Kalsifikasyon	15 (51,7)	8 (61,5)	7 (43,8)	0,34

- T1A, T1 ağırlıklı; T2A, T2 ağırlıklı
- Veriler ortalama±standart sapma veya sayı (yüzde) olarak verildi
- P değeri proksimal ve distal apendiküler yerleşim gösteren lezyonların karşılaştırmasını temsil etmektedir. P<0,05 olan sonuçlar istatistiksel olarak anlamlı kabul edildi.

TARTIŞMA

Bu çalışmada lezyon içerisindeki hemoraji odaklarına işaret eden T1 ağırlıklı serilerde yüksek sinyal olguların 2/3'ten fazlasında görüldükçe, lezyon içerisindeki kistik-nekrotik alanları temsil eden T2 ağırlıklı görüntülerdeki sıvı sinyali lezyonların yaklaşık 4/5'inde izlendi. Herhangi bir sekansta sıvı seviyelenmesi ise olguların yaklaşık 1/6'sında mevcuttu. Literatürde sinoviyal sarkom olgularında %47'sine varan oranda hemoraji varlığı ve çalışmamızdaki sonuçlara benzer şekilde de %10–25'inde de sıvı seviyelenmesi bildirilmiştir (1). Pediatrik yaş grubunda Farkas ve ark. tarafından yapılan çalışmada ise ekstremitte yerleşimli sinoviyal sarkom olgularının %62,5'inde T1 ağırlıklı sekanslarda hemoraji saptanırken, T2 ağırlıklı serilerde sıvı sinyali lezyonların %56,3'ünde izlenmiştir (15). Aynı çalışmada daha agresif seyir gösterdiği saptanan aksiyel iskelet yerleşimli sinoviyal sarkom olgularının ise %86'sında hemoraji ve sıvı sinyalinin varlığı bildirilmiştir (15). Çalışmamızda literatüre kıyasla daha yüksek oranda hemoraji ve kistik-nekrotik komponent saptanmasının nedeni olgularımızın yüksek dereceli olması ile açıklanabilir.

Çalışmamızda lezyonların yaklaşık 4/5'inde T2 ağırlıklı sekanslardaki solid sellüler kısım ile kanama/nekroz ve kalsifiye/fibrotik komponenti temsil eden 'triple sign' mevcut iken, olguların büyük çoğunluğu (28/29, %96,6) kontrastlı imajlarda heterojen olarak izlendi. Literatürde ise geniş serilerde 'triple sign' olguların %35–57'sinde bildirilmiştir (1). Liang ve ark. çalışmalarında olguların %87,5'inde heterojen kontrastlanma bildirirken (16), Farkas ve ark. pediatrik yaş grubunda apendiküler iskelette yerleşen lezyonların %62,5'inde ve aksiyel iskelette yerleşen daha kötü prognozlu lezyonların ise tamamında heterojen kontrastlanma tespit etmiştir (15). Heterojen kontrastlanma sinoviyal sarkomun ganglion kisti veya hematoma gibi benign yumuşak doku kitlelerinden ayırmada kullanılabilecek yararlı bir görüntüleme bulgusudur.

Perilezyonel ödem, Baheti ve ark.'nın çalışmasında olguların %45'inde saptanırken, metastaz gelişimi ile korelasyon gösterdiği saptanmıştır (17). Literatürde komşu kemikte erozyon veya kemik iliği invazyonu sinoviyal sarkomlu olguların %25'inde bildirilirken (1), sinoviyal sarkomlu olguların yaklaşık %27–59'unda kalsifikasyon varlığı gösterilmiştir (1,18,19). Çalışmamızın yüksek dereceli

lezyonlardan oluşmasını destekler şekilde olgularımızda perilezyonel ödem ve kemik tutulumu literatüre göre daha yüksek oranda izlenirken, kalsifikasyon ise benzer oranda saptandı.

Çalışmamızda proksimal ve distal apendiküler iskelette yerleşim gösteren sinoviyal sarkom lezyonlarının MRG bulguları arasında istatistiksel anlamlı farklılık izlenmedi. Benzer şekilde Farkas ve ark.'nın çalışmasında da proksimal ve distal apendiküler yerleşim gösteren lezyonların görüntüleme bulguları birbirine benzer olarak bildirilmiş olup (15), aksiyel iskelette yerleşen lezyonlara göre daha iyi seyir gösterdikleri belirtilmiştir. Ancak çalışmamızın yüksek dereceli lezyonlardan oluşması nedeniyle MRG bulguları, Farkas ve ark.'nın (15) çalışmasındaki aksiyel yerleşimli lezyonlar ile benzer olarak izlendi.

Çalışmamızın çeşitli sınırlılıkları bulunmaktadır. Bunlardan ilki çalışmanın retrospektif natürlü olup, bunun dışında görece küçük örneklem büyüklüğüne sahip olması da bir diğer sınırlılık olarak kabul edilebilir. Ancak çalışmamızın örneklem büyüklüğü literatürdeki çalışmalar ile benzer olarak izlenmektedir (9,15,19). Ek olarak çalışmamızın sadece yüksek dereceli lezyonlardan oluşması, düşük dereceli lezyonların MRG özellikleri hakkında yorum yapılmasına engel olmuştur. Ayrıca MRG bulguları için gözlemci içi ve gözlemciler arası uyum değerlendirilmemiştir.

SONUÇ

Sonuç olarak, bu çalışmada ekstremitte sinoviyal sarkomlarının MRG bulgularının lezyon yerleşim yeri ile ilişkisi incelenmiş olup, T1 ağırlıklı görüntülerde yüksek sinyal, T2 ağırlıklı görüntülerde 'triple sign' varlığı, kontrastlı imajlarda heterojen kontrastlanan solid ağırlıklı lezyon, lezyon içerisinde kalsifikasyon varlığı, perilezyonel ve fasyal ödem sinoviyal sarkomun çalışmamızda yaygın olarak tespit edilen MRG bulgularıdır. Ekstremitte sinoviyal sarkomlarının görüntüleme bulguları apendiküler iskeletteki yerleşimine göre değişkenlik göstermemektedir. Bu bulgulara farkındalığın artması, hastaların uygun klinik yönetim ile zamanında ve doğru tanı alması için önem taşımaktadır.

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Cyberchondria and quality of life in anxiety and obsessive-compulsive disorder: A cross-sectional assessment from a tertiary center

Anksiyete ve obsesif kompulsif bozuklukta siberkondri ve yaşam kalitesi: Bir üniversite kliniğinden kesitsel bir değerlendirme

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ABSTRACT

Aim: Cyberchondria refers to excessive internet searches for medical knowledge. Studies evaluating cyberchondria in the psychiatric clinical population are scarce. We aimed to investigate the relationship between cyberchondria and general anxiety, health anxiety, and quality of life scores of patients diagnosed with anxiety disorders or obsessive-compulsive disorder.

Materials and Methods: Sociodemographic, clinical, and internet use-related data of the patients who applied to the psychiatric outpatient clinic of a university hospital were collected. Sixty-one patients with anxiety disorders ($n = 34$), obsessive-compulsive disorder ($n = 22$), or both were included. Hamilton Anxiety Rating Scale (HARS), Cyberchondria Severity Scale (CSS), Anxiety Sensitivity Index-3 (ASI-3), Health Anxiety Inventory Short Version (SHAI), Adult Separation Anxiety Questionnaire (ASA), and Short Form 36 (SF-36) were applied.

Results: Most visited websites were social media (86.9%), more than half of the participants did online research about psychiatric disorders, half of those felt relief, and 10% changed or stopped their medication after online searches. CSS scores were associated with SHAI ($p = .007$), HARS ($p = .022$), absence of psychiatric comorbidity ($p = .005$), and average time spent on the internet per day ($p < .001$) in regression analysis. SF-36 social functioning scores were negatively correlated with CSS scores ($r = -.276$, $p = .036$). Anxiety disorders and obsessive-compulsive disorder patients did not differ significantly in terms of CSS scores ($p = .650$, $t = .457$).

Conclusion: Considering the increasing digitalization and internet use, research is needed to navigate the development of appropriate interventions by examining the concept of cyberchondria and its relationship with mental disorders.

Keywords: Cyberchondria, internet use, anxiety disorders, obsessive-compulsive disorder, quality of life

ÖZ

Amaç: Siberkondri, tıbbi bilgiye erişim amacıyla yapılan aşırı düzeyde internet araması anlamına gelir. Psikiyatrik klinik popülasyonda siberkondriyi değerlendiren çalışmalar az sayıdadır. Çalışmamızda anksiyete bozukluğu veya obsesif kompulsif bozukluk tanısı alan hastalarda siberkondri düzeyleri ile anksiyete, sağlık anksiyetesi ve yaşam kalitesi puanları arasındaki ilişkiyi araştırmayı amaçladık.

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Gereç ve Yöntem: Bir üniversite hastanesinin psikiyatri polikliniğine başvuran hastaların sosyodemografik, klinik ve internet kullanımına ilişkin verileri toplandı. Anksiyete bozukluğu ($n = 34$), obsesif kompulsif bozukluk ($n = 22$) veya her iki tanının birlikte görüldüğü toplam 61 hasta çalışmaya dahil edildi. Katılımcılara Hamilton Anksiyete Değerlendirme Ölçeği (HADÖ), Siberkondri Ciddiyet Ölçeği (SCÖ), Anksiyete Duyarlılığı İndeksi 3 (ASİ-3), Sağlık Anksiyetesi Ölçeği Kısa Formu (SAÖ-KF), Yetişkin Ayrılma Anksiyetesi Anketi (YAAA) ve Kısa Form 36 (SF-36) uygulandı.

Bulgular: En çok ziyaret edilen web sitelerinin sosyal medya (%86,9) olduğu, katılımcıların yarısından fazlasının psikiyatrik bozukluklarla ilgili çevrim içi araştırma yapmış olduğu, araştırma sonrasında yarısının rahatlatma hissi yaşadığı ve %10'unun çevrim içi aramalar sonrasında ilaçlarını değiştirmiş veya bırakmış olduğu saptandı. Regresyon analizinde SCÖ puanları SAÖ-KF ($p = .007$), HADÖ ($p = .022$), psikiyatrik komorbiditenin olmaması ($p = .005$) ve günlük internette geçirilen ortalama süre ($p < .001$) ile ilişkiliydi. SF-36 sosyal işlevsellik puanları ile SCÖ puanları arasında negatif korelasyon görüldü ($r = -.276$, $p = .036$). Anksiyete bozuklukları ve obsesif-kompulsif bozukluk tanı grupları arasında SCÖ puanları açısından anlamlı bir fark bulunmadı ($p = .650$, $t = .457$).

Sonuç: Artan dijitalleşme ve internet kullanımı göz önüne alındığında, siberkondri kavramını ve bunun psikiyatrik hastalıklarla ilişkisini inceleyerek uygun müdahalelerin geliştirilmesine yön verecek araştırmalara ihtiyaç vardır.

Anahtar Sözcükler: Siberkondri, internet kullanımı, anksiyete bozuklukları, obsesif kompulsif bozukluk, yaşam kalitesi

INTRODUCTION

With the widespread use of the internet, access to information on health-related issues has become more common than ever (1). Many users experience feelings of comfort and competence after conducting online health research. At the same time, some describe increased anxiety and stress levels after extensive or repetitive research, which might result in cyberchondria (2). Cyberchondria is derived from “cyber”, referring to internet use, and “hypochondriasis”, referring to pathological health anxiety (HA), without any causal implication (3). Several conceptualizations, including cyberchondria being an amplifier of HA, being a form of problematic internet use, or functioning as a safety behavior, exist (2, 4). Despite the view that cyberchondria is a modern version of hypochondriasis, a concept including both anxiety and compulsiveness, defining a syndrome-like phenomenon with multidimensional elements is increasingly supported (4).

Few studies were performed on clinical samples to investigate cyberchondria in the general population (5, 6). Meanwhile, to our knowledge, two studies investigated cyberchondria in psychiatric clinical populations (7, 8). Vismara et al. (2022) compared cyberchondria levels among patients with anxiety disorders (ADs), obsessive-compulsive disorder (OCD), and major depressive disorder (MDD), as well as a group of

healthy controls (8). They reported that patients with ADs and OCD showed higher cyberchondria symptom severity than healthy controls and a positive correlation between cyberchondria scores and HA measures. Newby and McElroy (2020) found that patients with illness anxiety disorder and/or somatic symptom disorder (i.e., hypochondriasis) who received internet-delivered cognitive behavioral therapy targeting HA showed improvement in cyberchondria levels after treatment compared to the baseline (7).

In our study, we aimed to examine the characteristics and relationship of cyberchondria with various psychopathological parameters in patients with ADs and/or OCD. We hypothesized that in a clinical sample, levels of cyberchondria would increase as general anxiety (GA), anxiety sensitivity (AS), and HA scores increased. Secondly, we investigated a possible negative relationship between cyberchondria levels and quality of life scores (QoL) of patients diagnosed with ADs and OCD. Finally, based on its compulsive pattern, we presumed that cyberchondria levels would be higher in patients with OCD than in patients with ADs.

MATERIALS AND METHODS

Participants and Sampling

This cross-sectional, analytical, and non-interventional study was conducted at the specialized outpatient clinic for ADs and OCD at Ege University School of Medicine Psychiatry

Department. The Declaration of Helsinki principles were followed. The patients provided written informed consent to participate in this study voluntarily.

All the patients followed up in the specialized outpatient unit were invited to the study sequentially when they attended their routine appointments. Inclusion criteria were being older than 18 years, being literate, and being followed up with any AD and/or OCD in the unit. Exclusion criteria were being pregnant, having active psychotic symptoms, being diagnosed with psychotic disorders, dementia, or bipolar disorders, having mental retardation, and non-voluntariness. The patient recruitment of the study was conducted between July 2018 and October 2019. Ethical approval was obtained from the local committee (Registration Date&Number: 01.06.2018, 18-6.1/28).

Clinical Evaluation and Data Collection Tools

A semi-structured interview was used to collect sociodemographic data, psychiatric history, and information on internet use. The following variables were investigated: age, gender, education level, relationship status, employment status, household members, psychiatric diagnosis, OCD diagnosis and obsession types, psychiatric comorbidities, alcohol consumption, drug consumption, family history for psychiatric disorders, and current medications. Most visited websites (social media, health-related, shopping, gaming, educational), health-related and psychiatry-related research on the internet, and any change in medication after a health-related internet search, as well as average time spent daily on the internet, were asked. Hamilton Anxiety Rating Scale (HARS) was applied to assess overall anxiety levels (9). HARS consists of 14 questions evaluating anxiety levels and is valid and reliable in Turkish (10).

The Turkish version of the Cyberchondria Severity Scale (CSS) was administered to measure cyberchondria severity. This self-report questionnaire consists of 33 items rated on a 5-point Likert scale from 1 (never) to 5 (always), constituting five subscales related to the multidimensional conceptualization of cyberchondria: compulsion, distress, excessiveness, reassurance-seeking, and mistrust of medical professionals (MMP) (11). Total score ranges from 33 to 165, with higher scores indicating higher levels of cyberchondria. Previous studies and a recent systematic review of the CSS found very good to excellent

psychometric properties (12). The scale was adapted to Turkish by Uzun and Zencir (2021) with a Cronbach alpha of 0.89 for the total score indicating adequate psychometric properties of validity and reliability (13). In some studies using the CSS, the MMP subfactor was found not to measure cyberchondria, hence removed from the CSS total scores (14). In the current study, both CSS scores were calculated (with and without MMP), and all the statistics were performed accordingly.

Turkish versions of the following self-report questionnaires were administered to evaluate the symptom severity of various cyberchondria-related constructs that pointed out an association with cyberchondria in previous studies. Health Anxiety Inventory Short Version (SHAI) consists of 18 items and is a self-report scale used to determine participants' HA levels (15). Anxiety Sensitivity Index 3 (ASI-3) is an 18-item scale evaluating AS effectively and multidimensionally (16). Adult Separation Anxiety Questionnaire (ASA) is a 27-item questionnaire assessing the symptoms of separation anxiety in adulthood (17). Finally, Short Form 36 (SF-36) is a self-report questionnaire that evaluates patients' QoL parameters within eight dimensions (vitality, physical functioning, bodily pain, general health perceptions, physical role functioning, emotional role functioning, social role functioning, and mental health) (18). All these questionnaires are valid and reliable in Turkish (19–22).

Statistical Analysis

Statistical Package for Social Sciences version 24 was used to analyze data (version 24.0; IBM Corp., Armonk, NY, ABD). The statistical significance threshold value was determined as $p < .05$. Numerical data with normal distribution were presented with mean and standard deviation (SD) values, and with skewed distribution were presented with median and inter-quartile range (IQR) values. Kolmogorov-Smirnov test was used to test normality. Normally distributed numerical data were analyzed using the Student's t-test and the Pearson correlation. Numerical data that failed the normality tests were assessed with the Mann-Whitney-U test and the Spearman correlation. Categorical data were analyzed using Chi-square and one-way analysis of variance tests. A multiple linear regression model was applied to the significantly correlated variables by specifying CSS scores as the dependent variable.

Ethical Considerations

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee at Ege University and with the 1964 Helsinki Declaration or comparable ethical standards. Informed consent was obtained from all individual adult participants included in the study.

RESULTS

Sample Characteristics

Sixty-one cases followed up with the diagnosis of any AD ($n = 34$), OCD ($n = 22$), or both AD and OCD ($n = 5$) were included in the study. Among the baseline characteristics, education duration was significantly longer in the ADs group (Table-1).

Clinical Characteristics

Among 34 patients whose primary diagnosis was any AD, 20 (58.8%) had panic disorder, eight (23.5%) had generalized anxiety disorder, and five (14.7%) had social anxiety disorder. Twenty-seven patients had OCD. The most frequent obsessions were about contamination (46%), religion (14%), and symmetry (10%). Psychiatric comorbidities were detected in 42.6% of the population, and depression (61%) was the primary comorbidity. 91.8% of the sample was currently on medication and using at least one type of antidepressant. Table-1 outlines the clinical characteristics.

Internet Use-related Characteristics

Table-1. shows the internet use-related variables of the sample in comparison between two diagnostic subgroups.

CSS Total and Subscale Scores

The mean CSS score of the sample was 75.95 ± 24.99 (Table-2). Age and CSS total scores were negatively correlated ($r = -.370$, $p = .004$). CSS total and subscale scores were not significantly different between gender groups. Total CSS scores were positively correlated with years of education ($\rho = .317$, $p = .015$). CSS scores were significantly higher in patients without children ($p = .005$, $t = -2.892$). There was no significant difference in CSS scores according to relationship, employment, or household status. CSS scores were analyzed regarding the psychiatric clinical features of the sample. Patients with comorbid psychiatric conditions showed significantly lower levels of CSS total ($p = .020$, $t = -2.402$). CSS scores did not

significantly differ regarding antidepressant usage, but patients currently under medication with antipsychotics showed lower levels of CSS ($p = .035$, $t = -2.535$). There was no significant difference in CSS scores regarding alcohol consumption, drug use, or psychiatric family history.

CSS total scores were evaluated according to internet-related features of the whole sample. Mean time spent on the internet per day was positively correlated with CSS total ($\rho = .597$, $p < .001$). Participants who previously searched about any disease online had significantly higher CSS total scores ($p < .001$, $Z = -4.899$). Patients who previously researched any psychiatric disease had significantly higher CSS total scores ($p = .029$, $Z = -2.178$) than the remaining. CSS total scores did not show any statistically significant difference between patients who felt relief after health-related online research and those who did not.

CSS Scores between ADs and OCD Groups

There was no significant difference between ADs and OCD groups for CSS (Table-2). In the OCD group, CSS total scores did not significantly differ by obsession type.

Correlations between CSS and SHAI, HARS, ASI-3, ASA Scores

CSS total scores were correlated with HARS ($r = .361$, $p = .005$), SHAI ($r = .362$, $p = .005$), and ASI-3 ($r = .320$, $p = .014$) scores (Table-3). The correlation statistics, which were performed for ADs and OCD groups individually, are presented in (Table-4).

Correlations between CSS and SF-36 Scores

There were no significant correlations regarding SF-36 except physical functioning, vitality, and social role functioning (Table-3). Correlations between SF-36 and CSS scores were evaluated according to diagnostic groups and indicated in (Table-5).

Multiple Linear Regression Model of CSS and Related Variables

A backward stepwise multiple linear regression model was applied, specifying CSS as the dependent variable. Among the related variables, the absence of psychiatric comorbidity ($p = .005$), higher average time spent on the internet ($p < .001$), higher SHAI ($p = .007$), and HARS ($p = .022$) scores predicted higher CSS scores (Table-6).

Table-1. Sociodemographic, Clinical, and Internet Use-Related Characteristics of the Sample

	All samples (<i>n</i> = 61)	ADs (<i>n</i> = 34)	OCD (<i>n</i> = 22)	Statistics
Age (years: mean $\pm$ <i>SD</i>)	38.06 $\pm$ 14.13	39.14 $\pm$ 2.72	35.86 $\pm$ 2.63	<i>p</i> = .415 ^a , <i>t</i> = 0.822
Gender % (<i>n</i>)				
Female	60.7 (37)	52.9 (18)	68.2 (15)	<i>p</i> = .258 ^b , χ^2 = 1.282
Male	39.3 (24)	47.1 (16)	31.8 (7)	
Education (median, IQR)	14.00, 4	15.00, 4	12.00, 4	<i>p</i> = .034 ^c, <i>Z</i> = -2.122
Employment % (<i>n</i>)				
Unemployed	42.6 (26)	41.2 (14)	50 (11)	<i>p</i> = .508, χ^2 = 1.356
Employed	42.6 (26)	38.2 (13)	40.9 (9)	
Retired	14.8 (9)	20.6 (7)	9.1 (2)	
Relationship status % (<i>n</i>)				
Single	32.8 (20)	38.2 (13)	27.3 (6)	<i>p</i> = .307, χ^2 = 2.363
Married	55.7 (34)	55.9 (19)	54.5 (12)	
Divorced	11.5 (7)	5.9 (2)	18.2 (4)	
Children % (<i>n</i>)				
Yes	50.8 (31)	44.1 (15)	54.5 (12)	<i>p</i> = .446, χ^2 = 0.582
No	49.2 (30)	55.9 (19)	45.5 (10)	
Household % (<i>n</i>)				
Family/Partner	59 (36)	52.9 (18)	68.2 (15)	<i>p</i> = .230, χ^2 = 2.936
Parents	26.2 (16)	26.5 (9)	27.3 (6)	
Alone	14.8 (9)	20.6 (7)	4.5 (1)	
Psychiatric comorbidity % (<i>n</i>)	42.6 (26)	35.3 (12)	40.9 (9)	<i>p</i> = .672, χ^2 = 0.180
Psychiatric family history % (<i>n</i>)	59 (36)	52.9 (18)	68.2 (15)	<i>p</i> = .258, χ^2 = 1.282
Current psychotropic medication % (<i>n</i>)				
Antidepressants	91.8 (56)	88.2 (30)	100 (22)	<i>p</i> = .095, χ^2 = 2.787 <i>p</i> = .045 ^d, χ^2 = 2.801
Antipsychotics	21.3 (13)	11.8 (4)	36.4 (8)	
Mood stabilizers	1.6 (1)	2.9 (1)	-	
Benzodiazepines	3.3 (2)	2.9 (1)	4.5 (1)	
Current alcohol consumption % (<i>n</i>)	23 (14)	20.6 (7)	22.7 (5)	<i>p</i> = .849, χ^2 = 0.036
Current drug use % (<i>n</i>)	4.9 (3)	5.9 (2)	4.5 (1)	-
HARS scores (mean $\pm$ <i>SD</i> / median, IQR)	11.02 $\pm$ 4.52	10.50, 5	10.00, 8	<i>p</i> = .680, <i>Z</i> = -0.412
HARS-psychic (median, IQR)	5.00, 3	6.00, 3	5.00, 3	<i>p</i> = .492, <i>Z</i> = -0.687
HARS-somatic (mean $\pm$ <i>SD</i>)	5.46 $\pm$ 2.68	5.47 $\pm$ 2.41	5.55 $\pm$ 3.29	<i>p</i> = .922, <i>t</i> = -0.098
Average time minutes spent on the Internet per day (median, IQR)	120.00, 210	120.00, 240	120.00, 210	<i>p</i> = .324, <i>Z</i> = -0.986
Most visited websites % (<i>n</i>)				
Social media	86.9 (53)	85.3 (29)	86.4 (19)	<i>p</i> = .911, χ^2 = 0.012
Health-related	32.8 (20)	29.4 (10)	36.4 (8)	<i>p</i> = .586, χ^2 = 0.296
Shopping	21.3 (13)	14.7 (5)	36.4 (8)	<i>p</i> = .061, χ^2 = 3.515
Gaming	23 (14)	26.5 (9)	4.5 (1)	<i>p</i> = .070, χ^2 = 4.376
Educational	37.7 (23)	44.1 (15)	31.8 (7)	<i>p</i> = .375, χ^2 = 0.847
Online research about any disease % (<i>n</i>)	80.3 (49)	85.3 (29)	72.7 (16)	<i>p</i> = .248, χ^2 = 1.336
Relief after online research % (<i>n</i>)	36.7 (18)	27.5 (8)	56.2 (9)	<i>p</i> = .058, χ^2 = 3.604
Which disease was researched? % (<i>n</i>)				
Anxiety disorders	28.5 (14)	37.9 (11)	12.5 (2)	<i>p</i> = .094 ^d , χ^2 = 3.246 <i>p</i> < .001 ^d, χ^2 = 20.391
Obsessive-compulsive disorder	18.3 (9)	-	56.2 (9)	
Heart diseases	14.2 (7)	24.1 (7)	-	<i>p</i> = .040 ^d, χ^2 = 4.574
Online research about psychiatric disorders % (<i>n</i>)	65.6 (40)	70.6 (24)	59.1 (13)	<i>p</i> = .375, χ^2 = 0.788

Relief after online research (about psychiatric disorders) %(n)	50 (20)	45.8 (11)	61.5 (8)	$p = .362, \chi^2 = 0.833$
Ever changed medication after online research % (n)	5 (2)	4.1 (1)	7.6 (1)	$p = .546^d, \chi^2 = 0.984$
Ever stopped medication after online research % (n)	5 (2)	-	15.3 (2)	$p = .125^d, \chi^2 = 4.517$

Notes. ADs: Anxiety Disorders, OCD: Obsessive-Compulsive Disorder, IQR: Interquartile Range, SD: Standard deviation, HARS: Hamilton Anxiety Rating Scale,

^a Independent Samples *t*-test, ^b Chi-square, ^c Mann Whitney-*U* test, ^d Fisher's Exact test.

Table-2. CSS Scores of All Sample, ADs, and OCD Groups

	All samples (n = 61)	ADs (n = 34)	OCD (n = 22)	Statistics
CSS total score (mean $\pm$ SD)	75.9 $\pm$ 24.9	78 $\pm$ 25.9	74.7 $\pm$ 23.9	$p = .650^a, t = 0.457$
CSS total score without MMP subscale (mean $\pm$ SD)	65.1 $\pm$ 23.4	67.1 $\pm$ 24.3	63.9 $\pm$ 22.3	$p = .631, t = 0.483$
CSS – compulsion (median, IQR)	10.0, 9	10.0, 9	11.0, 7.5	$p = .820^b, Z = -0.228$
CSS – distress (mean $\pm$ SD)	17.8 $\pm$ 6.6	18.8 $\pm$ 6.7	17.1 $\pm$ 6.2	$p = .361, t = 0.921$
CSS – excessiveness (mean $\pm$ SD)	21.1 $\pm$ 8.3	21.2 $\pm$ 8.4	21.1 $\pm$ 8.4	$p = .952, t = 0.060$
CSS – reassurance (mean $\pm$ SD)	13.1 $\pm$ 5.6	13.3 $\pm$ 5.8	13.2 $\pm$ 5.5	$p = .415, t = 0.822$
CSS – MMP (median, IQR)	12.0, 6	12.0, 4.5	12.0, 6.75	$p = .800, Z = -0.253$

Notes. ADs = Anxiety Disorders; OCD = Obsessive-Compulsive Disorder; CSS = Cyberchondria Severity Scale; IQR = Interquartile Range; MMP = Mistrust of Medical Professionals; SD = Standard Deviation.

^a Independent Samples *t*-test, ^b Mann Whitney *U*.

Table-3. Correlations between CSS and SHAI, HARS, ASI-3, ASA, SF-36 scores

		HARS			SHAI	ASI-3	ASA	SF-36		
		Total	Psychi c	Somati c				Physical Functioning	Vitality	Social Role Functioning
CSS total score	<i>r</i>	.361	.268	.290	.362	.320	.213	.252	-.119	-.276
	<i>p</i>	.005	.040	.026	.005	.014	.105	.056	.377	.036
CSS total score without MMP	<i>r</i>	.333	.283	.331	.370	.332	.209	.195	-.172	-.306
	<i>p</i>	.010	.030	.010	.004	.011	.112	.142	.200	.019
CSS compulsion	<i>r</i>	.183	.219	.161	.204	.264	.015	.023	-.670	-.297
	<i>p</i>	.165	.096	.222	.121	.045	.909	.866	.623	.024
CSS distress	<i>r</i>	.373	.259	.328	.386	.312	.257	.303	-.136	-,186
	<i>p</i>	.004	.047	.011	.002	.017	.049	.021	.315	.162
CSS excessiveness	<i>r</i>	.408	.321	.307	.355	.247	.150	.168	-.172	-.292
	<i>p</i>	.001	.013	.018	.006	.062	.258	.208	.200	.026
CSS reassurance	<i>r</i>	.334	.231	.269	.228	.353	.297	.205	-.111	-.212
	<i>p</i>	.010	.079	.039	.082	.007	.022	.122	.410	.110
CSS MMP	<i>r</i>	-.191	-.186	-.098	-.031	-.065	-.056	.345	.329	.051
	<i>p</i>	.147	.159	.458	.815	.627	.672	.008	.013	.701

Notes. CSS = Cyberchondria Severity Scale; MMP = Mistrust of Medical Professionals; HARS = Hamilton Anxiety Rating Scale; SHAI = Health Anxiety Inventory- Short Version; ASI-3 = Anxiety Sensitivity Index-3; ASA = Adult Separation Anxiety Questionnaire; SF-36 = Short Form-36. *r* = Pearson/Spearman Correlation Coefficient

Table-4. Correlations between CSS and SHAI, HARS, ASI-3, ASA Scores According to Diagnostic Groups

		HARS						SHAI		ASI-3		ASA	
		Total		Psychic		Somatic		ADs	OCD	ADs	OCD	ADs	OCD
		ADs	OCD	ADs	OCD	ADs	OCD						
CSS total score	<i>r</i>	.345	.294	.287	.209	.286	.248	.636	.306	.469	.323	.395	.195
	<i>p</i>	.046	.208	.099	.377	.101	.291	<.001	.189	.005	.177	.021	.411
CSS total score without MMP	<i>r</i>	.439	.330	.327	.220	.388	.358	.574	.336	.472	.354	.449	.272
	<i>p</i>	.009	.155	.059	.350	.023	.158	.001	.147	.005	.137	.008	.246
CSS compulsion	<i>r</i>	.094	.317	.093	.335	.075	.274	.272	.152	.346	.299	.017	.114
	<i>p</i>	.595	.173	.599	.148	.675	.242	.120	.523	.045	.213	.922	.632
CSS distress	<i>r</i>	.264	.425	.293	.153	.220	.441	.620	.256	.431	.291	.405	.262
	<i>p</i>	.131	.061	.093	.592	.212	.052	<.001	.277	.011	.227	.018	.265
CSS excessiveness	<i>r</i>	.391	.250	.390	.171	.269	.320	.617	.184	.390	.269	.366	.060
	<i>p</i>	.022	.288	.022	.471	.123	.169	<.001	.437	.023	.266	.034	.800
CSS reassurance	<i>r</i>	.418	.135	.362	.044	.367	.091	.386	.398	.383	.443	.431	.293
	<i>p</i>	.014	.570	.035	.854	.033	.702	.024	.082	.025	.057	.011	.211
CSS MMP	<i>r</i>	.035	-.520	-.042	-.406	.155	-.368	.171	-.222	.176	-.221	.249	-.381
	<i>p</i>	.845	.019	.812	.076	.380	.111	.332	.347	.319	.364	.155	.097

Notes. ADs = Anxiety Disorders; OCD = Obsessive-Compulsive Disorder; CSS = Cyberchondria Severity Scale; MMP = Mistrust of Medical Professionals; HARS = Hamilton Anxiety Rating Scale; SHAI = Health Anxiety Inventory- Short Version; ASI-3 = Anxiety Sensitivity Index-3; ASA = Adult Separation Anxiety Questionnaire. *r* = Pearson/Spearman Correlation Coefficient.

Table-5. Correlations between CSS and SF-36 subscale scores according to AdDs and OCD groups

		SF-36 bodily pain		SF-36 social role functioning		SF-36 physical functioning		SF-36 general health perceptions	
		ADs	OCD	ADs	OCD	ADs	OCD	ADs	OCD
CSS total score	<i>r</i>	.442	.308	.335	.114	.136	.305	.297	.047
	<i>p</i>	.010	.186	.057	.633	.450	.192	.093	.843
CSS total score without MMP	<i>r</i>	.459	.270	.310	.195	.065	.248	.372	.075
	<i>p</i>	.007	.250	.079	.410	.719	.291	.033	.754
CSS-compulsion	<i>r</i>	.402	.203	.365	.207	.034	.100	.177	.122
	<i>p</i>	.020	.391	.037	.381	.850	.674	.325	.609
CSS-distress	<i>r</i>	.368	.172	.141	.143	.311	.166	.293	.048
	<i>p</i>	.035	.468	.435	.548	.078	.485	.098	.841
CSS-excessiveness	<i>r</i>	.380	.209	.370	.237	.096	.139	.343	.124
	<i>p</i>	.029	.377	.034	.315	.597	.560	.051	.601
CSS-reassurance	<i>r</i>	.448	.425	.360	.059	.041	.443	.105	.030
	<i>p</i>	.009	.062	.040	.806	.822	.051	.560	.899
CSS-MMP	<i>r</i>	.002	.289	.391	.458	.091	.466	.180	.167
	<i>p</i>	.993	.217	.025	.043	.616	.038	.316	.482

Notes. ADs = Anxiety Disorders; OCD = Obsessive-Compulsive Disorder; CSS = Cyberchondria Severity Scale; MMP = Mistrust of Medical Professionals; SF-36 = Short Form-36. *R* = Pearson Correlation Coefficient; *p* = Spearman Correlation Coefficient.

Table-6. Regression coefficients and confidence intervals (CI) of variables that have a significant association with CSS scores

Factors ^a	Dependent variable CSS (<i>R</i> ² = 0.542)				Dependent variable CSS without MMP (<i>R</i> ² = 0.569)			
	Regression coefficient (B)		95% CI	<i>p</i>	Regression coefficient (B)		95% CI	<i>p</i>
	Unstandardized	Standardized			Unstandardized	Standardized		
Absence of psychiatric comorbidity	14.788	0.287	4.57-25.00	.005	14.321	0.296	5.04-23.59	.003
Average time on the internet per day	0.57	0.383	0.02-0.08	<.001	0.048	0.348	0.02-0.07	.001
SHAI scores	0.776	0.289	0.21-1.33	.007	0.754	0.299	0.24-1.26	.004
HARS scores	1.370	0.241	0.20-2.53	.022	1.620	0.304	0.56-2.67	.003

Notes. CSS = Cyberchondria Severity Scale; MMP = Mistrust of Medical Professionals; SHAI = Health Anxiety Inventory- Short Version; HARS = Hamilton Anxiety Rating Scale.

^a Backward stepwise multiple linear regression analyses.

DISCUSSION

To our knowledge, this study on health-related internet use and cyberchondria in a psychiatric clinical sample is the second of its kind, together with that of Vismara et al. (2022) (8). Our results demonstrated that cyberchondria severity was significantly associated with GA and HA levels. However, AS showed a correlation signal, which failed to come forward in the regression analysis. Our second hypothesis was almost completely unsupported since overall QoL scores were not associated with cyberchondria scores. Meanwhile, social role functioning, a subdomain of QoL, stood out, highlighting the possible impact of ADs, OCD, and related cyberchondria behavior on the social abilities of individuals with lived experience. Lastly, we did not find any difference regarding cyberchondria severity between patients with ADs and OCD.

The current results support previous findings and extend to assessing the impact of cyberchondria on the patients' QoL. Although cyberchondria has been shown to be associated with HA in the general population (2), it has not been studied in a sample with ADs. Cyberchondria may be closely related to compulsiveness, and one of its core features is repetitive and time-consuming online health research resembling obsessive-compulsive behavior (1, 12).

In line with the primary hypothesis, GA, HA, and AS scores increased as cyberchondria levels increased in the whole sample, and the multiple linear regression model showed that HA scores predicted cyberchondria levels, along with GA scores, absence of psychiatric comorbidity and average time spent on the internet per day. This finding is consistent with previous studies (2, 4). Many studies reported a moderate to strong relationship between HA and cyberchondria, with correlation coefficients ranging from 0.50 to 0.67 (12). In this study, the coefficient was 0.36, indicating a weaker correlation. However, a relationship between cyberchondria and anxiety levels was found in the ADs group but not in the OCD group. Considering that both cyberchondria and anxiety levels do not show a statistically significant difference between the two groups, there might be different factors associated with cyberchondria in the OCD group. A recent network analysis reported that the relationship between cyberchondria and HA showed variable values, and the two concepts were closely related but distinct structures (12). Some studies

emphasizing the compulsiveness of cyberchondria reported that contagion obsessions in OCD patients showed a stronger relationship with cyberchondria compared to symmetry obsessions (23). However, the findings of the current study do not support a relationship between obsession types and cyberchondria. There was no significant relationship between cyberchondria and GA, HA, and AS in this group, compared to significant correlations between those variables in the ADs group. Consequently, cyberchondria may indicate a distinct entity with different aspects and may not always be associated with HA. Based on these findings, it seems essential to evaluate the interaction of cyberchondria in cases with OCD by different associated psychopathological variables in future studies.

CSS total scores in this study were compared with the results of two other psychiatric clinical studies. Among patients with illness anxiety disorder and/or somatic symptom disorder (i.e., hypochondriasis), Newby and McElroy (2020) found a higher mean score of CSS than the current study's sample (102.2 ± 21.4) (7). On the other hand, Vismara et al. (2022) reported a CSS mean score of 62.8 ± 20.1 which was lower than in this study's sample (8). Comparing these current results with previous research findings, patients with ADs and/or OCD suffer a lower level of cyberchondria than patients primarily diagnosed with HA or hypochondriasis. Patients with higher levels of cyberchondria might have been referred to the tertiary psychiatric clinic in the present study, which could result in higher CSS means and symptom severity (24).

A critical feature of cyberchondria is a decrease in the person's other daily activities because of health-related internet searches (1). The present study determined that the QoL social functionality scores decreased as cyberchondria severity increased. Other areas of QoL were not related to cyberchondria. This finding is consistent with the results of a study in which cyberchondria and WHO-QOL scale ratings were negatively correlated in a non-clinical sample (25).

The relationship between cyberchondria and QoL is important but relatively understudied. It was reported that pain catastrophizing may contribute to cyberchondria, especially in the context of chronic pain (26). When the two diagnostic groups were examined regarding QoL, as the levels of cyberchondria increased in the group

with ADs, the patients' pain-related QoL decreased. This relationship was not found in the OCD group. Based on this result, it might be helpful to examine the relationship between cyberchondria and pain catastrophizing in future studies for ameliorating adequate interventions aiming at increasing the QoL of individuals with mental disorders.

Zavorotnyy et al. (2020) reported that in a patient group with MDD, extended exposure to health-related internet research was associated with poorer medication adherence (27). In the present study, very few patients who performed health-related internet use changed or stopped their medication. This finding might be interpreted as medication adherence levels were unaffected by cyberchondria and/or health-related internet use. This distinction may be due to the difference in primary diagnostic groups. Kalckreuth et al. (2014) reported that 36.2% of psychiatric patients having health-related internet searches believed that the internet could help them cope with their mental disorders (28). This study supports the current findings in which half of the patients who searched about mental illness on the internet reported relief afterward.

Along with the COVID-19 pandemic, several factors, including the heightened perception of threat and difficulty in coping with uncertainty faced by society and individuals all over the world, have paved the way for the increase in the presence of cyberchondria (29). Various vulnerability factors have been identified in terms of cyberchondria, including personal characteristics such as younger age, female gender, or a history of mental disorder, engagement in some forms of online behavior, such as increased acceptance of online information, increased use of social media, and information overload. In contrast, information about the pandemic and adaptive emotion regulation might serve as buffering factors for anxiety during the pandemic (30). In a recent study, cyberchondria presented a positive correlation with various phenomenology of anxiety (i.e., metacognitive beliefs about anxiety, GA, HA, anxiety about COVID-19) and with depression, OCD symptoms, and problematic

usage of the internet while conversely, QoL showed a negative correlation with cyberchondria (31). The present study was carried out in the pre-pandemic period, and it is important to have these results because the pandemic likely increased cyberchondria.

The limitations of this study should be considered. Due to its cross-sectional design, a causal relationship cannot be mentioned. Most assessment tools consisted of self-report scales, which may cause recall bias. Treatment and treatment response of the clinical sample is uncertain, and even if HARS is used to assess GA levels, it may not provide sufficient information about disease severity and remission status in the OCD group. The sample size is relatively limited, and psychiatric comorbidities might have impacted the results.

Despite all these limitations, the present study might be important in that it examines cyberchondria in a psychiatric clinical sample both as a whole and among different diagnostic groups. The exclusion of cases with comorbid ADs and OCD in the intergroup diagnostic evaluation can be considered one of the strengths of the study for this purpose. Statistical analysis of CSS total scores, both with and without MMP, might be another strength, considering the inclusion or exclusion of MMP did not lead to a significant difference. This study might be the first to evaluate the relationship between QoL and cyberchondria in a clinical psychiatric sample, as it reports guiding findings for future research on this subject.

CONCLUSION

Considering the rapidly progressing digitalization and the significant increase in internet use, especially during and after the COVID-19 pandemic, more research is needed that can shed light on the development of appropriate interventions by examining the concept of cyberchondria and its relationship with ADs, OCD, and other mental disorders.

Conflicts of interest: Authors declared no conflict of interest.

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Çoklu beyin metastazlarının stereotaktik radyoterapisi için tek izomerkezli dinamik konformal ark tedavi (DCAT) ve volumetrik ayarlı ark tedavi (VMAT) planlama tekniklerinin dozimetrik karşılaştırılması

Dosimetric comparison of single isocenter dynamic conformal arc therapy (DCAT) and volumetric modulated arc therapy (VMAT) planning techniques for stereotactic radiotherapy of multiple brain metastases

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ÖZ

Amaç: Çoklu beyin metastazlarında stereotaktik radyoterapi (SRT) için dinamik konformal ark tedavi (DCAT) ile volumetrik (hacimsel) ayarlı ark tedavi (VMAT) planlama tekniklerinin karşılaştırılmasıdır.

Gereç ve Yöntem: 2-5 metastazı olan 10 hastanın toplam metastaz sayısı 33 idi. DCAT planlarında BrainLab Elements, VMAT'da Eclipse tedavi planlama sistemleri kullanıldı. Planlanan hedef hacimlere 3-5 fraksiyonda 25-30 Gy doz verildi. Risk altındaki organlar için (OAR) ortalama ve maksimum dozlara ek olarak bazı organlar için de 0,5 ve 0,2 cm³ lük hacimlerin aldığı dozlar (D_{0,5} ve D_{0,2}), normal beyin V_{xGy} hacimleri (V_{5Gy} V_{8Gy} V_{10Gy} V_{12Gy} V_{18Gy} ve V_{24Gy} cm³) ve monitor unit (MU) karşılaştırıldı.

Bulgular: Plan kalitesi için konformite indeks değerlerinde DCAT VMAT'ye göre ($p=0,005$) üstünlük sağlarken, gradiyent indeks değerlerinde aradaki fark anlamsızdı ($p=0,359$). OAR'de sadece beyin sapı D_{ort}. ($p=0,009$) ve D_{0,5} ($p=0,047$) için fark DCAT lehine anlamlı iken, diğerlerinde benzer sonuçlar elde edildi. Normal beyin V_{10Gy} V_{12Gy} ve V_{18Gy} açısından VMAT planları DCAT'a göre önemli avantaj sağladı (sırasıyla, $p=0,028$, $p=0,028$, $p=0,047$). MU için planlar benzerdi ($p=0,059$).

Sonuç: DCAT ve VMAT ile beşe kadar beyin metastazı olan hastalarda klinik olarak kabul edilebilir planlar elde edildi. DCAT planı plan kalitesi ve beyin sapı dozlarında, VMAT planı daha iyi normal beyin koruması ve daha düşük bir ışınlama süresi açısından önemli avantaj sağladı.

Anahtar Sözcükler: Beyin metastazları, Stereotaktik radyoterapi, Tek izomerkezli çoklu hedefli radyoterapi, Dinamik konformal ark tedavi, Hacimsel ayarlı ark tedavi.

ABSTRACT

Aim: To compare dynamic conformal arc therapy (DCAT) and volumetric modulated arc therapy (VMAT) planning techniques for stereotactic radiotherapy (SRT) in multiple brain metastases.

Materials and Methods: The total number of metastases in 10 patients with 2-5 metastases was 33. BrainLab Elements treatment planning system (TPS) for DCAT plans and Eclipse TPS was used for VMAT. A dose of 25-30 Gy in 3-5 fractions was administered to the planning target volumes. In addition to the mean and maximum doses for organs at risk (OAR), doses received by 0.5 and 0.2 cm³ volumes for some organs (D_{0.5} and D_{0.2}), V_{xGy} volumes of the normal brain (V_{5Gy} V_{8Gy} V_{10Gy} V_{12Gy} V_{18Gy} and V_{24Gy} cm³) and monitor unit (MU) were compared.

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Results: For plan quality, DCAT was significantly superior to VMAT in conformity index values ($p= 0.005$), while the difference in gradient index values was not significant ($p= 0.359$). In OAR, the difference was significant in favor of DCAT only for brainstem D_{med} ($p= 0.009$) and $D_{0.5}$ ($p= 0.047$), while similar results were obtained in the others. VMAT plans provided significant advantages over DCAT in terms of normal brain V_{10Gy} , V_{12Gy} and V_{18Gy} ($p= 0.028$, $p= 0.028$, $p= 0.047$, respectively). Plans for MU were similar ($p=0,059$).

Conclusion: Clinically acceptable plans were obtained with DCAT and VMAT for patients with up to five brain metastases. The DCAT plan is significantly superior in terms of plan quality and brainstem doses, while the VMAT plan provided significant advantages in terms of better normal brain protection and a shorter irradiation time.

Keywords: Brain metastases, Stereotactic radiotherapy, Single isocenter multi-target radiotherapy, Dynamic conformal arc therapy, Volumetric modulated arc therapy.

GİRİŞ

Beyin metastazları erişkin kanser hastalarının yaklaşık %20-40'ında görülmektedir. Geçmişte beyin metastazlı hastalarda tüm beyin radyoterapisi (TBRT) standart tedavi iken, radyoterapi tekniklerindeki ilerlemelerle özellikle lineer hızlandırıcı bazlı stereotaktik radyocerrahi (SRS) veya fraksiyone stereotaktik radyoterapi (SRT) uygulamaları kullanıma girerek lokal kontrolde ve risk altındaki organların korunmasında önemli avantajlar sağlamıştır (1-3). Stereotaktik uygulamalarda planlanan hedef hacim (PTV) küçük olduğundan ve hedef dışında radyasyon dozunda hızlı düşüş sağlanabildiğinden hedefe yüksek doz uygulanırken hedef dışındaki sağlıklı dokuların dozları en aza indirgenerek tedavi planında yüksek doz konformitesi sağlanabilir (4, 5).

SRS, sınırlı sayıda ve büyüklükte hedefler için yüksek dozların (genellikle 20 Gy) tek fraksiyonda, SRT ise daha büyük hedefler için birden fazla fraksiyonda (genellikle 3-5 fraksiyonda 25-30 Gy) uygulandığı tedavi teknikleridir. Beyin metastazlarında stereotaktik radyoterapi uygulamalarının lokal kontrolü arttırdığı, beyin metastazlarının sıklığını ve nörobilişsel hasarı azalttığı, sağ kalımı artırma eğilimi gösterdiği bildirilmiştir (6, 7). Geçmişte SRS/SRT kullanımı düşük sayıda (<4) beyin metastazları ile sınırlıyken son çalışmalarda daha fazla sayıda beyin metastazı olan hastalar için de kullanılabileceği bildirilmiştir (4, 5, 8). Yamamoto ve ark. tarafından düşük hacimli beyin metastazları için SRS/SRT ile 5-10 beyin metastazında 2-4 beyin metastazına benzer sağ kalımın sağlandığı ve nörobilişsel fonksiyonun korunduğu rapor edilmiştir (9). Diğer çalışmasında ise, SRS/SRT'nin dikkatlice seçilmiş 10'dan fazla sayıda beyin metastazı olan

hastalarda da uygulanabileceği gözlemlenmiştir (10). Çalışmalarda daha büyük lezyonların tedavisinde, normal doku toksisite riskini en aza indirmek ve lokal kontrolü iyileştirmek için hipofraksiyone SRT yaklaşımı önerilmiştir (11-13). Büyük beyin metastazları için SRS ile karşılaştırıldığında radyonekroz riskinin daha düşük olduğu rapor edilmiştir (14-16).

Stereotaktik uygulamalarda reçete edilen hedef dozun planlanması ve uygulanması son derece hassas ve doğru olmalı, uygun planlama tekniği tercih edilmelidir. Lineer hızlandırıcı tabanlı tedavi modalitelerinde reçete edilen dozlar, hedefe genellikle dinamik konformal ark tedavi (DCAT) veya volumetrik (hacimsel) ayarlı ark tedavi (VMAT) teknikleri kullanılarak uygulanır. DCAT, çok yapraklı kolimatörlerin (MLC) gantrinin dönüşü esnasında hedefin yerleşimi ve şekline dinamik olarak uyduğu tedavi yaklaşımıdır. DCAT tekniğinde, Brainlab AG sistemleri tarafından geliştirilen Elements Multiple Brain Mets (Elements MBM SRS, BrainLab, Münih, Almanya) tedavi planlama sistemi (TPS), tedavi planını optimize etmek için belirli otomasyon biçimleri sunar (17-21). Geleneksel VMAT tekniğinde uygun doz dağılımları elde edebilmek için, gantri hasta etrafında tekli ya da çoklu ark açılarıyla sürekli hareket halinde iken, doz hızı, ışının şekli ve dönüş hızı gibi parametreler değişir. Önceleri birden fazla beyin metastazı olan hastalar, her lezyonun geometrik merkezine yerleştirilen bir izomerkez ile ayrı ayrı tedavi edilirken yakın zamanda tek bir izomerkez kullanılarak birden fazla lezyonun eşzamanlı tedavi yaklaşımı giderek yaygınlaşmıştır. Tek izomerkez kullanıldığında tedavi planlama ve tedavi süresinin azalmasının yanı sıra, tedavi cihazının verimi de artmaktadır. Önceki çalışmalarda, genellikle birden fazla eş düzlemli olmayan arkaların uygulandığı tekniğin etkili ve

güvenli bir yaklaşım olduğu belirtilmiştir (4, 5, 8, 22, 23). Çalışmalarda, çoklu beyin metastazlarında tekli ve çoklu DCAT planlarının plan kalitesi açısından benzerlik gösterdiği ve tek izomerkezli planın uygulama açısından daha avantajlı olduğu bildirilmiştir (8, 21).

Çalışmamızda, çoklu beyin metastazlarının tek izomerkezli SRT uygulamalarında otomasyon işlemleri içeren DCAT ile geleneksel manuel VMAT tedavi planlama tekniklerinin dozimetrik parametreler açısından karşılaştırılarak her iki tekniğinin avantaj ve dezavantajlarının belirlenmesi hedeflenmiştir.

GEREÇ ve YÖNTEM

Hasta Seçimi, Konturlama ve Doz

Etik kurul onayı alındıktan sonra (No: 24-7T/72) Mart 2022-Ocak 2024 tarihleri arasında kliniğimizde DCAT tekniği kullanılarak SRT uygulanan, farklı primer kanserlerden (8 akciğer ve 2 kolon) kaynaklanan çoklu beyin metastazı olan 10 hasta değerlendirilmiştir. Hastalardan tıbbi verilerinin yayınlanabileceğine ilişkin yazılı onam belgesi alınmıştır. Hastaların medyan yaşı 61 (44-82)'dir. Beyin metastazı sayısı medyan 3 (2-5), metastaz boyutu medyan 1,46 cm (0,83-3,76) idi. Tüm hastaların toplam metastaz sayısı 33'tür. Lezyonlar genellikle temporal, frontal, serebellar, parietal, oksipital yerleşimlidir. Tedavide Varian marka TrueBeam STx model lineer hızlandırıcı cihazı kullanılmıştır (Varian Medical Systems, Palo Alto, Kaliforniya, ABD).

Hastalar termoplastik maske sistemiyle tedavi pozisyonunda sabitlenerek yüksek çözünürlüklü bilgisayarlı tomografi (BT) (General Electric Discover RT marka, ABD) cihazıyla, 1,25 mm kesit aralığıyla tüm beyin görüntüleri elde edildi. Görüntüler Elements Multiple Brain Mets SRS (MBM SRS) (versiyon:3,00, Brainlab AG, Münih, Almanya) TPS'ye gönderildi. Füzyon ve konturlama için Brainlab Elements ImageFusion 4,0 programı kullanıldı. Hastaların planlama BT görüntüleri ile tedavi öncesi çekilen ince kesitli üç boyutlu kontrastlı manyetik rezonans görüntüleri (MRG) önce rijit füzyon yapıldı ardından distorsiyon düzeltilmesi yapılarak konturlamaya geçildi. Kontrastlı T1 ağırlıklı görüntüler kullanılarak beyin metastazları GTV (Gross Tümör Hacmi) olarak, beyin sapı, kiazma, koklea, göz, lens, optik sinirler ve hipokampus risk altındaki organlar (OAR) olarak aynı sekansta konturlandı. GTV'ye 1-2 mm güvenlik marjı eklenerek planlama hedef hacmi (PTV)

oluşturuldu. GTV ve PTV'nin medyan kümülatif hacimleri sırasıyla 0,20 (0,01-7,62) cm³ ve 1,06 (0,23-13,41) cm³ idi. Tüm beyin dokusundan toplam PTV'nin çıkarılmasıyla normal beyin dokusu tanımlandı. Metastazların sayısına, boyutuna ve konumuna göre PTV'lere 3-5 fraksiyonda 25-30 Gy doz verildi. Tedavi planları 6 MV FFF (flattening filter-free) foton ışınları kullanılarak, her PTV'nin maksimum dozu (%2) reçete edilen dozun %140'ından az tutuldu ve dozun en az %95'ini alması sağlanacak şekilde optimize edildi. Komplikasyon açısından önemli olan maksimum doz (D_{maks.}) için 3 ve 5 fraksiyondaki sınırlamalarda sırasıyla; beyin sapında D_{maks.} <23,1 Gy ve <23 Gy, kiazma ve optik sinirlerde D_{maks.} < 17,4 Gy, ve <22,5 Gy, koklealarda D_{maks.} <14,4 Gy ve <25 Gy), gözlerde D_{maks.} < 25) ve lenslerde D_{maks.} < 6 Gy dikkate alındı. Ortalama doz (D_{ort.}) ve ilgili OAR'nin 0,5 cm³ ve 0,2 cm³ hacimlerin aldığı dozların (D_{0,5} ve D_{0,2}) kısıtlamalarına da uyuldu (24- 27). DCAT ve VMAT planları en iyi hedef kapsamının yanı sıra, sağlıklı beyin ve OAR'lara verilen dozları en aza indirmek üzere optimize edildi.

DCAT Planlama

Brainlab Elements Multiple Brain Mets TPS ile yeniden DCAT SRT planları oluşturuldu. Planlar, tek izomerkezli eş düzlemlı olmayan birden fazla dinamik arkta (ışın sayısı) oluştu. Tedavi izomerkez konumu, tüm hedef hacimlerin kütle merkezine otomatik olarak yerleştirildi. İlk olarak, beyin metastazlarının sayısına ve konumlarına bağlı olarak ark sayısı önceden tanımlanmış şablonlardan otomatik olarak seçildi. Daha sonra her PTV'de optimal doz dağılımları elde edebilmek, hedef dışı OAR ve sağlıklı dokuları mümkün olduğunca koruyabilmek ve doz çakışma riskini en aza indirebilmek için, ark açısı ve uzunluğu, ışın ağırlığı, kolimatör dönüşü gibi ışın parametreleri optimizasyon sırasında otomatik olarak değiştirildi. Gerektiğinde OAR'lere ek doz kısıtlamaları eklendi. Planlarda farklı masa açılarında eş düzlemlı olmayan yarım ve yarımdan az arklar (5-10 ark, ark uzunlukları: 0-180°, 10-180°, 20-180°, 180-340° ve 180-350°) ve en düşük 4° olan farklı kolimatör açıları kullanıldı. Bir arkta gantri dönüşünün başlama ve bitiş açısı saat yönünde iken, diğer arkta bitiş yönünden saat yönünün tersine başladığı yerde sona ermektedir. Doz dağılımları Monte Carlo algoritması kullanılarak 1 mm grid boyutu üzerinden hesaplandı.

VMAT planlama

VMAT SRT planları Eclipse TPS'de (versiyon 16,1,0, Varian Medical Systems, Palo Alto, Kaliforniya, ABD) oluşturuldu. Planlarda DCAT'taki gibi PTV sayısına göre genellikle 5-10 eş düzlemlili olmayan yarım arklar kullanıldı. Tek izomerkez PTV hacimlerinin geometrik ortalamasına manuel olarak yerleştirildi. Planlarda PTV'den sonra mümkün olan en dik doz düşüşünü elde etmek için, PTV etrafında genellikle 3, 6 ve 9 mm marjla içi boş halka şeklinde yardımcı yapılar oluşturuldu. Dozimetrik parametrelerin karşılaştırılmasında daha doğru sonuçlar elde edebilmek için, uygulanan ışın konfigürasyonu (gantri, kolimatör ve masa açıları) DCAT planlarıyla aynı seçildi. Doz dağılımları Acuros XB (AXB) algoritma kullanılarak 1 mm grid aralığında hesaplandı.

Planların Değerlendirmesi

Her iki planlama tekniğinin sonucunda reçete edilen dozun %95'ini alan hacmin yüzdesi (%V₉₅), OAR ve tüm beyin için D_{ort.}, D_{maks.}; beyin sapı için D_{0,5}; kiazma, göz ve lensler için D_{0,2}; normal beyin için 5,8,10,12,18 ve 24 Gy doz alan hacimler (V_{5Gy}, V_{8Gy}, V_{10Gy}, V_{12Gy}, V_{18Gy} ve V_{24Gy}) gibi dozimetrik parametreler doz hacim histogramları kullanılarak analiz edildi. Her iki plan, tedavi planının kalitesini belirleyen konformite indeksi (CI) ile gradyent indeksi (GI) ve ışınlama süresini belirleyen monitor unit (MU) gibi diğer dozimetrik parametreler açısından da değerlendirildi.

CI, doz dağılımı ile tedavi edilen hedef hacmin şekli arasındaki yeterliliği temsil eder. İdeal değer 1'dir. CI için, DCAT'ta ters Paddick, VMAT'ta ise Radyasyon Tedavisi Onkoloji Grubu (RTOG) tarafından önerilen hesaplama metodları kullanıldı. CI_{RTOG} ile kabul edilebilir hedef konformitesi elde edilebilir, fakat hedef hacmin dışına çıkan dozu hesaba katmaz (28). Bu sorun modifiye edilmiş ters nCI_{Paddick} formülasyonu ile giderilmiştir (29, 30).

$$CI_{RTOG} = PIV / TV$$

(PIV: Tanımlanan izodoz hacmi; TV: Hedef hacim)

$$nCI_{Paddick} = TV \times PIV / (TV_{PIV})^2$$

TV: Hedef hacim; PIV: Tanımlanan izodoz hacmi; TV_{PIV}: Tanımlanan izodoz hacminin kapsadığı hedef hacim

GI, tanımlanan dozun tümör sonrası azalmanın tayininde kullanılır. GI, her iki planlama tekniğinde GI_{Paddick} formülü ile hesaplandı (31).

$$GI_{Paddick} = PIV_{\%50} / PIV_{\%100}$$

PIV_{%50}: Tanımlanan izodozun %50'sinin kapladığı hacim, PIV: Tanımlanan izodozun %100'ünün kapladığı hacim (tanımlanan izodoz hacmi). Tek lezyonlu hedefler için GI_{Paddick} <3 iken, çoklu lezyonlar ve birbirine yakın hedeflerde GI değeri artar.

İki tekniğin karşılaştırılmasında IBM SPSS istatistiksel 25 programı kullanılarak Wilcoxon Signed-Rank testi uygulandı ve p<0,05 anlamlı kabul edildi.

BULGULAR

On hastanın DCAT ve VMAT planları için, plan kalitesini değerlendirme parametreleri olan %V₉₅, CI ve GI ile ışınlama süresini ifade eden MU analizleri (Tablo-1)'de verilmiştir. Şekil-1'de her iki planın aksiyel, koronal ve sagittal (soldan sağa) kesitlerdeki doz dağılımları gösterilmiştir. Medyan V₉₅ değerleri DCAT planları için %99,60 (%97,90-100,0) iken, VMAT planları için %99,50 (%96,60-100,0) olup sonuçlar benzerdi. Ortalama CI değerleri DCAT'ta 1,22±0,09, VMAT'ta ise 0,96±0,02 olarak belirlenmiş olup aradaki fark anlamlıydı (p= 0,005). Farkın anlamlılığı DCAT planlama sisteminde CI_{Paddick}, VMAT planlama sisteminde ise CI_{RTOG} hesaplama yöntemlerinin kullanılmasından kaynaklanabilir. Her iki CI değerlerinin PTV'yi kapsadığı ve kabul edilebilir olduğu gözlemlendi. Ortalama GI_{Paddick} değerleri DCAT planında (3,61±0,61) VMAT'a (3,75±0,90) göre daha düşük olmasına rağmen aradaki fark anlamlı değildi (p=0,359). Ortalama GI değerleri DCAT ve VMAT için sırasıyla 2,77-4,40 ve 2,50-4,96 arasındaydı. Işınlama süresi DCAT planlarında VMAT planlarına kıyasla anlamlı derecede daha yüksek bulundu (p= 0,005). DCAT planında ortalama MU değeri 11880±3820 iken, VMAT'ta 9639±1864 idi.

Radyonekroz açısından değerlendirdiğimiz normal beyin hacimleri V_{5Gy}, V_{8Gy}, V_{10Gy}, V_{12Gy}, V_{18Gy}, V_{24Gy} için DCAT ve VMAT planlarında ortalama değerler sırasıyla 149,89±109,41, 58,72±40,70, 37,20±25,70, 10±18,21, 11,20±8,21, 4,67±4,04 ve 136,42±99,37, 49,63±33,49, 30,31±19,19, 21,16±13,20, 8,89±5,48, 3,48±2,36 cm³ idi (Tablo-2). Özellikle radyonekroz riski açısından önemli risk faktörleri olan V₁₀ ve V₁₂ yanı sıra V₁₈ açısından planlar

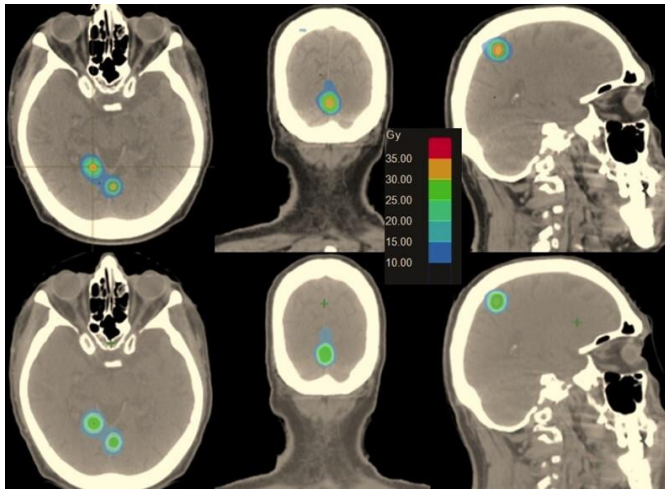
arasındaki fark anlamlıyken (sırasıyla $p=0,028$, $0,028$ ve $0,047$), V_{5Gy} , V_{8Gy} ve V_{24Gy} değerlerinde fark anlamsızdı ve VMAT planı DCAT planına kıyasla sağlıklı beyin koruması açısından daha üstündü. Tüm sağlıklı beyin aldığı maksimum dozlar VMAT planlarında önemli ölçüde düşüktü ve fark anlamlıydı (ortalama VMAT: $34,23\pm2,58$, DCAT: $40,11\pm2,79$, $p=0,005$). Ortalama dozlarda ($D_{ort.}$) önemli bir fark belirlenmedi (VMAT: $2,45\pm1,08$, DCAT: $2,52\pm1,16$) ve $p=0,445$).

Risk altındaki organlar olan beyin sapı, kiazma, koklea, göz, lens, optik sinir ve hipokampus değerleri açısından her iki planlama arasındaki anlamlı fark sadece DCAT lehine beyin sapı $D_{ort.}$ ve $D_{0,5}$ için gözlemlendi (sırasıyla $p=0,009$ ve $p=0,047$). DCAT planında $D_{ort.}$ ve $D_{0,5}$ değerleri sırasıyla $1,50\pm0,98$ Gy ve $4,33\pm2,60$ Gy iken, VMAT planında $2,47\pm1,51$ Gy ve $5,38\pm3,82$ Gy idi. Diğer kritik organlar için planlama teknikleri arasında sonuçlar benzerdi (Tablo-3).

Tablo-1. DCAT ve VMAT tekniklerinde plan kalitesi (% V_{95} , CI ve GI) ve MU parametrelerinin karşılaştırılması.

Parametreler	DCAT	VMAT	p
% V_{95} [medyan (min.-maks.)]	99,60 (97,90-100)	99,50 (96,60-100)	---
CI (ortalama $\pm$ SS)	1,22 $\pm$ 0,09	0,96 $\pm$ 0,02	0,005
GI (ortalama $\pm$ SS)	3,61 $\pm$ 0,61	3,75 $\pm$ 0,90	0,36
MU (ortalama $\pm$ SS)	11880 $\pm$ 3820	9639 $\pm$ 1864	0,059

DCAT: Dinamik konformal ark tedavi, VMAT: Hacimsel ayarlı ark tedavi, V_{95} dozun yüzde 95'ni alan hacim, CI: Konformite indeks, GI: Gradyent indeks, SS: Standart sapma, MU: Monitor unit.



Şekil-1. Dört metastazlı bir hasta için doz dağılımları. Reçete edilen izodoz 5 fraksiyonda 25 Gy idi. Üstteki üç panel DCAT planı için aksiyal, koronal ve sagittal (soldan sağa) kesitleri gösterirken, alttaki paneller VMAT planı için ilgili kesitleri göstermektedir.

Tablo-2. DCAT ve VMAT planlama teknikleri arasında sağlıklı beyin V_{5Gy} , V_{8Gy} , V_{10Gy} , V_{12Gy} , V_{18Gy} ve V_{24Gy} değerlerinin karşılaştırılması.

Parametreler	DCAT	VMAT	p
V_{5Gy} (cm ³)	149,89 $\pm$ 109,41	136,42 $\pm$ 99,37	0,114
V_{8Gy} (cm ³)	58,72 $\pm$ 40,70	49,63 $\pm$ 33,49	0,059
V_{10Gy} (cm ³)	37,20 $\pm$ 25,72	30,31 $\pm$ 19,19	0,028
V_{12Gy} (cm ³)	26,10 $\pm$ 18,22	21,16 $\pm$ 13,20	0,028
V_{18Gy} (cm ³)	11,20 $\pm$ 8,21	8,89 $\pm$ 5,48	0,047
V_{24Gy} (cm ³)	4,67 $\pm$ 4,04	3,48 $\pm$ 2,36	0,114

DCAT: Dinamik konformal ark tedavi, VMAT: Hacimsel ayarlı ark tedavi, V_{5Gy} , V_{8Gy} , V_{10Gy} , V_{12Gy} , V_{18Gy} ve V_{24Gy} : 5,8,10,12,18 ve 24 Gy doz alan hacimler.

Tablo-3. DCAT ve VMAT planlama teknikleri arasında risk altındaki organların dozimetrik parametrelerinin karşılaştırılması.

Parametreler	DCAT (Gy)	VMAT (Gy)	p
Beyin Sapı D _{maks.}	9,38±9,27	10,18±10,15	0,059
Beyin sapı D _{ort.}	1,50±0,98	2,47±1,51	0,009
Beyin sapı D _{0,5}	4,33±2,60	5,38±3,82	0,047
Kiazma D _{maks.}	1,97±0,97	2,80±1,46	0,203
Kiazma D _{ort.}	1,23±0,73	1,70±1,00	0,114
Kiazma D _{0,2}	1,40±0,81	1,17±1,18	0,508
Sol koklea D _{maks.}	2,35±3,85	2,09±2,96	0,444
Sağ koklea D _{maks.}	1,87±1,69	1,67±1,42	0,386
Sol koklea D _{ort.}	1,76±2,96	1,61±2,10	0,959
Sağ koklea D _{ort.}	1,44±1,32	1,32±1,17	0,683
Sol göz D _{maks.}	3,71±7,17	3,03±5,11	0,799
Sağ göz D _{maks.}	1,14±0,75	1,32±0,73	0,386
Sol göz D _{ort.}	0,83±0,87	0,96±0,88	0,114
Sağ göz D _{ort.}	0,43±0,28	0,60±0,37	0,074
Sol göz D _{0,2}	2,15±3,14	2,38±2,73	0,721
Sağ göz D _{0,2}	0,91±0,63	1,03±0,70	0,415
Sol lens D _{maks.}	0,97±1,11	1,05±0,85	0,201
Sağ lens D _{maks.}	0,52±0,34	0,64±0,42	0,169
Sol lens D _{ort.}	0,64±0,72	0,76±0,64	0,374
Sağ lens D _{ort.}	0,35±0,27	0,45±0,32	0,415
Sol optik sinir D _{maks.}	1,92±2,08	1,96±1,44	0,878
Sağ optik sinir D _{maks.}	1,23±0,85	1,61±0,86	0,203
Sol optik sinir D _{ort.}	0,97±0,78	1,18±0,91	0,074
Sağ optik sinir D _{ort.}	0,81±0,59	1,06±0,63	0,241
Sol optik sinir D _{0,2}	0,93±0,71	0,98±0,97	0,541
Sağ optik sinir D _{0,2}	0,79±0,60	0,78±0,72	0,799
Sol hipokampus D _{maks.}	4,40±4,08	4,51±2,59	0,575
Sağ hipokampus D _{maks.}	9,17±12,23	8,44±10,77	0,386
Sol hipokampus D _{ort.}	2,16±1,77	2,58±1,59	0,114
Sağ hipokampus D _{ort.}	3,63±4,82	3,52±4,27	0,953

DCAT: Dinamik konformal ark tedavi, VMAT: Hacimsel ayarlı ark tedavi,

D_{maks}: Maksimum doz, D_{ort}: Ortalama doz, D_{0,5}: 0,5 cm³ hacmin aldığı doz, D_{0,2}: 0,2 cm³ hacmin aldığı doz.

TARTIŞMA

Çalışmamızda birden fazla beyin metastazı olan hastaların tek izomerkezli SRT tedavi planlamalarında, otomasyon içeren DCAT ile manuel geleneksel VMAT'ın dozimetrik etkileri karşılaştırılmıştır. Çoklu beyin metastazlarında tek izomerkezli tekniğin kullanımı ilk kez DCAT'da Huang ve ark. (21) VMAT'da ise Clark ve ark. (3) tarafından geliştirilmiştir. Yapılan bir çalışmada, çoklu beyin metastazlarında tekli ve çoklu DCAT planlarının plan kalitesi açısından benzerlik gösterdiği ve tek izomerkezli planın uygulama açısından daha avantajlı olduğu bildirilmiştir (8, 21). Ancak, izomerkezden uzaktaki hedeflerin yerini değiştiren rotasyonel hataları en aza indirmek için, daha sıkı konumlandırma doğruluğu gerekmektedir (32-

34). Huang ve ark. tarafından tek izomerkezli DCAT'ın çok izomerkezli DCAT'a göre avantajlı olduğu ve çoklu metastazların tedavisinde tek izomerkezli DCAT, VMAT veya yoğunluk ayarlı radyoterapi (IMRT) arasındaki seçimde, doz uyumu, tedavi süresi ve periferik dozun yayılması gibi faktörlerin göz önüne alınması gerektiği belirtilmiştir (21).

Plan kalitesini değerlendirmek için kullanılan CI ve GI parametrelerinde, DCAT planlarında VMAT'a göre verilen dozun hedefi daha iyi kapsayarak daha avantajlı olduğu gözlemlendi. Tanımlanan dozun tümör sonrası azalmasının tayininde kullanılan GI değerlerinde her iki planlama tekniği benzerdi. Tümör hacmi küçük ve küreselliğe yakın lezyonlarda GI değerleri DCAT'ta daha düşüktü. Lezyonların sayısı,

lezyonlar arasındaki mesafe ve mutlak hacimleri GI değerlerini etkilemektedir. Plan kalite indekslerinden biri olan GI'daki herhangi bir azalma doğrudan radyonekroz riskiyle ilişkili olan V_{12} 'de bir azalmaya neden olur (1). Sonuçlarımız DCAT ve VMAT planlama tekniklerini karşılaştıran diğer çalışmalarda bildirilenlerle uyumludur. Hofmaier ve ark. 66 beyin lezyonu olan 20 hasta için 15-20 Gy'lik tek fraksiyon SRS çalışmasında DCAT için VMAT planlarına göre daha iyi CI (medyan DCAT: 0,75, VMAT: 0,73) ve daha düşük GI değerlerine (medyan, DCAT:5,99, VMAT:7,17) sahip olduğunu bildirmişlerdir (20). Narayanasamy ve ark. 3-7 lezyonu olan 8 hastanın DCAT SRS planları için BrainLab Multiple Metastases Elements (MME), VMAT SRS planları için Varian RapidArc uygulamasına sahip Eclipse tedavi planlama sistemlerinde CI ve GI değerlerinin benzer olduğunu gözlemlemişlerdir (35). Gevaert ve ark. 1-8 arasında değişen toplam 40 lezyonu olan 10 hasta için, DCAT ve VMAT planlarında CI'nın benzer, GI'nın ise VMAT'ta önemli ölçüde yüksek olduğunu bildirmişlerdir (36). Son zamanlarda Eclipse (Varian Inc.) TPS'de geliştirilmiş bir tür otomasyon içeren HyperArc VMAT ile Elements MBM ve geleneksel VMAT'ı karşılaştıran çalışmalar mevcuttur (1, 5, 18, 37, 38). Bu çalışmalarda HyperArc VMAT planlarının Elements MBM DCAT ve geleneksel VMAT'a göre önemli ölçüde daha iyi olduğu bildirilmiştir. Bir çalışmada ise HyperArc ve manuel VMAT'ın benzer dozimetrik kalite gösterdiği bildirilmiştir (39). HyperArc'da kolimatör açılarının seçiminden tedavi sunumuna kadar tedavi süreci, hedeflerin konumuna ve sayısına göre otomatik olarak tasarlanır. Bu özellikler plan kalitesine katkıda bulunabilir ve hedefin dışındaki yüksek doz düşüşlerini arttırabilir (40, 41). Çalışmamızda VMAT planlamasında kullandığımız Eclipse TPS sistemi HyperArc gibi özel sistemlerde mevcut olan çeşitli özelliklerden yoksundu. Işınlama süresi (MU) DCAT planlarında VMAT'a kıyasla önemli oranda yüksek bulunmasına rağmen fark anlamsızdı. MU'nun az olması periferik dozun azalması, cihazın yıpranmaması ve tedavi süresinin azalması gibi bazı avantajlar sağlar. Çalışmalarda MU açısından da çelişkili sonuçlar rapor edilmiştir. VMAT planlarındaki yüksek modülasyon nedeniyle genel MU sayısı DCAT için önemli ölçüde düşüktür (20). Noroyanasamy ve ark. BrainLab Multiple Metastases Elements ile RapidArc'ı karşılaştırdıkları çalışmalarında, DCAT ile MU sayısı açısından VMAT'a göre

önemli azalma olduğu belirtilmiştir (35). Ruggeri ve ark tarafından ise HyperArc VMAT ve Elements MBM DCAT planları arasında MU açısından anlamlı bir farkın olmadığı gözlemlenmiştir (1).

Radyonekroz gelişimiyle ilişkili faktörler, tedavi edilen hacim ve belirli bir doz alan beyin hacmidir (42). Çalışmamızda beyin V_{5Gy} , V_{8Gy} , V_{10Gy} , V_{12Gy} , V_{18Gy} ve V_{24Gy} açısından incelediğimizde, iki plan arasında anlamlı fark V_{10Gy} , V_{12Gy} ve V_{18Gy} ve tüm sağlıklı beyin aldığı D_{maks} değerlerinde gözlemlendi. VMAT planlarında dozlar önemli ölçüde daha düşüktü ve beyin koruması açısından avantaj sağlandı. Beyin koruması lezyonların sayısının ve hacimlerinin artmasıyla azalmaktadır. D_{ort} değerlerinde ise önemli bir fark belirlenmedi. Bazı çalışmalarda çalışmamıza benzer şekilde VMAT planlarının sağlıklı beyin korunması ve tedavi verimliliği açısından DCAT'tan daha üstün olduğu bildirilmiştir. Ruggiere ve ark. HyperArc VMAT planlarının daha düşük V_{12Gy} değeri sağladığını (DCAT: medyan 37,3 cm³, VMAT 23,7 cm³) bildirmişlerdir (1). Liu ve ark. 4 ile 10 beyin metastazlı 30 hastada VMAT tekniğinin DCAT'a göre daha iyi V_{12-8Gy} hacimleri sağladığını rapor etmişlerdir. V_{12Gy} için, PTV >2,1 cm³ olduğunda VMAT, PTV <2,1 cm³ olduğunda ise DCAT önerilmiştir (43). Bazı çalışmalarda ise DCAT planlarının VMAT'a göre daha uygun olduğu rapor edilmiştir. Raza ve ark. tarafından V_{8-12Gy} değerlerinin, 10-15 lezyonu olan hastalarda VMAT'a kıyasla DCAT'da anlamlı olarak daha düşük olduğu belirtilmiş; V_{12Gy} için en büyük fark DCAT ve VMAT planları arasında 10 veya daha fazla beyin metastazı olan ve toplam tümör hacmi <2 cm³ olan hastalarda görülmüş; düşük kümülatif tümör hacmi ve küreselliğe yakın lezyonlarda DCAT'ın, lezyonu küreden farklı, düzensiz şekilli hedef hacimlerde ise VMAT'ın üstün olabileceği bildirilmiştir (5). Hofmaier ve ark. V_{10Gy} ve V_{12Gy} değerlerinde DCAT planlarının önemli ölçüde daha avantajlı olduğunu göstermiş; V_{10Gy} ve V_{12Gy} deki küresellik ve farklılıklar arasında orta derecede bir korelasyon ile ilişkilendirmiştir. Düşük tümör hacmi olan hastalarda DCAT'ın daha iyi performans gösterdiği bildirilmiştir (20). Sonuçların farklılığı lezyonların sayısı ve boyutundaki farklılıklardan kaynaklanabilir.

Beyin sapı, kiazma, koklea, göz, lens, optik sinir ve hipokampus gibi OAR'ın doz değerleri her iki planlama tekniğinde literatürlerde (24-27)

belirtilen normal doku tolerans dozlarının altında kalarak korunmuştur. DCAT planlarında beyin sapı $D_{ort.}$ ve $D_{0.5}$ değerleri VMAT'a kıyasla daha düşük çıkarak önemli ölçüde üstünlük sağlamıştır. Tüm OAR'ların dozları doz sınırlamalarının altında kalmıştır. Sprowls ve ark. HyperArc'ın VMAT ile karşılaştırdığında hipokampus için avantajlı olduğunu göstermiştir (44). Raza ve ark. tarafından, ortalama hipokampus dozunun DCAT planlarında anlamlı derecede düşük olduğu, çoğu planlarda <3 Gy ortalama doz olduğu gözlemlenmiştir (5). Çalışmamızda hipokampus açısından her iki planlama arasında önemli fark yoktur ve dozlar kabul edilebilir doz sınırlamalarının altında kalmıştır.

SONUÇ

Çalışmamızda plan kalitesi göstergesi olan CI ve GI yanı sıra, beyin sapı $D_{ort.}$ ve $D_{0.5}$ için DCAT planlama tekniği önemli ölçüde daha iyi performans göstermiştir. Buna karşın normal beyin hacimleri V_{10Gy} , V_{12Gy} , V_{18Gy} değerleri ve ışınlama süresi (MU) açısından VMAT planı anlamlı derecede daha avantajlıdır. Bu radyasyon nekrozu riski olasılığının azalmasıyla sonuçlanabilir. Genellikle lezyonlar küreselliğe yakınsa DCAT planları üstünlük sağlayabilir. VMAT çoklu beyin metastazlı hastalar için özel bir radyocerrahi sistemine makul bir alternatif olabilir.

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The relationship of pregnancy losses with frequently-used hematologic parameters

Gebelik kayıplarının sık kullanılan hematolojik parametrelerle ilişkisi

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ABSTRACT

Aim: Recurrent pregnancy loss (RPL) is a common and undesirable condition in hematology and obstetrics practice. Pathogenesis for most cases remains undetermined. Thrombophilia may be a potential factor for these patients. Here, we aimed to investigate hematological parameters that have the potential to contribute to or predict RPL and to present our data as a single-center experience.

Materials and Methods: One hundred seventy female patients with at least one pregnancy loss between the years 2012 and 2024 were included retrospectively. The mean age of the patients was 34.48 ± 6.02 . Patients with antiphospholipid antibodies and miscarriage due to an identifiable etiology (maternal anatomic and endocrine abnormalities, infectious causes, trauma, chromosomal abnormalities) were excluded from the study. Patients were divided into two groups, defined as patients with one or two miscarriages and more than two miscarriages, respectively. Thrombophilia screening tests (mutations and protein C/S/antithrombin levels), blood count and coagulation parameters were recorded and investigated whether there was a significant correlation in terms of miscarriage in the two groups.

Results: Nine patients (5.3%), 71 patients (41.8%), 60 patients (35.3%), 21 patients (12.4%), four patients (2.4%), and five patients (2.9%) had a history of one, two, three, four, five, and six miscarriages, respectively. No significant difference was found between the genetic mutations investigated in terms of thrombophilia, protein C, S, and antithrombin levels, and the number of pregnancy losses in both groups. There was no significant difference between hemogram parameters and coagulation parameters except aPTT for both groups. The aPTT value of group B was found to be significantly lower than that of group A ($p=0.04$).

Conclusion: Conflicting results in hematologic parameters in case of RPL are common and it is not easy to obtain homogeneous results. However, a low aPTT value, a parameter that can be easily and quickly measured, may be a parameter that can help predict RPL.

Keywords: coagulation; miscarriage; pregnancy; thrombophilia; thrombosis

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ÖZ

Amaç: Tekrarlayan gebelik kaybı (TGK), hematoloji ve obstetrik alanında sık karşılaşılan ve endişe verici bir konudur; patogenezi belirsizdir. Bu çalışma, TGK ile potansiyel olarak ilişkili hematolojik faktörleri, özellikle trombofiliyi araştırmayı amaçlamaktadır.

Gereç ve Yöntem: 2012-2024 yılları arasında en az bir gebelik kaybı yaşamış 170 kadın hastayı retrospektif olarak analiz ettik; anrifosfolipit antikorları veya tanımlanabilir nedenleri olanlar dışlandı. Hastalar, bir veya iki düşük yapmış olanlar (grup A) ve ikiden fazla düşük yapmış olanlar (grup B) şeklinde iki gruba ayrıldı. Trombofili tarama testleri, hemogram ve koagülasyon parametrelerini değerlendirdik; analiz için SPSS 25.0 sürümü kullanıldı ve $p < 0,05$ değeri anlamlı kabul edildi.

Bulgular: Dokuz hastanın (%5,3) bir, 71 hastanın (%41,8) iki, 60 hastanın (%35,3) üç, 21 hastanın (%12,4) dört, dört hastanın (%2,4) beş ve beş hastanın (%2,4) altı düşük öyküsü mevcuttu. Trombofili, protein C, S ve antitrombin seviyeleri açısından araştırılan genetik mutasyonlar ile her iki gruptaki gebelik kaybı sayısı arasında anlamlı bir fark bulunamadı. Hemogram parametreleri ve koagülasyon parametreleri arasında, her iki grup için aPTT dışında anlamlı bir fark yoktu. Grup B'nin aPTT değeri, grup A'ninkinden anlamlı derecede düşük bulundu ($p = 0,04$).

Sonuç: TGK durumunda hematolojik parametrelerde çelişkili sonuçlar yaygındır ve homojen sonuçlar elde etmek kolay değildir. Kolay ve hızlı bir şekilde ölçülebilen düşük bir aPTT değeri, TGK'nin öngörülmesine yardımcı olabilecek bir parametre olabilir.

Anahtar Sözcükler: koagülasyon, düşük, gebelik, trombofili, tromboz

INTRODUCTION

Pregnancy loss is an undesired event occasionally resulting from various causes including thrombophilia. Pathogenesis for most cases remains undetermined (1). Causes related to thrombophilia may underlie these undetermined cases. Recurrent pregnancy loss (RPL) definition varies. While the Practice Committee of the American Society for Reproductive Medicine proposes two or more miscarriages, the European Society of Human Reproduction and Embryology proposes three or more miscarriages as the RPL definition (2-3). These patients are commonly evaluated by hematologists, by direct application to the outpatient clinic or consultation from the obstetrics. Besides factors responsible for thrombophilia such as inherited mutations (especially Factor V Leiden-FVLM and Prothrombin G20210A mutations-PGM), protein C/S/anti-thrombin deficiency and antiphospholipid antibodies, it is a matter of curiosity whether other hematological parameters such as blood count or coagulation parameters commonly used in hematology practice may be associated with the pathogenesis of RPL, at least related to thrombophilia, or have potential in predicting RPL.

Prothrombin time (PT) tests the extrinsic and common pathways of the coagulation cascade and represents the time, in seconds, for the patient's plasma to clot after the addition of calcium and extrinsic pathway activator (thromboplastin). Activated partial thromboplastin

time (aPTT) is a coagulation test used as a measure of the integrity of the intrinsic and common pathways of the cascade and represents the time, in seconds, for patient plasma to clot after the addition of phospholipid and calcium, an intrinsic pathway activator. The normal or reference range for PT and APTT tests varies from laboratory to laboratory (4). There are studies showing that the normal duration of aPTT and PT shortens during normal pregnancy and returns to normal after pregnancy (5,6). The relationship of these changes to pregnancy loss is not illustrated. Here, we would like to reveal whether these factors have value in predicting pregnancy loss(es) and present the retrospective data of these patients as a single-center experience.

MATERIALS AND METHODS

One hundred and seventy female patients who were evaluated due to at least one pregnancy loss in the hematology outpatient clinic of Aydın Adnan Menderes University between the years of 2012 and 2024 were included in the study. It was designed to be a single-center, retrospective, multidisciplinary, analytic, and cross-sectional study. Ethical committee approval has been obtained (Aydın Adnan Menderes University/Aydın, Türkiye. Date: 20.11.2023- Number: 453317). The research was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants prior to their involvement in the study.

Participants were provided with information about the purpose, methods, potential risks, and benefits of the research, and it was emphasized that their participation was voluntary. Furthermore, participants were informed of their right to withdraw from the study at any time.

The mean age of the patients was 34.48 ± 6.02 . Treatment management was carried out by gynecologists and/or hematologists according to gynecology and/or hematology-thrombosis guidelines and, in some patients, at the clinician's discretion (7,8). Patients with anticardiolipin antibodies IgG or IgM and anti-beta2 glycoprotein I antibodies IgG and IgM by enzyme-linked immunosorbent assay (ELISA) (above 40 GPL) or lupus anticoagulant positivity (above the upper limit of normal laboratory value), as well as patients with a known etiology for pregnancy loss (maternal anatomic and endocrine abnormalities, infectious causes, trauma, chromosomal abnormalities), were excluded from the study. Patients were divided into two groups: Groups A and B defined the patients with one or two miscarriages and more than two miscarriages, respectively. The distinction between Groups A and B was based on the European Society of Human Reproduction and Embryology's definition of RPL as three or more miscarriages (3). The parameters included in genetic thrombophilia screening (i.e. FVLM, PGM, Methylenetetrahydrofolate reductase-MHFR, Plasminogen activator inhibitor-PAI, Beta fibrinogen, and Factor-XIII gene mutations), and protein C, S and antithrombin levels of the patients were recorded for both groups, and their relationship with recurrent pregnancy loss was investigated. Laboratory parameters were recorded one month after the first abortion and reference values of the hospital laboratory were used to decide high or low values. Blood count parameters (leukocyte, neutrophil, lymphocyte, monocyte, eosinophil, basophil ($10^3/\text{mkrL}$ for each), hemoglobin (gr/dL), and platelet ($10^3/\text{mkrL}$) counts, mean corpuscular volume (fL), mean platelet volume (fL) and red cell distribution width (%)) were recorded for available patients. Regarding coagulation tests, aPTT, PT, international normalized ratio (INR), fibrinogen, d-dimer, and homocysteine were also recorded and the correlation between hemogram and coagulation parameters with pregnancy losses was investigated.

Statistical Analysis

SPSS version 25.0 for data analysis was used. The suitability of continuous variables to normal distribution was investigated using visual (histogram and probability plots) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). For the descriptive statistics of the research, mean and standard deviation were used. Chi-Square Test was used in the study to show whether there was a difference between categorical variables. The student-T Test was used to compare continuous variables with parametric properties in independent groups, and Mann Whitney U Test was used to compare continuous variables without parametric properties in independent groups. For statistical significance, a p-value of less than 0.05 was accepted.

RESULTS

The descriptive characteristics of the patients in groups A and B and the comparison of hematological parameters are shown in (Table 1). Nine patients (5.3%), 71 patients (41.8%), 60 patients (35.3%), 21 patients (12.4%), four patients (2.4%), and five patients (2.9%) had a history of one, two, three, four, five and six miscarriages, respectively. The number of patients that have homozygous FVLM and homozygous PGM were 2 and 0, respectively. Also, the number of patients with double heterozygosity for FVLM and PGM was 5. In 9 patients in group A and 17 in group B with FVL mutation; 7 and 16 of them, respectively, were accompanied by at least one other mutation (the mutations demonstrated in Table-1). In addition, of the 4 patients in group A and 5 patients in group B with PGM, at least one more mutation was detected in 2 and 5, respectively.

Forty-one patients (24.1%), 11 patients (6.5%), and 21 patients (12.4%) have a history of using low-molecular-weight heparin (LMWH), aspirin, and LMWH +aspirin during the last pregnancy period and/or the postpartum period, respectively. Ninety-three patients (54.7%) have not used any antiaggregant /anticoagulant treatment. We could not obtain any records regarding drug history for four patients (2.4%). Three patients (1.8%) and two patients (1.2%) had also a history of venous and arterial thrombosis in their lifetime, respectively. All arterial thrombosis cases were in the form of ischemic stroke. In terms of venous thrombosis type, it was deep vein thrombosis in 2 cases and intra-abdominal vein thrombosis in 1

case. The rest of the patients have not experienced any kind of documented thrombosis previously.

No significant difference was found between the genetic mutations investigated in terms of thrombophilia, protein C, S, and antithrombin levels and the number of pregnancy losses in both groups. Based on laboratory reference values, the number of patients with protein C, S, and AT deficiency were 0, 15, and 2, respectively, for group A and 3, 23, and 2, respectively, for group B.

Blood count parameters were evaluated in terms of their effect on miscarriage. Cut-off values were accepted as the cut-off values of our hospital laboratory and were examined by grouping as low/normal/high or normal/high according to the parameter. No significant correlation with leukocyte (WBC) ($<4,230/\text{mm}^3$ vs $4,230-$

$9,070/\text{mm}^3$ vs $9,070/\text{mm}^3 <$), neutrophil ($<1,780/\text{mm}^3$ vs $1,780-5,380/\text{mm}^3$ vs $5,380/\text{mm}^3 <$), monocyte ($<300/\text{mm}^3$, $300-820$ vs $820/\text{mm}^3 <$), hemoglobin (<13 gr/dL vs $13-16$ gr/dL vs 16 gr/dL<), mean corpuscular volume (MCV) (<79 fL vs $79-92$ fL vs 92 fL<), platelet count ($<100,000/\text{mcrL}$ vs $100,000-450,000/\text{mcrL}$ vs $450,000$ mcrL<), and mean platelet volume values (<9.4 fL vs $9.4-12.4$ fL vs 12.4 fL<) were detected between in group A and B. Also, there was no statistical significance regarding INR ($0.8 >$ vs $0.8-1.2$ vs $1.2 <$), prothrombin time (PT, <8 sec, $8-13.5$ sec and 13.5 sec<), fibrinogen (<284 mg/dL vs $284-580$ mg/dL vs 580 mg/dL<), homocysteine (<15 Umol/L vs 15 Umol/L<), d-dimer (<243 ng/ml DDU vs 243 ng/ml DDU <) values ($p>0.05$). However, the aPTT (<25.4 sec vs $25.4-38.4$ sec vs 38 sec <) value of group B was found to be significantly lower than that of group A. ($p=0.04$). (Table-1).

Table-1. The descriptive characteristics of the patients in groups A and B and the comparison of hematologic and coagulation parameters.

	Group A (n:80)	Group B (n:90)	p
Number of Miscarriages (mean$\pm$SD)	1.89 $\pm$ 0.31	3.49 $\pm$ 0.82	
Age (mean$\pm$SD)	34.18 $\pm$ 6.22	34.74 $\pm$ 5.85	p>0.05
History of previous thrombosis (n)	Venous:2 Arterial:1	Venous:1 Arterial:1	
Homozygous FVLM (n)	-	2	
Homozygous PGM (n)	-	-	
Double heterozygous for FVLM and PGM (n)	2	3	
MTHFR C677T	Homozygous:8 Heterozygous:26 Negative:40	Homozygous:16 Heterozygous:36 Negative:33	p>0.05
MTHFR A1298C	Homozygous:9 Heterozygous:25 Negative:40	Homozygous:12 Heterozygous:31 Negative:42	p>0.05
PAI gene mutation	4G4G: 3 4G5G: 3 5G5G: 2	4G4G: 3 4G5G: 3 5G5G: 3	p>0.05
Beta fibrinogen mutation	Homozygous:3 Heterozygous:15 Negative:8	Homozygous:2 Heterozygous:19 Negative:5	p>0.05
Factor-XIII gene mutation	Homozygous:1 Heterozygous:25 Negative:39	Homozygous:2 Heterozygous:19 Negative:57	p>0.05

Protein-C (%)*	92.05±20.83	100.28±25.38	p>0.05
Protein-S (%)*	69.51±22.90	67.11±20.93	p>0.05
Antithrombin (%)*	98.90±11.46	97.99±8.91	p>0.05
History of antiaggregant or anticoagulant use (n)	LMWH:15	LMWH:26	p>0.05
	ASA:5	ASA:6	
	LMWH+ASA:7	LMWH+ASA:14	
	Not used:50	Not used:43	
	Unknown:3	Unknown:1	
Blood count parameters(mean±SD)			
<i>WBC count (/mkrL)</i>	9138.87±2667.01	9449.74±3073.12	p>0.05
<i>Neutrophil count (/mkrL)</i>	6309.84±2678.57	6390.38±3157.79	
<i>Lymphocyte count(/mkrL)</i>	2115.97±653.54	2293.33±708.09	
<i>Monocyte count(/mkrL)</i>	536.61±197.26	542.18±180.06	
<i>Eosinophil count(/mkrL)</i>	147.90±143.23	172.82±183.07	
<i>Basophil count(/mkrL)</i>	32.26±16.63	30.00±14.68	
<i>MCV (fL)</i>	85.27±7.61	83.80±7.77	
<i>MPV (fL)</i>	10.18±1.18	10.45±1.19	
<i>RDW (%)</i>	13.87±1.35	14.12±1.94	
<i>Platelet (/mkrL)</i>	255,290.32±76,184.50	256,807.69±74,363.45	
<i>Hemoglobin (gr/dL)</i>	12.28±1.50	12.24±1.44	
Coagulation Tests (mean±SD)			
aPTT (sec.)	25.90±2.91	25.06±2.79	p<0.05
PT (sec.)	12.43±1.25	12.11±1.30	p>0.05
INR	0.94±0.08	0.94±0.08	p>0.05
Fibrinogen (mg/dL)	403.17±139.15	368.11±98.22	p>0.05
D-dimer (g/ml DDU)	981.54±1456.78	734.31±1094.48	p>0.05
Homocysteine (Umol/L)	12.91±7.62	11.69±6.39	p>0.05

* Normal range for Protein C, Protein S and Antithrombin as follows: 70-140%, 60-130%,83-118%, respectively.
ASA: acetylsalicylic acid, aPTT: activated partial thromboplastin time, FVLM: Factor V Leiden Mutation
INR: international normalized ratio, LMWH: Low-molecular-weight heparin, MCV: Mean Corpuscular Volume
MPV: Mean Platelet Volume, MTHFR: methylenetetrahydrofolate reductase, n: number
PAI: plasminogen activator inhibitor, PGM: Prothrombin Gene Mutation, PT: prothrombin time
RDW: red cell distribution width, SD: Standard Deviation, Sec.: second, WBC: White Blood Cell

DISCUSSION

RPL is an undesired event, and it is a condition that has psychological effects as well as physical health problems. The population prevalence of women who have had one miscarriage is 10.8% (10.3-11.4%), two miscarriages is 1.9% (1.8-2.1%), and three or more miscarriages is 0.7% (0.5-0.8%) (9). Several causes play a role in RPL including maternal age (9–75%), endocrine diseases (17–20%), uterine morphological pathologies (10–15%), chromosomal abnormalities (2–8%), thrombophilia, infectious agents (0.5–5%), and autoimmune disorders (20%). In approximately 50–75% of RPL cases, the exact cause is not clearly identified and, therefore, remains unexplained (10).

It is important to identify treatable causes of RPL which can be prevented by applying medical treatments such as referral to genetic counseling if there is a parental karyotype abnormality, surgery if there are correctable uterine anatomical abnormalities such as uterine septum, adhesions, submucosal myoma, aspirin, and heparin treatment if antiphospholipid antibody syndrome is present and medical treatment for these if there is thyroid dysfunction, hyperprolactinemia and/or diabetes mellitus (11). The combination of hereditary thrombophilia and pregnancy may mean a higher risk of RPL. However, due to the heterogeneity of samples in most studies, there is not much strong evidence for an association between inherited thrombophilia and RPL. Apart

from antiphospholipid antibody syndrome, whether the pregnant woman has thrombophilia or not; there is no evidence that aspirin or heparin treatment, which is frequently used in pregnant women, improves pregnancy outcomes (12,13). In most cases, the patient's expectation for the future after experiencing a pregnancy loss forces the physician to start medication, even though its effectiveness has not been proven. This approach, as with anticoagulant treatments, leaves the patient open to complications of the drug started, such as bleeding.

Various studies have examined the relationship between hematological parameters and pregnancy loss. In a case-control study by Erdem ZS et al, 50 patients between 18-40 years and a history of at least three consecutive recurrent pregnancy losses were included and it was suggested that high MPV, RDW, plateletcrit count, and low MCHC among blood count parameters could be an important indicator of recurrent miscarriages (14). In another prospective controlled study of 184 patients designed by Al-Husban et al., increased platelet and leukocyte counts in the first trimester are associated with an increased risk of miscarriage (15). We have not observed any correlation between RPL and blood count parameters in either group.

Regarding our study, we found that a decrease in aPTT was significantly correlated with the number of miscarriages. The effect of shortening aPTT value on pregnancy loss was supported by various studies. Ogasawara M, et al included 261 patients with a history of two consecutive first-trimester spontaneous abortions who had no antiphospholipid antibodies, and they were examined for aPTT, prothrombin time, and fibrinogen before the next pregnancy. Fifty-eight percent of 261 patients (22.2%) had a subsequent spontaneous abortion. Significantly elevated spontaneous abortion rates in patients with preconception-shortened aPTT were detected. The possible mechanism may be associated with thrombosis in syncytiotrophoblasts or the spiral artery induced by a hypercoagulable state. It was suggested that short aPTT values before pregnancy can indicate pregnancy loss (16). In the study conducted by Yao et al., 48 patients with recurrent abortion were divided into Group A (two miscarriages, n=21), Group B (three miscarriages, n=16), and Group C ($\geq$ four miscarriages, n=11) according to the number of miscarriages, and recurrent pregnancy loss was found to be

associated with decreased aPTT, prothrombin time and increased fibrinogen and D-Dimer levels (17). In another study by Ali A. et al, a significant relationship was found between recurrent miscarriages and increased factor VIII levels as well as decreased aPTT among 68 patients with RPL (18).

The assumption that thrombophilic status increases the risk of thrombosis at the low-flow maternal-placental interface, leading to placenta-mediated complications such as RPL, prevails among most clinicians. However, the role of hereditary deficiencies and mutations in the etiology of RPL has not been proven, and the risk is probably increased in high-risk thrombophilia (AT deficiency, FVL or Prothrombin G20210A variant homozygote mutations, concurrent heterozygosity for both FVL and Prothrombin G20210A variants, and rare combined thrombophilias such as AT deficiency plus FVL mutation) (19).

Although the effects of genetic mutations and protein C/S/AT deficiency on RPL have been investigated in various studies in different populations, conflicting results have been obtained regarding the presence of the correlation (20-27) In a cross-sectional multicenter study of 1384 patients conducted by Preston F. et al, it was emphasized that the probability of pregnancy loss (as both miscarriage and stillbirth) increased especially in those with protein C/S and antithrombin deficiency and that this situation increased even more clearly, especially in those with AT deficiency and in the patient group with multiple genetic thrombophilic defects (28). In a meta-analysis by Liu et al, 89 studies including 30,254 patients were compiled and found that FVL mutation, PGM and Protein S deficiency may increase the risk of RPL by 2.44-fold, 2.08-fold, and 3.45-fold, respectively. No significant correlation was found between antithrombin and protein C deficiency with RPL. The relationship with other genetic mutations (MTHFR, PAI, Beta fibrinogen, Factor XIII) was not investigated (29). Regarding FVL and PGM mutation; an association with these mutations and abortion has not been found in various studies (30-33). In our study, there were only two patients (all in group B) with FVL mutation (homozygous), while there were no patients with PGM homozygous mutation. There were two patients in group A and group B with double heterozygous mutations, respectively. In addition, there were no patients with protein

C/S/AT deficiency. Although it is not clear how much these mutations (FVL and PGM) seen in our patients affected the results, it would be reasonable to assume that this effect is limited since they are present in a small number of patients. Since there are not enough studies in the literature to investigate the contribution of other mutations (MTHFR, PAI, Beta fibrinogen, Factor-XIII gene mutations) in the etiology of RPL, it would not be appropriate to comment on their effect on our study and it is presented only as demographic data.

CONCLUSION

RPL continues to aggrieve pregnant women as an undesirable condition due to the multiple causes that lead to RPL, their different pathogenetic mechanisms, the difficulty of homogenization of patient groups, and ultimately the inability of studies to determine a parameter(s) that will predict this condition. As a parameter that can be easily and quickly assayed, a low aPTT value may be a parameter that can help predict RPL (30-33).

Limitations of the study

Although no risk factor is identified in 50-70% of couples with recurrent pregnancy loss; in our study, various parameters such as advanced maternal age, number of previous miscarriages, obesity (body mass index >30kg/m²), lifestyle factors (stress, smoking, and excessive alcohol consumption) may affect laboratory results as well as pregnancy outcomes, however, patients were not excluded from evaluation in this respect (34). Another limitation of the study is the patients who use antiaggregant and anticoagulant drugs due to

previous thrombotic profiles that may affect further miscarriages as well as laboratory values. These circumstances may show that a relatively heterogeneous population has been evaluated. In addition, patients in group A may have continued pregnancy loss in their subsequent pregnancies, if any, and therefore may have the potential to move to group B. This is a limitation arising from the retrospective and cross-sectional design of our study.

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Availability of data and material: The authors approve that all necessary papers regarding this report can be offered on request.

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Consent to participate: Informed consent form approving participation was obtained from the patients.

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Diyabetik ayak enfeksiyonu ile takipli hastalarda HBV seroprevalansı

HBV seroprevalence in patients with diabetic foot infection

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Öz

Amaç: Hepatit B virüsü (HBV), kronik karaciğer hastalıklarına ve hepatoselüler karsinoma neden olarak yılda 3 milyon kişinin ölümüne yol açan küresel bir sağlık sorunudur. Dünya Sağlık Örgütü 254 milyon kişinin kronik hepatit B ile yaşadığını tahmin etmektedir. Hastalık Kontrol ve Önleme Merkezleri, HBsAg prevalansının %2'yi aştığı bölgelerde HBV taraması yapılmasını önermektedir. Türkiye, %4,5 prevalans oranı ile orta endemisine bölgesindedir.

Diyabetik ayak enfeksiyonu olan hastalar, sık sağlık hizmeti başvuruları nedeniyle HBV'ye maruziyet açısından daha yüksek risk altındadır. Bu çalışma, bu hassas popülasyonda HBV seroprevalansını araştırmayı amaçlamaktadır.

Gereç ve Yöntem: 2016-2024 yılları arasında Ege Üniversitesi Enfeksiyon Hastalıkları kliniğinde diyabetik ayak enfeksiyonu nedeniyle yatırılarak takip ve tedavi edilen olgular, elektronik hasta kayıtları üzerinden retrospektif olarak incelenmiştir. Hastaların demografik verileri, HBsAg, Anti-HBs, Anti-HBc IgG ve gerekli durumlarda HBV DNA sonuçları kaydedildi.

Bulgular: Altı yüz otuz iki (n: 632, Erkek/Kadın: 449/183, ortalama yaş: 63,02± 11,429) hastanın, HBsAg, Anti-HBs ve Anti-HBc IgG pozitiflik oranları sırasıyla; %1,89, %36,07 ve %32,1 idi. Hastaların 164'ü enfeksiyonu doğal yolla geçirerek 79'u ise aşılama ile bağışıklık kazanmıştır. Yine hastaların 53'ünde izole Anti-HBc IgG pozitifliği tespit edilmişken, 12 hastada kronik hepatit B enfeksiyonu saptanmıştır. Hastaların 303'ünün (%47,9) ise HBV ile hiç karşılaşmamış ve aşısız olduğu dikkat çekmiştir. Beklediğimiz aksine HBsAg seropozitiflik oranları genel popülasyon ortalamasının altında bulunmuştur. Yirmi bir hastanın eksik verisi olması nedeni ile klinik sınıflaması yapılamamıştır.

Sonuç: Diyabetik ayak enfeksiyonu ile yaşayan hasta grubu HBV açısından çeşitli risk faktörlerine maruz kalmaktadır. Bu nedenle sistematik olarak taranmalı ve aşısız olanların aşılması yapılmalı, gerekli hastalarda tedavi başlanmalıdır.

Anahtar Sözcükler: Diabetes Mellitus, Diyabetik ayak enfeksiyonu, HBV prevalansı.

ABSTRACT

Aim: Hepatitis B virus (HBV) is a global health problem that causes chronic liver disease and hepatocellular carcinoma, killing 3 million people annually.

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The World Health Organization estimates that 254 million people are living with chronic hepatitis B. The Centers for Disease Control and Prevention recommends HBV screening in areas where HBsAg prevalence exceeds 2%. Turkey is in a medium endemicity area with a prevalence rate of 4.5%.

Patients with diabetic foot infection are at higher risk of HBV exposure due to frequent healthcare visits. This study aims to investigate HBV seroprevalence in this vulnerable population.

Materials and Methods: Patients hospitalized and treated for diabetic foot infection at ... University Infectious Diseases Clinic between 2016 and 2024 were retrospectively analyzed through electronic patient records. Demographic data, HBsAg, Anti-HBs, Anti-HBc IgG and HBV DNA results were recorded when necessary.

Results: HBsAg, Anti-HBs and Anti-HBc IgG positivity rates of six hundred and thirty-two (n: 632, Male/Female: 449/183, mean age: 63.02± 11.429) patients were 1.89%, 36.07% and 32.1%, respectively. 164 of the patients acquired the infection naturally and 79 of them were immunized by vaccination. Isolated anti-HBc IgG positivity was detected in 53 patients, while 12 patients had chronic hepatitis B infection. It was noteworthy that 303 (47.9%) of the patients had never encountered HBV and were not vaccinated. Contrary to our expectations, HBsAg seropositivity rates were below the general population average. Twenty-one patients could not be clinically classified due to missing data.

Conclusion: The patient group living with diabetic foot infection is exposed to various risk factors for HBV. Therefore, they should be systematically screened and those who are not vaccinated should be vaccinated, and treatment should be initiated in necessary patients.

Keywords: Diabetes Mellitus, Diabetic foot infection, HBV prevalence

GİRİŞ

Hepatit B virüsü (HBV) enfeksiyonu, kronik karaciğer hastalıkları, siroz ve hepatoselüler karsinom (HSK) gelişimine yol açan ciddi bir küresel sağlık sorunudur (1-4). Dünya Sağlık Örgütü'ne (WHO) göre, dünya çapında tahminen 254 milyon kişi kronik Hepatit B ile yaşamakta ve her yıl 1,2 milyon yeni enfeksiyon görüldüğü tahmin edilmektedir. Yılda en az 1,1 milyon ölüm HBV'nin neden olduğu kronik karaciğer hastalıklarına (siroz ve HSK) bağlanmaktadır (4). Ülkemizde de 2012-2016 yılları arasında gerçekleştirilen karaciğer transplantasyonlarının yaklaşık yarısının (%40-50) HBV enfeksiyonuna bağlı akut-kronik karaciğer yetmezliği ve HSK nedeniyle yapıldığı bildirilmiştir (5). Bu istatistikler, HBV enfeksiyonunun yaygın bir sağlık sorunu olarak ciddiyetinin altını çizmekte ve halk sağlığı üzerindeki etkisini azaltmak için rutin aşı şeması dışında kalan tüm popülasyonun taranması gerekliliğini vurgulamaktadır.

Diyabetik ayak enfeksiyonu olan hastalar, sık hastane ziyaretleri, çok sayıda enjeksiyon ve ameliyata maruz kalmaları ve hastalıkları sırasında kan tranfüzyonu olasılığı nedeniyle daha fazla risk altındadır. Bu bağlamda, bu bireylerin HBV'ye yakalanma riskinin daha yüksek olduğu düşünülmekte ve risk altındaki popülasyonlarda HBV enfeksiyonlarının yönetimi ve yaygınlığının azaltılmasında hedefe yönelik önleyici tedbirlerin

ve farkındalık kampanyalarının önemi vurgulanmaktadır (6-8). Bu çalışmanın amacı, diyabetik ayak enfeksiyonları nedeniyle izlenen hastalarda Hepatit B Virüsü (HBV) seroprevalansını araştırmaktır.

GEREKÇİ ve YÖNTEM

2016-2024 yılları arasında kliniğimizde yatarak takip ve tedavi edilen diyabetik ayak enfeksiyonlu olgular elektronik hasta kayıtları üzerinden retrospektif olarak incelendi. Hastaların demografik verileri, Hepatit B yüzey antijeni (HBsAg), Hepatit B yüzey antikoru (Anti-HBs), Hepatit B çekirdek antikoru immünoglobulin G (Anti-HBc IgG) ve HBV DNA düzeyleri (IU/ml) kaydedildi. HBsAg pozitif olan ancak alanin aminotransferaz (ALT) ve aspartat transaminaz (AST) seviyeleri normal olan ve HBV DNA'sı negatif olan hastalar HBV taşıyıcısı olarak kabul edilmiştir. HBsAg ve Anti-HBc IgG sonucu negatif, Anti-HBs pozitif olan hastalar aşılanmış kabul edilmiştir. İzole Anti-HBc IgG pozitifliğinin tek başına saptanması izole Anti-HBc IgG pozitifliği olarak kabul edilmiştir. Her üç parametre (HBsAg, Anti-HBs ve Anti-HBc) için negatif olan hastalar hepatit B ile hiç karşılaşmamış olarak kabul edilmiştir. Siroz varlığı; trombosit sayısı, INR değeri ve karaciğer USG'si ile değerlendirilmiştir.

Çalışmamız Helsinki Deklarasyonu kararlarına, hasta hakları yönetmeliğine uygun olarak planlandı ve Ege Üniversitesi Tıp Fakültesi Klinik Araştırmaları Etik kurulundan 12.12.24 tarih ve 24-12T/43 sayılı etik kurul onayı alındı.

İstatistiksel Analiz: Veriler, Excel ortamında düzenlenip SPSS'e aktarılmış ve tanımlayıcı istatistikler kullanılarak her bir değişkenin frekans ve yüzde dağılımları hesaplanmıştır. Eksik veriler kontrol edilmiş ve küçük eksiklikler için ortalama veya medyan imputasyonu uygulanmış, büyük oranlı eksiklikler ise analizden çıkarılmıştır.

BULGULAR

Kliniğimizde takip edilen diyabetik ayak enfeksiyonu olan 632 hastanın 183'ü (%29) kadın, 449'u (%71) ise erkekti, yaş ortalamaları ise

63.02± 11,42 yıl bulunmuştur. HBsAg (n=607), Anti-HBs (n=604) ve Anti-HBc IgG (n=577). HBsAg, Anti-HBs ve Anti-HBc IgG pozitifliği yüzdeleri sırasıyla %1,89, %36,07 ve %32,1 olarak bulunmuştur. 632 kişiden 303'ü Hepatit B ile hiç karşılaşmamış, 53'ünde izole Anti-HBc IgG pozitifliği tespit edilmiş ve 79'u aşılama yoluyla, 164'ü karşılaşarak bağışıklık kazanmıştır. 12 hasta hepatit B enfeksiyonu taşıyıcılığı ile takip edilmekte olup; iki hastada kronik HBV enfeksiyonu saptanmış ve entekavir tedavisi başlanmıştır (Tablo-1). Yirmi bir hastanın eksik verisi olması nedeni ile klinik sınıflaması yapılamamıştır. Trombosit değeri <150.000/μL altında olan 2 hastada siroz saptanmıştır. Bir hasta exitus olmuş. Diğer hasta ise gastroenteroloji bölümünde takiplerine devam etmektedir.

Tablo-1. HBsAg pozitif olan 12 hastanın özellikleri.

Hasta no.	YAŞ	CİNSİYET	HBV (IU/mL)	DNA	AST (U/L)	ALT (U/L)	PLT (10 ³ /μL)	INR	USG
1.	66	E	32		13	11	208	0,95	Grade 1-2 hepatosteatoz
2.	54	E	SAPTANMADI		6	5	280	1,1	Grade 2 hepatosteatoz
3.	58	E	46		25	18	271	1,26	OLAĞAN
4.	51	E	BAKILMAMIŞ		18	16	247	1,14	-
5.	65	E	136		12	9	272	1,05	OLAĞAN
6.	65	E	SAPTANMADI		16	26	277	1,01	Grade 2 hepatosteatoz
7.	70	E	BAKILMAMIŞ		10	25	475	1	-
8.	9	E	BAKILMAMIŞ		42	27	325	1	-
9.	52	E	135396389		115	52	120	1,5	
10.	67	E	170		12	12	225	0,9	Grade 1-2 hepatosteatoz
11.	59	E	439090056		41	27	89	1,24	Karaciğer parankim yapısı heterojen ve kaba görünümündedir
12.	33	E	1020		44	49	497	0,97	OLAĞAN

TARTIŞMA

HBV enfeksiyonu genellikle insandan insana kan ve kan ürünleri yoluyla bulaşır. Yaygın risk faktörleri arasında ortak enjektör kullanımı, damar içi uyuşturucu bağımlılığı, 1996'dan önce yapılan kan nakilleri veya organ nakilleri, hemodiyalize giren bireyler, dövmesi veya piercingi olanlar ve hapse girmiş bireyler yer almaktadır (9). Ayrıca, HBV ile enfekte annelerden doğan bebeklerde

genel popülasyona kıyasla daha yüksek enfeksiyon sıklığı görülmektedir.

Hastalık Kontrol ve Önleme Merkezleri (CDC), HBsAg (Hepatit B yüzey antijeni) prevalansının %2'yi aştığı ülkelerdeki bireylerde HBV enfeksiyonu için rutin tarama yapılmasını önermektedir (10). Bu tarama, enfeksiyonu erken teşhis etmek ve belirtilen eşikten daha yüksek prevalansa sahip bölgelerde yayılmasını kontrol etmek için önerilmektedir. Türkiye, Hepatit B

virüsünün (HBV) orta derecede endemik olduğu bir ülkedir ve prevalansı %4'tür (%2 ila %8 arasında değişmektedir) (11-14).

Çalışmamızda prevalansın Türkiye ortalamasının altında bulunmasını, hastanemizin TURHEP çalışmasında Türkiye'nin %2,3 ile en düşük KHB prelevansına sahip Ege Bölgesinde olması ve 3. Basamak olan hastanemizde seroloji takiplerine önem verilmesi ve Hepatit B ile karşılaşmamış ve aşısız hastalarının ilk başvurularında aşılama programına alınması olduğunu düşünmekteyiz (15).

Hepatit B virüsü (HBV) enfeksiyonunun karaciğer üzerinde dekompanseasyon, siroz ve hepatoselüler karsinom (HCC) dahil olmak üzere önemli zararlı etkileri vardır (16,17). Dahası, karaciğer glikoz homeostazında önemli bir rol oynadığından, karaciğer hasarı glikoz metabolizmasında bozukluklara yol açabilir ve böylece tip 2 diabetes mellitus (T2 DM) riskini artırabilir (18,19). Çin'de yapılan bir çalışmada 55.520 katılımcının yer aldığı prospektif bir kohort çalışmasında, KHB'si olan bireylerin, 5,6 yıllık ortalama bir takip süresi boyunca enfekte olmayan akranlarına göre T2DM geliştirme riskinin daha yüksek olduğu, bildirilmiştir (18).

Dünya çapında 20-79 yaşları arasındaki 537 milyon yetişkinin diyabetle yaşadığı tahmin edilmektedir. Bu veriler ışığında diyabetin komplikasyonları ile sık karşılaşılmaktadır (20).

Diyabetik ayak ülserleri diyabetin en sık hastaneye yatış gerektiren ve alt ekstremité amputasyonuna yol açan komplikasyondur (21,22). Diyabet hastalarında Hepatit B seroprevalansı ile ilgili çok sayıda yapılmış çalışma olmasına rağmen ülseri olan hastalarda yapılmış çalışma sayısı sınırlıdır. Türkiye'de yapılmış bir çalışmada ülseri olan ve olmayan hasta gruplarında HBsAg ve anti-HBs seropozitifliğinde anlamlı bir fark gözlenmemiş

ancak HbsAg seropozitifliğinin diyabet hastalarında genel popülasyondan daha yüksek olduğu bulunmuştur (23). Yapılan bir çalışmada ülser gelişen hastaların bir yıllık takiplerinde yalnızca %46'sı iyileşmiş, %10 hastada ülser tekrarlamış, %15'i ölmüş ve %17'sinin alt ekstremité amputasyonuna ihtiyacı olmuştur (24). Bu hasta grubun sık hastane başvurusu, tekrarlayan ameliyatlar ve kan tranfüzyonu ihtiyacı gelişmesi gibi risk faktörleri ile sık teması bu hasta grubunu hepatit B taranması için öncelikli gruplar içine sokmaktadır.

Bu bilgiler ışığında Kronik hepatit B hastaları diyabet için, diyabet hastaları ise hepatit B enfeksiyonu için risk altındadır. Günümüzde aşıyla korunabilir olması ve tedavisinin olması nedeni ile bu risk grubunun mutlaka taramasının yapılması gerektiğini düşünmekteyiz.

SONUÇ

Diyabetik ayak enfeksiyonu olan hastalar sıklıkla tıbbi yardım almakta, hastalıkları süresince sık sık hastane ziyaretleri, enjeksiyonlar, ameliyatlar geçirmekte ve kan transfüzyonlarına maruz kalmaktadır. Her ne kadar çalışmamız, bu grubun seropozitiflik oranının normal popülasyondan yüksek olduğunu istatistiksel olarak gösterememiş olsa da yukarıda belirtilen risk faktörleri göz önüne alındığında, bu grubun riskli bir popülasyon olduğu kanaatindeyiz. Bu bağlamda, HBV enfeksiyonu riski taşıyan bireylerin taranması ve gerekli tedavi süreçlerinin uygulanması elzemdir. Tarama sonuçlarına dayanarak, HBV'ye karşı aşılanmamış hastalar için uygun aşılama planlarının yapılması gerekmektedir. Dolayısıyla, diyabetik ayak enfeksiyonu ile yaşayan hasta grubunun HBV açısından sistematik olarak taranması büyük önem arz etmektedir.

Çıkar Çatışması: Yazarlar aralarında çıkar çatışması olmadığını beyan etmişlerdir.

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The role of biochemical markers in predicting pre-eclampsia

Preeklampsiyi öngörmeye biyokimyasal belirteçlerin rolü

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ABSTRACT

Aim: The aim of our study was to evaluate the role of biochemical markers in predicting preeclampsia.

Materials and Methods: 7240 women whose pregnancy follow-ups and deliveries were carried out by us between March 2018 and March 2024 were retrospectively screened. Patients in the study group consisted of 102 women diagnosed with preeclampsia at <34 weeks of gestation, and patients in the control group consisted of 118 women who gave birth at ≥37 weeks of gestation. Demographic characteristics and obstetric and biochemical outcomes of the two groups were compared with each other.

Results: The body mass index (BMI) value was significantly higher in the preeclampsia group compared with the controls group (p=0.016). The birth weight value was significantly lower in the preeclampsia group compared with the controls group (p<0.001). The gestational week at delivery was significantly lower in the preeclampsia group compared with the controls group (p<0.001). Lymphocyte and platelet counts were significantly lower in the preeclampsia group compared with the controls group (p<0.001 and p<0.001, respectively). NLR and SII value were significantly higher in the preeclampsia group compared with the controls group (p=0.022, p<0.001, respectively).

Conclusion: Our study reveals important clinical and laboratory differences in preeclamptic pregnancies. Increased inflammatory markers were found to be a feature of preeclamptic pregnancies and are consistent with the existing literature. Our findings can be used to determine early diagnosis and comprehensive management strategies to minimize the risks associated with preeclampsia.

Keywords: NLR, PLR, Pregnancy, Preeclampsia, SII, SIRI

ÖZ

Amaç: Çalışmamızın amacı preeklampsiyi öngörmeye biyokimyasal belirteçlerin rolünü değerlendirmektir.

Gereç ve Yöntem: Mart 2018-2024 tarihleri arasında gebelik takipleri ve doğumları tarafımızca gerçekleştirilen 7240 kadın retrospektif olarak tarandı.

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Çalışma grubundaki hastalar <34 gebelik haftasında preeklampsi tanısı konulan 102 kadından, kontrol grubundaki hastalar ise ≥37 gebelik haftasında doğum yapan 118 kadından oluştu. İki grubun demografik özellikleri, obstetrik ve biyokimyasal sonuçları birbirleriyle karşılaştırıldı.

Bulgular: Preeklampsi grubunda vücut kitle indeksi kontrol grubuna göre anlamlı yüksek saptandı ($p=0.016$). Doğum ağırlığı preeklampsi grubunda kontrol grubuna göre anlamlı düşük saptandı ($p<0.001$). Doğumdaki gebelik haftası preeklampsi grubunda kontrol grubuna göre anlamlı düşük saptandı ($p<0.001$). Lenfosit ve trombosit sayıları preeklampsi grubunda kontrol grubuna göre anlamlı düşük saptandı ($p<0.001$ ve $p<0.001$, sırasıyla). NLR ve SII değeri preeklampsi grubunda kontrol grubuna göre anlamlı yüksek saptandı ($p=0.022$, $p<0.001$, sırasıyla).

Sonuç: Çalışmamız, preeklampsi gebelerde önemli klinik ve laboratuvar farklılıklarını ortaya koymaktadır. Artmış inflammatuar belirteçlerin preeklampsi gebeliklerin bir özelliği olduğu bulunmuştur ve mevcut literatürle tutarlıdır. Bulgularımız, preeklampsi ile ilişkili riskleri en aza indirmek için erken tanı ve kapsamlı yönetim stratejilerini belirlemek için kullanılabilir.

Anahtar Sözcükler: Gebelik, NLR, PLR, Preeklampsi, SII, SIRI

INTRODUCTION

Preeclampsia is a pregnancy-specific health condition that is observed in approximately 6-8% of women, may recur in the next pregnancy, occurs after the 20th week of gestation and may significantly affect maternal and fetal health (1). Predicting this condition is very important for maternal and fetal health and is still being researched and there is no definitive method yet. Preeclampsia is characterized by widespread endothelial dysfunction in the body, often diagnosed by new-onset hypertension and concomitant disorders of various end organ disorders with or without new-onset proteinuria, and even seizures, and can have fatal consequences (2). The definitive treatment is delivery and if this delivery has to be preterm, it increases prematurity, perinatal mortality and morbidity. The etiology and pathogenesis of preeclampsia are considered multifactorial. Major pathophysiological processes include alterations in hemostatic-system, such as the endothelial-cell-damage, platelet activation and enhanced intravascular thrombin generation (3). Hyperactivation of inflammatory and immunologic responses in preeclampsia leads to a marked increase in the number of neutrophils and a change in neutrophils towards superoxide production leading to endothelial damage and dysfunction (4). Recently, there has been increased interest in predictive biomarkers for preeclampsia. An effective predictive test would facilitate early diagnosis and targeted treatment and contribute to the timing of delivery. There are many difficulties in the prevention and management of pre-eclampsia. Research has shown that the neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR), as well as

the ratio between blood and blood products, may be promising in diagnosing the prognosis of inflammation of chronic low-level associated diseases and in identifying cell subtypes (5–9). Leukocyte count is higher in pregnant women with PE compared to normal pregnant women, mainly due to an increase in neutrophil count as opposed to a decrease in leukocyte count (10). As a result of these changes, the neutrophil/lymphocyte ratio (NLR) is expected to increase in PE compared to normal pregnancies (10,11). Endothelial dysfunction in preeclampsia leads to increased platelet consumption in the maternal bloodstream and uncontrolled intravascular platelet activation. Some studies have reported a significant decrease in platelet count and an increase in mean platelet volume (MPV) and platelet distribution width (PDW) in pregnant women with preeclampsia (12). Some studies claim that platelet/lymphocyte ratio (PLR) and PDW may predict preeclampsia (13,14). The aim of our study was to evaluate the role of biochemical markers in predicting preeclampsia.

MATERIALS AND METHODS

The present study had a retrospective case-control design following the Principles of the Helsinki Declaration. Informed consent documents were received from all patients. The study received approval from our hospital's Ethics Committee (Date: 25/12/2024, Number: 2024/373). In our study, 7240 women whose pregnancy follow-ups and deliveries were carried out by us between March 2018 and March 2024 were retrospectively screened. The patients in the study group consisted of 102 women aged 18-45 years with a singleton pregnancy who were diagnosed with preeclampsia at <34 weeks of

gestation. The patients in the control group consisted of 118 women aged 18-45 years who had no complications during pregnancy, had a singleton pregnancy, and gave birth at ≥ 37 weeks of gestation. Women with a history of assisted reproductive technologies, body mass index (BMI) $>30\text{kg/m}^2$ at the time of pregnancy diagnosis, birth with chromosomal anomalies, history of preterm birth, history of complicated birth, multiple pregnancy, and history of chronic disease and immunosuppressive treatment for any reason were excluded. For the preeclampsia diagnosis, females having hypertension must also exhibit proteinuria, which is defined as the presence of minimum 300-mg of protein in a 24-hour urine collection. Patients who satisfy the requirements for hypertension associated with preeclampsia but do not exhibit proteinuria or any severe additional complications are diagnosed with GHT (15). Demographic characteristics and obstetric and biochemical outcomes of the two groups were compared with each other. Blood tests of all patients performed at first trimester were evaluated retrospectively. NLR, PLR, MLR, SII, and SIRI values of all patients were analyzed. SII was calculated using the (neutrophil $\times$ platelet)/lymphocyte formula, and SIRI was calculated using the (neutrophil $\times$ monocyte)/lymphocyte formula (16,17).

Statistical Analysis

Statistical analysis was carried out by employing the-SPSS 26.0 (IBM-Inc-Chicago-IL-USA). The distribution normality was measured with the Kolmogorov Smirnov Test. The quantitative data of the patients were reported as mean $\pm$ Standard Deviation (SD) (minimum-maximum). The Mann-Whitney U test was used in the comparison of groups. The results were evaluated at a 95% Confidence-Interval (CI). The p-value, <0.05 , was regarded as statistically significant.

RESULTS

The BMI value was significantly higher in the preeclampsia group compared with the controls group ($p=0.016$). The birth weight value was significantly lower in the preeclampsia group compared with the controls group ($p<0.001$). The gestational week at delivery was significantly lower in the preeclampsia group compared with the controls group ($p<0.001$) (Table-1).

Lymphocyte and platelet counts was significantly lower in the preeclampsia group compared with the controls group ($p<0.001$ and $p<0.001$, respectively). NLR was significantly higher in the preeclampsia group compared with the controls group ($p=0.022$). SII was significantly higher in the preeclampsia group compared with the controls group ($p<0.001$) (Table-2).

Table-1. Comparison of demographic and obstetric data according to the presence of pre-eclampsia

	Preeclampsia n=102	Controls n=118	p*
	Mean $\pm$ SD		
Age (years)	29.5 $\pm$ 3.8	28.6 $\pm$ 3.7	0.82
BMI (kg/m ²)	24.2 $\pm$ 3.6	23.3 $\pm$ 3.5	0.016
Smoking, n (%)	9 (8.8%)	10 (8.4%)	0.68
Gravidity	2 (1-3)	2 (1-4)	0.78
Parity	2 (1-3)	2 (1-3)	0.90
Birth weight (g)	2720 $\pm$ 280	3380 $\pm$ 430	<0.001
Gestational week	36.4 $\pm$ 1.2	39.1 $\pm$ 1.1	<0.001

* Mannn-Whitney U test, BMI: Body mass index

Table-2. Comparison of biochemical parameters according to the presence of preeclampsia

	Preeclampsia n=102	Controls n=118	p*
	mean ± SD		
Neutrophil (10 ³ /μL)	7.1±2.2	6.8±2.1	0.42
Lymphocyte (10 ³ /μL)	1.82±0.54	2.17±0.66	<0.001
Platelet (10 ³ /μL)	211.3±52.5	249.4±58.6	<0.001
NLR	3.88±1.16	3.12±1.24	0.022
PLR	116.2±54.4	115.8±53.8	0.76
MLR	0.19±0.04	0.18±0.05	0.68
MPV (fL)	9.2±1.28	9.4±1.22	0.46
SII	832.3±116.4	720.3±108.6	<0.001
SIRI	390.8±66.8	376.6±66.8	0.72

* Mann-Whitney U test, NLR: Neutrophil/lymphocyte-ratio, PLR: Platelet/lymphocyte-ratio, MLR: Monocyte/lymphocyte ratio, MPV: Mean platelet volume, SII: Systemic immuno-inflammation-index, SIRI: Systemic inflammatory response-index

DISCUSSION

Although there are many studies on pre-eclampsia in the literature, diagnostic tests to predict pre-eclampsia are limited. In our study, BMI values were significantly higher in the preeclampsia group compared to the control group, while birth weight and gestational age at birth were significantly lower. In addition, lymphocyte and platelet counts showed a significant lower in the preeclampsia group, while NLR and SII values showed a significant higher.

BMI was significantly higher in the preeclampsia group than in the control group, indicating that increased BMI may be a contributing factor to the development of preeclampsia. In the study conducted by Robillard et al., it was revealed that high BMI showed a linear relationship with late-onset preeclampsia in particular. It was also emphasized that BMI was an independent risk factor (18). The study by Sohlberg et al. showed that high BMI significantly increases the risk of preeclampsia (19). Our study supports previous research emphasizing the relationship between maternal obesity and hypertensive disorders in pregnancy.

In our study, when parameters such as neonatal outcomes, birth weight and gestational age were taken into account, pregnant women in the preeclampsia group were significantly negatively affected. Birth weight and gestational age at birth were significantly lower in the preeclampsia group, revealing the negative effects of

preeclampsia on fetal development and timing of birth. In the study conducted by An et al., it was revealed that preeclampsia significantly increases the risk of preterm birth (20). The meta-analysis by Bossung et al. confirmed that preeclampsia increases the risk of preterm birth, especially in cases of severe or early-onset preeclampsia (21). In the study conducted by Gunnarsdottir et al., it was emphasized that babies exposed to preeclampsia generally have lower birth weight and that this has a significant effect regardless of the gestational week (22). These findings highlight the need for careful prenatal care and timely intervention to optimize neonatal outcomes in pregnancies complicated by preeclampsia.

When biochemical parameters were evaluated, lymphocyte and platelet counts were significantly lower in the preeclampsia group. These changes reflect the systemic inflammatory response and endothelial dysfunction specific to preeclampsia. In the study conducted by Walle et al., a decrease in platelet counts was observed in the preeclampsia group and it was stated that this parameter was related to the severity of the diseases (23). In addition, unlike our study, lymphocyte counts were also evaluated but no significant difference was found (23). In the study conducted by Toptas et al., a decrease in PLR levels was detected in patients with preeclampsia. However, no statistical difference was found in lymphocyte levels (24).

In our study, an increase in NLR and SII values were found in the preeclampsia group, indicating an increased inflammatory condition in preeclampsia. In the study conducted by Oğlak et al., it was shown that NLR values in the first trimester were significantly higher in women with preeclampsia compared to healthy pregnant women. It has been stated that NLR may be a potential marker for the prediction of preeclampsia (25). In the study conducted by Zheng et al., it was reported that NLR has a moderate diagnostic value in predicting preeclampsia. It was stated that NLR can be a helpful tool in the diagnosis of preeclampsia because it reflects the inflammatory process (26). In the study conducted by Çallıoğlu et al., they found that SII could be a valuable biomarker in preeclampsia risk groups (27). In the study by Akdulum et al., SII was shown to be significantly higher in patients who developed preeclampsia. This emphasizes the importance of inflammation in the pathogenesis of preeclampsia (28). These markers can be used as potential indicators of the severity and progression of the condition, consistent with previous studies.

Overall, our findings are consistent with current evidence and reinforce the multifactorial pathophysiology of preeclampsia, including contributions from maternal obesity, systemic inflammation, and endothelial dysfunction. These

results highlight the importance of early diagnosis and targeted management strategies to reduce adverse maternal and neonatal outcomes associated with this condition. The retrospective design of the study and the small sample size can be shown as limitations. However, the evaluation of many biochemical parameters in terms of two groups can be shown as a strength of the study.

CONCLUSION

Our study reveals important clinical and laboratory differences between preeclamptic and normotensive pregnancies. Increased inflammatory markers have been found to be a feature of pregnancies complicated by preeclampsia and are consistent with existing literature. These findings can be used to determine early diagnosis and comprehensive management strategies to minimize the risks associated with preeclampsia and improve both maternal and neonatal outcomes. However, prospective studies with large sample sizes are needed to evaluate the usefulness of inflammatory markers in the early diagnosis and monitoring of preeclampsia.

Conflicts of interest: Authors declared no conflict of interest.

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Pregnancy outcomes and risks in over 40; a retrospective analysis from Turkey's highest birth rate region

40 yaş üstü kadınlarda gebelik sonuçları ve riskler; Türkiye'nin en yüksek doğum oranına sahip bölgesinin retrospektif analizi

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ABSTRACT

Aim: Pregnancies at advanced maternal age pose significant global health concerns, particularly in regions with high birth rates. This study evaluates the pregnancy outcomes, associated risks, and strategies to improve maternal and neonatal health among women aged 40 and above in Siverek, Şanlıurfa, Turkey—an area with the highest crude birth rate in the country.

Materials and Methods: The short-term pregnancy outcomes of 107 women aged 40 years and older, who delivered at a second level state hospital's obstetrics and gynecology clinic between 2020 and 2021, were retrospectively analyzed.

Results: The study revealed a high average gravida (6.4 ± 2.3) and parity (5.2 ± 2.2) among participants, reflecting the region's high fertility rates. Normal spontaneous vaginal delivery occurred in 69 (64.5%) cases, while 38 (35.5%) women underwent cesarean delivery, with prior cesarean section being the leading indication (31.6%). The mean gestational age was 38.7 ± 1.6 weeks, and the average birth weight was 3282 ± 488 grams. Neonatal outcomes were favorable, with mean Apgar scores of 7.7 ± 1.2 (at the first minute) and 8.8 ± 1.2 (at the fifth minute). However, 2 (1.9%) mothers required blood transfusions, and 5 (4.7%) neonates experienced asphyxia-related complications.

Conclusions: Pregnancies in women over 40 are associated with higher obstetric and neonatal risks, requiring meticulous monitoring and individualized care strategies. These findings emphasize the need for targeted interventions to enhance maternal and neonatal outcomes in regions with high fertility rates and limited healthcare resources.

Keywords: Multipar, Pregnant Women, Blood Transfusion, Apgar Score, Advanced Maternal Age

Öz

Amaç: İleri anne yaşı gebelikleri, özellikle yüksek doğum oranlarının görüldüğü bölgelerde önemli küresel sağlık sorunları oluşturmaktadır. Bu çalışma, Türkiye'nin en yüksek kaba doğum oranına sahip Siverek, Şanlıurfa'da 40 yaş ve üzeri kadınlar arasında gebelik sonuçlarını, ilgili riskleri ve anne ve yenidoğan sağlığını iyileştirmek için potansiyel stratejileri değerlendirmektedir.

Gereç ve Yöntem: 2020-2021 yılları arasında kırsal bir devlet hastanesinin kadın doğum kliniğinde doğum yapan 40 yaş ve üzeri 107 kadının kısa dönem gebelik sonuçları retrospektif olarak analiz edilmiştir.

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Bulgular: Çalışma, katılımcılar arasında yüksek ortalama gravida ($6,4 \pm 2,3$) ve parite ($5,2 \pm 2,2$) oranlarını ortaya koyarak bölgedeki yüksek doğurganlık oranlarını yansıtmıştır. Olguların 69'unda (%64,5) normal spontan vajinal doğum gerçekleşirken, 38'inde (%35,5) sezaryen doğum yapılmış, sezaryen endikasyonlarının başında önceki sezaryen öyküsü (%31,6) gelmiştir. Ortalama gebelik süresi $38,7 \pm 1,6$ hafta, ortalama doğum ağırlığı ise 3282 ± 488 gram olarak tespit edilmiştir. Yenidoğan sonuçları olumlu olup, ortalama Apgar skorları 1. dakikada $7,7 \pm 1,2$ ve 5. dakikada $8,8 \pm 1,2$ olarak kaydedilmiştir. Ancak 2 anne (%1,9) kan transfüzyonuna ihtiyaç duymuş ve 5 yenidoğan (%4,7) asfiksiye bağlı komplikasyonlar yaşamıştır.

Sonuç: 40 yaş üstü kadınlarda gebelikler, daha yüksek obstetrik ve neonatal risklerle ilişkilidir ve dikkatli izlem ile bireyselleştirilmiş bakım stratejileri gerektirir. Bu bulgular, yüksek doğurganlık oranlarına ve sınırlı sağlık hizmeti kaynaklarına sahip bölgelerde anne ve yenidoğan sonuçlarını iyileştirmek için hedefe yönelik müdahalelerin önemini vurgulamaktadır.

Anahtar Sözcükler: Multipar, Gebe , Kan Transfüzyonu, Apgar Skoru, İleri Anne Yaşı

INTRODUCTION

The International Federation of Gynecology and Obstetrics (FIGO) classifies pregnancies in women aged 35 and older as "advanced maternal age" and those aged 40 and older as "very advanced maternal age" to standardize pregnancy management and treatment protocols (1). Over the past decade, factors such as delayed marriage, second marriages, women's growing societal roles, extended education and career planning, effective contraception, and advancements in in vitro fertilization have contributed to delayed pregnancies (2-4). While fertility rates have declined in developed and industrialized cities, they remain considerably higher in non-industrialized regions where early marriage is common (5,6). For instance, in 2022, Şanlıurfa, a city in Turkey's southeastern region, recorded the highest total fertility rate in the country, averaging 3.59 children per woman. The province also reported the highest crude birth rate, with 27.3 births per thousand that year (7). Pregnancies among women of very advanced maternal age are notably more frequent in this region, driven by larger family sizes and elevated fertility rates. There is a significantly increased risk of fetal and neonatal complications in pregnancies of very advanced maternal age (8,9). Studies have shown that pregnancies in women of very advanced age are associated with increased risks of gestational diabetes mellitus (GDM), hypertension, postpartum hemorrhage, preterm birth, placenta previa, placental abruption, intrauterine growth restriction (IUGR), low birth weight (SGA), and macrosomia (10-15).

In this study, we aimed to examine the impact of very advanced maternal age on obstetric

outcomes in a rural population in southeastern Turkey, which has the highest fertility rate in the country. To our knowledge, no prior clinical study has specifically investigated obstetric outcomes in very advanced maternal age pregnancies within a population with such elevated fertility rates as our study cohort.

MATERIALS AND METHODS

In this retrospective study, pregnant women who presented for delivery at the Obstetrics and Gynecology Clinic of a second level state hospital between March 2020 and July 2021 were examined. The study included a total of 107 pregnant women aged 40 years or older. The patients' data were retrospectively reviewed and recorded from the hospital's digital records system and patient files. Pregnancies under 40 years of age were excluded from the study.

Obstetric histories, maternal and neonatal outcomes, as well as hemogram and biochemical results, were recorded. This study was approved by the Ethics Committee of University of Esenyurt (dated 07.09.2023, No. 2023/08-15).

Statistical Analysis

Statistical analyses were performed using SPSS 27. Descriptive statistics included means and standard deviations for continuous variables and frequencies and percentages for categorical variables. The Kolmogorov-Smirnov test was used to assess the normality of the data distribution. Parametric tests were applied to normally distributed data, while non-parametric tests were used for non-normally distributed data. ANOVA was utilized to evaluate the relationship between the number of abortions and maternal

age in the study population. A p-value of <0.05 was considered statistically significant.

RESULTS

In our study, the mean age of the participants was 41.5 ± 1.8 years. The mean gravida was 6.4 ± 2.3 , the mean parity was 5.2 ± 2.2 , and the mean number of abortions was 0.16 ± 0.42 . It was found that 69 women (64.5%) had normal spontaneous vaginal deliveries, while 38 women (35.5%) delivered by cesarean section. Among those who delivered by cesarean section, 12 cases (31.6%) had a history of previous cesarean as the indication. The indications for cesarean section and obstetric data are presented in (Table-1). No significant difference was found between the number of abortions and maternal age ($p=0.377$) (Figure-1).

For pregnancies in women aged 40 and older, the mean gestational age was 38.7 ± 1.6 weeks, the mean birth weight was 3282 ± 488 grams, and the mean length was 50.5 ± 2.1 cm. The mean head circumference was 35.0 ± 1.4 cm, the mean 1-minute Apgar score was 7.7 ± 1.2 , and the mean 5-minute Apgar score was 8.8 ± 1.2 . Two women (1.9%) required blood transfusions due to atony, but no complications were noted in their newborns. Additionally, 5 women (4.7%) had newborns with asphyxia-related complications (Table-2).

The mean hemoglobin level for women aged 40 and older was 12.0 ± 1.4 g/dL, the mean hematocrit was $35.4 \pm 4.3\%$, the mean platelet count was $246 \pm 61 \times 10^9/L$, and the mean leukocyte count was $11.9 \pm 3.4 \times 10^9/L$ (Table-3).

Table-1. Distribution of general characteristics of the study.

Variable (n=107)	Mean $\pm$ S.S.	
Age	41.58 ± 1.82	
Gravida	6.43 ± 2.30	
Parity	5.29 ± 2.29	
Abortion	0.16 ± 0.42	
	n	%
Delivery type		
NSVD	69	64.5
C/S	38	35.5
C/S indication		
Foot presentation	1	2.6
Detachment	2	5.3
Old C/S	12	31.6
FD	11	28.8
Unprogressed labor	3	7.9
Cord	2	5.3
Breast	5	13.2
Pre-eclampsia	2	5.3

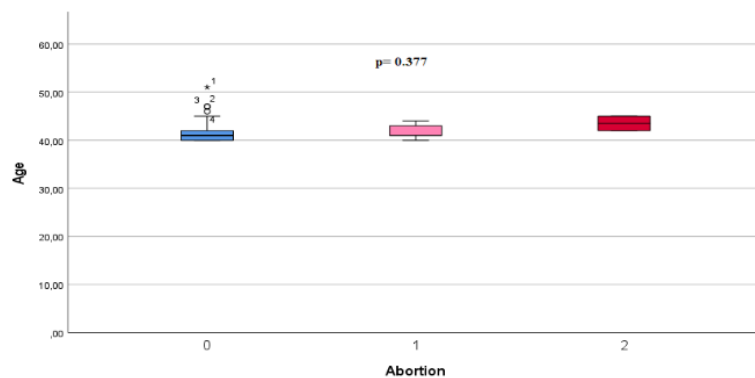


Figure-1. Relationship between the number of abortions and maternal age in the study population

Table-2. Distribution of some characteristics of the study

Variable (N=107)	Mean $\pm$ S. S.	
Gestational week	38.7 $\pm$ 1.6	
Weight	3282 $\pm$ 488	
Height	50.5 $\pm$ 2.1	
Head circumference	35.0 $\pm$ 1.4	
1st minute Apgar	7.7 $\pm$ 1.2	
5th minute Apgar	8.8 $\pm$ 1.21	
	n	%
Complication		
None	101	94.3
Transfusion (atony)	2	1.9
Preeclampsia	2	1.9
Placental abruption	2	1.9
Neonatal complication		
None	102	95.3
Asphyxia	5	4.7

Table-3. Distribution of biochemical characteristics related to the study

Variable (N=107)	Mean $\pm$ S. S.
Hgb	12.0 $\pm$ 1.4
Hct	35.4 $\pm$ 4.3
PLT	246 $\pm$ 6.7
WBC	11.9 $\pm$ 3.2

Abbreviations: Hgb: Hemoglobin, Hct: Hematocrit, PLT: Platelet Count, WBC: White Blood Cell Count

DISCUSSION

Women's increased participation in social and professional life, along with the availability of effective contraception, has contributed to delayed pregnancy planning (16,17). However, the drivers of advanced-age pregnancies differ across levels of societal development (18,19). In industrialized regions, delays are primarily due to women's active involvement in professional and social pursuits. In contrast, in non-industrialized regions, women continue to have children into advanced ages due to extended fertility periods and the influence of large family structures (20,21). Furthermore, the limited access to modern contraceptive methods for women in these regions is another contributing factor to advanced-age pregnancies. Our study cohort consisted of pregnant women aged 40 and above from an underdeveloped region in southeastern Anatolia known for its high birth rates.

In this study, the mean age of participants was 41.5 $\pm$ 1.8 years, with a mean gravida of 6.4 $\pm$ 2.3 and a mean parity of 5.2 $\pm$ 2.2. Similar studies in literature have reported mean ages of 41.6 $\pm$ 2 (22), 41.3 $\pm$ 1.8 (24), and 40.7 $\pm$ 2.4 years (24), which are consistent with our findings. However, compared to other studies (22,23), our study

demonstrated relatively higher gravida and parity values.

It is well known that increased parity and gravida at very advanced ages can be associated with adverse obstetric and perinatal mortality and morbidity (25). The elevated maternal age, gravida, and parity observed in our cohort, compared to other regions of Turkey, can be attributed to the unique socio-cultural and economic dynamics of the region. The region in question, Şanlıurfa, has a relatively weaker socio-cultural and economic profile compared to the rest of Turkey (26).

This may impact on family structure, reproductive motives, and socio-cultural perspectives within the study cohort, leading to a distinct demographic profile compared to other regions of the country

The rate of cesarean delivery increases with advancing maternal age. Eckner et al. reported a cesarean delivery rate of 43.1% in women aged 40 and older (27). Akarsu et al. reported a cesarean delivery rate of 63% in very advanced-age pregnancies (22). In our study, the cesarean delivery rate was 35% (38 cases). The lower cesarean delivery rate in this study, compared to the literature, may be attributed to a stronger

preference for larger families, the need for a quicker return to daily life, and a cultural inclination toward vaginal delivery in the region. The most common indication for cesarean delivery in our study was a prior cesarean section (31.6%, 12 patients), followed by fetal distress (28.8%, 11 patients). Other indications included breech presentation, placental abruption, failure to progress, cord prolapse, malpresentation, and preeclampsia.

In the study by Akarsu et al., the most common indication for cesarean delivery was a prior cesarean section (35%), followed by fetal distress (21%) (22). Similarly, another study reported a prior cesarean as the leading indication (55.3%), followed by fetal distress (12.1%) (23).

The literature presents conflicting findings on the relationship between fetal birth weight and advanced maternal age. Yogev et al. and Hsieh et al. reported that the number of low-birth-weight infants increases with advancing maternal age, while the same trend is not observed for infants with macrosomia (8,28). Other studies have reported no statistically significant differences in low birth weight among different maternal age groups (23,29). In our study, the mean birth weight was 3282 ± 488 grams, consistent with the literature (22), indicating that the impact of pregnancies in women aged 40 and older on birth weight may be minimal.

Advanced maternal age is associated with increased perinatal and obstetric complications, including preeclampsia, preterm birth, placental attachment abnormalities, and placental abruption (1-4). The relatively low incidence of these complications in our study may be attributed to the small sample size and the referral of high-risk and comorbid pregnant women to tertiary care hospitals. In our study, the rate of bleeding requiring transfusion was consistent with the findings reported in the literature (30). Studies have shown that while hemogram levels do not significantly differ between advanced maternal age pregnancies and other age groups, older mothers tend to experience greater blood loss and lower hemogram levels, which has been attributed to multiparity (31). There was no significant difference between the 1st and 5th-minute Apgar scores, aligning with findings in the literature (32). This suggests that the Apgar score may have a limited ability to predict perinatal mortality in pregnancies at very advanced maternal ages.

One of the notable findings of this study is the increased asphyxia rates associated with advanced maternal age (4.7%). It is thought that the physiological and biochemical changes that occur due to advanced maternal age render the necessary adaptation mechanisms inadequate during labour and thus make it difficult to tolerate the oxygenation and metabolic stress exposed during labour. This, together with physiological changes during pregnancy, may directly affect fetal oxygenation and the risk of neonatal asphyxia. It is thought that the limited biological abilities of the woman may affect the healthy delivery process and lead to an increase in complications such as asphyxia. Alsharif et al. In their study, they found that asphyxia rates increased significantly as maternal age increased (33). However, in another study, asphyxia was 2 times more common in the group with advanced maternal age compared to the reproductive period, and the risk of asphyxia increased 4 times (34). In contrast to these studies, Gündoğdu et al. considered maternal age of 35 years and older as a risk factor for asphyxia, and when the infants exposed to asphyxia were analyzed, it was reported that maternal age ranged between 17-48 years, and the mean age was 27.9 ± 6.6 years. In this study, no statistically significant relationship was found between maternal age and perinatal asphyxia (35). Awoyesuku et al. categorized maternal ages in infants with asphyxia and found no significant difference between ages (36).

CONCLUSION

This study is among the important to examine maternal and neonatal outcomes in pregnancies at very advanced maternal ages in a socioeconomically and culturally underdeveloped region of Turkey. Our findings, including higher rates of gravida and parity, increased cesarean delivery rates, and a rise in obstetric complications, indicate that women in this region continue to bear children actively throughout their entire reproductive period and into advanced ages. However, the most important finding of this study is the increased rates of asphyxia. Although normal delivery is more common at very advanced maternal age, it has been found to result in a consequence: increased asphyxia rates. Therefore, pregnancies in women of very advanced maternal age should be closely monitored due to the increased maternal and

neonatal risks, with careful attention to obstetric complications.

Conflicts of interest: Authors declared no conflict of interest.

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Restless leg syndrome in multiple sclerosis: relationship with restless legs syndrome and fatigue, quality of life, daytime sleepiness, sleep quality and anxiety-depression

Multipl sklerozda huzursuz bacak sendromu: huzursuz bacak sendromu ile yorgunluk, yaşam kalitesi, gündüz uykululuğu, uyku kalitesi ve anksiyete-depresyon arasındaki ilişki

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ABSTRACT

Aim: This study aimed to explore the association between Restless Legs Syndrome (RLS) severity and fatigue, quality of life, daytime sleepiness, sleep quality, and levels of anxiety and depression in people with multiple sclerosis (MS, pwMS).

Materials and Methods: A total of 73 pwMS were recruited from the MS clinic at Van Yüzüncü Yıl University. RLS was diagnosed based on the International RLS Study Group. Various scales were used to assess sleep quality, fatigue, daytime sleepiness, quality of life, and depression and anxiety, including the Pittsburgh Sleep Quality Index (PSQI), Modified Fatigue Impact Scale (MFIS), Epworth Sleepiness Scale (ESS), Preference-Based Multiple Sclerosis Index (PBMSI), and the Hospital Anxiety and Depression Scale (HADS).

Results: RLS was identified in 20 participants (27.40%). Significant differences were observed between pwMS with and without RLS in terms of subjective sleep quality, sleep disturbances, daytime dysfunction, sleep latency and habitual sleep efficiency sub-scores of the PSQI, total PSQI score, physical, cognitive, and total fatigue scores ($p < 0.05$). RLS severity showed a moderate positive correlation with MFIS-physical ($r = 0.535$), MFIS-total ($r = 0.494$), and PSQI-total scores ($r = 0.492$).

Conclusion: This study sheds light on the relationships between RLS severity and factors such as sleep quality and fatigue in pwMS.

Keywords: Multiple sclerosis, Restless leg syndrome, Sleep disorders, Quality of life

ÖZ

Amaç: Bu çalışma, multipl sklerozlu (MS) bireylerde huzursuz bacaklar sendromu (HBS) şiddeti ile yorgunluk, yaşam kalitesi, gündüz uykululuk, uyku kalitesi ve anksiyete-depresyon seviyeleri arasındaki ilişkiyi incelemeyi amaçlamaktadır.

Gereç ve Yöntem: Van Yüzüncü Yıl Üniversitesi MS kliniğinden toplam 73 MS'li birey çalışmaya dahil edildi. HBS tanısı, Uluslararası HBS Çalışma Grubu tarafından belirlenen kriterler esas alınarak konuldu.

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Uyku kalitesi, yorgunluk, gündüz uykululuğu, yaşam kalitesi, depresyon ve anksiyete düzeylerini değerlendirmek için Pittsburgh Uyku Kalitesi İndeksi (PUKİ), Modifiye Yorgunluk Etki Ölçeği (MYEÖ), Epworth Uykululuk Ölçeği (EUÖ), Tercihe Dayalı Multiple Skleroz İndeksi (TDMSİ) ve Hastane Anksiyete ve Depresyon Ölçeği (HADÖ) gibi çeşitli ölçekler kullanıldı.

Bulgular: HBS, 20 katılımcıda (%27,40) tespit edildi. HBS'li ve HBS'siz MS'li bireyler arasında fiziksel, bilişsel ve toplam yorgunluk skorları ile uyku kalitesi değerlendirmesinin subjektif uyku kalitesi, uyku bozuklukları, gündüz işlev bozukluğu, uykuya dalma süresi, efektif uyku alışkanlığı alt skorları ve total PUKİ skoru açısından anlamlı farklılıklar bulundu ($p<0,05$). HBS şiddeti, MYEÖ-fiziksel ($r=0.535$), MYEÖ-toplam ($r=0.494$) ve PUKİ-toplam skorları ile orta derecede pozitif korelasyon gösterdi ($r=0.492$).

Sonuç: Bu çalışma, HBS şiddeti ile uyku kalitesi, yorgunluk ve genel yaşam kalitesi arasındaki ilişkiler hakkında yeni bilgiler sunmaktadır.

Anahtar Sözcükler: Multipl skleroz, Huzursuz bacak sendromu, Uyku bozuklukları, Yaşam kalitesi

INTRODUCTION

Multiple Sclerosis (MS) is a chronic, progressive disease that affects the nervous system by causing demyelination, impacting over 2.5 million individuals worldwide. It primarily affects young adults between 20 and 40 years old, with a higher incidence in women(1) . Although precise data on the prevalence of MS in Turkey is lacking, estimates place it at roughly 96.4 per 100,000 (2). Recent epidemiological studies have noted a rising trend in both the incidence and prevalence of MS (3).

Restless Legs Syndrome (RLS), a sleep disorder, is characterized by uncontrollable, unpleasant sensations in the legs, typically worsening during rest or inactivity, accompanied by a strong urge to move the affected limbs. Meta-analyses have shown that the prevalence of RLS among people with MS (pwMS) ranges from 13.2% to 65.1% (4), and case-control studies reveal that pwMS have a 5.6-fold higher likelihood of developing RLS than the general population (5). Among pwMS, high disability levels and the presence of cervical cord lesions are notable risk factors for RLS (6).

In the general population, RLS tends to be more prevalent in women, older adults, and individuals with a family history of the condition. However, no conclusive evidence shows that this pattern holds in pwMS (7). While some research has found a link between MS duration and the presence of RLS (8,9), other studies have not confirmed this relationship (10,11). Cederberg et al. explored the association between RLS in pwMS and health-related quality of life (HRQoL), finding that those with RLS had lower physical and psychological HRQoL scores compared to pwMS

without RLS (12). Additionally, MS patients with RLS were found to experience more severe fatigue and more frequent anxiety symptoms. A moderate negative correlation was found between RLS severity and HRQoL, though this relationship weakened when factors such as fatigue, depression, and anxiety were considered (12).

The findings suggest that symptoms such as fatigue, depression, and anxiety can mediate the link between HRQoL and RLS severity. The study also highlighted the potential importance of anxiety in understanding the relationship between HRQoL and RLS severity, although the exact pathological connection between RLS and anxiety remains unclear. The possible explanation is that anxiety and RLS share several common features, such as muscle tension, restlessness, irritability, fatigue, sleep disturbances, and difficulty concentrating. Consequently, the uncontrollable and unpredictable nature of RLS symptoms may exacerbate anxiety, further diminishing the quality of life in pwMS (12).

While it is well established that RLS is more common in pwMS than in the general population, the precise relationship between RLS severity and other frequently occurring MS-related issues—such as fatigue, HRQoL, daytime sleepiness, sleep quality, and levels of anxiety and depression—remains unclear. This study aims to identify the factors related to RLS in pwMS, with the goal of informing effective treatment strategies to address this common symptom.

MATERIALS AND METHODS

Study Design

This research which has cross-sectional study design involved pwMS who voluntarily participate while attending their routine check-ups at the Neurology Department's MS Clinic of Van Yüzüncü Yıl University Medical Faculty Hospital. Ethic approval was received from Van Yüzüncü Yıl University Noninvasive Research Ethics Committee dated 24.06.2024 with approval number 2024/07-04.

Participants

In a previous study evaluating sleep quality among MS patients with and without RLS, the effect size between the two groups was calculated to be 0.57. Based on the prevalence of RLS in the MS population, the rate of pwMS with and without RLS was determined to be 1/3. Using the G*Power software (version 3.1), it was determined that a minimum of 72 patients (pwMS with RLS: 18, pwMS without RLS: 54) is needed to achieve a power of 85% and an error probability of 0.05.

Inclusion criteria have a definitive diagnosis of MS, aged between 18-65 years, no recent relapse within the last 3 months, willing to participate in the study. Also, pwMS having severe musculoskeletal, cardiovascular, pulmonary, metabolic, or any other conditions that would prevent participation, conditions known to be associated with RLS, such as anemia, rheumatoid arthritis, pregnancy, neuropathies, uremia, or neurological disorders other than MS, severe cognitive impairment as determined by a physician that would prevent the completion of tests, pregnancy are excluded from the study.

Outcomes

Demographic Data

Information such as age, gender, and clinical data (type of MS, EDSS score, year of MS diagnosis, duration, date of the last relapse, medications used by the participants) have been collected.

Expanded Disability Status Scale (EDSS) is a widely accepted tool for evaluating and tracking the level of neurological impairment in MS patients, based on clinical neurological assessments and ambulation abilities. The scale ranges from 0, indicating a normal neurological

examination, to 10.0, which signifies death caused by MS. Scores between 1.0 and 4.5 represent full ambulation, while scores from 5.0 to 9.5 indicate various degrees of mobility limitations. Scores of 7.0 or higher reflect dependency on a wheelchair or bed. The EDSS evaluates several functional systems, including the pyramidal, cerebellar, cerebral, sensory, brainstem, visual, bladder, and bowel functions (13).

Criteria for Restless Legs Syndrome (RLS) Diagnosis was initially established by the RLS Study Group in 1995 and have since been revised in 2003 and 2014 (14). Diagnosis is based on the presence of an urge to move the legs due to uncomfortable sensations, symptoms beginning or worsening during rest, relief with movement, and a pattern of symptoms that worsens in the evening or night. Patients meeting all these criteria are diagnosed with RLS by a neurologist.

The International Restless Legs Syndrome Study Group Rating Scale (IRLS): Symptom severity among RLS patients can vary widely, with some experiencing mild symptoms that are alleviated by movement, while others suffer from more severe issues that affect sleep and daily life. To measure the severity of RLS symptoms, the International RLS Study Group developed a scale in 2002. The Turkish version, validated by Ay et al. in 2019 (15). This scale consists of 10 questions, each rated from 0 to 4 (16).

The Epworth Sleepiness Scale (ESS) is used to evaluate daytime sleepiness. It comprises eight questions that ask the patient to rate the likelihood of dozing off in different situations during a typical day. Responses are scored from 0 to 3, and a total score of 10 or higher indicates excessive daytime sleepiness (17). The Turkish version of the scale has been validated as reliable (18).

The Modified Fatigue Impact Scale (MFIS) is commonly used to assess the impact of fatigue. It contains 21 questions that measure the physical, cognitive, and social side of fatigue. Lower scores reflect less fatigue [18]. The Turkish version has been validated, confirming that it is suitable for Turkish MS patients (19).

Buyse et al. developed the *Pittsburgh Sleep Quality Index (PSQI)*. In 1996, Ağargün et al. adapted it into Turkish and made it a self-report tool for assessing sleep quality and disturbances.

The 19-item questionnaire is divided into seven components, each scoring between 0 and 3, providing a total score between 0 and 21 (20,21).

The Hospital Anxiety and Depression Scale (HADS) is used to assess levels of anxiety and depression in participants. It consists of 14 items each scored on a 4-point Likert scale from 0 to 3. The higher scores indicate more severe symptoms (22). The Turkish version, validated by Aydemir et al. in 1997, has been proven to be a reliable scale for assessing anxiety and depression in MS patients (23).

Preference-Based Multiple Sclerosis Index (PBMSI) focuses on five domains: walking, concentration, fatigue, roles/responsibilities, and mood. Each domain offers three response options, with values ranging from 0, meaning the worst health state, to 1, meaning the best (24). The Turkish version of the PBMSI has been validated for reliability by Kahraman et al. (25).

Data Analysis

The data have been analyzed using IBM SPSS Statistics (Version 25.0, Armonk, NY: IBM Corp.). The normality of the data distribution has been checked using the Kolmogorov-Smirnov test and histograms. The median and interquartile ranges were used to present continuous variables. For categorical variables, frequency and percentage values were provided. The Mann-Whitney U test compared clinical and demographic factors and patient-reported outcomes between the groups (RLS positive and RLS negative participants). Effect sizes (ES) were calculated as Cohen's d for between-group comparisons. These effect sizes were interpreted as small (0.2), medium (0.5), and large (0.8) (26). The relationship between RLS severity and other variables in RLS-positive groups has been evaluated using Spearman correlation coefficients. Correlation has been considered small if ≤ 0.30 , moderate if between 0.31 and 0.59, and strong if ≥ 0.60 (27). Statistical significance has been set at $p < 0.05$.

RESULTS

A total of 73 pwMS, 20 with RLS and 53 without RLS, were included in the study. The distribution of the pwMS with RLS according to RLS severity assessed with IRLS was as follows: 3 participants had very severe, 4 participants had severe, and 13 participants had moderate RLS symptoms. The mean RLS severity was 19.95 ± 7.0 (min/max: 12/35). There were no significant differences between RLS-positive and RLS-negative pwMS in terms of age, gender, EDSS score, disease duration of MS, number of relapses, and the MS type ($p > 0.05$). (Table-1) provides comprehensive details on the participants' demographic and clinical characteristics.

There was a significant difference between RLS-positive and negative pwMS regarding perceived physical, cognitive and total fatigue scores, and sleep latency and habitual sleep efficiency sub-scores of the sleep quality questionnaire with a medium effect size ($p < 0.05$). Also, a significant difference was determined in subjective sleep quality, sleep disturbances, daytime dysfunction and total score of PSQI with a large effect size ($p < 0.05$). There were no significant differences between groups in quality of life, psychosocial parameters of the fatigue scale, depression and anxiety level, and sleep duration sub-score of the sleep quality assessment ($p > 0.05$). The detailed information is presented in (Table-2).

There was a moderately positive correlation between RLS severity, physical and total fatigue scores, and total sleep quality score ($p < 0.05$). Conversely, there was no significant correlation between RLS severity and EDSS score, disease duration of MS, number of relapses, cognitive and psychosocial sub-scores of MFIS, quality of life, daytime sleepiness, and depression and anxiety level ($p > 0.05$). The correlation coefficients are presented in (Table-3).

Table 1. Demographic and clinical characteristics of the participants

	PwMS with RLS (n=20)	PwMS without RLS (n=53)	<i>p</i>
Age (years)	33.0 (25.75; 44.25) Min/max: 22/59	29.0 (24.0; 35.0) Min/max: 18/58	0.109
Gender, n (%)			
Female	16 (80.0%)	38 (71.7%)	0.471
Male	4 (20.0%)	15 (28.3%)	

EDSS score	1.25 (0; 2.0) Min/max: 0/3.5	1.0 (0; 2.0)	0.947
Disease duration of MS (years)	8.0 (5.0; 13.0) Min/max: 1/29	6.0 (2.25; 9.0) Min/max: 0/19	0.052
Number of relapses	3.0 (2.0; 4.0) Min/max: 1/11	2.0 (1.25; 4.0) Min/max: 1/10	0.481
MS type, n (%)			
RRMS	20 (100%)	51 (96.2%)	0.378
PPMS	0 (0%)	2 (3.8%)	

EDSS: Expanded Disability Status Scale, MS: Multiple sclerosis, RRMS: Relapsing Remitting MS, PPMS: Primary Progressive MS

Data are expressed as median and interquartile range (IQR)

Table-2. Comparison of patient-reported outcome measures between RLS-positive and RLS-negative pwMS

	PwMS with RLS (n=20)	PwMS without RLS (n=53)	Cohen's d	p
PBMSI	0.50 (0.38; 0.86) Min/max: 0.07/1	0.68 (0.54; 0.89) Min/max: 0.20/1	0.33	0.075
MFIS-Physical	19.0 (10.50; 25.75) Min/max: 1/32	11.0 (5.0; 18.0) Min/max: 0/35	0.67	0.010
MFIS-Cognitive	20.0 (13.25; 30.75) Min/max: 4/36	11.0 (4.5; 22.5) Min/max: 0/38	0.71	0.007
MFIS-Psychosocial	4.0 (0.25; 6.0) Min/max: 0/8	1.0 (0; 4.0) Min/max: 0/8	0.41	0.124
MFIS-Total	49.0 (24.75; 58.0) Min/max: 11/76	26.0 (10.5; 37.0) Min/max: 0/77	0.72	0.006
PSQI- subjective sleep quality	2.0 (1.0; 2.0) Min/max: 0/3	1.0 (1.0; 1.0) Min/max: 0/3	1.03	<0.001
PSQI- sleep latency	2.0 (0.25; 3.0) Min/max: 0/3	1.0 (0; 2.0) Min/max: 0/3	0.64	0.023
PSQI- sleep duration	0 (0; 2.0) Min/max: 0/3	0 (0; 1.0) Min/max: 0/3	0.33	0.278
PSQI-habitual sleep efficiency	0 (0; 1.75) Min/max: 0/3	0 (0; 0) Min/max: 0/3	0.58	0.027
PSQI- sleep disturbances	2.0 (1.0; 2.0) Min/max: 0/3	1.0 (1.0; 1.0) Min/max: 0/2	0.93	0.001
PSQI- use of sleeping medication	0 (0; 0) Min/max: 0/3	0 (0; 0) Min/max: 0/0	NA	0.004
PSQI- daytime dysfunction	1.0 (0; 2.0) Min/max: 0/3	0 (0; 0.5) Min/max: 0/3	0.81	0.002
PSQI-total score	8.5 (3.25; 11.75) Min/max: 1/20	3.0 (2.0; 6.0) Min/max: 0/12	0.98	0.001
ESS	3.5 (1.25; 5.75) Min/max: 0/15	3.0 (0.5; 7.5) Min/max: 0/18	0.01	0.717
HADS-D	7.0 (1.5; 12.75) Min/max: 0/14	7.0 (4.0; 9.5) Min/max: 0/18	0.14	0.531
HADS-A	7.0 (4.25; 12.75) Min/max: 0/19	6.0 (3.0; 9.5) Min/max: 0/20	0.24	0.359

PBMSI: Preference-Based Multiple Sclerosis Index, MFIS: Modified Fatigue Impact Scale, PSQI: Pittsburgh Sleep Quality Index, ESS: Epworth Sleepiness Scale, HADS-D: Hospital Anxiety and Depression Scale-Depression, HADS-A: Hospital Anxiety and Depression Scale-Anxiety, RLS: Restless Legs Syndrome, MS: Multiple Sclerosis. Data are expressed as median and interquartile range (IQR)

Table-3. Correlation coefficients between the RLS-severity and patient-reported outcomes measurements and characteristics of MS

Variables	1	2	3	4	5	6	7	8	9	10	11	12	13
1. RLS severity	1												
2. Disease duration	-0.040	1											
3. EDSS	0.244	0.552*	1										
4. Number of relapses	0.002	0.266	0.133	1									
5. MFIS-Physical	0.535*	-0.122	0.185	-0.013	1								
6. MFIS-Cognitive	0.384	0.122	0.154	0.091	0.760**	1							
7. MFIS-Psychosocial	0.439	0.158	0.227	-0.095	0.758**	0.612**	1						
8. MFIS-Total	0.494*	-0.015	0.168	0.001	0.926**	0.926**	0.747**	1					
9. PBMSI	-0.307	0.140	-0.109	-0.287	-0.783**	-0.749**	-0.727**	-0.814**	1				
10. ESS	0.254	-0.014	0.134	-0.039	0.478*	0.620**	0.664**	0.630**	-0.668**	1			
11. PSQI-total	0.492*	-0.006	0.185	0.064	0.817**	0.734**	0.698**	0.794**	-0.730**	0.281	1		
12. HADS-D	0.182	0.232	0.131	-0.081	0.658**	0.692**	0.771**	0.671**	-0.613**	0.650**	0.547*	1	
13. HADS-A	0.346	0.025	0.112	-0.270	0.675	0.432	0.716**	0.594**	-0.456**	0.604**	0.439	0.748**	1

*Correlation is significant at the 0.05 level (2-tailed).

**Correlation is significant at the 0.01 level (2-tailed).

EDSS: Expanded Disability Status Scale, **PBMSI:** Preference-Based Multiple Sclerosis Index, **MFIS:** Modified Fatigue Impact Scale, **PSQI:** Pittsburgh Sleep Quality Index, **ESS:** Epworth Sleepiness Scale, **RLS:** Restless Legs Syndrome, **MS:** Multiple Sclerosis

DISCUSSION

This study provides important insights. Firstly, RLS-positive pwMS have worse sleep quality and fatigue levels than RLS-negative pwMS. Also, RLS severity is correlated with physical and general fatigue and sleep quality. On the other hand, RLS severity is not related to characteristics of MS such as disability level, disease duration, and number of relapses.

According to a meta-analysis conducted on RLS in MS, there are controversial results regarding the comparison of pwMS with and without RLS in terms of demographic and clinical characteristics such as EDSS score (17 studies), disease

duration (16 studies), MS types (11 studies), age (17 studies), and gender (17 studies). As a result, the rate of studies finding the relationship between RLS and demographic and clinical characteristics of pwMS was as follows: 52.9% for EDSS score, 18.8% for disease duration, 27.3% for MS types, 35.3% for age, and 18.8% for gender (4). Our study showed no significant differences between pwMS with and without RLS regarding EDSS score, disease duration, MS types, age, and gender. Even though there is a pattern for the presentation of RLS in the general population, the results are conflicted in MS. The reason for the conflicted results could be MS, which is a heterogeneous disease whose

presentation is changed from patient to patient. Also, studies use different assessment methods to diagnose RLS and evaluate RLS severity.

Cederberg et al. examined the relationship between RLS severity, and the common symptoms observed in MS. The study included a total of 275 pwMS; 74 (26.9%) were RLS-positive. When comparing RLS-positive MS individuals with RLS-negative ones, the RLS-positive group reported poorer physical and psychological health-related quality of life, higher perceived fatigue levels, and more anxiety symptoms. In the RLS-positive group, the severity of RLS was found to be associated with the physical and psychological sub-parameters of health-related quality of life, sleep quality, perceived fatigue, depression, and anxiety (12). Similarly, we found a significant difference between groups regarding sleep quality and fatigue. Also, there is a positive correlation between RLS severity and sleep quality. However, the groups showed parallel results in quality of life, depression, and anxiety levels in our study. This difference between studies could arise from participants with different disability levels. The average Patient Determined Disease Steps score was 3.5 in the Cederberg study (12), while the mean EDSS score was 1.24 in our study. The quality of life, depression, and anxiety levels could be worsened with disability level, and the presence of RLS is one of the factors affecting the deterioration at this point. Also, Sevim et al. conducted a nationwide, multicenter, cross-sectional study that assessed 1,068 MS patients using the Hamilton Anxiety and Depression Scales. Their results showed that patients with RLS had significantly higher anxiety and depression scores than those without. However, the percentage of secondary progressive MS, which is related to higher levels of anxiety and depression level compared to the other MS types (28), was higher than in our study.

Cederberg et al. investigated the relationship between physical function and RLS symptomology, such as presence, severity and frequency. They evaluated 22 pwMS with RLS and showed that physical function decreased as the severity of RLS increased (29). In another study, they enrolled 275 pwMS and divided them into two groups: pwMS with RLS (n=74) and without RLS (n=201). The authors assessed how cognitive functions are affected by the presence

and severity of RLS. The results revealed that pwMS with RLS have worse cognitive functions and increased RLS severity than without RLS, which is correlated to worse sleep quality and cognitive performance (29). In our study, even though we did not assess the physical and cognitive functions of the participants, we determined that pwMS with RLS have worse perceived physical and cognitive fatigue than pwMS without RLS. We hypothesize that the presence and severity of RLS causes worse sleep quality. The worse sleep quality beats a decline in cognitive and physical functions.

This study has both limitations and strengths. One limitation is that the participants' disability level was low, and most participants have relapsing-remitting MS, which may affect the generalizability of the findings. However, this is also a strength, as it reduces the potential confounding effects of other symptoms that may occur in individuals with higher levels of disability associated with MS. We did not assess the use of medication for restless legs syndrome (RLS) symptoms, which may have influenced the outcomes. Besides, we did not use objective sleep measurements. Although objective methods were not used, all selected assessment methods are valid, reliable, and have high psychometric properties in MS.

CONCLUSION

This study offers new insights into the connections between RLS severity, total sleep quality, physical component score, and total fatigue score in pwMS. Also, the total and most sub scores of the sleep quality and the fatigue scores (except psychosocial) showed the difference between pwMS with and without RLS. The findings suggest a potential approach to mitigating sleep quality and fatigue declines by addressing RLS. Understanding the intermediary factors linking RLS with sleep disturbances and fatigue in MS could provide important guidance for interventions.

Ethical Approval: Ethical approval was obtained from the Ethics Committee of Non-Interventional Clinical Trials of Van Yüzüncü Yıl University with the Approval Number: 2024/07-04, and Approval Date: 24.06.2024

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Evaluation of perioperative coagulation in patients undergoing brain and spine surgery

Beyin ve omurga cerrahisi geçiren hastalarda perioperatif koagülasyonun değerlendirilmesi

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ABSTRACT

Aim: Conditions during brain and spine surgery, such as trauma, surgery, general anesthesia, fluid infusions, hypothermia, and patient inactivity, may lead to coagulation disorders, increasing the risk of bleeding or thrombosis. This study aimed to evaluate the relationship between thromboelastography (TEG), coagulation, and perioperative transfusion.

Materials and Methods: In 2012, 58 adult ASA I–III patients undergoing brain and spine surgery were prospectively included after obtaining ethics committee approval and patient consent. Patients with coagulopathy or anticoagulants were excluded. Fluid requirements were managed with balanced crystalloids, and selected patients were randomly administered 6% hydroxyethyl starch or 4% gelatin solutions. Coagulation was monitored preoperatively, at the end of surgery, and 24 hours postoperatively using TEG and standard coagulation parameters.

Results: The mean age of the patients was 46.7 years, the mean BMI was 27.7, and 62.1% were female. Eleven patients (18.9%) required an average of 6.1 (± 4.1) mL/kg erythrocyte transfusion. Transfused patients had longer operative times ($p=0.022$), anesthesia durations ($p=0.014$), total fluid requirements ($p<0.001$), and crystalloid infusions ($p=0.039$). TEG data revealed no coagulation abnormalities causing clinical bleeding. However, postoperative Maximum Amplitude (MA) significantly increased in both groups. MA showed a positive correlation with age ($r=0.27$, $p=0.039$) and crystalloid volume ($r=0.29$, $p=0.027$), and a negative correlation with the lowest temperature ($r=-0.28$, $p=0.029$). Coagulation Index (CI) was positively correlated with preoperative fibrinogen levels ($r=0.28$, $p=0.045$).

Conclusion: TEG analysis showed similar coagulation profiles in transfused and non-transfused patients. Before drawing general conclusions about perioperative coagulation, these findings should be compared with studies using restrictive transfusion protocols.

Keywords: Coagulation, thromboelastography, neurosurgery, transfusion

ÖZ

Amaç: Beyin ve omurga cerrahisi sırasında travma, cerrahi, genel anestezi, sıvı infüzyonları, hipotermi ve hastanın hareketsizliği koagülasyon bozukluklarına yol açarak kanama veya tromboz riski oluşturabilir. Bu çalışmada, tromboelastografi (TEG) kullanılarak koagülasyon, perioperatif kanama ve transfüzyon arasındaki ilişki değerlendirildi.

Gereç ve Yöntem: Etik kurul onayı ve hasta onamı alındıktan sonra, 2012 yılında beyin ve omurga cerrahisi geçiren 58 yetişkin ASA I–III hasta prospektif olarak çalışmaya dahil edildi. Koagülopatisi veya antikoagülan kullanan hastalar dışlandı. Sıvı ihtiyacı dengeli kristalloidlerle karşılandı; rastgele seçilen hastalarda %6 hidroksietil-starch veya %4 jelatin solüsyonları kullanıldı.

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Koagülasyon, cerrahi öncesinde, sonunda ve 24 saat sonrasında TEG ve standart koagülasyon parametreleri ile takip edildi.

Bulgular: Hastaların yaş ortalaması 46,7, BMI ortalaması 27,7 olup %62,1'i kadındı. Transfüzyon gerektiren 11 hastaya ortalama 6,1 ($\pm 4,1$) mL/kg eritrosit verildi. Bu hastalarda operasyon süresi ($p=0,022$), anestezi süresi ($p=0,014$), toplam sıvı ($p<0,001$) ve kristaloit infüzyon miktarı ($p=0,039$) daha fazlaydı. TEG verilerine göre klinik kanamaya yol açacak koagülasyon anormalliği saptanmadı. Ancak, her iki grupta da postoperatif Maksimum Amplitüd (MA) anlamlı şekilde arttı. MA, yaş ($r=0,27$, $p=0,039$) ve kristaloit miktarı ($r=0,29$, $p=0,027$) ile pozitif; en düşük sıcaklık değeri ile negatif ($r=-0,28$, $p=0,029$) korelasyon gösterdi. Ayrıca Koagülasyon İndeksi (CI) preoperatif fibrinojen seviyesi ile pozitif korelasyon gösterdi ($r=0,28$, $p=0,045$).

Sonuç TEG analizleri, transfüzyon yapılan ve yapılmayan hastalarda koagülasyon profillerinin benzer olduğunu ortaya koydu. Perioperatif koagülasyon sürecine ilişkin genel kararlar vermeden önce, sonuçlarımızın restriktif transfüzyon rejimleri uygulanan çalışmalarla karşılaştırılması gerekmektedir.

Anahtar Sözcükler: Koagülasyon, nöroşirurji, transfüzyon, tromboelastografi

INTRODUCTION

Blood transfusion is a common occurrence during spinal surgery—prior studies have shown that anywhere from 6% to 32% of patients undergoing spine surgery require perioperative transfusion. Transfusion requirements for spine surgery patients vary depending on preoperative patient characteristics and surgical parameters (1). The decision to perform a transfusion is primarily influenced by factors such as the patient's age, preoperative hemoglobin levels, the length of surgery, and the surgeon's experience and preferences or factors like hypothermia and fluid infusions (2–6). Blood transfusion during neurosurgery is a typical procedure used to restore blood loss and maintain proper blood pressure and oxygenation of the patient. However, there is a strong relationship between perioperative blood transfusion and worsened outcomes following spine surgery. The authors reviewed that the main indicators for blood transfusions during intracranial meningioma surgery were blood loss and preoperative anemia. Perioperative hemostatic abnormalities can lead to either hemorrhagic or thrombotic complications. Neurosurgical patients are known to be particularly exposed to thromboembolic events, infection and longer hospital stays (1,7). Therefore, distinguishing bleeding due to surgery or coagulation disorders in the perioperative period and recognizing dynamic changes in hemostasis are necessary for safe patient management (4).

Nowadays, thromboelastography (TEG) has become widespread as a very useful and important method in understanding whether

perioperative bleeding is caused by coagulation disorder (8,9). In this study, we aimed to evaluate coagulation using TEG in patients undergoing brain and spine surgery with and without transfusion.

MATERIALS AND METHODS

Patient Selection

After the approval of the ethics committee (decision number 11-12/3 dated 18.01.2012) in our prospective, double-blind study, it was decided to include patients between the ages of 18 and 65 who underwent elective brain or spine surgery in the operating room of the Department of Brain and Nerve Surgery within 6 months, who were ASA (The American Society of Anesthesiologists) score I-III, did not have heart, kidney or liver failure, did not have hemorrhagic diathesis, did not have coagulation disorders and were not receiving anticoagulant treatment. During this period, 75 patients were reached. Twelve patients were excluded from the study due to exclusion criteria and 5 patients were not included in the study due to incomplete data. Fifty-eight volunteer patients who met the criteria were accepted into the study. Written consent was obtained from patients (or their legal guardians) stating that their medical data could be published.

Anesthesia Management

In the preoperative period, demographic data of patients (age, gender, weight, height, ASA score, preoperative laboratory data [hemoglobin, hematocrit (Hct), platelet count, fibrinogen level,

prothrombin time, INR and activated partial thromboplastin level]) were recorded.

All patients received inhalation based general anesthesia in combination with opioid and non-depolarizing neuromuscular blockers, with standard ASA monitoring and tracheal intubation. Blood pressure drops were occasionally intervened by titrating ephedrine or noradrenaline to keep the MAP at 65 mmHg and above. Total fluid replacement was performed according to the 4:2:1 rule. Intravenous fluids were determined as balanced crystalloids (Isolyte S®, Eczacıbaşı, İstanbul, Türkiye), 6% hydroxyethyl starch 130/0.4 (Voluven®, Fresenius Co, Bad, Hamburg, Germany) and 4% succinylated gelatin (Gelofusin®, B.Braun Co, Melsungen, Germany). Anesthetic drug dose changes according to the depth of anesthesia and the type and dose selection of intravenous fluids were left to the experience and interpretation of the experienced anesthesiologist. Red Blood Cell (RBC) transfusion was aimed at patients with a hematocrit level below 30% or with 1000 mL or more bleeding during intraoperative period. The Gross Formula was used to calculate the amount of intraoperative bleeding (10).

$$V_{\text{loss}} = BV \times (H_o - H_f) / H_{AV}$$

V_{loss} (mL) represents the patient's calculated blood loss, while the patient's total blood volume BV (mL) is determined using the Nadler formula. H_o refers to the patient's preoperative hematocrit value, H_f denotes the postoperative Hct value, and H_{AV} is the average of the preoperative and postoperative Hct values.

All patients were extubated in the operating room at the end of the operation. Intraoperative data

RESULTS

Demographic and intraoperative characteristics of patients are shown in (Table-1). The mean age of the patients in the study was 46.8, BMI was 28, the female ratio was 62.1%, and the operation time was 128 minutes. Intraoperative hypothermia (minimum 34.5 °C) was observed in 77.6% of the patients (45 patients). Eleven (19%) patients required a mean of 6.1 ($\pm$ 4.1) mL/kg erythrocyte transfusion. Comparing the characteristics of patients with and without transfusion (Table-2). Preoperative Hb ($p=0.002$), Htc ($p=0.003$) were lower in the transfused group. Total bleeding was 4.7 ± 3.6 mL/kg in those who were not transfused and 10.4 ± 6.2

including surgical site (brain, spine]) lowest body temperature, lowest hematocrit, total bleeding amount, total fluid volume administered, total crystalloid and colloid volumes, colloid type, blood product transfusion, anesthesia and operation duration were recorded at the end of the operation. Below 36°C was considered hypothermia.

The patient's coagulation course was evaluated with TEG-Haemoscope 5000 (Thromboelastograph® 5000 coagulation analyser "Haemoscope", Niles, Illinois, USA) by a physician who was unaware of the simultaneous patients' clinical course and applications. Blood samples were taken in three periods (preoperative, postoperative 1st and 24th hours) to determine for TEG variables as R (reaction time), K (coagulation time), α (α angle), MA (maximum amplitude), Ly30 (lysis rate) and CI (coagulation index).

In this study, we aimed to evaluate coagulation changes and their relationship with transfusion using thromboelastography (TEG) in patients undergoing cranial and spinal surgery.

Statistical Analysis

Data was evaluated with the SPSS 21.0 program. Mean, standard deviation, ratio, frequency values were used in descriptive statistics of the data. The distribution of variables was checked with the Kolmogorov-Smirnov test. Independent sample t-test and Mann-Whitney U test were used in quantitative data analysis. Paired sample t-test and Wilcoxon test were used in repeated measurements. Pearson and Spearman's correlation analysis identified the associations between the variables.

mL/kg in those who were transfused ($p<0.001$). The lowest hematocrit was 27.4 ± 1.9 % in patients with transfusion and 39.8 ± 4 % in those without ($p<0.001$). The duration of operation ($p=0.022$), duration of anesthesia ($p=0.014$), total fluid ($p<0.001$) and total crystalloid infusion ($p=0.039$) were higher in patients with transfusion. No coagulation-related blood products were administered to any patient. All patients were extubated in the operating room.

TEG data of patients who received and did not receive erythrocyte transfusion are shown in Table-3. According to these data, no coagulation abnormality that would lead to clinical bleeding was detected. A significant increase was found

only in postoperative MA in both transfused and non-transfused patients.

The relationship between the changes in TEG parameters and perioperative variables is given in Table-4 and Table 5. According to the postoperative TEG data, MA was significantly positively correlated with age and the amount of crystalloid administered ($r=0.27$, $p=0.039$ and $r=0.29$, $p=0.027$); and negatively correlated with the lowest temperature value ($r=-0.28$, $p=0.029$). A significant positive relationship was also found

between CI and preoperative fibrinogen levels during this period ($r=0.28$, $p=0.045$) (Table-4).

According to the postoperative 24th hour TEG data, R time was negatively correlated with preoperative fibrinogen ($r=-0.27$, $p=0.046$); MA was positively correlated with aPTZ ($r=0.28$, $p=0.041$); CI was found to be positively correlated with age and fibrinogen level ($r=0.27$, $p=0.043$ and $r=0.28$, $p=0.045$) (Table-5).

Table-1. Demographic and intraoperative data

		Median (Min-max)	Mean $\pm$ SD	N /%	
Age (year)		50 (18-65)	46.8 $\pm$ 13.3		
Gender	Male			22	37.9%
	Female			36	62.1%
Weight (kg)		80 (41-110)	78 $\pm$ 15		
Height (cm)		165 (150-191)	168 $\pm$ 11		
BMI		27.28 (17.06-39.06)	28 $\pm$ 5		
Baseline Hemoglobin (g/dL)		13.5 (11-18)	13.4 $\pm$ 1.6		
Baseline Hematocrit (%)		39.95 (33-51)	40.3 $\pm$ 4.2		
Baseline Platelet count (/10³)		246500 (104000-401000)	260362 $\pm$ 69727		
Baseline PT (sec)		11.6 (10-14)	11.6 $\pm$ 0.7		
Baseline APTT (sec)		25.4 (18.3-31.0)	25.4 $\pm$ 2.4		
Baseline INR		1 (0.9-1.2)	0.98 $\pm$ 0.07		
Fibrinogen (mg/dL)		305.5 (158-577)	327 $\pm$ 102		
Lowest temperature (°C)		35.6 (34.5-36.5)	35.6 $\pm$ 0.4		
Lowest Intraoperative Hematocrit value (%)		35 (23-49)	35.1 $\pm$ 5.3		
Total amount of bleeding (ml/kg)		4.29 (0.66-22.0)	5.8 $\pm$ 4.7		
Total fluid volume (mL/kg)		22.41 (11-68)	24.9 $\pm$ 11.7		
Amount of crystalloid (mL/kg)		18.97 (10-40)	20 $\pm$ 7.3		
Amount of colloid (mL/kg)		3.685 (0-14)	3.8 $\pm$ 4.2		
Red Blood Cell (mL/kg)		0 (0-17)	1.15 $\pm$ 2.95		
Anesthesia duration (min)		160 (65-330)	160 $\pm$ 70		
Operation duration (min)		125 (45-265)	128 $\pm$ 63		
ASA score	I			28	48.3%
	II			30	51.7%
Surgery area	Brain			29	50.0%
	Spine			29	50.0%
Colloid type	None			27	46.6%
	4% Gelatine			19	32.8%
	6% HES			12	20.7%
RBC transfusion	No			47	81.0%
	Yes			11	19.0%

BMI, Body Mass Index; PT, prothrombin time; APTT, activated partial thromboplastin time; INR, international normalized ratio; ASA, The American Society of Anesthesiologists; HES, hydroxyethyl starch; RBC, red blood cell

Table-2. The relationship of transfusion with demographic and intraoperative characteristics.

		RBC Transfusion (+) N=11	RBC Transfusion (-) N=47	p value
Age (year)		51.7 ± 13.6	45.6 ± 13	0.097
Gender	Female	9 (%81.8)	27 (57.4%)	0.138
	Male	2 (%18.2)	20 (42.6%)	
Height	(cm)	162 ± 7	169 ± 11	0.028*
Weight	(kg)	79 ± 19	78 ± 15	0.841
BMI		29.9 ± 7.3	27 ± 4.7	0.110
ASA	I / II / III	3 / 8 / 0	25 / 22 / 0	0.121
Surgery area	Brain	6	23	0.738
	Spine	5	24	
Anesthesia duration (min)		201 ± 53	150 ± 70	0.022*
Operation duration (min)		168 ± 47	118 ± 63	0.014*
Preoperative				
	Hemoglobin (g/dL)	12 ± 1.3	13.7 ± 1.5	0.002*
	Hematocrit (%)	36.9 ± 3.7	41 ± 3.9	0.003*
	Platelet (/10 ³)	260 ± 76	260 ± 69	0.977
	Fibrinogen (mg/dL)	365 ± 164	317 ± 121	0.329
	PT (sec)	11.5 ± 1.5	11.7 ± 0.7	0.584
	INR	0.98 ± 0.1	0.98 ± 0.06	0.679
	APTT (sec)	25 ± 2.5	25.5 ± 2.4	0.524
Intraoperative				
	Lowest temperature (°C)	35.6 ± 0.4	35.6 ± 0.4	0.537
	Lowest Hematocrit (%)	27.4 ± 1.9	39.8 ± 4	<0.001*
	Total amount of bleeding (mL/kg)	10.4 ± 6.2	4.7 ± 3.6	<0.001*
	Total fluid and blood volumes (mL/kg)	38 ± 15	22 ± 8.5	<0.001*
	Total crystalloid (mL/kg)	25 ± 9	19 ± 6.5	0.039*
	Total colloid (mL/kg)	7 ± 4	3 ± 4	0.546
	HES / Gelatin /None (number of patients)	4 / 6 / 1	8 /13 / 26	0.922
	Total RBC transfusion (mL/kg)	6.1 ± 4.1	-	

BMI (Body Mass Index); PT (Prothrombin time); APTT (Activated partial thromboplastin time); INR (International normalized ratio); ASA (The American Society of Anesthesiologists); HES (Hydroxyethyl starch); RBC (Red Blood Cells). * p<0.05

Table-3. Perioperative TEG Data

		Total N=58	RBC Transfusion (+) N = 11	RBC Transfusion (-) N = 47
R (min)	Preoperative	5.6 ± 1.5	5.9 ± 1.5	5.6 ± 1.5
	Postoperative 1 st hour	5.5 ± 2	5 ± 1.2	5.6 ± 2.2
	Postoperative 24th hour	5.8 ± 1.6	6 ± 1	5.7 ± 1.7
K (min)	Preoperative	2.4 ± 1.3	2.5 ± 1.5	2.3 ± 1.2
	Postoperative 1 st hour	2.5 ± 2	1.8 ± 0.6	2.6 ± 2.1
	Postoperative 24th hour	2.1 ± 1	1.9 ± 1	2 ± 1
Angle (α)°	Preoperative	60 ± 10	59 ± 11	60 ± 10
	Postoperative 1 st hour	61 ± 12	64 ± 9	60 ± 13
	Postoperative 24th hour	63 ± 10	63 ± 9	63 ± 10
MA (mm)	Preoperative	59 ± 7	59 ± 8	59 ± 7
	Postoperative 1 st hour	58 ± 8	63 ± 9 *	57 ± 7
	Postoperative 24th hour	61 ± 6 *†	61 ± 6	61 ± 6 *†
CI	Preoperative	-0.57 ± 2.6	-0.77 ± 2.9	-0.52 ± 2.6
	Postoperative 1 st hour	-0.54 ± 3.5	0.86 ± 1.9	-0.87 ± 3.7

Ly30 (%)	Postoperative 24th hour	-0.08 ± 2.5	-0.06 ± 2.1	-0.08 ± 2.7
	Preoperative	1 ± 1.3	0.52 ± 1.1	1 ± 1.4
	Postoperative 1 st hour	0.6 ± 0.9 *	0.21 ± 0.5	0.7 ± 0.9
	Postoperative 24th hour	0.6 ± 0.7	0.49 ± 0.7	1.6 ± 0.7

RBC, Red Blood Cells; R, reaction time; K, coagulation time; (α) alpha angle; MA, maximum amplitude; CI, Coagulation index; LY30, percentage of lysis 30 min after MA.

*Compared to preoperative period, †Compared to postoperative 1st hour period, $p < 0.05$

Table-4. Factors associated with preoperative and postoperative 1st hour TEG change.

		Preoperative/Postoperative 1 st hour Change					
		R (min)	K (min)	MA (mm)	Angle α (°)	CI	Ly 30 (%)
Age	r	-0.216	-0.102	0.271	0.174	0.236	-0.046
	p	0.104	0.447	0.039*	0.191	0.074	0.731
Weight	r	0.042	0.009	0.028	0.039	-0.009	0.049
	p	0.754	0.947	0.837	0.773	0.944	0.714
Height	r	0.081	-0.122	0.175	0.023	0.039	0.125
	p	0.547	0.360	0.188	0.865	0.769	0.351
BMI	r	-0.011	0.100	-0.087	0.025	-0.038	-0.03
	p	0.932	0.457	0.517	0.854	0.776	0.822
Baseline Hemoglobin	r	-0.012	0.023	0.058	-0.007	0.004	0.147
	p	0.928	0.864	0.663	0.958	0.977	0.271
Baseline Hematocrit	r	0.010	0.008	0.080	0.013	0.011	0.113
	p	0.941	0.951	0.551	0.921	0.934	0.397
Baseline Platelet Count	r	-0.064	-0.062	0.039	0.151	0.097	-0.072
	p	0.634	0.646	0.770	0.258	0.467	0.593
Baseline PT	r	-0.016	-0.181	0.02	0.094	0.070	0.181
	p	0.903	0.178	0.882	0.487	0.606	0.178
Baseline APTT	r	-0.084	-0.180	0.054	0.152	0.115	0.01
	p	0.534	0.181	0.688	0.259	0.394	0.939
Baseline INR	r	-0.039	-0.173	0.038	0.142	0.098	0.017
	p	0.772	0.194	0.778	0.287	0.463	0.897
Fibrinogen	r	-0.244	-0.206	0.230	0.24	0.279	0.216
	p	0.082	0.142	0.102	0.087	0.045*	0.123
Lowest temperature	r	0.085	0.220	-0.287	-0.145	-0.202	0.070
	p	0.527	0.098	0.029*	0.277	0.129	0.602
Lowest Intraoperative Hematocrit	r	0.073	0.084	-0.107	-0.029	-0.94	0.175
	p	0.586	0.532	0.423	0.832	0.481	0.190
Total amount of bleeding	r	-0.058	-0.052	0.158	-0.053	0.073	-0.08
	p	0.668	0.699	0.237	0.692	0.587	0.549
Total fluid volume	r	-0.192	-0.191	0.24	0.088	0.214	-0.082
	p	0.149	0.150	0.07	0.51	0.106	0.541
Amount of crystalloid	r	-0.149	-0.186	0.291	0.059	0.2	-0.025
	p	0.266	0.163	0.027*	0.661	0.132	0.854
Amount of colloid	r	-0.198	-0.146	0.102	0.120	0.182	-0.19
	p	0.136	0.274	0.447	0.370	0.171	0.154
Red Blood Cell Transfusion	r	-0.112	-0.092	0.085	0.035	0.095	0.004
	p	0.401	0.491	0.525	0.793	0.477	0.976
Anesthesia duration	r	-0.184	-0.142	0.143	0.135	0.187	-0.033
	p	0.166	0.288	0.286	0.312	0.160	0.805
Operation duration	r	-0.196	-0.155	0.140	0.158	0.200	-0.039
	p	0.140	0.247	0.296	0.236	0.133	0.769

R, reaction time; K, coagulation time; (α) alpha angle; MA, maximum amplitude; CI, Coagulation index; LY30, percentage of lysis 30 min after MA; BMI, Body Mass Index; PT, prothrombin time; APTT, activated partial thromboplastin time; INR, international normalized ratio.

Pearson/Spearman correlation; r, Correlation Coefficient; $p < 0.05$

Table-5. Factors associated with postoperative 24th hour TEG change.

		Preoperative/ Postoperative 24th hour Change					
		R (min)	K(min)	MA (mm)	Angle (α) ^o	CI	Ly 30(%)
Age	r	-0.214	-0.262	0.193	0.229	0.274	0.068
	p	0.117	0.053	0.159	0.093	0.043*	0.620
Weight	r	-0.175	-0.153	0.203	0.146	0.187	0.141
	p	0.200	0.263	0.137	0.289	0.172	0.303
Height	r	0.029	-0.103	0.260	-0.007	0.060	0.119
	p	0.833	0.454	0.055	0.961	0.664	0.385
BMI	r	-0.194	-0.100	0.053	0.159	0.158	0.079
	p	0.156	0.468	0.701	0.246	0.251	0.565
Baseline Hemoglobin	r	0.018	0.077	0.024	-0.063	-0.046	0.008
	p	0.895	0.578	0.864	0.650	0.736	0.954
Baseline hematocrit	r	-0.056	-0.029	0.053	0.033	0.045	0.015
	p	0.684	0.833	0.701	0.813	0.746	0.912
Baseline platelet count	r	-0.193	-0.235	0.027	0.330	0.248	-0.072
	p	0.158	0.085	0.845	0.014*	0.068	0.603
Baseline PT	r	0.107	-0.030	0.119	-0.105	-0.055	-0.063
	p	0.440	0.828	0.391	0.451	0.693	0.652
Baseline APTT	r	-0.114	-0.191	0.280	0.193	0.203	0.012
	p	0.411	0.168	0.041*	0.163	0.142	0.931
Baseline INR	r	0.177	-0.030	0.141	-0.099	-0.072	-0.080
	p	0.195	0.826	0.306	0.474	0.603	0.564
Fibrinogen	r	-0.278	-0.238	0.082	0.246	0.280	0.160
	p	0.046*	0.089	0.562	0.078	0.045*	0.258
Lowest temperature	r	0.001	0.001	-0.045	0.004	-0.012	0.093
	p	0.997	0.997	0.746	0.978	0.930	0.501
Perioperative lowest Hematocrit	r	-0.011	0.024	0.040	-0.016	-0.009	0.052
	p	0.936	0.863	0.775	0.907	0.950	0.705
Total amount of bleeding	r	-0.083	-0.012	-0.174	0.008	0.004	-0.071
	p	0.548	0.930	0.205	0.951	0.974	0.606
Total fluid volume	r	-0.053	-0.071	-0.050	0.030	0.035	-0.004
	p	0.699	0.606	0.719	0.828	0.799	0.977
Amount of crystalloid	r	-0.081	-0.109	0.078	0.055	0.092	0.045
	p	0.557	0.427	0.571	0.689	0.505	0.746
Amount of colloid	r	0.050	0.018	-0.158	-0.023	-0.062	-0.152
	p	0.715	0.896	0.249	0.869	0.654	0.267
RBC	r	-0.082	-0.037	-0.168	0.014	-0.001	0.088
	p	0.552	0.790	0.220	0.919	0.994	0.523
Anesthesia duration	r	-0.179	-0.201	-0.009	0.216	0.180	0.049
	p	0.190	0.141	0.947	0.113	0.189	0.722
Operation duration	r	-0.191	-0.206	0.006	0.227	0.191	0.045
	p	0.162	0.132	0.965	0.096	0.162	0.742

R, reaction time; K, coagulation time; (α) alpha angle; MA, maximum amplitude; CI, Coagulation index; LY30, percentage of lysis 30 min after MA, BMI, Body Mass Index; PT, prothrombin time; APTT, activated partial thromboplastin time; INR, international normalized ratio.

Pearson/Spearman correlation; r, Correlation Coefficient; **p <0.05**

DISCUSSION

In our study, coagulation changes and their relationship with transfusion were evaluated in cranial and spinal surgery cases. It was determined that 18.9% of the patients required

erythrocyte transfusion. When the characteristics of the patients who administered transfusion were compared with the patients who did not, it was seen that preoperative hemoglobin and hematocrit levels were lower, the durations of

surgery and anesthesia were longer. They had lower hematocrit measurements simultaneously with more intraoperative bleeding and total fluid requirement. The amount of bleeding and low hematocrit levels, based on the determined transfusion threshold, were the main reasons for transfusion in these patients. However, as the primary objective of our study, no significant change was found when TEG data of patients requiring transfusion were compared with those not receiving transfusion. In this case, it is not possible to blame coagulation disorders as the cause of bleeding and transfusion in our patients. During surgery, blood transfusion is performed to replace blood loss independent of coagulation. Here, it is important for teams to determine their own transfusion limits and approaches without creating a risk of decreased oxygen delivery to the tissues. In our model, during the study period, due to the logistical conditions of our institution, higher hematocrit levels were targeted compared to the restrictive approach applied today. Although the frequency of transfusion is not high in general, if lower hemoglobin levels were targeted, more patients could have been prevented from receiving transfusion. However, this issue does not change the aim of our study.

The issue that was evaluated in the study was to reveal how perioperative coagulation is affected in patients who underwent brain and neurosurgery. TEG evaluation revealed that coagulation parameters showed a similar profile in both transfused and non-transfused patients. Neither hypocoagulability nor hypercoagulability occurred in any patient. Pearson and Spearman's correlation tests also show that the coagulation of patients is not affected at a clinical level by factors such as preoperative characteristics, intraoperative infusion amount, fluid types, transfusion amount, body temperature and operation time. It should also be acknowledged that continuing surgical interventions in their normal course and without complications may have prevented adverse outcomes by ensuring that the factors remain within controllable limits. It appears that the preoperative fibrinogen level positively affects the postoperative CI, which is an indicator of clot quality. This relationship is not specific to our patients but is expected after most surgeries. Fibrinogen is a natural determinant of the coagulation index (11,12). This positive relationship continued at 24 hours

postoperatively. However, it remained within clinical limits.

Overall, TEG evaluation revealed the expected results of surgery on coagulation in our patients. Age is a factor that often leads to increased coagulation (13,14). Although there was no significant difference in age distribution between the two groups, the age factor had a positive effect on clot quality over the postoperative MA and 24th hour CI, but the hypercoagulability limit was not exceeded.

Hypothermia is also a factor known to be the cause of perioperative bleeding. Hushan et al. (15), unlike our study, found that MA and α angle values decreased under the effect of hypothermia and stated that hypothermia decreased platelet aggregation and prolonged reaction and coagulation times. Wenjun Zhou Martini observed in a study on pigs that hypothermia did not cause an increase in the R value, a decrease in the α angle, and a change in MA and Lys 30 values (16). Taggart et al. found in a study on dogs that there was an increase in the K time under 30 °C, a decrease in the α angle, but no significant change in MA and R times (17). However, in our patient group, hypothermia had no effect on coagulation or transfusion requirements. A negative relationship was detected between postoperative MA and the lowest temperature, but this was not significant enough to cause clinical bleeding.

The choice of colloid for intraoperative fluid replacement is also a factor known to have a negative effect on perioperative coagulation (5,18–20). The use of HES is considered contraindicated in coagulopathic patients due to its potential to exacerbate bleeding risks and impair coagulation. It is not a cause of bleeding or transfusion at the doses used in our study. There is no clear data showing a significant relationship between the amount of colloid used and TEG parameters. A positive relationship was found between MA and crystalloid volume at the end of the operation. This is related to the ability of low hemodilution rates to increase coagulation (19–21).

Boyd CJ et al. (22) stated that low hemodilution causes hypercoagulation and high hemodilution causes hypo coagulation. In our study, it was determined that the crystalloid we used in low volume at the end of the operation increased MA. However, this did not provide a distinction for

patients requiring transfusion. This effect probably disappeared with the removal of crystalloids at the 24th hour postoperatively (23). Although the duration of anesthesia and operation was also longer in cases where transfusion was performed, there was no significant relationship between both parameters and TEG data. In addition, the fact that fibrinolysis was not detected in any patient at the end of the operation or at the 24th hour postoperatively can be interpreted as the development of exposure to excessive coagulation activity.

It is difficult to explain the effect of bleeding during surgery on coagulation. Kenneth JT et al. (24) reported in a study using TEG that progressive blood loss in patients undergoing surgery under general anesthesia increased coagulation. The effects of additional factors such as trauma or similar stressful situations will affect the results. However, since it is not possible to avoid any fluid replacement in a patient who is bleeding during surgery, it is difficult to investigate the effect of isolated blood loss on coagulation. The study conducted by Ruttmann et al. on blood donors is also valuable (25). Blood samples taken from donors at certain intervals after 1 unit of blood was examined in TEG, and while the R period was shortened in the blood taken immediately after the blood was taken, it returned to normal values after 1 hour and did not change afterwards. In our study, RBC replacement was applied to approximately 19% of the patients. Interestingly, the postoperative MA value of this transfusion group, which cannot be considered as having a high transfusion volume, was found to be significantly higher than the preoperative MA value. In contrast, there was

no significant increase in postoperative MA in the group that did not receive transfusion, but rather a decrease was observed. It is difficult to attribute the MA increase in those who received transfusion only to transfusion due to the complicated effects of patient characteristics and perioperative surgical conditions on coagulation.

Our study has some limitations. Especially the small number of patients who underwent transfusion, the fact that the team targeted 30% hematocrit levels as a transfusion threshold instead of restrictive transfusion targets may seem restrictive. However, it should be emphasized again that, apart from determining the need for transfusion, since our general aim was to determine the perioperative coagulation profile and the affecting factors in these patients, the number of patients can be considered sufficient to evaluate TEG.

CONCLUSION

In conclusion, no data favoring hypo coagulation or hypercoagulation could be obtained in patients undergoing brain and neurosurgery under normal operating conditions using TEG. We believe that coagulation in these patients is balanced. However, before making a general decision about the course of perioperative coagulation in brain and spinal surgery patients, our results need to be compared with studies in which restrictive transfusion regimens were applied.

Conflict of Interest / (if present): The authors declare that there is no conflict of interest. All authors declare that they contributed to the design, execution, and analysis of the review and approved its final version.

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Spinal disrafizimli yenidoğanlarda cerrahi zamanlama ve gelişen hidrosefalide tedavi modalitesi

Surgical timing and treatment modalities for developing hydrocephalus in newborns with spinal dysraphism

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ÖZ

Amaç: Spinal disrafizm, embriyonik nöral tüpün kusurlu orta hat füzyonundan kaynaklanan çeşitli omurga anomalilerini kapsamaktadır. Gelişmekte olan ülkelerdeki bu anomaliler, acil ve ömür boyu tıbbi bakım gerektiren birçok morbiditeye sahiptir. Tedavide cerrahi önemli bir yer tutmakta ve gelişebilecek komplikasyonları önlemede etkili olmaktadır. Çalışmamızın amacı; spinal disrafizm cerrahisindeki zamanlamanın ve cerrahi olarak uygulanan yöntemlerin, gelişebilecek komplikasyonlar üzerindeki etkisini ortaya koymak ve gelişen komplikasyonları literatür eşliğinde tartışmaktır.

Gereç ve Yöntem: Bu çalışmaya, Sağlık Bakanlığı İzmir Tepecik Eğitim ve Araştırma Hastanesi Nöroşirürji Kliniği ile Çocuk Yenidoğan kliniğinde, 2007- 2010 yılları arasında spinal disrafizm tanısı alan 102 hasta dahil edildi. Dosyalar retrospektif olarak incelenerek, doğum haftası, kilosu, cinsiyeti, anne yaşı ve folik asit kullanım öyküleri, lezyonun lokalizasyonu, nörolojik muayenesi, ek hastalık varlığı, BT ve MR raporları, uygulanan cerrahi müdahale ve zamanlama, komplikasyonlar kaydedildi.

Bulgular: Çalışmadaki olguların (n=102) %55'i erkekti. Tüm olguların %42'sinde lezyon torako-lomber bölgedeydi ve %45'nde (n=56) hidrosefali saptandı. Olguların %34,3'ü ilk 6 saatte opere edildi. Operasyonu 6 saatten sonra yapılan olgularda anlamlı olarak nörolojik defisit oranı yüksek çıktı (p=0.026). Yine 6 saatten sonra opere edilen olgularda menenjit enfeksiyonu anlamı olarak yüksek saptandı (p=0.005). 3. Günde önce ve sonra opere edilen olgularda menenjit gelişme riski açısından anlamı farklılık saptanmadı (p=0.07). Primer defekt onarımı ile beraber şant takılan ve takılmayan olgular arasında enfeksiyon sıklığı, BOS fistülü riski, nörolojik defisit gelişme olasılığı açısından anlamlı bir farklılık saptanmadı (sırasıyla, p=1.00, p=0.74, p=0.098).

Sonuç: Spinal disrafizm olan yenidoğanlarda cerrahi sonrası komplikasyonları en aza indirmek için erken zamanda cerrahi planlama gereklidir. Erken cerrahi uygulama ileri dönem nörolojik defisit olasılığını da azaltacaktır.

Anahtar Sözcükler: Spinal Disrafizm, Meningomyelosel, Hidrosefali, Yenidoğan

ABSTRACT

Aim: Spinal dysraphism includes spinal anomalies caused by defective neural tube fusion, leading to significant morbidity, especially in developing countries. These conditions require urgent and lifelong medical care. Surgery plays a vital role in treatment and helps prevent complications.

This study evaluates the impact of surgical timing and techniques on complications, supported by a literature review.

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Materials and Methods: This retrospective study included 102 spinal dysraphism patients treated between 2007 and 2010 at İzmir Tepecik Training and Research Hospital. Patient records were analyzed for gestational age, birth weight, gender, maternal age, folic acid use, lesion location, neurological findings, comorbidities, imaging results, surgical procedures, timing, and complications.

Results: 55% of the cases in the study (n=102) were male. In 42% of all cases, the lesion was in the thoracolumbar region and hydrocephalus was detected in 45% (n=56). 34.3% of the cases were operated within the first 6 hours. The rate of neurological deficits was significantly higher in cases where the operation was performed after 6 hours (p=0.026). Again, meningitis infection was detected as high in cases operated after 6 hours (p=0.005). There was no significant difference in the risk of developing meningitis in cases operated before and after the 3rd day (p=0.07). There was no significant difference between the cases with and without shunt placement in terms of infection frequency, risk of CSF fistula, and probability of developing neurological deficit (p=1.00, p=0.74, p=0.098, respectively).

Conclusion: Early surgical planning is necessary to minimize post-surgical complications in newborns with spinal dysraphism. Early surgical applications will also reduce the possibility of late neurological deficits.

Keywords: Spinal dysraphism, Myelomeningocele, Hydrocephalus, Newborn

GİRİŞ

Spinal disrafizm (SD), normalde birleşmesi gereken iki nöral yapının tam kapanamaması ile oluşan orta hat gelişim anomalisidir. Spinal disrafizm sınıflandırması, derinin lezyonu kaplayıp kaplamadığına bağlıdır (1). Açık spinal disrafizmlerde meninksler, omurilik sıvısı ve omuriliği içeren nöral elementler, yenidoğanı büyük bir enfeksiyon, yaralanma ve müteakip nörolojik yetersizlik riskine sokar. Miyelomeningosel (MMS), açık spinal disrafizmlerin %98,8'ini oluşturur ve MMS'nin genel insidansı 1000 canlı doğumda 0,2 ila 0,4'tür (2). Etkilenen bebeklerin klinik durumu ve uzun vadeli sonuçları, lezyonun boyutuna, düzeyine, dahil olan nöral doku miktarına bağlıdır. Meningosel veya meningomyeloseli içeren SD olan olguların, %90'nda, cerrahi önce ve sonrasında beyin omurilik sıvısının (BOS) dolaşımının bozulması ile hidrosefali gelişmektedir. Gelişen hidrosefali tedavisinde ventriküloperitoneal şant (VP), en sık kullanılan yöntem olmakla birlikte hastaların %70'nde 10 yıl içinde şant işlev bozukluğu gelişmektedir (3). Yaşanılan şant disfonksiyonları sebebiyle değişik tedavi modaliteleri gündeme gelmiş ve nöroendoskopik gereçlerin gelişmesiyle birlikte endoskopik üçüncü ventrikülostomi, bu hastalarda kullanılmaya başlanmıştır. Bu hastalarda cerrahi zamanı ve kullanılan yöntemin belirlenmesi ayrıca hidrosefali olan SD'li olgularda hangi tedavi yolunun uygulanacağı hekimden hekime değişmektedir (4).

Çalışmamızın amacı, 2007-2010 yılları arasında SD sebebiyle opere olan hastalarda, hidrosefalinin gelişip gelişmediğini belirlemek, hidrosefali gelişen ve gelişmeyen olguların tedavisinde cerrahi zamanlamasının ve sonrasında gelişen komplikasyonların sonuçlarını ortaya koymaktır.

GEREÇ ve YÖNTEM

Çalışma grubu

Çalışmamıza S.B. Tepecik Eğitim Araştırma Hastanesi Beyin ve Sinir Cerrahisi Kliniği ile Çocuk Sağlığı Yenidoğan Kliniğinde 2007- 2010 yılları arasında klinik ve radyolojik olarak spinal disrafizm tanısı alan ve opere edilen 102 hasta dahil edildi.

Veri Toplanması

Retrospektif olarak hasta dosyalarından olguların anne yaşı, folik asit kullanım öyküleri, doğum kiloları, doğum haftaları, cinsiyetleri, lezyonun lokalizasyonu ve büyüklüğü, cerrahi öncesi ve sonrası nörolojik muayene, uygulanan cerrahi şekli ve zamanı, cerrahi önce ve sonrasındaki komplikasyonlar ile ek hastalıkların varlığı kaydedildi. Ayrıca hastaların cerrahiden önce ve sonra yapılan beyin ve spinal Bilgisayarlı Tomografi (BT) ve manyetik rezonans (MRG) görüntüleri değerlendirildi.

İstatistiksel Yöntem

Çalışmada SPSS istatistik programı (versiyon 10.1, Chicago, ABD) kullanıldı. Ölçüm ile elde edilen değişkenler normal dağılımda olduğunda t

test, normal olmayan dağılım için Mann-Whitney U test yapıldı. Grupların karşılaştırılmasında Ki-kare test kullanıldı. Tüm testlerde hata oranı (0.05) olarak belirlenip $p < 0.05$ olduğunda gruplar arası fark istatistiksel olarak anlamlı kabul edildi.

BULGULAR

Sosyodemografik ve hastalık özellikleri

Çalışmaya spinal disrafizm (MMS) tanısı alan 47'si (%46,1) kız, 55'i (%53,9) erkek 102 olgu alındı. Hastaların doğum haftası ortalama 37, doğum ağırlığı 2892 gr ve anne yaşı 26.2 idi. Annelerin 66'sı (%64,7) folik asit kullanmıştı.

Olguların lokalizasyonu en sık %41,2'si (42) torako-lomber olmak üzere %21,6'sı (22) lomber, %24,5 (25)'i lumbo-sakral, %9,8 (10)'i torakal, %2,9 (3) servikal bölgedeydi. Olguların %45,1'inde (46) hidrosefali saptandı.

Operasyon ile ilişkili klinik bulgular

Cerrahi zamanlamaya bakıldığında en sık %42,2 (43) olgu 6-24 saat arasında opere olmuş ve %34,3'ü (35) ilk 6 saatte, %13,7'si (14) 1-3 gün arasında ve %9,8 (10)'u 3.gün ve sonrasında opere edilmiştir.

Tüm olguların %78,4'e (80) primer onarım yapılırken %21,6 (22)'e primer onarım ile beraber ventrikülo-peritoneal şant ameliyatı uygulanmıştır.

Operasyon öncesinde 60 hastada (%58,8) nörolojik defisit varken operasyon sonrasında %8,8'inde nörolojik defisitte artış saptanmıştır.

Cerrahi komplikasyonlar ile ilişkili bulgular

Operasyon sonunda %17,6 menenjit, %14,7'sinde yara yeri enfeksiyonu, %15,7'sinde BOS fistülü ve %22,5'inde şant enfeksiyonu saptandı.

Cerrahi zamanlama ile komplikasyonların ilişkisi:

Olguların %34,3'ü (35) ilk 6 saatte opere edildi. Cerrahi zamanlama ile komplikasyonların ilişkisi değerlendirildiğinde ilk 6 saat içinde opere olan olgularda 6 saatten sonra opere olan olgulara göre nörolojik defisit, menenjit anlamlı olarak daha az saptandı (sırasıyla $p=0.026$, $p=0.005$).

İlk 24 saat içinde opere olan olgular ile 24 saat sonra opere olan olgular arasında menenjit, yara

yeri enfeksiyonu, BOS fistülü gibi komplikasyonlarda anlamlı farklılık saptanmadı.

Sadece 3 günden sonra opere olan olgularda yara yeri enfeksiyonu ve 24 saatten sonra opere olan olgularda şant enfeksiyonu anlamlı olarak yüksek saptandı (sırasıyla $p=0.038$, $p=0.045$)

Cerrahi yöntemin komplikasyonlar ile ilişkisi:

Sadece primer onarım yapılan olgular ile primer onarımla beraber şant takılan olgular karşılaştırıldığında, nörolojik defisitte artış, menenjit, yara yeri enfeksiyonu ve BOS fistülü, şant enfeksiyonu gibi komplikasyonlar açısından anlamlı farklılık saptanmamıştır (p sırasıyla $p=1.000$, $p=0.514$, $p=0.744$, $p=0.612$).

Tablo-1. Cerrahi sonrası komplikasyonların dağılımı

	Menenjit	Yara yeri enfeksiyonu	BOS fistülü	Şant enfeksiyonu
Hasta	18 (17.6)	15 (%14,7)	16 (%15,7)	23 (%22,5)

Tablo-2. Cerrahi zamanlama ile komplikasyonlar arasındaki ilişki.

	İlk 6 saatte cerrahi	6 saat sonra cerrahi	P*
Nörolojik defisitte artış	0 (%0)	9 (%100)	0.026
Menenjit	1 (%5,6)	17 (%94,4)	0.005
Yara yeri enfeksiyonu	4 (%26,7)	11 (%73,3)	0.499
BOS fistülü	4 (%25,0)	12 (%75,0)	0.393

*Ki-kare test

TARTIŞMA

Spinal açık ve kapalı orta hat gelişim anomalileri, gelişmekte olan ülkelerde hala görülmeye devam eden ve santral sinir sisteminin cerrahi gerektiren en sık konjenital anomalileri arasındadır (4). Cerrahi tedavide amaç, BOS sıvısına ve sağlam nöral dokuya zarar vermeyecek şekilde defektin kapatılmasıdır (5). Miyelomeningosel ile doğan altı bebekten yalnızca birinde doğumda kafa içi basıncında artış belirtileri görüldüğü, sekiz bebekten yalnızca birinin baş çevresinin 98 persantilin üzerinde olduğu bilinmektedir (6). Bu çocukların yalnızca %5 ila %25'inde belirgin hidrosefali mevcut olduğundan, MMS onarımı ile aynı seansta VP şant yapılmasının gerekliliği

tartışmalıdır (7). Bu anomali ile doğan bebeklerde tam görüş birliği olmasa da genel prensip, oluşabilecek komplikasyonları en aza indirmek için bir an önce opere edilmesinin yararlı olduğu şeklindedir (3).

Çalışmamızda hamileliğinde folik asit kullanmayanlarda (%64,7) literatüre uyumlu olarak nöral tüp defektlerinin daha fazla olduğu gözlemlendi. Ayrıca çalışmalarda belirtilen lumbosakral bölgeden ziyade en fazla defekt %41 ile torakolomber bölgedeydi (8). Operasyon sonunda %17,6 menenjit, %14,7'sinde yara yeri enfeksiyonu, %15,7'sinde BOS fistülü ve %22,5 şant enfeksiyonu saptandı. Cerrahi zamanlama ile komplikasyonların ilişkisi değerlendirildiğinde çalışmamızda, ilk altı saat içinde opere olan olgularda altı saatten sonra opere olan olgulara göre nörolojik defisit, menenjit anlamlı olarak daha az ve üç günden sonra opere olan olgularda yara yeri enfeksiyonu ve 24 saatten sonra opere olan olgularda şant enfeksiyonu anlamlı olarak yüksek saptandı. Ayrıca sadece primer onarım yapılan olgular ile primer onarımla beraber şant takılan olgular karşılaştırıldığında, komplikasyonlar açısından herhangi bir farklılık saptanmamıştır.

Postnatal spina bifida onarımı, cerrahiye engel bir durum olmadığı sürece doğumdan hemen sonra yapılmalıdır. Literatürde operasyonun genellikle yaşamın ilk 48 saatinde yapılması önerilmiştir. Cerrahi 72 saatten sonraya ertelenmemelidir. Yapılan çalışmalarda sonuçlar değişken olmakla birlikte çoğu araştırmacı, yaşamın ilk 24 ila 48 saatindeki MMS onarımının enfeksiyon riskini azalttığını öne sürmektedir (7). Öktem ve ark. ve Arslan ve ark. yaptığı çalışmalarda, erken (48 saatten önce) MMS onarımı yapılan hastalarda enfeksiyon oranlarının daha düşük olduğunu bulmuşlar ve her iki yazar grubu da 48 saat sonra yapılan MMS onarımının anlamlı derecede daha fazla mortalite ve morbidite ile ilişkili olduğunu belirtmişlerdir (9,10). Cerrahi müdahale sonrasında komplikasyonların araştırıldığı bir çalışmada, zamanında (ilk 48 saatte) opere edilen hastalarda ventrikülit riski %7 iken bu süreden sonra opere edilenlerde ventrikülit riskinin %37 olduğu gösterilmiştir (11). 1967'de Fernandez-Serrats ve ark., 48 saat içinde cerrahi müdahaleyi savunan 228 hastadan oluşan retrospektif bir seri yayınladılar ve ameliyat için 7 günden fazla bekleyen hastalardaki %40 enfeksiyon oranına karşılık erken cerrahi grubunda %8'lik bir enfeksiyon saptamışlardır

(12). Spina bifida operasyonu sonrasında menenjit oranlarına bakıldığında, Shehu ve arkadaşlarının yaptığı ve 77 olguyu içeren bir çalışmada post-op menenjit oranı %4, Siedel ve arkadaşlarının retrospektif olarak 65 hasta değerlendirdiği bir çalışmada %7.7 saptanmış olup çalışmamızdan literatürden daha fazla olarak %17,7 bulunmuştur (13,14). Ulusal bir kohort çalışmasında da 24 saat içinde veya 24 ile 48 saat arasında ameliyat edilen yenidoğanlarda genel perinatal enfeksiyon oranında hiçbir fark göstermedi. Ancak hastanın 48 saatten fazla beklemesi durumunda enfeksiyon riskinde anlamlı bir artış olduğu göstermişlerdir (15,16). Benzer çalışmalar, enfeksiyöz komplikasyonları önlemek için kapatmanın doğumdan sonraki 48-72 saat içinde tamamlanması gerektiği yönündeki klasik verilerin büyük kısmını temsil etmektedir.

Literatüre baktığımızda, yara enfeksiyonu, menenjit veya ventrikülit gibi komplikasyonlara yol açan miyelomeningosel defektinin operasyon zamanlaması konusunda fikir birliğine varamamaktadır (5, 8,17). Başka bir araştırmada, ilk 24 saat, 3-7 gün ve 1 haftadan sonra opere edilen olgular karşılaştırıldığında, hayatta kalım, ventrikülit oranı, gelişme geriliği ve motor fonksiyonların seviyesinde anlamlı farklılık saptanmamıştır (17). Charney ve ark.'nın 110 opere olan meningomyeloselli yenidoğanı retrospektif olarak değerlendirdiği bir çalışmada, hastalar ilk 48 saat (erken), 3-7 gün (gecikmiş), 1 hafta-10 ay geç cerrahi olarak ayırdıktan sonra cerrahi zamanlama ile komplikasyonlarına bakıldığında; ventrikülit oranı ve mortalite erken, gecikmiş veya geç cerrahi geçirenler arasında benzerdi; Ayrıca nöromotor gelişimde gruplar arasında anlamlı bir ilişki bulunmamıştır (18).

Hidrosefali, spina bifidanın iyi bilinen ve uzun dönem mortalite ve morbiditeyi etkileyen bir komorbiditesidir ve bu çocukların %80-85'i konjenital veya SB kapanmasından sonra gelişen şekilde etkilemektedir (19,20). Omurga defektinin ilk kapanmasını takiben etkilenen çocukların yaklaşık %65'inde iki veya üç haftalıkken hidrosefali gelişir. Hidrosefalini varlığı, aynı seansta hem primer kese tamirinin yapılmasını hem de ventriküloperitoneal şant (VP) takılmasını akla getirmiştir. MMS onarımı ve hidrosefali tedavisi aynı aşamada yapılması gerektiğinde en uygun zamanlama konusunda literatürde fikir birliği yoktur. Ama hidrosefali ile doğan MMS'li hastalarda aynı seansta şant ve kese onarımı cerrahisi olan hastalarda yara iyileşmesinin iyi

olduğu, BOS kaçağına bağlı komplikasyonların azaldığı, hidrosefali ile ilişkili komplikasyonların önleildiği ancak şant tıkanması ve şant enfeksiyonu gibi komplikasyonların görülme sıklığını arttırdığı düşünülmektedir. Şant enfeksiyonunun, VP şantların %3-25'ini komplekte ettiği gösterilmiştir [21].

Margaron ve ark.'nın 1997 ile 2007 yılları arasında spina bifida (SB) operasyonu ve şant uygulaması için cerrahi olan 276 olgu geriye yönelik değerlendirdiği bir çalışmada; genel SB yara enfeksiyonu oranı %8,3, şant enfeksiyon oranı %8,7 idi. 5 günden önce SB operasyonu ve şant uygulanan çocuklarda, SB'nin kapatılmasından 5-10 gün sonra şant uygulanan çocuklar karşılaştırıldığında, defekt kapatılmasından sonra şant takılan olgularda yara yeri enfeksiyonu anlamlı olarak daha düşük saptanmıştır (22). Benzer şekilde meningomiyelosele operasyonu ile beraber şant takılan hidroselli olgularda sadece defekt onarımı yapılan olgulara göre şant enfeksiyonu daha fazla saptanmıştır (23). 2020 yılında Istemen ve ark.'nın yayınladığı bir araştırmada, 80 meningomiyelosele tanılı hastaya retrospektif olarak bakıldığında, erken dönemde onarım ile beraber şant takılan hastalarda, şant enfeksiyonu, şant disfonksiyonu ve şant revizyonunun geç dönemde yerleştirilenlere göre daha yüksek olduğu belirlenmiştir (24). Aynı seansta şant yapılmayan, takiplerde hidrosefali gelişen, deri altında aşırı gerginlik ve birikim olan hastalarda şantın gecikmeden yerleştirilmesi yara iyileşmesini olumlu etkiler. Ancak yaradan BOS sızıntısı olan hastalarda şanta bağlı enfeksiyon riski yüksektir; bu nedenle yara kenarları tamamen iyileşene kadar ventriküler drenaj yapılmalıdır. 1995 ile 2017 yılları arasında 170 hastanın geriye yönelik değerlendirildiği bir çalışmada ise, meningomiyelosele operasyonu ile beraber şant takılmasının şant enfeksiyonu için anlamlı derecede riski azaltmadığı gösterilmiştir (25). Ayrıca Öktem ve arkadaşlarının retrospektif olarak 63 vakayı değerlendirdiği bir çalışmada, eş zamanlı işlem uygulanan bu hastaların %19,3'ünde menenjit geliştiğini, geç dönem ayrı seansta şant takılanlarda ise sadece %9,5'inde menenjit geliştiğini bulmuşlardır (9). Bu risk azalması birçok çalışmada, onarım sonrasında yapılan antibiyotik profilaksisi olarak

düşünülmüştür. Çalışmamızda da benzer şekilde cerrahi onarım ile beraber şant takılmasının primer onarımdan sonra şant takılması arasında şant enfeksiyonu, menenjit ve diğer komplikasyonlar açısından için anlamlı bir farklılık saptanmadı.

Sipina bifidalı olgularda yara yeri enfeksiyonu daha çok operasyondan sonra cildin aşırı gerilmesine bağlı kanlanma bozukluğu, gaita kontaminasyonu ve yenidoğanın daha düşük immunitesine bağlı gelişmektedir. Operasyon sonrası yara yeri enfeksiyonu çalışmalarda %9-%23 arasında değişmektedir (4,13). Cerrahi zamanlama ile yara yeri enfeksiyonu ilişkisi ile ilgili çalışmaların birçoğunda 48-72 saat sonrası yapılan cerrahilerde yara yeri enfeksiyonunun daha fazla olduğu belirtilmiştir (17,15). Dolayısıyla cerrahi tedavide gecikme ve sonrasında olan kolonizasyona bağlı olarak gelişebilecek yara enfeksiyonu ve diğer komplikasyonlar ek antibiyotik gerektirebilir, hastanede kalış süresini uzatabilir.

Çalışmamızda retrospektif olarak vaka alınması, hamilelik ve doğum sonrası süreç ile ilgili kayıtlardaki eksiklikler, vaka sayımızın fazla olmaması en önemli kısıtlılıklardır. Daha fazla hasta grupları içeren çok merkezli prospektif izlem ve kayıtları içeren çalışmaların literatür ve klinik izleme faydası olacaktır.

SONUÇ

Spinal disrafizm, bizim gibi gelişmekte olan ülkelerde hem tıbbi hem de sosyal yönden önemli bir sorundur. Bu olguların cerrahi tedavisinde klinik yanıt ve komplikasyonları en aza indirmek amaçlı ilk 24 saat gibi erken dönemde cerrahi önerilmektedir. Ayrıca çalışmamızda hidrosefali ile beraber şant takılmasının ayrı ayrı bir işlem olarak planlamaya göre herhangi bir üstünlüğü olmadığı ortaya konmuştur.

MMS'li hastalarını yenidoğan döneminden itibaren optimal yaşamsal işlevler ve hayatta kalmayı etkileyebilecek potansiyel komplikasyonlar açısından izlemek multidisipliner tedavi ve bakım gerektirmektedir.

Çıkar Çatışması: Yazarlar aralarında çıkar çatışması olmadığını beyan etmişlerdir.

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JZL184 modulates lung injury via occludin and TNF- α in two distinct ARDS models

JZL184 iki farklı ARDS modelinde okcludin ve TNF- α yoluyla akciğer hasarını modüle etmektedir

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ABSTRACT

Aim: Acute respiratory distress syndrome (ARDS) is a severe condition with high morbidity and mortality, currently treated with limited therapeutic options. Recent studies indicate that cannabinoids could play a protective role in preventing tissue damage, particularly in pulmonary disorders. While their benefits in lung pathologies are established, the specific role of cannabinoids in ARDS mechanisms remains underexplored. This study aims to investigate the protective effects of cannabinoids, particularly through occludin and TNF- α regulation, in two ARDS models with distinct pathophysiological mechanisms.

Materials and Methods: Rats were treated with LPS for the direct ARDS model or ANTU for the indirect ARDS model. The inhibitor of MAGL, the enzyme that degrades the endocannabinoid 2-AG, JZL184, was administered 30 minutes prior to the administration of LPS or ANTU. Lung tissue samples were collected 24 hours post-LPS and 4 hours post-ANTU administration. Biochemical analyses, including MDA, SOD and catalase, as well as immunohistochemical evaluations of occludin and TNF- α , were conducted on lung tissue samples.

Results: Biochemical analysis showed that MDA levels decreased in the ANTU and LPS groups, but increased in the ANTU+JZL184 group and decreased in the LPS+JZL184 group, reflecting paradoxical effects. SOD and catalase levels were higher in the ANTU group and lower in the ANTU+JZL184 group. Immunohistochemical analysis demonstrated the restoration of occludin expression in both endothelial and bronchiolar cells and a reduction in TNF- α levels following JZL184 treatment.

Conclusion: JZL184 attenuates inflammation and restores occludin expression in both ARDS models, indicating its possible use as a therapeutic strategy for ARDS.

Keywords: ANTU, JZL184, LPS, occludin, TNF- α

ÖZ

Amaç: Akut solunum sıkıntısı sendromu (ARDS), yüksek morbidite ve mortaliteye sahip ciddi bir durum olup, şu anda sınırlı terapötik seçenekler ile tedavi edilmektedir. Son veriler, kannabinoidlerin akciğer hastalıkları da dahil olmak üzere doku hasarına karşı koruyucu etkiler sunabileceğini göstermektedir.

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Akciğer patolojilerindeki faydaları kanıtlanmış olsa da kannabinoidlerin ARDS mekanizmalarındaki özgül rolü henüz yeterince araştırılmamıştır. Bu çalışma, farklı patofizyolojik mekanizmalara sahip iki ARDS modelinde, özellikle okludin ve TNF- α yoluyla kannabinoidlerin koruyucu etkilerini incelemeyi amaçlamaktadır.

Gereç ve Yöntem: Sıçanlar, doğrudan ARDS modeli için LPS ile veya dolaylı ARDS modeli için ANTU ile muamele edilmiştir. Endokannabinoid 2-AG'yi parçalayan MAGL enziminin inhibitörü olan JZL184, LPS veya ANTU verilmeden 30 dakika önce uygulanmıştır. Akciğer doku örnekleri, LPS uygulamasından 24 saat ve ANTU uygulamasından 4 saat sonra toplanmıştır. Akciğer doku örneklerinde MDA, SOD ve katalaz olmak üzere biyokimyasal analizler ile okludin ve TNF- α 'nın immünohistokimyasal değerlendirmeleri yapılmıştır.

Bulgular: Biyokimyasal analizler, MDA seviyelerinin ANTU ve LPS gruplarında azaldığını, ancak ANTU+JZL184 grubunda arttığını ve LPS+JZL184 grubunda azaldığını göstermiştir, bu da paradoksal etkileri yansıtmaktadır. SOD ve katalaz seviyeleri, ANTU grubunda daha yüksek ve ANTU+JZL184 grubunda daha düşük bulunmuştur. İmmünohistokimyasal analizler ise JZL184 tedavisi sonrasında, endotel ve bronşiyolar hücrelerin her ikisinde okludin ekspresyonunun düzeldiğini ve TNF- α seviyelerinin azaldığını göstermiştir.

Sonuç: JZL184, her iki ARDS modelinde inflamasyonu hafifletmekte ve okludin ekspresyonunu düzeltmektedir, bu da ARDS için terapötik bir yaklaşım olarak potansiyelini ortaya koymaktadır.

Anahtar Sözcükler: ANTU, JZL184, LPS, okludin, TNF- α

INTRODUCTION

Acute Respiratory Distress Syndrome (ARDS) represents a severe pulmonary disorder characterized by widespread alveolar damage, hypoxemia, and inflammation, often leading to respiratory failure. It is triggered by a variety of etiological factors, both direct, such as pneumonia and aspiration, and indirect, including sepsis and trauma (1,2). Despite its high incidence and substantial mortality rate, ARDS treatment remains limited to supportive care, with no effective pharmacological treatments yet established in clinical practice (3,4). This highlights the critical need for exploring novel therapeutic strategies to improve outcomes in ARDS patients.

The role of the endocannabinoid system in a wide range of diseases has positioned it as a promising therapeutic target. The discovery of CB1 and CB2 receptors, and their key ligands, anandamide and 2-arachidonoylglycerol (2-AG), as well as associated enzymes, has enhanced comprehension of this system. Given its widespread presence in mammals and its regulatory influence on essential physiological processes such as immune response and inflammation, it holds considerable pharmacological significance (5).

The two main ARDS models commonly used in experimental research are the lipopolysaccharide (LPS)-induced direct injury model and the alpha-naphthylthiourea (ANTU)-induced indirect injury model. LPS, a potent endotoxin from Gram-negative bacteria, triggers a strong inflammatory response and lung damage when administered intratracheally, mimicking the pathophysiology of direct lung injury in ARDS (6). In contrast, ANTU, a rodenticide, induces pulmonary edema and pleural effusion through indirect mechanisms, such as endothelial damage, leading to lung injury that is less directly associated with inflammation of the alveolar epithelium (7-12). These models provide valuable insights into the different mechanisms of ARDS and are essential for evaluating potential therapeutic agents.

The integrity of the alveolar barrier is crucial in ARDS pathogenesis. Tight junction proteins, such as occludin, play a pivotal role in maintaining this barrier. Studies have demonstrated that occludin expression is reduced in ARDS patients, leading to compromised alveolar barrier function (13). Therefore, interventions that preserve or restore occludin expression may offer therapeutic benefits.

This study aimed to investigate the effects of JZL184 in two distinct ARDS models, focusing on

its potential to attenuate pathological lung damage. Additionally, it seeks to uncover the underlying mechanisms by which cannabinoids may offer therapeutic benefits in ARDS by exploring the roles of occludin, a key protein essential for maintaining the integrity of epithelial and endothelial barriers, and tumor necrosis factor-alpha (TNF- α), a multifunctional cytokine involved in inflammation.

MATERIALS AND METHODS

Animals

This study utilized 50 male Wistar albino rats, weighing 200–250 g and aged 3–4 months. The animals were housed under standard laboratory conditions, including a controlled temperature of 22 ± 2 °C, a 12-hour light/dark cycle, and unrestricted access to water and a 21% protein pellet diet.

Experimental Groups

The rats were randomly divided into five distinct experimental groups, with each group comprising 10 animals (n = 10) (Table-1).

Table-1. Experimental groups.

Groups	Dose / Route of Application
1 Control	-
2 ANTU	10 mg/kg / i.p.
3 LPS	5 mg/kg / intratracheal
4 ANTU+JZL184	10 mg/kg + 10 mg/kg / i.p./i.p.
5 LPS+JZL184	5 mg/kg + 10 mg/kg / intratracheal/i.p.

ANTU-Induced Indirect ARDS Model

The indirect ARDS model was established using ANTU. ANTU was prepared as a suspension in olive oil at a concentration of 4 mg/ml and administered to the rats at a dose of 10 mg/kg via gavage. Four hours after ANTU administration, the rats were anesthetized with a combination of ketamine (75 mg/kg, intraperitoneal/i.p.) and xylazine (5 mg/kg, intramuscular). Following anesthesia, the animals were euthanized via exsanguination through the abdominal aorta. Once bleeding had ceased, the thoracic cavity

was carefully opened, and pleural effusion was collected using syringes (7-12).

Intratracheal LPS-Induced Direct ARDS Model

The direct ARDS model was induced using LPS. Under ketamine (75 mg/kg, i.p.) and xylazine (5 mg/kg, intramuscular) anesthesia, the neck area of each animal was shaved, and the rats were placed in a supine position, and a 1 cm midline incision was made to expose the trachea. LPS, dissolved in saline, was administered intratracheally at a dose of 5 mg/kg. Subsequent to the procedure, the rats were carefully rotated to a vertical position to ensure the uniform spread of LPS throughout the lungs. To minimize the risk of respiratory suppression, the rats were positioned within their cages at a 45-degree incline and closely monitored until they had fully regained consciousness from anesthesia. 24 hours following LPS administration, the rats were anesthetized and then euthanized for the assessment of pulmonary effects. The thoracic cavity was exposed, and the lungs were carefully removed and cleaned for further examination.

Biochemical Analyses

MDA Measurement (nmol/L)

Malondialdehyde (MDA) levels were determined using a thiobarbituric acid (TBA)-based assay. The reaction involved MDA or MDA-like substances reacting with TBA at 100 °C and pH 2–3 for 15 minutes, producing a pink pigment with maximum absorbance at 532 nm. Lung tissue samples were mixed with 10% trichloroacetic acid to precipitate proteins, followed by centrifugation. An aliquot of the supernatant was reacted with 0.67% TBA in a boiling water bath for 15 minutes. Absorbance was measured at 532 nm after cooling. Commercial kits (Rel Assay Diagnostics, Gaziantep, Turkey) were used for this analysis.

SOD Activity (U/ml)

Superoxide dismutase (SOD) activity, which catalyzes the dismutation of superoxide radicals into hydrogen peroxide (H₂O₂) and oxygen, was measured using a xanthine/xanthine oxidase system to generate superoxide radicals. The inhibition of the reaction forming a red formazan dye was used to quantify SOD activity. Measurements were performed using a biochemistry analyzer (Mindray BS-400, Ohio, USA).

Catalase Activity (U/ml)

Catalase activity was assessed by its ability to decompose H₂O₂, with the reaction stopped using ammonium molybdate. Residual H₂O₂ reacted with ammonium molybdate to form a yellow complex, quantified by absorbance at 405 nm. Commercial kits (Rel Assay Diagnostics, Gaziantep, Turkey) were employed for this analysis.

Immunohistochemical Analyses

Lung tissue sections (5 µm) were placed on charged slides, deparaffinized, and subjected to antigen retrieval with citrate buffer in a microwave. After cooling and washing with phosphate-buffered saline (PBS), endogenous peroxidase activity was blocked with 3% hydrogen peroxide, followed by Ultra V block to reduce non-specific binding. Sections were incubated with anti-occludin polyclonal primary antibodies (Thermo Fisher Scientific, Massachusetts, USA) and anti TNF-α polyclonal primary antibodies (Thermo Fisher Scientific, Massachusetts, USA), then treated with a biotinylated secondary antibody and streptavidin-peroxidase. Staining was performed using diaminobenzidine (DAB) chromogen, with hematoxylin as the counterstain. To minimize background staining, all slides were processed in a humidity chamber. Stained sections were examined under a Zeiss Axio Lab.A1 photomicroscope, with images captured for further analysis.

The intensity of TNF-α staining was evaluated using the H-score method. This score was calculated by multiplying the percentage of positively stained cells by their staining intensity, following the formula $H\text{-score} = \sum i \times P_i$, where i represents the intensity score and P_i the percentage of cells. For statistical accuracy, scoring was performed at 40× magnification, with 20 fields analyzed per section.

Chemicals

The following chemicals and reagents were used in this study: JZL184 (Santa Cruz Biotechnology, Texas, USA); olive oil (Sigma-Aldrich, St. Louis and Burlington, MA, USA); ANTU (Sigma-Aldrich, St. Louis and Burlington, MA, USA); LPS (Sigma-Aldrich, St. Louis and Burlington, MA, USA); xylazine (Bioveta, Komenského, Czech

Republic); ketamine (Pfizer, New York, USA); saline (Polifarma, Tekirdağ, Turkey).

Statistical Analysis of Data

Statistical analysis was conducted with Jamovi 2.3.21 (Computer Software, retrieved from <https://www.jamovi.org>, Sydney, Australia). Data are presented as mean ± standard deviation (SD). The normality of the data was assessed using the Shapiro-Wilk test. For variables that were not normally distributed, the Kruskal-Wallis test was performed, followed by Mann-Whitney U tests with Bonferroni correction for post-hoc analysis. A p-value of < 0.05 was considered statistically significant.

RESULTS

ELISA

MDA levels

Biochemical analysis revealed a statistically significant decrease in MDA levels in the lung tissue of the ANTU group compared to the control group. Although differences were observed in the LPS, ANTU+JZL184, and LPS+JZL184 groups compared to their respective controls, these changes were not statistically significant (Figure-1A).

SOD levels

In the ANTU group, SOD levels showed a significant increase compared to the control group. Additionally, SOD levels were significantly higher in the ANTU group than in the LPS group. In contrast, SOD levels in the LPS group did not differ significantly from the control group. Although variations in SOD levels were observed in the treatment groups (ANTU+JZL184 and LPS+JZL184) compared to their corresponding pathology groups, these changes were not statistically significant (Figure-1B).

CAT levels

Catalase levels exhibited variations in the ANTU group compared to the control group, with additional changes observed following JZL184 treatment. However, no statistically significant differences in catalase levels were observed between the groups (Figure-1C).

Immunohistochemistry

Occludin staining was observed in bronchiolar epithelial cells but not in endothelial cells in the ANTU group. In contrast, the ANTU+JZL184 group showed occludin staining in both

bronchiolar epithelial and endothelial cells. Similarly, in the LPS group, occludin staining was present in endothelial cells but absent in bronchiolar epithelial cells, whereas the LPS+JZL184 group exhibited staining in both cell types (Figure-2). Immunohistochemical analysis also revealed a significant increase in TNF- α levels in the alveolar septum and inflammatory cell cytoplasm infiltrating the expanded perivascular areas of the ANTU group. Similarly, the LPS group showed elevated TNF- α protein expression in the bronchiolar epithelium and in

the inflammatory cell cytoplasm infiltrating the alveolar septum and bronchiolar adventitia. In contrast, the ANTU+JZL184 and LPS+JZL184 treatment groups showed a statistically significant decrease in TNF- α staining compared to their respective pathology groups, indicating a potential therapeutic effect (Figure-3).

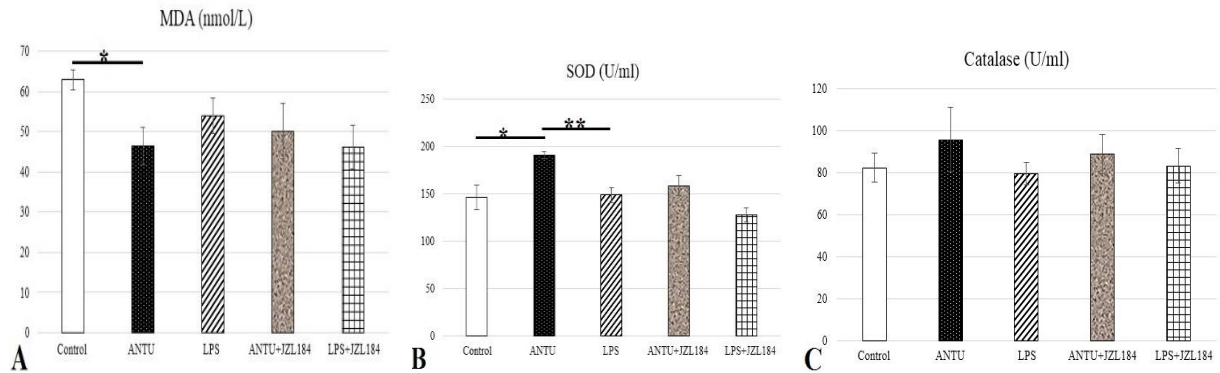


Figure-1. A-C: Biochemical parameters in ARDS models induced by ANTU and LPS, and the effects of JZL184 treatment. Data are shown as mean $\pm$ SD. *: Statistically significant difference between the ANTU and control groups ($p < 0.05$), **: Statistically significant difference between the ANTU and LPS groups ($p < 0.05$).

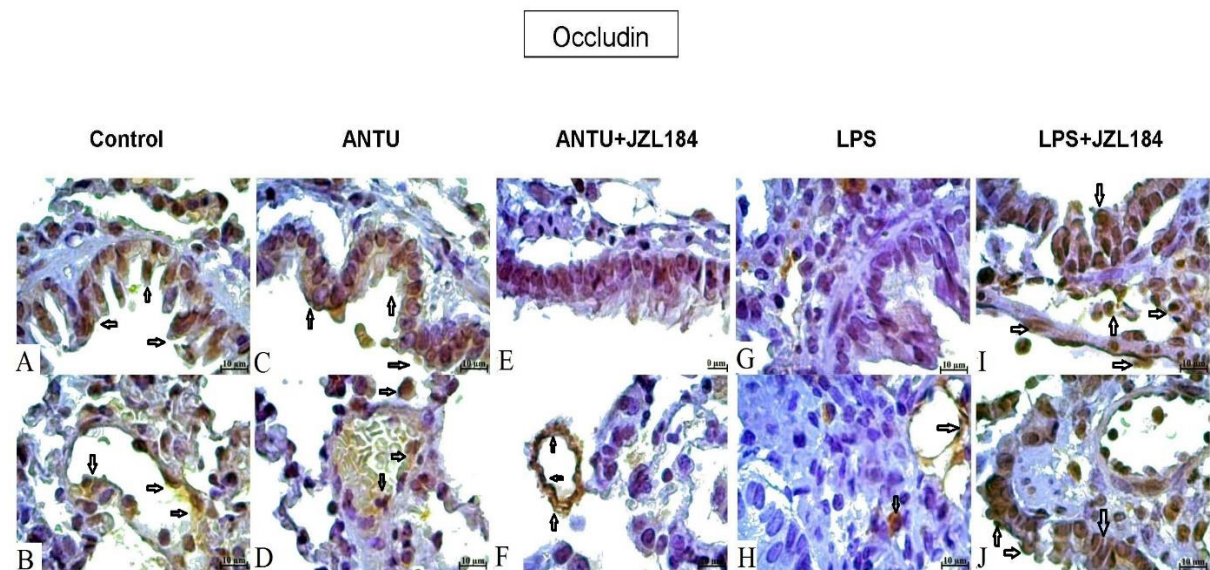


Figure-2. A-J: Immunohistochemical staining images to determine occludin staining intensity in all groups. Positive staining areas are indicated by arrows. Scale bar: 10 μ m

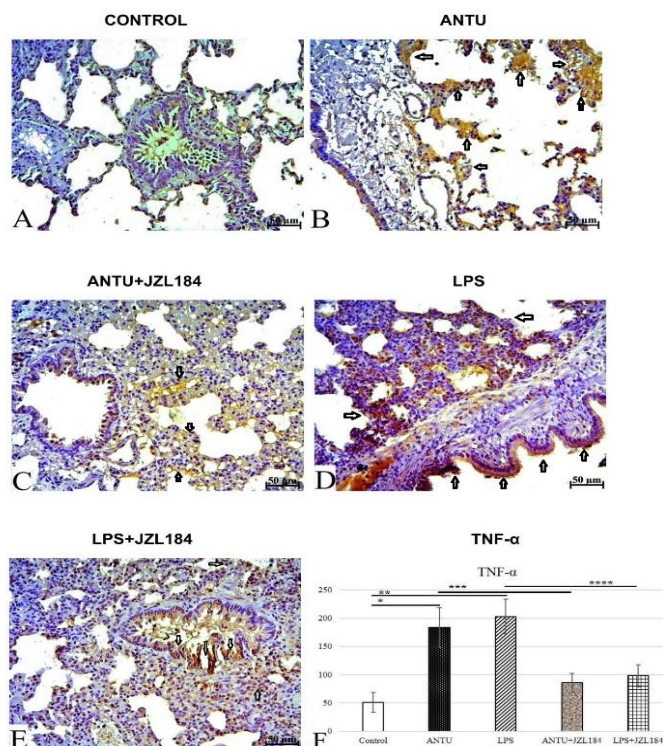


Figure-3. A-E. Immunohistochemical staining images to determine TNF- α expression in all groups. A: Control, B: ANTU, C: ANTU+JZL184, D: LPS, E: LPS+JZL184. Positive staining areas are indicated by arrows. Scale bar: 50 μ m. **F:** Statistical analysis of TNF- α levels across the groups. Data are shown as mean $\pm$ SD. * : Statistically significant difference between the ANTU and control groups ($p < 0.05$), ** : Statistically significant difference between the LPS and control groups ($p < 0.05$), *** : Statistically significant difference between the ANTU and ANTU+JZL184 groups ($p < 0.05$), **** : Statistically significant difference between the LPS and LPS+JZL184 groups ($p < 0.05$).

DISCUSSION

This is the first experimental study to demonstrate the efficacy of JZL184, which enhances endocannabinoid activity, in two distinct ARDS models by exerting anti-inflammatory and barrier-protective effects through the modulation of TNF- α and occludin expression. The findings indicate that JZL184 administration influences biochemical markers and immunohistochemical characteristics in these ARDS models, suggesting its potential therapeutic role in lung injury.

The endocannabinoid system (ECS), which plays a key role in regulating various physiological processes, has emerged as a promising target for treating inflammatory diseases, including lung injury. Cannabinoids, the active compounds derived from cannabis, have shown protective effects in conditions such as cardiovascular,

autoimmune, and neurodegenerative disorders (14-19). ECS, which includes cannabinoid receptors and a variety of metabolites, plays a role in regulating immune cell activity. Cannabinoid receptors are widely expressed in human lung tissue (20). Evidence has demonstrated the presence of CB1 and CB2 receptors within lung tissues, and a variety of cells release endocannabinoids in response to inflammatory stimuli. However, the exact effects of these endocannabinoids on lung health and diseases remain unclear. Research has indicated that the CB2 receptor agonist JWH133 has the potential to alleviate lung-associated complications in pathologies like RSV infection in both human and mouse models (21), can exert a protective effect against pulmonary fibrosis (22), can reduce the damage in brain, lung, liver and heart (23). These findings suggest that

cannabinoids could be promising agents for treating lung diseases.

Endocannabinoids such as 2-AG and anandamide are metabolized by the enzymes monoacylglycerol lipase (MAGL) and FAAH, respectively (24). JZL184, an inhibitor of MAGL, enhances 2-AG levels and exerts notable anti-inflammatory effects (25). Preclinical models have shown that JZL184 can also protect tissues from oxidative damage and exert anti-apoptotic effects (26,27).

In the current study, biochemical analysis showed a significant decrease in MDA levels in the ANTU and LPS groups compared to the control. In the treatment groups, MDA levels were altered but not significantly. SOD levels were higher in the ANTU group but reduced with JZL184 treatment. Catalase levels were elevated in the ANTU group but decreased with JZL184 treatment, with no significant differences between groups.

The changes in MDA levels across treatment groups reflect complex interactions between oxidative stress and JZL184's therapeutic effects. In the ANTU and LPS groups, MDA levels decreased. However, MDA levels increased in the ANTU+JZL184 group, suggesting a compensatory response or altered balance between oxidative stress and antioxidant defenses. In contrast, MDA levels decreased in the LPS+JZL184 group, indicating the potential anti-inflammatory and antioxidative properties of JZL184 in LPS-induced pathology. These differences may be attributed to the varying effects of JZL184 in ARDS models, which are based on different pathophysiological mechanisms.

Regarding SOD, the significant increase in the ANTU group reflects a compensatory response to oxidative stress, while the reduction in SOD levels in the ANTU+JZL184 group suggests that JZL184 modulates oxidative stress and inflammatory responses, reducing the need for excessive antioxidant production. In the LPS group, SOD levels remained unchanged, but the decrease in the LPS+JZL184 group, similar to the ANTU+JZL184 group, suggests that JZL184 modulates oxidative stress, reducing the need for excessive antioxidant production. Elevated catalase levels in the ANTU group indicate an adaptive response to oxidative stress. The decrease in catalase levels in the ANTU+JZL184

group suggests that JZL184 modulated oxidative stress, reducing the need for excessive antioxidant production, similar to its effect on SOD in the same group. Catalase levels in the LPS and LPS+JZL184 groups remained similar to the control, indicating that JZL184 had less effect on catalase activity in LPS-induced lung injury.

Occludin, a critical tight junction protein, plays a vital role in maintaining the integrity of the alveolar barrier. Reduced expression of occludin is associated with lung injury, and restoring its function has been shown to enhance barrier integrity and mitigate lung damage (28). Moreover, infections can further impair the alveolar epithelial barrier by decreasing the expression of occludin in lung tissues, exacerbating lung injury (29).

In the present study, occludin expression displayed distinct patterns in the two ARDS models. In the ANTU group, which primarily causes endothelial damage, occludin staining was present in bronchiolar epithelial cells but absent in endothelial cells, indicating a disruption in endothelial cell integrity. Conversely, in the LPS group, which induces direct epithelial injury, occludin staining was observed in endothelial cells but significantly reduced in bronchiolar epithelial cells, highlighting epithelial damage and compromised barrier function in this model.

JZL184 treatment demonstrated a therapeutic effect by restoring occludin expression in both cell types. In the ANTU+JZL184 group, occludin staining was observed in both bronchiolar epithelial and endothelial cells, suggesting that JZL184 mitigates the endothelial damage and preserves barrier integrity in the context of indirect lung injury. Similarly, in the LPS+JZL184 group, occludin expression was restored in both cell types, indicating the protective role of JZL184 in maintaining epithelial integrity in the direct lung injury model induced by LPS. These findings highlight that JZL184 treatment corresponds to the unique pathophysiological mechanisms of each model, offering potential therapeutic benefits by enhancing occludin expression and promoting the preservation of the tight junctions.

The current observation aligns with the findings of Wang et al., who reported that JZL184 enhances occludin expression in intestinal models, thereby improving epithelial barrier function under stress (30). Similarly, cannabidiol

treatment in the traumatic brain injury model has been shown to increase occludin expression and improve blood-brain barrier integrity, highlighting its potential for neuroprotection by restoring tight junction proteins (31). JWH133, a cannabinoid receptor 2 agonist, treatment in an intracerebral hemorrhage model has also been demonstrated to enhance occludin expression and protect the blood-brain barrier, suggesting that cannabinoid-based therapies may play a crucial role in maintaining barrier function across various injury models (32).

TNF- α is a versatile cytokine that plays a critical role in numerous physiological and pathological processes, including inflammation, cell death, and immune system regulation (33). Elevated levels of TNF- α have been implicated in the pathogenesis of various pulmonary inflammatory diseases, including asthma, chronic obstructive pulmonary disease, and ARDS, by inducing inflammation and tissue remodeling, and its inhibition has been shown to enhance lung function (34, 35).

The current analysis revealed distinct patterns of inflammation in the two ARDS models. In the ANTU group, elevated TNF- α levels in the alveolar septum and inflammatory cell cytoplasm infiltrating the expanded perivascular areas indicated enhanced endothelial damage, consistent with previous findings of TNF- α 's role in endothelial dysfunction. Similarly, the LPS group showed increased TNF- α expression in the bronchiolar epithelium and in the inflammatory cell cytoplasm infiltrating the bronchiolar adventitia, highlighting epithelial damage and disruption of the epithelial barrier.

Notably, treatment with JZL184 resulted in a significant reduction in TNF- α staining in both the ANTU+JZL184 and LPS+JZL184 groups. This suggests that JZL184 may reduce TNF- α -mediated inflammation and protect against endothelial and epithelial damage in these lung injury models. These findings suggest that JZL184 holds therapeutic potential by modulating the inflammatory response and preserving barrier integrity in ARDS.

Several studies have demonstrated the anti-inflammatory effects of cannabinoids, particularly in reducing TNF- α levels. It has been reported that cannabinoids, including CBD, decrease TNF- α and modulate inflammation (36). Similarly, a systematic review showed that both CBD and

CBD+THC combinations significantly lower TNF- α levels (37). Additionally, cannabinoids have been proposed to inhibit TNF- α and other inflammatory cytokines, providing potential therapeutic benefits in inflammatory diseases like SARS-CoV-2 infection (38). Furthermore, network-based pharmacology analysis identified key targets such as NF- κ B and TNF receptors in the anti-inflammatory activity of cannabinoids, supporting the therapeutic potential of compounds like JZL184 (39).

Occludin's expression and function are influenced by inflammatory cytokines, particularly TNF- α , which disrupts tight junctions by reducing occludin expression or altering its cellular localization. This leads to increased permeability and contributes to the development of various inflammatory diseases, such as inflammatory bowel disease, neuroinflammation, and sepsis. Modulating the occludin-TNF- α interaction presents a potential therapeutic strategy to restore barrier integrity and mitigate disease progression. Recent studies have demonstrated the protective effects of baicalin and rhubarb extract through the modulation of occludin and TNF- α . In aging mice with periodontitis, baicalin upregulated occludin expression, restored intestinal permeability, and reduced TNF- α levels, thereby alleviating inflammation and preventing alveolar bone loss (40). Similarly, rhubarb extract improved intestinal barrier integrity by increasing occludin expression and decreasing TNF- α levels in both serum and the hippocampus, offering protection against cerebral ischaemia-reperfusion injury (CIRI) (41).

In the current study, in accordance with the results presented above, a decrease in occludin protein levels was observed in parallel with elevated TNF- α levels in the pathological groups. This change was reversed following treatment with JZL184, highlighting the therapeutic potential of targeting the occludin-TNF- α pathway for the treatment of ARDS.

Limitations

Due to budget constraints, a single dose of JZL184 was used in the present study, which limits the ability to assess its dose-dependent effects. Additionally, various parameters potentially involved in the distinct pathophysiological mechanisms underlying ARDS could not be investigated for the same reason. Further research is needed to fully

elucidate these mechanisms and the therapeutic

CONCLUSION

The results of this study demonstrate that JZL184 offers significant therapeutic potential in both direct and indirect ARDS models. By enhancing occludin expression and restoring tight junction integrity in both endothelial and epithelial cells, JZL184 mitigates tissue damage and supports the preservation of alveolar barrier function. Additionally, JZL184 reduces TNF- α expression, thereby attenuating inflammation and consequently contributing to the protection of lung tissue. The differential effects observed in the ANTU- and LPS-induced injury models underscore the importance of understanding the specific pathophysiological mechanisms underlying ARDS. Overall, these findings suggest that JZL184 could be a promising candidate for further exploration in the treatment of ARDS,

potential of JZL184 in lung injury.

offering a novel approach to managing this challenging and complex condition.

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Conflicts of interest: Authors declared no conflict of interest.

Ethical Approval

All experimental procedures described in this study were previously approved by the Ethical Committee of Experimental Animals of Zonguldak Bulent Ecevit University (Protocol No: 2025-04-20/02) and are in accordance with the "Guide for the Care and Use of Laboratory Animals" (US National Institute of Health, revised 1996).

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Nefrolitiazis tanısında ve böbrek taşı boyut ölçümünde ultrasonografinin etkinliği ve doğruluğu

The effectiveness and accuracy of ultrasound in the diagnosis of nephrolithiasis and measurement of kidney stone size

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ÖZ

Amaç: Ultrasonografinin (US) nefrolitiazis tanısında ve böbrek taşı boyut ölçümündeki doğruluğunun ortaya konulması amaçlanmıştır.

Gereç ve Yöntem: Üriner sistem US tetkiki yapılan ve 1 ay içerisinde kontrastsız bilgisayarlı tomografi (BT) tetkiki de mevcut olan 193 hastanın, US raporları ve BT görüntüleri retrospektif olarak incelenerek üriner sistemde taş varlığı ve boyutu, lokalizasyonu ve eşlik eden hidronefroz varlığı yönünden değerlendirilip taş boyutları karşılaştırıldı.

Bulgular: Çalışmaya dahil edilen 193 hastada, US veya BT ile 318 adet taş tespit edildi. BT ile saptanan taşların %57,7'si US ile saptanabildi.

Taş boyut ölçümü için karşılaştırma 141 (%73,0) hastada, hem US hem de BT'de saptanan 183 (%57,5) taş için yapıldı. Taşların boyut ortalaması US ve BT'de sırasıyla $8,78 \pm 6,79$ mm ve $8,11 \pm 6,72$ mm olarak ölçüldü ($p < 0,001$). Taşlar boyutlarına göre (≤ 5 mm, $>5-10$ mm, >10 mm) ayrıldığında tüm gruplarda US ile ölçülen ortalama taş boyutları BT'ye kıyasla daha büyüktü ($p < 0,001$). Tüm lokalizasyonlarda US ile ölçülen taş boyutu BT'ye göre daha büyük saptandı ($p < 0,05$). Hidronefrozu olan ve olmayan her iki grupta da taş boyutu US'de, BT'den daha büyük ölçüldü ($p < 0,001$). Hastaların %15'inde US'de taş boyutu klinik olarak anlamlı kabul edilen 5mm'den büyük ölçülürken; BT'de bu taşların 5mm'den küçük olduğu anlaşıldı.

Sonuç: US'nin nefrolitiazis tanısında duyarlılığı BT'ye kıyasla daha düşük olup, boyut, yerleşim ve hidronefroz varlığına göre taşlar incelendiğinde tüm gruplarda US ile ölçülen taş boyutları BT'ye kıyasla daha büyük olarak saptandı.

Anahtar Sözcükler: Bilgisayarlı tomografi, Boyut ölçümü, Doğruluk, Nefrolitiazis, Ultrasonografi

ABSTRACT

Aim: To demonstrate the accuracy of ultrasonography (US) in diagnosing nephrolithiasis and measuring stone size.

Materials and Methods: Overall, 193 patients who underwent a urinary system US examination and had a non-contrast computed tomography (CT) examination within one month were retrospectively reviewed. US reports and CT images were evaluated regarding the presence and size of stones, their localization, and accompanying hydronephrosis, and the stone sizes were compared.

Results: In 193 patients included in the study, 318 stones were detected by US or CT. Overall, 57.7% of stones identified by CT were detected using US. The stone sizes measured were compared for 183 (57.5%) stones detected by both US and CT in 141 (73.0%) patients.

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The mean stone size was 8.78±6.79 mm in US and 8.11±6.72 mm in CT (p<0.001). The mean stone sizes measured by US were higher than those measured by CT in all size groups (≤5 mm, >5–10 mm, >10 mm; p<0.001) and all localizations (p<0.05). In both groups with and without hydronephrosis, the stone size measured by US was higher than that measured by CT (p<0.001). In 15% of the patients, the stone size measured by US was greater than 5mm, considered clinically significant, while on the CT, these stones were smaller than 5mm.

Conclusion: US is less sensitive than CT in diagnosing nephrolithiasis, and when stones are assessed per size, location, and the presence of hydronephrosis, the stone sizes measured using US were found to be larger than those measured using CT in all groups.

Keywords: Computed tomography, Accuracy, Nephrolithiasis, Size measurement, Ultrasonography

GİRİŞ

Ürolitiazis oldukça sık görülen bir hastalık olup yaşam boyunca insidansı %12 ve rekürrens oranı %50 olarak bildirilmiştir (1). Üriner sistem taşı tanısında altın standart görüntüleme yöntemi kontrastsız bilgisayarlı tomografi (BT) olup, duyarlılık ve özgüllüğü sırasıyla %95–98 ve %94–99 olarak bildirilmiştir (2). Ancak görece yüksek maliyet ve iyonizan radyasyon riski kontrastsız BT'nin yaygın kullanımının önüne geçmektedir (2). Bunun tersine kolay ulaşılabilir olması, iyonizan radyasyon içermemesi ve görece ucuz olması nedeniyle ultrasonografi (US) böbrek taşı tanısında ilk görüntüleme yöntemi olarak yaygın bir şekilde kullanılmaktadır. US'nin taş saptamadaki duyarlılık ve özgüllük değerleri çalışmalar arasında farklılık göstermekte olup, sırasıyla %24–81 ve %83–100 aralığında bildirilmiştir (3).

Böbrek taşının boyutu klinik yönetimde esas karar verici etken olarak kabul edilmektedir. Taşın boyutu 5mm'den daha küçük ise kendiliğinden düşme olasılığı daha yüksek olup, Coll ve ark. taşın boyutu arttıkça kendiliğinden düşme olasılığının azaldığını göstermiştir (4). Bu nedenle hangi hastaların gözlem ile takip edileceği ve hangi hastalara ürolojik girişim yapılacağı kararında taş boyutunun doğru tespiti kritik öneme sahiptir.

Böbrek taşı tanısında US'nin etkinliğini ve boyut ölçümündeki doğruluğunu değerlendiren çalışmalar farklı sonuçlar ortaya koymuştur (5–15). Bu çalışmada US'nin nefrolitiazis tanısında ve böbrek taşı boyut ölçümündeki etkinliği ve doğruluğunun saptanması, hangi boyuttaki taşların US ile doğru bir şekilde tespit edilebileceğinin ve hangi hastaların klinik yönetiminde US'nin etkin ve doğru bir

görüntüleme yöntemi olduğunun ortaya konulması amaçlanmıştır.

GEREÇ ve YÖNTEM

Etik kurul onayı alındıktan sonra, Aralık 2022 ile Temmuz 2024 tarihleri arasında İzmir Torbalı Devlet Hastanesi'nde aynı radyoloji hekimi tarafından üriner sistem US tetkiki yapılan ve aynı hastanede 1 ay içerisinde kontrastsız BT tetkiki de mevcut olan 18 yaş üzeri hastalar retrospektif olarak incelendi. US ve BT tetkikleri arasında geçen sürede taşı düşürdüğü veya bu sürede böbrek taşı için cerrahi girişim uygulandığı tespit edilen olgular, US ve BT tetkiki yetersiz tanısal kalitede olan olgular ve geyik boynuzu şeklinde taşı olan olgular çalışmadan dışlandı. Ayrıca US veya BT tetkiklerinde üreter taşı tespit edilen ancak iki tetkik arasındaki sürenin 1 haftadan daha uzun olduğu olgular interval dönemde üreter taşlarının yer değiştirme olasılığına bağlı oluşacak birebir karşılaştırma güçlüğü nedeniyle çalışmaya dahil edilmedi. Bu kriterler doğrultusunda çalışmaya 193 hasta dahil edildi.

Hastaların demografik özellikleri incelendikten sonra, kontrastsız BT görüntüleri US raporlarından bağımsız olarak değerlendirildi. Üriner sistemde taş varlığı ve boyutu, lokalizasyonu (böbrek üst pol, orta kesim, alt pol, üreter ve mesane) ve eşlik eden hidronefroz varlığı kaydedildi. Saptanan taşın en uzun boyutuna, yumuşak doku penceresine göre daha doğru sonuç verdiği bildirilen kemik penceresinde (16), üç planda (aksiyal, koronal ve sagittal) yapılan ölçümler sonucunda karar verildi. BT tetkiki 16 kesitli Toshiba Alexion (Toshiba Medical Systems Corporation, Houston, TX) cihazında 120 Kvp ile otomatik tüp akım modülasyonu kullanılarak elde olundu. Hastaların üriner sistem US tetkikleri aynı radyolog

tarafından LOGIQ™ P7 (GE Healthcare, Chicago, IL) ve V8 (Samsung Medison, Seoul, Korea) cihazlarında sırasıyla 1–5 ve 1–7 MHz konveks prob kullanılarak gerçekleştirildi. US raporunda taş varlığı ve en uzun boyutu, taşın lokalizasyonu, eşlik eden hidronefroz varlığı ayrıntılı şekilde kaydedildi ve raporlar elektronik hasta kayıt sisteminde arşivlendi.

BT görüntülerinden elde edilen taş boyutları altın standart kabul edilerek, US raporlarındaki veriler ile karşılaştırıldı. Ayrıca taşlar boyutlarına göre 3 gruba (≤ 5 mm, >5 – 10 mm, >10 mm) ayrılarak her bir alt grup için US ve BT'de saptanan taş boyut ortalamaları birbiri ile kıyaslandı. Benzer analiz taş lokalizasyonuna göre de yapıldı. Eşlik eden hidronefrozun taş boyut ölçümü üzerindeki etkisi de mesanede saptanan 5 adet taş analiz dışı bırakılarak değerlendirildi.

İstatistiksel analiz 'Statistical Package for the Social Sciences' (SPSS) versiyon 26 kullanılarak yapıldı. Normallik analizinde Shapiro-Wilk testi kullanıldı. Nümerik veriler ortalama ve standart sapma, ortanca (çeyrekler arası aralık) veya sayı (yüzde) değerleri olarak verildi. Normal dağılan veriler bağımlı veya bağımsız gruplar t testi, normal dağılmayan veriler Wilcoxon işaretli sıralar testi ile karşılaştırıldı. Kategorik veriler arasındaki ilişki ise ki kare testi ile değerlendirildi. P değeri 0,05'in altında saptanır ise istatistiksel olarak anlamlı kabul edildi.

BULGULAR

Çalışmaya dahil edilen 193 hastada, US veya BT ile 318 adet taş tespit edildi. Hastaların 125 (%64,8)'i erkek ve 68 (%35,2)'i kadındı. Tüm hasta grubunun yaş ortalaması $48,17 \pm 16,72$ olup, kadın ve erkeklerin yaş ortalamaları arasında anlamlı farklılık saptanmadı ($p=0,244$). Kadın ve erkeklerde US ile taş saptanma oranında anlamlı farklılık izlenmedi ($p=0,71$). BT tetkiki altın standart olarak kabul edildiğinde, US'nin taş saptamadaki duyarlılığı %57,7 (güven aralığı (GA); %52,1–%63,2) olarak bulundu.

Taş boyut ölçümü için karşılaştırma 141 (%73) hastada hem US hem de BT'de saptanan 183 taş için yapıldı. Sadece bir görüntüleme yöntemi ile taş tanısı alan 52 (%27) hastadan, 51

(%26,4)'inde taş yalnızca BT ile saptanırken, 1 (%0,6) hastada ise US'de saptanan taş BT ile izlenmedi. US ile saptanamayan 134 taşın ortalama boyutu $3,7 \pm 1,7$ mm olarak ölçüldü. Hem US hem de BT'de saptanan taşların boyut ortalaması US ve BT'de sırasıyla $8,78 \pm 6,79$ mm ve $8,11 \pm 6,72$ mm olarak ölçüldü ($p<0,001$). Bu taşların 118 (%64,5)'i US'de BT'ye kıyasla daha büyük, 54 (%29,5)'ü BT ile aynı ve 11 (%6,0)'i BT'den daha küçük boyutta ölçüldü.

Taşlar boyutlarına göre üç gruba (≤ 5 mm, >5 – 10 mm, >10 mm) ayrıldı. US'nin duyarlılığı, ≤ 5 mm boyutlu taşlar için %42,3 (GA; %35,3–%49,6), >5 – 10 mm boyutlu taşlar için %72,0 (GA; %60,4–%81,8), >10 mm olan taşlar için ise %100 (GA; %92,5–%100) olarak bulundu. Her üç grupta da US ile ölçülen ortalama taş boyutları BT'ye kıyasla istatistiksel anlamlı olarak büyük saptandı ($p<0,001$, Tablo-1, Şekil-1 ve 2).

US'nin taş yönetimi üzerine etkisini araştırmak için klinik olarak anlamlı taş boyutu önceki çalışmalara benzer şekilde 5 mm olarak kabul edildi (4,9). Bu boyuttan küçük taşlara herhangi bir işlem önerilmediği göz önüne alındığında, 21 (%15,0) hastada, US ile 5 mm'den büyük boyutta ölçülen 21 (%11,5) adet taşın BT'de 5 mm'den daha küçük boyutta olduğu anlaşıldı.

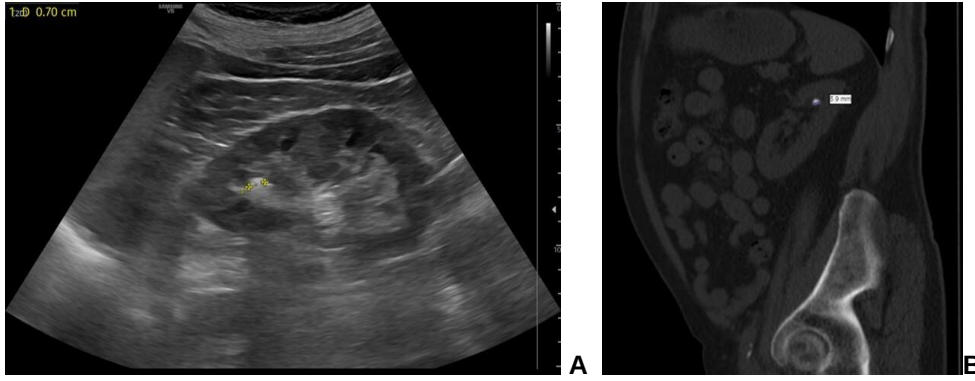
Taş yerleşiminin boyut ölçümü üzerindeki etkisini araştırmak için, taşlar yerleşimine göre dört gruba ayrıldı (böbrek üst pol, orta kesim, alt pol, üreter-mesane). Tüm lokalizasyonlarda US ile ölçülen taş boyutu BT'ye göre istatistiksel olarak anlamlı ölçüde büyük saptandı ($p<0,05$, Tablo-2).

Hidronefrozun taş boyut ölçümü üzerindeki etkisini araştırmak için böbrek ve üreter yerleşimli 178 adet taş için yapılan analizde, hidronefroz olan ve olmayan her iki grupta da US ile ölçülen taş boyutu BT ile ölçülenden daha büyük saptandı ($p<0,001$, Tablo-3). Hidronefroz eşlik ettiği 57 taş boyutuna göre 3 gruba (≤ 5 mm, >5 – 10 mm, >10 mm) ayrıldığında, ≤ 5 mm ve >10 mm boyutlu taşlarda US ile ölçülen taş boyutu BT'ye kıyasla anlamlı ölçüde büyük saptandı (sırasıyla $p<0,001$ ve $p=0,04$, Tablo-4). Boyutu >5 – 10 mm olan taşlarda ise ölçümler arasında anlamlı fark izlenmedi ($p=0,114$).

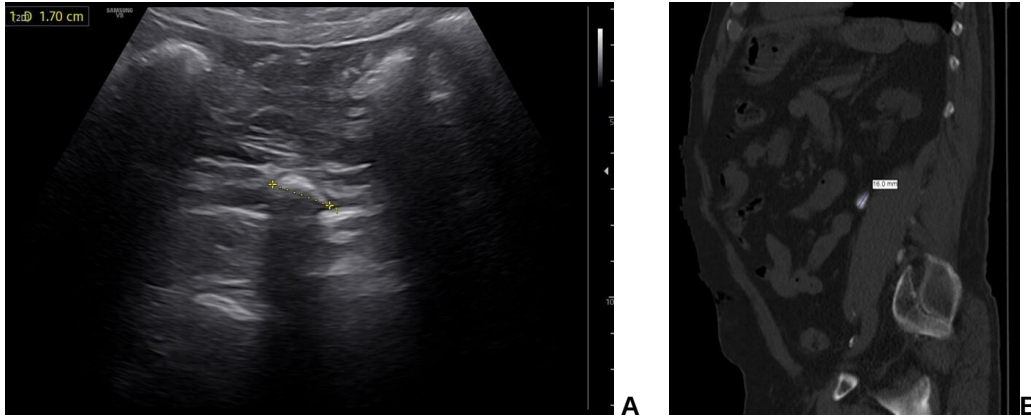
Tablo-1. Hem ultrasonografi (US) hem de bilgisayarlı tomografide (BT) saptanan taşların boyuta göre dağılımı ve boyut ortalamaları.

Taş boyut	Taş sayı	US'de boyutu daha büyük ölçülen taş sayısı	BT'de ölçülen taş boyut ortalaması	US'de ölçülen taş boyut ortalaması	p
≤5mm	82 (44,8)	52 (63,4)	3,63±0,98	4,33±1,20	<0,001
>5–10mm	54 (29,5)	32 (59,3)	7,26±1,40	7,83±1,72	<0,001
>10mm	47 (25,7)	34 (72,3)	16,89±7,70	17,63±7,74	<0,001

Veriler sayı (yüzde) veya ortalama ± standart sapma olarak gösterildi.



Şekil-1. 69 yaşında erkek hastanın yan ağrısı nedeniyle yapılan üriner sistem ultrasonografi tetkikinde sol böbrek üst pol düzeyinde 7 mm boyutunda taş ile uyumlu hiperekoik lezyon izlendi (1A). Olgunun elde olunan bilgisayarlı tomografi tetkikinde ise sagittal görüntülerde üst polde yerleşim gösteren taşın boyutu 5,9 mm olarak ölçüldü (1B).



Şekil-2. 72 yaşında erkek hastanın hematüri nedeniyle elde olunan üriner sistem ultrasonografi tetkikinde sol üreter orta kesim düzeyinde 17 mm boyutunda taş ile uyumlu hiperekoik lezyon ve arkasında akustik gölgelenme izlendi (2A).

Olgunun 1 hafta içerisinde elde olunan bilgisayarlı tomografi tetkikinde ise sagittal görüntülerde psoas kası anterior komşuluğunda üreter orta kesim düzeyinde taş ile uyumlu hiperdens lezyonun boyutu 16 mm olarak ölçüldü (2B).

Tablo-2. Hem ultrasonografi (US) hem de bilgisayarlı tomografide (BT) saptanan taşların yerleşimine göre dağılımı ve boyut ortalamaları.

Taş yerleşimi	Taş sayısı	US'de boyutu daha büyük ölçülen taş sayısı	BT'de ölçülen taş boyutu	US'de ölçülen taş boyutu	p
Böbrek üst pol	14 (7,7)	8 (57,1)	6,51±3,15	7,25±3,19	0,002
Böbrek orta kesim	66 (36,1)	48 (72,7)	5,75 (3,0–13,63)	6,25 (4,0–14,25)	<0,001
Böbrek alt pol	55 (30,0)	38 (69,1)	7,16±4,37	7,84±4,39	<0,001
Üreter-mesane	48 (26,2)	24 (50,0)	8,07±4,98	8,52±5,16	0,003

Veriler sayı (yüzde), ortalama ± standart sapma veya ortanca (çeyrekler arası aralık) olarak gösterildi.

Tablo-3. Hem ultrasonografi (US) hem de bilgisayarlı tomografide (BT) böbrek veya üreterde saptanan taşların eşlik eden hidronefroz varlığına göre dağılımı ve boyut ortalamaları.

Hidronefroz	Taş sayısı	BT'de ölçülen taş boyutu	US'de ölçülen taş boyutu	p
Var	57 (32,0)	7,18±4,55	7,68±4,58	<0,001
Yok	121 (68,0)	5,5 (3,85–10,25)	6,5 (4,75–11,25)	<0,001

Veriler sayı (yüzde), ortalama ± standart sapma veya ortanca (çeyrekler arası aralık) olarak gösterildi.

Tablo-4. Hem ultrasonografi (US) hem de bilgisayarlı tomografide (BT) böbrek veya üreterde saptanan ve hidronefroz eşlik eden taşların boyuta göre dağılımı ve boyut ortalamaları.

Taş boyut	Taş sayısı	BT'de ölçülen taş boyut ortalaması	US'de ölçülen taş boyut ortalaması	p
≤5mm	26 (45,6)	3,67±0,95	4,30±1,06	<0,001
>5–10mm	17 (29,8)	6,83±0,86	7,10±1,25	0,114
>10mm	14 (24,6)	14,12±3,15	14,67±3,25	0,040

Veriler sayı (yüzde) veya ortalama ± standart sapma olarak gösterildi.

TARTIŞMA

Bu çalışmada US'nin taş saptamadaki duyarlılığı BT'ye kıyasla düşük olup, taş boyutu ile ilişkili bulundu. Taşlar boyut, yerleşim ve eşlik eden hidronefroz varlığına göre gruplara ayrıldığında, tüm gruplarda US'de ölçülen taş boyutu BT'ye kıyasla istatistiksel olarak anlamlı ölçüde büyük saptandı. Hidronefrozun eşlik ettiği taşlar boyutlarına göre gruplandırıldığında yalnızca boyutu >5–10 mm olan taşlarda US ile BT boyut ölçümleri arasında anlamlı fark izlenmedi.

Literatürdeki çalışmalarda US'nin üriner sistem taşlarını saptamadaki duyarlılığı %12 ile %93 arasında değişkenlik göstermekte olup (10,17), bu çalışmada Ganesan ve ark.'nın (10)

çalışmasına benzer şekilde %57,7 olarak bulundu. US'nin taş saptamadaki duyarlılığı özellikle 5mm'den küçük boyutlu taşlarda düşük olmakla birlikte, daha önceki çalışmalara (10,11) paralel şekilde bu çalışmanın sonuçlarında da ortaya konulduğu gibi taş boyutu arttıkça US'nin duyarlılığı da artmaktadır.

US'nin taş boyut ölçümündeki doğruluğunu araştıran çalışmalar arasında görüş birliği bulunmamaktadır (6,8–12,15). Çalışmaların çoğunda US'nin BT'ye kıyasla taş boyutunu 2–3 mm kadar daha büyük ölçtüğü bildirilse de (6,8–10), bazı çalışmalarda istatistiksel olarak anlamlı fark bulunmazken (11,12), Abbas ve ark.'nın çalışmasında ise US'nin BT'ye kıyasla taş boyutunu daha küçük ölçtüğü tespit edilmiştir

(15). Bu çalışmada ise taş boyutuna, yerleşimine ve hidronefroz varlığına göre oluşturulan tüm alt gruplarda US ile ölçülen boyutlar BT'ye kıyasla daha büyük olarak ölçüldü. Yalnızca hidronefrozun eşlik ettiği taşlar boyutlarına göre de sınıflandırıldığında boyutu >5–10 mm olan taşlarda US ile BT boyut ortalamaları arasında anlamlı fark izlenmedi. BT'deki taş boyut ölçümünün taşın cinsinden etkilendiğini gösteren deneysel çalışma mevcut olup (16), çalışmalar arasındaki bu fark seçilen örneklemdeki taşların cinsi ve kullanılan US cihazları arasındaki fark nedeniyle ortaya çıkmış olabilir.

Nefrolitiazisin klinik yönetiminde, taş boyutu en önemli belirleyici faktör olarak kabul edilmektedir. Yapılan çalışmalarda 5 mm'den daha küçük boyutlu taşların kendiliğinden düşme olasılığının daha yüksek olduğu gösterilmiştir (4). Bu nedenle taş boyutunun US ile doğru olarak ölçülmesi kritik role sahiptir. Bu çalışmada taşı hem US hem de BT de tespit edilen hastaların %15'inde, BT ile gözlem önerilecek iken, US'de ise bu hastalarda taş boyutu 5 mm'den daha büyük ölçüldü. Benzer şekilde, Ganesan ve ark.'nın çalışmasında bu oran %14, Sternberg ve ark.'nın çalışmasında ise %26,8 olarak bildirilmiştir.

Nefrolitiazis tanısında BT altın standart olmakla birlikte, iyonizan radyasyon maruziyeti ve görece yüksek maliyeti nedeniyle US gibi alternatif görüntüleme yöntemlerine ihtiyaç duyulmaktadır. Ancak US'nin duyarlılığının düşük olması ve taş boyut ölçümündeki değişken doğruluk değerleri nefrolitiazis tanısında ve klinik yönetiminde tek başına kullanımının önüne geçmektedir. Yapılan çalışmalarda standartlaştırılmış taş yazılım programlarının kullanılması ve taşın kendisi yerine gölgesinin ölçülmesi, US'nin taş boyut ölçümündeki doğruluğunu arttırdığı gösterilmiştir (8,18). Ayrıca yazılımlar aracılığı ile hesaplanan

taş hacminin, yalnızca lineer boyut ölçümüne göre taşın kendiliğinden düşüp düşmeyeceğini daha doğru bir şekilde ön gördüğü bildirilmektedir (13). Çeşitli görüntü yeniden yapılandırma modelleri ile US'nin taş boyut ölçümündeki hata payının azaltılabileceği de gösterilmiştir (14).

Bu çalışmanın retrospektif dizaynına ek olarak çeşitli sınırlılıkları mevcuttur. Bunlardan ilki verilerin tek merkezden elde edilmesi bulguların genellenebilirliğini kısıtlamaktadır. İkinci olarak verilerin tek radyoloji hekimi tarafından değerlendirilmesi, gözlemciler arası uyumun değerlendirilmesine engel olmuştur. Benzer şekilde gözlemci içi uyum da bu çalışmada değerlendirilmemiştir. Son olarak, boyut ölçümü üzerine etkisi olduğu bildirilen taş natürünün analiz edilmemesi de çalışmanın bir diğer sınırlılığıdır.

SONUÇ

Böbrek taşı boyutunun doğru ölçümü hastaların klinik yönetiminde önemli role sahiptir. Bu çalışmada boyut, yerleşim ve eşlik eden hidronefroz varlığına göre taşlar incelendiğinde tüm gruplarda US ile ölçülen taş boyutları BT'ye kıyasla daha büyük olarak saptandı. Buna ek olarak, US'nin nefrolitiazis tanısında duyarlılığı BT'ye kıyasla daha düşük olup, bu fark ≤ 5 mm boyutlu taşlarda en belirgin olarak izlendi. Tanımlanan bu sınırlılıklar US'nin nefrolitiazis tanısı ve klinik yönetiminde tek başına kullanımını engellemektedir. Son yıllarda kullanılan çeşitli yazılımlar ve görüntü yeniden yapılandırma modelleri ise US'nin taş boyut ölçümündeki doğruluğunu artırmada umut vadetmektedir.

Çıkar Çatışması: Yazar çıkar çatışması olmadığını beyan etmişlerdir.

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Neurobehavioral effects of maternal triphenyl phosphite exposure: A comparative experimental study using the valproic acid model

Maternal trifenil fosfit maruziyetinin nörodavranışsal etkileri: valproik asit modeli ile karşılaştırmalı deneysel bir çalışma

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ABSTRACT

Aim: Neurodevelopmental disorders are characterized by impairments in one or more developmental domains originating in the embryonic period and persisting throughout life. Autism, experimentally induced by valproic acid (VPA) in animal models, is a neurodevelopmental disorder. This study aimed to compare the outcomes of maternal exposure to triphenyl phosphite (TPP), a neurotoxic organophosphorus compound, and VPA in terms of neurobehavioral alterations.

Materials and Methods: Pregnant rats were randomly assigned to three groups: VPA (n=3), TPP (n=3), and control (n=3). Each group received an intraperitoneal injection of either VPA, TPP, or saline (for the control group) on embryonic day 12.5. Male offspring (n=10 per group) were evaluated on postnatal days 33–34 using the Marble Burying Test and the Three-Chambered Social Interaction Test. Animals were perfused on postnatal day 35 for immunohistochemical (IHC) analysis.

Results: TPP group exhibited a significant increase in marble burying behavior ($p<0.05$). Also, Three-Chamber Social Interaction Test revealed significant impairments in social interaction in the VPA and TPP groups compared to the control ($p<0.05$). Histologic evaluation revealed higher nestin-positive cell density in the subgranular zone of the control group compared to VPA and TPP groups ($p<0.05$). Synaptophysin expression in the CA3 region was higher in the control group than in the VPA and TPP groups ($p<0.05$). Connexin-43 expression increased in the CA1 region of the TPP group compared to controls ($p<0.05$), while no significant change was observed in the VPA group.

Conclusion: Maternal TPP exposure may be regarded as a risk factor for autism-related neurodevelopmental disorders.

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ÖZ

Amaç: Nörogelişimsel bozukluklar, embriyonik dönemde ortaya çıkan ve yaşam boyunca devam eden bir veya daha fazla gelişimsel alandaki bozukluklarla karakterize edilmektedir. Nörogelişimsel bozukluklardan biri olan otizm, hayvan modellerinde valproik asit (VPA) ile indüklenebilmektedir. Bu çalışma, nörotoksik bir organofosfor bileşiği olan trifenil fosfit (TPP) ve VPA'ya maternal maruziyetin sonuçlarını nörodavranışsal değişiklikler açısından karşılaştırmak amacıyla planlanmıştır.

Gereç ve Yöntem: Gebe sıçanlar randomize şekilde üç gruba ayrılmıştır: VPA (n=3), TPP (n=3) ve kontrol (n=3). Her gruba embriyonik 12.5. günde intraperitoneal VPA, TPP veya salin (kontrol grubu için) enjeksiyonu yapılmıştır. Erkek yavrular (grup başına n=10) doğum sonrası 33-34. günlerde Üç Odacıklı Sosyal Etkileşim Testi ve Mermer Gömme Testi kullanılarak değerlendirilmiştir. Hayvanlar immünohistokimyasal (IHC) analiz için postnatal 35. günde perfüze edilmiştir.

Bulgular: Üç Odacıklı Sosyal Etkileşim Testi sonuçları; VPA ve TPP gruplarında kontrol grubuna kıyasla sosyal etkileşimde anlamlı bozulmalar olduğunu göstermiştir ($p<0.05$). Ayrıca, TPP grubunda bilye gömme davranışında anlamlı bir artış saptanmıştır ($p<0.05$). Histolojik analizler ile kontrol grubunun subgranüler zonunda (SGZ) VPA ve TPP gruplarına göre daha yüksek nestin-pozitif hücre yoğunluğu tespit edilmiştir ($p<0.05$). Connexin-43 ekspresyonunda, TPP grubunun CA1 bölgesinde kontrol grubuna kıyasla artış bulunmuş ($p<0.05$) ancak VPA grubunda ise anlamlı bir değişiklik olmamıştır. Sinaptofizin ise CA3 bölgesinde kontrol grubunda, VPA ve TPP gruplarına kıyasla daha yüksek eksprese edilmiştir ($p<0.05$).

Sonuç: Araştırmanın bulguları, TPP'nin maternal maruziyetinde yavruların nörogelişimsel bozukluklar açısından özellikle otizm için riskli olarak değerlendirilebileceğini ortaya koymuştur.

Anahtar Sözcükler: Nörogelişimsel Bozukluklar; Otizm Spektrum Bozukluğu; Maternal Maruziyet; Organofosfor Bileşikleri; Hipokampus.

INTRODUCTION

Neurodevelopmental disorders (NDs) are lifelong conditions characterized by impairments in social communication, dysfunctions in motor skills, and deficits in cognitive and sensory processes, commonly emerging in early childhood (1). The occurrence and severity of symptoms may differ among individuals (2). While these disorders are thought to have a genetic basis, non-genetic factors that are considered potential triggers have yet to be fully elucidated. Exposure to environmental factors during the embryonic period and early postnatal stages is recognized as a substantial risk factor for the onset of NDs. Such factors include socioeconomic status, malnutrition, advanced maternal age, stress, inflammation, exposure to high levels of air pollution, and contact with chemicals such as heavy metals and organophosphate pesticides (3–5).

Triphenyl phosphite (TPP) is an acetylcholinesterase inhibitor and an organophosphorus-derived neurotoxic agent

classified as Type II. It is commonly used in the agriculture sector as an insecticide and fungicide, in the plastic and rubber industry as a stabilizer, anti-wear additive, and epoxy resin. It causes delayed ataxia, paralysis, and changes in the nervous system prospectively (6). As a result of the administration of TPP at a dose of 1184 mg/kg, axonal and terminal degeneration was observed in the vestibular complex, cerebellum, superior and inferior colliculi, and cochlear nuclei. Degenerative axons and terminals were also identified in the cerebral cortex, hippocampus, thalamus, hypothalamus, septal region, substantia nigra, and subthalamic nucleus. Prominent degeneration was evident in the sensorimotor cortex, magnocellular preoptic and medial mammillary nuclei of the hypothalamus, as well as in the mediodorsal, ventromedial, and medial geniculate nuclei of the thalamus, while degeneration within the fasciculus gracilis and its associated nuclei appeared limited (7). Furthermore, TPP exposure at 250 mg/kg impaired learning abilities in male offspring (8). However, its effects on brain

development and behavioral outcomes have not been clarified yet.

Autism, one of the most common ND, is associated with an increased risk of occurrence subsequent to maternal exposure to detrimental environmental factors during critical developmental periods (9–12). Valproic Acid (VPA) is a histone deacetylase inhibitor and modulates neurotransmission and gene expression. It is an antiepileptic drug and is used in mood disorders (13). VPA exposure in the early embryonic period causes deterioration in the structure of the nervous system and behavior in rats (14). Rodier et al. proposed VPA as a model of autism in 1996, and the VPA model has become one of the most frequently studied models today (15,16).

This study sought to compare the neurobehavioral outcomes identified in juvenile offspring following maternal TPP exposure and the VPA-induced autism model.

MATERIALS AND METHODS

Approval of the experimental protocol was granted by the Local Ethics Committee for Animal Experiments affiliated with Ege University (Approval No: 2017-081).

Animals

Adult female Wistar albino rats (250–300 g, n=9) were first assessed for their estrous cycle stage using vaginal smears. Females at the appropriate stage were housed overnight with males, and the presence of vaginal plugs detected the following morning was used to indicate the onset of pregnancy, marking the first day of gestation. After delivery, only male offspring were included in all experimental procedures to minimize potential variability caused by hormonal fluctuations. Throughout the study, animals were maintained under controlled laboratory conditions ($22 \pm 2^\circ\text{C}$, $50 \pm 10\%$ humidity) with a 12-hour light/dark cycle and had ad libitum access to food and water.

Chemicals

VPA was used in the form of sodium valproate (DEPAKIN®, Sanofi-Aventis), which was diluted with 0.9% sodium chloride solution and administered at a dose of 500 mg/kg (13). TPP (Sigma-Aldrich, T84654) was initially administered at a non-toxic dose of 250 mg/kg, as reported by Levin et al. (8); however, due to mortality observed on the fourth day following administration, the

dose was reduced to 100 mg/kg and subsequently administered intraperitoneally in propylene glycol (1 ml/kg) in the main study.

Experimental design

Following confirmation of pregnancy by the detection of vaginal plugs, nine rats were randomly divided into three groups: TPP, VPA, and control. TPP and VPA were administered intraperitoneally on embryonic day 12.5 (E12.5), while the control group received an equivalent volume of saline. However, two of the pregnant rats receiving TPP at 250 mg/kg did not survive until the fourth day post-injection, and the remaining rat was excluded from the study. Subsequently, three pregnant rats, not previously subjected to experimental procedures, were randomly selected and administered TPP at a reduced dose of 100 mg/kg in propylene glycol (1 ml/kg) via intraperitoneal injection on E12.5. The revised dose did not significantly affect maternal weight gain during gestation, and offspring delivery occurred on gestational days 21 and 22. Offspring remained with their dams until weaning. Behavioral testing was conducted on P33–P34 (n=10 per group), and animals were perfused for immunohistochemical (IHC) analysis on P35 (n=5 per group).

Behavioral Tests

The male offspring (n=10/each group) were handled for 5 days before behavioral experiments which were conducted between 09:00 a.m. and 4:00 p.m in a room with dim light.

Marble Burying Test

Repetitive behaviors in rodent models of autism are commonly evaluated using the Marble Burying Test. Twenty regular glass marbles were placed on the odorless and unused sawdust with a depth of 5 cm in the same order for each cage. The rat was released from the corner of the cage not to disturb the placement of the marbles and the sawdust bed. The cages were covered after releasing and expected them to bury the marbles in 30 minutes. The animals were not fed or given water during the experiment and the marbles were cleaned with 70% alcohol between trials. After the test, animals were transferred to their home cages without disturbing the sawdust and marbles. If 2/3 of each marble is covered with sawdust, it was recorded as buried. The scores of three different researchers were averaged and the final score was determined (17).

Three-Chamber Social Interaction Test

Crawley's Three-Chamber Social Interaction Test was utilized to evaluate the social preference of rodents (18). In this study, the test was conducted on P33–P34 using a three-chambered rectangular apparatus, in which the central compartment provided two openings allowing access to the side chambers. In the habituation stage, the subject was released in the middle compartment with the blocked entrances for 5 minutes. In session I, a stranger animal (stranger 1) with similar characteristics (same sex, similar weight, and age) of the subject and had not encountered with the subject before was placed in one of the right or left chambers (changed systematically). The entrances were opened to allow the subject to explore other chambers for 10 minutes. In session II, another stranger animal (stranger 2) was placed in the other empty compartment and allowed the subject to explore for 10 minutes. A basket with holes was placed to prevent stranger rats from leaving the chamber and contact with the subject physically. Test materials were cleaned with 70% alcohol for each trial.

Histopathologic Evaluation

Perfusion procedures for histopathological analysis were initiated on postnatal day 35 (P35). Anesthetic agents Ketamine (80–100 mg/kg) and Xylazine (10–12.5 mg/kg) were administered to the animals. Following anesthesia, brains were removed and fixed in 4% formaldehyde for three days, then processed in Phosphate Buffer Solution (PBS) on the fourth day. The hippocampus was carefully removed following the stereotaxic coordinates described by Paxinos and Watson (19). Subsequently, the removed tissue was incubated for 24 hours in a solution containing 0.1 M cacodylate buffer and formalin. Hippocampal tissues were embedded in paraffin, sectioned at 5 µm thickness, and stained with routine Haematoxylin and Eosin (H&E).

Immunohistochemical Procedures

Based on the previously described protocol, sections were first incubated with 10% hydrogen peroxide (H₂O₂; Sigma-Aldrich, Inc., Saint Louis, MO, USA) for 30 minutes to block endogenous peroxidase activity, and subsequently treated with Super Block (ScyTek Laboratories, Inc., Logan, UT, USA) for 1 hour at room temperature to minimize non-specific antibody-antigen binding. Primary antibodies specific for nestin (Bioss Antibodies, Woburn, MA, USA), synaptophysin

(Sigma-Aldrich, Inc., Saint Louis, MO, USA), and connexin43 (Abcam, Cambridge, UK) were used at a dilution of 1:100. Tissue sections were incubated with these antibodies at +4 °C for 24 hours. Following incubation, a biotinylated secondary antibody (ScyTek Laboratories, Inc., Logan, UT, USA) was applied, followed by horseradish peroxidase (HRP)-conjugated streptavidin (ScyTek Laboratories, Inc., Logan, UT, USA). Finally, sections were washed and incubated with diaminobenzidine (DAB) and Mayer's hematoxylin (Merck KGaA, Darmstadt, Germany) for visualization under a light microscope (20).

Image Analysis

SYN and Cx-43 expression in CA1 and CA3 regions, along with the density of nestin-positive cells in the Subgranular zone (SGZ) (cells/mm²), were evaluated under a light microscope (Olympus BX-51) and imaged with a digital camera (Olympus C-5050). ImageJ (<https://imagej.nih.gov/ij>) and cellSens (Olympus Corporation, Japan) software were used for analysis. IHC evaluations were independently conducted by three blinded researchers, each assessing a minimum of 100 randomly selected neurons per rat. Immunoexpression levels were quantified using the H-score method, and the mean values of these scores were used for statistical analysis (21).

Data analysis

Statistical evaluations were conducted using IBM SPSS Statistics version 22. The Shapiro–Wilk test was applied to assess data normality and homogeneity. Datasets meeting these assumptions were analyzed through one-way ANOVA followed by Bonferroni post hoc comparisons, while non-normally distributed data were assessed using the Kruskal–Wallis test, with pairwise comparisons performed by the Mann–Whitney U test. Results are presented as mean ± standard error of the mean (SEM), and a threshold of p<0.05 was set for statistical significance.

RESULTS

Behavioral Tests Results

Marble Burying Test

Mann Whitney test revealed that there was a significant difference (p=0.029). Control group buried 10-15% of the marbles while TPP

($p=0.037$) and VPA ($p=0.012$) groups buried 30-35% regarding the repetitive behavior (Figure- 1).

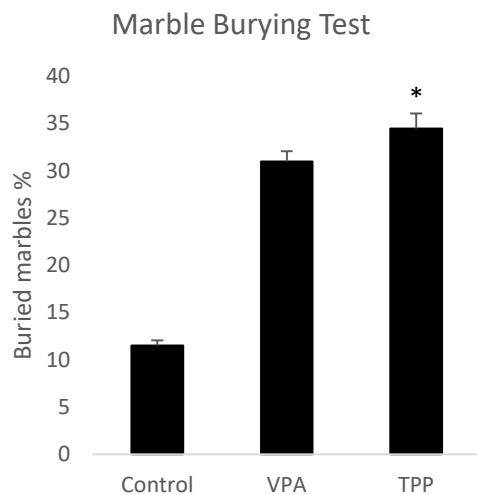


Figure-1. The percent of buried marbles was higher in TPP group than controls significantly ($n=10$ /each group).

* $p<0.05$ vs VPA and control

Three-Chamber Social Interaction Test

Kruskal-Wallis test demonstrated a significant difference between groups in session I ($p=0.009$). The duration in the empty chamber of VPA and TPP groups was longer than controls which were prone to explore the chamber of the stranger rat 1 significantly ($p=0.009$) (Figure-2A).

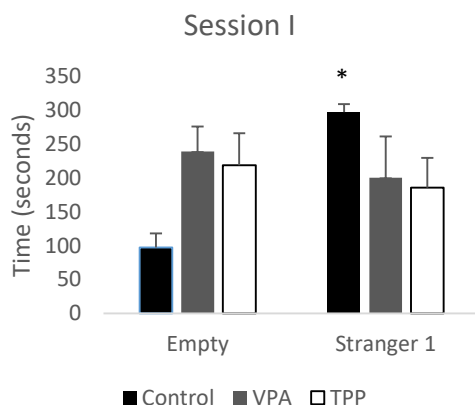


Figure-2. (A) Control rats preferred the chamber of the stranger rat 1 while they spent more time in the chamber of stranger rat 2 in session II.

In session II, control group spent time in the chamber of the stranger rat 2 more than the TPP group significantly ($p=0.005$). Moreover, the

findings showed that the TPP group preferred the chamber of the stranger rat 1 more than the VPA group ($p=0.048$) (Figure-2B).

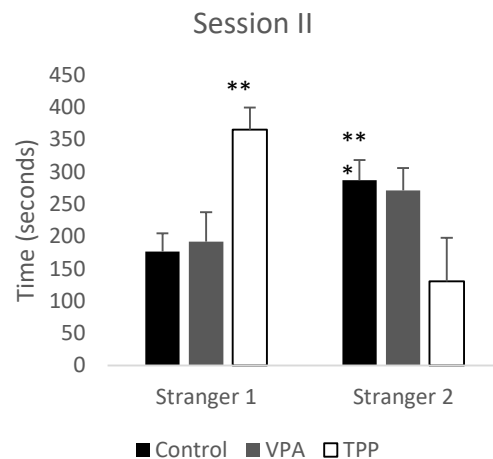


Figure-2. (B) Further, TPP group preferred the chamber of the stranger rat 1 rather than stranger rat 2 as a result of impaired social novelty.

$p<0.05$ vs VPA and TPP, $p^{**}<0.05$ vs TPP, $p^{***}<0.05$ vs TPP

Histopathological Results

Histological analysis of hippocampal tissue sections stained with H&E revealed notable histological differences between the experimental groups (Figure-3). The control group exhibited a typical hippocampal architecture, characterized by well-preserved pyramidal cell layers and normal neuronal morphology. In contrast, hippocampal sections from the VPA and TPP groups demonstrated neuronal abnormalities and evidence of apoptosis. These findings suggest that both VPA and TPP administration led to observable histopathological alterations in hippocampal structure compared to the control group.

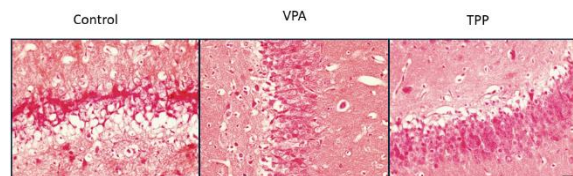


Figure-3. H&E-stained hippocampal tissue samples obtained from each experimental group (scale bar = 20 μ m). Typical histological architecture was observed in the hippocampal tissue of the control group, in contrast to the neuronal abnormalities and apoptotic changes detected in the VPA and TPP groups.

Immunohistochemical Results

Nestin expressions in SGZ

SGZ of hippocampus was examined by anti-nestin immunostaining. Nestin-positive cells were denser and more compact in control group as seen by arrows in Figure-4B while large cavity and disorganized cells were observed in the TPP group regarding neurodevelopmental delay. One-way ANOVA results showed a significant difference between groups ($F_{(2,15)}=18.536$, $p=0.000$). Post hoc analyses demonstrated that nestin expression was significantly higher in the control group (26.12 ± 3.07) compared to both the VPA (10.75 ± 1.05 , $p=0.001$) and TPP (9.74 ± 1.75 , $p=0.000$) groups (Figure-4A).

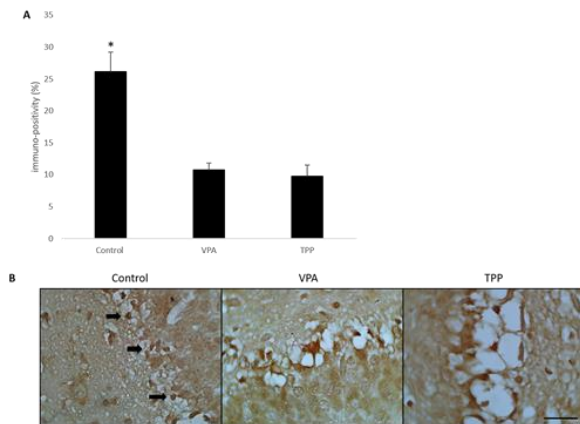


Figure-4. (A) Nestin expression in SGZ of control group was higher than other groups significantly. **(B)** Representing microphotographs of nestin expressions in the SGZ of hippocampus. The arrows indicate intense nestin-positive cells in SGZ ($n=6$ /each group, scale bar = 10 μ m, magnification x40). $p^*=0.001$ vs VPA, $p^*=0.000$ vs TPP

Synaptophysin and Connexin-43 expressions in CA1 and CA3

CA1 and CA3 areas of the hippocampus were examined to compare SYN and Cx-43 expressions within groups. Cx-43 expression of the TPP group (38.79 ± 2.46) was higher than control ($p=0.05$, 30.49 ± 3.07) in CA1 (Figure-5A). Furthermore, the scattered and impaired pyramidal neuron layer and Cx-43 immuno-expression are quite evident in the CA1 of the TPP group (Figure-5B).

Post hoc Bonferonni test following one-way ANOVA ($F_{(2,15)}=5.193$, $p=0.02$) revealed that SYN expression of the control group (37.75 ± 2.34) was

significantly higher than VPA (28.93 ± 2.07 , $p=0.04$) and TPP (28.21 ± 2.55 , $p=0.03$) groups in CA3 as seen in (Figure-5C). The impairment is prominent with dispersed neurons in CA3 of the TPP group and structural neuronal changes in the VPA group (Figure-5D).

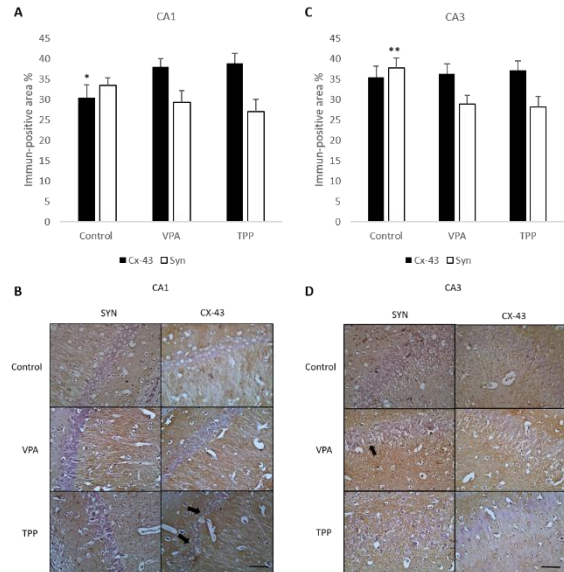


Figure-5. Cx-43 expression was higher in the TPP group than control significantly (A). The arrows indicate the impaired homogeneity of the CA1 pyramidal layer (B). SYN expression was higher in the control group more than VPA and TPP (C) and apoptotic-like neurons were seen in the CA3 of the VPA group as indicated by the arrow (D). $p^*=0.05$ vs TPP group, $p^{**}=0.02$ vs VPA and TPP groups (scale bar = 20 μ m)

DISCUSSION

Autism is a ND characterized by various neurological and behavioral impairments, showing significant differences in etiology, onset, and severity (22). It has been suggested that the behavioral changes seen in autism cannot be associated with a single region of the brain, but is related to many brain regions such as the limbic system, thalamus and cerebellum (23,24). One of the regions where structural changes seen in autism is the hippocampus (25). The embryonic maturation process of the hippocampus begins at day 12.5 and maternal VPA administration on that day causes autism-like behaviors in rats (26). The problems seen in the behaviors accompanying the developmental disorders of people with autism are caused by these abnormalities in hippocampus (27). Neuron loss was evident in the hippocampus

area in autism models of animals, and accordingly, impairments in learning skills were observed (28).

In this study, VPA model was compared with TPP, which is an environmental neurotoxic substance and behavioral changes and IHC differences in hippocampus were evaluated in juvenile rats.

Levin et al. (8) indicated that TPP (250 mg/kg) causes learning impairments in male offspring. However, in the present study, administration of TPP at a dose of 250 mg/kg resulted in maternal mortality, requiring a modification of the dose to 100 mg/kg for ensuing experiments. The Marble Burying Test was used to evaluate repetitive behaviors seen in autism. Wu et al. (29) showed that VPA-treated group showed more repetitive behavior by burying marbles compared to the control group. In a different model of autism studied by Degroote et al. (30) repetitive behaviors were measured with the Marble Burying Test, and a higher marble burying score was obtained in the experiment group than the controls. The number of marble burying increases in rodent models is associated with repetitive behaviors created by genetic manipulations (17). In present study, repetitive behaviors were observed in both VPA and TPP groups proving that TPP causes autism-like repetitive behavior.

The Three-Chamber Social Interaction Test was used to measure the deterioration in social communication seen in autism. The results in the VPA model of Dai et al. (31) demonstrated that the social interaction of the VPA group was impaired. A significant difference was found and social interaction impairment was also observed in the VPA group in the first session and second session scores of the three-chamber social interaction test performed by Kim et al. (32) and Wu et al. (33). In this study, VPA group was similarly dissociated and deteriorated and there was a decrease in social innovation preferences of the experimental group as compared to the control group. The effects of maternal TPP exposure on the social behavior were also found to be similar to VPA group. Maternal TPP exposure led to increased repetitive behaviors in the Marble Burying Test and impaired sociability and social novelty preferences in the Three-Chamber Social Interaction Test, which may reflect autism-like features.

The VPA model causes a delay in neurogenesis by substantially affecting interneurons, cell proliferation in SGZ and neuroplasticity of

hippocampus granular cells (17). In this study, nestin staining was used to show the differences of proliferation in SGZ. Cai et al. (34) showed a decrease in nestin-positive cells in SGZ in a mouse model of autism, and stated that this decrease was associated with a delay in migration towards the dentate gyrus. Similarly, the present study showed that maternal TPP exposure impaired nestin expression in the SGZ, suggesting a neurodevelopmental delay during the juvenile period, which may not only reflect reduced proliferation but also involve impairments in neuronal progenitor cells and neurogenesis processes.

Synaptic dysfunction is known to occur in animal models of autism induced by VPA (Iijima et al., 2016). Codagnone et al. (35) showed reduced SYN immunoreactivity in the hippocampal regions of VPA exposed rats. The present findings demonstrated scattered neurons and decreased SYN immunoreactivity in the CA3 region of TPP group refers to impaired synaptic transmission. Furthermore, studies have provided evidence of neuroglial responses and neuroinflammation in autism (36). The expression level of Cx-43, a major component protein in the astrocytic gap junction, is increased in the frontal cortex of post-mortem brain tissue from patients with autism (Mony et al., 2016). Chávez et al. (37) reported an increase in the activity of Cx-43 in the hippocampal regions in lipopolysaccharide model of autism associated with hippocampal neurotoxicity. In this study, increased Cx-43 expression, a marker of neuroinflammation and neurotoxicity implicated in the pathogenesis of NDs, particularly autism, was noticeably observed in the TPP group.

CONCLUSION

This study highlights the potential neurodevelopmental effects of maternal exposure to environmental toxicants. Maternal TPP administration may induce autism-like behaviors and IHC alterations in the hippocampus of juvenile rats, resembling changes observed in the VPA model. Although initial, these findings suggest that TPP exposure could be considered as a complementary approach in future ND models, particularly in studies focusing on autism. Further research is required to explore the effects of TPP at varying doses, on brain regions potentially associated with neurodevelopmental alterations,

additional behavioral outcomes, and across different developmental stages.

LIMITATIONS

The limitations of this study include the absence of early postnatal growth and maturation data, the unavailability of a separate propylene glycol vehicle control group, the lack of direct apoptosis measurement, and the possibility of an insufficient sample size.

Conflicts of interest: Authors declared no conflict of interest.

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Data Availability Statement

The data used to support the findings of study are included within the article.

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Important Note

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İntraoral lipom: 2 olgu sunumu

Intraoral lipoma: 2 case report

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Öz

Lipomlar yaygın yumuşak doku tümörleridir, ancak oral bölgede nadir görülürler. Matür yağ dokusundan oluşmuş, non-fonksiyone, selim tümörlerdir. Klinik seyir, yıllar içinde kademeli olarak genişleyen ve ağrısız bir kitle şeklinde gelişebilir. Dil altı, submandibular ve parafarengeal bölgelere uzanan dev lipom raporları bulunmaktadır. Lipom ön tanısı bulunan olguların cerrahi olarak eksizyonu ve post-operatif takibi kritik öneme sahiptir. Bu çalışmada, alt dudak bölgesinde zamanla büyümüş ve fonksiyon kaybına neden olan nadir görülen iki lipom vakası sunuyoruz.

Anahtar Sözcükler: İntraoral lipom, yağ tümörü, periferik lipom.

ABSTRACT

Lipomas are a common type of soft tissue tumour, occurring with greater frequency in other locations than in the oral region. They are non-functioning benign tumours formed from mature adipose tissue. The clinical course may manifest as a painless mass that gradually enlarges over years. There are reports of giant lipomas extending to the sublingual, submandibular and parapharyngeal regions. Surgical excision and post-operative follow-up are of critical importance in cases with a preliminary diagnosis of lipoma. In this study, we present two rare cases of lipomas in the lower lip region which enlarge over time and cause loss of function.

Keyword: Intraoral lipoma, fatty tumor, peripheral lipoma.

GİRİŞ

Lipomlar yumuşak dokuda en sık görülen mezenkimal neoplazmlardır. Atipi göstermeyen olgun beyaz adipositlerin çoğalması ile karakterizedir. Genellikle iyi sınırlı, ağrısız, lastik, soliter nodüller olarak bulunurlar ve ekstremitelerde nispeten sık görülürler. Buna karşılık baş ve boyunda nadiren lipomlara rastlanmaktadır. Oral lipom insidansının (OL) tüm benign oral lezyonların %1 ila %4'ü olduğu bildirilmiştir (1).

Her iki cinste ve tüm ırklarda eşit oranda rastlanılan lipomalar; çocuk ve adolesent bireylerde daha az görülürken 40-60 yaş grubu arasında sıklıkla görülür (1).

Oral lipomlar başlıca bukkal mukozada yer almakla birlikte, dil, ağız tabanı, dudaklar, damak ve dişeti mukozası içerisinde yer alabilmektedir. Bununla beraber retromolar ped ve tükürük bezleri daha az tutulan bölgelerdir. Mandibula ramus ve maksilla gövdesinde de nadiren intraosseöz lipomlar görülebilir (2).

Lezyonun boyutları değişkenlik göstermekte olup çoğu 1 cm'den küçüktür ve 3 cm'den büyük olanlar oldukça nadirdir (3). Lezyonlar sarımsı ve yumuşak yapıda, çevreden iyi ayrılmış, mobil, yavaş büyüyen ve çoğunlukla asemptomatik bir karakter sergilemektedir. Derin dokularda oluşan lipomalar vital bölgelerde basınç yaratabileceğinden önem gösterebilirler. Subkutanöz lipomalar bazı durumlarda estetik ya da fonksiyonel şikayetlere neden olabilmektedir (4).

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Lipomların oluşumunda kesin olarak bir neden belirtilemese de hiperkolesterolemi, obezite, endokrin bozukluklar, kromozomal anormallikler ve radyasyon etiyojisinde yer bulabilmektedir (2). Sporadik olarak meydana gelebilmenin yanında ailesel lipomatozis ile benign simetrik lipomatozisi de içeren bazı kalıtsal bozuklukları temsil edebilirler (3).

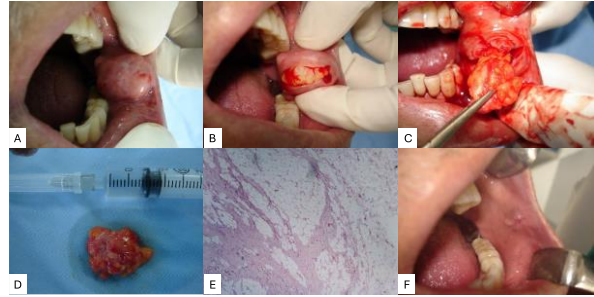
Radyolojik tanı yöntemleri arasında yer alan ultrason, yüksek çözünürlüklü BT ve MR görüntüleme gibi yöntemler lipom teşhisinde fayda sağlayabilirler. Histopatoloji ise oral lipomların teşhisinde altın standart olmayı sürdürmektedir (2).

Oral lipomalar konservatif cerrahi yaklaşım ile tedavi edilirler. Non-infiltratif tiplerinin rekürrens sıklığı düşüktür. Malign değişim çok nadirdir (5). Hastaların takip ve tedavilerinin planlanmasında, diğer muhtemel oral patolojilerden ayrımının yapılması önemlidir. Ayırıcı tanı bakımından lipomları incelediğimizde pannikülit, hematomlar, epidermoid kistler ile diğer adiposit tümörler gibi farklı lezyonlar düşünülebilir. Bunun yanında iyi huylu mezenkimal neoplazmalar ile tükürük bezi tümörleri de ayırıcı tanıda yer alabilirler (2).

Bu vaka raporunda oral mukozada yer alan iki oral lipom olgumuzu sunuyoruz.

Olgu Sunumu-1.

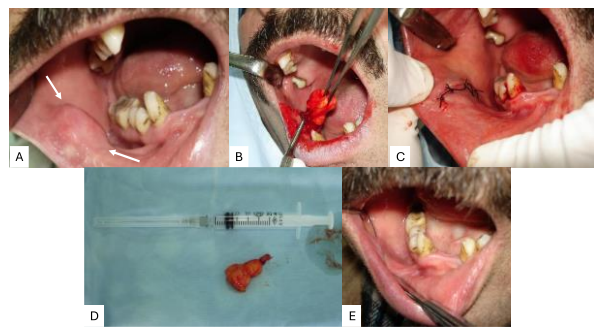
Kırk altı yaşındaki erkek hastanın sol oral komissür bölgesinde, yanak içi vestibüler mukozada uzun süredir var olan şişlik nedeniyle kliniğimize başvurmuştur. Hastadan alınan anamnezde kontrol altında olan diyabet dışında bir hastalığının olmadığı, sistemik durumunun stabil olduğu öğrenilmiştir. Yapılan klinik muayenesinde muhtemelen sol yanak yağ yastığından kaynaklanan, vestibüler mukozada yaklaşık 2 cm çapında düzgün sınırlı ekspansif lezyon tespit edilmiştir (Şekil-1A). Öncesinde küçük bir nodül şeklinde olan şişliğin özellikle son 1 yıl içinde hızla büyüdüğü öğrenilmiştir. Hastanın vücudunun başka bir bölgesinde benzer herhangi bir lezyona rastlanmamıştır. Lokal anestezi altında mukoza yüzeyinde yapılan insizyonun ardından künt diseksiyon ile oluşum eksize edilmiş, yara bölgesi primer kapatılmıştır (Şekil-1B, 1C, 1D). Yapılan histopatolojik inceleme sonucu lezyonun lipoma olduğu kesinleştirilmiştir (Resim-1E). Post-operatif 1 hafta sonra yapılan kontrolde operasyon bölgesinin sorunsuz bir şekilde iyileştiği gözlemlenmiştir (Şekil-1F).



Şekil-1. Olgu 1'e ait görseller. A: Sol dudak köşesindeki oral lipomun klinik görünümü. B: Lokal anestezi altında gerçekleştirilen insizyon ve sarı renkli lipomun görünümü. C,D: Eksize edilen materyalin görünümü. E: x40 büyütmede oral lipomun histolojik görünümü. F: Bir hafta sonra operasyon bölgesinin sorunsuz iyileştiği görülmekte.

Olgu Sunumu-2.

Kırk yaşındaki erkek hasta, sağ yanak mukozasında 5 yıldır yavaş büyüyen şişlik nedeniyle kliniğimize başvurmuştur. Yapılan intraoral muayenede 2,5 cm çapında hijyenik olmayan protez iritasyonuna bağlı geliştiği düşünülen ekspansif lezyon tespit edilmiştir (Şekil-2A). Lipom ön tanısıyla lokal anestezi ile yapılan operasyonda, lezyon kapsülü ile beraber eksize edilip, yara bölgesi primer olarak kapatılmıştır (Şekil-2B, 2C, 2D). Yapılan histopatolojik değerlendirmede lezyonun lipom olduğu doğrulanmıştır. Birinci hafta kontrolünde operasyon bölgesinin sorunsuz bir şekilde iyileştiği gözlemlenmiştir (Şekil-2E). Ayrıca hasta hijyenik olmayan protezinin yenilenmesi için Protetik Diş Tedavisi Anabilim Dalına yönlendirilmiştir.



Şekil-2. Olgu 2'ye ait görseller. A: Sağ dudak vestibüler mukozadaki lezyonun klinik görünümü (beyaz oklar). B: Lezyonun lokal anestezi altında eksizyonu. C: Operasyon bölgesinin primer kapatılması. D: Eksize edilen sarı renkli materyal. E: Bir hafta sonra elde edilen görselde operasyon alanının sorunsuz iyileştiği gözlenmektedir.

TARTIŞMA

Lipomalar oral mukozada sık görülen yumuşak doku mezenşimal neoplazileridir. Baş boyun bölgesindeki tüm mezenşimal lezyonların %15-20'sini ve oral kaviteyi ilgilendirenlerin %1-4'ünü oluştururlar. Genellikle yavaş gelişen, mukoza altında asemptomatik yumuşak şişlikler olarak görülürler. Sıklıkla bukkal mukozada, ağız tabanında, dilde ve dudaklarda yer alırlar. Fregnani ve arkadaşlarının yaptığı bir çalışmada 46 intraoral lipoma vakasını incelemiştir. Yirmi biri bukkal mukoza, 6'sı dil, 6'sı dudak, 5'i ağız tabanında tespit edilmiştir (6). Furlong ve ark. ise 125 lipom vakasını inceleyerek 30'unun parotis, 29'unun bukkal mukoza, 21'inin dudak, 17'sinin submandibular bölge, 15'inin dil, 6'sının palatina, 5'inin ağız tabanı ve 2'sinin vestibüler mukozada olduğunu bildirmiştir (7).

Lipomlar histoloji ve morfoloji bakımından basit lipom ve diğer lipomlar olarak sınıflandırılabilir. Diğer lipomları şu şekilde sıralayabiliriz; siyalolipom, miksoid lipom, intramüsküler lipom, iğsi hücreli lipom, pleomorfik lipom, anjiolipom, fibrolipom ve atipik lipom (8). Enzinger ve Harvey ilk olarak 1975 yılında iğsi hücreli lipomu tanımlamışlardır. Bu lezyonlar genelde sırt bölgesi ile boyun arka kısmında yer alan sert bir oluşum şeklinde yaşlı hastalarda görülmüştür. Histolojik incelemede olgun yağ hücreleri, iğsi hücreler ve mast hücreleri görülür. İntramüsküler lipomlar sınırları belirsiz, yavaş büyüyen, ağrı olmayan ve komşu kasların içine infiltre olan yağ hücrelerin görüldüğü lezyonlardır. Ağız içinde en çok dilde görülür. Daha yoğun damar yapısı görülen durumlarda lezyona angiolipoma adı verilir. Ağız içinde bukkal mukozada meydana gelirler ve genelde ağrısızdırlar. Pleomorfik lipomlar ise omuz ve boyunda olmak üzere sıklıkla 45 yaş üstü erkelerde karşılaşıldığı görülmüştür. İğsi hücreler ile olgun yağ dokusu içermesinin yanında çiçek görünümlü dev hücrelerin bulunması histolojik yapısında görülmektedir. Osteolipomlar; genelde büyük ve derin yumuşak dokulardan kaynaklanır, yağ dokusu yanında kemik dokusu da içerir (9).

Fregnani ve arkadaşlarının yukarıda bahsettiğimiz çalışmasında vakaların 21'i basit lipoma, 18'i fibrolipoma, 4'ü intramüsküler lipoma, 2'si tükrük bezi lipoması, 1'i mekik hücreli lipoma olarak tanımlanmış ve hiçbirisi rekürrens göstermemiştir (6). Furlong ve arkadaşlarının yayınladığı 125 vakadan oluşan hasta serisinde ise vakaların 62'si basit, 59'u pleomorfik, 2'si

fibrolipoma, 2'si kondroid lipoma olarak sınıflandırılmış, 23'ü intramüsküler yerleşim göstermiştir (7). Bizim 2 vakamızın patoloji sonucunda ise basit lipom olduğu görülmüştür.

Klinikte lipom, asemptomatik, yuvarlak, orta sertlikte, hareketli bir nodül şeklinde görülür. Genellikle ağrısız olan lipomlar nadiren sinire bası yapmaları halinde ağrı oluştururlar. Çapları 1-8 cm arasında olup, ortalama 6 ay gibi bir sürede belirgin hale gelirler (10). Lipomlar yavaş büyüyen agresif olmayan tümörler olmalarına rağmen büyük boyutlara ulaştıklarında çiğneme ve konuşma bozuklukları ve oluşturdukları basınca bağlı deformiteler oluşturabilmektedirler (5). Bizim vakalarımızdaki hastalarda da fonksiyon kaybı ve çiğneme bozukluğunun yanı sıra konuşma ile estetik bozuklukta görünmekteydi.

Histolojik açıdan lipomların normal yağ dokusundan farkı, lipoprotein lipaz aktivitesinin yüksekliğidir. Kalori alımında azalma lipom boyutunu etkilemez. Kapsüllü olan lipomlar çevre dokudan keskin sınırla ayrılırlar. Ayrıca lipomlar kas dokusuna yayılıp, infiltratif bir özellik gösterebilirler (10).

Ana tedavi yöntemi olarak lezyonun cerrahi olarak tamamen eksizyonu kabul edilmektedir. Genellikle başarılı bir eksizyondan sonra nüks görünmemekle birlikte basit lipomlar gibi kapsüllü olmayan infiltratif lipomlarda yeteri kadar eksizyon yapılmazsa lezyonun tekrarlama durumu ile karşılaşılabilir (2,3). Bizim vakamızda ise nüks görünmemiştir. Cerrahi olarak intraoral yaklaşımda insizyon bukkal oluğun üzerinden yapılır ve lipom eksize edilir. Derin yerleşimli lipom vakalarında lezyonun hepsini çıkartmak mümkün olamaz; bu vakalarda lezyonun bir kısmı çıkarılabilir ve nüks riski yüksektir (10). Steroid enjeksiyonu yapılması tümör boyutunu küçültten lokal yağ atrofisine sebep olarak lipomlar için bir başka tedavi yöntemi olarak kullanılabilir. 1 inçten küçük olan lipomların tedavisinde daha yaygın olarak tercih edilebilir. Tümörün merkezine yapılan 1:1 lidokain ve triamsinolon asetonid içerikli solüsyonun enjeksiyonu sonucu lezyon boyutlarında gerileme gözlemlenebilmektedir. Yara izi oluşumundan kaçınmak için 16 gauge iğne ve büyük şırıngayla yapılan yağ aspirasyonu, küçük ya da büyük lipomatöz büyümede yarar sağlayabilir. Son yıllarda özellikle lipomatozis gibi çok sayıda lipomun olduğu ve cerrahi tedavinin mümkün olmadığı durumlarda lipomları küçültmek amacı

ile lipoliz özelliği taşıyan fosfatidilkolin enjeksiyonları kullanılmaya da başlanmıştır (2).

SONUÇ

Sonuç olarak; lipomaların lokalizasyon, boyut, histolojik çeşitlilik ve semptom profili açısından farklılık gösterebileceği; tanı sürecinde klinik muayene, radyolojik yöntemler ve biyopsiyi takiben histopatolojik değerlendirmenin bütüncül bir yaklaşım gerektirdiği ortadadır. Sunmuş olduğumuz vakalarda da görüldüğü gibi cerrahi eksizyonun genel olarak etkin ve güvenilir bir

tedavi şekli olduğu söylenebilir. Bununla birlikte büyük boyutlarda ve derin yerleşim gösteren bazı durumlarda tedavi planlamasının zorlaşabileceği unutulmamalıdır. Daha çok sayıda hasta içeren, farklı lipoma türlerini ele alan geniş kapsamlı çalışmaların yapılması faydalı olacaktır.

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An atypical Stiff Person Syndrome patient presenting with epileptic seizures, brainstem involvement, and muscle stiffness of the lower body- a case report

Epileptik nöbetler, beyin sapı tutulumu ve alt vücudun kas sertliği ile seyreden atipik Stiff Person Sendromu - bir olgu sunumu

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ABSTRACT

Stiff Person syndrome (SPS) is a rare autoimmune neurological disorder presenting painful muscle spasms, and stiffness targeting mainly lower limbs, lower back, and abdomen. Antibodies such as Anti-GAD can be found positive. Electromyography is a valuable tool for diagnosing SPS, showing simultaneous contractions of the agonistic and the antagonistic muscle groups. Here in this case study, we present a 40-year-old male with SPS, presenting with usual symptoms of SPS like lower limb and lower back stiffness and painful muscle spasms, but unusual symptoms as well, like epileptic seizures, high amount of sweating and itching, difficulty in swallowing and urinary-stool retention. EMG findings were correlated with SPS, and anti-GAD levels were found positive. After Intravenous immunoglobulin treatment, the patient had a complete remission. In this paper, we will look at the unusual symptoms and findings in the SPS, and the important place of electromyography in diagnosing this disorder.

Keywords: Stiff person syndrome, epileptic seizures, electromyography, anti-GAD

Öz

Stiff Person Sendromu (SPS), genellikle alt ekstremiteler, bel ve karın bölgesine yönelik ağrılı kas spazmları ve sertlik ile kendini gösteren nadir bir otoimmün nörolojik hastalıktır. Anti-GAD gibi antikorlar pozitif bulunabilir. Elektromiyografi, SPS tanısında değerli bir araçtır ve agonist ve antagonist kas gruplarının eş zamanlı kasılmalarını gösterir. Bu olgu çalışmasında, SPS ile başvuran 40 yaşında bir erkek hasta sunulmuştur. Hasta, SPS'nin tipik belirtileri olan alt ekstremiteler ve bel sertliği ile ağrılı kas spazmlarının yanı sıra; epileptik nöbetler, aşırı terleme ve kaşıntı, yutma güçlüğü ve idrar-dışkı tutamama gibi alışılmadık semptomlar da göstermekteydi. EMG bulguları SPS ile uyumluydu ve anti-GAD seviyeleri pozitif bulunmuştu. İntravenöz immün globulin tedavisinin ardından hasta tam bir remisyona sağlandı. Bu sunumda, SPS'deki alışılmadık semptomlar ve bulgular ile bu hastalığın tanısında elektromiyografinin önemli rolüne değinilecektir.

Anahtar Sözcükler: Stiff Person Sendromu, epileptik nöbet, elektromiyografi, anti-GAD

INTRODUCTION

Stiff Person Syndrome (SPS) is a rare autoimmune neurological disorder that presents a variety of symptoms. The primary manifestations include painful muscle spasms and stiffness, particularly in the lower limbs, lower back, and abdomen.

However, some patients may also experience less common symptoms, such as epileptic seizures, excessive sweating, urinary retention, difficulty swallowing, and cranial neuropathies. The most frequently associated antibody in SPS is Anti-Glutamic Acid Decarboxylase (Anti-GAD). Electromyography (EMG) is a valuable diagnostic tool for SPS, as it demonstrates simultaneous muscle contractions in both agonist and antagonist muscle groups.

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In this case report, we discussed a 40-year-old SPS patient with uncommon symptoms,

CASE REPORT 1

A 40-year-old male with no history of chronic illness was admitted to our clinic with symptoms including numbness on the right side of his face, dysphagia, urinary retention, painful muscle spasms in the lower limbs, and difficulty walking. His symptoms began a year prior to admission, initially mild but gradually worsening over time. He also experienced excessive sweating followed by generalized itching. Notably, the patient had suffered two generalized tonic-clonic seizures before admission. Urinary catheterization was performed on the patient before his admission because of his urinary retention.

During the neurological examination, the patient was cooperative and oriented. Cranial nerves I–XII were intact except for the trigeminal nerve, where hypoesthesia was noted on the right side of the face. Muscle strength in the upper limbs was normal, but rigidity was observed in the lower limbs, predominantly in a flexor posture. Hyperreflexia was evident in the Achilles and patellar tendons. The patient had a low Body-Mass index (BMI) due to his dysphagia. Routine blood tests revealed no abnormalities, and cranial and spinal magnetic resonance imaging (MRI) findings were normal. Cerebrospinal fluid (CSF) analysis from a lumbar puncture was also unremarkable. The patient underwent screening for potential tumors, but all tests came back negative.

Electroencephalography (EEG) showed sharp slow-wave activity, consistent with epileptiform discharges in the right temporo-occipital region. Electromyography (EMG) revealed spontaneous yet normal motor unit potential (MUP) activity in both agonist and antagonist muscle groups in the proximal and distal lower limb and paraspinal muscles (Figure-1). There was no evidence of myoclonic activity, and the patient had no signs of encephalomyelopathy, so progressive encephalomyelitis with rigidity and myoclonus (PERM) was overruled. Blood tests confirmed the presence of anti-GAD antibodies, leading to a diagnosis of SPS.

The patient was treated with intravenous immunoglobulin (IVIG) at a dose of 2 g/kg. Within three days of treatment, the patient achieved complete remission. Painful spasms, sweating,

highlighting these rare features of the disorder.

and itching improved significantly over time. Rigidity in the lower limbs diminished, allowing the patient to walk unassisted, and the hypoesthesia in the right trigeminal nerve resolved. Follow-up EMG showed no abnormalities, and EEG revealed no epileptiform activity.

During the patient's follow-up examinations, similar symptoms were observed again, leading to the administration of IVIG treatment two more times at 3-month intervals. Since no remission was observed after the last IVIG treatment, it was decided to initiate rituximab therapy.

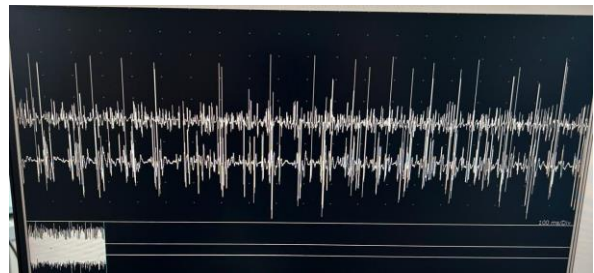


Figure-1. Both agonistic and antagonistic muscle contractions can be seen in this EMG finding.

DISCUSSION

Stiff Person Syndrome (SPS) is a rare autoimmune neurological disorder. Its pathogenesis involves the autoimmune disintegration of gamma-aminobutyric acid (GABA)-mediated inhibitory interneurons in the spinal cord (1). This disruption leads to hyperexcitability of anterior horn cells, resulting in simultaneous contractions of agonist and antagonist muscle groups (1). Consequently, patients experience painful muscle spasms and rigidity, primarily affecting the lower limbs, paraspinal muscles, and axial muscle groups. The antibody most commonly associated with SPS is Anti-GAD, detected in 88%-98% of patients with classical SPS (2). Studies showed that the higher the Anti-GAD levels are, the more resistant the SPS becomes. Our patient's serum Anti-GAD level was 11.28 IU/mL, presenting with refractory SPS, which contradicts existing research (1). However, it is important to note that the Anti-GAD test was performed after the patient received IV methylprednisolone, which may have lowered the Anti-GAD titers.

In classical SPS, prominent symptoms include rigidity and stiffness of the lower limbs, thoracolumbar, and abdominal muscles; painful spasms that may be triggered by stimuli such as noise, emotional stress, or tactile contact; and hyperlordosis-like deformity caused by stiffness and contractions of the thoracolumbar paravertebral muscles (3). However, studies have identified additional, less common symptoms associated with SPS, such as epileptic seizures, excessive sweating, persistent itching, urinary retention, constipation, vertigo, diplopia, dysphagia, and cranial neuropathies (3-5).

Epileptic seizures, while rare, can occur in SPS, particularly in patients with elevated anti-GAD antibody levels. (4). Generalized tonic-clonic seizures are most common, but focal seizures have also been reported. EEG findings in these patients often reveal epileptiform activity. The mechanism behind these epileptic seizures is mostly unclear. Still, it is suspected that the cause of these seizures is anti-GAD antibodies inducing hyperexcitability by inhibiting GABAergic pathways and decreasing the conversion of glutamate to GABA, resulting in excessive excitatory neurotransmission due to increased glutamate levels (1). In our case, EEG demonstrated sharp slow-wave activity in the right temporo-occipital region, which resolved following IVIG therapy.

EMG is a valuable diagnostic tool for SPS, as it detects characteristic electrophysiologic findings, including simultaneous contractions of agonist

and antagonist muscle groups (6). These contractions are spontaneous and simultaneous but exhibit normal motor unit characteristics. EMG abnormalities are typically localized to the lower extremities and lower lumbar paraspinal muscles. In our case, EMG played a crucial role in confirming the diagnosis.

The treatment of SPS can be divided into two categories: symptomatic therapy and immunotherapy. In symptomatic therapy, GABAergic treatments such as benzodiazepines or baclofen can be used to manage stiffness and painful spasms. In immunotherapy, the main treatment for SPS is IVIG. Research on plasma exchange therapy has shown conflicting results, with some patients experiencing improvement while others show no response (7). In more resistant cases, rituximab can be used as a long-term treatment.

CONCLUSION

SPS is a rare neurological disorder with a diverse range of symptoms. While rigidity and spasms are the primary features, clinicians should also consider less common manifestations such as epileptic seizures, brainstem involvement, and urinary retention when diagnosing or monitoring SPS.

Conflict of interest: None of the authors have conflict of interest to declare.

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Vesicouterine fistula after cesarean section: A case report

Sezaryen sonrası vezikouterin fistül olgusu: Olgu sunumu

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ABSTRACT

A vesicouterine fistula is an abnormal connection between the bladder and the uterus. It is a rare condition constituting 1-4% of urogenital fistulas. Its prevalence has increased in recent years due to the rising number of cesarean sections. Ultrasonography, cystoscopy, cystography, hysterosalpingography, computed tomography and magnetic resonance imaging methods are used in the diagnosis. Treatment options include conservative, medical and surgical methods. Surgical treatment is considered as the definitive solution. In this article, a case of uterovesical fistula after the third cesarean section was treated with surgical technique and presented with a review of the literature.

Keywords: Vesicouterine fistula, Cesarean, Incontinence, Complication

Öz

Vezikouterin fistül, mesane ve uterus arasındaki anormal bir bağlantıdır. Ürogenital fistüllerin %1-4'ünü oluşturan nadir bir durumdur. Son yıllarda artan sezaryen sayısı nedeniyle prevalansı artmıştır. Tanıda ultrasonografi, sistoskopi, sistografi, histerosalpingografi, bilgisayarlı tomografi ve manyetik rezonans görüntüleme yöntemleri kullanılmaktadır. Tedavi seçenekleri arasında konservatif, medikal ve cerrahi yöntemler yer almaktadır. Cerrahi tedavi kesin çözüm olarak kabul edilmektedir. Bu yazıda üçüncü sezaryen sonrası uterovezikal fistül gelişen bir olgu cerrahi teknikle tedavi edilmiş ve literatür gözden geçirilerek sunulmuştur.

Anahtar Sözcükler: Vezikouterine fistül, Sezaryen, İnkontinans, Komplikasyon

INTRODUCTION

Uterovesical fistulas are a group of rare diseases classified among genitourinary fistulas. These fistulas are mostly iatrogenic and commonly develop after obstetric and gynecologic interventions. A vesicouterine fistula is an abnormal connection between the posterior wall of the bladder and the anterior wall of the uterus. It accounts for 1-4% of all urogenital fistulas (1). In recent years, an increase in the prevalence of these fistulas has been observed worldwide with the increase in cesarean deliveries.

The most common cause of uterovesical fistulas is cesarean section operations. In addition, prolonged labor, use of forceps, vaginal delivery after cesarean section, uterine perforation, gynecologic injuries and genital tract infections may also play a role in fistula formation (2). Uterovesical fistulas developing after cesarean section and characterized with amenorrhea and cyclic hematuria without urinary incontinence are called Youssef syndrome (3). In this article, a case of uterovesical fistula which developed after the third cesarean section and was treated by open surgery is presented in the light of the literature.

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CASE REPORT 1

A 30-year-old patient, gravida 3, para 2, had a history of two previous cesarean sections. At 39 weeks of gestation, she presented to the emergency department with rupture of membranes. Due to a history of previous cesarean sections and prolonged rupture of membranes, the patient underwent an emergency cesarean section. Intraoperatively, it was observed that the bladder was adherent to the kerr line. Adhesions were opened with blunt and sharp dissections. The bladder was checked by instilling 350 cc of sterile methylene blue through a silicone catheter. No leakage was observed. The catheter was clear. The patient was discharged at 48th hour. On the 10th postoperative day, the patient presented to the outpatient clinic with a complaint of urine leakage from the vagina. Vesicouterine fistula was suspected on transvaginal ultrasonography (Figure-1A).

Cystoscopy was performed under general anesthesia. Cystoscopy revealed a 2cm vesicouterine fistula mouth in the dome of the bladder (Figure-1B).

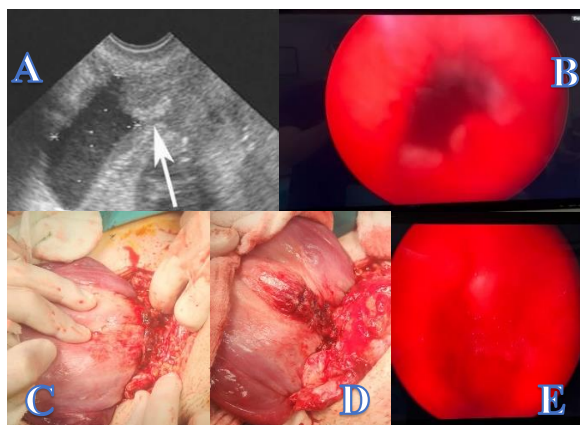
The vesicouterine fistula was passed through cystoscopy and the uterine cornu was visualized. The abdominal layers were opened through a Pfannenstiel incision after excising all sutures. Following the incision, the bladder was deperitonized. The fistula tract was then accessed at the kerr line anterior to the uterus (Figure-1C).

The fistula tract was accessed by adhesiolysis between the bladder dome and the anterior uterine wall. The fistula tract was excised including its connections in the bladder and uterus. The bladder wall was closed in two layers (Figure-1D).

Upon the patient's request, bilateral tubal ligation was performed simultaneously. Afterwards, cystoscopy was performed again (Figure-1E).

Postoperatively, bladder irrigation was performed with 50 cc of saline at 4-hour intervals for 48 hours. The catheter was kept in place for 15 days. At the end of this period, the catheter was clamped every 2 hours for 24 hours for bladder gymnastics. The catheter was withdrawn after the gymnastics was completed. The patient has no active complaints at the 2nd month postoperative follow-up.

Figure-1. Vesicouterine fistula images.



A: Vesicouterine fistula image on transvaginal ultrasonography. B: Image of a 2 cm vesicouterine fistula structure in the bladder dome. C: Image of fistula tract on the kerr line anterior to the uterus. D: Postoperative appearance of vesicouterine fistula repair. E: Closed view of the old fistula line in postoperative cystoscopy

DISCUSSION

Vesicouterine fistulas are rare genitourinary fistulas which are usually observed after obstetric procedures such as cesarean section operations. This condition, characterized by the absence of urinary incontinence and the presence of symptoms such as amenorrhea and cyclic hematuria, is also known as Youssef syndrome (3). In the literature, it has been reported that vesicouterine fistulas are mostly caused by bladder injury during cesarean section or iatrogenic damage in the kerr line region. In our case, it is thought that the fistula was formed due to adhesions between the bladder and uterus during the third cesarean section. In the diagnosis, cystoscopy is critical in determining the location and size of the fistula.

In our case, the diagnosis was made by transvaginal ultrasonography and cystoscopy and the fistula tract was evaluated in detail. Another recommendation is cystoscopic fulguration and laparoscopic approach instead of laparotomy (4). Surgical treatment is the most effective and definitive treatment for vesicouterine fistulas. Surgical intervention through transvesical, retroperitoneal, and transperitoneal approaches remains the preferred method for treating vesicouterine fistulae. The goal of the procedure is to close the bladder and uterine openings separately, with or without preserving the uterus.

(5) Using the Phannensteil incision and open surgical technique, the fistula between the bladder and uterus was successfully excised and the bladder wall was repaired in two layers. In addition, bilateral tubal ligation was performed simultaneously according to the patient's request. Porcaro et al. reported that emptying the bladder before obstetric surgeries and careful lower segment uterine dissections minimize the risk of uterovesical fistula formation (6). Bettez et al. reported that conservative treatment method can be applied if the diagnosis of uterovesical fistula is made immediately after delivery and successful results of early surgery in selected cases have been reported in the literature (7). Bonavina, et al. applied open surgery, laparoscopic or robotic methods in treatment, while conservative approaches may also be effective in small fistulas diagnosed early. In the study, it is emphasized that surgical techniques are generally successful, but complications can be prevented by reducing cesarean section rates and surgical attention. The author emphasizes the importance of preventing unnecessary cesarean sections and increasing the experience level of surgeons (8). Rao et al. emphasized that early diagnosis and appropriate surgical intervention increase recovery rates. Patient outcomes are generally satisfactory and complication rates are low (9). The study by Jóźwik et al. emphasizes that the most common cause of vesicouterine fistulas is cesarean

complications and menuria (menstrual bleeding with urine) is a distinguishing symptom in the diagnosis. Surgical repair has been determined as the most effective method of treatment and has been shown to have high success rates. Early diagnosis and appropriate surgical intervention provide complete recovery of the patients' symptoms. Bladder catheterization in the postoperative period is frequently preferred to support recovery in patients who have undergone fistula repair (10).

In our patient, the 15-day catheterization period was completed smoothly, and no complications were observed during follow-up.

CONCLUSION

Although vesicouterine fistulas are rare, they are a complication that should be carefully evaluated in patients at risk after cesarean section. Early diagnosis and appropriate surgical intervention play an important role in restoring the patient's quality of life. In this case, a successful fistula repair was performed by open surgery and the patient was discharged completely asymptomatic. While the surgical technique provided an effective solution in the management of vesicouterine fistulas, it was once again emphasized that careful preoperative planning and surgical execution are the keys to success.

Conflict of interest: None of the authors have conflict of interest to declare.

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The role of stem cells and biomaterials in peripheral nervous system regeneration

Periferik sinir sistemi yenilenmesinde kök hücre ve biyomateryallerin rolü

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ABSTRACT

Peripheral nerve injuries present significant challenges to patients and require intensive and costly treatments. The utilization of biomaterials is an effective treatment method. Personalized biomaterials are promising for the regeneration of damaged peripheral nerves. Stem cells have an important role in modern treatment methods because of their properties and promise to solve diseases requiring tissue regeneration, such as peripheral nerve damage. In this review, we aimed to investigate the relationship between stem cells and biomaterials, considering their different origins and potential compatibility in facilitating the regeneration of peripheral nerve injury.

Keywords: Peripheral Nervous System, Stem Cell, Biomaterials, Regeneration

Öz

Periferik sinir yaralanmaları hastalar için önemli zorluklar oluşturur ve yoğun ve maliyetli tedavi gerektirir. Biyomateryallerin kullanımı tedavide etkili bir yöntem sunmaktadır. Kişiselleştirilmiş biyomateryaller, hasar görmüş periferik sinirlerin yenilenmesi için umut vaat ediyor. Kök hücreler, sahip oldukları özellikler nedeniyle modern tedavi yöntemlerinde önemli bir yere sahip olup, periferik sinir hasarı gibi doku yenilenmesi gerektiren hastalıkların çözümünde umut vadediyor. Bu derlemede, kök hücreler ile biyomateryaller arasındaki ilişkiyi, bunların farklı kökenlerini ve periferik sinir hasarının yenilenmesini kolaylaştırmadaki potansiyel uyumluluğunu göz önünde bulundurarak araştırmayı amaçladık.

Anahtar Sözcükler: Periferik Sinir Sistemi, Kök Hücre, Biyomateryaller, Rejenerasyon

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INTRODUCTION

Peripheral nerve injuries can arise from accidents, trauma, or infections, and may lead to intense neuropathic pain, sensory impairment, and partial or complete loss of motor and autonomic functions depending on the type of injury. To reduce or eliminate these losses, researchers have conducted studies to enhance the quality of life of patients (1). Regeneration of the peripheral nervous system (PNS) has been a central research focus, yielding promising results. One of the defining characteristics of the peripheral nervous system (PNS) is its capacity for regeneration, which sets it apart from the central nervous system (CNS). Moreover, nerve regeneration occurs in target organs (2). Schwann cells (SC), which are associated with peripheral nerves, support repair in many tissues and contribute to regeneration through their migratory abilities (3).

The regeneration of fibers in the peripheral nervous system (PNS) allows for the repair of damaged nerves and restoration of their lost functions. This review discusses the structure of scientific research on the regeneration process and current treatment mechanisms involved. It examines the role of biomaterials and stem cells in the treatment of peripheral nerve injuries. It

also considers potential future treatment methods for peripheral nerve regeneration.

Central and Peripheral Nervous System: Basic Differences

The human nervous system or neural network is a system consisting of two parts: the CNS and PNS. Glial cells support neurons. Each neuron connects to other neurons to process sensory information and respond to signals. The CNS consists of the brain and spinal cord, whereas the PNS is a cranial, spinal, and peripheral nerve network. Neurons and glia are cells found in CNS and PNS. Neurons generally consist of three main parts: perikaryons, also called cell body, dendrite extensions extending from the perikaryon and supported by microtubules, and axons (4,5,6). The neurons were classified according to the number of extensions that emerge from their bodies (Table-1).

Glial cells are cell types that can proliferate and provide mechanical support to neurons. They are also involved in nutrition, myelin sheath formation, and protection. There are different types of glia in the CNS and the PNS. Neuroglia in the CNS are called central neuroglia and glia in the PNS are called peripheral neuroglia and are classified as follows (7,8) (Table-2).

Table-1. Classification of Neurons According to the Extensions Protruding from Their Body (8)

TYPES OF NEURON	NUMBER OF AXON	NUMBER OF DENDRITE
Multipolar Neuron	It has a single axon.	It has two or more dendrites.
Bipolar Neuron	It has a single axon.	It has a single dendrite.
Pseudounipolar Neuron	They have a single extension extending from the perikaryon region, one end extends to the peripheral ending and the other to the CNS.	
Anaxonic Neuron	It does not have a true axon. It has multiple dendrites. They do not produce action potentials; they regulate the electrical impulses of their neighbouring neurons.	

Table-2. Classification of Neuroglia.

CENTRAL NEUROGLIA	PERIPHERAL NEUROGLIA
Astrocytes	Schwann cells
Oligodendrocytes	Satellite cells
Ependymal cells	Enteric Neuroglia
Microglia	Teloglia

Astrocytes in the CNS are the largest neuroglial cells and have star-like cytoplasmic extensions. They play a role in forming the blood-brain barrier, substance transport, and neurogenesis (8,9). Oligodendrocytes are involved in myelin sheath production in the CNS. It is also known that these neuroglial cells provide nutritional support for neurons (8,10). Ependymal cells are neuroglial cells involved in the production of cerebrospinal fluid (CSF) and they line the canalis centralis in the spinal cord. Ependymal cells, which are ciliated epithelial glia, are involved in brain homeostasis and metabolism (8,11). Microglia are the smallest glial cells and although they are structurally different, they resemble macrophages in their antigen presentation and phagocytosis functions; they are small neuroglia involved in neuroinflammation and defense (8,12).

SCs, similar to oligodendrocytes, which are responsible for myelin sheath formation in the CNS, these cells are also responsible for myelin sheath formation in the PNS. Glia is responsible for the protection and nutrition of axons (8,13). Satellite cells, as the name suggests, form a microenvironment surrounding neuronal perikaryons and provide electrical insulation. In

Peripheral Nerve Structure and Function

The peripheral nerve consists of a ventral and a dorsal root, each of which originates from the spinal cord. It contains both the motor and sensory neurons. The nerve fibers in the PNS come together in bundles and form nerves. This nerve organization includes axons and SC. The outer layers of these cells contain reticular fibers, fibroblasts, and capillaries. Together, these structures form the endoneurium, which is the innermost layer of the peripheral nerve. The perineurium layer surrounds the nerve fibers and regulates the microenvironment of the fibers. The outermost layer of the nerves is the epineurium, which protects the nerve fibers by holding them together. Myelinated and unmyelinated fibers are present between these nerve fibers. The myelin sheath is a structure consisting predominantly of lipids that enable faster transmission of impulses in the nervous system. The conduction velocity in unmyelinated fibers is very low compared to that in myelinated fibers. PNS is divided into two as autonomic and somatic nervous systems. The autonomic nervous system is also divided into

the context of enteric neuroglia, it is known that these cells play a role in maintaining homeostasis and regulating digestive-related muscles associated with neurons in the ganglia of digestive organs within the autonomic nervous system (8,14,15). The teloglia is a neuroglial cell type that covers the nerve terminal at the end of the motor nerve fiber (8).

CNS and PNS are two parts of the vertebrate nervous system. There are differences between the cells of these systems and some of the tasks they fulfill. The CNS consists of the brain and spinal cord, while the PNS includes other nerves in the body - the nerves to the internal organs and the nerves to the limbs. The CNS is an information-processing mechanism that performs high-level neural activities such as memory and thinking. The peripheral neural network transmits signals and information generated by CNS activity to various parts of the body, as well as information generated by sensory activities such as touch and vision to the CNS. Both systems have glia with similar functions that provide support to neurons. With these similarities and differences, these two systems work interactively, providing neural control in the body and constituting the nervous system (16).

two parts as sympathetic and parasympathetic and these systems work together to regulate the involuntary functions of the internal organs and provide homeostasis, while the somatic nervous system controls voluntary responses that regulate muscle movements as well as sensory perception (6,17,18).

PNS establishes a connection between the CNS and the systems of the body. This communication occurs through the afferent and efferent fibers. While afferent fibers carry signals to the CNS, efferent fibers carry impulses from the CNS to the target organs. Depending on the direction of these impulses, sensory and autonomic ganglia function. The ganglia are oval structures that transmit signals to the nervous system. The PNS is a complex neural network system responsible for sensory transmission and motor function, control of reflexes, digestion of nutrients and regulation of respiration, control of smooth muscles, and regulation of homeostasis by working together with the CNS with all these structures (6,18,19) (Figure-1).

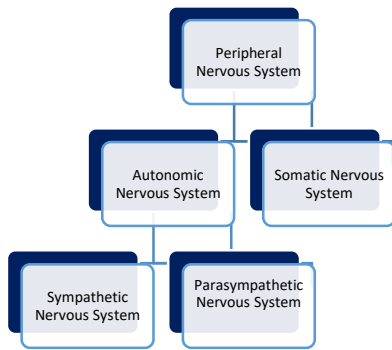


Figure-1. Peripheral Nervous System Subdivisions

Regeneration and Treatment of Peripheral Nervous System Injury

Peripheral Nerve Injury and Regeneration Process

Peripheral nerve injury is a common and life-threatening process that can occur as a result of traumatic situations and injuries such as traffic accidents, nerve compression, inflammation, and exposure to toxic chemicals such as alcohol and cigarettes. The incidence of peripheral nerve injuries in the community ranges from 13 to 23 per 100,000 people. A decline in the quality of life experienced by individuals with peripheral nerve damage has a detrimental impact on both the patient and their relatives, resulting in material and moral loss. In later periods, this nerve damage may result in loss of function (17,20,21).

Now, robust regeneration mechanisms of peripheral nerves have become operational. Zhang et al. delineated the peripheral nerve repair process into two phases: the remodeling of the organs by the CNS through the repaired peripheral nerve and the backward remodeling of the distal target organs by the CNS through the repair of the peripheral nerve. The regeneration process is initiated by phagocytosis of substances that cause nerve degradation by SC and macrophages. In the peripheral nerve, regeneration occurs from the proximal end to the distal end. Macrophages stimulate SCs for nerve growth by producing interleukin-1 (IL-1). SC are involved in axonal sprouting during regeneration. In this respect, SCs are an essential element in peripheral nerve regeneration (5,22).

During the process of peripheral nerve damage and repair, we encountered a condition called

Wallerian degeneration. Wallerian degeneration refers to the degeneration of the distal part as a result of the destruction of axons and is defined as one of the basic reactions in the PNS. This degeneration process generates an inflammatory response associated with the upregulation of signaling molecules such as cytokines and chemokines during the regeneration process (23,24).

Growth factors (GFs) are some of the molecules commonly involved in peripheral nerve regeneration. GFs are involved in various biological activities, especially tissue repair by regulating cellular communication and are protein molecules that provide cell growth and division. GFs are directed to the damaged area through various nerve guide channels (NGCs), and axon regeneration in the degenerated area is triggered by the long-term, controlled release of GFs at adequate concentrations. Considering the wide variety of studies conducted, growth factors such as vascular endothelial growth factor (VEGF), nerve growth factor (NGF), and neurotrophic factors from bone morphogenetic proteins (BMPs) increase peripheral nerve regeneration capacity. In this process, various signaling pathways are effective in supporting the survival of neurons and the regeneration process. In some of these pathways, the PI3K/Akt signaling pathway comes first and plays a role in regulating cell proliferation, structural and morphological changes, migration, and apoptosis. The MAPK/ERK signaling pathway is involved in axon growth and myelination of regenerating axons, whereas the JNK/c-Jun signaling pathway can also be induced by growth factors and is involved in the plasticity of axons with remyelination (25).

Laminin and fibronectin, called neurite promoting factor (NPF), are glycoprotein molecules that have a positive effect on growth in the regeneration zone. NPF molecules are found in the basal lamina of the SC, as in laminin, and adhesion molecules such as neuron adhesion molecule (NCAM) and N-cadherin, found in the membrane of the SC, have positive effects on the regeneration process (5,25). Additionally, different transcription factors are expressed following peripheral nerve injury. Transcription factors are regulatory protein molecules that mediate the transcription of the genetic information of DNA into RNA by interacting with various proteins and enzymes. As stated by

Zhang et al., the transcription factors known to affect regeneration are as follows:

Klf4 and c-Myc, when examined in mouse retinal ganglion cells (RGC), it was reported that Klf4 deficiency induced regeneration of the damaged sciatic nerve and c-Myc expression in the damaged optic nerve accelerated axon growth. After sciatic nerve injury, the expression of the c-Jun transcription factor increased in motor and sensory neurons, suggesting that it plays a role in the recovery of motor functions and is also an inducing force in SC reprogramming. It has been reported that the Krox-20 transcription factor is involved in the myelination process of peripheral nerves and is an initiator of SC reprogramming (25).

Current Treatments Used in Peripheral Nervous System Injuries

PNS injuries are traumas with severe damage and high persistence and are very difficult to treat. The most important reason for the difficulty in the successful repair of PNS injury is the very slow healing rate (26). Research on the development of different treatment methods and tools has been focused on the fact that post-traumatic damage in the PNS is financially challenging and can relieve the person in terms of spirituality. For example, studies on GFs, microsurgery, autograft and allograft therapies, NGC, neural stem cells, and SC, and the use of one or a combination of the two are being conducted. In none of the studies conducted to date, a treatment method that is expected to be effective in a way that will not cause financial difficulties to the individual and in addition to this, will completely eliminate the damage that has not been developed (27).

Re-functionalization of the PNS damage depends on many distinct factors such as the localization and size of the damage, the patient's condition, damage to the nerve cells, and the recovery capacity of the nerves are important in the choice and process of treatment (28).

Currently, the most widely accepted method is microsurgery, but the disadvantage of this method is that it cannot prevent degeneration in neural conduction bundles because it cannot reduce the production of neurotrophic factors and does not guarantee functional recovery. Which approach is chosen among microsurgical applications is important and several factors determine this choice, such as the condition of

the trauma, experience of the surgeon, patient-specific conditions (age, condition, etc.), and suitability of the trauma for treatment (27). If the gap in the nerve bundle is 1 cm or more, autologous or allogeneic nerve transplantation is performed to assist the microsurgical procedure; autologous nerve transplantation is more preferably used in PNS injury and is performed through SC and GF (29). Another procedure is to suture the two nerve endings from beginning to end, and this method attracts more attention from researchers owing to its more favorable clinical results. The most fundamental problem in allogeneic nerve transplantation is that the desired response cannot be obtained because of the rejection of the immune system. Although various immunomodulators have been used to eliminate immune system rejection, effective results have not been obtained (30).

The use of stem cells as another treatment method is common. It is generally aimed at supporting the nerve regeneration process in damaged areas by planting stem cells in appropriate polymers. Polymers are preferred owing to their high biocompatibility. Polypyrrole is the most preferred and widely studied polymer. Along with stem cells, neurotrophic factors and neural progenitor cells are also used because they have a positive effect on nerve regeneration owing to the improvement of action regeneration (26).

Some pharmacological treatment methods (4-aminopyridine (4-AP), erythropoietin and steroid hormones are some of the drugs mentioned) have been reported to improve myelination and nerve regeneration in peripheral nerve damage, but there are no clinically approved drugs (28).

The role of SC in nerve regeneration is to contribute to nerve regeneration resulting from peripheral damage by cooperating with stem cells and thus helping to repair peripheral nerve damage (31). SC is important for creating a suitable regeneration environment after peripheral damage by producing neurotrophic factors and for the regeneration of damaged axons (32). In addition, studies have shown that SC is a good option for the elimination of damages caused by surgical applications (31). Finally, the use of SC and various stem cells together in PNS damage is considered a promising approach in terms of bringing a different perspective to existing treatments or creating a new treatment process (33).

In conclusion, the most appropriate treatment for patients with peripheral nerve injury is the one that best suits the individual's condition. Although various treatment modalities are currently available, there is still no adequate and definite treatment modality that can obtain a definite result, because every treatment cannot be applied to every patient.

Stem Cells and Peripheral Nervous System

Stem Cell-Based Treatment Strategies

Stem cells possess the capacity to self-renew and differentiate into specialized cells. The primary rationale for utilizing stem cells in PNS injuries is to facilitate the generation of new cells to replace those that are dysfunctional or stimulate regeneration in the degenerated area. After repair, axonal regeneration at the distal end of the nerve requires a biochemically appropriate environment and the most suitable structure for this environment is the SC (34). The repair process progresses differently depending on the damage in the PNS. SC and mesenchymal stem cells are accepted as appropriate for treatment (17). It has been found that the increase or decrease in the proliferation of SC is effective in the repair of axons and it has been observed to be effective in the repair of large gaps in the distal nerve end denervation and proximal injuries in peripheral nerve damage (34). As a result of recent studies, the importance of SC in nerve autograft studies has been emphasized and alternative methods have been found (29).

Stem cells are used in tissue and bioengineering-based applications and are one of the most frequently preferred treatment methods for various diseases and PNS injuries, which are also the focus of this review. Utilizing the properties of stem cells, repair and regeneration of nerve damage and migration of new stem cells to the damaged area or target area are the main methods for the treatments used and current therapies are shaped around these abilities.

Stem Cell Types and Applications to Peripheral Nervous System

Stem cell types to be used in treatment methods can be obtained from different tissues and organs and are suitable for specific use. The source of the stem cells used in these applications varies due to the use of patient-specific treatment methods, considering the damage that each

patient has and the size and location of the damage. When transplanting stem cells to patients, they can be used with different biomaterials with high biocompatibility.

This review examines the various types of stem cells employed in the treatment of PNS injuries, their respective applications, and the relationship between stem cells and SCs.

Neural Stem Cells

Neural stem cells, which are the primitive cells of the nervous system and can differentiate into neurons and neuroglial cells, are stem cells that should be used in PNS injury. These are the most important sources of nerve regeneration (35). The subventricular and subgranular regions in the brain are the primary sites of neural stem cells (34). Various studies have shown that the implantation of neural stem cells into PNS damage has favorable effects (34). It has been observed that neural stem cells transplanted into damaged areas of the peripheral nervous system can differentiate into nervous system cells, including neurons and SC-like cells (35). They promote angiogenesis by synthesizing the trophic and growth factors required for nerve regeneration. They also repair and support nerve growth and myelination (35).

Stimulated/Induced Pluripotent Stem Cells (iPSC)

Although iPSCs are very similar to embryonic stem cells, they have some differences (34): they can differentiate into different cell lines, whereas undifferentiated iPSCs can differentiate into many different cell types, including myelinated SC and neural crest stem cells. The addition of iPSC to NGC enhances the recovery of the nerve gap and axonal growth (35).

One of the disadvantages of iPSC is that they may cause malignancy; since they originate from somatic cells, they may have epigenetic memories of these cells, which may make them more malignant (34,35).

Mesenchymal Stem Cells (MSCs)

The therapeutic use of MSCs for nerve regeneration is intensive. MSCs are multipotent stem cells derived from various tissues. The tissues from which they are obtained include the umbilical cord, bone marrow, dental pulp, and Wharton's jelly (36). Allogeneic transplantation is

possible in PNS damage because of their characteristics such as easy isolation and lack of differentiation (31). The repair capability of MSCs not only in PNS injury but also in cardiovascular disorders, neurological diseases, bone repair, etc. is preferred in terms of providing support to the treatment process (30). Simultaneously, because of their ability to synthesize neurotrophic factors, self-renewal, differentiation into cells such as SC, and migration, MSCs are one of the factors supporting regeneration in tissue damage, which causes them to play an important role in peripheral nerve damage (31).

In addition, mesenchymal stem cells can adhere to plastic and because of this ability, they are frequently used in studies using NGC made of polymers. These studies were usually performed by damaging the sciatic nerve in rats. Stem cells combined with appropriate GF were added to the surface of the NGC and placed in the damaged area to support the regeneration of the two ends of the cut nerve by stem cells.

Orofacial Stem Cells

The origins of orofacial stem cells vary; dental pulp, deciduous teeth, and gingiva are some of them and they are isolated from these tissues. Orofacial mesenchymal stem cells (MSCs) are multipotent stem cells that exert immunomodulatory effects. Orofacial stem cells share a common embryonic neural crest origin and form orofacial tissues by preserving certain features of neural cells. Some mesenchymal stem cells are a source of orofacial stem cells (29).

The relationship between orofacial stem cells and PNS damage is that orofacial mesenchymal stem cells express neurotrophic factors such as brain derived neurotrophic factor (BDNF) and nestin at higher rates than other MSCs and it is easier to differentiate into nerve cell types because they originate from neural cells. In this case, it shows us that orofacial MSCs are more suitable for use in regeneration-based treatment methods than other MSCs for PNS damage (29).

Mesenchymal Stem Cells Derived from Bone Marrow

Mesenchymal stem cells obtained from bone marrow can differentiate from all three embryonic layers - ectoderm, mesoderm, endoderm - and synthesize neurotrophic factors, myelin basic

protein, and GF differentiate into SC-like cells and participate in nerve regeneration for repair. One disadvantage of this method is that it is painful. In studies performed in combination with bone marrow stem cells, repair was faster because of the presence of bone marrow stem cells (37). At the same time, when compared with other stem cell sources, proliferation and differentiation ability is less, and stem cell potential is low (34).

Olfactory Mesenchymal Stem Cells

Olfactory mesenchymal stem cells are a type of mesenchymal stem cell that originates from the ectodermal germ layer and the neural crest, moreover they are easily collected due to their location in the nasal cavity (38). The biggest difficulty in the use of MSCs is collecting them from the anatomical niches where they are located, but this is not the case for olfactory mesenchymal stem cells due to their anatomical location. Olfactory mesenchymal stem cells are capable of immune modulation and can stimulate and modulate trophic factors required in damaged tissues (39).

Although there are a limited number of studies on olfactory mesenchymal stem cells *in vivo*, these stem cells have been shown to have therapeutic potential in neurodegenerative diseases and the treatment of spinal cord injury (38).

Mesenchymal Stem Cells Obtained from Umbilical Cord and Cord Blood (UC-MSCs)

Stem cell harvesting from the umbilical cord (UC) raises ethical questions because it is difficult to use and access. However, UC-MSCs have greater proliferation and differentiation capacity than MSCs, and studies using UC-MSCs have shown increased axon regeneration after PNS injury (30).

Cui et al., collagen nerve conduit and UC-MSCs were combined and used to stimulate regeneration in the damaged area of artificially induced sciatic nerve injury in dogs and as a result of the experiment, it was observed that the damage to the sciatic nerve positively affected regeneration (30).

Mesenchymal Stem Cells Derived from Gingiva and Dental Pulp

They can be isolated from various tooth tissues (dental pulp, gingiva, papilla, deciduous teeth,

etc.). Dental pulp stem cells (DPSC) obtained from molars do not cause some ethical controversies as in the harvesting of some stem cells (27), and they have higher immunomodulation and self-renewal abilities than other types of stem cells; therefore, they are used as an alternative source in studies in the field of regenerative medicine (36). They are multipotent stem cells originating from the neural crest, which demonstrate their capacity to undergo neurological differentiation when exposed to environments different from normal (27).

Recent studies have found that human DPSCs can differentiate into neural progenitor cells, which in turn can differentiate into SC-like cells. DPMSCs have also been found to support regeneration and repair in conditions such as spinal cord injury and nerve damage (36).

Adipose Stem Cells

Adipose stem cells have generally been found to have similar characteristics to bone marrow stem cells (40). They represent another source of multipotent stem cells capable of differentiating into all three germ layers (37). Since they are derived from the stroma of adipose tissue, their accessibility is high, they can be collected at higher rates compared to bone marrow stem cells, and their differentiation and proliferation abilities are considerably higher compared to other MSC types (17). Neurons and SC-like cells are produced from differentiated adipose stem cells and some studies have found that adipose stem cells may be as effective as autologous SC (34).

Stem Cells Derived from Skin and Hair Follicle

Skin-derived stem cells are found in the dermis and are multipotent, whereas stem cells derived from hair follicles are easily accessible pluripotent stem cells. Skin stem cells are very similar to neural crest cells and express significantly with SC when cultured with neuregulin, which is known to promote the proliferation and differentiation of SC (34).

Hair follicle stem cells originate from the neural crest and the follicle contains nestin, which stimulates the production of neural progenitor cells that can differentiate into neurons and neuroglial cells. In very few studies, undifferentiated hair follicle stem cells were used in sciatic nerve injury, and it was observed that

they differentiated into SC-like cells and participated in myelination (34).

Clinical Studies

Current (Experimental) Clinical Research and Findings on Biomaterials and Stem Cells in PNS Damage

Due to the decrease in the quality of life of patients with peripheral nerve damage and the occurrence of great material and moral losses, researchers have intensified studies on the repair and regeneration of peripheral nerve damage in recent years. The development of new materials and methods with advancements in science has increased the belief that promising treatments can be realized.

In a study conducted by Berrocal et al. in 2013 on the repair of peripheral nerve defects in rats, a collagen tube with the SC was used to successfully close long segmental gaps in the PNS. When we examined the findings of the study, it was found that in the group with collagen tubes, axons grew and extended to distal nerves. Thus, they stated that directing the SC to the nerve defect through a collagen channel realized nerve repair and that the repairable distance increased, and that they took the first steps in the use of the SC in peripheral nerve repair (41).

A clinical study conducted by Bozkurt et al. in 2014 evaluated the use of nerve guides in the proximal medial sural nerve biopsy model. In this study, a micro structured nerve guide (Neuromaix) was used. It was reported that regenerating axon clusters were formed in all 11 patients included in the study, and this model was proposed as a valid technique for reproducible peripheral nerve-guide evaluation in humans (42).

In 2018, Saeki et al. conducted a clinical trial to test the efficacy of new collagen channels filled with collagen filaments in patients with peripheral nerve injuries. Of the patients included in the study, 49 underwent artificial nerve tract grafting and 7 underwent autologous nerve grafting. Postoperative improvement in sensory function was found in 21 patients in the artificial nerve tract graft group and in 4 patients in the autologous nerve graft group. Collagen plays an important role in nerve regeneration by forming epineural and perineural sheaths, suggesting that an artificial nerve pathway graft is a suitable

alternative to autologous nerve grafts for peripheral nerve injury (43).

In 2018, Neubrech et al. investigated the role of chitosan nerve tubes in the healing of sensory nerve lesions in the hands. These studies concluded that the additional use of chitosan nerve tubes for primary nerve repair in digital nerve lesions enhances nerve healing and is effective in increasing sensory peripheral nerve regeneration ability as a result of tactile gnosis and sensitivity tests (44).

In a 2022 study by Dastagir et al., the effectiveness of nerve guides in healing the sural nerve was investigated. The results showed that

patients with vascular grafts had better lateral foot sensitivity regeneration compared to those without, leading to the conclusion that distal sural nerve biopsy can serve as a model for evaluating peripheral nerve regeneration (45).

Comparative Analysis of Treatment Approaches

Here the provided table explains a comparative analysis of various treatment approaches, highlighting key data on the effectiveness and side effects of each method. This information enhances our understanding of the differences between treatment options (Table-3).

Table-3. Various Biomaterials Used in Peripheral Nerve Injury.

Living Organisms Used in the Study	Material	Method	Conclusion
Fischer Rat	A collagen tube filled with SC	Through an incision made on the right iliac crest, the nerve part was resected to create a gap, and the NeuroGen tube was added.	Increase of the repairable distance between the two ends of the damaged nerve (41).
Human	Neuromaix, a nerve guide prepared for peripheral nerve defects	Nerve biopsy and placement of Neuromaix between nerve endings	A reproducible model has been suggested with the formation of regenerating axon clusters in patients (42).
Human	New collagen channels filled with collagen filament	Artificial nerve graft was applied to the patients and compared with patients who had autologous nerve grafts.	It has been stated that collagen offers an alternative treatment for peripheral nerve regeneration with its ability to repair peripheral nerve sheaths (43).
Human	Chitosan nerve tube	The efficiency of using a chitosan nerve tube in the sensory nerve lesion healing process was investigated with sensitivity and tactile gnosis.	It has been observed that the additional use of chitosan nerve tubes increases nerve healing (44).
Human	Vascular graft	A sural nerve biopsy was performed, and sensory tests were performed by placing the proximal and distal nerve endings into a vascular graft.	According to sensory test results, it has been reported that there is an increase in lateral foot sensitivity and better regeneration in patients in whom vascular grafts were used (45).
Human	Hyaloglide, a cross-linked and biocompatible hyaluronan gel	A microsurgical procedure was performed on the peripheral nerves in the hand and the gel was applied intraoperatively along the damaged nerve.	The application of hyaloglide supported the regeneration of nerve tissue and enabled the healing of lesions by creating a favorable microenvironment (46).

Limitations

Challenges in Research and Practice: Limitations of Current Treatment Methods

Every study brings along ethical and economic debates regarding its results. In studies of stem cells, the methods of obtaining stem cells are the most frequently discussed ethical issues. It is thought that expanding the field of study by considering the rights of individuals in ethical issues will bring discoveries and treatment methods. This situation obliges scientists who do not want to experience ethical conflicts to look for alternative ways and sometimes these alternative ways may force the research group economically. By increasing the budget allocated to scientific experiments, new scientific studies can be added to the literature by expanding the working areas of research groups and facilitating their access to materials. In addition to all these, another limitation is to ensure biocompatibility in biomaterial-based studies. Researchers expect that biomaterials produced from the polymers used in their studies, such as NGC, can become fully biodegradable in the living body after completing their function. However, no definitive solution has been found in current studies.

Conclusion and Expectations

Overview of Innovative Treatment Approaches

Peripheral nerve damage has severe and difficult consequences and affects the health of a living person physically and mentally. Many scientists have conducted various studies to reverse this situation. Scientists are working on current treatment methods such as stem cell therapy, biomaterials, and the combined use of stem cells and biomaterials. As we have frequently stated in the review, different perspectives should be added to current treatments with combinations that consider the patient's condition and damage. The diversity of treatment methods is important in terms of not having the same needs and damage for each patient.

Future of the Field and Possible Developments

Significant advances will be made in the field of peripheral nerve repair shortly. Furthermore, the increasing utilization of *in silico* studies, such as molecular docking, will facilitate the development of novel approaches to repair and regeneration, which will not only complement surgical

interventions but also enhance the efficacy of existing pharmacological agents. Consequently, treatment of peripheral nerve injury may be a more straightforward endeavor. However, it is postulated that the efficacy of these treatments will be optimized if they are tailored and specific.

ADDITIONAL SECTION

Firstly, literature databases such as PubMed and WoS (Web of Science) were searched for the last 10 years (2014-2024) by typing '*peripheral nerve injury, current treatments*', '*stem cells and peripheral nerve injury treatment*', '*(name of the relevant stem cell) and peripheral nerve injury*' and the appropriate articles were taken as reference.

Abbreviations Used in the Review:

PNS: Peripheral Nervous System

CNS: Central Nervous System

SC: Schwann Cell

CSF: Cerebrospinal fluid

GF: Growth factor

NGC: Nerve Guide Channel

VEGF: Vascular endothelial growth factor

NGF: Nerve growth factor

BMP: Bone morphogenetic protein

NPF: Neurite promoting factor

NCAM: Neuron adhesion molecule

RGC: Retinal ganglion cell

PPy: Polypyrrole

iPSC: Induced Pluripotent Stem Cells

MSC: Mesenchymal Stem Cells

BDNF: Brain Derived Neurotrophic Factor

UC: Umbilical cord

UC-MSCs: Mesenchymal stem cells obtained from umbilical cord and cord blood

DPSC: Dental Pulp Stem Cells

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Ethical Approval

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Cancer stem cells and the SHH pathway in malignant melanoma: Therapeutic implications

Malign melanomda kanser kök hücreleri ve SHH yolağı: Terapötik çıkarımlar

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ABSTRACT

Melanoma is an aggressive skin cancer arising from melanocytes. Melanoma is a complex disease, sourced from genetic and environmental factors. Within melanoma, cancer stem cells (CSCs) play a crucial role in tumor progression, therapeutic resistance, and recurrence due to their capabilities for self-renewal and differentiation. The Sonic Hedgehog (SHH) signaling pathway is an important regulator of CSCs and is essential for cell differentiation and proliferation. Due to the known role in embryonic development and involvement in cancers, SHH pathway significantly affects CSC behavior in malignant melanoma, promoting tumorigenicity, metastasis, and resistance to therapies. This pathway coordinates canonical mechanisms involving Gli transcription factors and non-canonical mechanisms affecting cell migration and cytoskeletal organization. Targeting the SHH pathway has emerged as a promising therapeutic strategy, with inhibitors focusing on components like Smoothened (Smo) and Gli proteins. However, resistance to these inhibitors necessitates further exploration of novel therapeutic combinations. Current research focuses on combining SHH inhibitors with immunotherapies for more effective, long-lasting responses. Targeted medicines, which disrupt SHH processes, attempt to eliminate the fundamental causes of carcinogenesis and increase melanoma patient survival rates.

Keywords: Melanoma, Cancer Stem Cells (CSC), Sonic Hedgehog (SHH) Signaling Pathway, Therapeutic Resistance, Metastasis

Öz

Melanom, melanositlerden kaynaklanan agresif bir cilt kanseridir. Melanom hem genetik hem de çevresel faktörlerden kaynaklanan karmaşık bir hastalıktır. Melanomda, kanser kök hücreleri (CSC'ler), kendini yenileme ve farklılaşma yetenekleri nedeniyle tümör ilerlemesinde, terapötik dirençte ve nüksde önemli bir rol oynar. Sonic Hedgehog (SHH) sinyal yolağı CSC'lerin önemli bir düzenleyicisidir ve hücre farklılaşması ve çoğalması için gereklidir. Embriyonik gelişimdeki ve kanserlerdeki rolü bilindiğinden, SHH yolağı melanomda CSC davranışını önemli ölçüde etkileyerek tümörjenisiteyi, metastazı ve tedavilere direnci teşvik eder. Bu yolak, Gli transkripsiyon faktörlerini içeren kanonik mekanizmaları ve hücre göçünü ve hücre iskeleti organizasyonunu etkileyen kanonik olmayan mekanizmaları koordine eder.

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SHH yolağının hedeflenmesi, Smoothened (Smo) ve Gli proteinleri gibi bileşenlere odaklanan inhibitörlerle umut verici bir terapötik strateji olarak ortaya çıkmıştır. Bununla birlikte, bu inhibitörlere karşı direnç, yeni terapötik kombinasyonların daha fazla araştırılmasını gerektirmektedir. Mevcut araştırmalar, daha etkili ve uzun süreli yanıtlar için SHH inhibitörlerini immünoterapilerle birleştirmeye odaklanmaktadır. SHH süreçlerini bozan hedefe yönelik ilaçlar, karsinogenezin temel nedenlerini ortadan kaldırmaya ve melanom hastalarının hayatta kalma oranlarını artırmaya çalışmaktadır.

Anahtar Sözcükler: Malign Melanom, Kanser Kök Hücreleri (CSC), Sonic Hedgehog (SHH) Sinyal Yolağı, Terapötik Direnç, Metastaz

INTRODUCTION

Melanocytes are the pigment-producing cells primarily found at the epidermal-dermal junction. Melanoma is an aggressive form of skin cancer originating from melanocytes. Environmental factors, particularly ultraviolet radiation (UVR) exposure, and various phenotypic risk factors link to the pathogenesis of melanoma.

Cancer stem cells (CSCs) within melanoma play a crucial role in tumor progression, therapeutic resistance, and recurrence due to their capabilities for self-renewal, differentiation, and immune evasion. One of the critical pathways implicated in the regulation of these CSCs is the Sonic Hedgehog (SHH) signaling pathway. The SHH signaling pathway is essential for the regulation of cell differentiation, proliferation, and tissue polarity. It is well-known for its role in embryonic development and its involvement in various cancers, including basal cell carcinoma and medulloblastoma. In the context of melanoma, the SHH pathway significantly influences CSC behavior, promoting tumorigenicity, metastasis, and resistance to conventional therapies. This pathway operates through canonical mechanisms involving the activation of Gli transcription factors, as well as non-canonical mechanisms that contribute to cell migration and cytoskeletal organization. Targeting the SHH pathway has emerged as a promising therapeutic strategy for treating melanoma. Preclinical and clinical settings have explored various inhibitors targeting components of the SHH pathway, such as Smoothened (Smo) and Gli proteins. However, the development of resistance to these inhibitors necessitates the ongoing investigation of new therapeutic combinations and novel inhibitors to improve patient outcomes. Current research is focused on combining SHH pathway inhibitors with other therapies, including immunotherapies, to achieve more effective and durable responses. The understanding of SHH signaling in CSCs

underscores the complexity of melanoma biology and the need for innovative treatment strategies. By disrupting the SHH signaling mechanisms that maintain CSC properties and contribute to tumor progression, targeted therapies aim to eliminate the root causes of tumorigenesis and improve long-term survival rates for melanoma patients.

Overview of Malignant Melanoma

Malignant melanoma stems from the pigment-producing cells called melanocytes. These cells originally derived from the neural crest cells and migrate to the basal layer of the epidermis of various parts of the body, including the skin, hair, uveal tract, meninges, and mucosa (1). These melanocytes are primarily located at the epidermal-dermal junction of the skin, which is where the majority of cutaneous melanomas develop (2). Malignant melanoma is one of the top five or six most common cancers and has the highest mortality rate among skin cancers. It accounts for 5% of cancer cases in men and 4% in women (3) and contributes to 75% of all skin cancer deaths (2,4,5). The formation of malignant melanoma is primarily driven by unrepaired DNA damage in skin cells, most commonly caused by ultraviolet radiation (UVR) from sun exposure or tanning beds (6). This DNA damage leads to genetic mutations that trigger rapid multiplication of the affected skin cells, resulting in the formation of malignant tumors (7). These tumors consist of heterogeneous populations of tumor cells, including CSC which possess highly tumorigenic and chemoresistant properties (8–10).

Cancer Stem Cells in Melanoma

CSCs play a pivotal role in the progression and treatment resistance of malignant melanoma. Identified through specific biomarkers such as ABCB5, NGFR (CD271), and ALDH, CSCs are instrumental in melanoma initiation and progression due to their prolonged self-renewal capacity, vasculogenic differentiation, and

immune evasion abilities (11,12). These CSCs contribute significantly to tumor heterogeneity. These cells are characterized by their highly tumorigenic and chemo resistant properties. They facilitate metastatic dissemination and therapeutic resistance, leading to malignant recurrence (13,14). The features of CSCs, such as low immunogenic profiles and the ability to escape immune detection, allow them to survive standard treatments and contribute to tumor relapse. Consequently, targeting this subpopulation is considered an attractive strategy to improve therapeutic outcomes (14). Recent research has provided pre-clinical proof-of-concept for the potential therapeutic utility of targeting CSCs in melanoma. Strategies that combine CSC-targeting agents with standard drugs may offer enhanced therapeutic options for melanoma patients (14). These approaches weight the unique properties of CSCs, such as quiescence and the expression of certain enzymes like ALDH, to overcome therapy resistance and prevent recurrence (13). CSCs in melanoma are also associated with unique metabolic reprogramming, which supports their survival and function within the tumor microenvironment. Advances in understanding the cellular and molecular biology of these cells have revealed their involvement in critical processes such as tumor dissemination, epithelial-to-mesenchymal transition (EMT), and angiogenesis, mediated by specific intracellular signaling pathways (14,15). Given the aggressive nature of malignant melanoma and its high drug resistance, innovative therapeutic strategies targeting CSCs hold promise. By disrupting the regulatory networks that maintain CSC characteristics and contribute to tumor metastasis, these therapies aim to eradicate the root causes of tumor progression and resistance. This will ultimately allow improving patient outcomes (16,17).

SHH Signalling in Melanoma

The SHH signaling pathway has been extensively studied in different cancer types, both because it is an important signaling pathway of developmental biology and because developmental processes are like cancer. In addition to chemical agents that inhibit the SHH pathway, such as cyclopamine, direct silencing of molecules in this pathway has been used to identify potential molecules and therapeutic targets. Cell culture-based melanoma studies

have shown that transient silencing of Smo and Gli1 in normoxia and hypoxia decreases the invasion capacity of cells (18). Another study reported that Gli1 gene expression is an important marker for melanoma and other cancer types. Silencing of Gli1 gene expression in an in vivo melanoma model suppresses the SHH pathway and is associated with the Kras/Akt signaling pathway (19). Kras/Mek/Erk signalling in SHH pathway regulates the localization and translocation of Gli1 to the nucleus (19). The mutations in Gli1 gene have been reported as a biomarker for melanoma susceptibility in melanoma patients (20).

Due to the Braf mutation in melanoma, Braf based studies are critical. PDGFR alpha, which is one of the molecules induced by SHH leads to the melanoma Braf insensitivity. With PDGFR alpha and Smo antagonist LDE225i increase the Braf sensitivity in melanoma (21).

SHH Signaling Pathways in Cancer Stem Cells

The SHH signaling pathway is fundamental to how cells grow, differentiate, and establish their organization within tissues. It plays an indispensable role in shaping the central nervous system (CNS) during vertebrate development, guiding the formation of complex patterns, determining cell fate, and directing axon growth (22). This pathway ensures not only the proliferation and survival of neural cells but also their specialization into different types of neurons. The key players in SHH signaling are the Gli transcription factors, Gli1, Gli2, and Gli3. Each of these molecules contributes differently to the pathway's functions (23). SHH signaling operates through two distinct mechanisms as the canonical and non-canonical pathways. Both mechanisms crucial but with specific and diverse roles in cellular regulation (22,23). Once SHH pathway is activated, Gli1 translocates to the nucleus. It drives the transcription of specific target genes. The activation of these genes is critical for various cellular processes. This pathway is not only essential for maintaining normal embryonic and somatic stem cell function but is also highly active in CSCs, contributing to their unique characteristics of self-renewal and tumor initiation. Gli1 activation in CSCs has been extensively studied and found to significantly upregulate several key regulatory molecules, including CD133, Hif1a, histone deacetylases (HDACs), and matrix metalloproteinases (MMPs).

These molecules play pivotal roles in maintaining CSC properties such as tumorigenicity, resistance to therapy, and metastatic potential,

making the SHH-Gli1 axis a critical target for therapeutic intervention in cancer treatment. (Figure-1) (24–29).

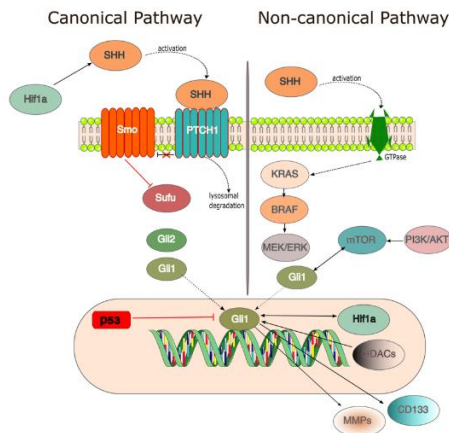


Figure-1. Canonical and non-canonical SHH pathways. Both types of pathways lead to the activation of the Gli1 protein, which translocates into the nucleus to drive target gene expression. This pathway is active in embryonic and somatic stem cells, as well as cancer stem cells. Gli1 activation has been shown to upregulate key molecules in cancer stem cell biology, including CD133, Hif1a, HDACs, and MMPs. The schematic diagram was created by Berrin Ozdil, using Inkscape, an open-source software.

In canonical SHH signaling, the pathway gets triggered when the SHH glycoprotein binds to the receptor Patched (Ptch1), which spans the cell membrane. Normally, without SHH, Ptch1 suppresses the activity of another membrane protein called Smo. However, when SHH attaches to Ptch1, this suppression is lifted, allowing Smo to accumulate and activate the pathway's downstream components, primarily the Gli proteins. The movement of Smo into the cell membrane is essential for activating the Gli proteins. Once activated, these proteins move to the cell nucleus, where they initiate the transcription of target genes like Ptch1 and Gli1. These molecules create both negative and positive feedback loops to regulate the pathway (23). Among the Gli proteins, Gli1 acts as a full-length transcriptional activator. In contrast, Gli2 and Gli3 can either activate or suppress gene expression depending on how they are processed after transcription. In the absence of SHH signaling, Gli2 and Gli3 are converted into their repressor forms, known as Gli2R and Gli3R, which help maintain a balance between turning the pathway on and off.

The non-canonical SHH signaling pathway operates independently of the primary Gli transcriptional activity. This involves other molecules such as G-protein-coupled receptors

(GPCRs) and small GTPases like RhoA. Emerging evidence suggests that the non-canonical pathway plays a role in processes such as cytoskeletal organization and cell migration (23). The selection mechanism between canonical and non-canonical routes remains a subject of investigation, indicating the complexity and versatility of the SHH pathway (23).

Aberrant activation of the SHH signaling pathway has been implicated in a variety of cancers, including basal cell carcinoma, medulloblastoma, and gliomas (13,30). The dysregulation of SHH signaling can promote tumorigenesis, tumor progression, and therapeutic resistance (30). One of the most significant components of this pathway in cancer is Smo. Smo mediates the transfer of transcription factors. Targeting Smo has become a primary strategy in cancer therapeutics, particularly for basal cell carcinoma and medulloblastoma, where Smo inhibitors have shown greater effectiveness (23).

CSCs are known for their capacity for self-renewal, differentiation, and tumorigenicity, which contribute to cancer initiation, progression, and resistance to therapy (11,12). These unique properties are maintained through the activation of several signaling pathways, including SHH, Wnt, and Notch, which are also essential for cancer and stem cell function (23) (Table 1). The

SHH signaling pathway is pivotal in regulating CSC behavior, particularly in melanoma. This pathway operates through the SHH ligand binding to its receptor Ptch, which subsequently activates Smo protein. Activated Smo leads to the translocation and activation of Gli transcription factors, which promote the expression of genes responsible for cell proliferation, survival, and stemness (31). SHH is particularly significant in coordinating CSC-driven processes such as tumor initiation, maintenance, and metastasis.

Similarly, the Wnt and Notch signaling pathways are crucial for CSC function. The Wnt pathway is involved in regulating cell fate determination, polarity, and migration (23). Aberrant Wnt signaling has been shown to promote tumorigenesis by sustaining CSC stemness and enhancing their metastatic potential. Wnt signaling not only supports CSC self-renewal but also contributes to resistance against therapies, particularly through β -catenin-mediated transcriptional activation of survival genes (32,33). The Notch pathway, on the other hand, plays a critical role in cell-cell communication and tumor microenvironment interactions. Notch signaling regulates CSC differentiation and quiescence, enabling these cells to evade immune surveillance and persist under therapeutic pressure. Dysregulation of Notch signaling has been associated with increased CSC-mediated tumor heterogeneity and adaptability (34). Interestingly, these pathways are not mutually exclusive, they often interact and converge on common downstream targets, amplifying their effects on CSC behavior. For instance, SHH and Wnt signaling are known to cooperate in maintaining the stem cell niche, while Notch signaling modulates the activity of both pathways to fine-tune CSC differentiation

and proliferation (35,36). In melanoma CSCs, the activation of SHH signaling has been linked to enhanced tumorigenicity and resistance to conventional therapies, such as chemotherapy and radiotherapy (17). CD133, a well-established cancer stem cell marker, has been associated with hypoxia-inducible factor 1-alpha (Hif1a), which is upregulated in tumors under hypoxic conditions. In response to hypoxia, elevated levels of Hif1a enhance the activation of the SHH signaling pathway via Gli transcription factors, leading to an increased expression of CD133 within the cell (37). This phenomenon has been demonstrated in hepatocellular carcinoma through SHH/Gli activation in cancer cells via SCUBE1 secreted by cancer-associated fibroblasts (38). The SHH signaling pathway plays a critical role in the regulation of CSCs, including those specifically identified in melanoma, as supported by our recent studies (29). Melanoma CSCs display higher expression levels of Gli1 and Ptch2 genes compared to non-stem cancer cells. Additionally, silencing SHH significantly reduces Gli1 and Ptch2 expression in CD133+ melanoma cells. Similarly, silencing Hif1a also leads to a decrease in both Gli1 and Ptch2 expression (29). Melanoma CSCs exhibit unique metabolic reprogramming and low immunogenic profiles, enabling them to evade the immune system. This is facilitated by the SHH pathway, which promotes the secretion of immunosuppressive factors and negative modulation of T cell functions, thus allowing CSCs to escape immune surveillance (16). Additionally, SHH signaling is implicated in processes such as epithelial-to-mesenchymal transition (EMT) and angiogenesis, which are critical for tumor dissemination and metastasis (13).

Table-1. Comparative Analysis of SHH, Notch, and Wnt Pathways in Cancer Biology

Feature	SHH Pathway	Wnt Pathway	Notch Pathway
Primary Function	Cell fate determination, stemness regulation	Cell differentiation, maintenance of stem cells.	Cell proliferation, polarity, and migration.
Key Components	SHH ligand, PTCH1, SMO, GLI transcription factors.	Notch receptors (Notch1-4), Delta/Jagged ligands.	Wnt ligands, Frizzled receptors, β -catenin.
Role in Cancer	Promotes tumor growth, stemness, and metastasis.	Enhances angiogenesis, drug resistance, and EMT.	Regulates proliferation, metastasis, and tumor microenvironment.
Pathway Crosstalk	Interacts with Wnt and Notch pathways.	Crosstalk with SHH and Wnt in stem cell niches.	Modulates SHH and Notch in developmental processes.

Therapeutic Implications

Targeting SHH pathway presents a promising strategy for eradicating CSCs in melanoma. However, single-pathway inhibitors often face challenges such as adaptive resistance and incomplete tumor eradication. Combining SHH pathway inhibitors with specific pathways (Wnt, Notch etc.) may enhance therapeutic efficacy by disrupting multiple facets of CSC regulation. Current research is exploring such combinatorial approaches, highlighting the potential for more durable clinical responses in melanoma treatment.

Melanoma has seen significant advancements in the realm of targeted therapies, specifically those aimed at inhibiting the Hedgehog (Hh) signaling pathway, which includes Smo and Gli inhibitors (23,39). The primary target for these inhibitors in clinical trials has been Smo. However, resistance mechanisms to Smo inhibitors have been identified, necessitating the exploration of new Hh pathway inhibitors to effectively control tumorigenesis (23). For example, the U.S. Food and Drug Administration (FDA) approved GDC-0449 (Vismodegib) in 2012 as a standard therapy for patients with locally advanced and metastatic basal cell carcinoma (BCC). Subsequent studies focused on combining GDC-0449 with temozolomide (TMZ) to evaluate its efficacy in both pediatric and adult patients with recurrent or refractory medulloblastoma (MB) (23).

The complexity of melanoma and its tendency to develop resistance to treatments have led researchers to explore combination therapies. Targeted therapies, while effective at first, often lead to quick but short-lived responses because of resistance. On the other hand, immunotherapies, like immune checkpoint inhibitors, tend to produce longer-lasting effects but can take time to work (40). By combining targeted therapies with immunotherapies, there's the potential to achieve both a fast and strong anti-tumor response along with long-term benefits for patients. Currently, clinical trials are investigating various combinations, such as dabrafenib (with or without trametinib) alongside ipilimumab for patients with Braf V600E/K-mutated metastatic melanoma. These trials aim to determine the best approach in terms of managing side effects, timing, and the sequence of administering these therapies. The goal is to develop strategies that can overcome treatment

resistance and provide lasting responses for melanoma patients(40).

The discovery of molecularly effective targeted therapies, particularly Hh inhibitors, has markedly improved the treatment landscape of melanoma. The focus has been on Smo and Gli inhibitors, with clinical trials validating their therapeutic potential in preclinical SHH-MB models (39). Despite the clinical use of Smo antagonists not being fully established, future Gli inhibitors and multitargeting approaches appear promising for MB patients (39). Evidence-based recommendations on the pharmacokinetics and pharmacodynamics of Hh inhibitors like sonidegib and vismodegib highlight their therapeutic considerations in special patient populations (41). Differences in their pharmacokinetic profiles may influence their efficacy and safety, suggesting a need for direct comparative clinical trials to determine their clinical relevance (41).

CONCLUSION

Malignant melanoma presents a significant challenge in skin cancer treatment due to its aggressive behavior and resistance to therapies. The critical role of cancer stem cells (CSCs) in driving melanoma progression, therapy resistance, and recurrence underscores the urgent need for innovative treatment strategies. A significant factor in this resistance is the presence of CSCs, which can renew themselves, differentiate into various cell types, and evade the immune system. The SHH pathway, with its dual roles in canonical and non-canonical signaling, emerges as a critical regulator of CSC properties, influencing tumorigenicity, metastasis, and resistance to conventional therapies. Targeting the SHH pathway has shown promise in preclinical and clinical settings. Besides, it is crucial to explore how SHH interacts with other pathways such as Wnt and Notch, which also regulate stemness, differentiation, and survival. The emergence of resistance to SHH inhibitors requires continuous exploration of novel therapeutic combinations and agents. A promising hypothesis is the integration of SHH pathway inhibitors with immunotherapies and targeted therapies that disrupt crosstalk with other molecules. This approach could reveal synergistic effects, overcoming resistance mechanisms and leading to more effective, durable responses in melanoma treatment. As the field advances, a deep understanding of the SHH signaling mechanisms and the development

of next-generation inhibitors hold the potential to disrupt the regulatory networks sustaining CSCs. By focusing on these innovative approaches, the goal is to eliminate the main causes of tumorigenesis and significantly improve long-term survival rates for patients with melanoma. Continuous efforts in this research area are vital for developing innovative treatments that can overcome the current challenges and provide better outcomes for melanoma patients.

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EGE TIP DERGİSİ Yazar Bilgi Formu

Ege Tıp Dergisi, Ege Üniversitesi Tıp Fakültesi'nin resmi yayın organı olup üç ayda bir yayımlanır ve Mart, Haziran, Eylül ve Aralık aylarında olmak üzere, dört sayı ile bir cilt tamamlanır. Dergi tüm tıp alanıyla ilgili güncel, nitelikli ve özgün çalışmaları yayımlamayı amaçlamaktadır.

Dergi sayfasına yüklenmiş olan başvurular dergi editörü veya onun belirlemiş olduğu bir alan editörü tarafında ön değerlendirmeye tabi tutulur. Ön değerlendirme sürecinde, uygun bulunan yazılar değerlendirme aşamasına geçirilirken, yayın koşullarına uymayan yazılar düzeltilmek üzere sorumlu yazara geri gönderilebilir, biçimce düzenlenebilir veya reddedilebilir. Değerlendirme aşamasında editör ya da alan editörü, yazıyı uygun gördüğü danışmanlara (hakemlere) incelenmek üzere gönderir. Hakemlik süreci çift kör olarak yürütülmektedir. Gerekli durumlarda, hakem ve editör görüşleri doğrultusunda sorumlu yazardan düzeltme/düzenleme yapması istenebilir. Yazardan düzeltme istenmesi, yazının yayımlanacağı anlamına gelmez. Bu düzeltmelerin en geç 21 gün içinde tamamlanıp dergiye gönderilmesi gereklidir. Sorumlu yazara yazının kabul veya reddedildiğine dair bilgi verilir.

Dergide yayımlanması kabul edilse de edilmese de sisteme yüklenmiş olan dosyalar arşivlenirler.

Ek Sayı: Ege Tıp Dergisi, talep olması durumunda Ek Sayı çıkartır. Ek Sayıda yer alacak olan yazıların bilimsel yönden değerlendirilmesi Ek Sayı konuk editör(lerinin)ün sorumluluğundadır. Ek Sayıda yer alacak olan yazıların hazırlanmasında derginin yazım kılavuzundaki kurallar esas alınır. Yazım kurallarına uygunluk dergi editörü ve yayın kurulunca kontrol edilir. Ek Sayı sadece elektronik olarak yayımlanır.

Açık Erişim ve Makale İşleme

Ege Tıp Dergisi, bilimsel yayınlara açık erişim sağlar. DOI numarasının belirlenmesinin ardından elektronik olarak yayımlanan sayıya ve içeriğinde yer alan yazıların tam metinlerine ücretsiz olarak ulaşılabilir.

Yazar(lar)dan yazılarının yayımı için herhangi bir ücret talep edilmez.

Okuyucular dergi içeriğini akademik veya eğitsel kullanım amaçlı olarak ücretsiz indirebilirler. Dergi herkese, her an ücretsizdir. Bunu sağlayabilmek için dergi Ege Üniversitesi'nin mali kaynaklarından, editörlerin ve hakemlerin süregelen gönüllü çabalarından yararlanmaktadır.

Telif Hakkı

Ege Tıp Dergisi, makalelerin Atıf-Gayri Ticari-Aynı Lisansla Paylaş 4.0 Uluslararası (CC BY-NC-SA 4.0) lisansına uygun bir şekilde paylaşılmasına izin verir. Buna göre yazarlar ve okurlar; uygun biçimde atıf vermek, materyali ticari amaçlarla kullanmamak ve uyarladıklarını aynı lisansla paylaşmak koşullarına uymaları halinde eserleri kopyalayabilir, çoğaltabilir ve uyarlayabilirler. Dergide yayımlanan yazılar için telif hakkı ödenmez.

Derginin Yazı Dili

Derginin yazı dilleri Türkçe ve İngilizcedir. Dili Türkçe olan yazılar İngilizce "abstract" ile, dili İngilizce olan yazılar da Türkçe özetleri ile yer alırlar. Öz ve "Abstract" bölümleri bire bir çevirileri şeklinde yer almalıdır. Yazının hazırlanması sırasında, Türkçe kelimeler için Türk Dil Kurumundan (www.tdk.gov.tr), teknik terimler için Türk Tıp Terminolojisinden (www.tipterimleri.com) yararlanılması önerilir. Dili İngilizce olan yazıların mutlaka yazım ve dilbilgisi açısından yeterliliklerinin kontrol edilmiş olması gereklidir. Dil açısından yetersiz görülen yazılar değerlendirmeye alınmazlar.

Yazarlık Kriterleri

Makalenin dergi sayfasına yüklenmesi sırasında, tüm yazarların adı, soyadı, ORCID numaraları ve tarih bilgisi ile ıslak imzalarının bulunduğu "Yayın Hakkı Devir Formu" ile yazarlık kriterlerinin

açıklandığı ve yazar katkılarının belirtildiği “Yazar Katkı Formu”nun doldurularak yüklenmesi zorunludur.

Ege Tıp Dergisi, Uluslararası Tıp Dergileri Editörleri Kurulu'nun (*International Committee of Medical Journal Editors*) standartlarını uygulamayı kabul etmiştir. Yazarlar “Biyomedikal Dergilere Gönderilen Makalelerin Uyması Gereken Standartlar: Biyomedikal Yayınların Yazımı ve Baskıya Hazırlanması (*Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication*)”daki yazarlık kriterlerini karşılamalıdır. Bu konudaki bilgiye www.icjme.org adresinden ulaşılabilir.

Etik Sorumluluk

Ege Tıp Dergisi, etik ve bilimsel standartlara uygun yazıları yayımlar. Dergide yayımlanan yazıların etik, bilimsel ve hukuki sorumluluğu yazar(lar)a ait olup editör ve yayın kurulu üyelerinin görüşlerini yansıtmaz.

Deney hayvanları ile yapılan çalışmalar dahil, tüm prospektif ve gerek görülen retrospektif çalışmalar için Etik Kurul Onayı alınmalı ve yazının “Gereç ve Yöntem” bölümünde Etik Kurul Onayının numarası ile birlikte alındığı tarih (gün-ay-yıl) belirtilmelidir. Hastanın mahremiyetinin korunmasının gerektiği tüm yazılarda etik ve yasal kurallar gereği, hastaların kimliğini tanımlayıcı bilgiler ve fotoğraflar, hastanın (ya da yasal vasisinin) yazılı bilgilendirilmiş onamı olmadan basılamadığından, **“Hastadan (ya da yasal vasisinden) tıbbi verilerinin yayınlanabileceğine ilişkin yazılı onam belgesi alındı”** cümlesinin “Gereç ve Yöntem” bölümünde (Gereç ve Yöntem bölümü olmayan yazılarda Giriş bölümünün sonuna) belirtilmesi gereklidir. Hayvanlar üzerinde yapılan çalışmalarda uluslararası etik kurallara uygunluğu gösteren komite onayı ilgili hayvan etik kurulundan alınmalıdır. Etik kurul onayı yanı sıra hayvanlara ağrı, acı ve rahatsızlık verilmemesi için yapılanlar açık olarak makalede belirtilmelidir (Bilgi için: www.nap.edu/catalog/5140.html).

Dergide yayımlanmak üzere gönderilen yazıların daha önce başka bir yerde yayımlanmamış veya yayımlanmak üzere gönderilmemiş olması gerekir. Daha önce kongrelerde sunulmuş çalışmaların Editöre gönderilen Ön Yazıda belirtilmesi gerekir. Makale, yazar(lar)ın daha önce yayımlanmış bir yazısındaki konuların bir kısmını içeriyorsa, bu durumun da Ön Yazıda belirtilmesi ve yeni başvuru dosyaları ile birlikte önceki makalenin bir kopyasının da dergi sayfasına yüklenmesi gereklidir.

Yazarlık kriterlerini karşılamayan ancak çalışmaya katkısı olan kişi, kurum veya kuruluşların isimlerine “Teşekkür” bölümünde yer verilebilir.

Çıkar çatışması: Çalışmaları ile ilgili taraf olabilecek tüm kişisel ve finansal ilişkilerin bildirilmesinden yazarlar sorumludur. Ticari bağlantı veya çalışma için maddi destek veren kurum(lar) varlığında kullanılan ticari ürün, ilaç, firma vb. ile nasıl bir ilişkinin olduğu veya herhangi bir çıkar çatışmasının olmadığı Çıkar Çatışması Formu’na doldurularak sisteme yüklenmeli ve metinde “Çıkar Çatışması” bölümünde belirtilmelidir. Çıkar çatışması formu <http://icmje.org/conflicts-of-interest/> adresinden edinilmelidir.

İntihal taraması: Ege Tıp Dergisi hiçbir şekilde intihale izin vermemektedir. Bu nedenle, dergiye gönderilen tüm yazılar ön değerlendirme sürecinde intihal tarama programı (*iThenticate* ve benzerleri) ile en az bir kez taranır. Belirlenen oranın üzerinde benzeşime sahip yazılar değerlendirmeye alınmadan yazara iade edilir.

YAZI TÜRLERİ

Yazılar, elektronik ortamda egetipdergisi.com.tr veya dergipark.gov.tr/etd adreslerinden birisi ile sisteme giriş yapılarak gönderilebilir. Yazı türlerinin içermesi gereken bölümler ile ilgili bilgilere “Yazının Hazırlanması” başlığı altında yer verilmiştir.

Araştırma Makalesi, yeni bilgiler içeren ve güncel konularda yapılmış olan orijinal çalışmaları tanımlar. Bu çalışmalar randomize kontrollü, gözlemsel, tanımlayıcı, teşhis veya tedavi doğrulayıcı, klinik, deneysel veya deney hayvanları ile yapılmış olabilirler. Kaynaklar, Öz-Abstract bölümleri ve Tablo/Şekil açıklamaları hariç, ana metin 3000 sözcük sayısını aşmamalıdır.

Olgu Sunumu, okuyucular için önemli olabilecek yeni bir bulgu veya nadir ve ilginç vaka veya durumları, tanı veya tedavi ile ilgili bir yaklaşımı içermelidir. En fazla beş yazar, Kaynaklar listesi hariç, 1000 sözcük ve 10 kaynak ile sınırlıdır. Sadece bir tablo ya da bir şekil ile desteklenebilir.

Klinik Görüntü, eğitsel önemi olduğu düşünülen, orijinal, ilginç ve yüksek kaliteli görüntü içermelidir. En fazla beş yazar, beş kaynak ve bir şekil (fotoğraf, görüntü, çizim, grafik vb.) içerebilir. Kaynaklar listesi hariç 500 kelimeyi geçmemeli, şekil alt yazısı 100 kelimeyi aşmamalıdır.

Teknik Not, eğitim, araştırma, tanı veya tedavi amaçlı gerçekleştirilmiş olan yeni ve orijinal bir uygulamayı, tekniği, alet veya cihazı tarif etmelidir. En fazla beş yazar, beş kaynak ve bir şekil (fotoğraf, görüntü, çizim, grafik vb.) veya tablo içerebilir. Kaynaklar listesi hariç 500 kelimeyi geçmemeli, şekil (varsa) alt yazısı veya tablo (varsa) açıklaması 50 kelimeyi aşmamalıdır.

Editöre Mektup, yayımlanan metinlerle veya mesleki konularla ilgili olarak 500 sözcüğü aşmayan ve beş kaynak ile bir tablo veya şekil içerecek şekilde yazılabilir. Ayrıca daha önce dergide yayınlanmış metinlerle ilişkili mektuplara cevap hakkı verilir.

Davetli Derleme Yazıları, Yayın Kurulunun daveti üzerine, tıpta özellikli konuların kapsamlı değerlendirmelerini içeren, konusunda deneyimli ve yetkin yazarların yazdığı derlemelerdir. Derleme yazıları da derginin değerlendirme sürecinden geçirilir. Kaynaklar, tablo ve şekil alt yazıları hariç 5000 kelimeyi geçmemelidir. En fazla beş yazar ve 80 kaynak ile sınırlıdır. Davetli yazılar dışında derleme yazıları kabul edilmez.

YAZININ HAZIRLANMASI

Ege Tıp Dergisine gönderilen tüm yazılar aşağıdaki kurallara uygun olarak hazırlanmalıdır.

Genel biçim

- Metin iki satır aralıklı olarak Arial 10 punto ile yazılmalıdır,
- Sayfa kenar boşlukları 2,5 cm olmalıdır,
- Sayfalar başlık sayfasından başlamak üzere, sağ üst köşesinden numaralandırılmalı ve satır numaraları eklenmelidir (Microsoft Office Word™ - Düzen - Satır numaraları - Sürekli)
- Kısaltmalar, metinde ilk olarak açık şekliyle yazılmış olanı takiben, yuvarlak parantez içinde yazılmalı ve tüm metin boyunca kısaltma aynı şekilde kullanılmalıdır. Başlık ve Öz bölümünde kısaltma kullanmaktan kaçınılmalı, metin içinde de gereksiz kısaltma kullanılmamasına özen gösterilmelidir. Cümleler kısaltma ile başlatılmamalıdır.
- Ana metin içerisinde belirtilen ürün (ilaç, cihaz, donanım veya yazılım vb.), ürünün adını takiben, üretici şirketin adı, şehri ve ülkesi parantez içinde yazılmalıdır. Örnek: Discovery St PET / CT tarayıcı (General Electric, Milwaukee, WI, ABD).
- Tüm ölçümlerin birimleri metrik sisteme (Uluslararası Birimler Sistemi, SI) göre yazılmalıdır. Örnek: mg/kg, µg/kg, mL/min, µL/h, mmHg, vb. Ölçümler ve istatistiksel veriler, cümle başında olmadıkları sürece rakamla belirtilmelidir.
- Eğer varsa, uygulanan istatistiksel yöntem, Gereç ve Yöntem bölümünde belirtilmelidir.
- Herhangi bir birimi ifade etmeyen ve 10'dan küçük sayılar ile cümle başında yer verilen sayılar yazı ile yazılmalıdır. Ondalık sayılar tam sayıdan Türkçe metinlerde virgül ile, İngilizce metinlerde nokta ile ayrılmalıdır.
- İlgili yazı, yazı türüne göre tarif edilmiş olan bölümler şeklinde hazırlanmış olmalıdır.

Ön Yazı

Editöre hitaben yazının başlığı, yazı türü, ilgili yazının neden Ege Tıp Dergisinde yayımlanması gerektiğini özetleyen kısa bir açıklama ile sorumlu yazar belirtilerek tüm yazarların adı-soyadı, ORCID numarası, kurum ve iletişim bilgileri (telefon, e-posta ve posta adresleri) yazılmalıdır. Yazının daha önce başka bir yerde yayımlanmadığına veya yayımlanmak üzere gönderilmediğine dair yazılı ifade içermelidir. Ege Tıp Dergisi başka bir dilde dahi olsa daha önce yayımlanmış, kabul edilmiş veya değerlendirme aşamasında olan hiçbir yazıyı yayımlamayı kabul etmemektedir. Yazı yazar(lar)ın daha

önce yayımlanmış bir yazısındaki konuların bir kısmını içeriyorsa, bu durumun da ön yazıda belirtilmelidir.

Daha önce bilimsel bir toplantıda sözlü veya poster bildiri şeklinde sunulmuş olan yazılar, sunumun gerçekleştirildiği toplantı ile ilgili bilgiler (tarih, yer, toplantının ismi) olacak şekilde Ön Yazıda belirtilmeli, Öz bölümünün sonuna da not olarak yazılmalıdır.

Ana Metin

Sisteme yüklenen Microsoft Office Word™ formatındaki ana metin dosyasında yazarlara ait isim ve kurum bilgileri yer almamalıdır. Ana metin yazı türüne göre aşağıdaki bölümlerden oluşmalıdır:

- Araştırma Makalesi: Türkçe başlık, Öz ve Anahtar Sözcükler / İngilizce başlık, *Abstract* ve *Keywords* / Giriş / Gereç ve Yöntem / Bulgular / Tartışma / Sonuç / Çıkar Çatışması / Teşekkür (varsa) / Kaynaklar / Tablolar (başlıkları ve açıklamalarıyla beraber) / Şekil Alt Yazıları.

- Olgu Sunumu: Türkçe başlık, Öz ve Anahtar Sözcükler / İngilizce başlık, *Abstract* ve *Keywords* / Giriş / Olgu Sunumu / Tartışma / Sonuç / Çıkar Çatışması / Kaynaklar / Tablo (başlıkları ve açıklamalarıyla beraber) / Şekil Alt Yazısı.

- Klinik Görüntü: Türkçe başlık / İngilizce başlık / Olgu / Çıkar Çatışması / Teşekkür (varsa) / Kaynaklar / Şekil Alt Yazısı.

- Teknik Not: Türkçe başlık / İngilizce başlık / Teknik not / Çıkar Çatışması / Teşekkür (varsa) / Kaynaklar / Tablo (başlıkları ve açıklamalarıyla beraber) (varsa) / Şekil Alt Yazısı (varsa).

Yazının Başlığı

Kısa, kolay anlaşılır ve yazının içeriğini tanımlar özellikte, kısaltma içermeyecek şekilde Türkçe ve İngilizce olarak yazılmalıdır.

Özler

Türkçe (Öz) ve İngilizce (*Abstract*) başlığı altında yazılmalıdır. Araştırma Makalelerinde Amaç, Gereç ve Yöntem, Bulgular ve Sonuç (*Aim, Materials and Methods, Results, Conclusion*) olmak üzere dört bölümden oluşmalı, en fazla 250 sözcük içermelidir. Araştırmanın amacı, yapılan işlemler, gözlemsel ve analitik yöntemler, temel bulgular ve ana sonuçlar belirtilmelidir. Öz metninde kaynak numarası ve mümkün olduğunca kısaltma kullanılmamalıdır. Olgu Sunumlarında bölümlere ayrılmamalı ve 200 sözcüğü aşmamalıdır. Klinik Görüntü, Teknik Not ve Editöre Mektup için öz gerekmemektedir.

Anahtar Sözcükler

Öz (*Abstract*) bölümünün sonunda, Anahtar Sözcükler (*Keywords*) başlığı altında, bilimsel yazının ana başlıklarını yakalayan, *Index Medicus Medical Subject Headings (MeSH)*'e uygun olarak yazılmış en az üç, en fazla beş anahtar sözcük olmalıdır. Türkçe anahtar sözcüklerin, Türkiye Bilim Terimlerinden (www.bilimterimleri.com) seçilmesine özen gösterilmelidir.

Metin

Yazı metni, yazının türüne göre yukarıda tanımlanan bölümlerden oluşmalıdır.

Kaynaklar

Ege Tıp Dergisi, ulusal kaynaklardan yararlanmaya özel önem verdiğini belirtir ve yazarların bu konuda duyarlı olmasını bekler.

Kaynaklar metinde, tablo açıklamaları ve şekil alt yazılarında yer aldıkları sırayla, cümle içinde atıfta bulunulan ad ya da cümle bitiminde, noktadan önce yuvarlak parantez “()” içinde, Arabik rakamlarla numaralandırılmalıdır. Birden fazla kaynak numarasının belirtilmesi durumunda rakamlar birbirlerinden virgül ve bir boşluk bırakılarak ayrılmalı ardışık ikiden fazla rakam olması durumunda en küçük ve en büyük rakamlar arasına tire işareti konarak yazılmalıdır. Örnekler: (2, 5, 7); (3-7).

Dergi isimleri, *Index Medicus (PUBMED)*'de kullanıldığı şekilde kısaltılmalıdır. Kısaltılmış yazar ve dergi adlarından sonra nokta olmamalıdır. Yazar sayısı altı veya daha az olan kaynaklarda tüm

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Tkacova R, Toth S, Sin DD. Inhaled corticosteroids and survival in COPD patients receiving long-term home oxygen therapy. *Respir Med* 2006;100(3):385-92.

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Solca M. Acute pain management: Unmet needs and new advances in pain management. *Eur J Anaesthesiol* 2002;19(Suppl 25):3-10.

Erken görünümde (E-pub) makale

Butterly SJ, Pillans P, Horn B, Miles R, Sturtevant J. Off-label use of rituximab in a tertiary Queensland hospital. *Intern Med J* doi: 10.1111/j.1445-5994.2009.01988.x

Kitap

Bilgehan H. Klinik Mikrobiyoloji. 2. Baskı. İzmir: Bilgehan Basımevi; 1986:137-40.

Kitap bölümü

McEwen WK, Goodner IK. Secretion of tears and blinking. In: Davson H (ed). *The Eye*. Vol. 3, 2nd ed. New York: Academic Press; 1969:34-78.

İnternet makalesi

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

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

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RESEARCH ARTICLES

- Anti-inflammatory effects of iturin A on the 6-OHDA-induced SH-SY5Y cell model of Parkinson's Disease** 193
Pinar Altın Celik Muazzez Derya Andeden Hamiyet Donmez Altuntas
- Corelation of the neuropathic pain and electrodiagnostic tests in earthquake-related peripheral nerve injuries** 201
Metin Balduz Halit Fidancı
- Investigation of aggression levels of methamphetamine users in probation** 209
Fatma Dilek Şeker Dilek Öker Keleş
- FoxM1 inhibition by Thioestrepton downregulates DNA damage response genes, enhancing sensitivity of breast cancer cells to therapy** 223
Funda Demirtas Korkmaz Zekeriya Duzgun Fadime Mutlu Icduygu
- GBA1 variants and Parkinson's Disease: A dual approach combining clinical data and literature review** 231
Sezin Canbek Goncagul Mert M. Fatih Gulseven M. Guney Senol
- Investigation of adults' attitudes towards protection from hypertension and affecting factors** 243
Güler Kayabaşı Kadriye Sayın Kasar
- Evaluation of the effect of PSA density on robotic radical prostatectomy outcomes** 256
Alp Akyol Kasım Emre Ergun Serdar Kalemci Adnan Şimsir Fuat Kızılay
- Heart failure in advanced age: comorbidities, treatment approaches, and survival outcomes** 262
Emre Demir
- Relationship with FIB-4, APRI scores and the clinical diagnosis in chronic liver diseases** 274
Pinar Peker Ali İbrahim Hatemi
- Magnetic resonance imaging findings of extremity synovial sarcomas** 283
İpek Tamsel İlhan Hekimsoy
- Cyberchondria and quality of life in anxiety and obsessive-compulsive disorder: A cross-sectional assessment from a tertiary center** 289
Selin Tanyeri Kayahan Özlem Kuman Tunçel Damla İşman Haznedaroğlu Şebnem Pırıldar
- Dosimetric comparison of single isocenter dynamic conformal arc therapy (DCAT) and volumetric modulated arc therapy (VMAT) planning techniques for stereotactic radiotherapy of multiple brain metastases** 300
Nezihat Olacak Sinan Hoca Mert Delikaya Yusuf Ziya Hazeral Deniz Yalman
- The relationship of pregnancy losses with frequently-used hematologic parameters** 310
Atakan Turgutkaya Ayşe Hilal Eroğlu Küçükçiler Niyazi Alper Seyhan
Ali Zahit Bolaman Zehra Manav Yiğit Gökay Bozkurt İrfan Yavaşoğlu
- HBV seroprevalence in patients with diabetic foot infection** 319
Nazlıhan Yalçın Gökçen Ünal Kocabaş Tansu Yamazhan Hüsni Pullukçu Anıl Murat Öztürk Meltem Işıkgöz Taşbakan
- The role of biochemical markers in predicting pre-eclampsia** 324
Ufuk Atlihan Begüm Ertan Mehmet Emre Peker Can Ata
Onur Yavuz Hüseyin Aytağ Aşar Alper İleri Tefik Berk Bildacı Selçuk Erkinç
- Pregnancy outcomes and risks in over 40; a retrospective analysis from Turkey's highest birth rate region** 330
Elif Ucar Melih Bestel
- Restless leg syndrome in multiple sclerosis: relationship with restless legs syndrome and fatigue, quality of life, daytime sleepiness, sleep quality and anxiety-depression** 337
Asiye Tuba Özdoğan Enes Aldemir Pervin Yeşiloğlu Vedat Çilingir
- Evaluation of perioperative coagulation in patients undergoing brain and spine surgery** 346
Alev Atalay Abdurrahim Derbent Mustafa Nuri Deniz Sezgin Ulukaya Sacittin Taner Balcıoğlu
- Surgical timing and treatment modalities for developing hydrocephalus in newborns with spinal dysraphism** 356
Hakan Korkmaz Ali Özcan Binatlı
- JZL184 modulates lung injury via occludin and TNF-α in two distinct ARDS models** 363
Yusuf Elma Emine Yılmaz Can Meryem Akpolat Ferah Mete Keçeci
- The effectiveness and accuracy of ultrasound in the diagnosis of nephrolithiasis and measurement of kidney stone size** 374
İlhan Hekimsoy
- Neurobehavioral effects of maternal triphenyl phosphite exposure: A comparative experimental study using the valproic acid model** 381
Petek Bilim Kübra Çelik Gurur Garip Ahmet Giray Yardımcı Buket Şura Yardımcı Meral Baka

CASE REPORT

- Intraoral lipoma: 2 case report** 390
Mehmet Ali Memişoğlu Mert Zeytinoğlu Ümrhan İnceoğlu Ekin Çelikten
- An atypical Stiff Person Syndrome patient presenting with epileptic seizures, brainstem involvement, and muscle stiffness of the lower body- a case report** 394
Göker Gören Abdullah Arı Hüseyin Nezi Özdemir Figen Gökçay
- Vesicouterine fistula after cesarean section: A case report** 398
Mehmet Emre Peker Ufuk Atlihan Duygu Uçar Kartal

REVIEWS

- The role of stem cells and biomaterials in peripheral nervous system regeneration** 402
Dila Hatun SAL Zeynep Simge YILMAZ Büşra HORUZ Aybike Berrak ÖZDEMİR Kubilay Doğan KILIÇ
- Cancer stem cells and the SHH pathway in malignant melanoma: Therapeutic implications** 415
Berrin Özdi Huseyin Aktug



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