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## CONTENTS

### Original Investigations - Özgün Araştırmalar

- 106 Seroprevalence and Trends in Human Immunodeficiency Virus Among Voluntary Non-Remunerated Blood Donors in a Teaching Hospital: Nine-year Retrospective Study**  
Bir Eğitim Hastanesindeki Gönüllü, Karşılıksız Kan Bağışçıları Arasında İnsan İmmün Yetmezlik Virüsü Seroprevalansı ve Eğilimleri: Dokuz Yıllık Retrospektif Çalışma  
*Muhamad Aidil Zahidin, Noor Haslina Mohd Noor, Marini Ramli, Mohd Nazri Hassan, Zefarina Zulkafli, Salfarina Iberahim, Rosnah Bahar, Shafini Mohd Yusoff, Wan Suriana Wan Abdul Rahman, Marne Abdullah*
- 111 Comparison of Harmonic Scalpel and Electrocautery During Breast Surgery: A Cohort Study**  
Meme Cerrahisinde Harmonik Neşter ve Elektrokoterin Karşılaştırılması: Bir Kohort Çalışması  
*Hassan Moaiery, Mohammad Yahya Faridi*
- 115 Evaluating the Effect of Chloroform and Water Extract of *Satureja khuzistanica* on the Expression of Bcl-2 and Bax in Gastric Cancer Cell Line**  
*Satureja khuzistanica* Kloroform ve Su Ekstraktının Mide Kanseri Hücre Hattında Bcl-2 ve Bax Ekspresyonu Üzerine Etkisinin Değerlendirilmesi  
*Bagher Biglak, Asghar Tanomand, Leyla Açık, Farzad Arjomandi Rad, Gholamreza Shahsavari, Saman Kazemi, Hossein Soltanzadeh*
- 121 Development of the COVID-19 Stigma Scale: Validity and Reliability Study**  
COVID-19 Damgalama Ölçeğinin Geliştirilmesi: Geçerlilik ve Güvenilirlik Çalışması  
*Bahadır Geniş, Mustafa Yıldız*
- 132 The Effect of Polyurethane Film Versus *Apis Dorsata* Honey Spray for Wound Dressing Following Long Bone Fractures Osteosynthesis**  
Uzun Kemik Kırıklarında Osteosentez Sonrası Yara Pansumanında Poliüretan Film ve *Apis Dorsata* Bal Spreyinin Etkisi  
*Surianty Shafei, Mohd Ariff Sharifudin, Shaifuzain Ab Rahman, Abdul Nawfar Sadagatullah*
- 139 Comparison of Use of Propiverine at 45 mg and Combined Treatment of Propiverine 30 mg and Mirabegron in Patients with Overactive Bladder Who did not Benefit from Propiverine 30 mg Treatment**  
Propiverin 30 mg Tedavisinden Fayda Görmeyen Aşırı Aktif Mesaneli Hastalarda Propiverin 45 mg Kullanımı ile Propiverin 30 mg ve Mirabegron Kombine Tedavisinin Karşılaştırılması  
*İrfan Şafak Barlas, Selamettin Demir*
- 145 Protective Effects of Curcumin and Resveratrol on Kidney Tissue on Cadmium-induced Oxidative Stress in Rats**  
Sıçanlarda Kadmiyum Kaynaklı Oksidatif Strese Karşı Kurkumin ve Resveratrolün Böbrek Dokusu Üzerindeki Koruyucu Etkileri  
*Suat Cakina, Latife Ceyda İrkin, Şamil Öztürk*
- 149 Mortality Predictors using Chest Computed Tomography Findings in COVID-19 Patients**  
COVID-19 Hastalarının Bilgisayarlı Göğüs Tomografisi Bulgularındaki Mortalite Belirteçleri  
*Ali Yavuz Uzun, Yılmaz Üçüncü, Nur Hürsoy, Fatma Beyazal Çeliker, Zihni Yazıcı*

## CONTENTS

- 156 Survey to Assess the Impact of the COVID-19 Pandemic on Tinnitus Perception**  
COVID-19 Pandemisinin Tinnitus Algısı Üzerindeki Etkisinin Değerlendirilmesi  
*Oğuz Yılmaz, Handan Yaman, Berna Özge Mutlu, Dilara Bayazıt, Serkan Eti, Hasan Demirhan, Yıldırım Ahmet Bayazıt*
- 161 Retrospective Analysis of the Factors Affecting Recurrence, Survival, and Effect of Hippocampus Radiotherapy Doses on Neurocognitive Functions in Patients Diagnosed with Glioblastoma Multiforme**  
Glioblastoma Multiforme Tanısıyla Tedavi Edilen Hastalarda Nüks, Sağkalımı Etkileyen Faktörlerin ve Hipokampus Radyoterapi Dozlarının Nörobilişsel Fonksiyonlara Etkisinin Retrospektif Analizi  
*Sevilcan İnan, Öznur Dönmez Tutanoğlu, Hülya Ertaş, Fatih Aydemir, Burcu Caner, Turgay Toksoy, Pınar Çona*
- 168 A Speckle-Tracking Echocardiography Study: Cardiac Effect of Sleep Duration**  
Speckle-Tracking Ekokardiyografi Çalışması: Uyku Süresinin Kalp Üzerine Etkileri  
*Gülsüm Bingöl, Fulya Avcı Demir, Özge Özden, Emre Özmen, Serkan Ünlü*
- 173 Wilson Disease in a Turkish Population: Molecular Insights into an Old Disease with Reported and Novel Variants**  
Bir Türk Popülasyonunda Wilson Hastalığı: Bildirilen ve Yeni ATP7B Varyantlarıyla Eski Bir Hastalığa Moleküler Bakış  
*Abdullatif Bakır, Vehap Topçu, Büşranur Çavdarlı*
- 179 Investigation of Prophylactic Drug Use Adherence in Patients with Migraine**  
Migren Hastalarında Profilaktik İlaç Kullanımına Olan Uyumun Araştırılması  
*Ezgi Can, Tuğba Tunç*
- 186 ChatGPT vs. Orthopedic Residents! Who is the Winner?**  
ChatGPT Ortopedi Asistanlarına Karşı! Kazanan Kim?  
*Semih Yaş, Asim Ahmadov, Alim Can Baymurat, Mehmet Ali Tokgöz, Secdegül Coşkun Yaş, Mustafa Odluyurt, Tolga Tolunay*
- 192 Kyphoplasty is not Superior to Vertebroplasty in Restoring Vertebral Height in the Long Term**  
Kifoplasti, Uzun Dönemde Vertebral Yüksekliği Geri Kazandırmada Vertebroplastiye Üstün Değildir  
*Alim Can Baymurat, Ferid Abdulaliyev, Asim Ahmadov, Muhammed Furkan Tosun, Mustafa Melik Can, Cem İsmail Güngör, Alpaslan Şenköylü*
- 200 Routine Histopathological Evaluation of Benign Anorectal Interventions: Essentiality or Excess?**  
Benign Anorektal Girişimlerde Rutin Histopatolojik Değerlendirme: Gereklilik mi, Fazlalık mı?  
*Ramazan Kozan, Enes Aksu, Kürşat Dikmen*
- Case Reports - Olgu Sunumları**
- 204 Surgical Treatment of the Oroantral Fistula in a Patient with Myasthenia Gravis: Clinical Considerations and Anesthetical Management**  
Miyastenia Gravisli Bir Hastada Oroantral Fistülün Cerrahi Tedavisi: Klinik ve Anestezi Yönetimi  
*Elif Betül Yıldırım, Turan Kazan, Yeliz Kılınc, Necmiye Şengel*

## CONTENTS

### 208 **A Rare Prenatal Case: Greig Cephalopolysyndactyly Syndrome**

Nadir Bir Prenatal Olgu: Greig Sefalopolisindaktili Sendromu

*Tilbe Hakçıl, Gülsüm Kayhan, Tuncay Nas, Pınar Tellı Celtemen, Meral Yirmibeş Karaoğuz*

### **Review - Derleme**

### 212 **A General Overview of Mesenchymal Stem Cell Therapies in Drug- and Chemical-Induced Liver Injury Models**

İlaç ve Kimyasal Kaynaklı Karaciğer Hasarı Modellerinde Mezenkimal Kök Hücre Tedavi Uygulamalarına Genel Bir Bakış

*Müşerref Şeyma Ceyhan, Suna Ömeroğlu, Zeynep Yiğman*

### **Original Image - Orijinal Görüntü**

### 222 **Rare Old Distal Carpal Row Dislocation: Wait, do not Reduce, or Operate**

Nadir Eski Distal Karpal Sıra Çıkığı: Bekleyin, Azaltmayın veya Çalıştırmayın

*Muath Mamdouh Mahmud Al-Chalabi, Abdul Nawfar Sadagatullah, Wan Azman Wan Sulaiman*

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## Seroprevalence and Trends in Human Immunodeficiency Virus Among Voluntary Non-Remunerated Blood Donors in a Teaching Hospital: Nine-year Retrospective Study

Bir Eğitim Hastanesindeki Gönüllü, Karşılıksız Kan Bağışçıları Arasında İnsan İmmün Yetmezlik Virüsü Seroprevalansı ve Eğilimleri: Dokuz Yıllık Retrospektif Çalışma

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### ABSTRACT

**Objective:** Human immunodeficiency virus (HIV) infection, which causes acquired immune deficiency syndrome, is a significant public health problem. HIV infection through blood transfusion remains at the top of any other risk exposure because blood carries a much higher level of HIV than other routes of infection. The aim of this study was to describe the seroprevalence of HIV infection from 2010 to 2019 among donors screened at the transfusion medicine unit in Northeastern Malaysia.

**Methods:** A retrospective study was conducted on voluntary blood donors at the Transfusion Medicine Unit, Hospital Universiti Sains Malaysia, from January 2011 to December 2019. Data such as age, gender, marital status, frequency of donations, and serological results were obtained from the computer system of the blood bank and analyzed.

**Results:** A total of 98,874 individuals donated blood and were screened for HIV infections. Only 29 donors were positive for HIV. Therefore, the overall seroprevalence of HIV infection in blood donors was only 0.03% in the nine consecutive years. The trend of HIV infection among our donors decreased initially but increased again from 2014 to 2019.

**Conclusion:** The prevalence in Northeastern Malaysia province was low compared with previous studies conducted in other regions worldwide. The application of standard operating procedures, with updated equipment, and planning for the use of molecular methods are necessary for the blood transfusion service to monitor transfusion-transmitted infections.

**Keywords:** Transfusion transmitted infections, human immunodeficiency virus, blood donors, seroprevalence, transfusion

### ÖZ

**Amaç:** Edinsel immün yetmezlik sendromuna neden olan insan immün yetmezlik virüsü (HIV) enfeksiyonu önemli bir halk sağlığı sorunudur. Kan nakli yoluyla HIV enfeksiyonu, diğer tüm risk maruziyetlerinin en üstünde yer alır çünkü kan, diğer enfeksiyon yollarından çok daha yüksek düzeyde HIV taşır. Bu çalışmanın amacı, Kuzeydoğu Malezya'daki transfüzyon tıbbi ünitesinde taranan bağışçılar arasında 2010'dan 2019'a kadar HIV enfeksiyonunun seroprevalansını tanımlamaktır.

**Yöntemler:** Ocak 2011'den Aralık 2019'a kadar, Sains Malaysia Üniversite Hastanesi Transfüzyon Tıbbi Birimi'nde gönüllü kan bağışçıları üzerinde retrospektif bir çalışma gerçekleştirildi. Kan bankasının bilgisayar sisteminden yaş, cinsiyet, medeni durum, bağış sıklığı ve serolojik sonuçlar gibi veriler elde edilerek analiz edildi.

**Bulgular:** Toplam 98.874 kişi kan bağışında bulundu ve HIV enfeksiyonları açısından tarandı. Sadece 29 donörde HIV pozitif çıktı. Bu nedenle, kan donörlerinde HIV enfeksiyonunun genel seroprevalansı art arda dokuz yılda yalnızca %0,03 idi. Bağışçılarımız arasındaki HIV enfeksiyonu eğilimi başlangıçta azaldı ancak 2014'ten 2019'a kadar yeniden arttı.

**Sonuç:** Kuzeydoğu Malezya eyaletindeki yaygınlık, dünya çapında diğer bölgelerde yürütülen önceki çalışmalarla karşılaştırıldığında düşüktü. Güncellenmiş ekipmanlarla standart operasyon prosedürlerinin uygulanması ve moleküler yöntemlerin kullanımına yönelik planlama, kan transfüzyonu hizmetinin transfüzyonla bulaşan enfeksiyonları izlemesi için gereklidir.

**Anahtar Sözcükler:** Transfüzyonla bulaşan enfeksiyonlar, insan immün yetmezlik virüsü, kan bağışçıları, seroprevalans, transfüzyon

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## INTRODUCTION

Blood transfusion is a routine therapeutic intervention in hospitals that can be lifesaving. However, this intervention is related to several transfusion-related infections, which are very serious complications of blood transfusion.

The human immunodeficiency virus (HIV) is an important viral agent and one of the significant public health problems associated with transfusion transmitted infection (TTI). HIV infections are still the main cause of morbidity and mortality in developed countries. Choosing healthy donors with a low risk of blood contamination is an important measure for protecting blood safety (1).

HIV/AIDS prevalence during more than a decade indicates that its prevalence is getting increasingly serious, and the rapid spread of HIV infection exists with the characteristics of regional and age differences (2).

The aim of this study was to describe the prevalence of HIV from 2010 to 2019 among donors screened at the transfusion medicine unit in northeastern Malaysia. These data from approximately 10,000 donors annually provide estimates of the temporal trends of HIV infection in a group of donors in this region.

## MATERIALS AND METHODS

A retrospective study was conducted at the Transfusion Medicine Unit, Hospital Universiti Sains Malaysia, from January 2011 to December 2019. The study population comprised all voluntary blood donors who donated blood at Transfusion Medicine Unit, Hospital Universiti Sains Malaysia during that period. The donors were those who weighed not less than 50 kg, were 18 years old, and fulfilled the standard donor criteria set by the Malaysia National Blood Centre.

Data from the volunteers who donated blood were included in the analysis. Sociodemographic data, such as age, gender, marital status, occupation, serological results, and frequency of donations, were obtained from the blood bank's computer system. To preserve the privacy of donors required by law, donor names were not disclosed by the blood bank and were individualized by donation number. Frequency of donation, i.e., first-time donors, lapsed donors, and regular donors, were also included in the study. First-time donors are those who donated for the first time at our unit, lapsed donors who donated blood twice or less and had not donated within the last 24 months, and regular donors are those who donated three or more times in a 12-month period.

Ethical clearance was obtained from the Universiti Sains Malaysia Ethical Committee (USM RUI grant; 1001/PPSP/812187), and verbal consent was obtained from the Blood Bank administration before the start of data collection. Confidentiality of the information was ensured as the blood donation number was registered on the data collection format instead of the names of the subjects.

### Statistical Analysis

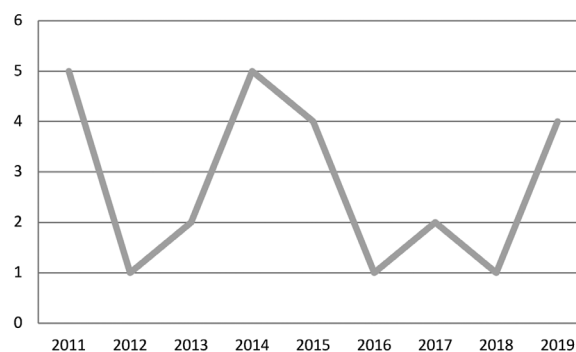
All tests were performed using the ARCHITECT HIV Ag/Ab Combo assay (Abbott Diagnostics, Chicago, IL) according to the manufacturer's instructions and validated standard operating procedures. The ARCHITECT HIV Ag/Ab Combo assay is a chemiluminescent microparticle immunoassay for the simultaneous qualitative detection of HIV p24 antigen and antibodies to HIV

type 1 and/or type 2 (HIV-1/HIV-2) in human serum or plasma. The ARCHITECT HIV Ag/Ab Combo assay is intended to be used as an aid in the diagnosis of HIV-1/HIV-2 infection and as a screening test for donated blood and plasma.

## RESULTS

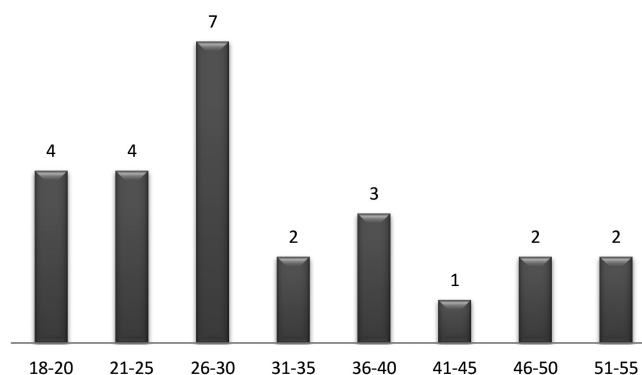
During the nine years, 98,874 individuals donated blood and were screened for HIV infections. The majority of the blood donors were aged 17-24 years with a male-to-female ratio of 1:1.21. Most of our donors were Malay (79.66%) and single (78.2%), with only 21.8% being married. Regular, lapsed and first-time donors were 12.89%, 27.54% and 59.55%, respectively.

The study showed that there were only 29 HIV-positive blood donors. Therefore, the overall seroprevalence of HIV infection in blood donors was found to be only 0.03% over nine consecutive years. The trend of HIV infection increased from 2010 to 2011, decreased from 2011 to 2013, and increased in 2013 and 2014. The trend decreased again from 2014 to 2018; however, it increased again in 2019 (Figure 1). Considering age, the age groups of 26-30 had the highest contribution of HIV infection (Chart 1). The male-to-female ratio was 4.8:1. The majority of our seroprevalence of HIV donors were from Malay, students, single and first-time donors, which was 75.9%, 41.4%, 62% and 55.2%, respectively (Chart 2). Twenty donors admitted they had a history of sexual promiscuity [MSM (n=10), multiple heterosexual partner (n=10)] and nine donors denied the risk factor.



**Figure 1.** Trends in HIV seroprevalence.

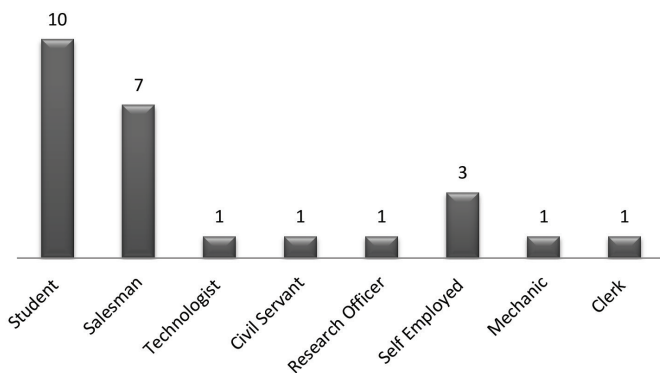
HIV: Human immunodeficiency virus.



**Chart 1.** Age of donors who are HIV positive.

HIV: Human immunodeficiency virus.





**Chart 2.** Occupation of donors who were HIV positive.

HIV: Human immunodeficiency virus.

## DISCUSSION

Blood transfusion is considered a potential risk factor for the transmission of viruses, such as HIV, which can be life-threatening and have global public health importance. A substantial percentage of blood donors harbor TTIs. Despite stringent donor selection and testing practices, safe blood free from TTIs remains an elusive goal because the threat of TTI agents entering the blood supply is not static (3). Voluntary donations and comprehensive screening of donors' blood for transfusion-transmitted viruses using standard methods to safeguard the blood recipient should be emphasized (4).

The overall prevalence of HIV infection among our blood donors was very low (0.03%). Low seroprevalence results were also seen among voluntary blood donors in Uttar Pradesh, southeast Iran, and Pakistan (1,4,5). The trend of HIV infection among our donors decreased initially but increased again from 2014 to 2019. Seroprevalence for HIV showed increasing patterns ranging from 6.34 to 16.74% per 100,000 per person-years, with only four cases detected in 2004 and 2005 to 12 cases in 2008 (three-fold increase) (6).

This variation in HIV prevalence could be due to differences in high-risk behavior, training programs, prevention methods, and the use of appropriate and safe methods in blood transfusion centers in different countries (7). Pre-donation counseling was recognized as one of the important strategies to reduce and prevent the donation of blood by individuals who might be at risk for HIV and other TTIs (8). Low seroprevalence of HIV infection among our donors might be due to the decreasing trend of HIV infection in Malaysia and/or that the donors who had risk behaviors related to HIV infection were screened before donation.

Considering age and sex, we found that the age groups 21-25 had the highest contribution of HIV infection, with a male to female ratio was 4.8:1. RN Makroo reported a high rate of HIV positivity in male donors (97.4%) compared with females in India, and 54.9% was seen in the age group of 18-30 years (9). A study among Chinese blood donors also reported more males (81% vs. 64%) and a higher proportion of 26-35 years old were found in HIV-positive than HIV-negative donors (41.9% vs. 26.9%) (10). Epidemiological analysis of HIV prevalence by Qiao et al. (2) reported higher risk existed in the young and middle-aged populations, especially in 30- to 40-year-old people. In Chiangmai, the proportion of male donors (80.6%) was more than female donors, with a mean age of 28.9 and 28.8 years,

respectively (11). However, in Nigeria, it was reported that an age range of 29 and below has a lower prevalence of HIV antibodies than other age groups, and this is an indication of success on the part of Nigerian government agencies and non-governmental organizations in the fight against new HIV infections (12). HIV-positive blood donors in Germany that were included in the analysis were significantly younger (median age; 28 years) and lived more frequently in a metropolis (13).

Qiao et al. (2) analyzed the epidemiologic characteristics and concluded that the regional and age differences in HIV prevalence were significant, and this result may provide basic data for the prevention and control of sexually transmitted diseases (STDs). More effective and efficient control programs with expanded geographical and aged population coverage are required (2).

We observed that the majority of our seroprevalence of HIV donors were from Malay, students, and single donors (75.9%, 41.4%, and 62% respectively). A study by the National Blood Centre reported that the majority of HIV-positive blood donors in their institution were students, unmarried, and first-time donors (41.4%, 62% and 55.2% respectively). This finding may be due to a low level of awareness among students regarding the TTI risk before blood donation, a current lifestyle that might contribute to the seropositivity, and an increasing number of donations made by students compared with others (6). The association of HIV status with younger age at sexual debut may be due to more lifetime partners. This increase could result from a longer sexual life. Prevention of HIV infection should include efforts to delay age at first sex (14).

A study by Rich et al. (10) observed that donors with less high school education (38.2% vs. 15.5%) and divorced/separated/widowed donors (14% vs. 4%) were higher in the HIV-positive than in the HIV-negative group. A study among Malawi blood donors reported that HIV-positive results were significantly associated with age above 25 years and being out of school (15). Nafishah et al. (6) observed that the higher the educational level, the lower the rate of seroconversion in TTI. Donors who finished primary education only had higher anti-HIV prevalence than donors who finished secondary level and higher education (1.82%, 0.52% and 0.22% respectively). This evidence supports that individuals with low education have higher risk behaviors, such as tattooing, intravenous drug abuse, and extramarital sex relations without condom use (16).

We reported higher HIV seroprevalence among donors who were single. A larger proportion of HIV-positive donors were also reported among divorced, separated, or widowed donors in China, suggesting the importance of understanding the psychosocial characteristics of HIV-positive donors and the implementation of behavioral and therapeutic interventions to prevent further spread of the infection (10). Donors recruited at workites and community halls had higher HIV seroprevalence (18.9% and 18.7%, respectively) than donors recruited at the National Blood Transfusion Service (NBTS) headquarters, schools, or clubs (7.9%) (17).

The majority of our seroprevalence of HIV donors were first-time donors, which was 55.2%. Regular and lapsed donors accounted for approximately 44.8% of HIV-positive donors. A study conducted on 130 first-time blood donors in Nigeria found that 6.2% were HIV-positive donors. This indicates that HIV is still being actively transmitted among the most productive age bracket in the Nigerian

community (12). The seroprevalence of HIV infection among voluntary non-remunerated blood donors in Malawi was also higher in first-time blood donors. The prevalence of HIV infections in repeat blood donors showed a reducing trend (15).

McFarland et al. (17) reported that HIV seropositivity among first-time blood donors in Zimbabwe was 51%. A study among blood donors in Brazil reported that the greatest number of cases with HIV infection was from the first-time donation (18). Among men, repeat donors had a lower overall HIV prevalence (2.47%) than first-time donors (3.64%;  $p < 0.0001$ ) (11).

We reported 69% of the HIV-positive donors admitted to having sexual promiscuity [MSM ( $n=10$ ) and multiple heterosexual partners ( $n=10$ )] and nine donors denied the risk factor. Sexual contacts were the most prominent reported risk factors among HIV-infected donors in Germany, reported as either MSM or heterosexual risk contacts, or both (13). Zeng et al. (19) reported that a man who has sex with men was associated with the highest odds of HIV infection. Not using a condom, having sex with HIV-infected individuals or partners with STDs, or having more than two sex partners were all associated with more than five times higher odds of having HIV (19). According to a study by Nafishah et al. (6), when asked regarding why they still donated even though they already knew they were involved in high-risk activities and could transmit infections through blood donations, the majority of respondents admitted they practiced safe sex and would never be infected, and most of the respondents thought that high-risk activities asked during the pre-donation interview only concerning recent activities. One of the preventive measures implemented at the National Blood Centre to prevent TTI is to delay donors with the potential to spread viruses, such as those involved in high-risk activities (6).

Wand and Ramjee (14) observed that an appropriate HIV/STD risk reduction program may be important in shaping the sexual behavior of young adolescents before or at the beginning of their sexual lives. Risk factors that serve as the most efficient donor deferral criteria may also change over time as the HIV epidemic evolves. Transfusion centers should not rely solely on exclusion by risk factors to ensure the safety of blood supply. An important part of a multifaceted strategy to maximize blood safety includes exclusion by HIV risk factors and universal HIV antibody screening (17).

Although most criteria for (temporary) deferral only target the prevention of window phase donations, improved recording of transmission risks could reduce the risk of undetected infections among donors (13). Another important issue is when the main source of replacement donors is recruited from a patient's family or acquaintances who hide their health conditions from their relatives. Thus, the selection of donors and their proper screening are key factors to ensure safe transfusion (3).

### Study Limitations

Nevertheless, there are some limitations to this study. As the study subjects involved only blood donors in a teaching hospital in Kelantan, the results may not be reflective toward the whole population of Malaysia. Furthermore, the ethnic population in this study were mostly Malay, is mainly reflected the general population

of Northeastern Malaysia whereas, other part of Malaysia, there are more indigenous ethnic groups such as Chinese, Indian, Kadazan, Dusun, Iban etc.

### CONCLUSION

The lower seroprevalence of TTI among our blood donors as compared to previous studies carried out in other regions worldwide could be due to stringent pre-donation selection, increased donor understanding regarding self-deferral policy, improved donors' understanding on safe blood donation, and recruitment of voluntary non-remunerated donors. Application of standard operating procedures, with updated equipment, as well as planning for the use of molecular methods are necessary for the Malaysia Blood Transfusion Organization to monitor blood-transmitted infections.

Health education to the community could strengthen their awareness about the mode of transmission and prevention of HIV infection. Conducting further community-based studies to identify societal risk factors of HIV infection could interrupt the transmission and be valuable in recruiting potential volunteer non-remunerated blood donors. Public awareness about blood-borne diseases should be increased, especially in citizens with a low level of education, and free experiments should be set up for patients with low-income levels.

The results and data generated would be useful in updating the policy regulation on donor testing in our center. We also advocate that a national surveillance program against TTI be established through the NBTS.

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### Ethics

**Ethics Committee Approval:** Ethical clearance was obtained from the Universiti Sains Malaysia Ethical Committee (USM RUI grant; 1001/PPSP/812187).

**Informed Consent:** Verbal consent was obtained from the Blood Bank administration before the start of data collection.

### Authorship Contributions

Surgical and Medical Practices: M.A.Z., N.H.M.N., M.R., M.N.H., Z.Z., S.I., R.B., S.M.Y., W.S.W.A.R., M.A., Concept: M.A.Z., N.H.M.N., M.R., M.N.H., Z.Z., S.I., R.B., S.M.Y., W.S.W.A.R., M.A., Design: M.A.Z., N.H.M.N., M.R., M.N.H., Z.Z., S.I., R.B., S.M.Y., W.S.W.A.R., M.A., Data Collection or Processing: M.A.Z., N.H.M.N., M.R., M.N.H., Z.Z., S.I., R.B., S.M.Y., W.S.W.A.R., M.A., Analysis or Interpretation: M.A.Z., N.H.M.N., M.R., M.N.H., Z.Z., S.I., R.B., S.M.Y., W.S.W.A.R., M.A., Literature Search: M.A.Z., N.H.M.N., M.R., M.N.H., Z.Z., S.I., R.B., S.M.Y., W.S.W.A.R., M.A., Writing: M.A.Z., N.H.M.N., M.R., M.N.H., Z.Z., S.I., R.B., S.M.Y., W.S.W.A.R., M.A.

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## REFERENCES

1. Mohsenizadeh M, Mollaei HR, Ghaziizadeh M. Seroepidemiological Study of Hepatitis B, C and HIV among Blood Donors in Kerman. *Asian Pac J Cancer Prev* 2017; 18: 3267-72.
2. Qiao YC, Xu Y, Jiang DX, Wang X, Wang F, Yang J, et al. Epidemiological analyses of regional and age differences of HIV/AIDS prevalence in China, 2004-2016. *Int J Infect Dis* 2019; 81: 210-20.
3. Attaullah S, Khan S, Khan J. Trend of transfusion transmitted infections frequency in blood donors: provide a road map for its prevention and control. *J Transl Med* 2012; 10: 20.
4. Arshad A, Borhany M, Anwar N, Naseer I, Ansari R, Boota S. Prevalence of transfusion transmissible infections in blood donors of Pakistan. *BMC Hematol* 2016; 16: 27.
5. Singh PJ, Bhatt H. Is there a need to phase out replacement blood donors by voluntary blood donors in hospital based blood transfusion services?. *Iraqi Journal of Hematology* 2017; 6: 43-8.
6. Nafishah A, Asiah MN, Syimah AT, Mohd Zahari TH, Yasmin A, Normi M. Rate of Seroconversion in Repeat Blood Donors at The National Blood Centre, Kuala Lumpur. *Indian J Hematol Blood Transfus* 2014; 30: 105-10.
7. Ramezani A, Amirmoezi R, Volk JE, Aghakhani A, Zarinfar N, McFarland W, et al. HCV, HBV, and HIV seroprevalence, coinfections, and related behaviors among male injection drug users in Arak, Iran. *AIDS Care* 2014; 26: 1122-6.
8. Dhingra N. *Blood Donor Counselling: Implementation Guidelines*. Switzerland: WHO press; 2014. p. 94.
9. Makroo RN, Chowdhry M, Bhatia A, Arora B, Rosamma NL. Prevalence of HIV among blood donors in a tertiary care centre of north India. *Indian J Med Res*. 2011; 134: 950-3.
10. Rich RR, Fleisher TA, Shearer WT, Schroeder HW, Frew AJ, Weyand CM. *Clinical Immunology: Principles and Practice* (Rich, Clinical Immunology). 4th edition. London; 2013.
11. Nantachit N, Robison V, Wongthanee A, Kamtorn N, Suriyanon V, Nelson KE. Temporal trends in the prevalence of HIV and other transfusion-transmissible infections among blood donors in northern Thailand, 1990 through 2001. *Transfusion* 2003; 43: 730-5.
12. Motayo BO, Faneye AO, Udo UA, Olusola BA, Ezeani I, Ogiogwa JI. Seroprevalence of transfusion transmissible infections (TTI), in first time blood donors in Abeokuta, Nigeria. *Afr Health Sci* 2015;15:19-24.
13. Preußel K, Offergeld R. Which Infectious Blood Donors Could Be Identified by the Donor History Questionnaire? - Comparison of Blood Donors Infected with HIV or HCV with Notified Cases from General Population in Germany. *Transfus Med Hemother* 2018; 45: 108-14.
14. Wand H, Ramjee G. The relationship between age of coital debut and HIV seroprevalence among women in Durban, South Africa: a cohort study. *BMJ Open* 2012; 2: e000285.
15. M'baya B, Jumbe V, Samuel V, M'bwana R, Mangani C. Seroprevalence and trends in transfusion transmissible infections among voluntary non-remunerated blood donors at the Malawi Blood Transfusion Service-a time trend study. *Malawi Med J* 2019; 31: 118-25.
16. Luksamijarulkul P, Thammata N, Tiloklurs M. Seroprevalence of hepatitis B, hepatitis C and human immunodeficiency virus among blood donors, Phitsanulok Regional Blood Center, Thailand. *Southeast Asian J Trop Med Public Health* 2002; 33: 272-9.
17. McFarland W, Mvere D, Shamu R, Katzenstein D. Risk factors for HIV seropositivity among first-time blood donors in Zimbabwe. *Transfusion* 2012; 38.
18. Andrade Neto JL, Pintarelli VL, Felchner PC, de Moraes RL, Nishimoto FL. HIV prevalence among blood donors in a blood bank in Curitiba (Brazil). *Braz J Infect Dis* 2002; 6: 15-21.
19. Zeng P, Liu J, Zhang C, Zhang B, Liu W, Huang M, et al. Current Risk Factors for HIV Infection Among Blood Donors in Seven Chinese Regions. *Transfusion* 2020; 60: 326-33.

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## Comparison of Harmonic Scalpel and Electrocautery During Breast Surgery: A Cohort Study

### Meme Cerrahisinde Harmonik Neşter ve Elektrokoterin Karşılaştırılması: Bir Kohort Çalışması

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#### ABSTRACT

**Objective:** As one of the most frequent procedures, breast surgery incurs high healthcare costs. The significance of surgical quality and complications before and after breast surgery entailed the use of methods with the highest outcomes and the least complications.

**Methods:** Throughout this research, postoperative complications and factors affecting the quality of surgery were evaluated in the Kowsar Hospital between 2019 and 2020 for patients requiring breast surgery. Patients undergoing breast surgery were randomly categorized into two groups: electrocautery (A) and harmonic scalpel (B).

**Results:** Of the 56 patients, 28 were in group A, and the remaining 28 were in group B. Intraoperative bleeding, drainage rate, period of drainage, and duration of hospitalization in group B were significantly lower ( $p<0.05$ ). Furthermore, the extent of pain in both groups was different ( $p<0.001$ ), so that there was less postoperative pain in women who experienced harmonic breast surgery. Patients throughout group A reported more complications than in group B, such that 7 (25%) patients underwent limb anesthesia and 3 (10.7%) patients experienced seroma, suggesting a higher risk of complications in group A; however, the difference between the two groups was not significant in terms of limb anesthesia ( $p=0.069$ ) and serum ( $p=0.075$ ).

**Conclusion:** Harmonic scalpel usage in breast surgery has fewer complications, better operation quality, and shorter hospital stay than other older methods; therefore, the use of harmonic scalpels may substitute for different surgical strategies.

**Keywords:** Breast mass, breast surgery, electrocautery, harmonic, complications

#### Öz

**Amaç:** En sık yapılan işlemlerden biri olan meme cerrahisi yüksek sağlık harcamalarına neden olur. Meme cerrahisi öncesi ve sonrası cerrahi kalitenin ve komplikasyonların önemi, sonuçları en yüksek ve komplikasyonları en az olan yöntemlerin kullanılmasını gerektiriyordu.

**Yöntemler:** Bu araştırma boyunca Kowsar Hastanesi'nde 2019-2020 yılları arasında meme ameliyatı gerektiren hastalarda ameliyat sonrası komplikasyonlar ve ameliyat kalitesini etkileyen faktörler değerlendirildi. Meme ameliyatı geçiren hastalar rastgele iki gruba ayrıldı: elektrokoter (A) ve harmonik neşter (B).

**Bulgular:** Elli altı hastanın 28'i A grubunda, geri kalan 28'i ise B grubundaydı. Grup B'de intraoperatif kanama, drenaj oranı, drenaj süresi ve hastanede kalış süresi anlamlı olarak daha düşüktü ( $p<0,05$ ). Ayrıca her iki grupta ağrı şiddeti farklıydı ( $p<0,001$ ), yani harmonik meme ameliyatı geçiren kadınlarda ameliyat sonrası ağrı daha azdı. Grup A'daki hastalar, grup B'ye göre daha fazla komplikasyon bildirdi; 7 (%25) hastaya ekstremitte anestezisi uygulandı ve 3 (%10,7) hastada seroma görüldü; bu, grup A'da komplikasyon riskinin daha yüksek olduğunu düşündürmektedir; ancak ekstremitte anestezisi ( $p=0,069$ ) ve serum ( $p=0,075$ ) açısından iki grup arasındaki fark anlamlı değildi.

**Sonuç:** Meme cerrahisinde harmonik neşter kullanımı diğer eski yöntemlere göre daha az komplikasyona, daha iyi operasyon kalitesine ve daha kısa hastanede kalış süresine sahiptir; bu nedenle harmonik neşterlerin kullanımı farklı cerrahi stratejilerin yerini alabilir.

**Anahtar Sözcükler:** Meme kitlesi, meme cerrahisi, elektrokoter, harmonik, komplikasyonlar

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## INTRODUCTION

Breast surgery is one of the most influential and popular procedures conducted today for several reasons, including remarkably benign and malignant masses and cosmetics. Breast masses are one of the most common and severe ailments in females. Breast cancer is less frequent than at the age of 30 years. In European and American nations, breast cancer is primarily found in women over the age of 50. According to research performed in Iran, the percentage of patients aged between 40 and 49 years is greater than that of other age categories. The rate of younger patients is still greater than that in Western nations (1). The standard of operation and problems that arose before and during breast surgery (bleeding, seroma, anesthesia, etc.) demanded the use of approaches for the optimum outcomes and the least complications (2). Because these patients have a lot of emotional strain, this doubles the sensitivity of work.

Consequently, if optimal outcomes are obtained throughout breast surgery, it will be better and more satisfying for our patients and will be effective in reducing patients' pain. Harmonic is a 55.5 kHz vibrating instrument that creates three synergistic results, comprising cavitation, coagulation, and cutting, to promote substantial homeostasis and tissue dissection at a defined stage, all of which minimize heat distribution to neighboring tissues (3). This instrument has been licensed by the US Food and Drug Administration for vessels with occlusion more significant than 5 mm in diameter (4-7). No systematic research and consensus were rendered on the advantages and disadvantages of breast surgery instruments. We also used a harmonic scalpel in breast surgery in this analysis and compared the effects of pre- and postoperative procedures employing electrocautery.

## MATERIALS AND METHODS

In Kowsar Hospital, Sanandaj (Iran), from 2019 to 2020, this prospective cohort analysis was conducted. Postoperative complications and variables influencing breast surgery quality were examined among patients.

The statistical population includes patients who underwent breast surgery at Kowsar Hospital. The exclusion criteria included patients with a background of radiotherapy and concurrent blood disorders (coagulation disturbances) or some other contraindication with breast surgery. In this analysis, patients were randomly classified into two categories, A (electrocautery) and B (harmonic), as standard surgical procedures. The patients' surgery was performed by one surgeon. Side effects and surgical performance were checked and followed up before and after surgery and after patients' discharge within 1 and 3 months (by telephone and examination at the clinic). The surgery length and volume of intraoperative bleeding were then compared based on the formulated checklist. Postoperative pain (VAS approach) dependent on ratings of 0 (no pain) to 10 (highest pain), duration of hospitalization, drainage, hematoma, infection, necrosis, seroma, and organ anesthesia were compared in both groups. The machines employed in this investigation were electrocautery (Avanteb smart-4, 2019, CF, I) and harmonic (Ethicon Endo-Surgery, 2019).

The approval of the Ethics Committee was obtained from the Kurdistan University of Medical Sciences for this study (approval number: IR.MUK.REC.1398.281, date: 04.02.2020).

## Statistical Analysis

Eventually, the collected data were evaluated using SPSS software version 22. An independent t-test was also used to compare the mean values, and Pearson's chi-squared test was used to compare the qualitative variables.

## RESULTS

A total of 56 patients were examined in this review, of which 28 were in the electrocautery group and 28 were in the harmonic group. The total patient age of 40.42 and 41.17 years was not statistically significant for both the electrocautery (A) and harmonics (B) groups. Breast size, method of procedures, position of the mass, and pathology were also matched. In group A, intraoperative bleeding was more, whereas in group B, the duration of surgery, extent of drainage during the initial 24 h, overall drainage, persistence of drainage, and period of hospitalization and pain were significantly lower than those in group A ( $p < 0.05$ ). The results of this study in the two groups of electrocautery and harmonic are shown in Table 1.

## DISCUSSION

Various studies have been conducted on oncology procedures and their complications with different surgical techniques (electrocautery and harmonics), but limited literature is accessible on breast surgery. Despite these variations, the advantages of employing harmonics are somewhat unclear. In the research of Hung et al. (3), the implementation of a harmonic scalpel during breast and axillary surgery was contrasted with the use of a harmonic scalpel combined with other surgical techniques such as electrocautery and scalpel in patients undergoing surgery. Following the current research, a study demonstrated that harmonic scalpel usage shortened the operation period and decreased the incidence of postoperative anesthesia throughout the axillary region (3). The use of harmonic scalpel and electrocautery procedures in radical mastectomy cases was studied in a clinical trial conducted by Ribeiro et al. (8). Throughout this analysis, seroma, hematoma, infection, tissue necrosis, surgery time, and intraoperative bleeding were evaluated between 7 and 14 days following surgery. In this examination, 46 patients underwent electrocautery, and 49 patients were exposed to harmonic surgery. According to this analysis, in the electrocautery group, the prevalence of postoperative complications was higher than that in the harmonic group, and there was no substantial change in postoperative seroma between the two groups (8). The complications of the electrocautery group were significantly higher in our research. Generally, in some studies, there is no significant difference between the use of electrocautery and harmonic, whereas in another number of studies, there are significant differences, especially during surgery and bleeding during and after surgery and the amount of pain in patients (2,8-16). A systematic review directed by Cheng et al. (11) investigated harmonic scalpel among patients undergoing oncosurgery. Breast, lung, head and neck, and colon cancers were studied in this analysis. The findings indicated that the length of the harmonic method had a shorter operating time of 25-29 min, which was less reported in breast cancers. The bleeding and drainage volumes in the harmonic community were smaller than those in other approaches in various experiments, varying from 42 to 141 and 42 to 292 mL. The hospitalization admission rate declined between 0.2 and 3.2 days in the harmonic group.

**Table 1.** Consequences and side effects of groups A and B

	Electrocauter			Harmonic			p-value
	n	Mean	Standard deviation	n	Mean	Standard deviation	Significant in less than 0.05
Bleeding during surgery (mL)	28	131.10	75.56	28	88.92	61.25	0.026
Surgery time (min)	28	90.53	36.90	28	63.57	30.24	0.004
Drain discharge volume (mL)	28	52.14	16.06	28	33.39	11.94	0.000
Number of days of drainage	28	3.71	1.48	28	2.53	1.31	0.003
Total volume of drainage (mL)	28	170.35	95.78	28	115.35	7368	0.019
Pain (score 0 to 10)	28	6.28	1.41	28	4.10	1.22	0.000
Number of hospitalization days	28	1.50	0.57	28	1.14	0.35	0.007
		<b>Electrocauter</b>			<b>Harmonic</b>		
Limb anesthesia	Yes	7 (25%)		Yes	2 (7.1%)		0.069
	No	21 (75%)		No	26 (92.9%)		
Infection at the operation site	Yes	0		Yes	0		-
	No	28 (100%)		No	28 (100%)		
Seroma	Yes	3 (10.7%)		Yes	0		0.075
	No	25 (89.3%)		No	28 (100%)		
Surgical scar	Low	9 (32.1%)		Low	12 (42.9%)		-
	Medium	10 (35.8%)		Medium	9 (32.1%)		
	High	9 (32.1%)		High	7(25%)		

The use of harmonic scalpels has, in general, been shown to minimize complications and optimize patient outcomes (11). The advantages of using harmonics in different experiments were explored in a systematic review of harmonic scalpels conducted by McCarus and Parnell (14). This analysis indicates a relatively lower risk of postoperative pain, intra- and postoperative bleeding, seroma, and hospitalization and a higher return to function. It seems that the older studies have different results concerning the use of older devices than the newer studies in this field, so that in our study, which was using the latest harmonic device, had better results. Overall, our research indicates that intra- and postoperative bleeding, postoperative pain, surgical period, duration of drainage, and hospitalization are significantly different among groups A and B ( $p < 0.05$ ). In group B, both seroma and anesthesia were lower than in group A, which was compatible with and validated by recent research (2,8-11,13-20).

### Study Limitations

The high expense of the instruments and the use of the devices, and restrictions on the use of harmonic devices due to the high cost of maintenance and repair.

### CONCLUSION

Using the harmonic method in breast surgery seems secure, reduces intra- and postoperative complications for patients, and significantly reduces postoperative pain. Consequently, it is advised that the harmonic approach should be utilized in breast surgery rather than conventional methods. Additional research using a broader sample can validate this analysis.

### Ethics

**Ethics Committee Approval:** The approval of the Ethics Committee was obtained from the Kurdistan University of Medical Sciences for this study (approval number: IR.MUK.REC.1398.281, date: 04.02.2020).

**Informed Consent:** It wasn't obtained.

### Authorship Contributions

Concept: H.M., M.Y.F., Design: H.M., M.Y.F., Data Collection or Processing: H.M., M.Y.F., Analysis or Interpretation: H.M., M.Y.F., Literature Search: H.M., M.Y.F., Writing: H.M., M.Y.F.,

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### REFERENCES

- Anborgh PH, Caria LB, Chambers AF, Tuck AB, Stitt LW, Brackstone M. Role of plasma osteopontin as a biomarker in locally advanced breast cancer. *Am J Transl Res* 2018; 7: 723.
- Selvendran S, Cheluvappa R, Trương VK, Yarrow S, Pang TC, Segara D, et al. Efficacy of harmonic focus scalpel in seroma prevention after axillary clearance. *Int J Surg Open* 2016; 30: 116-20.
- Hung SH, Chu D, Chen FM, Chen T, Chen RC. Evaluation of the harmonic scalpel in breast conserving and axillary staging surgery. *J Chin Med Assoc* 2012; 75: 519-23.
- Ecker T, Carvalho AL, Choe JH, Walosek G, Preuss KJ. Hemostasis in thyroid surgery: harmonic scalpel versus other techniques—a meta-analysis. *Otolaryngol Head Neck Surg* 2010; 143: 17-25.
- Jackson LL, Gourin CG, Thomas DS, Porubsky ES, Klippert FN, Terris DJ. Use of the harmonic scalpel in superficial and total

- parotidectomy for benign and malignant disease. *Laryngoscope* 2005; 115: 1070-3.
6. Kontos M, Kothari A, Hamed H. Effect of harmonic scalpel on seroma formation following surgery for breast cancer: a prospective randomized study. *J BUON* 2008; 13: 223.
  7. Sutton P, Awad S, Perkins A, Lobo D. Comparison of lateral thermal spread using monopolar and bipolar diathermy, the Harmonic Scalpel™ and the Ligasure™. *Br J Surg* 2010; 97: 428-33.
  8. Ribeiro GHFP, Kerr LM, Haikel RL, Peres SV, Matthes AGZ, Michelli RAD, et al. Modified radical mastectomy: a pilot clinical trial comparing the use of conventional electric scalpel and harmonic scalpel. *Int J Surg* 2013; 11: 496-500.
  9. Archana A, Sureshkumar S, Vijayakumar C, Palanivel C. Comparing the harmonic scalpel with electrocautery in reducing postoperative flap necrosis and seroma formation after modified radical mastectomy in carcinoma breast patients: a double-blind prospective randomized Control Trail. *Cureus* 2018; 10: e2476.
  10. Burdette TE, Kerrigan CL, Homa KA. Harmonic scalpel versus electrocautery in breast reduction surgery: a randomized controlled trial. *Plast Reconstr Surg* 2011; 128: 243e-9e.
  11. Cheng H, Clymer JW, Sadeghirad B, Ferko NC, Cameron CG, Amaral JF. Performance of Harmonic devices in surgical oncology: an umbrella review of the evidence. *World J Surg Oncol* 2018; 16: 2.
  12. D'Hont A, Rao P, Feldmann P, Grivet L, Islam-Faridi N, Taylor P, et al. Identification and characterisation of sugarcane intergeneric hybrids, *Saccharum officinarum* x *Erianthus arundinaceus*, with molecular markers and DNA in situ hybridisation. *Theor Appl Genet* 1995; 91: 320-6.
  13. Faisal M, Fathy H, Shaban H, Abuelela ST, Marie A, Khaled I. A novel technique of harmonic tissue dissection reduces seroma formation after modified radical mastectomy compared to conventional electrocautery: a single-blind randomized controlled trial. *Patient Saf Surg* 2018; 12: 1-12.
  14. McCarus SD, Parnell LK. The Origin and Evolution of the HARMONIC® Scalpel. *Surg Technol Int* 2019; 35: 201-13.
  15. Militello G, De Marco P, Falco N, Kabhuli K, Mascolino A, Licari L, et al. Is it really useful the Harmonic scalpel in axillary dissection for locally advanced breast cancer? A case series. *G Chir* 2016; 37: 262.
  16. Mittal P, Kumar A, Kaur S, Pandove PK, Singla RL, Singh J. A comparative study of the use of harmonic scalpel versus unipolar cautery in modified radical mastectomy. *Niger J Surg* 2017; 23: 20-5.
  17. Zhang Z, Li L, Pang Y, Li Q, Guo C, Wang Y, et al. Comparison of harmonic scalpel and conventional technique in the surgery for breast cancer: A systematic review and meta-analysis. *Indian J Cancer* 2018; 55: 348.
  18. Anborgh PH, Caria LB, Chambers AF, Tuck AB, Stitt LW, Brackstone M. Role of plasma osteopontin as a biomarker in locally advanced breast cancer. *Am J Transl Res* 2015; 7: 723.
  19. Huang J, Yu Y, Wei C, Qin Q, Mo Q, Yang W. Harmonic scalpel versus electrocautery dissection in modified radical mastectomy for breast cancer: a meta-analysis. *PLoS One* 2015; 10: e0142271.
  20. Khan S, Khan S, Chawla T, Murtaza G. Harmonic scalpel versus electrocautery dissection in modified radical mastectomy: a randomized controlled trial. *Ann Surg Oncol* 2014; 21: 808-14.

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## Evaluating the Effect of Chloroform and Water Extract of *Satureja khuzistanica* on the Expression of Bcl-2 and Bax in Gastric Cancer Cell Line

*Satureja khuzistanica* Kloroform ve Su Ekstraktının Mide Kanseri Hücre Hattında Bcl-2 ve Bax Ekspresyonu Üzerine Etkisinin Değerlendirilmesi

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### ABSTRACT

**Objective:** Gastric cancer is a complex disease. Natural antioxidant sources without toxic side effects are suitable for the treatment and management of diseases such as cancer. *Satureja khuzistanica* (*S. khuzistanica*) is an endemic plant in southern Iran. Despite numerous articles published about this plant, a limited number of studies have been conducted on its anticancer effects. Therefore, this study was conducted to evaluate the cytotoxic and apoptotic effects of this plant on the gastric cancer cell line.

**Methods:** The antimicrobial activities of chloroform and water extracts were screened against pathogenic bacteria [*Helicobacter pylori* (*H. pylori*)]. The cytotoxicity effect of the extracts was evaluated using an microculture tetrazolium assay on gastric cancer cell lines (AGS and KATO III) and normal gingival fibroblasts. The expression of Bax and Bcl-2 was evaluated using real-time polymerase chain reaction. Finally, all data were analyzed using REST software.

**Results:** Both extracts showed good inhibitory activity against *H. pylori*. In addition, extracts showed cytotoxic activity against gastric cancer cell lines. Treatment of *S. khuzistanica* cells increased and decreased the expression of Bax and Bcl-2 apoptotic genes, respectively ( $p < 0.05$ ). In addition, in HGF cells, there were no cytotoxic effects or apoptosis induction triggered by the extracts.

**Conclusion:** The chloroform extract had more cytotoxic and apoptotic effects than the water extract. The extracts could have anticancer and apoptotic effects on the gastric cancer cell line.

**Keywords:** AGS and KATO-III cell line, apoptotic effect, gastric cancer, *Satureja khuzistanica*

### ÖZ

**Amaç:** Mide kanseri karmaşık bir hastalıktır. Toksik yan etkileri olmayan doğal antioksidan kaynakları kanser gibi hastalıkların tedavisi ve yönetimi için uygundur. *Satureja khuzistanica* (*S. khuzistanica*), İran'ın güneyindeki endemik bir bitkidir. Bu bitki hakkında çok sayıda makale yayınlanmış olmasına rağmen antikanser etkileri konusunda sınırlı sayıda çalışma yapılmıştır. Bu nedenle bu çalışma, bu bitkinin mide kanseri hücre hattı üzerindeki sitotoksik ve apoptotik etkilerini değerlendirmek amacıyla yapılmıştır.

**Yöntemler:** Kloroform ve su ekstraktlarının antimikrobiyal aktiviteleri patojen bakterilere [*Helicobacter pylori* (*H. pylori*)] karşı tarandı. Ekstraktların sitotoksikite etkisi, mide kanseri hücre dizileri (AGS ve KATO III) ve normal diş eti fibroblastları üzerinde bir mikrokültür tetrazolyum tahlili kullanılarak değerlendirildi. Bax ve Bcl-2'nin ifadesi, gerçek zamanlı polimeraz zincir reaksiyonu kullanılarak değerlendirildi. Son olarak tüm veriler REST yazılımı kullanılarak analiz edildi.

**Bulgular:** Her iki ekstrakt da *H. pylori*'ye karşı iyi bir inhibitör aktivite gösterdi. Ek olarak ekstraktlar mide kanseri hücre dizilerine karşı sitotoksik aktivite gösterdi. *S. khuzistanica* hücrelerinin tedavisi sırasıyla Bax ve Bcl-2 apoptotik genlerinin ekspresyonunu artırdı ve azalttı ( $p < 0.05$ ). Ayrıca HGF hücrelerinde ekstraktların tetiklediği herhangi bir sitotoksik etki veya apoptoz induksiyonu görülmemiştir.

**Sonuç:** Kloroform ekstraktının su ekstraktına göre daha fazla sitotoksik ve apoptotik etkiye sahip olduğu görülmüştür. Ekstraktların mide kanseri hücre dizisi üzerinde antikanser ve apoptotik etkileri olabilir.

**Anahtar Sözcükler:** AGS ve KATO-III hücre dizisi, apoptotik etki, mide kanseri, *Satureja khuzistanica*

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## INTRODUCTION

Japan ranks first in terms of the incidence of gastric cancer within the world and South Korea, Costa Rica, the former Soviet republics, and Japan rank first to fourth in the world in terms of mortality caused by gastric cancer. Its highest prevalence has been observed in the Far East. In Iran, cancer is the third most common cause of death, and more than 30,000 people die each year due to this disease (1,2). Gastric cancer is a common disease in Azerbaijan and Iran. Ardebil has the highest incidence of gastric cancer in Iran after Gilan province. Genetic factors, environmental factors, and food habits are among the causes of gastric cancer in these provinces. Gastric cancer can be divided into completely separate pathologic subtypes with different epidemiological and prognostic features (3). The spread of gastric cancer is a complex and multistage process. It involves genetic and epigenetic changes in cancer-producing genes, tumor suppressor genes, DNA-modifying genes, cell cycle regulators, and signaling molecules. Gastric cancer may be caused by genomic instability, which can be in the form of microsatellite instability or chromosomal instability (4,5).

The main cause of most cancers is the overexpression of anti-apoptotic proteins, such as Bcl-2, and lack of apoptosis. Increasing the expression of these proteins increases resistance to chemotherapy. Thus, inhibiting the expression or function of these proteins in cancer cells can induce apoptosis. The *Bax* and *Bcl-2* genes, other apoptotic genes, and programmed cell deaths are involved in the incidence and development of gastric cancer (6). Chemotherapy is used to control many cancer diseases. However, side effects (e.g., neurological damage and kidney failure) of chemotherapy and the drugs used are sometimes more hazardous than cancer itself. Thus, to reduce these complications and given its resistance to chemotherapy, modern methods are needed to prevent and increase the chance of treating cancer patients (7). *Satureja khuzistanica* (*S. khuzistanica*) is a plant with two main effective ingredients, namely thymol and carvacrol, which have anticancer, antioxidant, and antimicrobial effects (8-11). A study on the percentage and ingredients of essential oil of *S. khuzistanica* showed that the mean essential oil content of *S. khuzistanica* was 18.3%. Additionally, 43 compounds were identified in the essential oil of *S. khuzistanica*, which contains 90% of the total carvacrol (11). The objective of this study was to evaluate the antimicrobial, cytotoxic, and anticancer effects of chloroform and water extracts of *S. khuzistanica* on gastric cancer.

## MATERIALS AND METHODS

*S. khuzistanica* was collected from the mountains around Khuzestan from June to August. The plant was identified and sent by the botanists of Khoraman Pharmaceutical Plant Company (Dr. Reza Shahsavari). Approximately 120 g of *S. khuzistanica* powder was mixed with 1 L of chloroform and water. After 2 weeks, extraction was performed by distillation using a rotary evaporator.

### Antimicrobial Activity

The antimicrobial activities of chloroform and water extracts were screened against pathogenic bacteria [*Helicobacter pylori* (*H. pylori*)]. Concentrations of chloroform and water extract of *S. khuzistanica* (25 and 50 mg/mL) were tested against *H. pylori* using the disc diffusion method. About 50  $\mu$ L of each concentration

of the extracts was added to each sterile blank disc (Padtan Teb, Iran). From the fresh culture of *H. pylori*, 1 McFarland solution in brucella broth (Himedia) was prepared, and 100  $\mu$ L of the solution was taken by sterile swab. Next, it was cultured on a brucella agar medium containing 5% defibrinated sheep blood and 7% inactivated fetal bovine serum. The dried discs of the extracts were placed on the culture at appropriate distances. After placing the discs, the media were kept in a "CO<sub>2</sub>" incubator (with 10% "CO<sub>2</sub>") at 37 °C for 72 h. Antibacterial activity was determined by measuring the zone of inhibition around the test discs. The growth inhibition diameter was the average of three different measurements. The result was expressed as the inhibition zone diameter and was compared with the standard antibiotics ampicillin (Amp, 10  $\mu$ g) and chloramphenicol (C, 30  $\mu$ g).

### Preparation of the Cell Culture Medium

In the present study, AGS and KATO III gastric cancer cell lines and gingival fibroblasts were prepared from Pasteur institute and cultured in RPMI enriched with 10% fetal calf serum, 1% Pen strep, and 5% CO<sub>2</sub> at 37 °C.

### MTT Test

Microculture tetrazolium (MTT) test is a colorimetric method performed based on the reduction and breakdown of tetrazolium yellow crystals with the chemical formula of 3-(4, 5-dimethylthiazol-2-yl) -2, 5-diphenyltetrazolium bromide (MTT). The test was conducted using succinate dehydrogenase enzyme and the formation of insoluble blue crystals. In the present study, the MTT test was performed to evaluate the effect of *S. khuzistanica* on the viability of AGS and KATO III cells and gingival fibroblasts. Accordingly, the cells cultured in a 96-well plate at concentrations of 100, 500, 250, 125, 62.5, and 31.25  $\mu$ g/mL of the plant extract were treated on normal and cancer cells over 24 h. Then, the medium was replaced with MTT (Sigma Aldrich) solution. After 4 h of incubation in the dark, the MTT solution was replaced with DMSO. The light absorption was then read at 570 nm in an ELISA device. In the next step, the percentage of live cells was calculated using the formula (%) = (ODexp/ODcon)  $\times$  100. In this formula, ODexp and ODcon are the light absorption of the exposed and control groups, respectively. All experiments were performed in triplicate.

Generally, to evaluate the gene expression changes, total cell RNA was extracted from the gastric cancer cell line using a solution (Ribospin304-15 Gene All, Korea) according to the manufacturer's instructions. Spectrophotometer and agarose gel electrophoresis were used for quantitative and qualitative analysis of the concentration and purity of the extracted RNA. In this study, cDNA was synthesized from mRNA using the Sina Colon First Starandn cDNA kit according to the manufacturer's instructions. To produce cDNAs with similar concentrations, we measured the volume of each sample so that 1000 ng of RNA was available. Eventually, the cDNA samples were produced and stored at -20 °C until the real-time polymerase chain reaction (RT-PCR).

### Primers

Each gene fragment was proliferated by selecting a pair of specific primers for each gene, including forward and reverse prime (Table 1).

RT-PCR was performed using a magnetic induction cycler (MIC) device and RealQ Plus 2x Master Mix Green. For this purpose, 12.5  $\mu$ L of cyber green mixer master, 3  $\mu$ L of the cDNA sample, 0.5  $\mu$ L of forward and reverse primers (10 pmol) in water, and 8.5  $\mu$ L of nuclease-free water were mixed in 25  $\mu$ L of the reaction mixture. The *beta-actin* ( *$\beta$ -actin*) gene was amplified as an internal control, and the fold change in the relative expression of each target mRNA was calculated using the comparative Ct ( $2^{-\Delta\Delta Ct}$ ) method.

### Real-time PCR Procedures

In this research, the heat schedule of the RT-PCR device (MIC model) was 95 °C for 15 min in the first stage, 95 °C for 10 s in the second stage, and 49-51-60 °C, respectively, for the *Bax*, *Bcl-2*, and  *$\beta$ -actin* genes C for 15 s, and 72 °C for 20 s in 40 cycles in the last stage. Finally, the data were analyzed by comparing the threshold cycle and the difference in the obtained threshold from the treated cells with the extracts and untreated cells with the target gene ratio (Table 2, 3).

### Melting Curve Analysis

Once PCR is completed, the device can plot the melting curve of each sample. Thus, by analyzing the melting curve, it is possible to identify the presence of non-specific bands and the primer dimer (Figure 1).

### Statistical Analysis

Statistical analysis of the data was performed using REST software at a significance level of <0.05.

## RESULTS

According to the following equation, the percentage of chloroform and water extracts of the *S. khuzistanica* plants is 7.5%.

$$\frac{\text{the weight of the extract obtained based on gram}}{\text{the weight of the plant used based on gram}} \times 100 = \text{percentage of the extracts obtained}$$

$$\frac{9.53}{120} \times 100 = 7.5$$

**Table 1.** The sequence of primers used along with the length of the proliferated fragment

Primer name	Primer sequence	Primer binding temperature	Base pair length
Bax-F	GGTTGTCGCCCTTTTCTA	48.84	108
Bax-R	CGGAGGAAGTCCAATGTC	49.1	108
B-actin-F	GCGAGAAGATGACCCAGAT	50.87	88
B-actin-R	GAGGCGTACAGGGATAGC	50.97	88
Bcl-2-F	GATGTGATGCCTCTGCGAAG	65	93
Bcl-2-R	CATGCTGATGTCTCTGGAATCT	64	93

**Table 2.** Contents of RT-PCR-master mix per sample for examining a gene as performed according to the time and temperature schedule of Table 3 of RT-PCR

Value based on $\mu$ L	Substance
12.5	Master mix
8.5	Distilled water
0.5	Forward primer
0.5	Reverse primer
3	cDNA
25	Total volume

RT-PCR: Real-time polymerase chain reaction.

**Table 3.** RT-PCR test schedule and temperature for genes

Gene	Step	Temperature	Time	Number of cycles
Bax & Bcl-2 & $\beta$ -actine	Initialization	95 °C	15 min	40
$\beta$ -actine	Denaturation	95 °C	10 sec.	
$\beta$ -actine	Annealing	51 °C	15 sec.	
Bax	Annealing	49 °C	15 sec.	
Bcl-2	Annealing	64 °C	15 sec.	
Bax & Bcl-2 & $\beta$ -actine	Extension	72 °C	20 sec	

RT-PCR: Real-time polymerase chain reaction.

Images taken from the growth of AGS gastric cancer cells before and after treatment with *S. khuzistanica* extract are illustrated in Figure 2.

### Antimicrobial Activity

The antimicrobial activity of the extracts was measured using the disk diffusion method. The antimicrobial activity of the extracts was found within the 25-50 mg/mL concentration range. The inhibition zones of the bacteria were in the range of  $18 \pm 0.4$  mm. According to the disk diffusion method, all the concentrations used were inhibitory for all the reference bacterial strains.

### MTT Results

The MTT assay was performed to find a minimum of 50% killing extract in  $\mu\text{g/mL}$ , examine the cytotoxic effect of the extract, and determine the  $\text{IC}_{50}$  in AGS cells. This test was performed in three replicates. The results of the MTT test at different concentrations of the extract in  $\mu\text{g/mL}$  were obtained as a linear chart. In addition, the results showed no significant cytotoxic properties in normal cells. The results for the cancer cells are presented in Figure 3.

### RNA Electrophoresis Results

In order to evaluate the quality of the extracted RNAs, three samples were electrophoresed randomly on Agarose gel. For this purpose, 1.5% gel was made and the congealed samples were loaded onto gel and electrophoresed at V100 for 2 min (Figure 4).

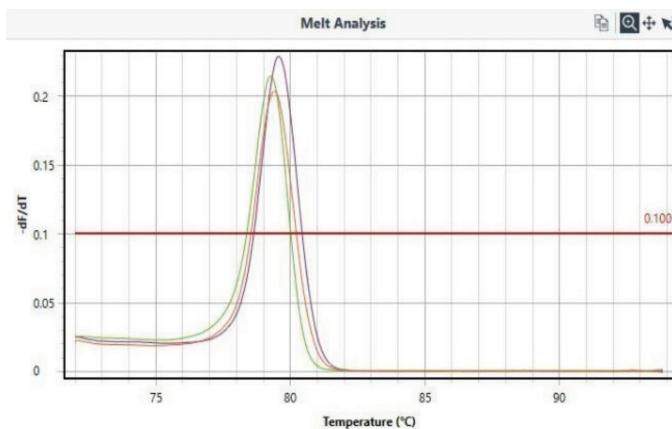


Figure 1. Melting curve for beta-actin gene.

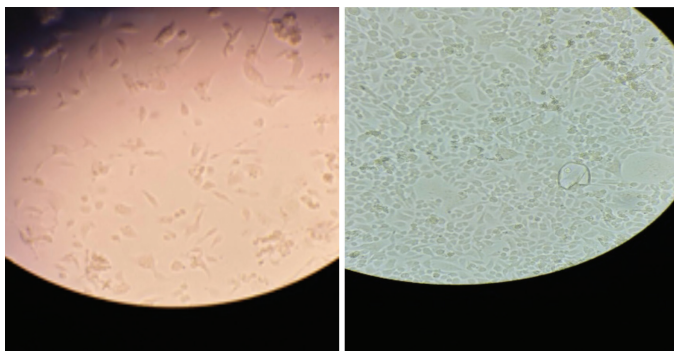


Figure 2. AGS gastric cancer cells before (A) and after (B) treatment with extract.

### Results of Real-Time PCR

After obtaining the mean, CTs were analyzed using the following formula: in addition, the results were analyzed using REST software, and the following charts were obtained.

$$\text{Ratio} = \frac{(E_{\text{target}})^{\Delta Ct_{\text{target}}(\text{control} - \text{sample})}}{(E_{\text{ref}})^{\Delta Ct_{\text{ref}}(\text{control} - \text{sample})}}$$

Results of relative expression of the *Bcl-2* gene and *Bax* relative to the  $\beta$ -actin gene.

According to the charts of  $\beta$ -actin genes as a reference and *Bcl-2* gene and *Bax* as a target, the expression of *Bax* and *Bcl-2* genes after applying *S. khuzistanica* extract increased and decreased, respectively. Therefore, after applying a dose of 125  $\mu\text{g/mL}$ , the expression rate of these genes increased and decreased by approximately 1.9 and 0.227 times, respectively, at a significance level of 0.000.

The RT-PCR results show the relative expression of *Bax* to *Bcl-2* (Figure 5).

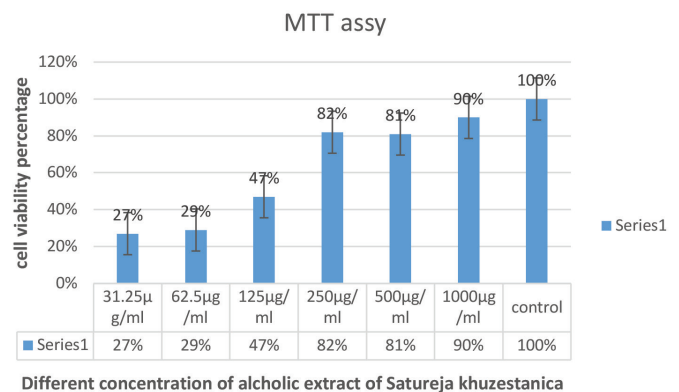


Figure 3. The result of the MTT test to determine the concentration of the extract which killed 50% of the cells.

MTT: Microculture tetrazolium.

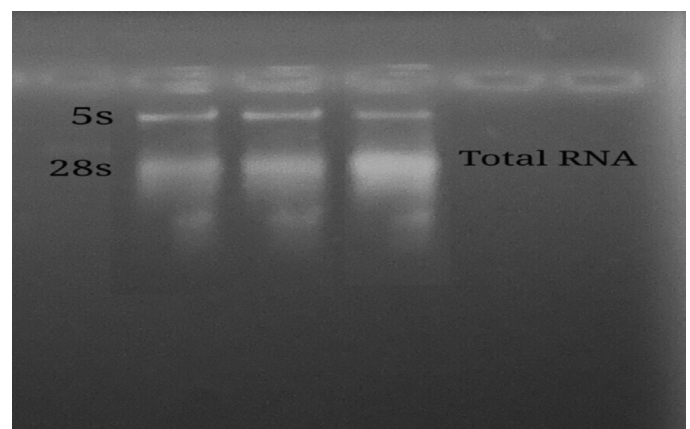
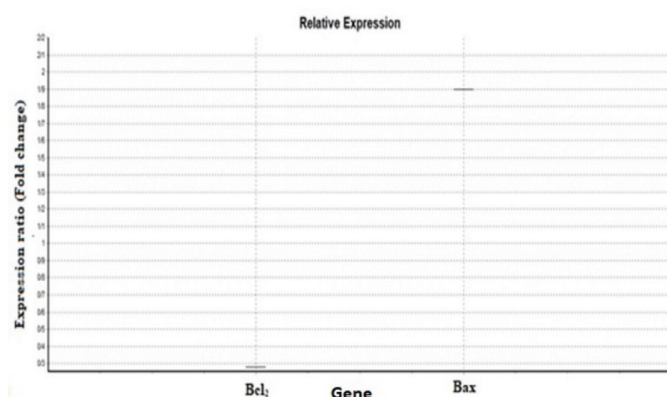


Figure 4. Gel electrophoresis of total RNA extracted from the samples; The quality of extracted RNAs was controlled by putting samples on a 2% agarose gel.



**Figure 5.** RT-PCR results of relative expression of Bax to BCL-2.

RT-PCR: Real-time polymerase chain reaction.

## DISCUSSION

Traditional medicine is effective in many diseases, and its use for treating diseases has been proven. Anxiolytic sources without toxic side effects are a good approach for the treatment and control of diseases such as cancer. Several studies have been conducted on the benefits of *S. khuzistanica* in medicine. For example, Rezvanfar et al. (12) investigated the benefits and protective effects of *S. khuzistanica* on cyclophosphamide-induced hemorrhagic cystitis in a rat model. The results showed that the *S. khuzistanica* extract protects against cyclophosphamide-induced hemorrhagic cystitis in a rat model by reducing radical-induced toxic stress (12).

In another study, Malmir et al. (13) studied new flavonoid monoterpenes in *S. khuzistanica*. The results showed that *S. khuzistanica* had a beneficial antidiabetic and antioxidant effect. In addition, Saidi (14) studied *S. khuzistanica* and its chemical and antioxidant properties and showed that this plant is a rich source of antioxidant compounds. They also examined the effects of *S. khuzistanica* on inhibiting the expression of iNOS in the macrophage cell line J774A.1. The results showed that *S. khuzistanica* had anti-inflammatory effects through its effect on iNOS gene expression (15).

Esmaili-Mahani et al. (16,17) investigated the effect of *S. khuzistanica* on the tolerance of pain induced by morphine by inhibiting the activation of glial cells. According to their results, *S. khuzistanica* can have beneficial effects in inhibiting drug tolerance and reducing glial cell activation. Sharafati-Chaleshtori et al. (18) showed that carvacrol of *S. khuzistanica* reduces the viability rate of cancer cell degeneration of the genome, and genome degradation at concentrations close to  $IC_{50}$  is more significant. Saffari studied the effect of *S. khuzistanica* on MCF-7 breast cancer cells. These authors showed that the rate of cell apoptosis increased with increasing concentration and time. Furthermore, the alcoholic extract of MCF-7 breast cancer cells showed anticancer activity (19).

Ahmadi et al. (20) evaluated the protective effect of *S. khuzistanica* essential oil on ovarian failure caused by busulfan. The results showed that busulfan significantly reduced the number and quality of oocytes, fertilization rate, embryo growth before implantation, and embryo quality. *S. khuzistanica* essential oil significantly reduced the adverse effects of busulfan. According to their results,

*S. khuzistanica* essential oil can protect the fertility of the female sex against damages caused by busulfan (20). Sazgar et al. (2017), combining the hydroalcoholic extract of celeriac and Satureja, showed that it had a toxic effect on HeLa cancer cells, whereas this extract did not have a toxic effect on normal fibroblasts. Shirali and Alizadeh (21) evaluated the antimicrobial properties of the essential oil of *S. khuzistanica*. The results showed that phenolic compounds such as carocrole, thymol, and gamma-terpinene in essential oil compounds have antimicrobial properties in this plant. Moreover, an increase in phenolic compounds of the essential oil is directly associated with an increase in antimicrobial activity, which prevents lipid oxidation and heart coronary and cancer diseases (21).

Loizzo et al. (22) investigated the anticancer effects of the essential oil of Satureja. They studied the cellular cytotoxicity of this essential oil on various cancer cells, including breast cancer. They reported that Satureja essential oils could inhibit breast cancer cells, and this ability increases with increasing concentration (22). This study showed that the treatment of cells with *S. khuzistanica* leads to an increase and reduction in the expression of apoptotic *Bax* and *Bcl-2* genes. In addition, they reported that the extract of this plant had significantly increased anti-apoptotic properties against the AGS cell line. Therefore, the expression of *Bax* and *Bcl-2* genes changes in gastric cancer cells. A significant reduction in *Bax* gene expression as a pro-apoptotic regulator of apoptosis and an increase in *Bcl-2* gene expression as an anti-apoptotic regulator demonstrate the high capacity of *S. khuzistanica* to reduce the incidence of cell apoptosis. *S. khuzistanica* significantly increases apoptosis by reducing the expression of Bcl-2 and increasing the expression of Bax (23). In addition, this study provided significant information on the correlation between busulfan and apoptosis due to oxidative stress in sperm and testicular tissue in adult rats. An imbalance between the production of ROS and the reduction of antioxidant mechanisms resulted in the toxicity induced by oxidative stress in adult male rats after treatment with busulfan. However, a 1-week treatment with *S. khuzistanica* in rats treated with busulfan-*S. khuzistanica* reduced the apoptosis induced by oxidative stress, cytotoxicity, and genotoxicity in sperm [Nasimi et al. (23), 2018]. Natural substances in *S. khuzistanica* extract can increase and decrease the relative expression of *Bax* and *Bcl-2* genes, respectively. This study showed that the expression of *Bax* and *Bcl-2* genes after application of *S. khuzistanica* extract (125  $\mu\text{g}/\text{mL}$ ) increased and decreased, respectively. These results suggest that the expression of *Bax* and *Bcl-2* genes can be changed by the application of *S. khuzistanica* extract. Data from a study show that *S. khuzistanica* has anticancer effects on MCF-7 cancer cells and shows synergistic effects in combination with vincristine (17).

In a previous study, the methanol extract of *S. khuzistanica* induced cytotoxic effects in a dose-dependent manner. In this regard, almost half of the cells were destroyed during 24 h with 125  $\mu\text{g}/\text{mL}$ , and MCF-7 was detected as the most sensitive cell line (24,25).

## CONCLUSION

In our study, the results of antimicrobial activity showed that among the extracts, chloroform extract had a more powerful antimicrobial effect than water extracts against *H. pylori* bacteria. The antimicrobial effect of thymol and carvacrol is due to the damage to membrane integrity induced by a change in pH hemostasis.

In the cytotoxic assay, chloroform and water extracts exhibited cytotoxic effects on the gastric cancer cell lines (AGS and KATO III). Chloroform extract had a more powerful cytotoxic effect than water extract on gastric cancer cell lines.

Dysregulation of the mitochondrial apoptotic pathway is one of the most important events during carcinogenesis. The Bcl-2 protein family, including anti-apoptotic (Bcl-2 and Bcl-xl) and pro-apoptotic (Bax and Bak) members, plays an essential role in regulating this pathway. According to our results, the Bax/Bcl-2 ratio was significantly ( $p < 0.05$ ) correlated with the gastric cancer cell line.

This is the first report demonstrating the potential pro-apoptotic effects of *S. khuzistanica* extracts on gastric cancer cell lines (AGS and KATO III). However, further investigations of the extracts in animal models with gastric cancer are needed for additional understanding of their *in vivo* activity.

**Acknowledgement:** We thereby appreciate the Khoraman Company for their cooperation in providing the *S. khuzistanica* plant.

#### Ethics

**Ethics Committee Approval:** This study was approved by the Bonab Islamic Azad University (approval number: 1394.625).

**Informed Consent:** It wasn't obtained.

#### Author Contributions

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

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

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## REFERENCES

- Kolahdozan S, Sadjadi A, Radmard AR, Khademi H. Five common cancers in Iran. Arch Iran Med 2010; 13: 143-6.
- Almasi Z, Rafiemanesh H, Salehinyi H. Epidemiology characteristics and trends of incidence and morphology of stomach cancer in Iran. Asian Pac J Cancer Prev 2015; 16: 2757-61.
- Rugge M, Fassan M, Graham, DY. Epidemiology of gastric cancer. Gastric Cancer Springer. 2015; p. 23-34.
- Huntsman DG, Ladanyi M. The molecular pathology of cancer: from pan-genomics to post-genomics. J Pathol 2018; 244: 509-11.
- Harris TJ, McCormick F. The molecular pathology of cancer. Nat Rev Clin Oncol 2010; 7: 251-65.
- Hu B, El Hajj N, Sittler S, Lammert N, Barnes R, Meloni-Ehrig A. Gastric cancer: classification, histology and application of molecular pathology. J Gastrointest Oncol 2012; 3: 251-61.
- Ahanjan M, Ghaffari J, Mohammadpour G, Nasrolahie M, Haghshenas MR, Mirabi AM. Antibacterial activity of satureja bakhtiarica bung essential oil against some human pathogenic bacteria. African Journal of Microbiology Research, 2011; 5: 4764-8.
- Abdollahi A, Hassani A, Ghosta Y, Bernousi I, Meshkatsadat MH, Shabani R, et al. Evaluation of essential oils for maintaining postharvest quality of thompson seedless table grape. Nat Prod Res 2012; 26: 77-83.
- Adiguzel A, Ozer H, Kilic H, Cetin B. Screening of antimicrobial activity of essential oil and methanol extract of satureja hortensis on foodborne bacteria and fungi. Czech J Food Sci 2007; 25: 81-9.
- Alizadeh A, Khoshkhui M, Javidnia K, Firuzi O, Tafazoli E, Khalighi A. Effects of fertilizer on yield, essential oil composition, total phenolic content and antioxidant activity in satureja hortensis l.(lamiaceae) cultivated in iran. Journal of Medicinal Plants Research, 2010; 4: 33-40.
- Sharifi-Rad J, Sharifi-Rad M, Hoseini-Alfatemi SM, Iriti M, Sharifi-Rad M, Sharifi-Rad M. Composition, cytotoxic and antimicrobial activities of satureja intermedia ca mey essential oil. Int J Mol Sci 2015; 16: 17812-25.
- Rezvanfar MA, Farshid AA, Sadrkhanlou RA, Ahmadi A, Rezvanfar MA, Salehnia A, et al. Benefit of Satureja khuzestanica in subchronically rat model of cyclophosphamide-induced hemorrhagic cystitis. Exp Toxicol Pathol 2010; 62: 323-30.
- Malmir M, Gohari AR, Saeidnia S, Silva O. A new bioactive monoterpene-flavonoid from satureja khuzistanica. Fitoterapia 2015; 105: 107-12.
- Saidi M. Antioxidant activities and chemical composition of essential oils from Satureja khuzestanica, oliveria decumbens and thymus daenensis. Journal of Essential Oil Bearing Plants 2014; 17: 513-21.
- Jalalvand M, Shahsavari R, Sheikhan A, Mosayebi G. Satureja khuzestanica essential oil (SKEO) inhibits iNOS gene expression in lipopolysaccharide-stimulated J774A.1 macrophage cell line. Life Science Journal, 2013; 10: 230-5.
- Esmaeili-Mahani S, Ebrahimi B, Abbasnejad M, Rasoulia B, Sheibani V. 2015. Satureja khuzestanica prevents the development of morphine analgesic tolerance through suppression of spinal glial cell activation in rats. J Nat Med 2015; 69: 165-70.
- Esmaeili-Mahani S, Samandari-Bahraseman MR, Yaghoobi MM. 2018. In-Vitro Anti-Proliferative and Pro-Apoptotic Properties of Satureja Khuzestanica on Human Breast Cancer Cell Line (MCF-7) and Its Synergic Effects with Anticancer Drug Vincristine. Iran J Pharm Res 2018; 17: 343-52.
- Sharafati-Chaleshtori R, Rokni N, Rafieian-Kopaei M, Drees F, Salehi E. Antioxidant and antibacterial activity of basil (ocimum basilicum l.) Essential oil in beef burger. J Agr Sci Tech 2015; 17: 817-26.
- Saffari-Chaleshtori J, Nikoukar M, Heidarian E, Keloushadi M, Ghasemi-Dehkordi P, Gholami M. The effect of carvacrol on the growth inhibition and genomic destruction in prostatic cancer cells using comet assay technique. Journal of Shahrekord University of Medical Sciences 2015; 17: 57.
- Ahmadi A, Bamohabat Chafjiri S, Sadrkhanlou RA. Effect of Satureja khuzestanica essential oil against fertility disorders induced by busulfan in female mice. Vet Res Forum 2017; 8: 281-6.
- Shirali RB, Alizadeh R. Ingredient compounds, phenolic compounds, antioxidant and antimicrobial properties of essential oils of Satureja khuzestanica bunge in southern iran. 2015.
- Loizzo MR, Saab AM, Tundis R, Statti GA, Menichini F, Lampronti I. Phytochemical analysis and in vitro antiviral activities of the essential oils of seven Lebanon species. Chem Biodivers 2008; 5: 461-70.
- Nasimi P, Tabandeh MR, Roohi S. Busulfan-mediated oxidative stress and genotoxicity decrease in sperm of Satureja Khuzestanica essential oil-administered mice. Syst Biol Reprod Med 2018; 64: 348-57.
- Soltanzadeh H, Acik L, Türk M, Houshmand M, Shahsavari G. Antimicrobial, antioxidant, cytotoxic and apoptotic activities of Satureja khuzestanica GMJ 2018; 29: 264-70.
- Panahzadeh M, Soltanzade H, Tanomand A, Shahsavari G, The layered double hydroxide (LDH) nanosheets decrease anticancer potential of Satureja khuzestanica in HepG2 hepatocellular carcinoma cells. Gene Reports 2021; 25: 101325.

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## Development of the COVID-19 Stigma Scale: Validity and Reliability Study

### COVID-19 Damgalama Ölçeğinin Geliştirilmesi: Geçerlilik ve Güvenilirlik Çalışması

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#### ABSTRACT

**Objective:** Stigmatization leads to severe consequences both individually and socially. This situation can also occur in epidemics and complicate the treatment process. Therefore, the objective evaluation of stigma and the creation of an action plan on this issue are the most basic steps in the fight against the epidemic. This study aimed to develop and study the reliability and validity of a scale that evaluates stigma against individuals who experienced coronavirus disease-2019 (COVID-19).

**Methods:** A 24-item draft scale was prepared by considering the stigma experienced by individuals who experienced COVID-19 both in their close relations and social environment. The study was conducted on the relatives of patients referred to Kocaeli University Faculty of Medicine Hospital's different polyclinics. In addition to the developed COVID-19 Stigma Scale (CSS), the COVID-19 Information Questionnaire (CIQ) was used.

**Results:** The sample of the study consisted of 323 healthy individuals who were 54.5% male, with a mean age of 39.0±13.7 years. In the factor analysis, which included 24 items, five items with a factor load below 0.40 were eliminated. The final version of the scale consisted of three factors, and these factors (rejection, discrimination, and emotional reactions) explained 61% of the total variance. Cronbach's alpha coefficient for the total CSS was calculated as 0.91. A significant negative correlation was found between the CSS total and CIQ total scores ( $r=-0.301$ ,  $p<0.001$ ).

**Conclusion:** The CSS is valid and reliable in assessing the stigmatization of individuals in the healthy population toward COVID-19 patients.

**Keywords:** COVID-19, social stigma, scale, validity, reliability

#### ÖZ

**Amaç:** Damgalama hem bireysel hem de toplumsal olarak ciddi sonuçlara yol açmaktadır. Bu durum salgınlarda da ortaya çıkabilir ve tedavi sürecini zorlaştırabilir. Bu nedenle damgalanmanın objektif olarak değerlendirilmesi ve bu konuda bir eylem planının oluşturulması salgınla mücadelede en temel adımlardır. Bu çalışma, koronavirüs hastalığı-2019 (COVID-19) geçirmiş bireylere yönelik damgalamayı değerlendiren bir ölçeğin geliştirilmesini, güvenilirliğini ve geçerliliğini incelemeyi amaçlamaktadır.

**Yöntemler:** COVID-19 geçirmiş bireylerin hem yakın ilişkilerinde hem de sosyal çevrelerinde yaşadıkları damgalanma dikkate alınarak 24 maddelik taslak ölçek hazırlandı. Araştırma Kocaeli Üniversitesi Tıp Fakültesi Hastanesi'nin farklı polikliniklerine başvuran hastaların yakınlarında yapıldı. Geliştirilen COVID-19 Damgalama Ölçeğine (CDÖ) ek olarak COVID-19 Bilgi Anketi (CBA) uygulandı.

**Bulgular:** Araştırmanın örneklemini yaş ortalaması 39,0±13,7 yıl olan %54,5'i erkek 323 sağlıklı birey oluşturdu. Yirmi dört maddenin yer aldığı faktör analizinde faktör yükü 0,40'ın altında olan beş madde elendi. Ölçeğin son halinin üç faktörden oluştuğu ve bu faktörlerin (reddetme, ayrımcılık ve duygusal tepkiler) toplam varyansın %61'ini açıkladığı belirlendi. Toplam CDÖ için Cronbach alfa katsayısı 0,91 olarak hesaplandı. CDÖ toplam ve CBA toplam puanları arasında anlamlı bir negatif bağıntı bulundu ( $r=-0,301$ ,  $p<0,001$ ).

**Sonuç:** CDÖ, sağlıklı nüfustaki bireylerin COVID-19 hastalarına yönelik damgalanmalarını değerlendirmede geçerli ve güvenilirirdir.

**Anahtar Sözcükler:** COVID-19, toplumsal damgalama, ölçek, geçerlik, güvenilirlik

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## INTRODUCTION

According to the World Health Organization, social stigmatization means when individuals or groups in the immediate environment of an individual are labeled, discriminated against, or lose their status because of conditions related to a disease (1). Individuals can be subject to stigmatization for being close to an individual with a disease even when they are not sick themselves. Family members who undertake the care of the patient and healthcare employees who maintain the treatment are the groups that are subject to stigmatization (2). Stigmatization leads to severe consequences both individually and socially. It might cause stigmatized individuals to avoid treatment and thus increase mortality and morbidity rates (3,4). Additionally, it is well known that stigmatization is correlated with higher depression rates and lower self-regard in these individuals (5). Stigmatization is widespread during epidemics and that used to manifest itself for tuberculosis (TBC), human immunodeficiency virus (HIV), severe acute respiratory syndrome (SARS), and swine flu is present today for coronavirus disease-2019 (COVID-19) (5,6). Stigmatization of COVID-19 poses a severe threat to healthcare employees, patients, elders, and patients' relatives (7). physical and verbal violence toward healthcare employees who play a key role in the fight against epidemics increased during the COVID-19 pandemic (6). This makes it even harder for healthcare employees to continue with their duties under already challenging working conditions.

In addition to the direct negative effects of the epidemic on physical health, there are many indirect negative social effects. Stigma creates divisions in the mind, leading to discrimination. In this case, a loss of status may occur according to the social, economic, and political power of the stigmatized group (8). It seems that there are two types of stigma in the stigma studies conducted on people who have had COVID-19. While the first is self-stigmatization, the second is society's stigmatization of people with COVID-19 (9). People with COVID-19 may feel differentiated in their physical or mental abilities. This differentiation can cause social isolation and some psychiatric disorders (10). The second type of stigma, social stigma, can increase the self-stigma of people with COVID-19. As a result, existing mental problems may intensify and the functionality of individuals may deteriorate (11).

The psychometric assessment tools used in studies on stigmatization in epidemics are very rich. The literature on stigmatization of COVID-19 gets richer every day, while there are some shortcomings in terms of psychometric measurements. The researchers used the scales frequently used in past epidemics by adopting COVID-19 (7,12). However, it should be noted that this method can be problematic because every epidemic has its specific development and perception created by this development in people's minds. For example, in any diseases such as TBC, bird flu, swine flu (H5N1), or SARS transmitted through the respiratory tract, there has never been a period in which worldwide precautions were taken and habit changes were experienced as in the period of COVID-19. This period brought many new practices, including shutting down airports to flights, closing down cafes, restaurants, and stadiums, transforming in-person education into online education, and modifying measures taken depending on age groups. Therefore, it can be assumed that developing disease-specific scales will be more effective in

determining the size of stigmatization and in terms of measures to be taken. It is noteworthy that generally, a limited number of items are used in the assessment of stigmatization about COVID-19 in studies.

Moreover, as such assessments that lack validity and reliability analyses might lead to erroneous and missing results, it can be suggested that these methods are insufficient. The purpose of this study is to develop a scale that evaluates stigmatization of COVID-19 and analyzes its validity and reliability, considering the shortcomings in the literature. We believe that this scale will be important in evaluating the problems of stigmatization in the literature and developing solution recommendations.

## MATERIALS AND METHODS

### *Development of the COVID-19 Stigma Scale*

While developing the COVID-19 Stigma Scale (CSS), stigmatizations experienced by people with COVID-19 in both their close interpersonal contacts and social life were addressed. Studies on stigmatization attitudes about COVID-19 were reviewed. In addition, studies on infections with high contagiousness, such as TBC, HIV, and H5N1, with a stigmatization attitude were reviewed. While deciding on the scale items, attention was paid to making the items plain and understandable and ensuring that a single item only has a single idea or judgment. Because of the evaluations made among the researchers, areas such as labeling, stigmatization in close contact people, stigmatization in social relations, inequality, discrimination, loss of social status, and emotional responses were identified as main dimensions. It was observed that the scales developed for stigmatization were especially combined on three conceptual fields: labeling, emotional response, and loss of social status. In this framework, a 39-item form was created initially. Although these items were created considering the dimensions in design, it was predicted that every item could occur in another dimension. For example, the item "The society should respect fewer people who experienced COVID-19" includes both labeling (disrespect) and loss of social status concepts. These items corresponded to multiple situations regarding labeling (stigmatizing the individual, healthcare employees, foreigners, people in close contact, and people who experienced COVID-19). As the study's main purpose was to determine social stigmatization against patients, in other words, stigmatization against COVID-19 patients, items outside this framework were excluded. Some similar items were combined. In the final assessment among the researchers, the number of items in the draft scale dropped to 25.

In the second stage, for content validity, opinions were sought from three academic members, two from psychiatry and one from adolescent mental health, and a nurse specializing in public health. An item was removed, and corrections were made to make the items easier to understand on the basis of the recommendations of these persons who have sufficient background and expertise in their respective fields and are outside the study team. The Davis technique was used in content validity analysis. Because of this technique, the content validity index of the scale was determined to be 91.6%.

After these arrangements, a pilot study was conducted with 10 participants. The items that were not understood well or might have

caused a misunderstanding were corrected again. Thus, face validity was guaranteed for the form of 24-item CSS.

It was considered appropriate to use a Likert-type evaluation for the items on the scale. Because individuals might have demonstrated an attitude with which they might show less or partial participation rather than exhibiting a sharp attitude about the items. For every item, the participation level was configured as *completely disagree (0)*, *disagree (1)*, *slightly agree (2)*, *agree (3)*, and *completely agree (4)*. The total scale score ranges from 0 to 76-higher scores in the scale indicate a higher stigmatizing attitude toward COVID-19 patients. The Turkish and English versions of the final version of the scale are shown as an additional file (Appendix 1A).

### **Inclusion and Exclusion Criteria**

Among the relatives of the patients who applied to the hospital, at least primary school graduates volunteered for the study, and over 18 were included in the study. As exclusion criteria, relatives who applied for infectious diseases, chest diseases, and COVID-19 polyclinics were accepted.

### **Sample**

Three hundred twenty-nine people voluntarily agreed to be involved in the study. Three hundred twenty-three forms filled out with complete data were evaluated. The sample's mean age was  $39.04 \pm 13.74$ , and mean years of education were  $12.69 \pm 4.38$ . Most of the participants were female ( $n=176$ , 54.5%), married ( $n=202$ , 62.5%), and employed ( $n=188$ , 58.2%).

### **Assessment Tools**

**Sociodemographic Information Form:** A form prepared by the researchers to evaluate the patients' population and other characteristics (age, gender, educational level, marital status, employment status, alcohol use and smoking habits, sickness status, and living environment) was used.

**COVID-19-Related Experience Form:** Information form with variables including having experienced COVID-19 and having been affected by the pandemic. It was prepared by the researchers.

**COVID-19 Information Questionnaire:** The COVID-19 Information Questionnaire (CIQ) was prepared by the researchers in light of the information in the literature. In this questionnaire comprising 15 items, every item has a right or wrong option. Some items are reverse scored-higher scores in this questionnaire indicate a higher level of knowledge about COVID-19. This questionnaire is attached (Appendix 1B).

### **Ethics Approval and Consent to Participate**

Ethical permission for the study was obtained from the Kocaeli University Non-invasive Clinical Research Ethical Committee (approval number: KÜ GOKAEK-2021/7.18, project number: 2021/134). After obtaining ethical permission, the study was conducted between 15.04.2021 and 10.06.2021. Permission was also obtained from the Republic of Türkiye, Ministry of Health, Directorate of Healthcare Services Management. The participants were explained the purposes of the study and were told they could withdraw from the study at any stage without any reason. Written consent was obtained from all participants who agreed to participate in the study.

### **Statistical Analysis**

SPSS 22 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.) was used for statistical analysis. Descriptive statistics are given in numbers, percentages, mean, and standard deviation (SD). Because the skewness and kurtosis values of the numeric variables were between ( $\pm 1$ ), it was assumed that the data were evenly distributed (13). The correlation between the numeric variables was analyzed using Pearson's correlation test. The independent samples t-test was used to compare the numeric variables between the two groups, and One-Way analysis of variance was used to compare the numeric variables between three and more groups. Exploratory factor analysis (EFA) was used to determine construct validity. In this analysis, the Kaiser-Meyer-Olkin (KMO) test was used to evaluate the sufficiency of the sample size, and the Barlett sphericity test was used to evaluate the conformity of the scale items to the factor analysis. Principal component analysis was used in EFA, and direct Oblimin was preferred for the factor rotation technique. While determining the factor items, the item factor load was accepted as  $>0.40$ . For their validity together, the correlation coefficient between the CIQ and CSS scores was examined. In the reliability analysis, both the total score and the internal reliability coefficient of the CSS subdimensions were determined using Cronbach's alpha value. In addition, when item-total scores and items were excluded, Cronbach's alpha value was calculated. For statistical analyses, the significance level was taken as  $p < 0.05$ .

## **RESULTS**

### **Validity of the Scale**

#### **Construct Validity**

An EFA was performed using the principal component method and the direct Oblimin rotation technique for the 24-item scale. Because of the KMO and Bartlett's tests, the KMO value was 0.929, and Bartlett's test ( $\chi^2=4827.63$ ; SD: 276;  $p < 0.001$ ) was significant. A four-factor structure that had an eigenvalue greater than one and explained 62.65% of the total variance was identified. Three items with a factor load below 0.40 were identified (items 7, 11, and 17). Factor analysis was renewed after removing these items. The scale explained 59.33% of the variance in a three-factor structure. However, with the new analysis, two more items in the scale had a factor load below 0.40 (items 12 and 23). These two items were also removed from the scale. With the renewed analysis after removing these items, a structurally valid scale comprising 19 items and three factors that explained 61.85% of the variance was obtained. The first factor explained 43.08%, the second factor explained 11.75%, and the third factor explained 7.02% of the total variance. Considering the items that constituted the factors, the first factor (1, 2, 3, 4, 5, 16, 22, 24) was called the rejection dimension, the second factor (8, 13, 14, 15, 18, 19, 20) the discrimination dimension, and the third factor (6, 9, 10, 21) the emotional reaction (Table 1).

Considering the items that failed to provide a sufficient factor load in the factor analysis made in the first stage (items 7, 11, and 17), it was observed that perception of riskiness about persons who experienced COVID-19, shopping restrictions, and feeling angry about the bad news on the media were not correlated with stigmatization. This situation suggests that there is a limit between



fundamental rights, needs, and stigmatization. Additionally, people might not see other people who experienced COVID-19 as risky, or even if they do so, it seems to be not in correlation with stigma. The items that were excluded from the scale (items 12 and 23) look similar. Both items meet at a common point based on insufficiency. Removing these similar items together shows the fact that scale validity was obtained. Individuals do not establish a relationship between receiving COVID-19 and insufficiency (physical weakness) or insufficiency in work performance after receiving COVID-19 and stigmatization.

### Content Validity

For this type of validity, the correlation between the total score of the scale and its subscales was calculated (Table 2). There was

a significant high correlation between the CSS total score and rejection ( $r=0.929$ ,  $p<0.001$ ), discrimination ( $r=0.789$ ,  $p<0.001$ ), and emotional reaction sub-scales ( $r=0.733$ ,  $p<0.001$ ).

### Concurrent Validity

The concurrent validity of CSS was evaluated with its correlation with CIQ. The results are presented in Table 2. There was a significant negative relationship between the CSS total score and the CIQ total score ( $r=-0.301$ ,  $p<0.001$ ). Similarly, there was a significant negative relationship between the CSS subscale scores and the CIQ total score (Table 2).

### Discriminant Validity

In terms of discriminant characteristics, past studies have

**Table 1.** Explanatory factor analysis and reliability analysis results of CSS

CSS items	Factor loading	Corrected item-total correlation	Cronbach's alpha when the item is removed
<b>Factor 1: Rejection</b>			
1. People who experienced COVID-19 cannot be considered "normal" even if they have recovered	0.598	0.537	0.912
2. I would feel uncomfortable near a person who has experienced COVID-19	0.884	0.795	0.903
3. I don't want my kid to be in the same environment as a person who has experienced COVID-19	0.902	0.711	0.906
4. I would be extremely concerned if a person who experienced COVID-19 coughs around me	0.894	0.655	0.908
5. I do not want to live, work, or travel with someone who has experienced COVID-19 in the same environment	0.856	0.718	0.906
16. People who have experienced COVID-19 should not stroll around in society	0.499	0.623	0.908
22. People who have experienced COVID-19 should be kept at a more physical distance	0.610	0.725	0.906
24. People who have experienced COVID-19 should not be in close contact with family members	0.462	0.711	0.907
<b>Factor 2: Discrimination</b>			
8. Society should have less respect for people with COVID-19	0.820	0.595	0.911
13. Even if my friend who has COVID-19 has recovered, I would not want to meet her/him	0.582	0.592	0.910
14. People who have experienced COVID-19 should be ashamed of their illness	0.817	0.492	0.912
15. If my partner experienced COVID-19, I would re-evaluate our relationship	0.845	0.545	0.912
18. It is normal not to employ people who have experienced COVID-19	0.685	0.458	0.912
19. People who experienced COVID-19 should not eat in crowded places	0.571	0.722	0.907
20. People who experienced COVID-19 are paying for their sins	0.745	0.575	0.911
<b>Factor 3: Emotional reactions</b>			
6. People who experienced COVID-19 were inattentive and careless	0.497	0.544	0.911
9. People who experienced COVID-19 are responsible for their fault	0.794	0.385	0.915
10. Patients with COVID-19 are the primary worry of society	0.702	0.426	0.914
21. The main reason for our recent unhappiness is the COVID-19 patients	0.584	0.453	0.914
<b>Items with a factor load below 0.40</b>			
7. My anger builds up toward COVID-19 patients as I see bad news about COVID-19 in the media	-	-	-
11. People should not buy from shops or markets run by people who have experienced COVID-19	-	-	-
12. Experiencing COVID-19 as an indicator of physical insufficiency	-	-	-
17. People who have experienced COVID-19 are dangerous	-	-	-
23. People who experienced COVID-19 had low work performance	-	-	-

COVID-19: Coronavirus disease-2019, CSS: COVID-19 Stigma Scale.

demonstrated a clear relationship between education status and stigmatization attitudes (9,10). Our study reinforces this outcome (Table 3). There is a significant negative relationship between education level and CSS total score ( $r=-0.254$ ,  $p<0.001$ ). The relationship between the CSS total score and pandemic experiences is shown in Table 4. The CSS total score was significantly high in those whose income level dropped post-pandemic ( $F=5.580$ ,  $p=0.004$ ), significantly low in those who experienced COVID-19

[ $t(321)=3.174$ ,  $p=0.002$ ], and significantly low in those whose family member experienced COVID-19 [ $t(321)=2.082$ ,  $p=0.038$ ]. These results reinforce the fact that the scale has a discriminant character.

### CSS Reliability Analysis

#### Internal Consistency

In the internal consistency analyses, the item-total item correlation coefficients were used, and when the item was removed, Cronbach's

**Table 2.** Correlation coefficients between the CSS subdimension and total score and the CIQ total score

Variable	Mean	SD	1	2	3	4	5
1 CIQ	11.87	2.08	-				
2 CSS-total	13.30	11.94	-0.301**	-			
3 CSS-rejection	7.47	7.38	-0.332**	0.929**	-		
4 CSS-discrimination	2.30	3.46	-0.216**	0.789**	0.591**	-	
5 CSS-emotional reactions	3.52	3.20	-0.124*	0.733**	0.519**	0.498**	-

\*Correlation is significant at the 0.05 level (2-tailed). \*\*Correlation is significant at the 0.01 level (2-tailed). CSS: COVID-19 Stigma Scale, CIQ: COVID-19 Information Questionnaire.

**Table 3.** Sociodemographic characteristics of the participants and their relationship with CSS scores

Characteristics	Number (%)	CSS scores t/F/r	Statistical analysis	
			p	
<sup>1</sup> Age (mean ± SD)	39.04±13.74	13.30±11.94	0.129	<b>0.020</b>
<sup>1</sup> Education years (mean ± SD)	12.69±4.38	13.30±11.94	-0.254	<b>&lt;0.001</b>
<sup>2</sup> Gender	Female	176 (54.5)	13.48±11.45	0.290
	Male	147 (45.5)	13.09±12.54	
<sup>3</sup> Occupation	Unemployed	71 (22)	14.22±13.17	1.546
	Employed	188 (58.2)	13.04±11.70	
	Student	31 (9.6)	9.93±8.75	
	Retired	33 (10.2)	15.96±12.75	
<sup>2</sup> Marital status	Married	202 (62.5)	14.27±12.25	1.894
	Single	121 (37.5)	11.68±11.27	
<sup>2</sup> Alcohol use	No	226 (70.0)	14.98±12.46	3.933
	Yes	97 (30.0)	9.40±9.63	
<sup>2</sup> Smoking	No	234 (72.4)	13.47±11.99	0.398
	Yes	89 (27.6)	12.87±11.88	
<sup>2</sup> Somatic illness	No	265 (82.0)	13.52±12.24	0.701
	Yes	58 (18.0)	12.31±10.51	
<sup>2</sup> Psychiatric illness	No	285 (88.2)	13.15±11.79	-0.626
	Yes	38 (11.8)	14.44±13.13	
<sup>2</sup> Children of age <18 in family	No	205 (63.5)	12.75±12.28	-1.092
	Yes	118 (36.5)	14.26±11.31	
<sup>2</sup> Elderly of age >65 years in family	No	287 (88.9)	12.85±12.05	-1.932
	Yes	36 (11.1)	16.91±10.52	
<sup>2</sup> Number of family members	≤3	194 (60.1)	12.89±12.27	-0.765
	≥4	129 (39.9)	13.93±11.45	

<sup>1</sup>The Pearson correlation test was used. <sup>2</sup>Independent samples t-test was used. <sup>3</sup>One-Way analysis of variance was used. Bold characters indicate statistical significance. SD: Standard deviation, CSS: COVID-19 Stigma Scale.

alpha coefficients were used, as shown in Table 1. Of the 19-item scale, the corrected item-total item correlation coefficients were between 0.385 and 0.795. The scale's internal consistency Cronbach's alpha coefficient was calculated as 0.91. Cronbach's alpha coefficient was 0.90 in the rejection sub-dimension, 0.88 in the discrimination sub-dimension, and 0.68 in the emotional reaction sub-dimension.

### **Sociodemographic Characteristics of the Participants**

The sociodemographic characteristics of the participants and their relationship with the CSS scores are shown in Table 3. While there was a significant positive relationship between CSS scores and age ( $r=0.129$ ,  $p=0.020$ ), a significant negative relationship was found between CSS scores and education years ( $r=-0.254$ ,  $p<0.001$ ). The CSS score was significantly lower in alcohol users than in the non-users [ $t(321)=3.933$ ,  $p<0.001$ ].

## **DISCUSSION**

To the best of our knowledge, our study is the first community-based scale development study that intends to evaluate stigmatization against patients who experienced COVID-19. The adoption of scales that are not COVID-19 specific (5,7) or stigmatization in the items created by the researchers (15) was evaluated. Furthermore, stigma scales are presented as recommendations (16). However, these studies' validity and reliability analyses were either not done at all or not on the desired level. The scale we developed based on this gap in the literature was shown to be a valid and reliable tool for evaluating stigmatization against patients who experienced COVID-19.

The CSS, which was completed through in-person interviews with the participants, revealed a three-factor configuration that was found to be valid and reliable for psychometric assessment. The factor constructs of the scale are generally in conformity with the conceptual foundation of stigmatized beliefs, attitudes, and behaviors. The first factor we call rejection in our scale evaluates avoiding both close and social relations, in other words, rejecting the establishing relationships with the patients. Not approving being in a close relationship with people who experienced COVID-19, not wanting to live and travel in the same environment, not wanting their kid to be in the same environment, and being concerned or uncomfortable while in the same environment are suitable for the denomination of this factor. Rejection was defined as the first stage in Rosenberg's (17) study, which attempts to determine the social stages of epidemics. The researcher who took inspiration from the book of Albert Camus while identifying these phases suggested that society, in the first place, attempts to reject the presence of epidemics to secure themselves and protect their financial interests (14). From this perspective, rejection is the first stage in which society begins to stigmatize.

Meanwhile, this rejection continues and becomes an individual attitude in further stages. Link and Phalen (8) in 2001 reported that another important component of stigmatization is ironically "power". It was stated that the main structure of this power comprised factors such as not approving, excluding, discriminating, categorizing, and rejecting (18,19). Considering the items under the second factor

**Table 4.** Relationship of scale with pandemic experiences

Variables	n (%)	CSS scores	Statistical analysis		
			t/F	p	
Post-pandemic income level	Reduced	95 (29.4)	15.12±12.39	5.580	<b>0.004</b>
	Not changed	194 (60.1)	13.46±11.87		
	Increased	34 (10.5)	7.29±9.13		
Post-pandemic job loss	No	277 (85.8)	13.15±12.16	-0.558	0.577
	Yes	46 (14.2)	14.21±10.64		
Experiencing COVID-19 infection	No	67 (20.7)	14.37±12.55	3.174	<b>0.002</b>
	Yes	256 (79.3)	9.23±8.14		
Experiencing COVID-19 infection in the family	No	118 (36.5)	14.35±13.00	2.082	<b>0.038</b>
	Yes	205 (63.5)	11.49±9.61		
Experiencing COVID-19 infection in a close environment	No	277 (85.8)	15.71±14.43	1.481	0.140
	Yes	46 (14.2)	12.90±11.46		
Have a relative who died of COVID-19	No	252 (78)	13.17±12.35	1.344	0.511
	Yes, a first-degree relative	11 (3.4)	15.54±13.37		
	Yes, a second- or third-degree relative	60 (18.6)	13.45±9.88		
How much did the pandemic affect you?	Not at all	17 (5.3)	12.70±14.52	0.604	0.660
	Slightly	38 (11.8)	14.73±14.61		
	Moderately	114 (35.3)	14.26±11.40		
	Significantly	72 (22.3)	12.09±11.42		
	Very much	82 (25.4)	12.50±11.31		

Bold characters indicate statistical significance. CSS: COVID-19 Stigma Scale, COVID-19: Coronavirus disease-2019.

of the scale, it was found appropriate to call this sub-dimension discrimination. Items such as not wanting to meet friends who experienced COVID-19, thinking about re-evaluating the relationship after the partner experiences COVID-19, not employing people who experienced COVID-19, and showing less respect by the society are placed in a common structure about discrimination. While discrimination is an important part of all types of stigmatization, it is one of the main components of social stigmatization. Social stigmatization exists based on society's stereotypes, prejudices, and discriminatory beliefs (18). On the other hand, a main component of discrimination, "social stigmatization" is closely related to other types of stigmatizations (primary, secondary, corporate, and structural stigmatization), and social stigmatization can be a result of and a reason for other types of stigmatizations (20). Because the COVID-19 pandemic is not limited to an individual level and impacts social habits, attitudes, and perceptions, stigmatization of COVID-19 is clearly a type of social stigmatization. Discrimination is also a component of stigmatization.

Because of the studies in the literature, it was concluded that stigmatization has phases such as "labeling", "stereotyping", "prejudice", "loss of social status and discrimination" (19). These phases are not distinguished from each other with clear borders, and it is possible to see that one of them can embody one another or others at the same time. Although emotional reactions were not discussed much in the initial studies on stigmatization, Link et al. (21) emphasized that emotional reactions are an important part of the stigmatization process. When stigmatization occurs, emotions emerge in both the stigmatizer and the stigmatized. The severity of these emotions is closely related to the depth of stigmatization. The third factor in our scale clearly demonstrates the emotional reactions that emerge despite stigmatization. Items such as blaming people who experienced COVID-19, seeing them as the source of concern of society, and as the main reason for the recent unhappiness they experienced explicitly include these emotional reactions. Considering the scale factors in light of the information from the literature, it seems suitable for the conceptual content of stigmatization. On the other hand, as mentioned by the researchers, the relationship between these factors is highly dynamic, and its boundaries are not clear.

It is a known fact that humans can marginalize others with an instinct to protect themselves despite situations or events that they are not knowledgeable about (22). In other words, people create a border with another by stigmatizing them. Therefore, they feel safe in their inner world. The relationship between stigmatization and lack of information is clearly observed in both psychiatric and medical illnesses (23). For example, the less knowledgeable individuals are about schizophrenia, the more they tend to stigmatize them (22,23). It has been demonstrated that providing information about diseases reduces negative opinions and stigmatization about the disease (24,25). The moderately significant negative correlation ( $r=-0.301$ ,  $p<0.001$ ) between the CSS scale developed in our study and the total scores in the CIQ shows the validity of the scale. In addition, stigmatization level was high in individuals with lower levels of education (Table 3). Studies on stigmatization of the COVID-19 pandemic demonstrate that individuals with low educational levels are under social media impression and engage in stigmatization to a higher degree (10).

Behaviors such as stigmatization, discrimination, marginalization, and avoidance emerged in past epidemics such as the COVID-19 pandemic (26). Because of the potentially lethal situations, new diseases and incurable/unknown diseases are defined as factors related to an increased risk of stigmatization (27). The COVID-19 pandemic is the first pandemic that occurred in an era in which communication networks were developed to such an extent, despite having similarities to other pandemics. This pandemic we live through has created many specific conditions because of the period we live in.

Investigating the impact of these conditions, Yuan et al. (28) recently compared individuals who experienced COVID-19 with healthy controls in terms of self-stigma. This study yielded very striking outcomes. For individuals who experienced COVID-19, stigmatization areas such as social rejection, financial insecurity, internalized embarrassment, and social isolation were significantly higher than those in healthy controls. The researchers who evaluated factors related to stigmatization stated that male gender, low educational level (high school and below), unemployment, experiencing COVID-19 by a family member, and economic loss variables were closely related to stigmatization. Another study was conducted with 7411 participants from 173 countries (29). This study had a cross-sectional design, and it was found that 27.3% of the participants believed that people spoke ill or spread rumors about individuals who experienced COVID-19. However, as one of the most important study results, one out of five persons in the sample (21.9%) believed that people who experienced COVID-19 lost their dignity and status in society (29). In our study, the total score on the scale was significantly higher in people who had lower income levels in the post-pandemic period, people who did not experience COVID-19 infection, and people whose family did not experience COVID-19 than in other categories (Table 4). These results demonstrate that the scale has a predictive character. Additionally, the level of stigmatization against people who experienced COVID-19 by people who used alcohol was significantly lower than that against those who did not use alcohol (Table 3). These results can be explained by the higher education level of individuals who use alcohol ( $14.82\pm 3.52$  vs.  $11.78\pm 4.40$ ,  $p<0.001$ ). The lack of a detailed investigation of alcohol use makes it harder to interpret the outcomes obtained in the respective field.

Conducting the study face-to-face and reaching a sample beyond the necessary sample for developing the scale are the strengths of the study. We believe that our study will provide important contributions to the literature in measuring COVID-19-related stigma, which is also considered a public health problem. These evaluations will provide a basis for further studies.

### **Study Limitations**

However, our study has certain limitations. The COVID-19 pandemic is a dynamic process in which individuals' perceptions and attitudes can change periodically. Conducting this study in a specific period might be a particular limitation. It should also be noted that although there were individuals from each education group in our study, the average years of education were above the country average, and the study represents the results of a single center.

## CONCLUSION

With this study attended by enough persons from every educational level in society and conducted by in-person interviews, the 19-item three-factor CSS was found to be valid and reliable. For both the total and scale factors, Cronbach's alpha internal consistency coefficients were found to be sufficient. Evaluating the stigmatization attitude against people who experienced COVID-19 and taking necessary measures in this context will play a key role in preventing possible psychiatric disorders and trauma experienced by society.

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## Ethics

**Ethics Committee Approval:** Ethical permission for the study was obtained from the Kocaeli University Non-invasive Clinical Research Ethical Committee (approval number: KÜ GOKAEK-2021/7.18, project number: 2021/134).

**Informed Consent:** Written consent was obtained from all participants who agreed to participate in the study.

## Authorship Contributions

Concept: B.G., M.Y., Design: B.G., M.Y., Data Collection or Processing: B.G., M.Y., Analysis or Interpretation: B.G., Literature Search: B.G., M.Y., Writing: B.G., M.Y., Critical Review: B.G., M.Y.

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## REFERENCES

1. WHO. Social Stigma associated with COVID-19-World Health Organization, 2020. <https://www.who.int/docs/default-source/coronaviruse/covid19-stigma-guide.pdf> (accessed September 6, 2020)
2. Chopra KK, Arora VK. COVID-19 and social stigma: Role of scientific community. *Indian J Tuberc* 2020; 67: 284-5.
3. Peprah P, Gyasi RM. Stigma and COVID-19 crisis: A wake-up call. *Int J Health Plann Manage* 2021; 36: 215-8.
4. Lasalvia A. Epidemic emergencies and social stigma. Which lessons should we learn from SARS and Ebola epidemics for the current CoViD-19 pandemics?. *Riv Psichiatr* 2020; 55: 250-3.
5. Rueda S, Mitra S, Chen S, Gogolishvili D, Globerman J, Chambers L, et al. Examining the associations between HIV-related stigma and health outcomes in people living with HIV/AIDS: a series of meta-analyses. *BMJ Open* 2016; 6: e011453.
6. Bagcchi S. Stigma during the COVID-19 pandemic. *Lancet Infect Dis* 2020; 20: 782.
7. Duan W, Bu H, Chen Z. COVID-19-related stigma profiles and risk factors among people who are at high risk of contagion. *Soc Sci Med* 2020; 266: 113425.
8. Link BG, Phelan JC. Conceptualizing stigma. *Annu Rev Sociol* 2001; 27: 363-85.
9. Turner-Musa J, Ajayi O, Kemp L. Examining Social Determinants of Health, Stigma, and COVID-19 Disparities. *Healthcare (Basel, Switzerland)* 2020; 8: 168.
10. Bruns DP, Kraguljac NV, Bruns TR. COVID-19: Facts, cultural considerations, and risk of stigmatization. *J Transcult Nurs* 2020; 31: 326-32.
11. Baldassarre A, Giorgi G, Alessio F, Lulli LG, Arcangeli G, Mucci N. Stigma and Discrimination (SAD) at the Time of the SARS-CoV-2 Pandemic. *Int J Environ Res Public Health* 2020; 17: 6341.
12. Cassiani-Miranda CA, Campo-Arias A, Tirado-Otálvaro AF, Botero-Tobón LA, Upegui-Arango LD, Rodríguez-Verdugo MS, et al. Stigmatisation associated with COVID-19 in the general Colombian population. *Int J Soc Psychiatry* 2020; 67: 728-36.
13. Tabachnick B, Fidell L. Using multivariate statistics (6 edition). United States: Pearson Education; 2013.
14. Des Jarlais DC, Galea S, Tracy M, Tross S, Vlahov D. Stigmatization of newly emerging infectious diseases: AIDS and SARS. *Am J Public Health* 2006; 96: 561-7.
15. Teksin G, Uluyol OB, Onur OS, Teksin MG, Ozdemir HM. Stigma-related factors and their effects on health-care workers during COVID-19 pandemics in Turkey: A multicenter study. *Sisli Etfal Hastan Tip Bul* 2020; 54: 281-290.
16. Campo-Arias A, Álvarez-Solorza I, Tirado-Otálvaro AF, Cassiani-Miranda CA. Proposal of a scale for COVID-19 stigma-discrimination toward health workers. *J Investig Med* 2021; 69: 100-1.
17. Rosenberg CE. What is an epidemic? AIDS in historical perspective. *Daedalus* 1989; 118: 1-17.
18. Lee S, Chan LY, Chau AM, Kwok KP, Kleinman A. The experience of SARS-related stigma at Amoy Gardens. *Soc Sci Med* 2005; 61: 2038-46.
19. Ozmen S, Erdem R. Conceptual framework of the stigmatization. *Suleyman Demirel University The Journal of Faculty of Economics and Administrative Sciences* 2018; 23: 185-208.
20. Soltan EM, Salama HM, Aboelmagd MA. Assessment of stigma among patients infected with hepatitis C virus in Suez City, Egypt. *J Public Health* 2018; 26: 279-88.
21. Link BG, Yang LH, Phelan JC, Collins PY. Measuring mental illness stigma. *Schizophr Bull* 2004; 30: 511-41.
22. Economou M, Richardson C, Gramandani C, Stalikas A, Stefanis C. Knowledge about schizophrenia and attitudes towards people with schizophrenia in Greece. *Int J Soc Psychiatry* 2009; 55: 361-71.
23. Thornicroft G, Rose D, Kassam A, Sartorius N. Stigma: Ignorance, prejudice or discrimination?. *Br J Psychiatry* 2007; 190: 192-3.
24. Lanfredi M, Macis A, Ferrari C, Rillosi L, Ughi EC, Fanetti A, et al. Effects of education and social contact on mental health-related stigma among high-school students. *Psychiatry Res* 2019; 281: 112581.
25. Wolff G, Pathare S, Craig T, Leff J. Public education for community care. A new approach. *Br J Psychiatry* 1996; 168: 441-7.
26. Taylor S. The psychology of pandemics: Preparing for the next global outbreak of infectious disease. Newcastle: Cambridge Scholars Publishing; 2019.
27. Brewis A, Wutich A, Mahdavi P. Stigma, pandemics, and human biology: Looking back, looking forward. *Am J Hum Biol* 2020; 32: e23480.
28. Yuan Y, Zhao YJ, Zhang QE, Zhang L, Cheung T, Jackson T, et al. COVID-19-related stigma and its sociodemographic correlates: a comparative study. *Global Health* 2021; 17: 54.
29. Dye TD, Alcantara L, Siddiqi S, Barbosu M, Sharma S, Panko T, et al. Risk of COVID-19-related bullying, harassment and stigma among healthcare workers: an analytical cross-sectional global study. *BMJ Open* 2020; 10: e046620.

**Appendix 1A. COVID-19 Stigma Scale****Name:** .....**Date:** .... / .... / .....

Below are the thoughts and attitudes regarding COVID-19 patients. Please indicate at what level you agree with the ideas in each item by ticking (✓) the most appropriate option. Thank you for participating in this study.

Completely  
disagree

Disagree

Slightly  
agree

Agree

Completely  
agree

- 1 People who experienced COVID-19 cannot be considered “normal” even if they have recovered.
- 2 I would feel uncomfortable near a person who has experienced COVID-19.
- 3 I don’t want my kid to be in the same environment as a person who has experienced COVID-19.
- 4 I would be extremely concerned if a person who experienced COVID-19 coughs around me.
- 5 I do not want to live, work, or travel with someone who has experienced COVID-19 in the same environment.
- 6 People who have experienced COVID-19 should not stroll around in society.
- 7 People who have experienced COVID-19 should be kept at a more physical distance.
- 8 People who have experienced COVID-19 should not be in close contact with family members.
- 9 Society should have less respect for people with COVID-19.
- 10 Even if my friend who has COVID-19 has recovered, I would not want to meet her/him.
- 11 People who have experienced COVID-19 should be ashamed of their illness.
- 12 If my partner experienced COVID-19, I would re-evaluate our relationship.
- 13 It is normal not to employ people who have experienced COVID-19.
- 14 People who experienced COVID-19 should not eat in crowded places.
- 15 People who experienced COVID-19 are paying for their sins.
- 16 People who experienced COVID-19 were inattentive and careless.
- 17 People who experienced COVID-19 are responsible for their illness.
- 18 Patients with COVID-19 are the primary worry of society.
- 19 The main reason for our recent unhappiness is that COVID-19 patients.

**Appendix 1A.** Turkish version of COVID-19 Stigma Scale

İsim: .....

Tarih: .... / .... / .....

Aşağıda COVID-19 hastalarıyla ilgili düşünce ve tutumlar yer almaktadır. Lütfen her bir maddedeki fikirlere hangi düzeyde katıldığınızı en uygun seçeneği (✓) işaretleyerek belirtiniz. Çalışmaya katıldığınız için teşekkür ederiz.

Kesinlikle Katılmıyorum Biraz Katılıyorum  
katılmıyorum Katılıyorum Tamamen Katılıyorum

- 1 COVID-19 geçiren kişiler iyileşmiş olsalar bile "normal" değerlendirilmez.
- 2 COVID-19 geçirmiş bir kişinin yanında kendimi huzursuz hissedirim.
- 3 Çocuğumun COVID-19 geçirmiş bir kişiyle aynı ortamda olmasını istemem.
- 4 COVID-19 geçirmiş bir kişi yanımda öksürürse çok endişelenirim.
- 5 COVID-19 geçirmiş biriyle aynı ortamda yaşamak, çalışmak veya seyahat etmek istemem.
- 6 COVID-19 geçirmiş kişiler toplumda dolaşmamalıdır.
- 7 COVID-19 geçirmiş kişilere karşı daha fazla fiziksel mesafe koyulmalıdır.
- 8 COVID-19 geçirmiş kişiler aile üyeleriyle yakın temasta bulunmamalıdır.
- 9 Toplum, COVID-19 geçirmiş kişilere daha az saygı duymalıdır.
- 10 COVID-19 olan bir arkadaşım iyileşse bile onunla görüşmek istemem.
- 11 COVID-19 geçirmiş insanlar hastalıklarından utanmalıdır.
- 12 Partnerim COVID-19 geçirirse ilişkimizi yeniden değerlendiririm.
- 13 COVID-19 geçirmiş kişilere iş vermemek normaldir.
- 14 COVID-19 geçirmiş kişiler kalabalık ortamlarda yemek yememelidir.
- 15 COVID-19 geçirmiş insanlar günahlarının bedelini ödüyor.
- 16 COVID-19 geçirmiş insanlar dikkatsiz ve özensizdir.
- 17 COVID-19 geçirmiş kişiler hastalıklarından kendileri sorumludur.
- 18 COVID-19 hastaları toplumun birincil endişe kaynağıdır.
- 19 Son zamanlardaki mutsuzluğumuzun asıl sebebi COVID-19 hastalarıdır.

**Appendix 1B. COVID-19 Information Questionnaire**

No	Item	True	False
1	COVID-19 is a virus-transmitted disease.		
2	COVID-19 is transmitted by both droplets and direct contact.		
3	COVID-19 improves with antibiotics.		
4	Pets and street animals are a risky group in terms of transmission.		
5	Children do not easily catch COVID-19.		
6	COVID-19 is more severe in the elderly.		
7	COVID-19 often leads to death.		
8	Those with COVID-19 often have sequela that worsens their quality of life.		
9	Headache, fever, cough, and sore throat are the main symptoms of COVID-19.		
10	Frequent gargling kills the coronavirus.		
11	Malaria drugs are the most effective in the fight against COVID-19.		
12	In the fight against COVID-19, frequent hand washing is sufficient.		
13	Sun rays kill coronavirus.		
14	Wind is an important factor in the spread of coronavirus.		
15	A person who catches COVID-19 will not get COVID-19 again.		

**Scoring:**

The answers to the above statements are given below.

1: True, 2: True, 3: False, 4: False, 5: False, 6: True, 7: False, 8: False, 9: True, 10: False, 11: False, 12: False, 13: False, 14: False, 15: False. In questions "1<sup>st</sup>, 2<sup>nd</sup>, 6<sup>th</sup>, and 9<sup>th</sup>", the "True" answer is "1" point, and marking the "False" answer is "0". In questions "3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup>, 7<sup>th</sup>, 8<sup>th</sup>, 10<sup>th</sup>, 11<sup>th</sup>, 12<sup>th</sup>, 13<sup>th</sup>, 14<sup>th</sup>, and 15<sup>th</sup>", the "False" answer is "1" and marking the "True" answer is "0." Higher scores suggest that the person has more accurate information about COVID-19. COVID-19: Coronavirus disease-2019.

**Appendix 1B. Turkish version of COVID-19 Information Questionnaire**

No	İfade	Doğru	Yanlış
1	COVID-19 virüsle bulaşan bir hastalıktır.		
2	COVID-19 hem damlacık yoluyla hem de doğrudan temas yoluyla bulaşır.		
3	COVID-19 antibiyotiklerle iyileşme gösterir.		
4	Evcil hayvanlar ve sokak hayvanlar bulaş açısından riskli bir gruptur.		
5	Çocuklar COVID-19'a kolay kolay yakalanmazlar.		
6	Yaşlılarda COVID-19 daha ağır seyretmektedir.		
7	COVID-19 sıklıkla ölüme yol açar.		
8	COVID-19 hastaları genellikle yaşam kalitelerini kötüleştiren bir sekel yaşarlar.		
9	Baş ağrısı, ateş, öksürük ve boğaz ağrısı COVID-19'un temel semptomlarıdır.		
10	Sık sık gargara yapmak koronavirüsü öldürür.		
11	Sıtma ilaçları COVID-19 ile mücadelede en etkin ilaçlardır.		
12	COVID-19 ile mücadelede sık el yıkamak yeterlidir.		
13	Güneş ışınları koronavirüsü öldürür.		
14	Rüzgar, koronavirüsün yayılmasında önemli bir etkidir.		
15	COVID-19'a yakalanan bir kişi bir daha COVID-19 geçirmez.		

**Puanlama:**

Yukarıda yer alan ifadelerin yanıtları aşağıda belirtilmiştir.

1: Doğru, 2: Doğru, 3: Yanlış, 4: Yanlış, 5: Yanlış, 6: Doğru, 7: Yanlış, 8: Yanlış, 9: Doğru, 10: Yanlış, 11: Yanlış, 12: Yanlış, 13: Yanlış, 14: Yanlış, 15: Yanlış. "1., 2., 6. ve 9." sorularda doğru yanıt "1" puan, yanlış yanıtın işaretlenmesi ise "0" puandır. "3., 4., 5., 7., 8., 10., 11., 12., 13., 14. ve 15." sorularda yanlış yanıt "1" puan, doğru yanıtın işaretlenmesi ise "0" puandır. Puanların yükselmesi kişinin COVID-19 hakkında daha fazla doğru bilgiye sahip olduğunu düşündürmektedir. COVID-19: Koronavirüs hastalığı-2019.





## The Effect of Polyurethane Film Versus *Apis Dorsata* Honey Spray for Wound Dressing Following Long Bone Fractures Osteosynthesis

Uzun Kemik Kırıklarında Osteosentez Sonrası Yara Pansumanında Poliüretan Film ve *Apis Dorsata* Bal Spreyinin Etkisi

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### ABSTRACT

**Objective:** Surgical site infection (SSI) following implant-related fracture osteosynthesis remains a burden and challenging for orthopedic surgeons. Honey-based dressings can be used as prophylactic agents.

**Methods:** This prospective, randomized clinical study was designed to compare the effect of conventional polyurethane film and *Apis dorsata* honey spray as dressing materials after long bone fracture osteosynthesis.

**Results:** Forty participants with closed tibial or femoral diaphyseal fracture treated with open reduction and internal osteosynthesis with intramedullary implants or plates and screws were randomly divided into 3 groups: 16 were dressed with polyurethane film (group A), 13 with *Apis dorsata* honey spray (group B), and 11 as controls. Wounds of the two groups were dressed using a similar wound protocol immediately (D0) and three days (D3) after surgery. In the control group, wounds were treated with non-adhesive film only. All wounds were evaluated on day 14 (D14) and day 42 (D42) for local complications and the effects on skin commensals. On D42, wound dehiscence and scar formation were also evaluated. *Acinobacter* species were isolated from a control.

**Conclusion:** One patient from group A had superficial SSI. There was no significant association between wound healing and the dressing materials used. Honey dressing reduced the risk of hypertrophic scar formation. Other outcomes were comparable between groups A and B. *Apis dorsata* honey is a safe alternative dressing that is comparable to polyurethane film as a dressing material following long bone fracture osteosynthesis.

**Keywords:** Dressing material, fracture fixation, honey dressing, Tualang honey, wound healing

### ÖZ

**Amaç:** İmplantla ilişkili kırık osteosentezini takip eden cerrahi alan enfeksiyonu (CAE), ortopedik cerrahlar için bir yük ve zorluk olmaya devam etmektedir. Bal bazlı pansumanlar profilaktik ajan olarak kullanılabilir.

**Yöntemler:** Bu prospektif, randomize klinik çalışma, uzun kemik kırığı osteosentezi sonrasında pansuman malzemesi olarak geleneksel poliüretan film ve *Apis dorsata* bal spreynin etkisini karşılaştırmak için tasarlanmıştır.

**Bulgular:** Açık redüksiyon ve intramedüller implantlar veya plaklar ve vidalarla internal osteosentez ile tedavi edilen kapalı tibial veya femur diyafiz kırığı olan kırık katılımcı rastgele 3 gruba ayrıldı: 16'sına poliüretan film (grup A), 13'üne *Apis dorsata* bal spreyi (grup B) ve 11'ine kontrol grubu uygulandı. İki grubun yaraları ameliyattan hemen sonra (D0) ve ameliyattan üç gün sonra (D3) benzer bir yara protokolü kullanılarak pansuman yapıldı. Kontrol grubunda yaralar sadece yapışkan olmayan filmle tedavi edildi. Tüm yaralar, lokal komplikasyonlar ve deri komensalleri üzerindeki etkiler açısından 14. günde (D14) ve 42. günde (D42) değerlendirildi. D42'de yara açılması ve skar oluşumu da değerlendirildi. *Acinobacter* türleri bir kontrolden izole edildi.

**Sonuç:** Grup A'dan bir hastada yüzeysel CAE vardı. Yara iyileşmesi ile kullanılan pansuman malzemeleri arasında anlamlı bir ilişki yoktu. Ballı pansuman hipertrofik skar oluşumu riskini azalttı. Diğer sonuçlar Grup A ve B arasında benzerdi. *Apis dorsata* balı, uzun kemik kırığı osteosentezini takiben pansuman malzemesi olarak poliüretan filmle karşılaştırılabilecek güvenli bir alternatif pansumandır.

**Anahtar Sözcükler:** Pansuman malzemesi, kırık tespiti, ballı pansuman, Tualang balı, yara iyileşmesi

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## INTRODUCTION

Surgical site infection (SSI) carries potential devastating sequelae following implant-related fracture osteosynthesis. These complications remain a challenge, particularly in terms of costs, prolonged hospital stay, morbidity, and even mortality (1-3). Post-surgical wound dressing remains one of the contributing factors to the final wound outcome that needs special attention in reducing SSI (1,4,5). Other factors that may also influence the wound outcome include the severity of the injury, duration of the surgery, and the patient's co-morbidities (2,3,6-9). Wounds involving the lower limbs differ from those involving the trunk in terms of skin tension and involve wider exposed areas with a higher risk of infection. Furthermore, the presence of implants in the wound warrants extra attention in terms of dressing and wound care. Appropriate post-surgical dressing material is paramount in fracture osteosynthesis because any wound infection with an implant beneath may lead to osteomyelitis and result in chronic morbidities to the patient as well as a financial burden to the society (2,3,7,9,10).

Dressing materials are used on surgical wounds to provide a waterproof sealer and to prevent adhesion during wound dressing; hence, they may reduce pain as well. It may also provide a moist environment conducive to wound healing (5,11). Several dressing materials are commonly used, including polyurethane-based film dressing and paraffin-based materials. Polyurethane film spray, commonly known as Opsite\* spray, is a convenient and innovative, quick-drying transparent film dressing spray for surface and surgical wounds. This product is widely used in local hospitals as a dressing material for post-surgical wounds. In orthopedic surgery, polyurethane film spray is a common dressing applied after long bone fracture osteosynthesis.

Honey is a semi-solid or saturated solution of natural sugars synthesized from flower nectar by honeybees (12,13). Although honey of various types shares the same general content and properties, each type has different potential and uses depending on the type, honeybee species, purity, and geographical origin (12-15). *Apis dorsata* or Asian rock bees produce honey in hives built high up in the Tualang tree (*Koompassia excelsa*), one of the tallest trees in Malaysia (12,13,15-17). Thus, the honey is also famously known as the Malaysian Tualang honey. It is one of the examples of local honey that can act as a potent anti-inflammatory agent with effective antimicrobial and antifungal effects, attributed to its high level of phenolic acids and flavonoids (13-15,18,19). Various studies have reported its effectiveness in treating diabetic wounds, burns, and other types of infected wounds (4,12,13,15,19,20).

To the best of our knowledge, there has been no study conducted on the potential of honey as a wound dressing post-osteosynthesis of fractures. Hence, the present study aimed to compare the effect of using a conventional polyurethane film (Opsite\* spray) with *Apis dorsata* honey spray as a dressing material following internal osteosynthesis of long bone fractures.

## MATERIALS AND METHODS

The study was designed as a prospective, randomized, unblinded, controlled study recruiting patients with closed tibial or femoral diaphyseal fracture treated with open reduction and internal osteosynthesis using intramedullary implants or plates and screws.

The participants were selected among trauma patients aged between 15 and 45 years at the time of injury within a 12-month study period at a single tertiary center. Only patients who underwent primary closure of the surgical wound following the surgical procedures were included in the study. Patients who sustained any previous open fractures or osteomyelitis involving the ipsilateral bone were excluded from the study. Those with a history of allergic reactions to any honey-based products, a history of bronchial asthma, or a history of developing allergic reaction to any of the materials used in the study were also excluded. Other exclusion criteria were immunocompromised patients, prolonged use of corticosteroids, chemotherapy treatment, or a history of renal failure or diabetes mellitus.

The study was commenced after approval by the Human Research Ethics Committee USM (HREC) [approval number: USMKK/PPP/JEPeM/254.3(7.2)]. The participants were provided with complete information about the aims and the flow of the study. Written informed consent was required to participate in the study. The routine clinical assessment and treatment for which the participants came were conducted as usual, and none of the participants abandoned their right to receive the best possible consultation despite being part of the study.

The participants' basic demographics and detailed current and previous medical histories were recorded in a study proforma. This includes the severity of soft tissue injury sustained along with the long bone fracture. The severity of soft tissue injury associated with closed fractures was graded using the Tscherne and Oestern classification (21) (Table 1).

### Randomization and Dressing Materials Used

Based on the dressing materials used following the intended internal osteosynthesis, the participants were divided randomly into 3 groups. In group A, the patients were subjected to wound dressing with polyurethane film (Opsite\* spray), a moisture vapour permeable dressing spray that contains an acrylic copolymer, isopropyl alcohol, acetone, ethyl acetate, and propellants (dimethyl ether and butane 40), supplied by Smith & Nephew Malaysia. *Apis dorsata* honey spray was used for wound dressing in group B. Honey spray was supplied by the Federal Agricultural Marketing Authorities in a single batch in collaboration with the School of Pharmaceutical Sciences laboratory. The participants' wounds in the control group were dressed only with a breathable, self-adhesive, and absorbent film dressing (Mepore®) supplied by Molnlycke Health Care US. It is widely available in operation theatres as a standard dressing following Opsite\* spray application.

Randomization was performed via elective operating theatre that available during working hours. The selection of dressing material for the patients was based on their operation day. The participants who were operated on Mondays and Thursdays were subjected to polyurethane film dressing. Honey spray dressing was used on the participants operated on Tuesdays and Fridays. Participants who were operated on Wednesdays were grouped as controls. The decisions on which operation day was chosen were based on the availability of the empty slot in the elective lists and the semi-emergency lists. The participants who were admitted earlier had priority to be listed in the operation lists.

All participants in group B were screened for allergy to the honey spray dressing one day before surgery. The dressing material was applied to the dorsum area of the participants' hands. Any form of allergic reactions, such as rashes, itchiness, or others, was observed and documented. This step was clearly stated in the written consent form and explained in detail to all participants. Participants who developed adverse reactions to the dressing material were excluded from the study.

### Wound Closure and Dressing Technique Protocol

Following open reduction and internal osteosynthesis performed in the standard manner, all surgical wounds were primarily closed with Vicryl 2/0 and Dafilon 3/0 sutures (Figure 1A). The length of the surgical wounds was recorded in centimeter (cm) units (Figure 1B) and the operative time in hours and minutes. For the patients in groups A and B, a thin layer of the respective dressing materials was applied to the surgical wound immediately after wound closure (D0), followed by application of the non-adhesive film (Mepore®). The control group was treated with film (Mepore®) only.

### Wound Assessment

The first wound inspection for all wounds was performed on D3 post-surgery, followed by the second assessment on D14 during surgical suture removal (Figure 2). Assessment included the presence of local changes such as redness around the wound, local oedema, and serous or purulent discharge. Signs of adverse reactions, such as itchiness and blister formations, were observed and documented, if any. After wound inspection on D3, a swab for culture and sensitivity was taken, and the wounds were re-applied with similar dressing materials used immediately after the surgery.

The third and final assessment was performed during follow-up on D42 post-surgery. Any wound dehiscence or discharge was observed and recorded. The surgical scars were evaluated and documented as a normal scar appearance or a hypertrophic scar (protruding scar resembling a fibroma or collagen nevus).

## RESULTS

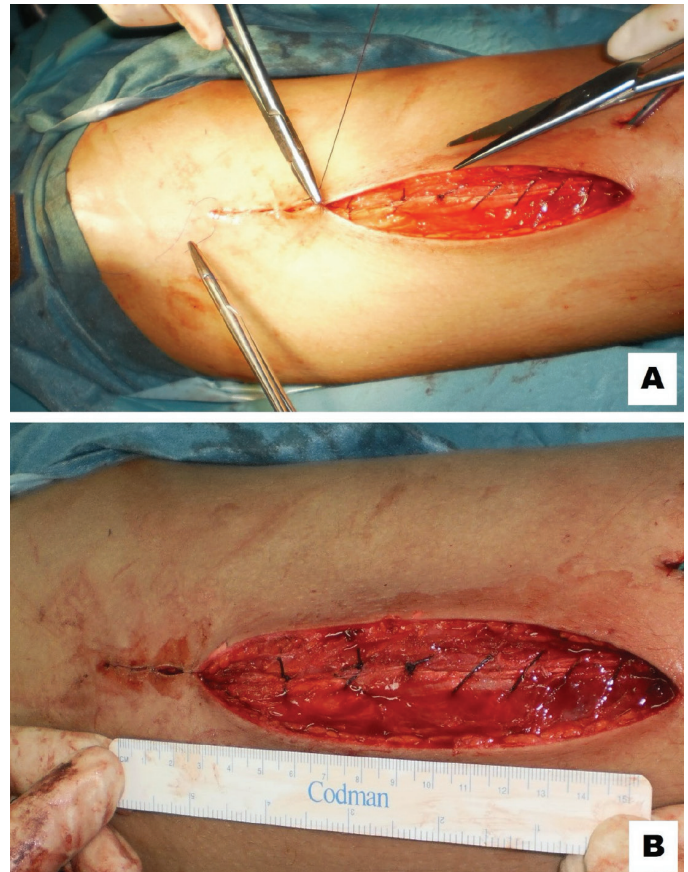
A total of 42 participants fulfilled the study criteria. The following randomization, there were 16 participants in group A, 14 participants in group B, and 12 participants as controls. One participant from group B and the control group defaulted to follow-up and was excluded from the study. The remaining participants (n=40) consisted of 17 female and 23 male participants with a mean age of 19 years. None of the participants in group B developed an allergic reaction to the *Apis dorsata* honey dressing spray.

Femoral fractures were fixed in 33 participants (82%) and only 7 participants (18%) had tibial fractures. One participant from group A had bilateral femoral fractures. Thirty-eight participants had undergone open reduction and plating compared with intramedullary nailing in 2 participants. Associated injuries included fracture of the radius and scapula, avulsion fracture of the posterior cruciate ligaments, and concomitant fracture of the femur or tibia. There were 3 participants with polytrauma, one in each group. Only 3 participants (7.5%) had grade C2 soft tissue injury. The remaining participants either had grade C0 [19 (47.5%)] or C1 [18 (45%)]. None had grade C3 soft tissue injury. The mean operating time in all cases

was 80 min. The mean length of the surgical wounds was 16.11 cm. The mean duration from the time of injury to the time of surgery was 5 days and 19 hours.

### Wound Complications

None of the participants in all the 3 groups had clinical signs of



**Figure 1.** (A) Fascia closed with vicryl 2/0 and skin closure with dafilon 3/0 suture. (B) The surgical wound was measured using a sterile ruler in centimeter units.



**Figure 2.** Wound appearance of a patient from group B.

infection on D3 post-surgery. However, a participant from group A had a 2 cm superficial wound dehiscence with serous discharge identified during surgical suture removal. The wound healed well following dressing with normal saline and sterile strip application. No further antibiotics or debridement was required. Analysis using Fisher's exact test found no significant correlation between wound complications and the dressing materials used ( $p>0.950$ ).

### Wound Commensal Microorganisms

*Acinobacter* species was isolated via swab culture from the surgical wound of a participant from the control group on D3 post-surgery day 3. However, Fisher's exact test analysis also found no significant correlation between the dressing materials used and their effect on wound commensal microorganisms ( $p>0.275$ ).

### Wound Assessment on D42

All wounds healed without any clinical signs of infection or dehiscence on D42 post-surgery. Only 3 out of 13 patients (23.1%) in group B had a hypertrophic scar compared with group A (43.8%) and the control group (72.7%) (Figure 3). Table 2 summarizes the association between the dressing material used and wound healing.

### Factors Influencing Wound Healing

Simple logistic regression showed that none of the factors analyzed except for the dressing material used was significant in influencing wound healing in all patients. Multiple logistic regression analysis showed that the use of honey dressing spray had a significant influence on wound healing. These are summarized in Tables 3, 4.

## DISCUSSION

The majority of participants (82%) in the present study had femoral fractures, although the tibia is the most common long bone fracture of the lower limbs (6,7,21). One of the criteria of patients who were recruited for this study was any fracture of the femoral or tibial shaft that required open reduction and was internally fixed with an implant device. Thus, the smaller number of participants with tibial fracture included in the study was because this type of fracture may be treated conservatively with a full-length cast or intramedullary device with a close reduction method. Compared with femoral fractures in adults, it is rarely indicated for conservative treatment with closed reduction method and casting. Almost all participants were treated with plating of the fractures compared with intramedullary fixation. This finding corresponds with the standard technique of surgery that ordinary compression plates generally need an open reduction method compared with intramedullary fixation, which can be performed using the closed method or indirect reduction (7,21). The recommended fixation of closed diaphyseal femoral and tibial fractures generally involves either intramedullary nailing or plating. However, plating usually has a narrow indication of usage, for example in diaphyseal fracture with concurrent neck of femur fracture.

Most participants (80%) had other associated injuries, which may contribute to the mean duration before surgery. It will influence the urgency of the surgery compared with elective surgery in a stable trauma case. The severity of soft tissue injury contributes to the risk

of wound infection and the healing process (6,7,21). There was an equal distribution of participants in the groups with regard to the severity of soft tissue injuries. Thus, each group of participants had a similar risk of infection and outcome.

The duration of the wound exposed to the environment and the length of the wound will influence the rate of SSI (2,7,9). The entire cohort of participants had a mean operating time of 2 h and a mean wound length of 16.11 cm. The complexity and difficulty in the internal osteosynthesis method were also determined by the types of fracture, availability of an appropriate implant, and surgeon's experience. There is a standard surgical exposure for open reduction and osteosynthesis of femoral and tibial diaphyseal fractures, but no standard wound length is recommended in the literature. Adequate exposure for assisting in the surgical procedure is generally quoted (7,22).

There was an isolated case of superficial wound infection in group A, as evident by the presence of wound dehiscence and serous discharge at D14 post-surgery. No intervention was needed as the wound healed well until the final assessment on D42. The participant underwent open reduction with intramedullary femoral osteosynthesis at 3 weeks after trauma because of a limited slot for elective operation. The decision to use the open reduction method was made due to the difficulty in fracture reduction as soft callus had already formed. The surgery was completed within 130 min. No other associated injuries were noted. The longer operating time with difficult reduction were the possible factors that influenced wound healing in the patient.

One of the aims of wound assessment was to identify local adverse reactions to honey spray. None was detected in all participants in group B. Although the participants in group B had yellowish skin staining surrounding the wound from the honey dressing, it did not affect wound healing, and the stain was easily resolved with normal saline irrigation. There was no significant correlation between the dressing materials used and wound complications. These results may be due to the strict selection of participants and the small sample size. Young and fit individuals, mild to moderate soft tissue injuries, and simple fracture patterns also have a good prognosis in wound healing (21). Thus, the dressing method is not the only factor that affects the wound healing process.

The thin film formed on the wounds in groups A and B acted as a barrier to the external environment. Limited studies are available to prove whether the film may reduce the number of commensals on the skin. In a study by Wille and Blussé van Oud Alblas (23), polyurethane

**Table 1.** Tscherne and Oestern classification for closed fractures (24)

Grade	Energy	Typical fracture pattern	Typical soft tissue damage
C0	Low	Spiral	None to minimal
C1	Mild to moderate	Rotational ankle fractures and dislocations	Superficial abrasion/contusion
C2	High	Transverse segmental complex	Deep abrasions
C3	High	Complex	Extensive skin contusion



**Figure 3.** Example of hypertrophic scar formation in a patient in group A.

film with chlorohexidine possessed the most antimicrobial activity with regard to the microflora of the skin. However, this study showed no significant correlation between the dressings used and their effects on wound commensals. The only microorganism that was isolated from one of the participants' wounds (from the control group) was *Acinobacter* species. *Acinetobacter* species is a Gram-negative *Coccobacillus* that is found within environmental soils, water, sewage, and foodstuffs (as spoilage organisms) such as milk products, meat, poultry, and fish. Microorganisms also present as commensals in human skin. In immunocompromised patients, it may become virulence and responsible for SSI and other healthcare-associated infections in intensive care units (8,9). In the case of a control in this study, the participant remained asymptomatic because he was a healthy and fit individual. His wound also healed without any complications.

**Table 2.** Association between dressing materials and wound healing

Group	Wound assessment on D42		c <sup>2</sup> stat (df)	p-value*
	Healed with a normal scar	Healed with a hypertrophic scar		
Group A	9	7	5.951 (2)	0.051
Group B	10	3		
Control group	3	8		

\*Chi-square test.

**Table 3.** Clinical factors influencing wound healing analyzed using simple logistic regression

Clinical Factors	Crude OR (95% CI)	p-value
Age	1.020 (0.923; 1.127)	0.702
Gender		
Male	0.865 (0.246; 3.050)	0.822
Female	1	
Type of dressing material		
Polyurethane film (group A)	0.292 (0.056; 1.525)	0.144
Honey spray dressing (group B)	0.133 (0.018; 0.716)	0.021
Control group	1	
Site of the fracture		
Diaphysis of the right tibia	0.000 (0.000; 0.000)	0.950
Diaphysis of the right femur	0.675 (0.168; 2.709)	0.579
Diaphysis of the left tibia	0.250 (0.021; 3.041)	0.277
Diaphysis of the left femur	1	
Type of implant		
Intramedullary nails	1.235 (0.072; 21.241)	0.884
Plates and screws	1	
Associated injuries		
No	0.680 (0.139; 3.337)	0.635
Yes	1	
Soft tissue injury		
Grade C2	4.333 (0.326; 57.649)	0.267
Grade C1	2.708 (0.708; 10.360)	0.146
Grade C0	1	

**Table 3.** Continued

Clinical factors	Crude OR (95% CI)	p-value
Duration of injury to surgery	0.999 (0.996; 1.003)	0.707
Duration of surgery	1.003 (0.982; 1.026)	0.757
Surgical wound length	1.183 (0.888; 1.577)	0.251

OR: Odds ratio, CI: Confidence interval.

**Table 4.** Clinical factors influencing wound healing analyzed using multiple logistic regression

Clinical factors	B	Adjusted OR (95% CI)	p-value
<b>Type of dressing material</b>			
Polyurethane film (group A)	-1.232	0.292 (0.056, 1.525)	0.144
Honey spray dressing (group B)	-2.185	0.133 (0.018, 0.716)	0.021
Control group		1	

Backward LR method applied. Hosmer and Lemeshow test,  $p=0.866$ . Classification table 67.5% correctly classified. The area under the receiver operating characteristic curve was 0.708. OR: Odds ratio, CI: Confidence interval.

None of the participants had wound dehiscence at the end of the study period. The rate of SSI in a clean surgery is 1 to 2% (2,3,24). The possible reason for no case of wound infection in the current study was the strict study criteria of healthy individuals without comorbidities. Surgical prophylactic antibiotic usage also plays a role in reducing wound infection (1-3,8-10). All patients in this study were administered intravenous cefuroxime 1.5 g as a prophylactic antibiotic before surgery.

Another finding that was recorded during the final wound assessment was the presence of a hypertrophic scar. The current study showed not significant association between the type of dressing used and healing. Although in group B, most patients' wounds healed with a normal healthy scar compared with the wounds of the other two groups, this cannot reflect the advantages of honey dressing as the result was not statistically significant (Table 2). The possible reasons were limited sample size and crude assessment of the scars at D2 post-surgery.

### Study Limitations

A better scar assessment should involve an expert plastic surgeon or team with more objective scar evaluation, which was one of the limitations of the study. Nevertheless, multiple logistic regression analysis revealed that patients dressed with honey spray dressing had a lower risk of developing hypertrophic scars compared with the other groups (Table 4). This result was attributed to the anti-inflammatory and antimicrobial potential of honey, and its effects on reducing wound contracture (13,16,25). Mohamad Shah et al. (26) reported that apart from the antimicrobial effects, the active components of *Apis dorsata* honey also demonstrated an antiproliferative effect on human keloid fibroblasts, with the potential to be used for keloid treatment.

### CONCLUSION

*Apis dorsata* honey spray has comparable effects with the widely used polyurethane film for wound dressing following long bone osteosynthesis. Apart from promoting healing and preventing SSI, the risk of hypertrophic scar formation was lower.

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### Ethics

**Ethics Committee Approval:** The study was commenced after approval by the Human Research Ethics Committee USM (HREC) [approval number: USM/KK/PPP/JEPeM/254.3(7.2)].

**Informed Consent:** Written informed consent was required to participate in the study.

### Authorship Contributions

Concept: S.S., M.A.S., S.A.R., A.N.S., Design: S.S., M.A.S., S.A.R., A.N.S., Data Collection or Processing: S.S., M.A.S., S.A.R., A.N.S., Analysis or Interpretation: S.S., M.A.S., S.A.R., A.N.S., Literature Search: S.S., M.A.S., S.A.R., A.N.S., Writing: S.S., M.A.S., S.A.R., A.N.S.

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

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### REFERENCES

- Gupta A, Dev A, Nigam VK, Padmanabhan P, Singh S. A review on next-generation nano-antimicrobials in orthopedics: prospects and concerns. In: Prasad R, Siddhardha B, Dyavaiah M, editors. Nanostructures for Antimicrobial and Antibiofilm Applications. Nanotechnology in the Life Sciences. Switzerland: Springer; 2020. p. 33-62.
- Suranigi SM, Ramya SR, Devi CS, Kanungo R, Najimudeen S. Risk factors, bacteriological profile and outcomes of surgical site infections following orthopaedic surgery. Iranian J Microbiol 2021; 13: 171-7.
- Tucci G, Romanini E, Zanolli G, Pavan L, Fantoni M, Venditti M. Prevention of surgical site infections in orthopaedic surgery: a synthesis of current recommendations. Eur Rev Med Pharmacol Sci 2019; 23(Suppl 2): 224-39.

4. Aznan MI, Khan OH, Unar AO, Tuan Sharif SE, Khan AH, Syed Abd Aziz SH, et al. Effect of Tualang honey on the anastomotic wound healing in large bowel anastomosis in rats - A randomized controlled trial. *BMC Complement Altern Med* 2016; 16: 28.
5. Mat Saad AZ, Wan Sulaiman WA, Halim AS, Mohd Yussof SJ, Jaafar H. The efficacy of Tualang honey in comparison to silver in dressing wounds in rats. *JAAS* 2011; 3: 45-53.
6. Halvachizadeh S, Pfeifer R, Kalbas Y, Schuerle S, Cinelli P, Pape HC. Assessment of alternative techniques to quantify the effect of injury on soft tissue in closed ankle and pilon fractures. *PLoS One* 2022; 17: e0268359.
7. Luo P, Zhang Y, Wang X, Wang J, Chen H, Cai L. A nomogram for predicting skin necrosis risk after open reduction and internal fixation for tibia fractures. *Int Wound J* 2022; 19: 1551-60.
8. Misha G, Chelkeba L, Melaku T. Incidence, risk factors and outcomes of surgical site infections among patients admitted to Jimma Medical Center, South West Ethiopia: prospective cohort study. *Ann Med Surg* 2021; 65: 102247.
9. Gottrup F, Melling A, Hollander DA. An overview of surgical site infections: aetiology, incidence, and risk factors. *EWMA Journal* 2005; 5: 11-15.
10. Leaper DJ, Van Goor H, Reilly J, Petrosillo N, Geiss HK, Torres AJ, et al. Surgical site infection - a European perspective of incidence and economical burden. *Int Wound J* 2004; 1: 247-73.
11. Molan PC. The evidence supporting the use of honey as a wound dressing. *Int J Low Extrem Wounds* 2006; 5: 40-54.
12. McLoone P, Warnock M, Fyfe L. Honey: a realistic antimicrobial for disorders of the skin. *J Microbiol Immunol Infect* 2016; 49: 161-7.
13. Mohd Nasir NA, Halim AS, Singh KK, Dorai AA, Haneef MN. Antibacterial properties of tualang honey and its effect in burn wound management: A comparative study. *BMC Complement Altern Med* 2010; 10: 31.
14. Al-Kafaween MA, Al-Jamal HAN, Mohd Hilmi AB. Antibacterial activity of selected varieties of Malaysian honey against *Escherichia coli*: a comparative study. *Int Arab J Antimicrob Agents* 2021; 11: 3.
15. Ahmed S, Othman NH. Review of the medicinal effects of Tualang honey and a comparison with Manuka honey. *Malays J Med Sci* 2013; 20: 6-13.
16. Mohd Kamal DA, Ibrahim SF, Kamal H, Mohd Kashim MIA, Mokhtar MH. Physicochemical and medicinal properties of Tualang, Gelam and Kelulut honeys: a comprehensive review. *Nutrients* 2021; 13: 197.
17. Shehu A, Ismail S, Rohin MAK, Harun A, Abd Aziz A, Haque M. Antifungal properties of Malaysian Tualang honey and stingless bee propolis against *Candida albicans* and *Cryptococcus neoformans*. *J Appl Pharm Sci* 2016; 6: 44-50.
18. Chew CY, Chua LS, Soontorngun N, Lee CT. Discovering potential bioactive compounds from Tualang honey. *Agric Nat Resour* 2018; 52: 361-5.
19. Devasvaran K, Yong YK. Anti-inflammatory and wound healing properties of Malaysia Tualang honey. *Curr Sci* 2016; 110: 47-51.
20. Jull AB, Cullum N, Dumville JC, Westby MJ, Deshpande S, Walker N. Honey as a topical treatment for wounds. *Cochrane Database Syst Rev* 2015; 3: CD005083.
21. Ibrahim DA, Swenson A, Sassoon A, Fernando ND. Classifications in brief: the Tschernie classification of soft tissue injury. *Clin Orthop Relat Res* 2017; 475: 560-4.
22. Hoppenfeld S, De Boer P, Buckley R. *Surgical Exposures in Orthopaedics: The Anatomic Approach*, 4th Edition: Philadelphia: Walters Kluwer-Lippincott Williams & Wilkins; 2009.
23. Wille JC, Blussé van Oud Alblas A. A comparison of four film-type dressings by their anti-microbial effect on the flora of the skin. *J Hosp Infect* 1989; 14: 153-8.
24. Culver DH, Horan TC, Gaynes RP, Martone WJ, Jarvis WR, Emori TG, et al. Surgical wound infection rates by wound class, operative procedure, and patient risk index. *National Nosocomial Infections Surveillance System. Am J Med* 1991; 91(3B): 152S-7S.
25. Vazhacharickal PJ. A review on health benefits and biological action of honey, propolis and royal jelly. *J Med Plants Stud* 2021; 9: 1-13.
26. Mohamad Shah NS, Halim AS, Gan SH, Shamsuddin S. Antiproliferative effect of methanolic extraction of Tualang honey on human keloid fibroblasts. *BMC Complement Altern Med* 2011; 11: 82.

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## Comparison of Use of Propiverine at 45 mg and Combined Treatment of Propiverine 30 mg and Mirabegron in Patients with Overactive Bladder Who did not Benefit from Propiverine 30 mg Treatment

Propiverin 30 mg Tedavisinden Fayda Görmeyen Aşırı Aktif Mesaneli Hastalarda Propiverin 45 mg Kullanımı ile Propiverin 30 mg ve Mirabegron Kombine Tedavisinin Karşılaştırılması

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### ABSTRACT

**Objective:** There are many anti-cholinergic drugs that are used to treat overactive bladder. Mirabegron was created to lessen the many side effects of these drugs, especially acute urinary retention. The use of mirabegron along with anti-cholinergic is a safe and effective treatment method. In addition, anti-cholinergic therapies have been developed that reduce bladder overactivity with side effects similar to those of lower dose approaches and with higher efficacy.

**Methods:** In this study, data were collected for 35 male patients (group 1) in whom propiverine 45 mg was administered instead of propiverine 30 mg treatment; the other 31 patients (group 2) were given mirabegron 50 mg on propiverine 30 mg treatment. Patients were called for control at 1 and 6 months; adherence and persistence were determined; residual urine volumes were measured by ultrasonography; and complaints were evaluated using the Overactive Bladder Assessment Form (OAB-V8).

**Results:** When the ages and chronic diseases of the patients in groups 1 and 2 were compared, there was no significant difference between the groups. Similar rates of adherence and persistence were observed between the groups. There were no statistically significant differences between the groups in terms of daily micturition and weekly incontinence frequencies, post-voiding residual urine volumes, side effects (especially dry mouth), and responses and scores on the OAB-V8 forms.

**Conclusion:** Propiverine 45 mg monotherapy or propiverine 30 mg and mirabegron combined treatment can be safely administered with similar efficacy and limitations.

### Öz

**Amaç:** Mesane aşırı aktivitesinin yaşam tarzı değişikliği ve davranışların düzenlenmesi gibi birinci basamak tedavilerinden fayda görmeyen hastalarda kullanılan medikal tedavi için birçok antikolinergik ilaç geliştirilmiş olmasına rağmen, mirabegron antikolinergik ilaçlara bağlı birçok yan etkiyi özellikle akut üriner retansiyonu azaltmak için kullanılmaya başlanmıştır. Mirabegronun antikolinergiklerle kombinasyon tedavisinde kullanılmasının güvenli ve etkili bir tedavi yöntemi olduğu gösterilmiştir. Antikolinergik ilaçlardan propiverin de düşük doz antikolinergik ilaçlardan kısıtlı fayda gören hastalarda kullanılması amacıyla 45 mg uzatılmış salınlı formunun kullanımının da etkin ve güvenli olduğu gösterilmiştir.

**Yöntemler:** Bu çalışmada propiverin 30 mg tedavisi yerine propiverin 45 mg propiverin uygulanan 35 erkek hastanın (grup 1) ve 30 mg propiverin tedavisine ek olarak mirabegron 50 mg tedavisi uygulanan 31 erkek hastanın (grup 2) verileri toplandı. Hastalara ilk başvurularında üroflowmetri yapıldı, semptomları ve işeme günlükleri incelendi. Hastalar 1. ve 6. ayda kontrole çağırıldı. Tedavi uyumu ve memnuniyet belirlendi. Ultrasonografi ile rezidü idrar hacimleri ölçüldü ve Aşırı Aktif Mesane Değerlendirme Formu (OAB-V8) ile şikayetler değerlendirildi.

**Bulgular:** Her iki gruptaki hastaların yaşları ve kronik hastalıkları karşılaştırıldığında gruplar arasında anlamlı fark yoktu. Gruplar arasında günlük işeme ve haftalık inkontinans sıklıkları, işeme sonrası rezidüel idrar hacimleri, yan etkiler (özellikle ağız kuruluğu) ve OAB-V8 formlarındaki yanıtlar ve puanlar açısından istatistiksel olarak anlamlı fark yoktu.

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## ABSTRACT

**Keywords:** Anti-cholinergic, beta 3 receptor agonist, propiverine, sustained release, high dose, combination therapy, bladder overactivity, post-micturition residual urine, acute urinary retention, OAB-V8 Overactive Bladder Questionnaire, adherence, persistence

## Öz

**Sonuç:** Propiverin 45 mg monoterapisi veya propiverin 30 mg ve mirabegron kombine tedavisi benzer etkinlik ve sınırlamalarla güvenle uygulanabilir.

**Anahtar Sözcükler:** Antikolinergik, beta 3 reseptör agonisti, propiverin, sürekli salım, yüksek doz, kombinasyon terapisi, mesane aşırı aktivitesi, işeme sonrası kalan idrar, akut idrar retansiyonu, OAB-V8 Aşırı Aktif Mesane Anketi, bağlılık, kalıcılık

## INTRODUCTION

Overactive bladder is a clinical disorder with urgency with or without urinary incontinence, and it is often accompanied by frequency and nocturia without any detectable pathology, such as urinary tract infection (1). Studies on its frequency have shown that it occurs in 12-17% of the population, and its frequency is known to increase with age (2,3). In cases of bladder overactivity, the main treatment has been accepted as anti-cholinergic drugs, and although many have been developed for this purpose, side effects often limit their use, especially acute urinary retention; this has led to the search for different drugs (1,4,5). As a result, the beta-3 adrenergic receptor agonist drug mirabegron, which is in a different drug group from anti-cholinergic therapy, was developed to reduce the use of anti-cholinergic drugs and avoid their side effects, especially acute urinary retention (6).

Acute urinary retention is caused by the inability to empty the bladder due to different pathologies, and it is an important health problem that can cause serious complications if not treated (7). This condition is sudden and manifests as severe pain and discomfort caused by urgency (8). It usually occurs in elderly male patients with benign prostatic hypertrophy due to infection or drug use. Spinal trauma and operations such as pelvic surgery that may impair the neurological coordination of voiding can also cause acute urinary retention. Acute urinary retention is seen at a rate of 0.45-0.68% in men over the age of 40 years, with a corresponding rate of 0.007% in women (9-11). While the risk increases from the age of 40 years, it is especially common at older ages (8). Similar to complaints related to bladder storage phases and bladder overactivity, the risk of urinary retention increases with age (12).

The measurement of residual urine volume by ultrasound imaging or catheterization is recommended in patients with risk factors for acute urinary retention, such as residual urinary symptoms, incontinence, or a history of neurological diseases and previous pelvic surgery (1). Although a residual urine volume of up to 100 mL can be monitored without intervention, a residual urine volume above 200 mL is considered pathological. Although there is no absolute standardization of these numbers, if there is a residual urine volume above 500 mL, the patient is considered to have acute urinary retention (11,13).

In patients with acute urinary retention risk, care should be taken in dose and drug selection because anti-cholinergic drugs, especially when used in overactive bladder treatment, relax the bladder muscle and reduce contractions. Bladder muscle contractility decreases over time, especially in elderly patients. In addition, polypharmacy and comorbidities increase with age and reduce bladder muscle

contraction, and the amount of residual urine in these patients increases with obstruction (11,14).

Studies that have investigated propiverine treatment in patients who had not benefited from anti-cholinergic therapy have shown a decrease in complaints due to its calcium antagonistic effect. There are also studies showing that propiverine 45 mg sustained release high doses reduce complaints due to bladder overactivity with similar side effects and higher efficiency when compared with propiverine 30 mg (15). As a beta 3 receptor agonist, mirabegron, unlike anti-cholinergic drugs, relaxes bladder smooth muscles in the storage phase without decreasing contractility in the voiding phase; therefore, some studies have shown that side effects in the voiding phase are less common. The use of mirabegron in combination therapy with anti-cholinergics has also been shown to be safe and preferential because it increases the success rate of the treatment. However, although high-dose (45 mg) propiverine and mirabegron combined with anti-cholinergic therapy are the preferred methods for treating patients who do not benefit from low-dose anti-cholinergic therapy, no study has compared these two treatment options (16).

## MATERIALS AND METHODS

This prospective study follows the rules of the Declaration of Helsinki regarding patients' rights and ethical guidelines and was approved by the Ethics Committee of Van Training and Research Hospital (approval number: 2021/06). All patients involved in this study provided written informed consent.

In our clinic, 178 adult male patients, whose complaints continued despite propiverine 30 mg treatment for a month, presented with bladder storage phase complaints between September 2019 and January 2021. Patients under the age of 18 years were excluded from the study. Patients with fewer bladder storage phase complaints, more voiding phase complaints, alpha blocker treatment with a pre-diagnosis of benign prostatic hyperplasia, and known neurological pathology were not included in the study.

A total of 66 patients met the study criteria, and their maximum urinary flow rate was above 15 mL/min and post-voiding residue urine volume was below 100 mL in uroflowmetry analysis. In 35 of the 66 patients (group 1), propiverine 45 mg was started instead of 30 mg propiverine treatment; mirabegron 50 mg was added to the 30 mg propiverine treatment of the other 31 patients (group 2). Patients were called for control 1 and 6 months after the treatment began; at this time, adherence and persistence were determined. Post-voiding residual volumes were evaluated by ultrasonography, and complaints were assessed using the Overactive Bladder Assessment Form (OAB-V8). Patient information and results were retrospectively

analyzed. Patients with a post-voiding residual volume above 500 mL were evaluated as having acute urinary retention.

### Statistical Analysis

For statistical analysis, Mann-Whitney U, chi-square, and McNemar tests were performed to evaluate numerical variables and categorical data between groups, respectively. P-values less than 0.05 were considered significant.

## RESULTS

In our study, the ages of 35 patients who received propiverine 45 mg in group 1 and 31 patients who received propiverine 30 mg and mirabegron combination therapies in group 2 ( $48.26 \pm 19.45$  vs.  $45.74 \pm 18.36$ ;  $p=0.592$ ) and their comorbid chronic diseases were compared ( $p=0.229$ ); no significant differences were found between the groups. Furthermore, there were no significant differences between the daily micturition counts ( $7.70 \pm 1.59$  vs.  $7.40 \pm 1.59$ ;  $p=0.488$ ) and weekly incontinence counts ( $2.57 \pm 2.67$  vs.  $2.00 \pm 1.65$ ;

$p=0.307$ ) of the patients in the groups. Although there was no significant difference between the groups in terms of post-voiding residual volumes, one patient in group 1 developed acute urinary retention two weeks after using propiverine 45 mg treatment. This caused us to determine the standard deviation rate, which was high in group 1 ( $65.00 \pm 117.44$  vs.  $64.69 \pm 39.17$ ;  $p=0.323$ ). When dry mouth developed after treatment, its advent was not statistically significant, although 12 patients (34.29%) in group 1 had new reports of dry mouth, and five patients (16.13%) reported the condition in group 2 ( $p=0.092$ ) (Table 1).

It was observed that there was no significant difference between the two groups in persistence during the first month (45.71% vs. 32.26%;  $p=0.267$ ) and the sixth month after treatment (25.71% vs. 22.58%;  $p=0.875$ ), as well as adherence during the first month (62.86% vs. 64.52%;  $p=0.889$ ) and the sixth month after the treatment (40.00% vs. 41.94%;  $p=0.891$ ). It was found that the patients who were not satisfied with the treatment after one month of use were not satisfied after six months of use. Similarly, it

**Table 1.** Comparison of demographic characteristics, micturations, post-voiding residual volumes, incontinence, and dry mouth between the groups

	Group 1; (n=35)	Group 2; (n=31)	p
Age	48.26±19.45	45.74±18.36	0.592
<b>Comorbidity</b>			
Positive	10 (28.57%)	5 (16.13%)	0.229
Negative	25 (71.43%)	26 (83.87%)	
Micturations/d	7.70±1.59	7.40±1.59	0.488
Post-voiding residual volumes	65.00±117.44	64.69±39.17	0.323
Incontinence/w	2.57±2.67	2.00±1.65	0.307
<b>Dry mouth</b>			
Positive	12 (34.29%)	5 (16.13%)	0.092
Negative	23 (65.71%)	26 (83.87%)	

Group 1: Patients using propiverine (45 mg). Group 2: Patients using propiverine (30 mg) and mirabegron combination treatment. Mann-Whitney U and chi-square tests were utilized for statistical analyses.

**Table 2.** Comparison of adherence and persistence between the groups

	Group 1; (n=35)	Group 2; (n=31)	p
<b>Adherence (first month)</b>			
Positive	22 (62.86%)	20 (64.52%)	0.889
Negative	13 (37.14%)	11 (35.48%)	
<b>Persistence (first month)</b>			
Positive	16 (45.71%)	10 (32.26%)	0.267
Negative	19 (54.29%)	21 (67.74%)	
<b>Adherence (sixth month)</b>			
Positive	14 (40.00%)	13 (41.94%)	0.891
Negative	21 (60.00%)	18 (58.06%)	
<b>Persistence (sixth month)</b>			
Positive	9 (25.71%)	7 (22.58%)	0.875
Negative	26 (74.29%)	24 (77.42%)	

Group 1: Patients using propiverine (45 mg), Group 2: Patients using propiverine (30 mg) and mirabegron combination treatment. Chi-square test.

was observed that the adherence and persistence rates decreased from the first-month control to the sixth-month control (Table 2). In the group using propiverine 45 mg treatment, the adherence (62.86% vs. 42.86%;  $p=0.008$ ) and persistence (45.71% vs. 25.71%;  $p=0.016$ ) rates decreased significantly in the sixth month of the treatment compared with the first month of the treatment. In the group using combined propiverine 30 mg and mirabegron treatment, the adherence rate (64.52% vs. 38.71%;  $p=0.016$ ) decreased significantly in the sixth month of the treatment compared with the first month, whereas the decrease in the persistence rate (32.26% vs. 22.58%;  $p=0.250$ ) was not statistically significant (Table 3).

The complaints of the patients after treatment in both groups were evaluated using the OAB-V8 form; the average scores for each of the questions and the total scores on the OAB-V8 forms were compared between the two groups. Both groups were similar in their reports of frequent urination during the daytime hours ( $4.64\pm0.59$  vs.  $4.43\pm0.90$ ;  $p=0.288$ ), an uncomfortable urge to urinate ( $4.36\pm0.80$  vs.  $4.17\pm0.91$ ;  $p=0.359$ ), a sudden urge to urinate with little or no warning ( $4.31\pm0.95$  vs.  $4.13\pm0.86$ ;  $p=0.447$ ), accidental loss of small amounts of urine ( $2.22\pm1.90$  vs.  $2.20\pm1.60$ ;  $p=0.960$ ), night-time urination ( $3.22\pm1.25$  vs.  $3.00\pm1.53$ ;  $p=0.518$ ), waking up at night to urinate ( $2.72\pm1.49$  vs.  $2.40\pm1.73$ ;  $p=0.419$ ), an uncontrollable urge to urinate ( $3.89\pm1.09$  vs.  $3.63\pm1.10$ ;  $p=0.348$ ) and urine loss associated with a strong desire to urinate ( $2.22\pm1.90$  vs.  $2.17\pm1.60$ ;  $p=0.899$ ). There was no statistically significant difference between

the mean values ( $29.58\pm7.06$  vs.  $28.13\pm7.13$ ;  $p=0.411$ ) of the total OAB-V8 scores of both groups (Table 4).

## DISCUSSION

Although beta 3 receptor agonists and anti-cholinergic drugs are the most important treatment modalities for overactive bladder, their success is limited. For this reason, sustained release forms of medication administered in higher doses and combination therapies, which have a synergistic effect on the bladder through different receptors, can be given to increase the effectiveness of anti-cholinergic drugs used for this purpose while keeping the side effects at tolerable levels (6,15,16). In the study of Sussman et al. (17), in which the 12-month treatment adherence and persistence levels of 71,980 patients who received anti-cholinergic and mirabegron treatment were examined, the adherence rate was 44% for those receiving mirabegron treatment and 31% for those receiving anti-cholinergic treatment. A persistence rate of 19% was reported for patients using mirabegron and 12% for those receiving anti-cholinergic treatment. Although the adherence rates in our study were much higher after one month of treatment, they appeared to be similar after six months of treatment. In the study by Sussman et al. (17), adherence was observed to be much higher in patients using anti-cholinergics because there were patients who received combination therapy and mirabegron alone in the group using mirabegron. However, in our study, all patients in the group using mirabegron received combination therapy; therefore,

**Table 3.** Comparison of the adherence and persistence of the groups in the first and sixth months

	Group 1; (n=35)	p	Group 2; (n=31)	p
<b>Adherence</b>				
First month	22 (62.86%)	<b>0.008</b>	20 (64.52%)	<b>0.016</b>
Sixth month	14 (42.86%)		13 (38.71%)	
<b>Persistence</b>				
First month	16 (45.71%)	<b>0.016</b>	10 (32.26%)	0.250
Sixth month	9 (25.71%)		7 (22.58%)	

Group 1: Patients using propiverine (45 mg), Group 2: Patients using propiverine (30 mg) and mirabegron combination treatment, McNemartest.

**Table 4.** OAB-V8 Overactive Bladder Questionnaire for patients

	Group 1; (n=35)	Group 2; (n=35)	p
Frequent urination during daytime hours	$4.64\pm0.59$	$4.43\pm0.90$	0.288
Uncomfortable urge to urinate	$4.36\pm0.80$	$4.17\pm0.91$	0.359
Sudden urge to urinate with little or no warning	$4.31\pm0.95$	$4.13\pm0.86$	0.447
Accidental loss of small amounts of urine	$2.22\pm1.90$	$2.20\pm1.60$	0.960
Night-time urination	$3.22\pm1.25$	$3.00\pm1.53$	0.518
Waking up at night because you had to urinate	$2.72\pm1.49$	$2.40\pm1.73$	0.419
Uncontrollable urge to urinate	$3.89\pm1.09$	$3.63\pm1.10$	0.348
Urine loss associated with a strong desire to urinate	$2.22\pm1.90$	$2.17\pm1.60$	0.899
OAB-V8 score	$29.58\pm7.06$	$28.13\pm7.13$	0.411

Group 1: Patients using propiverine (45 mg), Group 2: Patients using propiverine (30 mg) and mirabegron combination treatment, Mann-Whitney U test, OAB-V8: Overactive Bladder Assessment Form.

we found that the use of multidrugs reduced patient compliance, but because of fewer anti-cholinergic side effects, adherence was similar between the two groups. When we called for control after one month in our study, we found that the patients in our study had higher persistence rates, but after six months of treatment, the rates were similar. Sussman et al. (17) showed that persistence rates decreased after an average of three months, and many patients discontinued the treatment. In our study, it was observed that the adherence and persistence rates decreased between 1 and 6 months.

In a study conducted by Stöhrer et al. (16), 66 patients with neurogenic detrusor hyperactivity who were administered sustained release propiverine (45 mg) were called as controls to determine the efficacy and side effects after 21 days, and they found a persistence rate of 39%. In addition, 36% of the patients had side effects such as dry mouth, which is similar to our study (16). In a review article evaluating 64 studies and 46,666 patients in total to examine the efficacy and effects of anti-cholinergic drug therapy after mirabegron treatment, it was shown that, similar to our study, mirabegron and anti-cholinergic treatment can be safely used with high efficiency and a similar side effect profile in patients who do not benefit from anti-cholinergic monotherapy (18). In their studies with patients using anti-cholinergic monotherapy such as solifenacin or tolterodine, Drake et al. (19) investigated the effects of mirabegron and solifenacin or mirabegron and tolterodine combination therapies on residual urine volume and the risk of acute urinary retention. As with our study, they showed that it can be safely used without increasing post-voiding residual volumes and the risks of acute urinary retention, especially in patients without obstructive pathologies such as benign prostatic hypertrophy (19).

In all studies that evaluated mirabegron combination therapy, combination therapy was compared with mirabegron combined with the same dose of anti-cholinergic monotherapy. Similarly, all studies that evaluated high-dose sustained release propiverine 45 mg treatment were compared with low-dose propiverine treatment (15-19). Although there are different studies evaluating mirabegron combination therapy and high-dose sustained release anti-cholinergic treatment in patients who do not benefit from anti-cholinergic therapy, there is no study comparing the two treatments in patients who do not benefit from low-dose anti-cholinergic therapy to determine which of the mirabegron combination and high-dose anti-cholinergic therapies should be preferred. In our study, we compared the effectiveness and limitations of these two treatment modalities.

### Study Limitations

The limitations of our study were the insufficient number of patients and the fact that it was not possible to completely exclude patients who had obstructive diseases, especially older male patients, because the study was conducted with male patients.

### CONCLUSION

According to the findings we obtained in our study, in male patients with bladder overactivity without obstructive pathologies who did not benefit from low-dose anti-cholinergic treatment, propiverine 45 mg monotherapy or propiverine 30 mg and mirabegron

combined treatment can be safely preferred with similar efficacy and limitations.

### Ethics

**Ethics Committee Approval:** This study was reviewed and approved by the Medical Ethics Committee of Van Training and Research Hospital (approval number: 2021/06).

**Informed Consent:** All patients involved in this study provided written informed consent.

### Authorship Contributions

Surgical and Medical Practices: İ.Ş.B., S.D., Concept: İ.Ş.B., S.D., Design: İ.Ş.B., S.D., Data Collection or Processing: İ.Ş.B., S.D., Analysis or Interpretation: İ.Ş.B., S.D., Literature Search: İ.Ş.B., S.D., Writing: İ.Ş.B., S.D.

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

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### REFERENCES

- Gormley EA, Lightner DJ, Burgio KL, Chai TC, Clemens JQ, Culkin DJ, et al. Diagnosis and treatment of overactive bladder (non-neurogenic) in adults: AUA/SUFU guideline. *J Urol* 2012; 188: 2455-63.
- Coyne KS, Sexton CC, Vats V, Thompson C, Kopp ZS, Milsom I. National community prevalence of overactive bladder in the United States stratified by sex and age. *Urology* 2011; 77: 1081-7.
- Moon SJ, Kim YT, Lee TY, Moon H, Kim MJ, Kim SA, et al. The influence of an overactive bladder on falling: a study of females aged 40 and older in the community. *Int Neurourol J* 2011; 15: 41-7.
- Benner JS, Nichol MB, Rovner ES, Jumadilova Z, Alvir J, Hussein M, et al. Patient-reported reasons for discontinuing overactive bladder medication. *BJU Int* 2010; 105: 1276-82.
- Brubaker L, Fanning K, Goldberg EL, Benner JS, Trocio JN, Bavendam T, et al. Predictors of discontinuing overactive bladder medications. *BJU Int* 2010; 105: 1283-90.
- Parasuraman S, Muralidharan S, Jaya Raja Kumar K. Mirabegron - a beta-3 adrenergic agonist for overactive bladder syndrome: a review. *Int J Res Pharm Sci* 2013; 4: 593-6.
- Kaplan S, Wein A, Staskin R, Roehrborn C, Steers W. Urinary retention and post-void residual urine in men: separating truth from tradition. *J Urol* 2008; 180: 47-54.
- Choong S, Emberton M. Acute urinary retention. *BJU Int* 2000; 85: 186-201.
- Meigs JB, Barry MJ, Giovannucci E, Rimm EB, Stampfer MJ, Kawachi I. Incidence rates and risk factors for acute urinary retention: the health professionals follow up study. *J Urol* 1999; 162: 376-82.
- Jacobsen SJ, Jacobson DJ, Girman CJ, Roberts RO, Rhodes T, Guess HA, et al. Natural history of prostatism: risk factors for acute urinary retention. *J Urol* 1997; 158: 481-7.
- Klarskov P, Andersen JT, Asmussen CF, Brenøe J, Jensen SK, Jensen IL, et al. Acute urinary retention in women: a prospective study of 18 consecutive cases. *Scand J Urol Nephrol* 1987; 21: 29-31.
- Milsom I, Abrams P, Cardozo L, Roberts RG, Thüroff J, Wein AJ. How wide spread are the symptoms of an overactive bladder and how are they managed? A population-based prevalence study. *BJU Int* 2001; 87: 760-6.
- Kelly CE. Evaluation of voiding dysfunction and measurement of bladder volume. *Rev Urol* 2004; 6: 32-37.

14. Verhamme K, Sturkenboom M, Stricker B, Bosch R. Drug-induced urinary retention: incidence, management and prevention. *Drug Saf* 2008; 31: 373-88.
15. Masumori N, Funato Y, Yamaguchi Y, Itoh K. Evaluation of Usefulness of Propiverine Hydrochloride in Poor Responders to Previous Anticholinergics. *LUTS* 2018; 10: 116-21.
16. Stöhrer M, Mürtz G, Kramer G, Warnack W, Primus G, Jinga V, et al. Efficacy and tolerability of propiverine hydrochloride extended-release compared with immediate-release in patients with neurogenic detrusor overactivity. *Spinal Cord* 2013; 51: 419-23.
17. Sussman D, Yehoshua A, Kowalski J, Lee W, Kish J, Chaudhari S, et al. Adherence and persistence of mirabegron and anticholinergic therapies in patients with overactive bladder: a real-world claims data analysis. *Int J Clin Pract* 2017; 71: e12824.
18. Kelleher C, Hakimi Z, Zur R, Siddiqui E, Maman K, Aballéa S, et al. Efficacy and Tolerability of Mirabegron Compared with Antimuscarinic Monotherapy or Combination Therapies for Overactive Bladder: A Systematic Review and Network Meta-analysis. *European Urology* 2018; 74: 324-33.
19. Drake MJ, MacDiarmid S, Al-Shukri S, Barkin J, Fianu-Jonasson A, Herschorn, et al. Adding Mirabegron to Solifenacin to Treat Overactive Bladder Has Little Impact on Postvoid Residual Volume or Urinary Retention Risk. *J Urol* 2017; 104: 1-4.

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## Protective Effects of Curcumin and Resveratrol on Kidney Tissue on Cadmium-induced Oxidative Stress in Rats

Sıçanlarda Kadmiyum Kaynaklı Oksidatif Strese Karşı Kurkumin ve Resveratrolün Böbrek Dokusu Üzerindeki Koruyucu Etkileri

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### ABSTRACT

**Objective:** Cadmium (Cd) is a well-known widespread environmental pollutant and is not rapidly excreted by the kidneys; instead, it accumulates and causes kidney damage. This study aimed to compare the effects of antioxidant curcumin and resveratrol on antioxidant defense in Cd-induced rat kidney tissue.

**Methods:** In the study, 36 (200-250 gr) Wistar albino rats were divided into 6 (n=6) groups. Group 1: control; group 2: CdCl<sub>2</sub>; group 3: curcumin; group 4: CdCl<sub>2</sub> + curcumin; group 5: resveratrol; group 6: CdCl<sub>2</sub> + resveratrol. At the end of the experiment, malondialdehyde (MDA), total oxidant capacity (TOC), and total antioxidant capacity (TAC) activities were measured in kidney tissues.

**Results:** In the CdCl<sub>2</sub>-treated group, oxidative stress index (OSI), TOC, and MDA levels increased compared with the control group, and TAC values decreased (p<0.05). In the case of resveratrol or curcumin administered with Cd, TAC levels increased, MDA levels, and OSI values decreased compared with the group administered only Cd (p<0.05).

**Conclusion:** Both resveratrol and curcumin may have protective effects in the kidneys against CdCl<sub>2</sub>-induced oxidative damage.

**Keywords:** Cadmium, curcumin, resveratrol, kidney damage

### ÖZ

**Amaç:** Kadmiyum (Cd) bilinen yaygın bir çevre kirleticidir ve böbrekler tarafından hızla atılmaz, birikir ve böbrek hasarına neden olur. Bu çalışma, Cd kaynaklı sıçan böbrek dokusunda antioksidan kurkumin ve resveratrolün antioksidan savunma üzerindeki etkilerini karşılaştırmayı amaçlamaktadır.

**Yöntem:** Çalışmada 42 (200-250 gr) Wistar albino rat 6 (n=6) gruba ayrıldı. Grup 1: kontrol; grup 2: CdCl<sub>2</sub>; grup 3: kurkumin; grup 4: CdCl<sub>2</sub> + kurkumin; grup 5: resveratrol; grup 6: CdCl<sub>2</sub> + resveratrol oluşturuldu. Deney sonunda böbrek dokularında malondialdehit (MDA), toplam oksidan kapasite (TOK) ve toplam antioksidan kapasite (TAK) aktiviteleri ölçüldü.

**Bulgular:** CdCl<sub>2</sub> verilen grupta oksidatif stres indeksi (OSI), TOK ve MDA seviyeleri kontrol grubuna göre arttı, TAK değerleri azaldı (p<0,05). Cd ile birlikte resveratrol veya kurkumin verilmesi durumunda, sadece Cd verilen gruba göre TAK düzeyleri yükselmiş, MDA düzeyleri ve OSI değerleri düşmüştür (p<0,05).

**Sonuç:** Hem resveratrol hem de kurkumin böbreklerde CdCl<sub>2</sub>'nin neden olduğu oksidatif hasara karşı koruyucu etkilere sahip olabilir.

**Anahtar Sözcükler:** Kadmiyum, kurkumin, resveratrol, böbrek hasarı

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## INTRODUCTION

Cadmium is widely used in the manufacture of paints, plastics, nickel-cadmium batteries, and in the galvanic coating industry. Exposure sources for living organisms are water, air, and soil. The sources of inhalation exposure are industrial activities, the burning of fossil fuels, and smoking (1,2). Cadmium is highly accumulated in the kidney, liver, pancreas, and lung. Cadmium indirectly generates reactive oxygen-nitrogen species, including superoxide, hydroxyl, and nitric oxide radicals. The indirect role of this metal in free radical formation is its replacement by iron and copper found in cytoplasmic and membrane proteins. The free and weakly bound copper and iron ion levels increase in the Fenton reaction. Copper plays a role in the degradation of hydrogen peroxide through the Fenton reaction and causes oxidative stress and pathological disorders in the liver, kidney, and brain. Lipid peroxidation is the primary mechanism of cadmium poisoning resulting from oxidative stress. Free radicals invade the cell membrane, rendering it unstable, and disrupt the cell membrane structure because of lipid peroxidation (3-5). The mechanisms of acute poisoning with cadmium are in the form of depletion of glutathione, binding to sulfhydryl groups in the protein structure, formation of superoxide ions, and increase of reactive oxygen species (ROS). Cadmium-mediated increased free oxygen groups cause lipid peroxidation and subsequent DNA destruction. Cadmium is not rapidly excreted by the kidneys, but accumulates and causes kidney damage. It also increases the tendency for kidney stone formation. There is a defense mechanism called an antioxidant that prevents the harmful effects of ROS (6-8).

Curcumin is obtained from turmeric (Indian saffron), which is a yellow spice. Curcumin has a wide spectrum of effects, including anti-inflammatory, antioxidant, anticarcinogenic, antidiabetic, antiviral, and neuroprotective effects. It facilitates the removal of many reactive oxygen radicals, especially superoxide anions. In addition, it has been reported to scavenge ROS, inhibit lipid peroxidation, and protect cellular macromolecules from oxidative damage (9).

Resveratrol is a powerful antioxidant, and its osteogenic, anti-inflammatory, and analgesic effects have been described. Resveratrol prevents free radical formation. Its antioxidant activity is attributed to the ribonucleotide, reductase inhibition ability, and cyclooxygenase transcription ability in DNA polymerase activity. Scavenges hydroxyl and superoxide radicals inhibit lipid peroxidation caused by hydroxyl radicals, preventing DNA damage and LDL oxidation. Studies have shown that resveratrol plays a regulatory role in inflammatory events, atherosclerosis, and carcinogenesis. In addition, the antioxidant, anti-cyclooxygenase, lipid, and lipoprotein metabolism-regulating effects of resveratrol have also been demonstrated (10-14).

The objective of this project is to determine the protective effect of curcumin and resveratrol, which have antioxidant properties, in kidney tissue against cadmium-induced oxidative stress in rats. In recent years, studies have focused on researching and developing new drugs with antioxidant properties against cadmium toxicity. In the literature review, no study compared the protective effect of curcumin and resveratrol against cadmium toxicity. In this project, we determined the protective effect of curcumin and resveratrol against cadmium-induced oxidative stress in kidney tissue by comparing them.

## MATERIALS AND METHODS

### Chemicals

The rats received CdCl<sub>2</sub> (Merck Millipore, Billerica, Massachusetts, United States) intraperitoneally (I.P.) at a dose of 5 mg/kg/day (15), curcumin (Sigma Co., MO, USA) I.P. at a dose of 200 mg/kg/day for 4 weeks (16) and Resveratrol (Tocris Bioscience, Bristol, UK) of 10 mg/kg/day was given to the group 5 and group 6 through gavage for 4 weeks (17).

### Study Design and Animals

The study procedures were conducted under the guidelines approved by the Local Ethics Committee for animal experiments at the University of Çanakkale Onsekiz Mart Faculty of Medicine (approval number: 2021-02-07). This work was supported by the Çanakkale Onsekiz Mart University Scientific Research Coordination Unit (project number: THD-2021-3626). Animal housing and experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals. Wistar albino rats weighing (200±25 g) were maintained in clean plastic cages under standard temperature and humidity conditions. The animals were fed a standard laboratory pellet diet and sterile water. The animals were kept at 25 °C under a 12-h light/12-h dark cycle, with free access to water and food. Inclusion criteria in this study were (a) healthy rats and no abnormalities, (b) 4-month-old female, and (c) weight >250 g. The exclusion criteria were (a) disability or disorder rats and (b) the dead rats after treatment, and (c) male rats.

Sample size calculations were made using the G\*Power 3.1.9.4 program, considering the studies in the literature (18). To achieve power =0.8 and alpha =0.05 to detect this difference would require a total of 36 animals.

There was a randomized design into six groups as follows (six rats in each group):

- Group 1: control,
- Group 2: CdCl<sub>2</sub> (5 mg/kg, I.P.) for 10 days,
- Group 3: Curcumin (200 mg/kg/day gavage) for 4 weeks,
- Group 4: CdCl<sub>2</sub> (5 mg/kg, I.P.) 10 days + curcumin (200 mg/kg/day gavage) for 4 weeks (from the day of cadmium administration),
- Group 5: Resveratrol (10 mg/kg/day gavage) for 4 weeks,
- Group 6: CdCl<sub>2</sub> (5 mg/kg, I.P.) 10 days + resveratrol (10 mg/kg/day gavage) for 4 weeks (from the day of cadmium administration).

All experimental procedures were performed under ketamine/xylazine anesthesia. At the end of the treatment, rats were sacrificed under ketamine/xylazine anesthesia (19). No animals died because of medication.

### Spectrophotometric Analysis

The kidneys were washed in ice-cold 1.15% KCl and homogenized. The homogenate was centrifuged at 14,000 rpm for 30 min, and assays were performed on the resultant supernatant. Protein concentration was estimated using the method of Lowry et al. (20). Tissue samples taken for malondialdehyde determination were homogenized and subjected to procedures as outlined previously (21). TAC and TOC levels were measured by a spectrophotometric assay using commercially available kits (Rel Assay Diagnostics,

**Table 1.** Spectrophotometric analysis results

Group	TAC ( $\mu\text{mole H}_2\text{O}_2$ Equiv./gram protein)	TOC ( $\mu\text{mole H}_2\text{O}_2$ Equiv./gram protein)	OSI	MDA (nmol/g)
1	3.52±0.59	10.88±0.41	0.31±0.05	1.63±0.47
2	2.68±0.48 <sup>5p</sup>	13.48±1.10 <sup>5p</sup>	0.51±0.09 <sup>5p</sup>	3.16±0.41 <sup>5p</sup>
3	3.30±0.67	12.43±0.54 <sup>1p</sup>	0.38±0.07	1.48±0.22
4	3.68±0.98 <sup>2p</sup>	12.66±1.56	0.37±0.09 <sup>2p</sup>	2.55±0.28 <sup>2p</sup>
5	4.07±0.33	13.27±0.53 <sup>2p</sup>	0.32±0.03	1.51±0.24
6	3.89±0.30*	12.02±1.53	0.30±0.06*	2.52±0.16*

Group 1: Control group, Group 2: CdCl<sub>2</sub>, Group 3: Curcumin, Group 4: CdCl<sub>2</sub> + Curcumin, Group 5: Resveratrol, Group 6: CdCl<sub>2</sub> + Resveratrol, OSI:  $[(\text{TOC}, \mu\text{mole H}_2\text{O}_2 \text{ Equiv./gram protein})/(\text{TAC}, \mu\text{mole H}_2\text{O}_2 \text{ Equiv./gram protein})] \times 100$ . Group comparisons: <sup>5p</sup>: Group1 and group 2, <sup>1p</sup>: Group 1 and group 3, <sup>2p</sup>: Group1 and group 5, \*p: Group 2 and group 4, \*Group 2 and group 6. TAC: Total antioxidant capacity, TOC: Total oxidant capacity, OSI: Oxidative stress index, MDA: Malondialdehyde.

Türkiye). OSI was defined as the ratio of the TAC level to the TOC level.

### Statistical Analysis

Values are presented as means ± standard deviation. Statistical analysis was performed using SPSS, version 19.0 (SPSS, IBM Company). Comparison between the two groups for continuous variables was performed using the Mann-Whitney U test. Multiple comparisons were performed by One-Way analysis of variance (ANOVA). P-values 0.05 were accepted as the significance level.

## RESULTS

In group 2 given CdCl<sub>2</sub>, TOC, OSI, and MDA levels increased compared with the control group, and TAC values decreased (p<0.05). TAC levels increased, MDA levels, and OSI values decreased in group 4 and group 6 compared with group 2 (p<0.05). TOC value increased in group 3 and group 5 compared with group 1 (p<0.05) (Table 1).

## DISCUSSION

Cadmium is a heavy metal that is associated with pathological changes in target organs, including the lung, liver, and kidney, and causes serious health problems, even at low exposure levels. In many studies, the toxic and carcinogenic effects of cadmium on human health have been investigated. Heavy metals such as Cd+2 cause oxidative stress by disrupting the redox balance in cells. Many studies have reported that Cd+2 toxicity causes damage to biological components of the cell in humans and animals. Cd+2 reduces the GSH content of the cell and the activities of enzymes such as SOD, peroxidases, and CAT, causing ROS accumulation and oxidative stress increment. In other studies, it has been stated that cadmium causes an increase in malondialdehyde levels, which is an indicator of lipid peroxidation, and a decrease in superoxide dismutase and glutathione peroxidase values, which are antioxidant enzymes in organs such as the liver and lungs (6,7,22,23). In our study, TOC, OSI, and MDA levels increased in the Cd+2 administered group compared with the control group, whereas the TAC value decreased. This explains the increase in ROS formation and the inadequacy of the antioxidant defense system among the toxic action mechanisms of Cd+2.

In recent studies, curcumin has attracted attention for its potential antioxidant or anti-apoptotic properties. Curcumin has many beneficial properties, including antioxidant and anti-inflammatory actions (24-26). In this study, it was observed that the TAC level increased and the MDA level and OSI value decreased in the group administered curcumin with Cd. On the basis of our results, we can say that curcumin may benefit kidney tissue in cadmium-induced oxidative stress.

It has been reported that resveratrol prevents oxidative stress-induced tissue damage by preventing the oxidation of membrane lipids and enhancing antioxidant capacity. It has been reported that resveratrol scavenges free radicals (O<sub>2</sub><sup>-</sup>, OH<sup>·</sup>) in the cell culture medium and prevents the peroxidation of membrane lipids, which develops due to the radical production increased by chromium exposure (27-29). In this study, it was observed that the TAC level increased and the MDA level and OSI value decreased in the group administered resveratrol with Cd. Because of the hydroxyl groups it has, resveratrol donates a hydrogen electron and becomes OH, and prevents peroxidation of cell membranes by scavenging O<sub>2</sub> radicals. According to our results, resveratrol contributes to the cell defense system by both reducing the increased radical production caused by Cd and increasing the expression of antioxidant enzymes.

### Study Limitations

It is important to acknowledge the limitations of the current study. Because only female rats used in the experiments, these findings might not apply to male rats. Further studies that include different genders, ages, etc. are necessary. Future research should consider this difference.

## CONCLUSION

As a result, both resveratrol and curcumin support the defense system of cells by scavenging free radicals that increase the oxidative damage caused by Cd in the kidneys. More extensive studies are required on this subject.

**Acknowledgments:** This work was approved by Çanakkale Onsekiz Mart University, Scientific Research Unit (THD-2021-3626).



## Ethics

**Ethics Committee Approval:** The study procedures were conducted under the guidelines approved by the Local Ethics Committee for animal experiments at the University of Çanakkale Onsekiz Mart Faculty of Medicine (approval number: 2021-02-07).

**Informed Consent:** This study does not apply because it involves animal subjects.

## Authorship Contributions

Surgical and Medical Practices: S.C., Ş.Ö., Concept: S.C., L.C.İ., Ş.Ö., Design: S.C., L.C.İ., Ş.Ö., Data Collection or Processing: S.C., L.C.İ., Ş.Ö., Analysis or Interpretation: S.C., L.C.İ., Ş.Ö., Literature Search: S.C., L.C.İ., Ş.Ö., Writing: S.C., L.C.İ., Ş.Ö.

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

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## REFERENCES

- Patrick L. Toxic metals and antioxidants: part II. The role of antioxidants in arsenic and cadmium toxicity. *Altern Med Rev* 2003; 8: 106-28.
- Rani A, Kumar A, Lal A, Pant M. Cellular mechanisms of cadmium-induced toxicity: a review. *Int J Environ Health Res* 2014; 24: 378-99.
- Ingawale DK, Mandlik SK, Naik SR. Models of hepatotoxicity and the underlying cellular, biochemical and immunological mechanism(s): a critical discussion. *Environ Toxicol Pharmacol* 2014; 37: 118-33.
- Jomova K, Valko M. Advances in metal-induced oxidative stress and human disease. *Toxicology* 2011; 283: 65-87.
- Wiwaniitkit V. Minor heavy metal: A review on occupational and environmental intoxication. *Indian J Occup Environ Med* 2008; 12: 116-21.
- Liu J, Qian SY, Guo Q, Jiang J, Waalkes MP, Mason RP, et al. Cadmium generates reactive oxygen- and carbon-centered radical species in rats: insights from in vivo spin-trapping studies. *Free Radic Biol Med* 2008; 45: 475-81.
- Liu J, Qu W, Kadiiska MB. Role of oxidative stress in cadmium toxicity and carcinogenesis. *Toxicol Appl Pharmacol* 2009; 238: 209-14.
- Wu X, Cobbina SJ, Mao G, Xu H, Zhang Z, Yang L. A review of toxicity and mechanisms of individual and mixtures of heavy metals in the environment. *Environ Sci Pollut Res Int* 2016; 23: 8244-59.
- Naik SR, Thakare VN, Patil SR. Protective effect of curcumin on experimentally induced inflammation, hepatotoxicity and cardiotoxicity in rats: evidence of its antioxidant property. *Exp Toxicol Pathol* 2011; 63: 419-31.
- Cho J. Antioxidant and neuroprotective effects of hesperidin and its aglycone hesperetin. *Arch Pharm Res* 2006; 29: 699-706.
- Cottart CH, Nivet-Antoine V, Laguillier-Morizot C, Beaudeau JL. Resveratrol bioavailability and toxicity in humans. *Mol Nutr Food Res* 2010; 54: 7-16.
- Das S, Lin HS, Ho PC, Ng KY. The impact of aqueous solubility and dose on the pharmacokinetic profiles of resveratrol. *Pharm Res* 2008; 25: 2593-600.
- Dimpfel W. Different anticonvulsive effects of hesperidin and its aglycone hesperetin on electrical activity in the rat hippocampus in vitro. *J Pharm Pharmacol* 2006; 58: 375-9.
- Kumar A, Lalitha S, Mishra J. Hesperidin potentiates the neuroprotective effects of diazepam and gabapentin against pentylenetetrazole-induced convulsions in mice: Possible behavioral, biochemical and mitochondrial alterations. *Indian J Pharmacol* 2014; 46: 309-15.
- Hu J, Zhang B, Du L, Chen J, Lu Q. Resveratrol ameliorates cadmium induced renal oxidative damage and inflammation. *Int J Clin Exp Med* 2017; 10: 7563-72.
- Sankrityayan H, Majumdar AS. Curcumin and folic acid abrogated methotrexate induced vascular endothelial dysfunction. *Can J Physiol Pharmacol* 2016; 94: 89-96.
- Wiciński M, Malinowski B, Węclewicz MM, Grzešek E, Grzešek G. Anti-atherogenic properties of resveratrol: 4-week resveratrol administration associated with serum concentrations of SIRT1, adiponectin, S100A8/A9 and VSMCs contractility in a rat model. *Exp Ther Med* 2017; 13: 2071-8.
- Othman MS, Nada A, Zaki HS, Abdel Moneim AE. Effect of Physalis peruviana L. on cadmium-induced testicular toxicity in rats. *Biol Trace Elem Res* 2014; 159: 278-87.
- Cosar R, Yurut-Caloglu V, Eskiocak S, Ozen A, Altaner S, Ibis K, et al. Radiation-induced chronic oxidative renal damage can be reduced by amifostine. *Med Oncol* 2012; 29: 768-75.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193: 265-75.
- Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; 95: 351-8.
- Anetor JI. Rising Environmental Cadmium Levels in Developing Countries: Threat to Genome Stability and Health. *Niger J Physiol Sci* 2012; 27: 103-15.
- Lee JC, Son YO, Pratheeshkumar P, Shi X. Oxidative stress and metal carcinogenesis. *Free Radic Biol Med* 2012; 53: 742-57.
- Biswas SK, McClure D, Jimenez LA, Megson IL, Rahman I. Curcumin induces glutathione biosynthesis and inhibits NF-kappaB activation and interleukin-8 release in alveolar epithelial cells: mechanism of free radical scavenging activity. *Antioxid Redox Signal* 2005; 7: 32-41.
- Corona-Rivera A, Urbina-Cano P, Bobadilla-Morales L, Vargas-Lares Jde J, Ramirez-Herrera MA, Mendoza-Magaua ML, et al. Protective in vivo effect of curcumin on copper genotoxicity evaluated by comet and micronucleus assays. *J Appl Genet* 2007; 48: 389-96.
- Menon VP, Sudheer AR. Antioxidant and anti-inflammatory properties of curcumin. *Adv Exp Med Biol* 2007; 595: 105-25.
- Gerogiannaki-Christopoulou M, Athanasopoulos P, Kyriakidis N, Gerogiannaki IA, Spanos M. Trans Resveratrol in wines from the major Greek red and white grape varieties. *Food Control* 2006; 17: 700-6.
- Signorelli P, Ghidoni R. Resveratrol as an anticancer nutrient: molecular basis, open questions and promises. *J Nutr Biochem* 2005; 16: 449-66.
- Soleas GJ, Diamandis EP, Goldberg DM. Resveratrol: a molecule whose time has come? And gone? *Clin Biochem* 1997; 30: 91-113.

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## Mortality Predictors using Chest Computed Tomography Findings in COVID-19 Patients

### COVID-19 Hastalarının Bilgisayarlı Göğüs Tomografisi Bulgularındaki Mortalite Belirteçleri

© Ali Yavuz Uzun<sup>1</sup>, © Yılmaz Üçüncü<sup>1</sup>, © Nur Hürsoy<sup>2</sup>, © Fatma Beyazal Çeliker<sup>2</sup>, © Zihni Yazıcı<sup>3</sup>

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#### ABSTRACT

**Objective:** The coronavirus disease (COVID) pandemic is still ongoing. Computed tomography (CT) is widely used in coronavirus disease-2019 (COVID-19) patients for lung damage determination. The aim of this study was to investigate the relationship between mortality rates and measurements of intrathoracic anatomical structures using CT images.

**Methods:** This retrospective study was conducted in a single center and included a total of 322 cases, namely, 147 deceased and 175 surviving patients. All patients were diagnosed with COVID-19 on the basis of a positive polymerase chain reaction test. Total lung volumes, diameters of major vascular structures, comorbidity status, and laboratory blood tests were measured or determined. Total lung volumes were calculated using the range of -1024 to -300 Hounsfield Unit in the Slicer application.

**Results:** A decrease in total lung volume was associated with a higher mortality rate ( $p<0.001$ ). Increases in right and left pulmonary artery diameters were associated with high mortality rate ( $p=0.002$  and  $0.001$ , respectively). Increase in cardiothoracic ratio and decrease in thorax diameter were associated with high mortality rate in female patients ( $p=0.013$  and  $p<0.001$ , respectively). It was found that patients with cardiovascular disease ( $p=0.043$ ), chronic lung disease ( $p=0.005$ ) and renal failure ( $p<0.001$ ) had a significant mortal course. Elevated values of white blood cell count ( $p=0.018$ ), aspartate aminotransferase ( $p<0.001$ ), lactate dehydrogenase ( $p<0.001$ ) and C-reactive protein ( $p<0.001$ ) were found to be associated with high mortality rates.

**Conclusion:** Total lung volume and intrathoracic main vascular sizes can be obtained from CT images using computer applications, and these measurements can provide an idea of the mortality rate in COVID-19 patients. In addition, comorbidity status and laboratory blood parameters can be used as prognostic markers.

**Keywords:** COVID-19, tomography, lung volumetry, mortality, pulmonary arteries

#### Öz

**Amaç:** Koronavirüs hastalığı (COVID) salgını etkilerini sürdürmektedir. Bilgisayarlı tomografi (BT), koronavirüs hastalığı-2019 (COVID-19) hastalarında akciğer hasarının belirlenmesinde yaygın olarak kullanılmaktadır. Bu çalışmanın amacı, BT görüntüleri kullanılarak intratorasik anatomik yapıların ölçümleri ile mortalite oranları arasındaki ilişkinin araştırılmasıdır.

**Yöntemler:** Bu retrospektif çalışma tek merkezde gerçekleştirildi ve 147'si ölen, 175'i hayatta kalan toplam 322 olguyu içeriyordu. Tüm hastalara pozitif polimeraz zincir reaksiyonu testi temelinde COVID-19 tanısı konuldu. Toplam akciğer hacimleri, ana damar yapılarının çapları, komorbidite durumu ve laboratuvar kan testleri ölçüldü veya belirlendi. Toplam akciğer hacimleri Slicer uygulamasında -1024 ila -300 Hounsfield Birimi aralığı kullanılarak hesaplandı.

**Bulgular:** Total akciğer hacmindeki azalma, daha yüksek mortalite oranıyla ilişkiliydi ( $p<0,001$ ). Sağ ve sol pulmoner arter çaplarındaki artış mortalite oranıyla ilişkiliydi (sırasıyla  $p=0,002$  ve  $0,001$ ). Kadın hastalarda kardiyotorasik oranın artması ve toraks çapındaki azalmanın yüksek mortalite oranı ile ilişkili olduğu görüldü (sırasıyla  $p=0,013$  ve  $p<0,001$ ). Kardiyovasküler hastalığı ( $p=0,043$ ), kronik akciğer hastalığı ( $p=0,005$ ) ve böbrek yetmezliği ( $p<0,001$ ) olan hastaların yüksek mortalite riskinin olduğu belirlendi. Beyaz küre sayısı ( $p=0,018$ ), aspartat aminotransferaz ( $p<0,001$ ), laktat dehidrojenaz ( $p<0,001$ ) ve C-reaktif protein ( $p<0,001$ ) değerlerindeki artışın, yüksek ölüm oranlarıyla ilişkili olduğu belirlendi.

**Sonuç:** Bilgisayar uygulamaları kullanılarak BT görüntülerinden toplam akciğer hacmi ve intratorasik ana damar boyutları elde edilebilmekte ve bu ölçümler, COVID-19 hastalarındaki ölüm oranı hakkında fikir verebilmektedir. Ayrıca komorbidite durumu ve laboratuvar kan parametreleri de prognostik belirteç olarak kullanılabilir.

**Anahtar Sözcükler:** COVID-19, tomografi, akciğer hacmi, mortalite, pulmoner arterler

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## INTRODUCTION

Coronaviruses are a family of viruses with two subgroups belonging to the Nidovirales family and the Torovirina group. Coronaviruses (CoVs) are enveloped RNA viruses with a wide disease network that infect mammals and birds. Human pathogens generally affect the respiratory tract. There are six known subgroups that affect humans (1). Four of them, 229E, OC42, NL63, and HKU1, cause common cold, whereas the remaining two types, severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) and middle east respiratory syndrome-coronavirus (MERS-CoV), can cause serious and in some cases fatal infections (2). In December 2019, a new type was identified in the Wuhan region of China, which was different from MERS-CoV and SARS-CoV and was named SARS-CoV-2 (2). The diagnosis of coronavirus disease-2019 (COVID-19) infection is mainly based on the detection of the virus in respiratory samples by reverse transcriptase polymerase chain reaction (PCR) (3). Chest computed tomography (CT) has become a powerful method for the diagnosis of COVID-19 (4). The aim of this study was to determine whether there are some differences in mortality rates between deceased and surviving patients using chest CT. In the case of obtaining meaningful data, chest CT may be useful in determining prognosis or prompt management such as treatment.

## MATERIALS AND METHODS

**Study design:** This study was conducted with the approval of the Ethics Committee of Recep Tayyip Erdoğan University Faculty of Medicine (approval number: 2021/73, date: 26.04.2021). Because the study was conducted retrospectively, no volunteers were used.

**Data sources:** The study was retrospectively conducted on 147 deceased and 175 surviving patients, aged 50 and over, who visited the adult COVID polyclinic of Recep Tayyip Erdoğan University Faculty of Medicine Training and Research Hospital between March 2020 and May 2021. Patients who were diagnosed with COVID-19 on a positive PCR test and had lung involvement on CT imaging were included in the study. Patients with pleural effusion, pneumothorax, intrathoracic mass, and pulmonary hypertension were not included in the study. In addition, patients diagnosed with non-COVID pneumonia or lacking chest CT imaging at the first admission to the hospital were excluded. Blood parameters obtained at the time of admission or, if hospitalized, the blood parameters within the first 24 hours were included in the study. The measured parameters included leukocytes, neutrophils, lymphocytes, hemoglobin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), C-reactive protein (CRP), D-dimer, fibrinogen, international normalized ratio (INR), and neutrophil-to-lymphocyte ratio (NLR). AST in one patient, LDH in five patients, CRP in one patient, D-dimer in 23 patients, fibrinogen in 34 patients, and INR in 21 patients were observed to be deficient. The comorbidity status of the cases, cardiovascular system disease, diabetes mellitus (DM), chronic lung disease, malignancy, renal failure, and hepatic failure, was determined and recorded (5). Patients were allocated into the deceased and surviving groups. For the survivor group, patients with a history of intensive care unit admission were not included.

**Chest CT and quantitative analysis:** The images were taken with a 16-detector tomography device (Toshiba Alexion, Japan) in the hospital emergency unit, in the supine position, and in deep

inhalation. The drawing characteristics were as follows: tube current (50-300 mA), voltage (120 kV), and cross-sections (between 0.625 and 5 mm). CT images of the patients were taken from the radiology system as DICOM files. Later, these images were opened on the application Slicer (ver 4.11.20210226) (6). By selecting the range of -1024 to -300 Hounsfield unit on the slicer application, the three-dimensional shape of the lungs was extracted, and the volume of the lungs was calculated by deleting the large airways (Figure 1) (6-8). Then, the images of the patients were opened through the application called RadiAnt DICOM viewer (ver 2020.2.3), and their vascular, tracheal, and cardiothoracic measurements were made through this program. The diameters of the right pulmonary artery (RPA), left pulmonary artery (LPA), ascending aorta (AA), and pulmonary trunk (PT) were measured from the bifurcation point of the PT (Figure 2) (9-11). The ratio of the diameter of the PT to that of the AA was also recorded. Transversely, thoracic diameters were measured from the widest measurable distance between the inner walls of the thorax, and cardiac diameters were measured from the outer surface of the heart where the widest value was captured (Figure 3) (12). Transverse and anteroposterior diameters of the trachea were measured along the inner lumen of the trachea

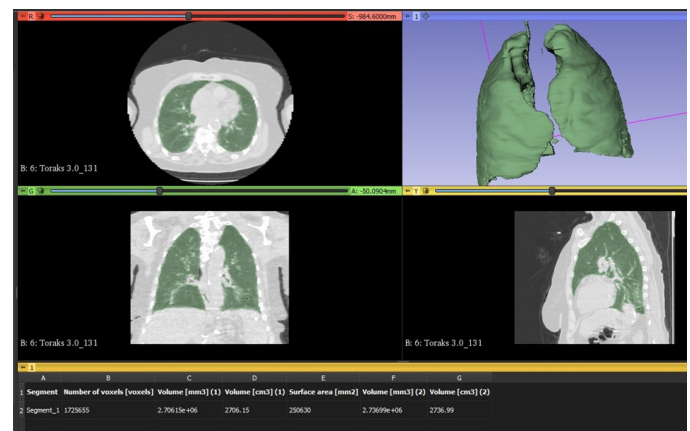


Figure 1. Lung volume measurement with slicer application.

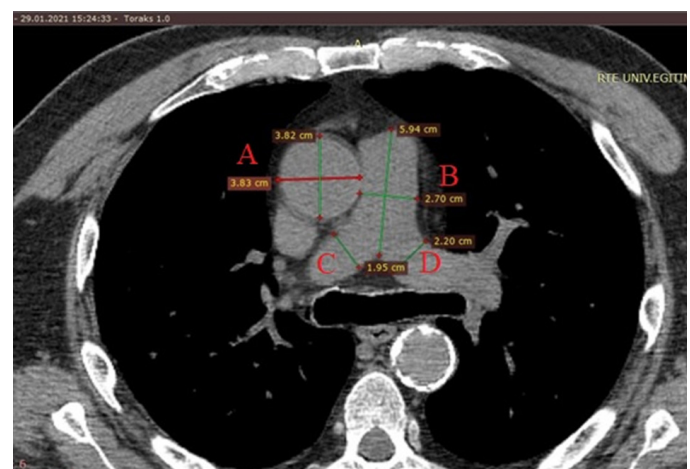
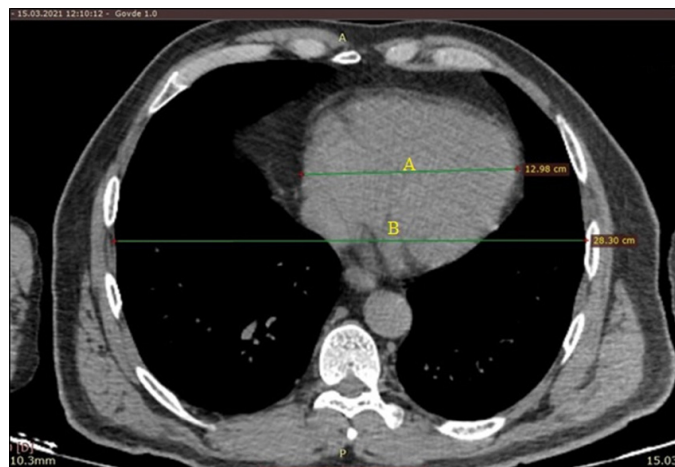


Figure 2. Vascular measurements. (A) Ascending aorta, (B) pulmonary trunk, (C) right pulmonary artery, (D) left pulmonary artery (measurements were made at the bifurcation level of the pulmonary artery).

from axial sections passing 2 cm above the apex of the arcus aorta in the sagittal images. The ratio of the transverse diameter to the anteroposterior diameter was also recorded (Figure 4) (12,13). Because the imaging of 13 of the cases was taken in external centers on the same day, they could not be recorded in the computer environment and their measurements could not be made. These cases were evaluated using only laboratory parameters. In addition, an error was encountered while calculating the volumes of 12 cases using DICOM images, and the Slicer application and volume measurement could not be performed. The imaging thickness of cross-sections: one case 0.625 mm, six cases 5 mm, and all the remaining cases 1 mm.

### Statistical Analysis

IBM SPSS Statistics 25.0 (New York, USA) program was used for statistical calculations. For parametric tests, it was first checked whether it showed a normal distribution using the Kolmogorov-Smirnov test. Then, those with normal distribution were evaluated using the independent samples t-test, whereas those without

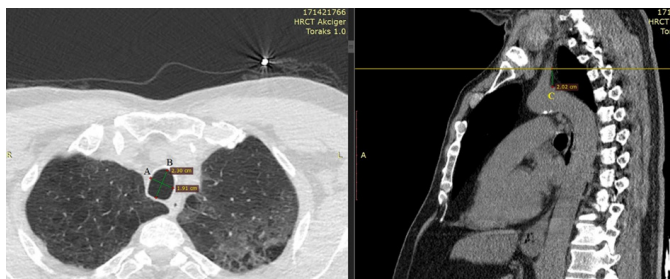


**Figure 3.** Thorax and cardiac diameters. (A) Cardiac diameter, (B) Thorax diameter, (A, B) Cardiothoracic ratio.

normal distribution were evaluated using the Mann-Whitney U test. Non-parametric tests were evaluated using the chi-square test. Pearson's chi-square test or Fisher's test was used according to the number of cells. Normally distributed parameters are given as mean  $\pm$  standard deviation. Those that do not fit the normal distribution are given as the median value and the 25% and 75% percentile values in parentheses. SPSS receiver operating characteristic (ROC) curve analysis was used to determine the cut-off value for the parameters. Any p-value of  $<0.05$  was considered statistically significant.

### RESULTS

**Patient characteristics:** The characteristics, comorbidities, and laboratory findings of the patients are listed in Tables 1, 2. A total of 322 people, 147 deceased and 175 surviving patients, were included in the study. The age range was 51-89, with a mean of  $71.07 \pm 10.88$  and a median value of 71. The age range of the deceased group was between 53-96, with a mean of  $74.39 \pm 10.24$  and a median of 75, while the age range of the surviving group was 51-95, with a mean of  $68.28 \pm 10.64$  and a median value of 66. The mortality rate increased with age and was found to be statistically significant ( $p < 0.001$ ). While there were 97 (66%) men and 50 (34%) women in the deceased group, there were 93 (53.1%) men and 82 (46.9%) women in the surviving group. There was a significant difference in favor of males between those who died and surviving patients



**Figure 4.** Tracheal measurements. (A) Trachea transverse diameter, (B) Trachea anteroposterior diameter, (C) detecting the level 2 cm above the aortic arch, (A, B) Tracheal index.

**Table 1.** Clinical features and comorbidities of patients

Parameters		Patients			
		All, (n=322)	Deceased, (n=147)	Survived, (n=175)	p-value
Age	All	71.07 $\pm$ 10.88	74.39 $\pm$ 10.24	68.28 $\pm$ 10.64	<0.001*
	Male	69.96 $\pm$ 10.26	71.86 $\pm$ 9.68	67.99 $\pm$ 10.53	0.009*
	Female	72.67 $\pm$ 11.57	79.32 $\pm$ 9.58	68.61 $\pm$ 10.82	<0.001*
Gender	Male	190	97	93	
	Female	132	50	82	0.02*
Comorbidity status	Cardiovascular disease	225 (69.8%)	111 (75%)	114 (65%)	0.043*
	Diabetes mellitus	108 (33.5%)	54 (26%)	53 (30%)	0.22
	Chronic lung disease	39 (17.5%)	26 (17.6%)	13 (7.4%)	0.005*
	Renal failure	41 (12.7%)	30 (20.4%)	11 (6.2%)	<0.001*
	Malignancy	32 (9.9%)	19 (12.9%)	13 (7.4%)	0.101
	Hepatic failure	3 (0.1%)	2 (1.3%)	1 (0.6%)	0.594

\* $p < 0.05$  indicates a significant difference.

( $p=0.02$ ). It was found that patients with cardiovascular system disease ( $p=0.043$ ), chronic lung disease ( $p=0.005$ ), and renal failure ( $p<0.001$ ) had a more mortal course. No significant difference was observed between the deceased and surviving groups for DM, malignancy, and hepatic failure.

**Laboratory findings:** Increased white blood cell ( $p=0.018$ ), increased neutrophil ( $p<0.001$ ), decreased lymphocyte ( $p<0.001$ ), increased NLR ( $p<0.001$ ), increased AST ( $p<0.001$ ), increased LDH ( $p<0.001$ ), increased CRP ( $p<0.001$ ), high D-dimer ( $p=0.003$ ) and high INR ( $p<0.001$ ) were found to be associated with mortality rate. There was no significant difference between the two groups for platelet, ALT, hemoglobin, and fibrinogen values. Details are given in Table 2.

**Lung volumes and morphometric measurements:** Details of the measurements are given in Table 3. Total lung volumes were found to be lower in deceased patients than in surviving patients in both sexes ( $p<0.001$ ). Right and LPA diameters were found to be greater in deceased female patients than in surviving female patients ( $p<0.001$ ). In male patients, there was no significant difference in the right and LPA diameters between the deceased and surviving patients, unlike female patients. No significant difference was observed between the deceased and surviving patients in terms of PT diameter, AA diameter, ratio of PT to AA, cardiac diameter, transverse tracheal diameter, and tracheal index values. Thorax diameters were found to be significantly lower in the deceased than in the surviving female patients ( $p<0.001$ ). The cardiothoracic ratio was found to be significantly higher in the deceased than in the surviving female patients ( $p=0.013$ ). All numerical parameters considered in the study were evaluated with ROC analysis in terms of death and survival status, and only the total lung volumes of female patients were found as the parameter providing area under the curve (AUC)  $>0.70$ . According to the statistical analysis, the cut-off value was found to be 2547 mL, but the sensitivity was 70% and the specificity was 61%. In the ROC analysis for male patients, AUC=0.69 was calculated, and the cut-off value was 3961 mL. The sensitivity

value was 68% and the specificity was 60%. Running ROC analysis for the remaining parameters did not yield an AUC  $>0.7$ .

## DISCUSSION

The COVID-19 pandemic, which emerged toward the end of 2019, persists at the time of writing, even after the introduction of specific vaccines. Although the gold standard diagnosis of the disease is based on PCR, thoracic imaging methods are frequently used to provide an early treatment approach, especially in patients with respiratory distress, because of their long-term observations. At the same time, blood tests from patients are also studied, and their clinical course is arranged accordingly.

Jin et al. (14) reported that male patients showed a worse prognosis in their study on gender factors. Colombi et al. (5) did not find patients with chronic lung disease to have a more mortal course, unlike our study. Eslami et al. (12) did not find patients with hypertension and cardiovascular system disease to have a poor prognosis, unlike our study. Although there are differences between studies, in general, patients with cardiovascular system disease, DM, chronic lung disease, and renal failure have a poor prognosis. The different interpretations of comorbidity conditions among studies may be related to the small number of cases included.

Liao et al. (15) found platelet counts and fibrin degradation products to predict the severity and prognosis of patients with COVID-19. While the decrease in platelet counts was not found to be significant regarding mortality rate in our study, there are many studies in the literature that find the decrease to be significant. According to the meta-analysis study of Henry et al. (16), the increase in white blood cell counts and the decrease in platelet counts were found to be significant. While the increase in ALT value was found to be significant in some studies, it was found to be insignificant in our study. It should be noted that in other studies, blood parameters were categorized as normal, decreased, or increased. In our study, these parameters were handled as direct numerical values, and a

**Table 2.** Laboratory findings of the patients

Parameters	Deceased	Survivor	Total	p-value
WBC ( $\times 10^3/\mu\text{L}$ )	6.77 (5.04/9.61)	6.03 (4.8/7.7)	6.26 (5.0/8.51)	0.018*
Neutrophil ( $\times 10^3/\mu\text{L}$ )	4.93 (3.56/7.93)	4.07 (3.2/5.61)	4.33 (3.32/6.67)	$<0.001^*$
Lymphocyte ( $\times 10^3/\mu\text{L}$ )	0.88 (0.62/1.42)	1.3 (0.92/1.66)	1.1 (0.76/1.6)	$<0.001^*$
NLR (%)	6 (3.24/9.38)	3.21 (2.23/4.9)	4.2 (2.46/6.89)	$<0.001^*$
Platelet ( $\times 10^3/\mu\text{L}$ )	166 (137/224)	184 (150/235)	175 (143/231)	0.051
Hemoglobin (mg/dL)	12.94 $\pm$ 1.94	13.21 $\pm$ 1.49	13.09 $\pm$ 1.71	0.153
AST (IU/L)	40 (28/58)	31 (23/39)	33 (25/51)	$<0.001^*$
ALT (IU/L)	22 (16/33)	21 (14/32)	22 (15.8/32.3)	0.252
LDH (IU/L)	361 (260/519)	275 (218/343)	300 (233/418)	$<0.001^*$
CRP (mg/L)	122.8 (59/193.6)	60.5(18.9/119.5)	81.9 (27.2/147.8)	$<0.001^*$
D-Dimer ( $\mu\text{gFEU/mL}$ )	0.79 (0.44/1.82)	0.67 (0.37/0.91)	0.68 (0.40/1.14)	0.003*
Fibrinogen(mg/dL)	498 (439/610)	490 (415/580)	492 (422/659)	0.176
INR	1.05 (0.97/1.15)	0.99 (0.94/1.08)	1.01 (0.95/1.12)	$<0.001^*$

WBC: White blood cell, NLR: Neutrophil-to-lymphocyte ratio, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, LDH: Lactate dehydrogenase, CRP: C-reactive protein; INR: International normalized ratio, \* $p<0.05$  indicates a significant difference.

comparison was performed between the two groups. The difference in the significance of blood parameters between our study and other studies may be due to the statistical method used.

Li et al. (17) stated that a decrease in the total lung volume indicated a poor prognosis, which is consistent with our study. Lanza et al. (6) found that the total lung volumes in COVID patients were lower

in those who needed intubation. Carvalho et al. (18) showed that the severity of the disease increased as lung volume decreased. Contrary to most studies, Ippolito et al. (19) did not find the decrease lung volumes to be associated with poor prognosis. There may be technical differences in the three-dimensional applications and devices used in these studies conducted at different centers.

**Table 3.** Measurements of the intrathoracic structures

Parameters	All (n=309)	Deceased (n=134)	Survivor (n=175)	p-value	
TLV (mL)	All	3177 (2467/4185)	2847 (2227/3773)	3399 (2637/4398)	<0.001*
	Men	3998 (3028/4820)	3408 (2771/4316)	4228 (3531/5177)	<0.001*
	Women	2574 (2108/3078)	2253 (1830/2668)	2775 (2304/3301)	<0.001*
PT (cm)	All	2.89 (2.64/3.13)	2.89 (2.68/3.11)	2.89 (2.63/3.14)	0.571
	Men	2.89 (2.67/3.10)	2.87 (2.64/3.1)	2.92 (2.67/3.09)	0.918
	Women	2.89 (2.63/3.17)	2.93 (2.73/3.13)	2.87 (2.6/3.21)	0.318
RPA (cm)	All	2.01 (1.81/2.23)	2.06 (1.87/2.27)	1.96 (1.76/2.19)	0.002*
	Men	2.04 (1.86/2.25)	2.03 (1.87/2.27)	2.06 (1.83/2.23)	0.483
	Women	1.93 (1.76/2.20)	2.08 (1.83/2.29)	1.87 (1.68/2.08)	0.001*
LPA (cm)	All	2.09 (1.91/2.29)	2.14 (2.01/2.38)	2.05 (1.89/2.23)	0.001*
	Men	2.1 (1.94/2.28)	2.11 (1.98/2.36)	2.07 (1.94/2.24)	0.142
	Women	2.1 ± 0.34	2.23±0.32	2.03±0.33	0.001*
AA (cm)	All	3.72±0.41	3.73±0.39	3.72±0.43	0.707
	Men	3.76±0.41	3.74±0.41	3.79±0.41	0.460
	Women	3.67±0.42	3.73±0.39	3.64±0.43	0.255
PT/AA (%)	All	0.78 (0.71/0.86)	0.79 (0.72/0.86)	0.78 (0.71/0.86)	0.696
	Men	0.78 (0.71/0.85)	0.78 (0.71/0.86)	0.79 (0.7/0.84)	0.440
	Women	0.78 (0.73/0.88)	0.79 (0.73/0.90)	0.78 (0.73/0.87)	0.903
Cardiac diameter (cm)	All	13.32±1.61	13.5±1.67	13.2±1.56	0.12
	Men	13.7±1.56	13.85±1.44	13.59±1.65	0.30
	Women	12.8±1.56	12.87±1.9	12.77±1.33	0.724
Thorax diameter (cm)	All	25.52±2.35	25.43±2.63	25.59±2.13	0.557
	Men	26.87±1.83	26.78±1.91	26.95±1.75	0.538
	Women	23.63±1.59	22.92±1.79	24.05±1.31	<0.001*
CTR (%)	All	0.53±0.07	0.53±0.07	0.52±0.07	0.065
	Men	0.51±0.07	0.52±0.06	0.51±0.08	0.279
	Women	0.54±0.06	0.56±0.07	0.53±0.06	0.013*
TTR (cm)	All	1.83±0.31	1.84±0.27	1.82±0.33	0.534
	Men	1.99±0.27	1.95±0.27	2.02±0.27	0.064
	Women	1.61±0.21	1.65±0.2	1.59±0.22	0.139
TAP (cm)	All	2.22 (1.82/2.58)	2.34 (1.86/2.65)	2.14 (1.8/2.53)	0.034*
	Men	2.5 (2.24/2.77)	2.5 (2.25/2.79)	2.49 (2.17/2.75)	0.606
	Women	1.84±0.31	1.87±0.36	1.82±0.28	0.350
TTR/TAP (TI %)	All	0.83 (0.74/0.93)	0.82 (0.7/0.92)	0.83 (0.76/0.93)	0.167
	Men	0.8 (0.71/0.88)	0.8 (0.66/0.88)	0.81 (0.75/0.89)	0.179
	Women	0.88 (0.76/0.98)	0.92 (0.75/1)	0.87 (0.78/0.98)	0.606

TLV: Total lung volume, PT: Pulmonary trunk, LPA: Left pulmonary artery, RPA: Right pulmonary artery, AA: Ascending aorta, CTR: Cardiothoracic ratio, TTR: Trachea transverse diameter, TAP: Trachea anteroposterior diameter, TI: Tracheal index, \*p<0.05 indicates a significant difference.

Lung volumes can also be affected by parameters such as height, weight, orthopedic problems, and ethnic characteristics, which were not included in our study. Considering that the patient population we studied was the patient group with respiratory distress, the patients may not have been able to inhale deeply enough during the extraction, and this may be one of the factors that may have affected our measurements. In the literature, different parameters such as well-ventilated lung volume and the ratio of this volume to the total volume have been studied, and more meaningful data may have been obtained.

Hayama et al. (10) stated that the diameter of the PT and the PT/AA ratio were higher in severe patients. Spagnolo et al. (9) reported that increased PT diameter and increased PT/AA ratio were associated with poor prognosis. Eslami et al. (12) found no significant effect of the PT and PT/AA ratio on the mortality rate. Esposito et al. (20) found the increases in PT, LPA, and RPA diameters to be significant regarding mortality rate. Yildiz et al. (21) found the increase in PT diameter to be significant, whereas the increase in the diameters of the AA, LPA, and RPA was not found to be significant. Erdoğan et al. (22) found that increased PT diameter, AA diameter, and PT/AA ratio were all associated with poor prognosis. Planek et al. (23) did not find the PT/AA ratio to be clinically significant, which is similar to our study. Spagnolo et al. (9) showed that an increase in the diameter of the PT and PT/AA were significantly associated with death. In general, many studies have found vascular measurements to be meaningful. However, none focused on gender. In our study, the increase in the diameters of the right and LPA was found to be significant regarding mortality rate when gender factor was not considered. However, it was found to be significant only in female cases when the gender factor was considered.

In addition, the increase in the diameters of the PT and AA was found to be significant in most studies, which is not similar to our study.

Eslami et al. (12) stated that an increase in CTR was significantly associated with mortality, whereas the tracheal index was not significant. In the study by Ünlü et al. (24), which classified 326 patients according to the percentage of pneumonic lung involvement, a significant relationship was found between the percentage of lung involvement and the diameter of the trachea. They stated that the percentage of lung involvement in patients increased along with the increase in both the transverse and anteroposterior diameters of the trachea, and this could indicate a poor prognosis (24). Tai et al. (25) reported that tracheal measurements were affected by height, gender, and race. In our study, information about height was not recorded. In our study, the thorax diameters were found to be significantly lower in the deceased than in the surviving female patients. There are very few studies on mortality rate in COVID-19 infection related to thorax diameter or cardiothoracic ratio in the literature.

### Study Limitations

In our study, height and weight values, which may affect our measurements, could not be included. Adding a third group of non-COVID-19 patients would have yielded more efficient results.

## CONCLUSION

In other studies, the mortality rate in COVID-19 patients was associated with some blood parameters and comorbidity status, unlike our study. Similarly, in other studies, some main intrathoracic vascular sizes were associated with mortality rate, unlike in our study. However, the number of cases in most of these studies was less than that in our study. In addition, in most of these studies, the examination according to gender has not been accomplished. Therefore, increasing the number of cases and examining by gender may yield new results, as in our study.

### Ethics

**Ethics Committee Approval:** This study was conducted with the approval of the Ethics Committee of Recep Tayyip Erdoğan University Faculty of Medicine (approval number: 2021/73, date: 26.04.2021).

**Informed Consent:** Because the study was conducted retrospectively, no volunteers were used.

### Authorship Contributions

Concept: A.Y.U., Y.Ü., N.H., Design: Y.Ü., A.Y.U., N.H., Resources: N.H., F.B.Ç., Materials: N.H., F.B.Ç., Data Collection or Processing: A.Y.U., N.H., Analysis or Interpretation: A.Y.U., N.H., Z.Y., Literature Search: A.Y.U., Z.Y., Y.Ü., Writing: A.Y.U., Z.Y., Y.Ü., Critical Review: A.Y.U., Z.Y., Y.Ü.

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

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## REFERENCES

- McIntosh K, Perlman S. Coronaviruses, Including Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS). Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases 2015: 1928-36.
- Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A Novel Coronavirus from Patients with Pneumonia in China, 2019. *N Engl J Med* 2020; 382: 727-33.
- Carter LJ, Garner LV, Smoot JW, Li Y, Zhou Q, Saveson CJ, et al. Assay Techniques and Test Development for COVID-19 Diagnosis. *ACS Cent Sci* 2020; 6: 591-605.
- Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, et al. Correlation of Chest CT and RT-PCR Testing for Coronavirus Disease 2019 (COVID-19) in China: A Report of 1014 Cases. *Radiology* 2020; 296: E32-E40.
- Colombi D, Bodini FC, Petrini M, Maffi G, Morelli N, Milanese G, et al. Well-aerated Lung on Admitting Chest CT to Predict Adverse Outcome in COVID-19 Pneumonia. *Radiology*.2020; 296: E86-E96.
- Lanza E, Muglia R, Bolengo I, Santonocito OG, Lisi C, Angelotti G, et al. Quantitative chest CT analysis in COVID-19 to predict the need for oxygenation support and intubation. *Eur Radiol* 2020; 30: 6770-8.
- Rorat M, Jurek T, Simon K, Guziński M. Value of quantitative analysis in lung computed tomography in patients severely ill with COVID-19. *PLoS One* 2021; 16: e0251946.
- Balbi M, Conti C, Imeri G, Caroli A, Surace A, Corsi A, et al. Post-discharge chest CT findings and pulmonary function tests in severe COVID-19 patients. *Eur J Radiol* 2021: 138.

9. Spagnolo P, Cozzi A, Foà RA, Spinazzola A, Monfardini L, Brà C, et al. CT-derived pulmonary vascular metrics and clinical outcome in COVID-19 patients. *Quant Imaging Med Surg* 2020; 10: 1325-33.
10. Hayama H, Ishikane M, Sato R, Kanda K, Kinoshita N, Izumi S, et al. Association of plain computed tomography-determined pulmonary artery-to-aorta ratio with clinical severity of coronavirus disease 2019. *Pulm Circ* 2020; 10: 5-7.
11. Mahammedi A, Oshmyansky A, Hassoun PM, Thiemann DR, Siegelman SS. Pulmonary Artery Measurements in Pulmonary Hypertension the Role of Computed Tomography. *J Thorac Imaging* 2013; 28: 96-103.
12. Eslami V, Abrishami A, Zarei E, Khalili N, Baharvand Z, Sanei-Taheri M. The Association of CT-measured Cardiac Indices with Lung Involvement and Clinical Outcome in Patients with COVID-19. *Acad Radiol* 2021; 28: 8-17.
13. Eom JS, Lee G, Lee HY, Oh JY, Woo SY, Jeon K, et al. The relationships between tracheal index and lung volume parameters in mild-to-moderate COPD. *Eur J Radiol* 2013; 82: e867-72.
14. Jin JM, Bai P, He W, Wu F, Liu XF, Han DM, et al. Gender Differences in Patients With COVID-19: Focus on Severity and Mortality. *Front Public Health* 2020; 8: 152.
15. Liao D, Zhou F, Luo L, Xu M, Wang H, Xia J, et al. Haematological characteristics and risk factors in the classification and prognosis evaluation of COVID-19: a retrospective cohort study. *Lancet Haematol* 2020; 7: e671-8.
16. Henry BM, de Oliveira MHS, Benoit S, Plebani M, Lippi G. Hematologic, biochemical and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. *Clin Chem Lab Med* 2020; 58: 1021-8.
17. Li K, Chen D, Chen S, Feng Y, Chang C, Wang Z, et al. Predictors of fatality including radiographic findings in adults with COVID-19. *Respir Res* 2020; 21: 146.
18. Carvalho ARS, Guimarães A, Werberich GM, de Castro SN, Pinto JSF, Schmitt WR, et al. COVID-19 Chest Computed Tomography to Stratify Severity and Disease Extension by Artificial Neural Network Computer-Aided Diagnosis. *Front Med (Lausanne)* 2020; 7: 577609.
19. Ippolito D, Ragusi M, Gandola D, Maino C, Pecorelli A, Terrani S, et al. Computed tomography semi-automated lung volume quantification in SARS-CoV-2-related pneumonia. *Eur Radiol* 2021; 31: 2726-36.
20. Esposito A, Palmisano A, Toselli M, Vignale D, Cereda A, Maria P, et al. Chest CT-derived pulmonary artery enlargement at the admission predicts overall survival in COVID-19 patients: insight from 1461 consecutive patients in Italy. *Eur Radiol* 2021; 31: 4031-41.
21. Yıldız M, Yadigar S, Yıldız BŞ, Aladag NB, Keskin O, Ozer RS, et al. Evaluation of the relationship between COVID-19 pneumonia severity and pulmonary artery diameter measurement. *Herz* 2021; 46: 56-62.
22. Erdoğan M, Öztürk S, Erdöl MA, Kasapkara A, Beşler MS, Kayaaslan B, et al. Prognostic utility of pulmonary artery and ascending aorta diameters derived from computed tomography in COVID-19 patients. *Echocardiography* 2021; 38: 1543-51.
23. Planek MIC, Ruge M, du Fay de Lavallaz JM, Kyung SB, Gomez JMD, Suboc TM, et al. Cardiovascular findings on chest computed tomography associated with COVID-19 adverse clinical outcomes. *Am Heart J Plus* 2021; 11: 100052.
24. Ünlü S, İlgar M, Akçiçek M. The evaluation of the trachea as a new parameter in determining the prognosis of COVID-19: First pilot study. *Eur Rev Med Pharmacol Sci* 2021; 25: 4835-40.
25. Tai A, Corke C, Joynt GM, Griffith J, Lunn D, Tong P. A comparative study of tracheal diameter in Caucasian and Chinese patients. *Anaesth Intensive Care* 2016; 44: 719-23.



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## Survey to Assess the Impact of the COVID-19 Pandemic on Tinnitus Perception

### COVID-19 Pandemisinin Tinnitus Algısı Üzerindeki Etkisinin Değerlendirilmesi

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#### ABSTRACT

**Objective:** This study aimed to evaluate the effect of coronavirus disease-2019 (COVID-19) on the perception of tinnitus in the population.

**Methods:** A web-based Google survey was performed between May and April 2020, and 1895 volunteers answered questions mainly related to demographic data, daily lifestyle during the pandemic, aural problems, and the presence of tinnitus. Participants with tinnitus were further evaluated using a visual analog scale (VAS) and Tinnitus Handicap Inventory (THI).

**Results:** Of 1895 participants, 184 (9.4%) had tinnitus. Of 184 participants with tinnitus, 64.7% had tinnitus onset before the pandemic, whereas 35.3% had tinnitus onset after the pandemic. There was no significant difference between the parameters of the participants who had no tinnitus and those who had tinnitus onset before and after the pandemic ( $p>0.05$ ). There was no significant difference between the THI results of the participants with tinnitus onset before and after the pandemic ( $p>0.05$ ). The VAS scores of the participants with tinnitus onset after the pandemic were higher than those of the participants with tinnitus onset before the pandemic ( $p<0.05$ ). The participants who had a tinnitus onset before the pandemic described alterations in their tinnitus perception as increased, decreased and unchanged tinnitus perception in 28.8%, 13.4% and 57.8%, respectively.

**Conclusion:** Because of this study, we observed that the COVID-19 pandemic did not increase tinnitus and tinnitus-related problems. However, because of the life-threatening risks that come with the pandemic, people may not consider the effects of tinnitus as a primary problem.

**Keywords:** COVID-19, perception, SARS-CoV-2, survey, tinnitus

#### ÖZ

**Amaç:** Bu çalışma ile toplumda koronavirüs hastalığı-2019'un (COVID-19) tinnitus algısına etkisinin değerlendirilmesi amaçlanmıştır.

**Yöntemler:** Mayıs ve Nisan 2020 arasında web tabanlı bir Google anketi yapıldı ve 1895 gönüllü, temel olarak demografik veriler, pandemi sırasında günlük yaşam tarzı ve kulak sorunları ile kulak çınlaması varlığı ile ilgili soruları yanıtladı. Kulak çınlaması olan katılımcılar visual analog skala (VAS) ve Tinnitus Handikap Envanteri (THI) ile ayrıca değerlendirildi.

**Bulgular:** Bin sekiz yüz doksan beş katılımcının 184'ünde (%9,4) tinnitus vardı. Tinnituslu 184 katılımcının %64,7'sinde tinnitus pandemiden önce, %35,3'ünde ise pandemiden sonra tinnitus başlamıştır. Kulak çınlaması olmayan ve pandemi öncesi ve sonrasında tinnitus başlayan katılımcıların parametreleri arasında anlamlı fark bulunmadı ( $p>0,05$ ). Pandemi öncesi ve sonrası tinnitus başlangıcı olan katılımcıların THI sonuçları arasında anlamlı fark bulunmadı ( $p>0,05$ ). Pandemi sonrası tinnitus başlayan katılımcıların VAS skorları, tinnitus pandemi sonrası başlayanlara göre daha yüksekti ( $p<0,05$ ). Pandemi öncesi tinnitus başlangıcı olan katılımcılar tinnitus algısındaki değişiklikleri sırasıyla %28,8, %13,4 ve %57,8 oranında artmış, azalmış ve değişmemiş tinnitus algısı olarak tanımlandılar.

**Sonuç:** Bu çalışma sonucunda COVID-19 pandemisinin kulak çınlaması ve kulak çınlamasına bağlı problemlerde artışa yol açmadığını gözlemledik. Ancak pandemi ile birlikte gelen hayatı tehdit eden riskler nedeniyle insanlar kulak çınlamasının etkilerini birincil sorun olarak görmeyebilirler.

**Anahtar Sözcükler:** COVID-19, algı, SARS-CoV-2, anket, tinnitus

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## INTRODUCTION

Tinnitus can be defined as the conscious perception of an auditory sensation in the absence of acoustic stimuli, with a prevalence of 8-15% in adults (1). Tinnitus can be associated with various conditions such as hearing impairment, noise exposure, ototoxicity, and head and neck trauma (2-4). Emotional factors and stress play a role in tinnitus (5,6). Symptoms such as frustration, annoyance, irritability, anxiety, depression, hyperacusis, insomnia, and concentration difficulties are also associated with the perception of tinnitus (7-9).

An increased prevalence of psychological distress in the population was reported during epidemic quarantine (10-13). Mask use is another phenomenon that enters our life during this period. Mask use reduces the air quality down because of the pressure on the nose and increase of the carbon dioxide in ventilated air. Both social shortages and mask use have negative effects on human psychology, which may cause anxiety and depression related to tinnitus etiology.

Coronavirus disease-2019 (COVID-19) dependent tinnitus cases and increased tinnitus perception were also defined in previous studies (14-18). Tinnitus severity increased by 40%, remained unchanged by 54%, and decreased by 6% because of the effects of social distinction and loneliness experience on tinnitus perception during the pandemic (10). It is possible that different populations may react differently to pandemic conditions (19). Several health questionnaires are available to assess the effects of tinnitus. The Tinnitus Handicap Inventory (THI) is the one that is mostly used worldwide. It has been translated into Turkish by (20). The validity and reliability of the study have been confirmed (Cronbach's alpha coefficient 0.88) (20).

In this study, we created a survey form to assess the effects of COVID-19-related pandemic lifestyle changes on tinnitus perception. In addition, we aimed to compare the discomfort levels of patients with tinnitus onset before and during the COVID-19 pandemic.

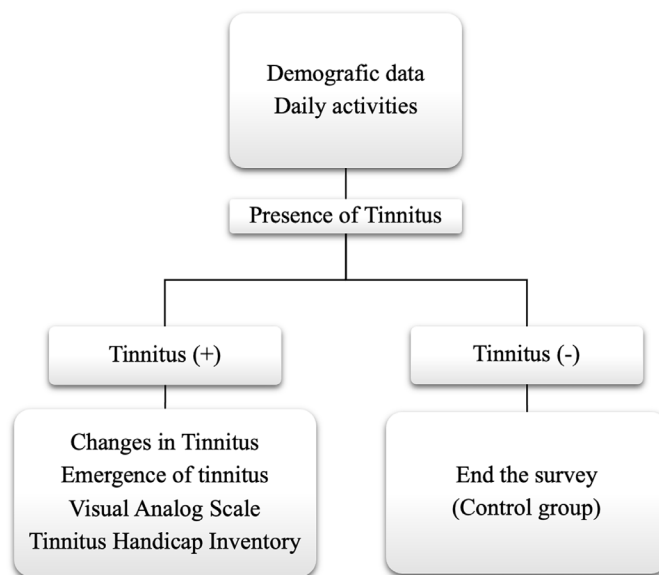
## MATERIALS AND METHODS

To evaluate tinnitus perception and pandemic-related changes in the population, the study was designed as a descriptive cross-sectional model. A Web-based Google Survey was conducted between April and May 2020 after obtaining ethical approval from İstanbul Medipol University (approval number: 525, date: 25.06.2020). Data from 1,337 female and 558 male patients with tinnitus were assessed. The patients were 18-70 years of age. All volunteers read and approved the informed consent before starting the survey. A total of 1895 volunteers were accepted to answer questions that were mainly related to demographic data, daily lifestyle during the pandemic, aural problems, and the presence of tinnitus.

The "Google Survey" application was used to obtain the survey data, and the answers of the participants were recorded. Answering the questions took a total of 5-7 minutes.

The flow chart is shown in Table 1. In the first part of the survey, which consists of three main parts, the daily life activities of the participants during the pandemic period and the presence of tinnitus were questioned. Participants with tinnitus completed the second and third parts of the questionnaire. For the participants without

**Table 1.** Survey flow chart



tinnitus, the questionnaire ended in the first part. The participants who had tinnitus were further evaluated with a second part of the survey, which included questions related to changes in tinnitus severity or the emergence of tinnitus since the beginning of the pandemic and a visual analog scale (VAS). Six main problem areas (discomfort level before and during pandemic, sleep disturbance, concentration problems, general comfort and hearing problems) for the patients evaluated with VAS. All the VAS questions were evaluated as 0 points means no and 10 points means very severe.

The third part of the questionnaire included the THI. Participants with tinnitus were also asked to complete the THI, which included 25 questions with a score range of 0 to 100. Accordingly, reactions to tinnitus were graded as slight (grade 1), mild (grade 2), moderate (grade 3), severe (grade 4), and catastrophic (grade 5) when the THI scores ranged between (0 to 16), (18 to 36), (38 to 56), (58 to 76) and (78 to 100), respectively (20).

### Statistical Analysis

The Statistical Package for Social Sciences version 23.0 was used for quantitative analysis. Descriptive data were evaluated. Chi-square and Spearman correlation coefficient tests were used to assess correlations. Paired samples t-test was used to compare the VAS scores between "tinnitus onset before" and "tinnitus onset after" in the evaluation of the tinnitus discomfort effect of the participants.

## RESULTS

Of 1895 participants, 184 (9.4%) had tinnitus complaints. Of 184 participants with tinnitus, 64.7% had tinnitus onset before the pandemic, whereas 35.3% had tinnitus onset after the pandemic. There was no significant difference between the demographic, lifestyle, and mask usage parameters of the participants who had no tinnitus and those who had tinnitus onset before and after the pandemic ( $p > 0.05$ ) (Table 2). There was no significant difference between the THI results of the participants with tinnitus onset

**Table 2.** Survey responses of participants with and without tinnitus

Questions	Parameters	Non-tinnitus (%)	Tinnitus onset before the pandemic (%)	Tinnitus onset after the pandemic (%)	p
Age (years)	18 to 30	45.5	45.2	45.9	
	30 to 50	54.5	54.8	54.1	
Gender	Man	47.2	48.7	44.3	
	Woman	57.8	51.3	55.7	
How many days did you go out in the last 2 months?	<10	27.3	21.7	37.7	
	10 to 20	26.7	29.6	21.3	
	21 to 30	13.6	16.5	8.2	
	>30	32.4	32.2	32.8	
How many hours do you go out a day?	0 to 2	40.3	34.8	50.8	
	2 to 4	17.6	20.9	11.5	
	4 to 6	42.0	44.3	37.7	
	>6	-	-	-	
Do you have a known disease?	COVID-19	0.6	-	1.6	>0.05
	Other	12.5	12.2	13.1	
	None	51.7	47.8	59.0	
	Cardiac	2.3	1.7	3.3	
	Otologic	17.0	20.9	9.8	
	Neurologic	2.3	3.5	-	
	Endocrine	13.6	13.9	13.1	
How many hours did you wear a mask per day in the last 2 months?	<1 h	24.4	22.6	27.9	
	1 to 4 h	36.4	36.5	36.1	
	4 to 8 h	17.0	18.3	14.8	
	>8 h	19.9	20.0	19.7	
What type of mask do you wear?	Surgical	69.9	71.3	67.2	
	Other (cloths etc.)	25.0	21.7	31.1	
	N95	5.1	7.0	1.6	
Do you have nasal congestion?	No	59.7	55.7	67.2	
	Bilateral	12.5	13.9	9.8	
	Unilateral	27.8	30.5	23	
Do you have allergic rhinitis?	Yes	35.8	40.0	27.9	
	No	64.2	60.0	72.1	
Do you have a hearing loss?	Bilateral	10.8	8.7	14.8	
	No	69.3	71.3	65.6	
	Unilateral	19.9	20	19.6	
Do you experience pain or discharge in your ear?	Bilateral	8.5	7.8	9.8	
	No	82.4	86.1	75.4	
	Unilateral	9.1	6.1	14.8	

before and after the pandemic ( $p>0.05$ ) (Table 3).

Participants with post-pandemic tinnitus had higher VAS scores than those with pre-pandemic tinnitus ( $p<0.05$ ) (Figure 1). The participants who had a tinnitus onset before the pandemic described alterations in their tinnitus perception as increased, decreased and unchanged tinnitus perception in 28.8%, 13.4% and 57.8%, respectively (Figure 2). The tinnitus characteristics of the participants who complained of

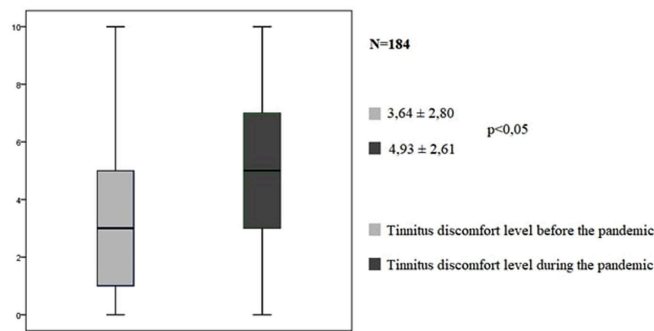
tinnitus in the last 2 months are shown in Figure 3.

## DISCUSSION

Tinnitus was reported in 9.4% of the population in our study, and tinnitus onset occurred after the pandemic in one-third of them. These rates seem within acceptable ranges since tinnitus can affect up to 30% of the adult population (21). Psychosocial stress

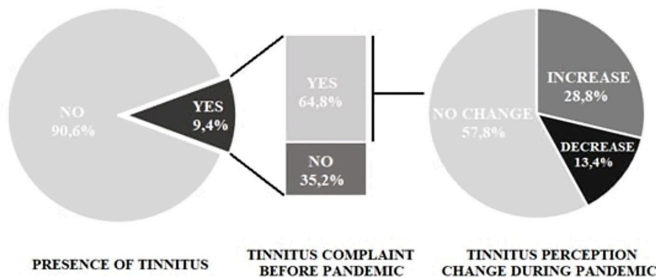
**Table 3.** Tinnitus Handicap Inventory results of participants with tinnitus onset before and after the pandemic

Grade	Tinnitus onset before the pandemic, n (%)	Tinnitus onset after the pandemic, n (%)	p
1	4 (3.3)	1 (1.5)	>0.05
2	16 (13.5)	10 (15.3)	
3	78 (65.5)	41 (63)	
4	7 (5.9)	3 (4.6)	
5	14 (11.8)	10 (15.3)	
Total	119 (100)	65 (100)	

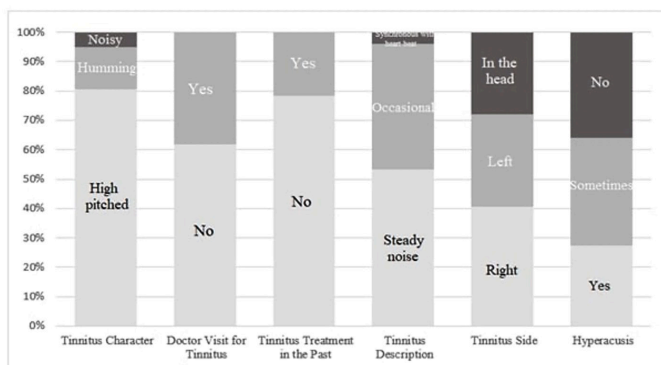


**Figure 1.** Total VAS scores of participants with tinnitus onset before and after the pandemic.

VAS: Visual analog scale.



**Figure 2.** Tinnitus perception change in people with tinnitus onset after pandemic.



**Figure 3.** General features related to tinnitus.

can trigger tinnitus according to epidemiological studies (22,23). We believe that although the initial period of the pandemic was a stressful state, it was associated with the occurrence of tinnitus. Our study covers a 2-month period after the pandemic life conditions begin. Further studies should evaluate the long-term effects of the pandemic on the onset of tinnitus.

Personality, different coping abilities, cognitive characteristics, and stress may affect tinnitus perception (24,25). There is a linear correlation between tinnitus and the duration and magnitude of stress (23). According to a national study in the UK, tinnitus is moderately and severely annoying in 2.8% and 1.6% of people and severely affects daily life in 0.5% of people (26). In studies, it was observed that there was a significant difference between VAS and THI scores before and during the pandemic, and it was determined that the 2019 coronavirus disease pandemic negatively affected tinnitus perceptions and quality of life (27). These results were also supported by other studies performed in different countries (28-30). Hence, psychological stress increased in the population during quarantine could increase the perception of tinnitus severity (7-10,16). An increase in the THI scores of the participants was also reported during the pandemic (31). It was reported that tinnitus severity was mostly unchanged in 54% or decreased in 6% of the people during the pandemic (10).

The primary purpose of this study was to investigate the effect of stress on the perception of tinnitus, which occurs because of the isolation of people during the COVID-19 pandemic. Despite this, it has been shown that COVID-19 disease can cause tinnitus perception depending on the effect it has on the central nervous system and auditory mechanisms (32,33). In our study, some of the participants may have had a perception of tinnitus after these effects; however, due to the lack of PCR results of these patients, the patients could not be fully evaluated in this respect. Patients in this group need to be evaluated in more detail.

A small proportion of the participants in the study had unilateral or bilateral earaches. Earache and discharge are often associated with external auditory canal and middle ear pathologies. These pathologies also can cause tinnitus. Especially during the pandemic period, the rate of unilateral ear discharge increases up to 14.8% in patients with tinnitus. These patients should be evaluated in terms of external ear canal and middle ear pathologies.

**Study Limitations**

However, there is no such opportunity within the scope of the study, and this appears to be a limitation of the study. According to the results of this study, the THI scores of the participants with tinnitus onset before and after the pandemic were similar, although an increase in VAS scores was encountered. Accordingly, it is plausible to say that although people could have experienced increased tinnitus discomfort during the pandemic, that discomfort may have been neglected because of the presence of a life-threatening condition, COVID-19.

**CONCLUSION**

As a result of this study, we observed that the short-term effects of the changes that come with the sudden change in the daily life conditions of the people affected by the COVID-19 pandemic do not lead to an increase in tinnitus or problems related to tinnitus.

Because of the life-threatening risks that come with the pandemic, people may not see the effects of tinnitus as a primary problem. This study covers a short-term effect that needs to be supported by studies that will show the long-term consequences.

### Ethics

**Ethics Committee Approval:** Approval was granted by the Ethics Committee of İstanbul Medipol University (approval number: 525, date: 25.06.2020).

**Informed Consent:** All volunteers read and approved the informed consent before starting the survey.

### Authorship Contributions

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

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

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### REFERENCES

- Hoffmann HJ, Reed GW. Epidemiology of Tinnitus. In: Snow JB, editor. Tinnitus: Theory and management. Hamilton: BC Decker; 2004. p. 16-41
- Nicolas-Puel C, Akbaraly T, Lloyd R, Berr C, Uziel A, Rebillard G, et al. Characteristics of tinnitus in a population of 555 patients: specificities of tinnitus induced by noise trauma. *Int Tinnitus J* 2006; 12: 64-70.
- Seligmann H, Podoshin L, Ben-David J, Fradis M, Goldsher M. Drug-induced tinnitus and other hearing disorders. *Drug Saf* 1996; 14: 198-212.
- Folmer RL, Griest SE. Chronic tinnitus resulting from head or neck injuries. *Laryngoscope* 2003; 113: 821-7.
- Alpini D, Cesarani A. Tinnitus as an alarm bell: Stress reaction tinnitus model. *ORL J Otorhinolaryngol Relat Spec* 2006: 31-6.
- Hinton DE, Chhean D, Pich V, Hofmann SG, Barlow DH. Tinnitus among Cambodian refugees: Relationship to PTSD severity. *J Trauma Stress* 2006; 19: 541-6.
- McKenna L, Hallam RS, Hinchcliff R. The prevalence of psychological disturbance in neuro-otology outpatients. *Clin Otolaryngol Allied Sci* 1991; 16: 452-6.
- Langguth B. A review of tinnitus symptoms beyond ringing in the ears: A call to action. *Curr Med Res Opin* 2011: 1635-43.
- Si E. Psychological profiles of tinnitus patients. *Tinnitus handbook*. Thomson Learning 2000: 25-57.
- Brooks SK, Webster RK, Smith LE, Woodland L, Wessely S, Greenberg N, et al. The psychological impact of quarantine and how to reduce it: rapid review of the evidence. *Lancet* 2020; 395: 912-20.
- Hawryluck L, Gold WL, Robinson S, Pogorski S, Galea S, Styra R. SARS control and psychological effects of quarantine, Toronto, Canada. *Emerg Infect Dis* 2004; 10: 1206-12.
- Jeong H, Yim HW, Song YJ, Ki M, Min JA, Cho J, et al. Mental health status of people isolated due to Middle East Respiratory Syndrome. *Epidemiol Health* 2016; 38: e2016048.
- Vindegard N, Benros ME. COVID-19 pandemic and mental health consequences: Systematic review of the current evidence. *Brain Behav Immun* 2020; 89: 531-42.
- Elibol E. Otolaryngological symptoms in COVID-19. *Eur Arch Otorhinolaryngol* 2021; 278: 1233-6.
- Lamounier P, Gonçalves VF, Ramos HVL, Gobbo DA, Teixeira RP, Dos Reis PC, et al. A 67-year-old woman with sudden hearing loss associated with SARS-CoV-2 infection. *Am J Case Rep* 2020; 21: 1-6.
- Naylor G, Burke LA, Holman JA. Covid-19 Lockdown Affects Hearing Disability and Handicap in Diverse Ways: A Rapid Online Survey Study. *Ear Hear* 2020; 41: 1442-9.
- Özçelik Korkmaz M, Eğilmez OK, Özçelik MA, Güven M. Otolaryngological manifestations of hospitalised patients with confirmed COVID-19 infection. *Eur Arch Otorhinolaryngol* 2021; 278: 1675-85.
- Viola P, Ralli M, Pisani D, Malanga D, Sculco D, Messina L, et al. Tinnitus and equilibrium disorders in COVID-19 patients: preliminary results. *Eur Arch Otorhinolaryngol* 2021; 278: 3725-30.
- Rawson T, Fioretti A, Jafari Z, Beukes EW, Baguley DM, Jacquemin L, et al. Changes in Tinnitus Experiences During the COVID-19 Pandemic. *Front Public Heal* 2020; 8: 592878.
- Aksoy S, Firat Y, Alpar R. The Tinnitus Handicap Inventory: a study of validity and reliability. *Int Tinnitus J* 2007; 13: 94-8.
- McCormack A, Edmondson-Jones M, Somerset S, Hall D. A systematic review of the reporting of tinnitus prevalence and severity. *Hear Res* 2016; 337: 70-9.
- Baigi A, Oden A, Almlid-Larsen V, Barrenäs ML, Holgers KM. Tinnitus in the general population with a focus on noise and stress: a public health study. *Ear Hear* 2011; 32: 787-9.
- Hasson D, Theorell T, Wallén MB, Leineweber C, Canlon B. Stress and prevalence of hearing problems in the Swedish working population. *BMC Public Health* 2011; 11: 130.
- Kirsch CA, Blanchard EB, Parnes SM. Psychological characteristics of individuals high and low in their ability to cope with tinnitus. *Psychosom Med* 1989; 51: 209-17.
- Welch D, Dawes PJ. Personality and perception of tinnitus. *Ear Hear* 2008; 29: 684-92.
- Refaie A El, Davis A. Epidemiology of tinnitus. *Tinnitus handbook*. San Diego (Calif): Thomson Learning 2000: 1-23.
- Aydoğan Z, Çınar Satekin M, Ocak E, Tokgoz Yılmaz S. Effects of the coronavirus disease 2019 pandemic on subjective tinnitus perception. *J Laryngol Otol* 2022; 136: 410-3.
- Krog NH, Engdahl B, Tambs K. The association between tinnitus and mental health in a general population sample: results from the HUNT Study. *J Psychosom Res* 2010; 69: 289-98.
- Michikawa T, Nishiwaki Y, Kikuchi Y, Saito H, Mizutani K, Okamoto M, et al. Prevalence and factors associated with tinnitus: a community-based study of Japanese elders. *J Epidemiol* 2010; 20: 271-6.
- Khedr EM, Ahmed MA, Shawky OA, Mohamed ES, El Attar GS, Mohammad KA. Epidemiological study of chronic tinnitus in Assiut, Egypt. *Neuroepidemiology* 2010; 35: 45-52.
- Schlee W, Hølleland S, Bulla J, Simoes J, Neff P, Schoiswohl S, et al. The Effect of Environmental Stressors on Tinnitus: A Prospective Longitudinal Study on the Impact of the COVID-19 Pandemic. *J Clin Med* 2020; 9: 2756.
- Chirakkal P, Al Hail AN, Zada N, Vijayakumar DS. COVID-19 and Tinnitus. *Ear Nose Throat J* 2021; 100: 1605-2.
- Beukes E, Ulep AJ, Eubank T, Manchaiah V. The Impact of COVID-19 and the Pandemic on Tinnitus: A Systematic Review. *J Clin Med* 2021; 10: 2763.



## Retrospective Analysis of the Factors Affecting Recurrence, Survival, and Effect of Hippocampus Radiotherapy Doses on Neurocognitive Functions in Patients Diagnosed with Glioblastoma Multiforme

Glioblastoma Multiforme Tanısıyla Tedavi Edilen Hastalarda Nüks, Sağkalımı Etkileyen Faktörlerin ve Hipokampus Radyoterapi Dozlarının Nörobilişsel Fonksiyonlara Etkisinin Retrospektif Analizi

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### ABSTRACT

**Objective:** This study aimed to evaluate radiotherapy (RT) doses, RT effects on neurocognitive functions, and possible factors that may affect recurrence or death in patients with glioblastoma multiforme (GBM).

**Methods:** The data of 21 patients with GBM were retrospectively analyzed. RT treatment plans and doses and hippocampus ipsilateral and contralateral doses were recorded. The Mini-Mental State Examination (MMSE) is used to assess neurocognitive functions. The time of recurrence and death, if any, of the patients was recorded. Factors such as gender, age, patient performance status, tumor size, tumor localization, type of surgery, and time between surgery and RT were analyzed to determine any effect on the risk of recurrence or death.

**Results:** The median planning target volume dose was 59.86 gray (Gy). The maximum ipsilateral hippocampus dose was 51.85 Gy, and the maximum contralateral hippocampus dose was 46.25 Gy. With the MMSE, 3 of 4 patients had cognitive impairment. At the end of follow-up, 16 patients had recurrence and died. The median disease-free survival was 10 months [95% confidence interval (CI): 5.7-14.2], and the median overall survival was 24 months (95% CI: 16.0-31.9). Only poor performance status increased the risk of recurrence (hazard ratio: 4.31, 95% CI: 1.26-14.70, p=0.02).

### Öz

**Amaç:** Bu çalışmada glioblastoma multiforme (GBM) tanılı hastalarda radyoterapi (RT) dozları ve RT'nin nörobilişsel etkileri ile nüks veya ölümü etkileyebilecek olası faktörlerin değerlendirilmesi amaçlanmıştır.

**Yöntemler:** GBM tanılı 21 hastanın verilerini retrospektif olarak inceledik. RT tedavi plan ve dozları ile hipokampus ipsilateral ve kontralateral dozları kaydedildi. Nörobilişsel işlevleri değerlendirmek için Mini-Mental Durum Muayenesi (MMDM) yapıldı. Hastaların varsa nüks ve ölüm zamanları kaydedildi. Yaş, cinsiyet, hasta performans durumu, tümör boyutu, cerrahi tipi, tümör lokalizasyonu ve cerrahi ile RT arasındaki süre gibi faktörlerin nüks veya ölüm riski üzerindeki etkisini belirlemek için analiz yapıldı.

**Bulgular:** Hastaların medyan planlama tedavi volümü dozu 59,86 gray (Gy) idi. Maksimum ipsilateral hipokampus dozu 51,85 Gy ve maksimum kontralateral hipokampus dozu 46,25 Gy idi. MMDM ile 4 hastanın 3'ünde bilişsel işlev bozukluğu vardı. İzlem sonunda 16 hastada nüks ve ölüm saptandı. Medyan hastaliksız sağkalım 10 aydı [%95 güven aralığı (GA): 5,7-14,2], medyan genel sağkalım 24 aydı (%95 GA: 16,0-31,9). Sadece kötü performans durumunun nüks riskini artırdığı saptandı (tehlike oranı: 4,31, %95 GA: 1,26-14,70, p=0,02).

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**ABSTRACT**

**Conclusion:** Because hippocampus shielding was not performed, our hippocampus doses were high. Hippocampal-sparing RT is essential for the preservation of neurocognitive functions. The increased risk of recurrence in patients with poor performance status is possibly related to treatment dose reduction, delay, or discontinuation.

**Keywords:** Glioblastoma, radiotherapy, hippocampus, cognitive dysfunction

**ÖZ**

**Sonuç:** Hipokampus koruması yapılmadığı için hipokampus dozlarımız yüksekti. Hipokampal koruyucu RT, nörobilişsel işlevlerin korunması için gereklidir. Performans durumu kötü olan hastalarda artmış nüks riski, muhtemelen tedavi dozunun azaltılması, tedavinin geciktirilmesi veya kesilmesi ile ilişkilidir.

**Anahtar Sözcükler:** Glioblastoma, radyoterapi, hipokampus, bilişsel bozukluk

**INTRODUCTION**

Primary brain tumors constitute approximately 2% of all cancers. In adults, glioblastoma multiforme (GBM) and grade 3 anaplastic astrocytoma (AA) are the two most common histological types (1,2). GBM, the most common and aggressive primary malignant intracranial tumor in adults, accounts for 50-60% of all astrocytic tumors. The patients are usually between 45 and 70 years of age. Diagnosis in childhood is rare. The male/female ratio is approximately 1.5/1 (3-5).

GBM is an infiltrating tumor located in the cerebral hemisphere. It is commonly at the borders of the parietal, occipital, and frontal lobes. They usually occupy more than one functional brain region because of their diffuse and deep localization, which is one of the main factors that make surgery difficult. They can also be localized outside the cerebral hemispheres. Almost half of brainstem glial tumors exhibit high malignancy characteristics. Furthermore, approximately 10% of GBMs are not deeply localized and may mimic cerebral metastases by localizing on the white-gray matter border (6-9).

The standard treatment is the Stupp protocol, which involves surgical resection of the tumor, followed by regional radiotherapy (RT) with concurrent temozolomide (TMZ) chemotherapy and six adjuvant cycles of TMZ (10). A randomized phase III study revealed that RT and TMZ combination prolongs survival in GBM. In this study, 573 patients were randomized to receive RT only or RT concurrent with TMZ, followed by adjuvant TMZ. RT was administered in 30 fractions [2 gray (Gy)/day] totaling 60 Gy. Concurrent TMZ was administered at a dose of 75 mg/m<sup>2</sup>/day continuously during RT, including weekends. Adjuvant TMZ was initiated 28 days after the completion of RT and was administered for 6 cycles (150-200 mg/m<sup>2</sup>/day, on 1-5 days every 28 days). In this study, the median overall survival (OS) was 12 months in the RT-alone arm and 15 months in the RT-TMZ arm. The 2-year survival rates were 10% and 26%, respectively. The median disease-free survival (DFS) was 5 months and 6.9 months. The 1- and 2-year DFS rates were 9% and 2% in the RT-alone arm and 27% and 11% in the RT-TMZ arm (p<0.0001). In this phase III study, the RT-TMZ combination demonstrated a remarkable improvement in survival (11).

In a phase II study by Athanassiou et al. (12), among 110 patients, RT alone (60 Gy) was compared with RT-TMZ treatment. The results of the study favored the combined treatment arm: the median DFS was 5.2 months vs. 10.8 months; the 1-year DFS rates were 7.7% vs. 36.6%; the median OS was 7.7 months vs. 13.4 months; and the 1-year OS rates were 15.7% vs. 56.3%, respectively. Toxicity was mostly hematological, and 1 patient was reported to have died due to febrile neutropenia.

The hippocampus plays an important role in emotional learning and memory consolidation (13,14). Whole-brain RT can lead to various side effects, such as the development of cerebellar and neurocognitive dysfunctions and impaired short-term memory and learning ability (15,16). Interruption of neurogenesis in the subgranular region can lead to memory impairment (17,18). The function of the hippocampus is negatively affected by radiation. Therefore, improving techniques to protect the hippocampus from radiation is vital. Dosimetric studies performed with intensity-modulated radiotherapy (IMRT) or tomotherapy in the literature show promise.

In our study, we evaluated RT doses and hippocampal neurocognitive effects in patients with GBM treated with the Stupp protocol. We also evaluated treatment tolerance, disease-free and OS times, and possible factors that may affect recurrence or death.

**MATERIALS AND METHODS**

In our study, we retrospectively evaluated 21 patients treated with a histologically confirmed diagnosis of GBM between April 2015 and October 2020. Inclusion criteria were as follows: being over 17 years of age, having Eastern Cooperative Oncology Group (ECOG) performance score ≤3, and having normal bone marrow, renal, and liver function (defined as hemoglobin ≥10 g/dL, thrombocyte count ≥100,000/μL, absolute neutrophil count ≥1,500/μL; serum creatinine level <1.5 mg/dL; alanine aminotransferase and aspartate aminotransferase levels <2.5 times the upper limit of normal, total bilirubin <1.5 times the upper limit of normal). Patients with poor medical condition due to other comorbidities or infections were excluded from the study.

The treatment protocol was TMZ concurrent RT with a median of 60 Gy (2 Gy-60 Gy) with standard 6 mV photon energy applied according to the Stupp protocol. In RT, we used VMAT and IMRT techniques in 30 fractions as planning target volume 1 (PTV1)=GTV+2 cm=40 Gy, PTV2=GTV+1 cm=60 Gy as standard. TMZ was administered concurrently with RT as 75 mg/m<sup>2</sup>/day throughout RT; and it was administered 150-200 mg/m<sup>2</sup>/day, on 1-5 days every 28 days for 6 cycles, following.

When evaluating neurocognitive functions, we first drew the hippocampus retrospectively to determine RT doses. We further evaluated the ipsilateral and contralateral hippocampus doses. Subsequently, the Mini-Mental State Examination (MMSE) was performed on 4 alive patients in the outpatient setting. The MMSE evaluates orientation, registration (short-term memory), recall, attention and calculation, language, and the ability to understand and follow verbal and written commands. A score equal to or

greater than 24 indicates normal cognition. Scores below 24 indicate cognitive impairment (19-23 points: mild, 10-18 points: moderate,  $\leq 9$  points: severe impairment). The MMSE test was obtained from the internet (19).

Disease recurrence and death data were obtained from patient files. DFS was defined as the length of time between the end of primary treatment for cancer and the time of disease recurrence or death. OS was defined as the length of time from GBM diagnosis to death.

This study was conducted in accordance with the Declaration of Helsinki. Ethics committee approval was obtained from the Bursa City Hospital Institutional Clinical Research Ethics Committee for the study (approval number: 2019-KAEK-140, 2021-19/7, date: 20.10.2021).

### Statistical Analysis

Statistical analysis was performed using SPSS software version 28.0 (IBM Corp., Armonk, NY, USA). The variables' distribution is evaluated using the Shapiro-Wilk test, and the variables are given as median (minimum-maximum) values and frequency values. Categorical variables were compared using the chi-square test and Fisher's exact test. The Kaplan-Meier test, with comparisons made with the log-rank test, was used for survival analyses. Cox regression analysis was used to evaluate possible factors on the risk of recurrence and death. A p-value  $<0.05$  is considered statistically significant.

## RESULTS

Twenty-one patients were involved in the study. After the median follow-up period of 66 months, the median DFS was 10 months [95% confidence interval (CI): 5.7-14.2 months] and the median OS was 24 months (95% CI: 16.0-31.9 months). The patients' clinical characteristics are given in Table 1.

We administered TMZ to 21 patients concomitantly with RT. 4 patients could not complete RT because of ECOG performance status deterioration. Two patients died during RT. TMZ-induced pancytopenia developed in one patient, which required treatment delay. In the first-month control MRI after RT, 2 patients had residual/recurrent mass images and were referred for surgery. Adjuvant TMZ could not be administered to 5 patients because of ECOG performance status deterioration. In 1 patient, the dose was reduced by 10% because of grade 2 thrombocytopenia. Four patients had recurrence on adjuvant treatment. Three of them were referred for surgery. One patient underwent reirradiation and was administered second-line chemotherapy following. Seventeen of 21 patients who died during the follow-up period, 4 patients remained alive.

In the statistical analysis, we examined the factors that may alter the risk of recurrence or death. Upon evaluation for the risk of recurrence, being older than 50 years of age, being male, having poor ECOG performance score, smaller tumor size, total excision, tumors on right cerebral hemisphere or non-frontal location, and the interval between surgery and RT being more than 4 weeks were found as factors increasing the risk, with only poor ECOG performance score being statistically significant ( $p=0.02$ ). Factors increasing the risk of death were the same as those for the risk of recurrence, except that having a left cerebral tumor seemed to increase the risk of death, and none of the factors were statistically significant. The Cox regression

analysis results are given in Tables 2, 3.

RT critical organ doses are given in Table 4. We further examined the ipsilateral and contralateral hippocampus doses of 19 patients. The median ipsilateral maximum hippocampus dose was 61.21 Gy (minimum-maximum: 26.53-63.06), and the median contralateral maximum hippocampus dose was 48.01 Gy (minimum-maximum: 16.03-62.89). The ipsilateral and contralateral hippocampus doses are given in Table 5.

In our evaluation of 4 living patients with MMSE, we found 1 patient with normal cognitive function, one with mild cognitive function, one with moderate cognitive function, and one with severe cognitive dysfunction. The MMSE is shown in Figure 1.

## DISCUSSION

In high-grade astrocytomas, the 5-year survival after treatment with surgery and RT is less than 10-20%; the median OS is 27 months in AA and 8 months in GBM (20).

The treatment of GBM consists of surgery, followed by RT and chemotherapy. Chemotherapy has been used in GBM treatment

**Table 1.** Clinical characteristics of the patients

	Number (%)
<b>Age</b>	
<50 years	5 (24%)
$\geq 50$ years	16 (76%)
<b>Gender</b>	
Male	17 (81%)
Female	4 (19%)
<b>ECOG* performance score</b>	
ECOG 0, 1	12 (57%)
ECOG 2, 3	9 (43%)
<b>Location of the tumor</b>	
Temporal	3 (14%)
Frontal	6 (28%)
Parietal	5 (24%)
Temporoparietal	2 (10%)
Temporooccipital	1 (5%)
Frontoparietal	3 (14%)
Parietooccipital	1 (5%)
<b>Tumor size</b>	
<4 cm	6 (29%)
$\geq 4$ cm	15 (71%)
<b>Surgery</b>	
Total excision	18 (85%)
Subtotal excision	3 (15%)
<b>Surgery-radiotherapy interval</b>	
<4 weeks	3 (14%)
$\geq 4$ weeks	18 (86%)

\*ECOG: Eastern Cooperative Oncology Group.



since the 1970s. Nitrosoureas are alkylating agents and are the oldest drugs used in the treatment of central nervous system malignancies. Carmustine (BCNU) is still one of the most effective chemotherapeutics, with a response rate of approximately 40%. In

recent years, TMZ, a second-generation alkylating agent, has been used in GBM and AA and has been found to be as effective as BCNU, and has been included in standard post-RT treatment protocols (21,22). In a multicenter study, in 525 patients with recurrent GBM,

**Table 2.** Cox regression analysis of factors for recurrence

		Univariate analysis		
		HR	95% CI	p-value
Age	<50 years (R) vs. ≥50 years	3.11	0.82-11.72	0.09
Gender	Female (R) vs. male	1.11	0.24-5.00	0.88
ECOG	ECOG score 0, 1 (R) vs. ECOG score 2, 3	4.31	1.26-14.70	0.02
Tumor size	<4 cm (R) vs. >4 cm	0.83	0.25-2.66	0.75
Surgery	Total excision (R) vs. subtotal excision	0.70	0.15-3.18	0.65
Location of the tumor	Left cerebral (R) vs. right cerebral	1.01	0.34-3.00	0.97
Location of the tumor	Frontal (R) vs. others	1.36	0.48-3.83	0.55
Surgery-radiotherapy interval	<4 weeks (R) vs. ≥4 weeks	4.34	0.56-33.10	0.15

HR: Hazard ratio, CI: Confidence interval, ECOG: Eastern Cooperative Oncology Group.

**Table 3.** Cox regression analysis of factors for death

		Univariate analysis		
		HR	95% CI	p-value
Age	<50 years (R) vs. ≥50 years	1.55	0.43-5.50	0.49
Gender	Female (R) vs. Male	1.91	0.42-8.68	0.39
ECOG	ECOG score 0, 1 (R) vs. ECOG score 2, 3	382.87	0.28-512995.00	0.10
Tumor size	<4 cm (R) vs. >4 cm	0.68	0.20-2.24	0.53
Surgery	Total excision (R) vs. subtotal excision	0.52	0.11-2.35	0.39
Location of the tumor	Left cerebral (R) vs. right cerebral	0.79	0.26-2.35	0.67
Location of the tumor	Frontal (R) vs. others	1.87	0.64-5.51	0.25
Surgery-radiotherapy interval	<4 weeks (R) vs. ≥4 weeks	2.46	0.32-18.98	0.38

HR: Hazard ratio, CI: Confidence interval, ECOG: Eastern Cooperative Oncology Group.

**Table 4.** Critical organ radiation doses during radiotherapy

	Median dose (minimum-maximum) (gray)
PTV V95* dose	59.86 (57.90-60.55)
PTV** dose	60.67 (60.00-61.40)
Right lens maximum dose	3.35 (1.35-8.41)
Left lens maximum dose	2.90 (0.88-6.95)
Right eye maximum dose	17.17 (5.30-55.00)
Left eye maximum dose	17.25 (1.76-49.08)
Right optic nerve maximum dose	18.28 (1.66-54.39)
Left optic nerve maximum dose	20.20 (1.10-54.42)
Optic chiasm maximum dose	31.45 (1.68-54.25)
Whole brain maximum dose	62.78 (55.78-65.24)
Brain stem maximum dose	46.72 (2.55-60.87)

\*PTV V95: Planning target volume enclosed by 95% isodose, \*\*PTV: Planning target volume.


**Table 5.** Ipsilateral and contralateral hippocampus radiation doses (gray)

Mean dose ipsilateral	Maximum dose ipsilateral	Mean contralateral dose	Maximum dose contralateral
60.29	62.25	29.05	39.78
52.17	56.78	32.41	34.39
40.11	56.30	19.87	32.05
47.58	61.21	26.82	46.25
56.88	61.58	36.19	54.32
58.41	61.48	42.84	62.89
45.70	50.93	30.41	48.01
58.26	61.60	33.25	40.50
44.28	60.94	30.85	58.48
49.72	62.07	20.92	35.71
60.12	62.61	38.88	53.76
11.86	26.53	8.67	20.05
60.91	63.06	36.07	49.56
15.2	47.07	15.87	48.51
6.82	42.49	3.11	16.03
19.85	49.51	16.3	36.32
60.99	62.28	40.22	57.31
57.35	62.45	29.91	55.1
51.85	60.57	29.91	55.1

### Mini-Mental State Examination (MMSE)

Patient's Name: \_\_\_\_\_ Date: \_\_\_\_\_

**Instructions:** Ask the questions in the order listed. Score one point for each correct response within each question or activity.

Maximum Score	Patient's Score	Questions
5		"What is the year? Season? Date? Day of the week? Month?"
5		"Where are we now: State? County? Town/city? Hospital? Floor?"
3		The examiner names three unrelated objects clearly and slowly, then asks the patient to name all three of them. The patient's response is used for scoring. The examiner repeats them until patient learns all of them, if possible. Number of trials: _____
5		"I would like you to count backward from 100 by sevens." (93, 86, 79, 72, 65, ...) Stop after five answers. Alternative: "Spell WORLD backwards." (D-L-R-O-W)
3		"Earlier I told you the names of three things. Can you tell me what those were?"
2		Show the patient two simple objects, such as a wristwatch and a pencil, and ask the patient to name them.
1		"Repeat the phrase: 'No ifs, ands, or buts.'"
3		"Take the paper in your right hand, fold it in half, and put it on the floor." (The examiner gives the patient a piece of blank paper.)
1		"Please read this and do what it says." (Written instruction is "Close your eyes.")
1		"Make up and write a sentence about anything." (This sentence must contain a noun and a verb.)
1		"Please copy this picture." (The examiner gives the patient a blank piece of paper and asks him/her to draw the symbol below. All 10 angles must be present and two must intersect.) 
30		<b>TOTAL</b>

**Figure 1.** Mini-Mental State Examination.

with TMZ, a 6-month DFS rate of 46% and a 12-month OS rate of 24% were observed (23).

TMZ is a per oral applied, rapidly absorbed agent and shows nearly 100% bioavailability. TMZ can cross the blood– brain barrier and reach effective concentrations in the central nervous system, with a cerebrospinal fluid/plasma ratio of approximately 30-40% (24). TMZ improves the quality of life and prolongs DFS and OS (25). In older studies, the median OS was around 1-1.5 years. However, with improvements in cancer care, some patients with GBM have an OS of nearly 2 years. For example, in the GEINO 14-01 trial published in 2020, the median OS of patients who received 6 months of adjuvant TMZ was 23.3 months (26). Similarly, in a study comparing different RT techniques, the median OS of patients was around 18-25 months (27). In our study, the patients' median DFS was 10 months, and the median OS was 24 months, which is consistent with recent data. In contrast, isocitrate dehydrogenase 1 (IDH1) mutation and O6-methylguanine-DNA methyltransferase (MGMT) methylation are known prognostic factors for GBM. The OS of class 1 patients (class 1=MGMT methylated/IDH1 mutant or MGMT methylated/IDH1 wild type/Gross Total Resection/Karnofsky Performance Status ≥90) reaches 67 months (28). The mutation status of our patients is unknown. The longer OS in our study could be the result of the high MGMT methylated percentage of patients in the study group.

While administering RT to the patients, we tried to keep our PTV 95 (PTV enclosed by the 95% isodose) and risk organs within the RTOG 0933 range, as shown in Table 2. However, we did not draw the hippocampus. In recent studies, it has been reported that deficits in learning and memory, especially in patients receiving whole-brain RT, are associated with the radiation-affected hippocampus (29,30). In a study using linac-based IMRT with hippocampus sparing for whole-brain RT, Gondi et al. (31) reported that they delivered 30 Gy to the whole brain. They determined the shape of the hippocampus and created hippocampal avoidance zones using a volumetric expansion of 5 mm around the hippocampus. According to their results, the maximum dose received by the hippocampus was 15.3 Gy, whereas the mean dose was 7.8 Gy. They reported that modern IMRT techniques provide hippocampus preservation with acceptable target coverage and homogeneity (31). According to RTOG 0933, the hippocampus maximum dose should be ≤16 Gy. Because we did not perform hippocampus protection, our hippocampus RT doses were high.

While it is possible to protect both hippocampi in whole brain RT, it is difficult to protect the same side of the hippocampus in tumors to which higher doses are administered, such as GBM. Wee et al. (32) showed that hippocampus preservation does not increase the risk of relapse.

#### Study Limitations

One of the major limitations of this study is the limited number of patients. Statistical significance could not be reached for the factors of disease recurrence or death. In addition, the genetic profile of patients for prognostic changes is unknown. The retrospective design of the study is another limitation. MMSE could not be performed on all patients because 16 patients included had already died at the time of evaluation.

## CONCLUSION

In our study, the Stuppe protocol for the treatment of GBM was well tolerated. Because we did not preserve the hippocampus during RT, our patients showed loss of neurocognitive functions. We have thereby understood that we should pay more attention to protecting at least the opposite hippocampus according to the tumor location.

## Ethics

**Ethics Committee Approval:** Ethics committee approval was obtained from the Bursa City Hospital Institutional Clinical Research Ethics Committee for the study (approval number: 2019-KAEK-140, 2021-19/7, date: 20.10.2021).

**Informed Consent:** Prospective study.

## Authorship Contributions

Surgical and Medical Practices: S.İ., Ö.D.T., H.E., F.A., T.T., P.Ç. Concept: S.İ., Ö.D.T., H.E., Design: S.İ., Ö.D.T., H.E., B.C., Data Collection or Processing: S.İ., Ö.D.T., H.E., F.A., T.T., P.Ç., Analysis or Interpretation: B.C., Ö.D.T., Literature Search: S.İ., Ö.D.T., B.C., Writing: S.İ., Ö.D.T., B.C.

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## REFERENCES

- Kleihues P, Burger PC, Scheithauer BW. The new WHO classification of brain tumours. *Brain Pathol* 1993; 3: 255-68.
- Burger PC, Vogel FS, Green SB, Strike TA. Glioblastoma multiforme and anaplastic astrocytoma. Pathologic criteria and prognostic implications. *Cancer* 1985; 56: 1106-11.
- Karlsson UL, Leibel SA, Wallner K, Davis LW, Brady LW. *Brain*. Philadelphia; 2004; 28: 791-838.
- Levin VA, Leibel SA, Gutin PH: Neoplasms of the central nervous system. In: DeVita VT Jr, Hellmar S, Rosenberg SA, (eds): *Cancer: Principles and Practice of Oncology*. 6th ed. Philadelphia, Pa: Lippincott Williams & Wilkins; 2001; p. 2100-2160.
- Kleihues P, Cavenee WK, International Agency for Research on Cancer. *Pathology and Genetics of Tumours of the Nervous System*. Lyon, France: International Agency for Research on Cancer, 2000.
- Ringertz N. Grading of gliomas. *Acta Pathol Microbiol Scand* 1950; 27: 51-64.
- Michelsen JJ, New PF. Brain tumour and pregnancy. *J Neurol Neurosurg Psychiatry* 1969; 32: 305-7.
- Burger PC, Green SB. Patient age, histologic features, and length of survival in patients with glioblastoma multiforme. *Cancer* 1987; 59: 1617-25.
- DAVIS L, MARTIN J, et al. A study of 211 patients with verified glioblastoma multiforme. *J Neurosurg* 1949; 6: 33-44.
- Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005; 352: 987-96.
- Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5- year analysis of the EORTC-NCIC trial. *Lancet Oncol* 2009; 10: 459-66.
- Athanassiou H, Synodinou M, Maragoudakis E, Paraskevaidis M, Verigos C, Misailidou D, et al. Randomized phase II study of temozolomide and radiotherapy compared with radiotherapy alone in newly diagnosed glioblastoma multiforme. *J Clin Oncol* 2005; 23: 2372-7.
- Marsh JC, Gielda BT, Herskovic AM, Wendt JA, Turian JV. Sparing of the hippocampus and limbic circuit during whole brain radiation therapy: A dosimetric study using helical tomotherapy. *J Med Imaging Radiat Oncol* 2010; 54: 375-82.
- Raineki C, Holman PJ, Debiec J, Bugg M, Beasley A, Sullivan RM. Functional emergence of the hippocampus in context fear learning in infant rats. *Hippocampus* 2010; 20: 1037-46.
- De Angelis LM, Delattre JY, Posner JB. Radiation induced dementia in patients cured of brain metastases. *Neurology* 1989; 39: 789-96.
- Roman DD, Sperduto PW. Neuropsychological effects of cranial radiation: current knowledge and future directions. *Int J Radiat Oncol Biol Phys* 1995; 31: 983-98.
- Jeneson A, Mauldin KN, Hopkins RO, Squire LR. The role of the hippocampus in retaining relational information across short delays: the importance of memory load. *Learn Mem* 2011; 18: 301-5.
- Hamilton GF, Murawski NJ, St Cyr SA, Jablonski SA, Schiffino FL, Stanton ME, et al. Neonatal alcohol exposure disrupts hippocampal neurogenesis and contextual fear conditioning in adult rats. *Brain Res* 2011; 1412: 88-101.
- Mini-Mental State Examination (MMSE), <https://medworksmidia.com/product/mini-mental-state-examination-mmse>. (Accessed 01.11.2022).
- Sherman CD. *UICC Klinik onkoloji*. 4. Baskı, Ankara: Başbakanlık Basimevi, 1990.
- Karadeniz AN, Frazer I. Erişkin Yaş Oligodendrogliomalı Hastalarda Kombine Tedavi ve Radyoterapi Sonuçları. *Türk Onkoloji Dergisi* 1987; 3: 605-11.
- Levin VA, Silver P, Hannigan J, Wara WM, Gutin PH, Davis RL, et al. Superiority of post-radiotherapy adjuvant chemotherapy with CCNU, procarbazine, and vincristine (PCV) over BCNU for anaplastic gliomas: NCOG 6G61 final report. *Int J Radiat Oncol Biol Phys* 1990; 18: 321-4.
- Newlands ES, Foster T, Zaknoen S. Phase I study of temozolomide (TMZ) combined with procarbazine (PCB) in patients with gliomas. *Br J Cancer* 2003; 89: 248-25.
- Brada M, Hoang-Xuan K, Rampling R, Dietrich PY, Dirix LY, Macdonald D, et al. Multicenter phase II trial of temozolomide in patients with glioblastoma multiforme at first relapse. *Ann Oncol* 2001; 12: 259-66.
- Stupp R, Dietrich PY, Ostermann Kraljevic S, Pica A, Maillard I, Maeder P, et al. Promising survival for patients with newly diagnosed glioblastoma multiforme treated with concomitant radiation plus temozolomide followed by adjuvant temozolomide. *J Clin Oncol* 2002; 20: 1375-82.
- Balana C, Vaz MA, Sepúlveda JM, Mesia C, del Barco S, Pineda E, et al. A phase II randomized, multicenter, open-label trial of continuing adjuvant temozolomide beyond six cycles in patients with glioblastoma (GEINO 14–01). *Neuro Oncol* 2020; 22: 1851.
- Mallick S, Kunhiparambath H, Gupta S, Benson R, Sharma S, Laviraj MA, et al. Hypofractionated accelerated radiotherapy (HART) with concurrent and adjuvant temozolomide in newly diagnosed glioblastoma: a phase II randomized trial (HART-GBM trial). *J Neurooncol* 2018; 140: 75-82.
- Wee CW, Kim E, Kim N, Kim IA, Kim TM, Kim YJ, et al. Novel recursive partitioning analysis classification for newly diagnosed glioblastoma: a multi-institutional study highlighting the MGMT promoter methylation and IDH1 gene mutation status. *Radiother Oncol* 2017;

- 123: 106-11.
29. Yung WK, Albright RE, Olson J, Fredericks R, Fink K, Prados MD, et al. A phase II study of temozolomide vs. procarbazine in patients with glioblastoma multiforme at first relapse. *Br J Cancer* 2000; 83: 588-93.
30. Monje ML, Mizumatsu S, Fike JR, Palmer TD. Irradiation induces neural precursor-cell dysfunction. *Nat Med* 2002; 8: 955-62.
31. Gondi V, Tolakanahalli R, Mehta MP, Tewatia D, Rowley H, Kuo JS, et al. Hippocampal-sparing whole-brain radiotherapy: a “how-to” technique using helical tomotherapy and linear accelerator-based intensity-modulated radiotherapy. *Int J Radiat Oncol Biol Phys* 2010; 78: 1244-52.
32. Wee CW, Kim KS, Kim CY, Han JH, Kim YJ, Kim IA. Feasibility of hippocampus-sparing VMAT for newly diagnosed glioblastoma treated by chemoradiation: pattern of failure analysis. *Radiat Oncol* 2020; 15: 98.

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## A Speckle-Tracking Echocardiography Study: Cardiac Effect of Sleep Duration

### Speckle-Tracking Ekokardiyografi Çalışması: Uyku Süresinin Kalp Üzerine Etkileri

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#### ABSTRACT

**Objective:** Sleep is a complex event involving various physiological processes. Recent studies during the last few years have shed light on the effects of sleep duration on health. We investigated the cardiac effects of sleep duration on speckle-tracking echocardiography. We divided the patients into 2 groups who slept more or less than 7 hours and evaluated all of them with transthoracic echocardiography.

**Methods:** This study was a retrospective study was conducted to investigate the cardiac effects of sleep duration. Between June 2021 and February 2022, 129 individuals who were admitted to our hospital for routine check-up with no known chronic disease and with normal and good quality echocardiographic examination were included in the study. The patients who were eligible for the study design were called by the investigator to obtain information on average sleep duration, and two groups were defined by the self-reported sleep duration ( $\leq 7$  hours,  $> 7$  hours per day) and were used in the statistical analyses.

**Results:** A total of 129 patients [ $\leq 7$  hours ( $n=80$ ),  $> 7$  hours ( $n=49$ ) per day] were enrolled in this study. All echocardiographic parameters were compared with sleep duration. Among these parameters, left ventricular (LV) global longitudinal strain (GLS) was found to be greater in the group who slept less. ( $-21.5 \pm 2.6$  vs.  $-20.3 \pm 2.2$   $p=0.005$ ). However, being within normal limits, LV end diastolic diameter and right heart chambers were found to be more enlarged in those who slept 7 hours or less than 7 hours. It was also observed that the right atrial volume was higher in the less sleeper group

**Conclusion:** As a result, GLS, which is one of the parameters showing LV function, was found to be less in healthy people with a sleep time above a certain period, and we believe that this may be a hypothesis to explain the relationship of long sleep time with cardiovascular events.

**Keywords:** Echocardiography, sleep, speckle-tracking imaging

#### Öz

**Amaç:** Uyku, çeşitli fizyolojik süreçleri içeren karmaşık bir olaydır. Yakın zamanda yapılan çalışmalar, uyku süresinin sağlık üzerindeki etkilerine ışık tutmuştur. Biz de çalışmamızda uyku süresinin kalpte speckle-tracking ekokardiyografi bulguları üzerine etkisini araştırdık. Hastaları 7 saatten fazla uyuyan ve az uyuyan olarak 2 gruba ayırdık ve hepsini transtorasik ekokardiyografi ile değerlendirdik.

**Yöntemler:** Bu çalışma, uyku süresinin kardiyak etkilerini araştırmak amacıyla yapılan retrospektif bir çalışmadır. Hastanemize Haziran 2021-Şubat 2022 tarihleri arasında kontrol amaçlı başvuran, bilinen kronik bir hastalığı olmayan, normal ve görüntü kalitesi iyi ekokardiyografiye sahip 129 kişi çalışmaya alındı. Çalışmaya dahil edilmeye uygun hastalar, ortalama uyku süresi hakkında bilgi almak amacıyla bir araştırmacı tarafından arandı ve hastalar kendilerinin sözel olarak belirttiği uyku sürelerine istatistiksel analizlerde kullanılmak üzere iki gruba (günde  $\leq 7$  saat,  $> 7$  saat) ayrıldı.

**Bulgular:** Bu çalışmaya toplam 129 hasta [günde  $\leq 7$  saat ( $n=80$ ),  $> 7$  saat ( $n= 49$ )] alındı. Tüm ekokardiyografik parametreler uyku süresi ile karşılaştırıldı. Bu parametreler arasında sol ventrikül (LV) global longitudinal strain (GLS) daha az uyuyan grupta daha fazla bulundu ( $-21,5 \pm 2,6$  vs.  $-20,3 \pm 2,2$   $p=0,005$ ) Ayrıca LV end diastolik çapı ve sağ kalp boşluklarının çapları 7 saat veya 7 saatten az uyuyanlarda daha fazla genişlemiş olarak bulundu. Yine daha az uyuyan grupta sağ atriyal hacmin daha yüksek olduğu gözlemlendi.

**Sonuç:** Sonuç olarak, LV fonksiyonunu gösteren parametrelerden biri olan GLS değerinin, uyku süresi belirli bir sürenin üzerinde olan sağlıklı kişilerde daha az olduğunu saptadık. Bunun uzun uyku süresinin kardiyovasküler olaylarla ilişkisini açıklamak için bir hipotez olabileceğini düşünmekteyiz.

**Anahtar Sözcükler:** Ekokardiyografi, uyku, speckle-tracking görüntüleme

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## INTRODUCTION

Sleep is a complex event involving various physiological processes. Recent studies during the last few years have shed light on the effects of sleep duration on health (1). Currently, there is no evidence of harm and long-term consequences of sleeping between 6-8 hours a day, but there are data on the benefits of consistent sleep between 6 and 8 h per night (2). Many factors such as the variety of studies, how sleep duration is determined, the diversity in the studied populations, and the selection of parameters that are considered to be affected by sleep duration affect the results, which makes it challenging to obtain a clear result about healthy sleep duration and its effects (2). Over recent years, there has been a good amount of evidence suggesting that too little or too much sleep is related to adverse health outcomes such as mortality (3,4), cardiovascular disease (5-7), respiratory disorder (8), type 2 diabetes (9) hypertension (10,11), and obesity (12,13). Unlike the cardiovascular adverse effects of short sleep duration, there are no compelling hypotheses regarding the mechanism of the relationship between long sleep and illness. In addition, there is only little empirical data to make clear inferences. Myocardial strain imaging can measure left ventricular (LV) global longitudinal strain (GLS) and provides objective quantification of LV myocardial deformation (14). In our current study, we divided the patients into 2 groups who slept more or less than 7 h and evaluated all of them with speckle-tracking echocardiography (STE).

## MATERIALS AND METHODS

This retrospective study was conducted to investigate the cardiac effects of sleep duration. Echocardiographic assessment was performed by two experienced echocardiography specialists. The study protocol was approved by the Local Ethics Committee of Memorial Bahçelievler Hospital (approval number: 34, date: 23.03.2022). Written and verbal consent was obtained from all the patients. Between June 2021 and February 2022, 160 individuals who were admitted to our hospital for routine check-up with no known chronic disease were scanned, and patients with normal and good quality echocardiographic examination were included in the study. Patients with poor image quality and whose images were unsuitable for strain examination on echocardiography, who did not answer our phone calls, who had a chronic disease such as asthma, coronary heart disease, hypertension, diabetes, or obstructive sleep apnea, and who were on regular medical treatment were excluded from the study. After all patients were evaluated according to the exclusion criteria, 129 patients were enrolled. The patients who were eligible for the study design were called by the investigator to obtain information on average sleep duration, and two groups were defined by the self-reported sleep duration ( $\leq 7$  h,  $> 7$  h per day) and were used in the statistical analyses.

### *Echocardiographic Evaluation*

Transthoracic echocardiography examinations were performed according to the recommendations of the American Society of Echocardiography. M-mode, two-dimensional imaging, conventional, tissue Doppler evaluation at the septal and lateral mitral annulus, and strain imaging were obtained in all patients at rest in the left decubitus position (15). The echocardiography machine that was

used for all patients was a "Philips Epiq 7C." Patients without optimal image quality to perform strain analysis were excluded from the study. The echocardiographic images were analyzed by one experienced cardiologist using the workstation "QLAB version 13."

### *Data Analysis*

STE was performed on four consecutive cardiac cycles of two-dimensional left ventricle (LV) images from the three standard apical views for LV and left atrium (LA); right ventricle (RV) focused apical 4 chamber according to the latest guidelines and a novel 2D strain analytical software (AutoStrain, Philips) was used for strain analysis (16). This software is able to define the region for tracking around a line automatically, and it allows manual changes afterwards. In segments with poor tracking, the border was re-adjusted manually until the best tracking was obtained. Furthermore, RV global longitudinal strain and RV free wall strain, components of left atrial strain (LAS) (atrial reservoir (LAS-r), conduit (LAS-cd), and contractile (LAS-ct) function) were examined according to the recommendations of the European Association of Cardiovascular Imaging (16).

### *Statistical Analysis*

Continuous variables are presented as mean  $\pm$  standard deviation, and categorical data are presented as percentages or frequencies. Continuous variables were examined by the Kolmogorov-Smirnov test to check for normality of distribution. The patient population was categorized on the basis of sleep duration. Baseline characteristics were compared between groups using Student's t-test or the  $\chi^2$  test. A two-tailed p-value of  $\leq 0.05$  was considered statistically significant. All data were analyzed using SPSS version 23.0.

## RESULTS

We included 129 individuals (71 male and 58 female). The basal clinical characteristics of the patients are presented in Table 1. The mean age of participants who slept 7 hours or less than 7 hours was  $36.2 \pm 9.2$ , while the mean age of those who slept for more than 7 hours were  $33 \pm 11$ .

All echocardiographic parameters were compared with sleep duration. Among these parameters, LV-GLS was found to be greater in the group who slept less. ( $-21.5 \pm 2.6$  vs.  $-20.3 \pm 2.2$   $p=0.005$ ). However, being within normal limits, left ventricular end diastolic diameter (LVEDD) and right heart chambers were found to be more enlarged in those who slept 7 hours or less than 7 hours. It was also observed that the right atrial volume was higher in the less sleeper group. The analysis of standart echocardiographic parameters and STE findings according to sleep duration is summarized in Table 2, 3 respectively.

## DISCUSSION

In the current study, we evaluated the effects of sleep duration on cardiac functions with STE. The main result of our study was that the LV-GLS value was lower in people who slept more than 7 h than in those who slept for 7 h or less. According to the statistical results, although LV-GLS values were within normal limits in both groups, they were significantly lower in those who slept more than 7 h.

**Table 1.** Clinical and basic biochemical features of the patients

	Sleep duration <7 hours (n=80)	Sleep duration ≥7 hours (n=49)	p
Age (years)	36.2±9.2	33±11	0.078
Height (cm)	169.2±9.3	166.1±9.8	0.074
Weight (kg)	72.5±12.7	69.8±12.7	0.230
Haemoglobin (gr/dL)	14.2±1.9	13.6±2.3	0.097
Haematocrit (%)	43±5	42±4.1	0.243
Creatinine (mg/dL)	0.8±0.2	0.8±0.2	0.689
ALT (U/L)	19±11.5	18.8±21.1	0.931
AST (U/L)	19.3±12.9	23±34.2	0.388
Serum fasting glucose (mg/dL)	97.4±20	100±13.7	0.419
TSH (mU/L)	2±1.2	2.2±1.1	0.478
Estimated GFR (mL/min)	109.9±19.1	107.9±18.6	0.566

ALT: Alanine aminotransferase, AST: Aspartate transaminase, TSH: Thyroid stimulant hormone, GFR: Glomerular filtration rate.

**Table 2.** Standard echocardiographic parameters according to sleep duration

	Sleep duration <7 hour	Sleep duration ≥7 hour	p
LVEF (%)	65.1±4.1	65.5±3.9	0.677
RVEF (%)	58.7±6.7	58.9±6.9	0.870
LVEDD (cm)	4.6±0.4	4.5±0.4	<b>0.039</b>
LVESD (cm)	2.7±0.4	2.5±0.4	0.056
LVEDV (mL)	79.2±20.1	73.2±17.9	0.112
LVESV (mL)	27.5±7.9	25.6±6.8	0.206
LA volume (mL)	45±18.9	41.9±16.3	0.375
RA volume (mL)	39.1±12.1	33.6±14.9	0.035
LA diameter (cm)	3.3±0.5	3.1±0.4	0.099
RVEDD (cm)	3.4±0.5	3.2±0.5	<b>0.018</b>
RA diameter (cm)	3.5±0.5	3.1±0.6	<b>0.001</b>
TAPSE (cm)	2.3±0.4	2.3±0.4	0.228
MAPSE (cm)	1.6±0.3	1.6±0.3	0.601
Mitral inflow E wave (m/sec)	0.7±0.2	0.8±0.2	0.052
Mitral inflow A wave (m/sec)	0.6±0.1	0.6±0.2	0.104
Septal e' (m/sec)	10.3±2.1	10.7±2.6	0.404
Septal a' (m/sec)	7.5±2	7.1±1.8	0.351
Septal s' (m/sec)	7.1±1.2	7±1.1	0.961
Lateral e' (m/sec)	14.1±3.8	14.8±3.9	0.365
Lateral a' (m/sec)	7.5±1.9	7.8±2.3	0.521
Lateral s' (m/sec)	9.8±2.3	9.5±2.3	0.404
IVRT (msec)	88.9±20.7	84.3±21.3	0.268
DT (msec)	128.5±37.5	136.8±36.1	0.273
TR maximum velocity (m/sec)	2.3±0.2	2.2±0.3	0.067
Estimated PAPs (mmHg)	25.8±4.4	24.4±3.9	0.107

LVEF: Left ventricular ejection fraction, RVEF: Right ventricular ejection fraction, LVEDD: Left ventricular end-diastolic diameter, LVESD: Left ventricular end-systolic diameter, LA: Left atrium, RA: Right atrium, RVEDD: Right ventricular end-diastolic diameter, TAPSE: Tricuspid annular plane systolic excursion, MAPSE: Mitral annular plane systolic excursion, IVRT: Isovolumetric relaxation time, DT: Deceleration time, TR: Tricuspid regurgitation, PAPs: Systolic pulmonary artery pressure.

**Table 3.** STE components according to sleep duration

	Sleep duration <7 hour	Sleep duration ≥7 hour	p
LV-GLS (%)	-21.5±2.6	-20.3±2.2	<b>0.005</b>
LA-r (%)	49.4±15.3	50.1±16.4	0.822
LA-cd (%)	-34.8±11.7	-33.3±12.1	0.488
LA-ct (%)	-14.5±11	-16.8±9.6	0.242
RV-FWSL (%)	-29.3±5.5	-27.8±5.7	0.121
RV-GLS (%)	-25±7.3	-24.7±5	0.780

STE: Speckle-tracking echocardiography, RV-FWSL: Right ventricular free wall longitudinal strain, RV-GLS: Right ventricular global longitudinal strain, LV-GLS: Left ventricular global longitudinal strain, LAS-R: Left atrial reservoir strain, LAS-cd: Left atrial conduit strain, LAS-ct: Left atrial contractile strain.

Although various studies have shown the effects of long sleep duration on cardiovascular health, to the best of our knowledge, there is no study in the literature showing its relationship with LV-GLS. LV-GLS is a measure of LV myocardial shortening, which is a good marker for LV dysfunction and determining cardiovascular events in patients with various diseases and healthy people (17).

Sleep is an indispensable physiological activity for people to maintain their lives optimally. Many studies conducted to date have shown that sleep duration is associated with morbidity and mortality (9,18).

In our study, we found that LV-GLS was lower in people who slept more than 7 hours compared to the people who slept 7 hours or less. In a multicenter study by Wang et al. (18), the relationship between sleep duration, death, and major cardiovascular events was investigated. As a result of this study, it was observed that the risk of death and major cardiovascular events was increased in people who slept 6 hours or less and people who slept for more than 8 hours. In this study, it was observed that the risk increase in people who slept 6 h or less was less than that in those who slept 9-10 hours, and this risk was even higher in people who slept 10 hours or more [ $<6$  hours hazard ratio (HR): 1.09, 9-10 hours HR: 1.17,  $>10$  hours HR: 1.41] (18). In a meta-analysis, Cappuccio et al. (9) investigated the relationship between sleep duration and coronary heart disease, cardiovascular causes, and stroke-related death. Because of this meta-analysis, the risk of death from coronary artery disease and stroke was found to be high in both groups, but a significant increase in cardiovascular mortality was not found in the group with short sleep duration, unlike long sleep duration (9). In another meta-analysis, Yin et al. (19) compared people who slept 7 h or less with those who slept more than 7 hours. As a result of this analysis, it was shown that the risk of coronary heart disease, cardiovascular disease, stroke, and total mortality increased with each hour of decrease and/or increase in sleep time. They also found that each hour of oversleeping increased the risk of these events by more than one hour of less sleep (19).

As seen in the above-mentioned studies and meta-analyses, long sleep duration increases the risk of cardiovascular morbidity and mortality. Due to this situation, a clear mechanism has not yet been revealed. One of the most prominent hypotheses at the moment is that long sleep duration may be a marker of one or more underlying diseases. In a study by Patel et al. (20), it was observed that in conditions such as systemic lupus erythematosus and various psychiatric pathologies such as depression, excessive alcohol intake, and obesity, the rate of sleep for 7 hours or more was

higher. Similarly, in another study, Stranges et al. (21) showed that people who slept for 8 hours or more had less physical activity and worse results in the SF-36 questionnaire, which indicates physical health. Krueger and Friedman (22), on the other hand, compared people who sleep 9 hours or more per day with those who sleep 7 hours. Compared with the other group, the prevalence of diabetes, cardiovascular diseases, and smoking history was higher in people who slept a lot (22). As seen in the aforementioned studies, it should be kept in mind that long sleep duration may not be a cause of adverse cardiovascular events in the studies but a reflection of the comorbidities that cause these events.

Another result of our study was that LVEDD and the diameters of the right heart chambers were larger in the group with a short sleep duration. Similar to our study, Lee et al. (23) demonstrated that LVEDD was significantly higher in people who slept for 7 h or less compared with other groups, yet no evaluation of right heart chambers was performed in this study. Although there is no clear explanation for the short sleep duration and the presence of enlargement of the heart chambers, various hypotheses can be put forward. It was first shown in studies that short sleep duration was associated with increased adrenergic tone (24,25) that may have increased the release of renin in the macula densa in the kidney via beta receptors, leading to sodium retention and consequently an increase in intravascular volume. This may explain the increase in the right and left ventricles, especially because of increased preload. This can also be explained by the increase in adrenergic tone, which may cause an increase in LV end-diastolic volume because of increased blood pressure in patients and a consequent increase in afterload. Therefore, we believe that more studies are needed on this subject.

### Study Limitations

Our study has several limitations. The first limitation is that the study was a single-center study and the number of patients included was small. Second, verbal learning of sleep duration and not using polysomnography may have affected the results of the study. Some sleep disorders and sleep quality, particularly those detected by polysomnography, affect cardiovascular health.

### CONCLUSION

As a result, GLS, which is one of the parameters showing LV function, was found to be less in healthy people with a sleep time above a certain period, and we think that this may be a hypothesis to explain



the relationship of long sleep time with cardiovascular events. In addition, there may be significant changes in the geometry of the left and right heart chambers due to increased adrenergic tone during short sleep. We think that further studies are needed to reflect this situation in the clinic.

### Ethics

**Ethics Committee Approval:** The study protocol was approved by the Local Ethics Committee of Memorial Bahçelievler Hospital (approval number: 34, date: 23.03.2022).

**Informed Consent:** Written and verbal consent was obtained from all the patients.

### Authorship Contributions

Concept: G.B., Ö.Ö., Design: G.B., Ö.Ö., Supervision: G.B., Resources: G.B., Ö.Ö., Materials: G.B., Ö.Ö., Data Collection and/or Processing: G.B., Ö.Ö., Analysis and/or Interpretation: G.B., F.A.D., E.Ö., S.Ü., Literature Search: G.B., F.A.D., E.Ö., S.Ü., Writing: G.B., Ö.Ö., E.Ö., Critical review: F.A.D., E.Ö., S.Ü.

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

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### REFERENCES

- Montano N, Fiorelli E, Tobaldini E. Sleep Duration and the Heart: I Sleep, Therefore I Beat. *J Am Coll Cardiol* 2019; 74: 1315-6.
- Cappuccio FP, D'Elia L, Strazzullo P, Miller MA. Sleep duration and all-cause mortality: a systematic review and meta-analysis of prospective studies. *Sleep* 2010; 33: 585-92.
- Ferrie JE, Shipley MJ, Cappuccio FP, Brunner E, Miller MA, Kumari M, et al. A prospective study of change in sleep duration: associations with mortality in the Whitehall II cohort. *Sleep* 2007; 30: 1659-66.
- Kripke DF, Garfinkel L, Wingard DL, Klauber MR, Marler MR. Mortality associated with sleep duration and insomnia. *Arch Gen Psychiatry* 2002; 59: 131-6.
- Meisinger C, Heier M, Lowel H, Schneider A, Doring A. Sleep duration and sleep complaints and risk of myocardial infarction in middle-aged men and women from the general population: the MONICA/KORA Augsburg Cohort Study. *Sleep* 2007; 30: 1121-7.
- Ikehara S, Iso H, Date C, Kikuchi S, Watanabe Y, Wada Y, et al. Association of sleep duration with mortality from cardiovascular disease and other causes for Japanese men and women: the JACC study. *Sleep* 2009; 32: 295-301.
- Stone KL, Ewing SK, Ancoli-Israel S, Ensrud KE, Redline S, Bauer DC, et al. Self-reported sleep and nap habits and risk of mortality in a large cohort of older women. *J Am Geriatr Soc* 2009; 57: 604-11.
- Bliwise DL. Sleep-related respiratory disturbances. *J Gerontol* 1984; 39: 255.
- Cappuccio FP, D'Elia L, Strazzullo P, Miller MA. Quantity and quality of sleep and incidence of type 2 diabetes: a systematic review and meta-analysis. *Diabetes Care* 2010; 33: 414-20.
- Gangwisch JE, Heymsfield SB, Boden-Albala B, Buijs RM, Kreier F, Pickering TG, et al. Short sleep duration as a risk factor for hypertension: analyses of the first National Health and Nutrition Examination Survey. *Hypertension* 2006; 47: 833-9.
- Cappuccio FP, Stranges S, Kandala NB, Miller MA, Taggart FM, Kumari M, et al. Gender-specific associations of short sleep duration with prevalent and incident hypertension: the Whitehall II Study. *Hypertension* 2007; 50: 693-700.
- Cappuccio FP, Taggart FM, Kandala NB, Currie A, Peile E, Stranges S, et al. Meta-analysis of short sleep duration and obesity in children and adults. *Sleep* 2008; 31: 619-26.
- Stranges S, Cappuccio FP, Kandala NB, Miller MA, Taggart FM, Kumari M, et al. Cross-sectional versus prospective associations of sleep duration with changes in relative weight and body fat distribution: the Whitehall II Study. *Am J Epidemiol* 2008; 167: 321-9.
- Collier P, Phelan D, Klein A. A Test in Context: Myocardial Strain Measured by Speckle-Tracking Echocardiography. *J Am Coll Cardiol* 2017; 69: 1043-56.
- Mitchell C, Rahko PS, Blauwet LA, Canaday B, Finstuen JA, Foster MC, et al. Guidelines for Performing a Comprehensive Transthoracic Echocardiographic Examination in Adults: Recommendations from the American Society of Echocardiography. *J Am Soc Echocardiogr* 2019; 32: 1-64.
- Badano LP, Koliás TJ, Muraru D, Abraham TP, Aurigemma G, Edvardsen T, et al. Standardization of left atrial, right ventricular, and right atrial deformation imaging using two-dimensional speckle tracking echocardiography: a consensus document of the EACVI/ASE/Industry Task Force to standardize deformation imaging. *Eur Heart J Cardiovasc Imaging* 2018; 19: 591-600.
- Russo C, Jin Z, Elkind MS, Rundek T, Homma S, Sacco RL, et al. Prevalence and prognostic value of subclinical left ventricular systolic dysfunction by global longitudinal strain in a community-based cohort. *Eur J Heart Fail* 2014; 16: 1301-9.
- Wang C, Bangdiwala SI, Rangarajan S, Lear SA, AlHabib KF, Mohan V, et al. Association of estimated sleep duration and naps with mortality and cardiovascular events: a study of 116 632 people from 21 countries. *Eur Heart J* 2019; 40: 1620-9.
- Yin J, Jin X, Shan Z, Li S, Huang H, Li P, et al. Relationship of Sleep Duration with All-Cause Mortality and Cardiovascular Events: A Systematic Review and Dose-Response Meta-Analysis of Prospective Cohort Studies. *J Am Heart Assoc* 2017; 6: e005947.
- Patel SR, Malhotra A, Gottlieb DJ, White DP, Hu FB. Correlates of long sleep duration. *Sleep* 2006; 29: 881-9.
- Stranges S, Dorn JM, Shipley MJ, Kandala NB, Trevisan M, Miller MA, et al. Correlates of short and long sleep duration: a cross-cultural comparison between the United Kingdom and the United States: the Whitehall II Study and the Western New York Health Study. *Am J Epidemiol* 2008; 168: 1353-64.
- Krueger PM, Friedman EM. Sleep duration in the United States: a cross-sectional population-based study. *Am J Epidemiol* 2009; 169: 1052-63.
- Lee JH, Park SK, Ryoo JH, Oh CM, Kang JG, Mansur RB, et al. Sleep Duration and Quality as Related to Left Ventricular Structure and Function. *Psychosom Med* 2018; 80: 78-86.
- Zhong X, Hilton HJ, Gates GJ, Jelic S, Stern Y, Bartels MN, et al. Increased sympathetic and decreased parasympathetic cardiovascular modulation in normal humans with acute sleep deprivation. *J Appl Physiol* 2005; 98: 2024-32.
- Tochikubo O, Ikeda A, Miyajima E, Ishii M. Effects of Insufficient Sleep on Blood Pressure Monitored by a New Multibiomedical Recorder. *Hypertension* 1996; 27: 1318-24.

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## Wilson Disease in a Turkish Population: Molecular Insights into an Old Disease with Reported and Novel Variants

Bir Türk Popülasyonunda Wilson Hastalığı: Bildirilen ve Yeni Varyantlarıyla Eski Bir Hastalığa Moleküler Bakış

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### ABSTRACT

**Objective:** Wilson's disease (WD) is a rare autosomal recessive genetic liver disorder with hepatic, neurological, or psychiatric manifestations between 1<sup>st</sup> to 5<sup>th</sup> decades. WD is caused by homozygous or compound heterozygous pathogenic variants in the *ATP7B* gene. In this study, we aimed to contribute to the *ATP7B* gene variant spectrum in a Turkish population of WD patients.

**Methods:** We investigated 49 patients from 46 families to determine the underlying molecular etiology of WD. DNA samples were extracted from peripheral blood. The molecular genetic diagnosis was performed using the next-generation sequencing method.

**Results:** Molecular genetic analysis revealed 26 different variants, two of which were novel c.1707+4A>G (IVS4+4A>G) and p.M497K in 34 patients from 31 different families. p.M769Hfs\*26 was the variant with the highest allele frequency at 11.3%, followed by the p.H1069Q variant (8%). The classification of the variants according to the molecular mechanism was as follows: missense 61.5%, splice site and frameshift 15.4%, and non-sense 0.08%.

**Conclusion:** In this study, we aimed to contribute the variant spectrum of the *ATP7B* gene in the Turkish population and the genetic profile of WD with the obtained data.

**Keywords:** Wilson disease, *ATP7B* gene, variants

### Öz

**Amaç:** Wilson hastalığı (WH), 1. ve 5. dekatlar arasında hepatik, nörolojik veya psikiyatrik belirtiler gösteren, otozomal resesif geçişli, nadir görülen bir genetik karaciğer hastalığıdır. WH, *ATP7B* genindeki homozigot veya bileşik heterozigot patojenik varyantlardan kaynaklanır. Bu çalışmada, WH hastalarının Türk popülasyonunda *ATP7B* gen varyant spektrumuna katkıda bulunmayı amaçladık.

**Yöntemler:** WH'nin altında yatan moleküler etiyojijiyi belirlemek için 46 aileden 49 hastayı inceledik. DNA örnekleri periferik kandan ekstrakte edildi. Moleküler genetik tanı, yeni nesil dizileme yöntemi kullanılarak gerçekleştirildi.

**Bulgular:** Moleküler genetik analizde, 31 farklı aileden 34 hastada ikisi yeni olmak üzere c.1707+4A>G (IVS4+4A>G) ve M497K) 26 farklı varyant ortaya çıkardık. p.M769Hfs\*26 %11,3 ile en yüksek alel frekansına sahip varyan idi ve onu p.H1069Q varyantı (%8) takip ediyordu. Varyantların moleküler mekanizmaya göre sınıflandırılması şu şekildeydi; yanlış anlamlı %61,5, splay ve çerçeve kayması %15,4, anlamsız %0,08.

**Sonuç:** Bu çalışmada elde edilen verilerle *ATP7B* geninin Türk popülasyonundaki varyant spektrumuna ve WH'nin genetik profiline katkıda bulunmayı amaçladık.

**Anahtar Sözcükler:** Wilson hastalığı, *ATP7B* geni, varyantlar

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## INTRODUCTION

Wilson's disease [(WD), MIM#277900] is a rare autosomal recessive inherited metabolic disease of copper metabolism (1). It manifests as hepatic, neurological, or psychiatric disorders, or a combination thereof, in individuals aged three to fifty years. The disease is diagnosed on the basis of typical clinical and laboratory findings, such as low ceruloplasmin concentration and high urinary copper excretion (2). Early symptoms of WD are usually observed between the ages of 5 and 35, but in some cases, the disease can manifest in both infancy and old age (3,4). In most cases, clinical manifestations are usually latent and may not be noticeable until progressive liver failure and/or neurological dysfunction (5). Although the Kayser-Fleischer ring is a diagnostic ophthalmologic feature, it can be seen in a limited number of patients with WD (6). Biochemical indicators for early diagnosis may not be helpful for WD, particularly in children (7). The prevalence of WD is considered to be 1/30,000 to 1/100,000 live births and a carrier frequency of 1 in 90 (8). According to some studies on the European population, the disease is observed much more commonly than previous estimates, with an estimated prevalence of 1:10,000 to 1:7,000 (9,10). This discrepancy probably originates from difficulties in the clinical diagnosis of WD, the presence of latent forms, and limitations of diagnostic methods.

WD is typically inherited in an autosomal recessive manner and is caused by either homozygous or compound heterozygous pathogenic variants in the *ATP7B* gene, which encodes a copper-transporting ATPase. The P-type copper-transporting ATPase ensures that copper is incorporated into ceruloplasmin and excreted into the bile via the apical membrane of hepatocytes. *ATP7B* dysfunction causes hepatocyte damage due to the failure of hepatic excretion of copper into the bile (11). Free copper enters the bloodstream and causes damage to many organs and tissues, predominantly in the liver, brain, cornea, and kidney. With an early diagnosis and treatment of WD, liver cirrhosis and other complications can be prevented before they cause disability or death (12).

The *ATP7B* gene (MIM#606882) is located on chromosome 13q14.3 and is approximately 80 kb in size, consisting of 21 exons, 20 introns, and 4.3 kb open reading frames (13). It encodes a protein called copper-transporting ATPase 2 that contains 1464 amino acids. Copper binding sites in the NH<sub>2</sub>-terminal domain, the ATP-binding domain that converts the energy of ATP (containing the nucleotide binding and phosphorylation subdomains), and the six transmembrane proteins are the domains of this protein. In WD patients, most pathogenic variants are missense.

Although the *ATP7B* gene is frequently studied worldwide, there is a lack of data from Türkiye. In addition, the spectrum of *ATP7B* variants differs significantly between populations. This study contributes to the variant distribution of the *ATP7B* gene in the Turkish population.

## MATERIALS AND METHODS

All procedures were performed following the tenets of the Declaration of Helsinki. Informed consent was obtained from the participants' parents for molecular genetic analysis and the publication of patient data before their enrollment in the study. This study was approved by the Ankara City Hospital Ethics Committee (approval number: E1/878/2020, date: 02.07.2020).

## Patients

Between 2016 and 2019, 49 index cases from 46 different families were referred to our clinic for molecular diagnosis of WD.

The clinical diagnosis process included physical examination, anamnesis, complete blood count, urine analysis, biochemical analysis of blood (alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase, CE), parameters of copper metabolism ceruloplasmin, and 24-h urine copper excretion). The patients were evaluated as WD patients according to clinical findings (presence of Kayser-Fleischer ring, neurological abnormalities, abnormal liver function) and laboratory findings; (low serum ceruloplasmin, increased urinary copper excretion). The exclusion criteria were chronic viral hepatitis, autoimmune hepatitis, and other metabolic liver diseases.

## Genetic Analyses

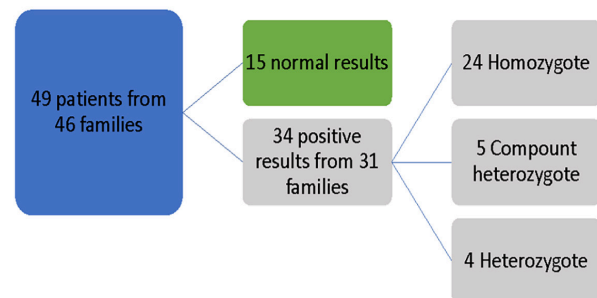
Genetic analyses were performed for diagnosis using a parental informed consent form. DNA was extracted from peripheral blood using a QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. All exons of the *eATP7B* (NM\_000053.4) gene all exons and exon-intron boundaries were sequenced using in-house designed primers on the MiSeq system (Illumina, San Diego, CA, USA) with next-generation sequencing according to the manufacturer's instructions.

## In Silico Bioinformatics Analyses

The potential functional effects of novel missense variants were predicted using Alamut® Visual 2.4 Software (SIFT, Polyphen-2, and Variant Taster). Information about the location of AA and predicted transmembrane domains was obtained from UniProt (<http://www.uniprot.org/>). The classification of variants was made according to the ACMG 2015 criteria (14).

## RESULTS

Between 2016 and 2019, 49 patients from 46 families were referred to our clinic for molecular genetic diagnosis of WD. In this study, we confirmed the molecular diagnosis of WD in 34 of 49 patients (34/49, 69%) (Figure 1). The average age of patients was 16.6. Twenty-two of the patients were between 6 and 18 years old, 5 of them were between 18-30, and the remaining 3 were over 30 years old. The age information of 4 patients was not available (Figure 2). In the molecular genetic analyses positive group, the number of male patients was 22, and the number of female patients was 12, which indicates male dominance (65%) (Figure 3).



**Figure 1.** Classification of patients according to the results of molecular genetic analyses.

In this series, we found 26 unique genetic variants, two of which were novel. In terms of variant effect, missense type was the most common (61.5%, 16/26). The splice site and frameshift frequency were the same at 15.4% (4/26), and non-sense was 0.08% (2/26). While 24 of the 34 patients were homozygous, 5 were compound heterozygous and 4 were heterozygous states. All compound heterozygous results were confirmed by parental molecular genetic analyses. In addition, all variants are classified as pathogenic or likely pathogenic according to the ACMG 2015 classification criteria (Table 1).

Variants were relatively frequent in the exon 8 region. The distribution of the variants tended to be balanced when exon 8 was excluded (Figure 4). p.M769Hfs\*26 and p.H1069Q were the most common variants with allele frequencies of 7/62 (11.3%) and 5/62 (8%), respectively. Patients 31 and 32 were siblings and both had c.1285+5G>T (IVS2+5G>T) alterations, which were also shared by unrelated patient 22. Moreover, p.R778G and p.L1327V were also shared variants by two unrelated families. Patients 7, 8 (c.1707+4>G or IVS4+4>G) and 24, 25 (Y1384\*) were affected siblings from two different families, respectively. p.M297K and c.1707+4>G were the

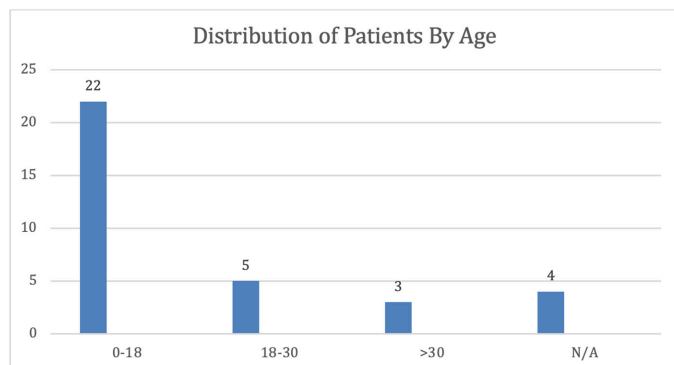
only 2 novel variants in the study, and both were classified as likely pathogenic according to ACMG criteria.

## DISCUSSION

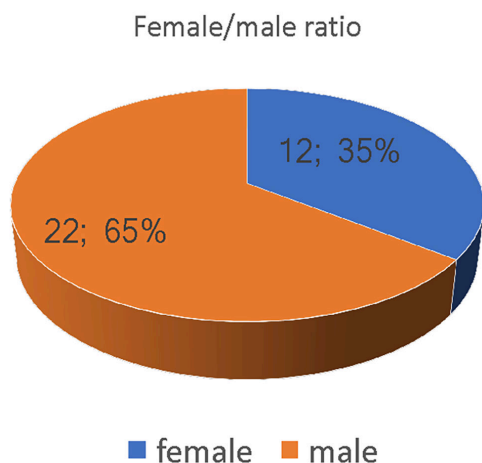
To the best of our knowledge, this is the largest study conducted in Türkiye. A total of 26 unique variants were detected in 34 index cases from 31 different families with a 67% (31/46) diagnostic rate, which is much lower than that reported in previous studies reporting variants in approximately 80% of all clinically confirmed WD (15). The highest mutation detection rates reported in the literature are 98% and 97.35% in cohorts from the United Kingdom and Vietnam, respectively (9,16). Higher variant detection rates may be related to complementary techniques implemented in DNA analysis (such as MLPA) to detect large deletions/duplications, the varying prevalence of WD in different populations, or specific patient selection criteria used in clinical diagnosis. Similar previous cohorts from Türkiye reported variant detection rates of approximately 65% (30/46) and 71% (23/32) using Sanger sequencing, which is comparable to the present study (17,18). Considering the number of patients included in our study and other studies from Türkiye, the differences could be interpreted as insignificant. In our study, the age of the patients evaluated ranged 6 to 39 years at the time of molecular diagnosis. The age range of the evaluated patients was highly variable, consistent with other studies in the literature.

As a typical feature of WD, the common variant responsible for the disease differs among populations. In Europe, Mediterranean countries, and North America, the most common variant is p.H1069Q with a prevalence varying from 10% to 40%, whereas in Asian populations, R778L is more prevalent, with a frequency of 20% to 44% (19). Based on data from Eastern, Central, and Northern Europe, p.H1069Q is suggested to be the most frequent variant with an allele frequency of 30% to 70% in molecularly confirmed WD patients (20). However, in this study, p.M769Hfs\*26 was the most frequent variant with an allele frequency of 11.3% (7/62), followed by p.H1069Q with a frequency of 8% (5/62). p.H1069Q was previously reported as the most common variant in other studies from Türkiye with a 17.39% and 15.625% allele frequency (17,18). The number of patients in our cohort was insufficient to infer common WD allele in the Turkish community. A larger group of patients with molecularly identified WD might reveal a higher allele frequency in favor of the H1069Q variant. Nevertheless, we specify p.M769Hfs\*26 as one of the frequent alleles in Turkish patients with WD. The p.M769Hfs\*26 variant is mentioned in the list of major causative mutations in some other European countries (21). Our study is the first to suggest that p.M769Hfs\*26 is also a common allele in the Turkish WD population. The other recurrent variants detected in different families included c.1285+5G>T, p.R778G, and p.L1327V.

According to previous studies, missense variants were observed as a more common molecular mechanism compared with non-sense and frameshift effects (50% vs. 16.7%) in WD (22). Similar rates were found in our study (missense type 61.5%, splice site, and frameshift 15.4%) except for non-sense variants, which we found only twice with a 0.08% (2/26) prevalence. WD appears to have high allelic heterogeneity between different populations. The distributions of variants within the gene were evaluated, and the most common variants were seen in exon 8, which we thought could be the hot-



**Figure 2.** Distribution of admitted patients by age group All patients were divided into three groups: 0-18, 18-30, and over 30. The age information of 4 patients was not available.



**Figure 3.** Female/male ratio of the patients.

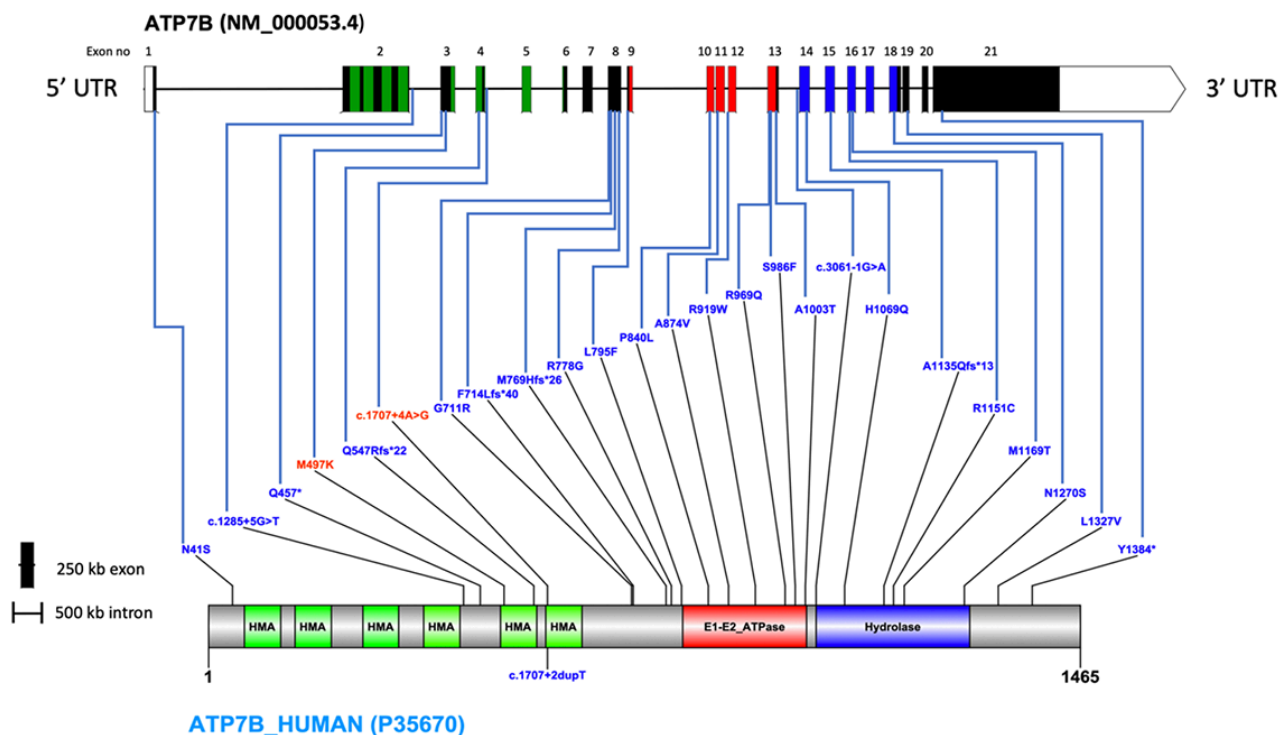
**Table 1.** The entire list of index cases and all identified variants

Patient	Age (year)	Gender	Variants*	Zygoty	Classification <sup>†</sup>	Variant ID**
1	29	F	c.2131G>A (p. G711R)	Het	P	CM992817
2	13	M	c.3007G>A (p. A1003T) /c.3207C>A (H1069Q)	Comp Het	P	CM980180/CM930059
3	39	M	c.3402delC (A1135Qfs*13)	Hom	P	CM930912/rs137853281
4	13	F	c.1369C>T p. Q457*	Hom	P	CM992591
5	18	M	c.3979C>G (p. L1327V)	Hom	P	CM993113
6	19	M	c.3979C>G (p. L1327V)	Hom	P	CM993113
7	17	F	IVS4+4>G (c.1707+4A>G)	Hom	LP	NOVEL
8	6	M	IVS4+4A>G (c.1707+4A>G)	Hom	LP	NOVEL
9	N/A	M	c.2304_2305insC (p. M769Hfs*26)	Hom	P	CI951903
10	15	M	c.3506T>C (p. M1169T)	Hom	P	CM992826
11	6	M	c.3207C>A (p. H1069Q)	Hom	P	CM012441
12	6	M	c.2141_2142delTC (p. P714Lfs*40)	Het	P	NOVEL
13	27	F	c.2332C>G (p. R778G)	Hom	P	CM950112/ rs137853284
14	28	M	IVS13-1G>A (c.3061-1G>A)	Hom	P	CS961487
15	11	F	c.122A>G (p. N41S)	Het	P	CM040679).
16	36	M	c.1490T>A p. M497K	Hom	LP	NOVEL
17	18	F	c.2304_2305insC (p. M769Hfs*26)	Het	P	CI951903
18	10	M	c.2332C>G (p. R778G)	Hom	P	CSM950112
19	8	F	c.1639delC (p. G547Rfs*22)	Hom	P	CD982472
20	16	M	c.2304_2305insC (p. M769Hfs*26)	Hom	P	CI951903
21	11	M	c.2383C>T p. L795F/c.2519C>T p. P840L	Comp Het	P	CM970141/CM980172
22	17	F	IVS2+5G>T (c.1285+5G>T) /c.2957C>T p. S986F/ c.3451C>T p. R1151C	Comp Het	P	CS982084/CM107540/CM076004
23	9	M	c.2621C>T (p. A874V)	Hom	P	CM980173
24	9	F	c.2141T>G p. Y1384*	Hom	P	rs1431633756
25	18	M	c.2141T>G p. Y1384*	Hom	P	rs1431633756
26	11	F	IVS2+2dupT (c.1707+2dupT)	Hom	P	CI992786
27	11	M	c.1639delC (p. Q547Rfs*22)	Hom	P	CD982472
28	37	M	c.2906G>A p. R969Q	Hom	P	rs121907996
29	19	F	c.2557C>T p. R919W	Hom	P	CM980176
30	N/A	M	c.2621C>T (p. Ala874Val) /c.3809A>G (p. A1270S)	Comp Het	P	CM980173/930060
31	N/A	F	IVS2+5G>T (c.1285+5G>T) / IVS13-1G>A (c.3061 1G>A)	Comp Het	P	CS982084/ CS961487
32	15	M	IVS2+5G>T (c.1285+5G>T) /IVS13-1G>A c.3061-1G>A	Comp Het	P	CS982084/CS961487
33	6	M	c.3207C>A (p. H1069Q)	Hom	P	CM012441???
34	N/A	M	c.2304_2305insC (p. M769Hfs*26)	Hom	P	rs137853287

N/A: Not available, F: Female, M: Male, P: Pathogenic, LP: Likely pathogenic, <sup>†</sup>ACMG 2015, <sup>\*</sup>*ATPTB* (NM\_000053.4), <sup>\*\*</sup>Human Gene Mutation Database (HGMD), and dbSNP.

spot region (Figure 4). Previous studies have reported different exons for potential hot spot regions. For example, while Coffey et al. (9) reported exons 8, 14, and 18 as hot spot regions for the United Kingdom population, Huong et al. (16) pointed to exon 2 as a hot spot region for Vietnamese populations. Also, Balashova et al. (22) considered exons 7, 8, and 17 as hot spots for the Russian

Federation. This confirms the thesis that “hotspots” of the *ATPTB* gene vary significantly by geographic region. Because relatively frequent variants were observed in exon 8 (4/26, 15.4%), this region was thought to be a potential hot spot region for the *ATPTB* gene within the Turkish population. However, this is a low rate to provide an advantage in early molecular genetic diagnosis, and variants in



**Figure 4.** The locations of the variants on the *ATP7B* gene and protein domains were visualized. The novel variants are shown in red.

the *ATP7B* gene were shown to be distributed throughout the gene when exon 8 was excluded. Therefore, despite studies suggesting that a certain exon of the gene should be examined first, the most appropriate first step option for the molecular genetic diagnosis of WD in the Turkish population could be DNA sequence analysis of the whole gene.

Previous studies have shown that there is no significant difference in the prevalence of the disease between the genders in WD. Conversely, in our study, the ratio of male patients to female patients was significantly higher (22/34, 65%) (Figure 3), which is an unexpected situation in autosomal inherited genetic diseases (23). When we look at the other studies from Türkiye, we see slight male dominance there as well (18/30, 60% and 12/23, 52%) (17,18). This may be due to the limited number of patients evaluated in the studies from Türkiye, but it may also be due to the different genetic backgrounds of the Turkish population. In addition, although there is no difference in the occurrence of the disease between the sexes, there appears to be a significant gender difference in the clinical presentation of WD patients. It has been reported that liver findings in women and neuropsychiatric findings in men are more common in WD than in the opposite sex. This situation may also be reflected in the selected patient group in studies conducted in Türkiye.

Although WD was clinically considered, no variant was detected in the molecular genetic analysis of 15 of 49 patients. Possible explanations for the non-detection of variants include unknown variants in regulatory regions such as promoters or enhancers, deeper introns, or other DNA control regions outside the scope of DNA sequencing, and large deletions/duplications. In addition, other disease-related cellular factors may be responsible for the clinical findings. On the other hand, 4 patients had only one potential

disease-causing variant. The reason for this could be a variant in the other allele that remains undetected because of the same reasons specified for completely normal molecular genetic analyses. RNA-seq and large deletion/duplication analysis should be performed in such patients. Further studies are needed to determine the cause of WD in patients without a variant in the *ATP7B* gene in molecular genetic analysis.

## CONCLUSION

In most patients with WD, clinical manifestations are usually latent and may not be noticed until progressive liver failure and/or neurological dysfunction. Therefore, molecular genetic analysis of the *ATP7B* gene is important for the early diagnosis and treatment of clinically suspected patients. The molecular genetic spectrum of the *ATP7B* gene in WD differs between populations. In this study, it was observed that there were differences in the Turkish population. The variants we detected will contribute to our understanding of the molecular genetic structure of the disease in the Turkish population. In addition, the new variants detected will be added to the international databases of the disease. Thus, it will help to fully reveal the genetic mechanisms underlying the disease.

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## Ethics

**Ethics Committee Approval:** This study was approved by the Ankara City Hospital Ethics Committee (approval number: EI/878/2020, date: 02.07.2020).

**Informed Consent:** Informed consent was obtained from the participants.

**Authorship Contributions**

Surgical and Medical Practices: A.B., V.T., B.Ç., Concept: A.B., V.T., B.Ç., Design: A.B., V.T., B.Ç., Data Collection or Processing: A.B., V.T., B.Ç., Analysis or Interpretation: A.B., V.T., B.Ç., Literature Search: A.B., V.T., B.Ç., Writing: A.B., V.T., B.Ç.

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

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**REFERENCES**

1. Wilson S. Word Cloud contest. *Brain* 1912; 34: 295-507.
2. Sternlieb I. Perspectives on Wilson's disease. *Hepatology* 1990; 12: 1234-9.
3. Ala A, Walker AP, Ashkan K, Dooley JS, Schilsky ML. Wilson's disease. *Lancet* 2007; 369: 397-408.
4. Perri RE, Hahn SH, Ferber MJ, Kamath PS. Wilson Disease--keeping the bar for diagnosis raised. *Hepatology* 2005; 42: 974.
5. Valentino PL, Roberts EA, Beer S, Miloh T, Arnon R, Vittorio JM, et al. Management of Wilson Disease Diagnosed in Infancy: An Appraisal of Available Experience to Generate Discussion. *J Pediatr Gastroenterol Nutr* 2020; 70: 547-54.
6. Chandra A, Bhattacharjee MS. Kayser-Fleischer Rings in Wilson's Disease. *N Engl J Med* 2021; 385: e46.
7. Ferenci P. Diagnosis of Wilson disease. *Handb Clin Neurol* 2017; 142: 171-80.
8. Figus A, Angius A, Loudianos G, Bertini C, Dessi V, Loi A, et al. Molecular pathology and haplotype analysis of Wilson disease in Mediterranean populations. *Am J Hum Genet* 1995; 57: 1318-24.
9. Coffey AJ, Durkie M, Hague S, McLay K, Emmerson J, Lo C, et al. A genetic study of Wilson's disease in the United Kingdom. *Brain* 2013; 136: 1476-87.
10. Kulkarni A, Sharma VK. Wilson's disease; 2017.
11. Peña MM, Lee J, Thiele DJ. A delicate balance: homeostatic control of copper uptake and distribution. *J Nutr* 1999; 129: 1251-60.
12. Rodriguez-Castro KI, Hevia-Urrutia FJ, Sturniolo GC. Wilson's disease: A review of what we have learned. *World J Hepatol* 2015; 7: 2859-70.
13. Tanzi RE, Petrukhin K, Chernov I, Pellequer JL, Wasco W, Ross B, et al. The Wilson disease gene is a copper-transporting ATPase with homology to the Menkes disease gene. *Nat Genet* 1993; 5: 344-50.
14. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015; 17: 405-23.
15. Schmidt HH-J. Role of genotyping in Wilson's disease. *J Hepatol* 2009; 50: 449-52.
16. Huong NTM, Hoa NPA, Ngoc ND, Mai NTP, Yen PH, Văn Anh HT, et al. Mutation spectrum of ATP7B gene in pediatric patients with Wilson disease in Vietnam. *Mol Genet Metab Rep* 2022; 31: 100861.
17. Simsek Papur O, Akman SA, Cakmur R, Terzioğlu O. Mutation analysis of ATP7B gene in Turkish Wilson disease patients: identification of five novel mutations. *Eur J Med Genet* 2013; 56: 175-9.
18. Terzioğlu O. Clinical and genetic analysis of pediatric patients with Wilson disease. *Turk J Gastroenterol* 2015; 26: 397-403.
19. Zhang Y, Wu Z-Y. Wilson's disease in Asia. *Neurology Asia* 2011; 16: 103-9.
20. Ferenci P. Regional distribution of mutations of the ATP7B gene in patients with Wilson disease: impact on genetic testing. *Hum Genet* 2006; 120: 151-9.
21. Gomes A, Dedoussis GV. Geographic distribution of ATP7B mutations in Wilson disease. *Ann Hum Biol* 2016; 43: 1-8.
22. Balashova MS, Tuluzanovskaya IG, Glotov OS, Glotov AS, Barbitoff YA, Fedyakov MA, et al. The spectrum of pathogenic variants of the ATP7B gene in Wilson disease in the Russian Federation. *J Trace Elem Med Biol* 2020; 59: 126420.
23. Litwin T, Gromadzka G, Członkowska A. Gender differences in Wilson's disease. *J Neurol Sci* 2012; 312: 31-5.

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## Investigation of Prophylactic Drug Use Adherence in Patients with Migraine

### Migren Hastalarında Profilaktik İlaç Kullanımına Olan Uyumun Araştırılması

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#### ABSTRACT

**Objective:** Adherence to drug use; it is a crucial part of patient care and clinical briefing. Non-compliance with prescribed treatment regimens is an important and common behavioral health problem for treating chronic diseases. In our study, we aimed to investigate the rate of adherence to medication use in migraine patients taking prophylactic drugs, the factors affecting it, and the reasons for decreased compliance.

**Methods:** Eighty patients diagnosed with migraine and using prophylactic treatment for migraine were included in the study. Detailed sociodemographic and clinical histories were obtained at the first interview. The scores obtained by applying the Beck Anxiety and Depression Inventory were recorded for each patient. The patients were evaluated with weekly visits for four weeks; adherence was examined using the tablet counting method.

**Results:** In our study, 70% of our patients who received prophylactic drugs for migraine were non-compliant with the treatment; it was observed that the three most common causes were forgetting to take medication, drug side effects, and drug expiration. It was observed that low education level, being married, diet and exercise incompatibility, and high sleep problems, depression, and anxiety symptoms affected the adherence to prophylactic drug use in a statistically significant and negative way.

**Conclusion:** To increase the patient's compliance with the treatment regimen, all potential barriers to compliance must be considered. Measures that consider the factors under the patient's control and the interaction between patient and physician and patient-health care will have the greatest impact on improving adherence. This will lead to better clinical outcomes and decrease morbidity and health expenditures.

**Keywords:** Migraine, drug, treatment, prophylactic treatment, compliance, tablet counting method, health

#### ÖZ

**Amaç:** İlaç kullanımına olan bağlılık; hasta bakımının ve klinik bilgilendirmenin çok önemli bir parçasıdır. Reçete edilen tedavi rejimlerine uyumsuzluk, kronik hastalıkların tedavisinde önemli ve yaygın bir davranışsal sağlık sorunudur. Çalışmamızda profilaktik ilaç kullanan migren hastalarında ilaca uyum oranları, etkileyen faktörler ve azalan uyum nedenlerinin araştırılması amaçlandı.

**Yöntemler:** Çalışmaya migren tanısı alan ve migren için profilaktik tedavi kullanan 80 hasta dahil edildi. İlk görüşmede detaylı sosyodemografik ve klinik öykü alındı. Beck Anksiyete ve Depresyon Envanteri uygulanarak elde edilen puanlar her hasta için kaydedildi. Hastalar dört hafta boyunca haftalık ziyaretlerle değerlendirildi; adherans tablet sayma yöntemi ile incelendi.

**Bulgular:** Çalışmamızın sonucunda migren nedeniyle profilaktik tedavi alan hastalarımızın %70'inin tedaviye uyumsuz olduğu; ilaç kullanımında uyumsuzluğa yol açan en sık 3 sebebin ilaç almayı unutma, ilaç yan etkisi ve ilacın bitmesi olduğu görüldü. Uyuma etki eden faktörlere bakıldığında; eğitim seviyesinin düşüklüğü, evli olmak, diyet ve egzersiz uyumsuzluğu ve uyku problemi, depresyon ve anksiyete belirtilerinin fazla olmasının profilaktik ilaç kullanımına olan uyumu istatistiksel olarak anlamlı şekilde etkilediği görüldü.

**Sonuç:** Hastanın tedavi rejimini takip etme uyumunu artırmak için uyumun önündeki tüm olası engeller göz önünde bulundurulmalıdır. Uyumun iyileştirilmesinde en büyük etkiyi, hastanın kontrolünde olan faktörleri ve hasta-hekim ve hasta-sağlık hizmetleri arasındaki etkileşimi dikkate alan önlemler olacaktır. Bu, daha iyi klinik sonuçlara ve morbidite ve sağlık harcamalarında azalmaya yol açacaktır.

**Anahtar Sözcükler:** Migren, ilaç, tedavi, profilaktik tedavi, uyum, tablet sayma yöntemi, sağlık

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## INTRODUCTION

Migraine is a multifactorial neurovascular syndrome characterized by headache attacks in individuals with genetic susceptibility (1). Migraine headache, which occurs in approximately 10% of the adult population, is the most common type of headache and ranks first in prevalence among semi-headaches (2). Migraine is seen approximately three times more frequently in women than in men (3). It is most commonly encountered in the second and third decades of life, and the age range within which the attacks are most common is 35-45 years. While the disease usually progresses in attacks, migraines become chronic in approximately 4% of patients (4). Migraine affects people in the most productive period of their lives and therefore causes significant loss of the workforce (5). According to data from the World Health Organization (WHO), migraine is among the top 20 diseases that cause severe loss of work force in the world (6). Migraine imposes heavy financial obligations for countries because of both the loss of workforce and the high cost of treatment. Patients with migraine describe pain attacks of varying severity and frequency. Various treatments, including antihypertensive beta-blockers, antidepressants, anti-epileptic drugs, and anti-CGRP monoclonal antibodies, can be administered for prophylaxis. There is no universally accepted guideline for prophylactic treatment, but patients with 3 attacks per month are generally candidates for prophylactic treatment (4,7). Patient tolerance is an important component of prophylactic treatment success.

Compliance or adherence refers to the course of a patient's prescribed and planned treatment in terms of time, dose, and frequency. Compliance with drug use in chronic treatments has been investigated for many chronic diseases, and poor compliance (<80% of the recommended treatment) has been associated with poor prognosis in many disease groups (8). Treatment compliance is a crucial part of patient care and is indispensable for achieving clinical goals. In a 2003 report on drug compliance published by the WHO, it was concluded that increasing the effectiveness of compliance has positive effects on health and may have a greater impact than specific medical treatments (10). Non-adherence to prophylactic treatments and behavioral lifestyle changes complicate treatment plans and are known barriers to successful treatment responses (11). Adherence or compliance is determined by the proportion of doses received in the schedule, often compared with the drug doses prescribed in a specific period. This rate is observed in observational studies and can be determined by researchers recording the number of drug doses taken by patients or by monitoring systems that determine drug intake. Drug counting by comparing the doses of drugs taken

by patients with the amount of drugs prescribed in a given period contributes to accurate measurement of compliance (9). Treatment compliance is important for the success of the treatment of migraine and other headaches, but studies of compliance in cases of migraine and other headaches are insufficient. Research conducted to date has shown that the rates of compliance with both acute and prophylactic migraine treatment are low (12).

According to the WHO, there are many factors that lead to poor treatment compliance and they can be grouped into 5 categories: socio-economic factors, treatment-related factors, patient-related factors, health system- and healthcare-related factors, and disease-related factors (10) (Table 1).

It is difficult to objectively determine the compliance with drug therapy. In defining compliance, direct and indirect methods are used, each with different advantages and disadvantages. Direct methods include the evaluation of blood pressure levels, urinary wastes and breakdown products, drug metabolites, and various other markers, whereas indirect methods include the review of treatment outcome, patient notification, records of the number of drug doses or compliance with appointments, prescription follow-up, and the physician's opinion (13,14).

In the present study, to evaluate the compliance of patients receiving prophylactic migraine treatment, drug counting (number of tablets consumed) was used as an indirect and objective method. This approach makes it possible to cooperate more closely with patients, and if a decrease in compliance was observed upon monitoring drug intake, the reasons for non-compliance were sought.

## MATERIALS AND METHODS

Our study was evaluated by the Gazi University Faculty of Medicine Clinical Research Ethics Committee, and ethics committee approval was obtained (approval number: 207, date: 11.04.2016).

Among patients aged 18 years who presented to the General Neurology Outpatient Clinic and Headache Outpatient Clinic of the Gazi University Faculty of Medicine's Neurology Department between 2016 and 2017, 80 patients who were diagnosed with migraine according to the ICHD-III beta diagnostic criteria and received prophylactic treatment for migraine were included in the study. Patients who did not require prophylactic treatment for migraine headaches and received only acute treatment were not included in the study. Patients with secondary headaches who could not attend follow-up appointments or could not be contacted at specified times during the study period were also excluded.

**Table 1.** Factors affecting adherence to treatment

Categories	Factors
Demographics features	Age, race, gender, occupation, educational status, and health information
Disease	Disease type, duration and severity, presence of complications, frequency of hospital use, satisfaction with healthcare providers, and quality of care
Treatment	Dose, type of drug, presence of other drugs, drug supply, diet required by the treatment, and presence of side effects
Behaviour	Doctor- patient interaction, patient's level of knowledge, illness, and treatment beliefs, caregiver knowledge, and beliefs
Economic features	Economic situation, health insurance and insurance type, and treatment and drug prices

Eighty patients diagnosed with migraine who met the inclusion criteria were included in the study. Informed consent was first obtained in writing from the patients who agreed to be included in the study, and detailed clinical information was obtained. In the first appointment, patients were interviewed according to this form, which included questions about age, gender, educational status, marital status, duration of illness, frequency of migraine attacks, presence of chronic migraine, duration of headaches, factors that trigger or alleviate headaches, presence of aura and/or prodrome, presence of concomitant diseases, sleep patterns, previous surgery or serious illnesses, smoking, compliance with exercise and healthy diet, total number of drugs used daily, migraine drugs used, and total doses for all drugs for 1 week. Neurological examinations of the patients were performed, and any abnormal findings were recorded. At the same time, the presence of the greater occipital nerve (GON) and trigger point tenderness were examined for each patient. Scores obtained from applications of the Beck Anxiety Scale and Beck Depression Scale were recorded for all patients in the same interviews. One week after the first meeting, patients were interviewed again and the number of drug dosages remaining in their pill boxes or bottles was determined. The same interviews were repeated three additional times at 1-week intervals, and patients were asked the number of remaining drug doses each time. If there were any variations in the patients remaining drug doses during this period, the reasons for the variations were learned and recorded. These reasons included forgetting to take one's medicine, leaving home without drugs, not taking drugs due to side effects, running out of drugs, misunderstanding directions, difficulty in swallowing medicine, doubting the necessity of the drugs, difficult drug use, and dislike of taking medicine. The weekly doses of the drugs used by the patients for migraine headache were calculated and recorded.

### **Statistical Analysis**

Statistical evaluation of the data was performed using IBM SPSS Statistics 23.0. Descriptive and frequency assessments were used to evaluate the patients' demographic data. For age, gender, occupation, marital status, educational status, duration of illness, total number of drugs used daily, number of migraine drugs used daily, types of migraine drugs used, headache duration, and monthly frequency of headache attack, Pearson's chi-square test, Yates' corrected chi-square test, and Fisher's chi-square test were used to compare relationships with migraine type, reasons for dose adjustment, comorbidities, causes of headache relief, exercise and diet compliance, and smoking. The conformity of continuous variables to normal distribution was evaluated using visual methods (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk tests). While statistical analysis of patient groups with and without treatment compliance was performed according to Beck Depression Scale scores, Beck Anxiety Scale scores, and total number of drugs used daily, the Mann-Whitney U test, a non-parametric test, was used for variables that did not show normal distribution. Statistical significance was set at  $p < 0.05$ .

## **RESULTS**

### **Descriptive Findings**

Eighty patients, including 69 women (86.2%) and 11 men (13.8%) aged 20-66 years were included in our study. In terms of occupation, 52.5% of the patients were housewives, 32.5% were employed, 12.5% were students, and 2.5% were retired. Furthermore, 71.2% of the patients were married and 28.8% were single, and 48.7% of the patients were primary school graduates, 20% were high school graduates, and 31.3% were higher education graduates. While 55% of the patients had migraine headaches for  $\leq 10$  years, 22.5% had experienced migraines for 11-19 years and 22.5% for 20 years or more. Furthermore, 77.5% of the patients reported migraines without auras and 22.5% had migraines with auras. While migraine headaches progressed as attacks in 90% of the patients, they were continuous in 10%. Prophylactic migraine medication was used by 57.5% of the patients as a single medication per day, and the remaining patients were using additional drugs due to comorbid diseases in addition to preventive migraine medication. In the first clinical interview, 73.8% of the patients who underwent neurological examinations had tenderness in the GON region and many trigger points. It was determined that 48.8% of the patients did not comply with their diets and 51.3% did not exercise. The smoking prevalence was 16.2%. Sleep problems were reported by 56.2% of the patients; most these problems were difficulty in falling asleep, waking up early in the morning, and frequent interruption of sleep during the night. Another comorbid condition accompanied migraines in 37.5% of the patients. Beck Depression Scale scores indicated high-level depressive symptoms for 17.5% of the patients, moderate-level symptoms for 10%, and low-level symptoms for 30%. While 55% of the patients had low anxiety scores, high anxiety scores were obtained for 28.7% and moderate anxiety scores for 16.3%. Among patients using preventive migraine treatment, the most commonly used medication was amitriptyline at a rate of 29.1%, whereas 21.5% of the patients used topiramate, 19.3% used duloxetine, 16.1% used propranolol, and 7.5% used sodium valproate.

Patients were followed for 4 weeks, and it was observed that 30% of the patients had some remaining drug dosages in their pill boxes on a weekly basis that was compatible with appropriate drug use for 4 weeks. The remaining 70% were considered to have had non-compliance with their treatment because they took fewer drug dosages than they should have on a weekly basis (Table 2). During the 4-week follow-up period, patients with treatment non-compliance were asked about the reasons for the fluctuations in their drug dosages. Forgetting to take the medication was the most common reason at a rate of 55.8%. This was followed by drug side effects at a rate of 14.7%, the drug having ended at a rate of 11.3%, leaving home without the medication at a rate of 9%, and dislike of taking medication at a rate of 5.6% (Table 3).

### **Comparative Statistics**

The gender, age, occupation, disease duration, presence of aura, monthly attack frequency, duration of attacks, presence of chronic migraine, smoking, presence of concomitant diseases, presence

**Table 2.** Distribution of adherence to drug use by individuals participating in the study

Number		(%)
<b>Compliance with drug use (n=80)</b>		
Non-adherens	56	70.0
Adherens	24	30.0

**Table 3.** Distribution of reasons for changes in drug dose among individuals participating in the study

Reasons for dose change	Number	(%)
Forget	50	56.8
Side effects	13	14.7
End of the drug	10	11.3
Leaving the house without medication	8	9
Unlike taking medication	5	5.6
Misunderstanding directives	1	1.1
Doubts about the necessity of the drug	1	1.1
Total	88	100

of additional drugs used, and presence of GON and/or trigger point sensitivity were compared between the compliant and non-compliant patient groups. No statistically significant differences were found for any of these variables ( $p>0.05$ ).

When the compliance statuses of the patients were compared according to marital status, 77.2% of married patients and 52.2% of single patients were found to be non-compliant with the proper usage of medication. Thus, single patients had a higher rate of treatment compliance, and marital status had a statistically significant effect on compliance ( $p=0.030$ ). Comparing the level of education and compliance with treatment, it was found that 82.1% of primary school graduates and 44% of higher education graduates were non-compliant. The relationship between educational status and compliance was statistically significant, and treatment compliance increased as the level of education increased ( $p=0.003$ ). While 82.2% of the patients who reported that they had sleep problems were non-compliant with the proper use of medication, 54.3% of the patients without sleep problems were found to be non-compliant, and the relationship between sleep patterns and compliance with preventive drug use was statistically significant ( $p=0.007$ ). When the compliance statuses of the patients were evaluated according to their diet and exercise habits, 84.6% of the patients who did not pay attention to their diets were found to be non-compliant, and this difference was statistically significant ( $p=0.021$ ). Similarly, 85.4% of migraine patients who did not exercise were non-compliant with the appropriate use of preventive medication, and there was a statistically significant difference when these patients were compared with those who exercised ( $p=0.009$ ) (Table 4).

## DISCUSSION

In this study, patients who required prophylactic treatment for migraine headaches were evaluated in terms of treatment compliance. We determined compliance with drug use in these patients, the factors affecting compliance, and the reasons for non-compliance. Therefore, we applied the tablet counting method

to the drug dosages of migraine patients receiving prophylactic treatment. Because of the evaluations performed for 4 weeks, it was determined that 70% of our patients were non-compliant with their treatments.

We concluded that the education levels of the patients affected compliance in a statistically significant way. As the level of education increased, treatment compliance also increased ( $p=0.003$ ). Our findings are in agreement with the results of many other studies. In most previous studies, it was reported that education level affected adherence to medication schedules. In a study conducted by Leopold et al. (15) with patients with Parkinson's disease, many sociodemographic and disease-related factors were examined, as in our study, and it was reported that only gender and education level had significant effects on compliance. As the level of education increased, compliance with appropriate drug dosages also increased (15).

When our patients were evaluated in terms of compliance according to marital status, it was observed that single patients were statistically significantly more compliant with appropriate drug dosages than married patients ( $p=0.03$ ). We know of no similar study in the literature to date addressing the effect of marital status on treatment compliance.

We also evaluated our patients in terms of the presence of sleep problems, exercise, and dietary habits. In accordance with the literature, the presence of sleep problems, low levels of exercise, and failure to maintain healthy dietary habits decreased treatment compliance among our patients. These findings were statistically significant for sleep problems, exercise, and diet ( $p=0.007$ ,  $p=0.009$ , and  $p=0.021$ , respectively). Sleep disorders are common among individuals with migraines and have been found to increase the frequency of migraine attacks. Various sleep problems are frequently reported, particularly in patients with chronic migraine (16). Furthermore, one of the most common modifiable risk factors among migraine patients is obesity, which has been associated with the risk

**Table 4.** Comparison of patient groups who are compatible and non-compliant with drug use according to educational status, marital status, sleep patterns, and exercise and diet

	Compliance with drug use			
	Non-adherence		Adherence	
	Number	%	Number	%
<b>Educational status (n=80)</b>				
Primary school graduate	32	82.1	7	17.9
High school graduate	13	81.3	3	18.7
High education	11	44.0	14	56.0
<b>p=0.003</b>				
<b>Marital status (n=80)</b>				
Married	44	77.2	13	22.8
Single	12	52.2	11	47.8
<b>p=0.030</b>				
<b>Sleep pattern (n=80)</b>				
No problem	19	54.3	16	45.7
Problem	37	82.2	8	17.8
<b>p=0.007</b>				
<b>Diet compliance status (n=80)</b>				
Not attention	33	84.6	6	15.4
Partially	18	56.3	14	43.8
Attentive	5	55.6	4	44.4
<b>p=0.021</b>				
<b>Exercise status (n=80)</b>				
Not attention	35	85.4	6	14.6
Partially	17	54.8	14	45.2
Attentive	4	50.0	4	50.0
<b>p=0.009</b>				

of chronic migraine, especially in women of reproductive age. There are data supporting the argument that obesity leads to migraine chronicity by causing systemic inflammation and insulin resistance (17). Because obesity is also related to exercise habits, a sedentary lifestyle poses an indirect risk of migraines. Risk factors such as obesity, sedentary lifestyle, and sleep disturbances that complicate migraine control should be recognized and managed immediately to break the vicious cycle that accelerates the recurrence of these headaches. This is even more important considering the low levels of adherence to acute and/or preventive treatment among many migraine patients (18). Cognitive and behavioral interventions can improve patients' quality of life by promoting treatment compliance. In a study by Hepp et al. (19), regardless of the prophylactic agent prescribed for patients with chronic migraine, poor adherence to treatment was reported. At the end of 1 year, the patients' rate of compliance with treatment was found to be between 17% and 20%. Compared with previous studies evaluating compliance among patients with episodic migraines, compliance appeared to be lower among those with chronic migraines (19). Poor habits in terms of diet and exercise and the consequences of those habits increase susceptibility to chronic migraines, as do sleep disturbances, and

make it more difficult for patients to comply with treatment. Overall, the results obtained in this study were compatible with those reported in the literature. Although most our patients had episodic migraines, the high levels of risk factors among our patients seemed to worsen compliance.

Compliance with prophylactic treatment was lower among patients with anxiety and depressive symptoms. The Beck Anxiety Scale and Beck Depression Scale scores of non-compliant patients were higher than those of compliant patients, and the differences were statistically significant ( $p=0.01$  for both). Our findings are consistent with the data of many previous studies. Anxiety and depression adversely affect health and complicate the treatment of different illnesses. Mood disorders that impair an individual's energy, motivation, and cognitive functions may adversely affect the desire to be treated, the ability to continue treatment, and treatment compliance (20). Therefore, cognitive and behavioral interventions may improve treatment compliance and quality of life by improving patients' strategies for coping with psychosocial stress factors (18).

In our study, the reasons for non-compliance with prophylactic treatment among migraine patients were also investigated. The

most common reason for non-compliance was forgetting to take one's medication, as reported by 56.8% of our patients. This was followed by aversion to the drug's side effects, reported by 14.7%, and expiration of the drug, reported by 11.3%. In a previous survey study, reasons for non-compliance with the appropriate use of drugs in general were questioned and results similar to ours were obtained. The most common reason for non-compliance was forgetting to take the medication (30%), and other reasons were the existence of other priorities (16%), knowingly skipping a dose (11%), lack of knowledge (9%), and emotional factors (7%) (21). In addition, the prescription of complex treatment regimens, insufficient information about side effects and benefits, patient lifestyle, and drug cost have been cited as reasons for non-compliance (22).

### Study Limitations

Although the patients included in the present study showed typical demographic and clinical features of migraine patients and thus represented the target population well, the small sample size is a limitation of the study. Another limitation of this study is the tablet counting method used to measure compliance. Regardless of the measurement method chosen, no effort to monitor patients' drug usage at home perfect. Surveys may not accurately capture missed drug dosages or identify dosages taken at inappropriate times. Compliance measurements performed by counting the number of tablets, as we did in the present study, will indicate missed dosages but do not reflect the timing of the dosages (15). In future studies, the use of a combination of measurement methods would help reduce the effect of this limitation.

### CONCLUSION

Appropriate compliance with prescribed medications is a crucial part of patient care and an indispensable point in achieving clinical goals because non-compliance leads to inferior clinical outcomes, increased morbidity and mortality rates, and unnecessary health expenditures. In our study, the tablet counting method, which is an indirect and objective measurement method, was used to evaluate the compliance of migraine patients with prophylactic drug treatment. Approximately 25-50% of adult patients with headaches does not comply with preventive treatment. In this study, non-compliance was observed in 70% of migraine patients receiving prophylactic treatment. The higher rate of non-compliance obtained in this study in comparison with the literature may be attributed to the low level of education of most of our patients.

All potential barriers to compliance must be considered to increase the patient's ability to follow the prescribed treatment regimen. Interventions that take into account factors under the patient's control and interactions among the patient, physician, and healthcare system will have the greatest impact on improved compliance. This will lead to better clinical outcomes and decrease mortality, morbidity, and unnecessary health expenditures.

### Ethics

**Ethics Committee Approval:** Our study was evaluated by the Gazi University Faculty of Medicine Clinical Research Ethics Committee, and ethics committee approval was obtained (approval number: 207, date: 11.04.2016).

**Informed Consent:** Informed consent was first obtained in writing from the patients who agreed to be included in the study.

### Authorship Contributions

Concept: E.C., T.T., Design: E.C., T.T., Data Collection or Processing: E.C., Analysis or Interpretation: T.T., Literature Search: E.C., T.T., Writing: E.C., T.T.

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

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### REFERENCES

- Saper JR. Diagnosis and symptomatic treatment of migraine. *Headache* 1997; 37 Suppl 1: S1-14.
- Obermann M, Katsarava Z. Epidemiology of unilateral headaches. *Expert Rev Neurother* 2008; 8: 1313-20.
- Téllez-Zenteno JF, García-Ramos G, Zermeño-Pöhls F, Velazquez A; GGSM. Demographic, clinical and comorbidity data in a large sample of 1,147 patients with migraine in Mexico City. *J Headache Pain* 2005; 6: 128-34.
- İnan LE. Nörolojide Yeni Ufuklar: Başağrıları. Ankara: Ayrıntı basımevi, 2011.
- Leonardi M, Steiner TJ, Scher AT, Lipton RB. The global burden of migraine: measuring disability in headache disorders with WHO's Classification of Functioning, Disability and Health (ICF). *J Headache Pain* 2005; 6: 429-40.
- Leonardi M, Steiner TJ, Scher AT, Lipton RB. The global burden of migraine: measuring disability in headache disorders with WHO's Classification of Functioning, Disability and Health (ICF). *J Headache Pain* 2005; 6: 429-40.
- Chiang CC, Schwedt TJ. Calcitonin gene-related peptide (CGRP)-targeted therapies as preventive and acute treatments for migraine-The monoclonal antibodies and gepants. *Prog Brain Res* 2020; 255: 143-70.
- Hepp Z, Bloudek LM, Varon SF. Systematic review of migraine prophylaxis adherence and persistence. *J Manag Care Pharm* 2014; 20: 22-33.
- Berger A, Bloudek LM, Varon SF, Oster G. Adherence with migraine prophylaxis in clinical practice. *Pain Pract* 2012; 12: 541-9.
- Burkhart PV, Sabaté E. Adherence to long-term therapies: evidence for action. *J Nurs Scholarsh* 2003; 35: 207.
- Sokol MC, McGuigan KA, Verbrugge RR, Epstein RS. Impact of medication adherence on hospitalization risk and healthcare cost. *Med Care* 2005; 43: 521-30.
- Stovner Lj, Hagen K, Jensen R, Katsarava Z, Lipton R, Scher A, et al. The global burden of headache: a documentation of headache prevalence and disability worldwide. *Cephalalgia* 2007; 27: 193-210.
- Lee JK, Grace KA, Foster TG, Crawley MJ, Erowele GI, Sun HJ, et al. How should we measure medication adherence in clinical trials and practice? *Ther Clin Risk Manag* 2007; 3: 685-90.
- Farmer KC. Methods for measuring and monitoring medication regimen adherence in clinical trials and clinical practice. *Clin Ther* 1999; 21: 1074-90.
- Leopold NA, Polansky M, Hurka MR. Drug adherence in Parkinson's disease. *Mov Disord* 2004; 19: 513-7.
- Andress-Rothrock D, King W, Rothrock J. An analysis of migraine triggers in a clinic-based population. *Headache* 2010; 50: 1366-70.

17. Evans RW, Williams MA, Rapoport AM, Peterlin BL. The association of obesity with episodic and chronic migraine. *Headache* 2012; 52: 663-71.
18. Pistoia F, Sacco S, Carolei A. Behavioral therapy for chronic migraine. *Curr Pain Headache Rep* 2013; 17: 304.
19. Hepp Z, Dodick DW, Varon SF, Gillard P, Hansen RN, Devine EB. Adherence to oral migraine-preventive medications among patients with chronic migraine. *Cephalalgia* 2015; 35: 478-88.
20. DiMatteo MR, Lepper HS, Croghan TW. Depression is a risk factor for noncompliance with medical treatment: meta-analysis of the effects of anxiety and depression on patient adherence. *Arch Intern Med* 2000; 160: 2101-7.
21. Morisky DE, Green LW, Levine DM. Concurrent and predictive validity of a self-reported measure of medication adherence. *Med Care* 1986; 24: 67-74.
22. Ickovics JR, Meade CS. Adherence to HAART among patients with HIV: breakthroughs and barriers. *AIDS Care* 2002; 14: 309-18.

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## ChatGPT vs. Orthopedic Residents! Who is the Winner?

### ChatGPT Ortopedi Asistanlarına Karşı! Kazanan Kim?

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#### ABSTRACT

**Objective:** In recent advancements in artificial intelligence, ChatGPT by OpenAI has emerged as a versatile tool capable of performing various tasks; however, its application in medicine is challenged by complexities and limitations in accuracy. This article aims to compare ChatGPT's performance with orthopedic residents at Gazi University in a multiple-choice exam to assess its applicability and reliability in the field of orthopedics.

**Methods:** In this observational study conducted at Gazi University, 31 orthopedic residents were stratified by experience level and assessed using a 50-question multiple-choice test on various orthopedic topics. The study also evaluated ChatGPT 3.5's responses to the same questions, focusing on both the correctness and reasoning behind the answers.

**Results:** Orthopedic residents tested, ranging from 6 months to 5 years in experience, scored between 23 and 40 out of 50 in a multiple-choice exam, with a mean score of 30.81, varying by seniority. ChatGPT provided correct answers for 25 out of 50 questions, showing consistency in different languages and times, but also exhibited limitations by giving incorrect responses or stating that the correct answer was not among the choices for some questions.

**Conclusion:** While ChatGPT can accurately answer some theoretical questions, its effectiveness is limited in interpretive scenarios and in situations with multiple variables, although its accuracy may improve with updates over time.

**Keywords:** ChatGPT, artificial intelligence, orthopedics, traumatology

#### ÖZ

**Amaç:** Yapay zeka alanındaki son gelişmelerde, OpenAI tarafından geliştirilen ChatGPT çeşitli görevleri yerine getirebilen çok yönlü bir araç olarak ortaya çıkmıştır, ancak tıp alanındaki uygulaması karmaşıklıklar ve doğruluk sınırlamaları nedeniyle zorlanmaktadır. Bu makale, ChatGPT'nin performansını Gazi Üniversitesi'ndeki ortopedi asistanları ile çoktan seçmeli bir sınavda karşılaştırarak ortopedi alanında uygulanabilirliğini ve güvenilirliğini değerlendirmeyi amaçlamaktadır.

**Yöntemler:** Gazi Üniversitesi'nde yapılan bu gözlemsel çalışmada, 31 ortopedi asistanı deneyim düzeyine göre sınıflandırılmış ve çeşitli ortopedik konularda 50 soruluk çoktan seçmeli bir test kullanılarak değerlendirilmiştir. Çalışmada ayrıca ChatGPT 3.5'in aynı sorulara verdiği yanıtlar, yanıtların hem doğruluğuna hem de arkasındaki mantığa odaklanılmıştır.

**Bulgular:** Deneyimleri 6 ay ile 5 yıl arasında değişen ortopedi asistanları, çoktan seçmeli bir sınavda 50 üzerinden 23 ile 40 arasında puan aldı ve kıdeme göre değişmekle birlikte ortalama puan 30,81 oldu. ChatGPT, farklı dillerde ve zamanlarda tutarlılık göstererek 50 sorudan 25'ine doğru yanıt vermiş, ancak yanlış yanıtlar vererek veya bazı sorular için doğru yanıtın seçenekler arasında olmadığını belirterek sınırlamalar da sergilemiştir.

**Sonuç:** Çalışma, ChatGPT'nin bazı teorik soruları doğru bir şekilde yanıtlayabilse de, yorumlayıcı senaryolarda ve çok değişkenli durumlarda etkinliğinin sınırlı olduğu, ancak zaman içinde kişiselleştirilmiş güncellemelerle doğruluğunun artabileceği sonucuna varmaktadır.

**Anahtar Sözcükler:** ChatGPT, yapay zeka, ortopedi, travmatoloji

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## INTRODUCTION

In recent years, advancements in the field of artificial intelligence have experienced an upsurge in the scientific community. Of particular note, Chat Generative Pre-trained Transformer (ChatGPT) developed by OpenAI and endowed with a chatbot capability has been described as a groundbreaking development in this domain. Launched in November 2022, ChatGPT, unlike other chatbots, can respond to questions very quickly and can be used for different purposes (1). For example, it can write code for computer software, create a film script or a story, and answer multiple-choice questions in written form (2). However, despite all these impressive features, the use of artificial intelligence programs in the field of medicine can be limited compared with other areas because of the large number of variables involved. The impact of these developments on academic life is still a topic of study that has not yet been clearly defined.

In some journals, publications have begun to emerge where ChatGPT is recognized as a co-author (3,4). In contrast to the journals that have recognized ChatGPT as a co-author, some publications have raised concerns over the ethical implications of attributing authorship to an AI language model such as ChatGPT (5).

Despite its many impressive capabilities, ChatGPT has certain limitations and undesirable features. According to information provided by OpenAI, the company that developed the program, ChatGPT is capable of citing non-existent articles and processing non-existent data. Given the risk of introducing not only erroneous information but also plagiarism into academic publications, this raises concerns over the reliability of scientific records. In addition, it should be noted that ChatGPT's responses to questions may be incorrect, yet presented in a coherent manner, potentially creating a dangerous situation for non-healthcare professionals reliant on the program's output. The provision of inaccurate data by ChatGPT could lead to negative outcomes in future research or healthcare decisions.

The use of artificial intelligence programs to search internet data and find answers to many questions is increasing daily. As evidenced by publications related to ChatGPT in 2023, studies across various scientific fields, including public health and orthopedic surgery, have been conducted (6). It is still a matter of debate whether passing grades can be obtained in some written exams using this program (7,8). This situation has led to restrictions on the use of the program in some countries and universities.

The aim of this study was to demonstrate the level of success of ChatGPT, which has recently become a popular topic and is gaining popularity in academic circles, in a multiple-choice orthopedic exam by comparing it with the answers of orthopedic residents.

## MATERIALS AND METHODS

### *Study Design*

This observational study is planned to be conducted at a tertiary hospital that is actively involved in resident training. The study participants comprised 31 orthopedic residents from the Department of Orthopedics and Traumatology. These residents

were selected based on voluntary participation and were stratified into five groups according to their level of experience: 6 months to 1 year, 1-2 years, 2-3 years, 3-4 years, and 4-5 years. This stratification ensured a diverse range of expertise and perspectives within the field of orthopedics.

### *Test Design*

A comprehensive test consisting of 50 multiple-choice questions was designed to assess knowledge in various domains of orthopedics, including basic orthopedics, trauma, spine, orthopedic tumors, arthroplasty, and pediatric orthopedics. The questions, each with only one correct answer, were meticulously crafted by a working group of senior orthopedic professors, ensuring the validity and relevance of the content. Some examples of the questions asked to ChatGPT are presented in Figures 1-3.

### *Data Collection: Residents*

The test was conducted by the residents under fair, controlled conditions to maintain the integrity of the responses. The time allocated, environment, and mode of answer submission were standardized for all participants. Responses were collected and anonymized for further analysis.

### *Data collection - ChatGPT*

The same set of questions was presented to the ChatGPT 3.5 program, developed by OpenAI, at two different times to evaluate consistency in responses. For scenario-based questions, we used the same ChatGPT session to benefit from the AI's memory retention capabilities. For independent questions, a new session was initiated for each question to simulate a fresh interaction, mimicking a real-world clinical query scenario.

### *Ethics*

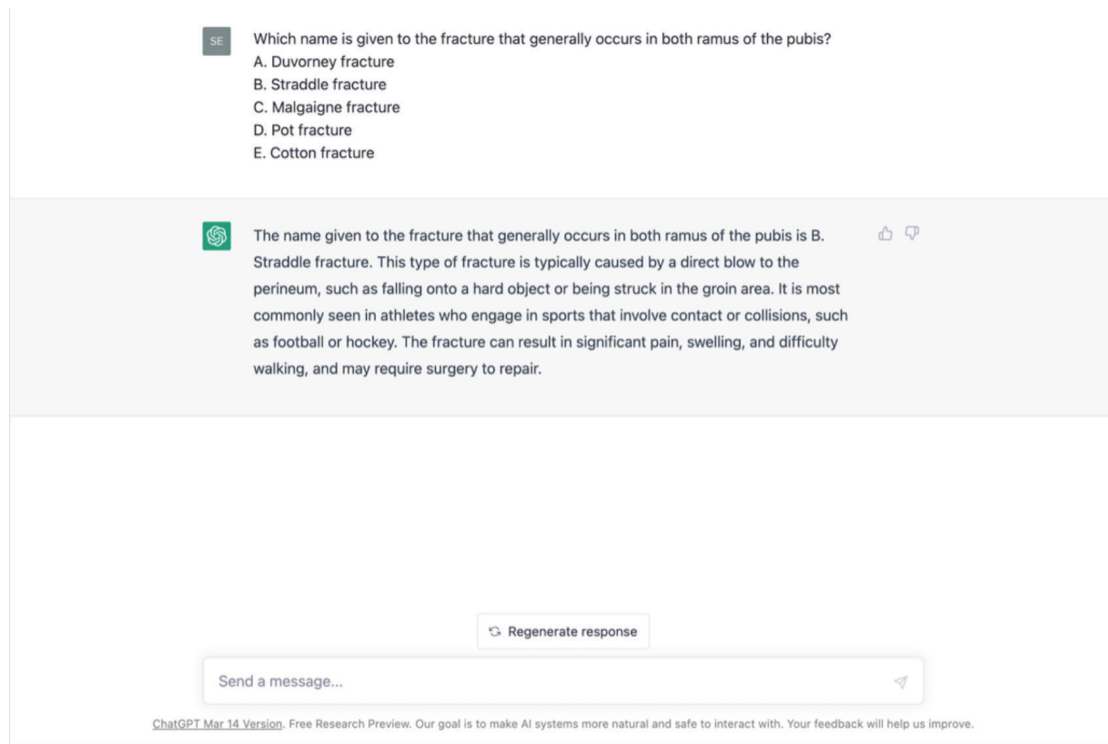
The study received ethical approval from the Ethical Committee of Gazi University (approval number: E-77082166-604.01.02-643268, date: 27.04.2023). The research team ensured that all aspects of the study were conducted in accordance with the highest standards of academic integrity and ethical research practice.

### *Statistical Analysis*

Data from both the residents' exams and ChatGPT responses were collated and coded for analysis. Responses were categorized as "correct", "incorrect", or "invalid/no answer". For ChatGPT, additional categorization was done for "consistent response" and "different explanations."

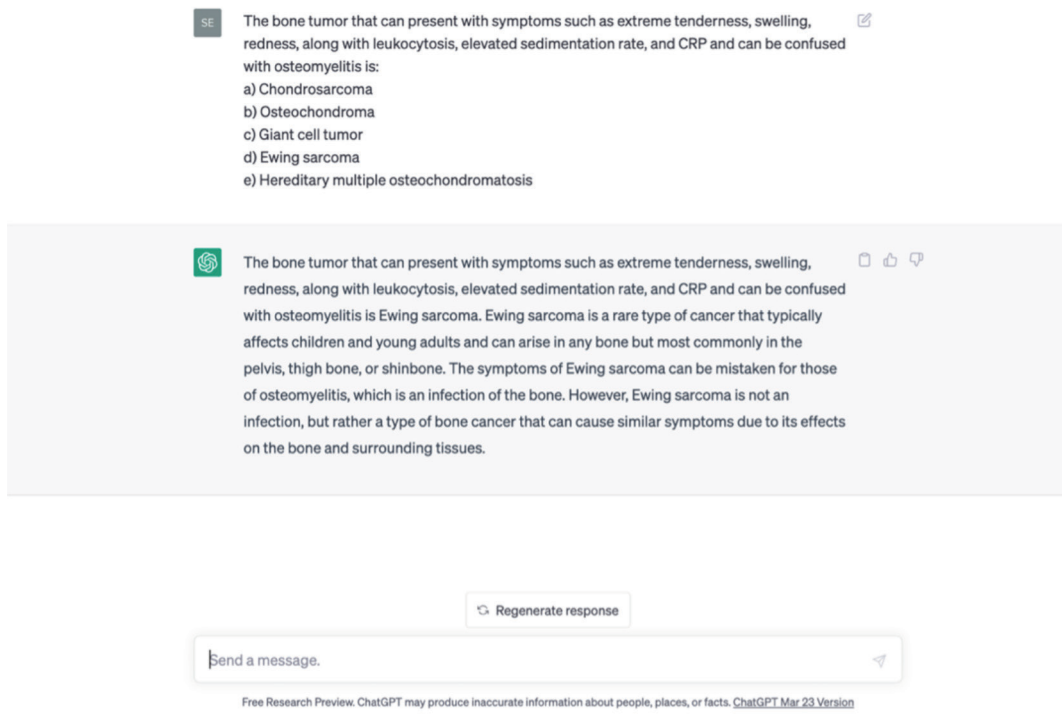
Statistical analysis was conducted using IBM SPSS (IBM Corp. Released 2020. IBM SPSS Statistics for Macintosh, Version 27.0. Armonk, NY: IBM Corp. Descriptive statistics were generated to summarize the basic features of the data. This included computation of means, standard deviations, and ranges for the number of correct answers. A comparison was then made between the answers provided by the AI and those given by the orthopedic residents. This comparison focused on not only the correctness of the answers but also the reasoning and explanation provided, especially for complex or scenario-based questions.





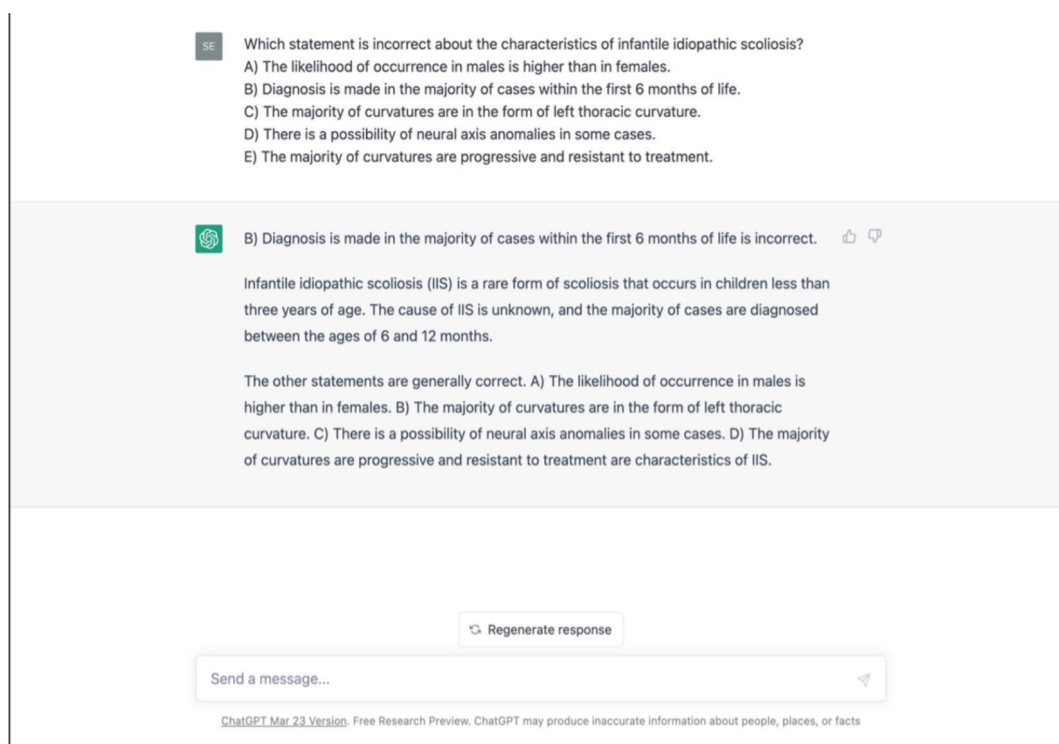
**Figure 1.** Example question that ChatGPT answered correctly.

ChatGPT: Chat Generative Pre-trained Transformer.



**Figure 2.** An English question example that ChatGPT answered correctly.

ChatGPT: Chat Generative Pre-trained Transformer.



**Figure 3.** Example question that ChatGPT answered incorrectly.

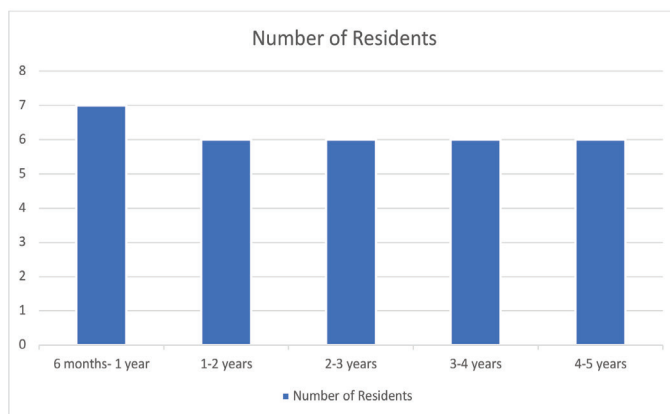
ChatGPT: Chat Generative Pre-trained Transformer.

## RESULTS

The exam results of 31 orthopedic resident doctors with a seniority ranging from 6 months to 5 years were included. Among the 31 orthopedic residents, 7 of them (22.6%) had seniority between 6 months and 1 year, 6 of them (19.35%) had seniority between 1 and 2 years, 6 of them (19.35%) had seniority between 2 and 3 years, 6 of them (19.35%) had seniority between 3 and 4 years, and the remaining 6 of them (19.35%) had seniority between 4 and 5 years (Figure 4).

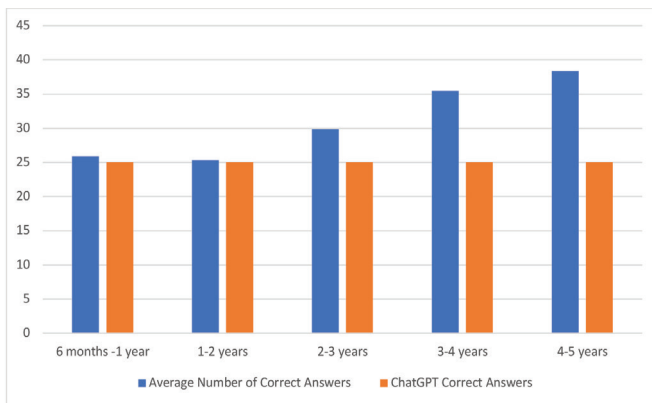
The number of correct answers obtained by 31 orthopedic resident doctors who took the exam was calculated to have a minimum of 23 and a maximum of 40 out of 50, with a mean of 30.81. The mean score of orthopedic residents with seniority between 6 months and 1 year was calculated to be 25.86 ( $\pm 2.26$ ) correct out of 50 multiple-choice questions. The mean score of residents with seniority between 1 and 2 years was also determined to be 25.33 ( $\pm 3.67$ ). The mean of correct answers for residents with a seniority between 2 and 3 years was 29.89 ( $\pm 5.49$ ). The mean of correct answers for residents with a seniority between 3 and 4 years was 35.5 ( $\pm 2.42$ ). The mean number of correct responses for the most experienced orthopedic residents with a seniority of 4 to 5 years was computed as 38.33 ( $\pm 1.5$ ).

The ChatGPT was asked 50 multiple-choice orthopedic questions via the chatbot link <https://chat.openai.com/chat> in both Turkish and English at different times. Consistent answers were provided



**Figure 4.** Number of residents.

by the program regardless of the language or time of questioning. However, the program provided different explanations for the same answer when the questions were asked at different times. The program's answers were internally consistent in different languages and at different times. ChatGPT provided the correct answer for 25 of the 50 multiple-choice questions. It indicated that two questions were incorrect, stating that the correct answer was not among the choices. It gave incorrect answers to 23 questions (Figure 5).



**Figure 5.** Comparison of residents' and ChatGPT's correct answers.

ChatGPT: Chat Generative Pre-trained Transformer.

## DISCUSSION

Our study adds to the growing body of research evaluating the capabilities of AI, specifically ChatGPT, in the medical field. In our analysis, ChatGPT demonstrated a level of knowledge comparable to that of orthopedic residents with 6 months to 2 years of experience, correctly answering 50% of the questions. However, it showed limitations in questions requiring interpretation or inference, and there were concerns about the accuracy and reliability of its sources.

A study highlighted that ChatGPT 3.5, along with ChatGPT 4, was prone to generate fabricated bibliographic citations, a phenomenon categorized as a type of "hallucination" (9). This issue was obvious in our study as well, where ChatGPT provided false information with fabricated sources. This phenomenon poses significant concerns for the use of AI in academic and clinical settings where the accuracy of sources is paramount.

Upon examination of its responses, it can be considered a potential danger that ChatGPT presents false information in a fluent and well-formed manner, even when it is incorrect. In addition, ChatGPT's success rate in a multiple-choice orthopedic exam was found to be inadequate. Upon reviewing the literature, it is possible for the ChatGPT artificial intelligence program to achieve near-passing grades in certain exams.

In the study conducted by Fijačko et al. (7), the questions from two distinct exams developed by the American Heart Association were directed to ChatGPT for analysis. ChatGPT answered 68.4% and 76% of the questions correctly in these exams. In this study, ChatGPT could not answer a few questions correctly, exceeding the passing threshold of the exams. In our study, ChatGPT answered 50% of the questions correctly.

In another research study, the "United States Medical Licensing Exam" questions consisting of three stages were presented to ChatGPT, and ChatGPT approached the passing score in almost all stages (8). In a research conducted in a non-medical domain, ChatGPT was exposed to four distinct final exam questions from a law faculty, and it successfully achieved a passing grade for all of the exams (10). In our study, ChatGPT answered a similar number of questions correctly as the first-year resident. This may indicate that ChatGPT has more knowledge in certain areas.

Sahin et al. (11) reported that ChatGPT is a successful study assistant; however, the way the questions are asked is important in the success of ChatGPT. Yapar et al. (12) mentioned in their study that ChatGPT can provide strong support for patients in home care in the early period after orthopedic procedures.

In another study evaluating the success of ChatGPT-3.5, ChatGPT-4, and orthopedic residents, it was shown that orthopedic residents were more successful than ChatGPT and ChatGPT-4 was more successful than ChatGPT-3.5 (13). This was similar to the result in our study.

A study in orthopedics showed that the ChatGPT answered approximately 65% of the questions about anterior cruciate ligament surgery correctly (14). However, although ChatGPT provides guidance and effectively adapts to different target audiences, it cannot replace the expertise of orthopedic surgeons in diagnosis and treatment planning because of its limited knowledge in orthopedics and potential for inaccurate answers.

Analyzing these studies, it can be concluded that ChatGPT can produce more positive results in non-medical fields, but it may not provide sufficient results due to the large number of variables involved in medical subjects. Considering the results of our study, the performance of ChatGPT is limited, and although it seems to be helpful in solving some exam questions, it is not sufficient to provide accurate information. Despite its potential to produce different answers to the same questions at different times with different explanations, it should not be overlooked that ChatGPT can be used in academic settings and multiple-choice exams, albeit in a limited way. Although its current medical use appears to be limited, the accuracy of the information provided by the program may increase over time with further research and development. However, it should also be noted that there is a risk that both positive and negative practices may increase as the program improves, raising ethical concerns.

### Study Limitations

Our study was limited by the sample size and scope of the questions. Future studies could utilize a larger pool of participants and questions from standardized exams such as the orthopedic board exam for a more comprehensive evaluation.

## CONCLUSION

ChatGPT was found to have entry-level knowledge compared with orthopedic residents. It may provide accurate information in answering certain theoretical questions, but the information it provides for questions requiring interpretation and inference may not be at the desired level. However, the accuracy of the theoretical knowledge may increase with updates developed over time.

### Ethics

**Ethics Committee Approval:** The study received ethical approval from the Ethical Committee of Gazi University (approval number: E-77082166-604.01.02-643268, date: 27.04.2023).

**Informed Consent:** It was obtained.

### Authorship Contributions

Surgical and Medical Practices: S.Y., Concept: S.Y., Design: S.Y., Supervision: T.T., Data Collection or Processing: M.O., Analysis or Interpretation: S.C.Y., T.T., Literature Search: A.A., A.C.B., M.A.T., S.C.Y., Writing: S.Y., A.A., S.C.Y.

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

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### REFERENCES

- Rudolph J, Tan S, Tan S. ChatGPT: Bullshit spewer or the end of traditional assessments in higher education? *Journal of Applied Learning & Teaching*, 2023.
- Rivas P, Zhao L. Marketing with ChatGPT: Navigating the Ethical Terrain of GPT-Based Chatbot Technology. *AI* 2023; 4: 375-84.
- Polonsky MJ, Rotman JD. Should Artificial Intelligent Agents be Your Co-author? Arguments in Favour, Informed by ChatGPT. *Australasian Marketing Journal* 2023; 31: 91-6.
- Biswas S. ChatGPT and the Future of Medical Writing. *Radiology* 2023; 307: e223312.
- Thorp HH. ChatGPT is fun, but not an author. *Science* 2023; 379: 313.
- Biswas SS. Role of Chat GPT in Public Health. *Ann Biomed Eng* 2023; 51: 868-9.
- Fijačko N, Gosak L, Štiglic G, Picard CT, John Douma M. Can ChatGPT pass the life support exams without entering the American heart association course? *Resuscitation* 2023; 185: 109732.
- Kung TH, Cheatham M, Medenilla A, Sillos C, De Leon L, Elepaño C, et al. Performance of ChatGPT on USMLE: Potential for AI-assisted medical education using large language models. *PLOS Digit Health* 2023; 2: e0000198.
- Walters WH, Wilder EI. Fabrication and errors in the bibliographic citations generated by ChatGPT. *Sci Rep* 2023; 13: 14045.
- Choi JH, Hickman KE, Monahan A, Schwarcz D. ChatGPT goes to law school. Available at SSRN, 2023.
- Sahin MC, Sozer A, Kuzucu P, Turkmen T, Sahin MB, Sozer E, et al. Beyond human in neurosurgical exams: ChatGPT's success in the Turkish neurosurgical society proficiency board exams. *Comput Biol Med* 2024; 169: 107807.
- Yapar D, Demir Avci Y, Tokur Sonuvar E, Egerci ÖF, Yapar A. ChatGPT's potential to support home care for patients in the early period after orthopedic interventions and enhance public health. *Jt Dis Relat Surg* 2024; 35: 169-76.
- Massey PA, Montgomery C, Zhang AS. Comparison of ChatGPT-3.5, ChatGPT-4, and Orthopaedic Resident Performance on Orthopaedic Assessment Examinations. *J Am Acad Orthop Surg* 2023; 31: 1173-9.
- Kaarre J, Feldt R, Keeling LE, Dadoo S, Zsidai B, Hughes JD, et al. Exploring the potential of ChatGPT as a supplementary tool for providing orthopaedic information. *Knee Surg Sports Traumatol Arthrosc* 2023; 31: 5190-8.



## Kyphoplasty is not Superior to Vertebroplasty in Restoring Vertebral Height in the Long Term

Kifoplasti, Uzun Dönemde Vertebral Yüksekliği Geri Kazandırmada Vertebroplastiye Üstün Değildir

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### ABSTRACT

**Objective:** The aim of this study was to evaluate whether kyphoplasty (KP) is superior to vertebroplasty (VP) in restoring spinal height in the long term.

**Methods:** The study encompassed a cohort of 33 patients aged between 42 and 90 years, with a follow-up period of at least 5 years, who had undergone either KP (n=16) or VP (n=17) for the diagnosis of osteoporotic vertebral fractures at our institution. Clinical comparisons were conducted on the basis of Oswestry scores, EuroQol-5 Dimension (EQ-5D), and visual analog scale (VAS) scores, while radiological assessments were performed considering fractured vertebral height and local kyphosis angle values. Evaluations were conducted across preoperative, postoperative, and last control radiographs.

**Results:** In both cohorts, the mean age was comparable, and there was no significant difference in the follow-up duration (p=0.126). Regarding radiological assessments during the early postoperative phase, KP patients exhibited a noteworthy enhancement in the anterior vertebral column height (mean, from 1.3471 mm to 2.0941 mm), middle vertebral column height (mean, from 1.3375 mm to 1.6437 mm), and local kyphosis angle improvement (mean, from 17.88° to 7.81°). However, the last control values demonstrated similar outcomes in both groups (KP patients: 1.4412 mm, 1.4063 mm, 13.69°; VP patients: 1.2813 mm, 1.3176 mm, 17.18°). In addition, there were no statistically significant differences in Oswestry scores, EQ-5D index, and VAS scores between the two groups.

**Conclusion:** According to our study, KP appears to be an effective method in the early treatment of painful collapsed vertebral fractures, but it was not observed to be superior to VP in the long term.

**Keywords:** Osteoporotic vertebral fracture, kyphoplasty, vertebroplasty, vertebral column height, local kyphotic angle

### ÖZ

**Amaç:** Bu çalışmanın amacı, kifoplastinin (KP) uzun dönemde omurga yüksekliğini geri kazanmada vertebroplastiye (VP) üstün olup olmadığını değerlendirmektir.

**Yöntemler:** Çalışma, kurumumuzda osteoporotik vertebra kırığı tanısıyla KP (n=16) veya VP (n=17) uygulanan, takip süresi en az 5 yıl olan, yaşları 40 ile 90 arasında değişen 33 hastadan oluşan bir kohortu kapsamaktadır. Klinik karşılaştırmalar Oswestry skorları, EuroQol-5 Dimension (EQ-5D) ve VAS skorlarına göre yapılırken, radyolojik değerlendirmeler kırık vertebra yüksekliği ve lokal kifoz açısı değerleri dikkate alınarak yapıldı. Bu değerlendirmeler ameliyat öncesi, ameliyat sonrası ve son kontrol radyografileri üzerinden yapıldı.

**Bulgular:** Her iki grupta yaş ortalaması benzerdi ve takip süresinde anlamlı bir fark yoktu (p=0,126). Ameliyat sonrası erken dönemdeki radyolojik değerlendirmelerle ilgili olarak, KP hastaları ön vertebral kolon yüksekliğinde (ortalama 1,3471 mm'den 2,0941 mm'ye), orta vertebral kolon yüksekliğinde (ortalama 1,3375 mm'den 1,6437 mm'ye) ve lokal kifoz açısında (ortalama 17,88°den 7,81°ye) kayda değer bir iyileşme sergilemiştir. Bununla birlikte, son kontrol değerleri her iki grupta da benzer sonuçlar göstermiştir (KP hastaları: 1,4412 mm, 1,4063 mm, 13,69°; VP hastaları: 1,2813 mm, 1,3176 mm, 17,18°). Ayrıca, Oswestry skorları, EQ-5D indeksi ve VAS skorları açısından iki grup arasında istatistiksel olarak anlamlı bir fark bulunmadı.

**Sonuç:** Çalışmamıza göre, KP ağırlı çökmüş vertebra kırıklarının erken tedavisinde etkili bir yöntem gibi görünmektedir, ancak uzun vadede vertebroplastiye üstünlüğü gözlenmemiştir.

**Anahtar Sözcükler:** Osteoporotik vertebra kırığı, kifoplasti, vertebroplasti, vertebral kolon yüksekliği, lokal kifotik açı

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## INTRODUCTION

Osteoporotic vertebral compression fractures are a significant healthcare concern, affecting a substantial portion of the elderly population. These fractures often lead to chronic pain, decreased mobility, and diminished quality of life. Kyphoplasty (KP) and vertebroplasty (VP) are two minimally invasive surgical techniques widely employed for stabilizing these fractures and alleviating associated symptoms (1). Although both procedures have demonstrated efficacy in pain relief and fracture stabilization, there remains a debate regarding their comparative effectiveness. Some studies suggest that KP may be superior in reducing cement leakage and improving postoperative vertebral body height, although it is more expensive and time-consuming than VP (2). Other research indicates no significant differences in long-term pain and disability outcomes between the two methods, highlighting the need for further comparative studies (3).

Recent advancements in materials and techniques, including the use of alternative cements to polymethylmethacrylate (PMMA), have opened new avenues for improving the safety and efficacy of these procedures (4). Both methods are effective in controlling pain in patients with osteoporotic vertebral fractures. Although KP is a more effective method for restoring collapsed vertebral fractures, its long-term results are controversial.

In this study, we compared the long-term clinical and radiological outcomes of patients with osteoporotic vertebral fractures who underwent KP and VP and specifically evaluated whether KP was superior to VP in terms of long-term vertebral height restoration.

## MATERIALS AND METHODS

### Study Design and Population

Ethics committee approval was obtained from the Ethics Committee of Gazi University for this retrospective study (approval number: E-77082166-604.01.02-830153, date: 19.12.2023). This retrospective study encompassed 66 patients who underwent either KP or VP for vertebral compression fractures at Gazi University Hospital, Ankara, of whom 33 were excluded because of the lack of

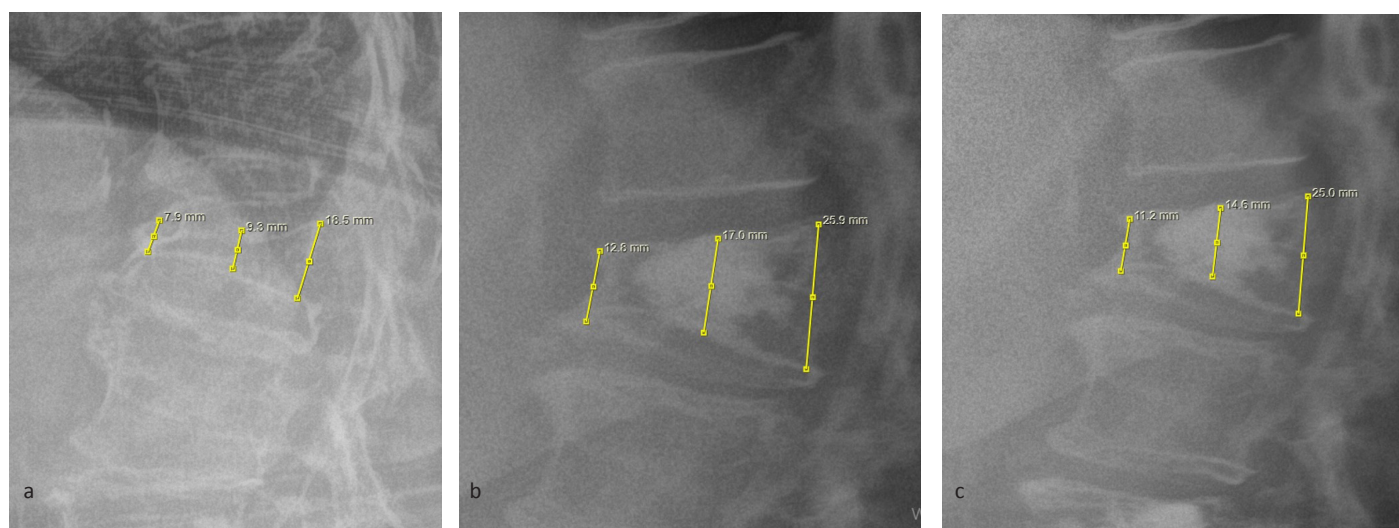
pre- and postoperative clinical and radiological data. The final cohort comprised 33 patients who underwent both preoperative and postoperative assessments and maintained a follow-up period of at least 24 months. Patients with metastatic spinal fractures, those undergoing surgery for fractures and/or degenerative causes of the spine, and those with instrumentation in the spine were excluded from the study.

The 33 patients included in the study were divided into two groups according to the surgical procedure performed: KP (n=16) and VP (n=17).

### Data Collection

Clinical assessments were performed using the Oswestry Disability Score, visual analog scale (VAS), and EuroQoL- 5 Dimension (EQ-5D) scores. Radiological evaluations encompassed the measurement of anterior and middle column height, along with the assessment of the kyphotic angle of the fractured vertebrae. All radiological evaluations were performed on plain radiographs of the spine taken in the standing position. The local kyphosis angle was obtained by measuring the Cobb angle between the inferior endplate of the vertebra above the fractured vertebra and the inferior endplate of the fractured vertebra. These evaluations were conducted at preoperative, early postoperative, and final follow-up visits (Figure 1-3). These measurements were obtained using the hospital's PACS system (ExtremePACS 2015 version 4.3).

The Oswestry Disability Index (ODI) is a scale used to assess disability in relation to back pain and quality of life. It consists of 10 questions. Each question has five possible answers, and a percentage of the answers is calculated. Patients with a score of 0-20 have mild back problems, whereas patients with a score of 80-100 have severe back problems and are bedridden. The EQ-5D is a preference-based measure of health-related quality of life that includes one question for each of 5 dimensions, including mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. The EQ-5D questionnaire also includes a Visual Analogue Scale (VAS), which allows respondents to report their perceived health on a scale from 0 (worst possible health) to 100 (best possible health) (5).



**Figure 1.** Radiological evaluation of the kyphoplasty patient (a) preoperative; (b) early postoperative; (c) last control.

Subsequent to data collection, rigorous statistical analyses were performed to draw meaningful conclusions. Comparative analyses were specifically conducted between the results obtained from the KP and VP patient groups.

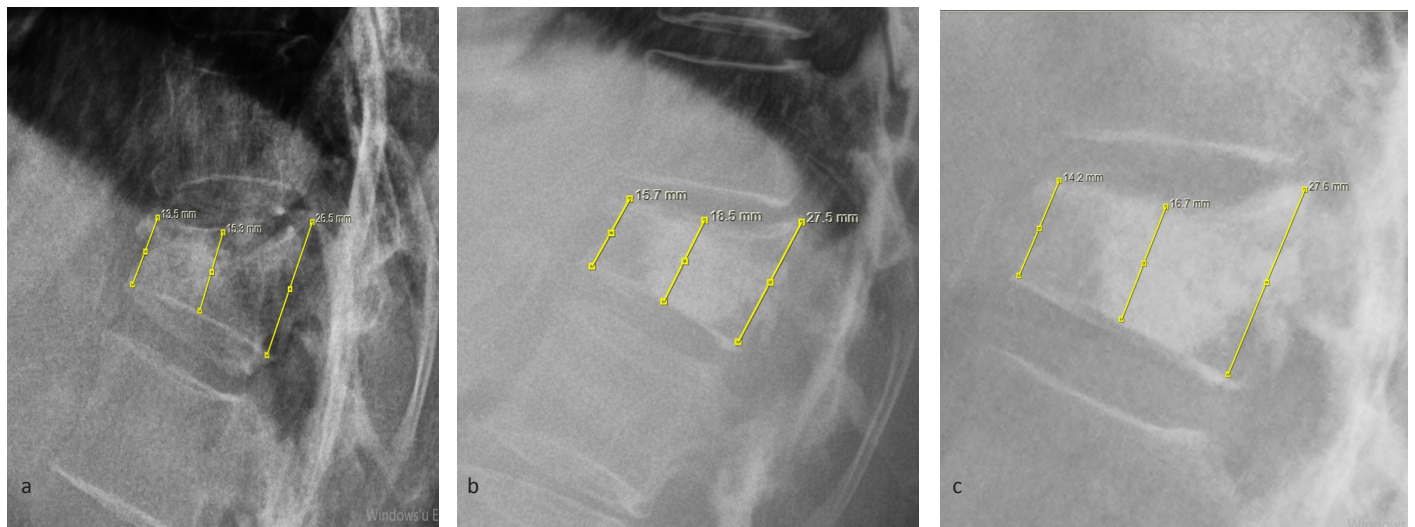
### Surgical Techniques

In KP, patients were positioned prone under general anesthesia.

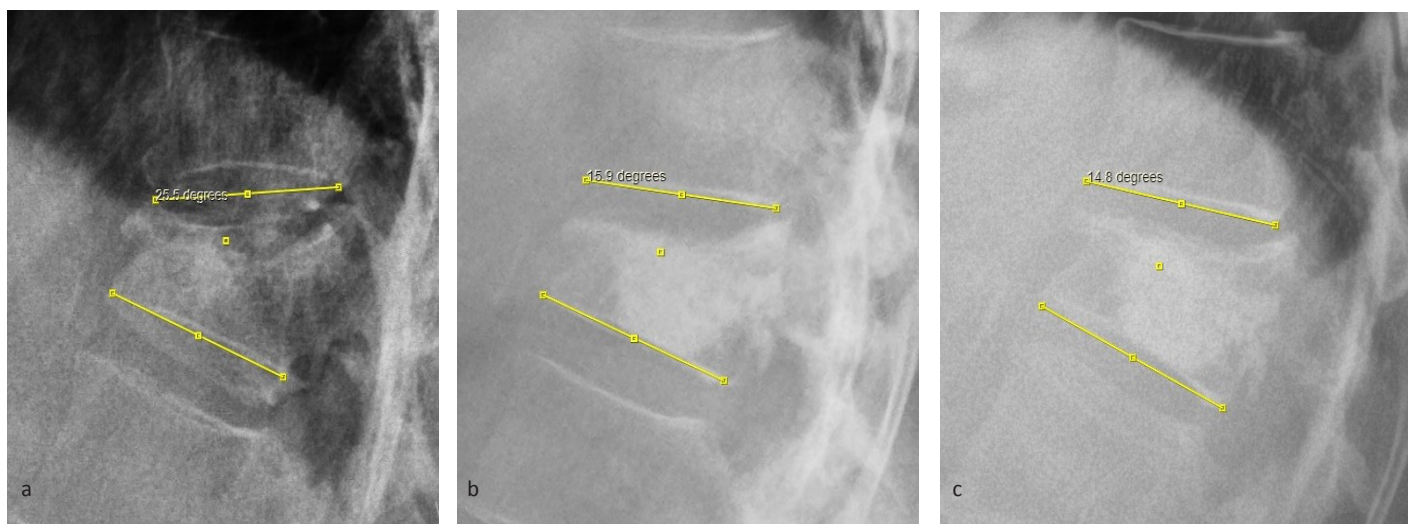
The procedures were conducted under fluoroscopic guidance. For each targeted vertebra, a 1 cm skin and subcutaneous incision was made approximately 1.5-2 cm lateral to the pedicle. A trocar was inserted between the center and edge of the pedicle (between 1-3 o'clock on the right side pedicle and 9-11 o'clock on the left side pedicle). Under posteroanterior (PA) fluoroscopic guidance, the trocar was advanced approximately 2-3 cm, ensuring that it did not cross the medial line of the pedicle. Lateral fluoroscopic images of the vertebral column were obtained. Once it was confirmed that the tip of the trocar had passed the posterior wall of the vertebral corpus and was inside the corpus, the trocar was further advanced.

Upon reaching approximately one-fourth of the anterior part of the vertebral corpus, the trocar was removed, and its position within the corpus was confirmed using a probe. A working cannula was then placed in the trocar position. KP balloon instruments were inserted through the working cannula and inflated with contrast material. The expansion of the kyphotic vertebra was monitored by fluoroscopy. This procedure was performed bilaterally. After sufficient correction of the vertebra was observed, the KP balloon was deflated and removed. Before injection of PMMA cement, a contrast material was used to check for vascular leakage. Then, PMMA cement was slowly injected, ensuring that its consistency was not too fluid. Approximately 3-4 cc of PMMA cement was used in the thoracic region and 5-6 cc in the lumbar region. During the injection, both lateral and PA images were taken to ensure that the PMMA cement did not leak outside the corpus, especially into the spinal canal.

VP was performed similarly to KP under general anesthesia in the prone position and guided by fluoroscopy. Following the placement of the working cannula into the vertebral corpus, PMMA cement



**Figure 2.** Radiological evaluation of vertebroplasty patient (a) preoperative; (b) early postoperative; (c) last control.



**Figure 3.** Radiological evaluation of the local kyphosis angle (a) preoperative; (b) early postoperative; (c) last control.

was directly injected without balloon KP. Care was taken to ensure that the PMMA cement did not leak, especially into the spinal canal, during fluoroscopic monitoring.

To prevent possible complications, we evaluated venous extravasation by injecting contrast material before VP and KP. We also check the fluidity of PMMA and inject PMMA after it has a consistency similar to toothpaste.

Both KP and VP patients were mobilized at the 4<sup>th</sup> hour after surgery. None of the patients required the use of a brace.

### Statistical Analysis

The analysis was conducted using SPSS version 27. Descriptive statistics were computed, and the Shapiro-Wilk test was used to evaluate the normality of distributions. Pearson's and Spearman's correlation analyses were performed as appropriate. The Wilcoxon signed-rank test assessed differences in vertebral height measurements preoperatively and postoperatively. The Mann-Whitney U test was used to compare the KP and VP groups in terms of measurements and scores.

## RESULTS

The KP patients ranged in age from 43 to 84 years with a mean age of 62.81 [standard deviation (SD)=20.06] years and a mean follow-up of 95.65 (SD=39.80) months. The VP group ranged in age from 42 to 90 years with a mean age of 68.29 (SD=18.06) years and a mean follow-up of 89.72 (SD=43.75) months. There were no significant differences in the mean age and follow-up between KP and VP patients (Table 1). Fracture levels were generally located in the thoracolumbar region (Table 2).

**Table 1.** Demographic characteristics and basic information of patients

	KP (mean ± SD)	VP (mean ± SD)	p
Number of patients (n)	16	17	0.596
Age (years)	62.81±20.06	68.29±18.06	0.407
Gender F/M	7/9	9/8	0.336
Follow-up period (month)	95.65±39.80	89.72±43.75	0.126

KP: Kyphoplasty, VP: Vertebroplasty, SD: Standard deviation, F: Female, M: Male.

**Table 2.** Fracture levels of the vertebrae

	n	%
T6	1	3.0
T8	1	3.0
T9	1	3.0
T10	4	12.1
T11	5	15.2
T12	7	21.2
L1	6	18.2
L2	4	12.1
L3	1	3.0
L4	3	9.1

The mean functional status of the patients in the KP group as measured by the ODI was 26.88 (SD=13.31), indicating moderate disability. The quality of life, as assessed by the EQ-5D index, had a mean value of 0.865 (SD=0.099). Preoperative vertebral anterior column height measurements averaged 1.15 cm (SD=0.61), early postoperative measurements averaged 1.89 cm (SD=0.36) and at the last follow-up, 1.44 cm (SD=0.415). The vertebral mid-column height was 1.33 cm (SD=0.381) preoperatively, 1.91 cm (SD=0.322) early postoperatively, and 1.40 cm (SD=0.312) at the last follow-up (Table 3).

In the VP group, the ODI indicated a higher level of disability with a mean score of 31.53 (SD=21.02), whereas the EQ-5D index was comparable to the KP group with a mean score of 0.871 (SD=0.100). The preoperative vertebral measurements in this group averaged 1.25 cm (SD=0.55), the early postoperative measurements demonstrated a significant increase, averaging 1.27 cm (SD=0.362) and at the last control 1.28 cm (SD=0.34). The vertebral mid-column height was 1.41 cm (SD=0.478) preoperatively, 1.61 cm (SD=0.488) early postoperatively, and 1.41 cm (SD=0.483) at the last control (Table 3).

There was no significant change in vertebral posterior column height in either group preoperatively, early postoperatively, or at final follow-up.

For both groups, the collected scores and measurements displayed a range of distributions, with some variables exhibiting non-normal distribution characteristics, as indicated by their skewness and kurtosis values. This data variability underscores the heterogeneity within the patient population and the outcomes of the surgical procedures.

### Normality of the Data

Shapiro-Wilk tests showed that most variables in the KP and VP groups did not deviate significantly from a normal distribution. However, certain variables in each group, such as age distribution and specific preoperative and postoperative measurements, showed significant deviations from normality (Table 4).

### Correlation Analysis

Pearson correlation analyses within the KP group indicated significant negative correlations between age and specific postoperative measurements and strong relationships between disability, quality of life, and pain levels. The VP group showed similar patterns, with age negatively correlated with certain preoperative and postoperative measurements and significant interrelations among disability, quality of life, and pain scores.

### Wilcoxon Signed-Rank Tests

In the KP group (n=16), a Wilcoxon signed-rank test revealed significant changes in vertebral measurements from the preoperative to postoperative stages. There was a significant increase in early postoperative vertebral anterior column height measurement (Z=-2.699, p=0.007) and vertebral mid-column height measurement (Z=-3.319, p=0.001), but no significant change in vertebral posterior column height measurement (Z=-0.586, p=0.558). A significant decrease was observed in the postoperative local kyphosis angle measurement (Z=-3.527, p<0.001) (Table 5).



**Table 3.** Descriptive statistics were computed separately for patients who underwent kyphoplasty and vertebroplasty

		Minimum	Maximum	Mean	SD
KP	Oswestry	10	54	26.88	13.306
	EQ-5D index	0.703	1.000	0.86463	0.098828
	VAS	50	90	73.75	12.042
	Preop VACH	0.40	1.30	1.1471	0.61842
	Postop VACH	0.50	1.70	1.8941	0.36423
	Last Control VACH	0.70	1.60	1.4412	0.41508
	Preop VMCH	0.60	1.80	1.3375	0.38101
	Postop VMCH	0.70	2.30	1.9137	0.32243
	Last control VMCH (mm)	0.70	1.80	1.4063	0.31298
	Preop VPCH	2.50	3.30	2.9750	0.25690
	Postop VPCH	2.50	3.40	2.9938	0.23514
	Last control VPCH	2.5	3.3	2.919	0.2287
	Preop LKA	11	22	17.88	2.872
	Postop LKA	5	12	7.81	2.257
Last control LKA	8	20	13.69	3.027	
VP	Oswestry	10	78	31.53	21.018
	EQ-5D index	0.699	1.000	0.87065	0.100091
	VAS	30	100	69.41	17.843
	Preop VACH	0.60	1.40	1.2500	0.55466
	Postop VACH	0.70	1.50	1.2750	0.36231
	Last control VACH	0.60	1.40	1.2813	0.34294
	Preop VMCH	0.30	1.60	1.4118	0.47812
	Postop VMCH	0.70	1.70	1.6188	0.48870
	Last control VMCH	0.50	1.60	1.4176	0.48378
	Preop VPCH	2.20	3.40	2.9647	0.29779
	Postop VPCH	2.20	3.50	2.9471	0.33563
	Last control VPCH	2.0	3.4	2.824	0.3270
	Preop LKA	8	29	17.82	5.480
	Postop LKA	7	25	16.76	4.893
Last control LKA	6	26	17.18	5.637	

VACH: Vertebral anterior column height (cm), VMCH: Vertebral mid-column height (cm), VPCH: Vertebral posterior column height (cm), LKA: Local kyphosis angle (°), VAS: Visual analog scale, KP: Kyphoplasty, VP: Vertebroplasty, SD: Standard deviation, Preop: Preoperative, Postop: Postoperative.

In the VP group (n=17), the test also indicated significant changes. There was a significant increase in the postoperative vertebral anterior column height measurement ( $Z=-3.461$ ,  $p=0.001$ ) and vertebral mid-column height measurement ( $Z=-2.126$ ,  $p=0.033$ ), whereas no significant change was observed in the vertebral posterior column height measurement ( $Z=-0.577$ ,  $p=0.564$ ). A significant decrease was noted in the postoperative local kyphosis angle measurement ( $Z=-2.812$ ,  $p=0.005$ ) (Table 6).

#### **Mann-Whitney U Test Findings**

The Mann-Whitney U test demonstrated significant differences between the KP and VP groups in terms of ODI scores, postoperative anterior column, mid-column height, and local kyphosis angle. No significant differences were found between the groups in other

variables, including age, EQ-5D index, VAS, and various preoperative and postoperative measurements (Table 7).

## **DISCUSSION**

Our study compared the outcomes of KP and VP in terms of restored vertebral height, quality of life, disability, and pain scores. The findings indicate that both procedures are effective in managing vertebral compression fractures although there are nuanced differences between them.

#### **Comparative Efficacy in Pain and Disability Management**

The results of our study align with those found in previous research. A systematic review and meta-analysis included 29 studies with 2,838 patients, and found no significant differences in mean pain scores

**Table 4.** Normality analysis of vertebroplasty and kyphoplasty was performed separately using the Shapiro-Wilk test

	KP			VP		
	Stat.	df	p	Stat.	df	p
Age	0.817	16	0.005	0.824	17	0.004
Oswestry	0.935	16	0.287	0.963	17	0.681
EQ-5D index	0.929	16	0.239	0.915	17	0.122
VAS	0.922	16	0.180	0.946	17	0.399
Preop VACH	0.478	16	0.000	0.917	17	0.132
Postop VACH	0.872	16	0.029	0.398	17	<0.001
Last control VACH	0.907	16	0.104	0.400	17	<0.001
Preop VMCH	0.876	16	0.033	0.946	17	0.395
Postop VMCH	0.877	16	0.035	0.929	17	0.211
Last control VMCH	0.900	16	0.081	0.943	17	0.354
Preop VPCH	0.916	16	0.147	0.907	17	0.089
Postop VPCH	0.943	16	0.387	0.963	17	0.682
Last control VPCH	0.897	16	0.071	0.902	17	0.075
Preop LKA	0.946	16	0.426	0.983	17	0.978
Postop LKA	0.899	16	0.077	0.962	17	0.674
Last control LKA	0.946	16	0.427	0.959	17	0.620

VACH: Vertebral anterior column height (cm), VMCH: Vertebral mid-column height (cm), VPCH: Vertebral posterior column height (cm), LKA: Local kyphosis angle (°), VAS: Visual analog scale, KP: Kyphoplasty, VP: Vertebroplasty, Preop: Preoperative; Postop: Postoperative, p-value of significance, Stat.: Shapiro-Wilk test statistical value, df: Degrees of freedom.

postoperatively and at 12 months between the KP and VP groups. This study also reported similar disability scores postoperatively and at 12 months for both groups (3). These findings suggest that both KP and VP are equally effective in managing pain and disability associated with vertebral compression fractures. In another study from Türkiye, both KP and VP were found to be effective in improving functional recovery and pain relief in patients with osteoporotic vertebral fractures. While KP showed slightly better radiological outcomes, this difference was not clinically significant, leading the authors to recommend VP for its simpler management (6).

A study examining long-term outcomes over a 5-year period showed only subtle differences between KP and VP, suggesting the use of VP over KP considering treatment costs (7). Another study assessing KP outcomes over 3 years found significant improvements in pain and mobility compared with controls, as well as a reduced risk of new vertebral fractures (8). Additionally, a study evaluating VP outcomes over a 29-month period reported significant reductions in pain and disability scores, with less intense analgesic use compared with conservative therapy (9). In our study, the mean follow-up time was 96 (SD=39.80) months, which is consistent with the results of other long-term studies. Our findings show that there were no significant differences in pain and quality of life scores between patients undergoing both KP and VP procedures.

**Table 5.** Wilcoxon signed-rank test results for the kyphoplasty group

	Preop/Postop VACH	Preop/Postop VAMH	Preop/Postop VPCH	Preop/Postop LKA
Z	-2.699 <sup>c</sup>	-3.319 <sup>c</sup>	-0.586 <sup>c</sup>	-3.527 <sup>d</sup>
p	0.007	0.001	0.558	<0.001

<sup>c</sup>Based on negative ranks. <sup>d</sup>Based on positive ranks. p: Asymptotic significance, Z: Wilcoxon test statistics, Preop: Preoperative, Postop: Postoperative, VACH: Vertebral anterior column height, VMCH: Vertebral mid-column height, VPCH: Vertebral posterior column height, LKA: Local kyphosis angle.

**Table 6.** Wilcoxon signed-rank test results for the vertebroplasty group

	Preop/Postop VACH	Preop/Postop VAMH	Preop/Postop VPCH	Preop/Postop LKA
Z	-3.461 <sup>c</sup>	-2.126 <sup>c</sup>	-0.577 <sup>d</sup>	-2.812 <sup>d</sup>
p	0.001	0.033	0.564	0.005

<sup>c</sup>Based on negative ranks. <sup>d</sup>Based on positive ranks. p: Asymptotic significance, Z: Wilcoxon test statistics, Preop: Preoperative, Postop: Postoperative, VACH: Vertebral anterior column height, VMCH: Vertebral mid-column height, VPCH: Vertebral posterior column height, LKA: local kyphosis angle.

**Table 7.** Mann-Whitney U tests showing differences between the kyphoplasty and vertebroplasty groups

	Age	Oswestry	EQ-5D index	VAS	Preop VACH	Postop VACH	Last control VACH			
Mann-Whitney U	113.000	80.000	133.500	118.000	118.000	126.000	135.500			
Z	-0.829	-2.019	-0.090	-0.665	-0.650	-0.362	-0.018			
p	0.407	0.123	0.928	0.506	0.515	0.018	0.986			
	Preop VMCH	Postop VMCH	Last control VMCH	Preop VPCH	Postop VPCH	Last control VPCH	Preop LKA	Postop LKA	Last control LKA	
Mann-Whitney U	119.000	122.000	121.500	132.500	129.000	111.000	132.000	17.500	73.500	
Z	-0.618	-0.508	-0.527	-0.128	-0.256	-0.931	-0.145	-4.288	-2.259	
p	0.536	0.011	0.598	0.898	0.798	0.352	0.885	0.000	0.064	

p: Asymptotic significance, Z: test statistics (measure of the magnitude of difference between two groups), Preop: Preoperative, Postop: Postoperative, VACH: Vertebral anterior column height, VMCH: Vertebral mid-column height, VPCH: Vertebral posterior column height, LKA: Local kyphosis angle.

### **Vertebral Height Restoration and Kyphosis Reduction**

Our findings on vertebral height restoration are particularly noteworthy. We observed significant changes in both vertebral height measurements and local kyphosis angle in the early postoperative period in the KP group. This is consistent with another study, which found that KP was superior to VP in terms of increasing postoperative vertebral body height (2,10). Although the final follow-up radiographs of KP patients showed better results in terms of both vertebral height and local kyphosis angle, there was a significant loss of both vertebral height and local kyphosis angle in KP patients compared with early postoperative values. Biomechanical studies also support the initial superiority of KP in increasing vertebral body height and reducing kyphosis although these gains may diminish with repetitive loading (11).

### **Complications and Cost-Effectiveness**

There were no serious intraoperative or early postoperative complications in either group of patients. Two patients who underwent KP had adjacent vertebral fractures at 3 and 6 months postoperatively. Three patients who underwent VP had adjacent vertebral fractures at 8 and 13 months of age. None of the patients underwent surgery for an adjacent vertebral fracture. The fractures healed conservatively during follow-up. With the exception of adjacent vertebral fractures, no complications were observed in the patients included in this study.

While KP has advantages in vertebral height restoration, it is important to consider the balance of benefits and risks. KP is associated with lower odds of new fractures and less extraosseous cement leakage than VP. However, the more complex nature of KP, including its higher cost and longer operative time, raises questions about its cost-effectiveness, especially when the differences in pain and disability outcomes between the two procedures are statistically insignificant (2,3). Complications related to cement extravasation, such as compression of neural elements and venous embolism, are rare but more common with VP (11). In our study, no serious complications related to PMMA cement leakage were observed. Cement leakage into the intervertebral disk space occurred in one patient who underwent KP and in three patients who underwent VP. However, because these patients did not exhibit any clinical problems during follow-up, no additional procedures were necessary. This finding highlights the relative safety of both procedures in terms of cement leakage, with clinical outcomes remaining unaffected despite this occurrence.

### **Study Limitations**

One of the main limitations of our study is that it was retrospective and the number of patients included in the study was relatively small. The lack of data on patients' body mass index, comorbidities, and bone mineral density are also limitations of the study. In addition, the wide age range of the patients included in the study and the inability to classify them according to age are also important limitations of our study.

### **CONCLUSION**

In conclusion, our study contributes to the ongoing debate on the comparative efficacy of KP and VP. While both procedures are effective in managing pain and disability, KP appears to offer better outcomes in terms of vertebral height restoration and reduced complications related to cement leakage. However, its higher cost and longer operative time necessitate further research to establish its cost-effectiveness. Future studies should focus on long-term outcomes and identify specific patient subgroups that may benefit more from one procedure than the other. This would enable more personalized treatment approaches and optimize outcomes for patients suffering from vertebral compression fractures.

### **Ethics**

**Ethics Committee Approval:** Ethics committee approval was obtained from the Ethics Committee of Gazi University for this retrospective study (approval number: E-77082166-604.01.02-830153, date: 19.12.2023).

**Informed Consent:** Retrospective study.

### **Authorship Contributions**

Surgical and Medical Practices: A.C.B., F.A., A.A., A.Ş., Concept: A.C.B., F.A., A.Ş., Design: A.C.B., A.A., A.Ş., Data Collection or Processing: M.F.T., M.M.C., C.İ.G., Analysis or Interpretation: A.A., M.F.T., M.M.C., Literature Search: A.A., Writing: A.C.B., F.A., A.A.

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

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### **REFERENCES**

1. Patel A, Petrone B, Carter KR. Percutaneous Vertebroplasty and Kyphoplasty. 2023 May 30. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024.
2. Daher M, Kreichati G, Kharrat K, Sebaaly A. Vertebroplasty versus Kyphoplasty in the Treatment of Osteoporotic Vertebral Compression Fractures: A Meta-Analysis. *World Neurosurg* 2023; 171: 65-71.
3. Gu CN, Brinjikji W, Evans AJ, Murad MH, Kallmes DF. Outcomes of vertebroplasty compared with kyphoplasty: a systematic review and meta-analysis. *J Neurointerv Surg* 2016; 8: 636-42.
4. Filippiadis DK, Marcia S, Masala S, Deschamps F, Kelekis A. Percutaneous Vertebroplasty and Kyphoplasty: Current Status, New Developments and Old Controversies. *Cardiovasc Intervent Radiol* 2017; 40: 1815-23.
5. Kumar K, Verma AK, Wilson J, LaFontaine A. Vertebroplasty in osteoporotic spine fractures: a quality of life assessment. *Can J Neurol Sci* 2005; 32: 487-95.
6. Ateş A, Gemalmaz HC, Deveci MA, Şimşek SA, Çetin E, Şenköylü A. Comparison of effectiveness of kyphoplasty and vertebroplasty in patients with osteoporotic vertebra fractures. *Acta Orthop Traumatol Turc* 2016; 50: 619-22.
7. Liu JT, Li CS, Chang CS, Liao WJ. Long-term follow-up study of osteoporotic vertebral compression fracture treated using balloon kyphoplasty and vertebroplasty. *J Neurosurg Spine* 2015; 23: 94-8.

8. Kasperk C, Grafe IA, Schmitt S, Nöldge G, Weiss C, Da Fonseca K, et al. Three-year outcomes after kyphoplasty in patients with osteoporosis with painful vertebral fractures. *J Vasc Interv Radiol* 2010; 21: 701-9.
9. Thillainadesan J, Schlaphoff G, Gibson KA, Hassett GM, McNeil HP. Long-term outcomes of vertebroplasty for osteoporotic compression fractures. *J Med Imaging Radiat Oncol* 2010; 54: 307-14.
10. Patel N, Jacobs D, John J, Fayed M, Nerusu L, Tandron M, et al. Balloon Kyphoplasty vs Vertebroplasty: A Systematic Review of Height Restoration in Osteoporotic Vertebral Compression Fractures. *J Pain Res* 2022; 15: 1233-45.
11. McCall T, Cole C, Dailey A. Vertebroplasty and kyphoplasty: a comparative review of efficacy and adverse events. *Curr Rev Musculoskelet Med* 2008; 1: 17-23.

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## Routine Histopathological Evaluation of Benign Anorectal Interventions: Essentiality or Excess?

Benign Anorektal Girişimlerde Rutin Histopatolojik Değerlendirme: Gereklik mi, Fazlalık mı?

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### ABSTRACT

**Objective:** To evaluate the necessity of routine histopathological examination in benign anorectal surgeries and to explore the potential for a selective approach based on patient characteristics.

**Methods:** Retrospective analysis of patients who underwent surgery for benign anorectal diseases between January 2013 and December 2023. Demographic data, surgical procedures, and histopathological findings were assessed.

**Results:** Among 830 patients, 482 underwent histopathological examination. No malignancy was detected. However, human papilloma virus (HPV)-related lesions were diagnosed in 2.1% of patients. A retrospective design and limited patient population were identified as major limitations.

**Conclusion:** Routine histopathological examination may be unnecessary for benign anorectal surgeries in patients without HPV-related risk factors. Further large-scale studies are required to determine the criteria for selective examination, optimizing resource usage in clinical practice.

**Keywords:** Anal fistula, anal fissure, hemorrhoid, human papilloma virus, pathology, routine histopathologic examination

### ÖZ

**Amaç:** Benign anorektal cerrahilerde rutin histopatolojik incelemenin gerekliliğini değerlendirmek ve hasta özelliklerine göre seçici bir yaklaşım potansiyelini araştırmaktır.

**Yöntemler:** Ocak 2013 ile Aralık 2023 tarihleri arasında benign anorektal hastalıklar nedeniyle ameliyat edilen hastaların verileri retrospektif analiz edildi. Demografik veriler, cerrahi prosedürler ve histopatoloji bulguları değerlendirildi.

**Bulgular:** Sekiz yüz otuz hastanın 482'sine histopatolojik inceleme yapıldı. Malignite tespit edilmedi. Ancak hastaların %2,1'inde human papilloma virüs (HPV) ile ilişkili lezyonlar teşhis edildi. Retrospektif dizayn ve sınırlı hasta popülasyonu çalışmanın önemli sınırlamalar olarak tanımlandı.

**Sonuç:** HPV ile ilişkili risk faktörleri olmayan hastalarda benign anorektal cerrahi sonrası rutin histopatolojik inceleme gereksiz olabilir. Seçici inceleme kriterlerini belirlemek ve klinik uygulamada kaynak kullanımını optimize etmek için daha büyük ölçekli çalışmalara ihtiyaç vardır.

**Anahtar Sözcükler:** Anal fistül, anal fissür, hemoroid, human papilloma virüsü, patoloji, rutin histopatolojik inceleme

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## INTRODUCTION

Histopathological examination is indispensable in all surgical resections performed when there is suspicion of malignancy or when the diagnosis is unclear (1). When evaluated in terms of cost, routine histopathological examination constitutes a significant financial burden for many procedures. Additionally, it holds an important place in healthcare practices in terms of time and workforce (1). The growing world population, increasing life expectancy, and the rising number of surgical interventions each year justify examining measures to reduce the financial burdens on the healthcare system (2). The rationale against routine histopathology includes the rarity of malignancy in benign lesions, low clinical significance of these malignancies, and high procedural costs (3).

In clinical practice, histopathological examination is routine for patients undergoing surgery for benign anorectal diseases; however, its value and necessity are not fully understood. From this perspective, it is inevitable to question the necessity of routine histopathological examination in surgical procedures for benign anorectal diseases, which generally fall within the definition of ambulatory surgery and constitute a significant portion of our clinical practice, is inevitable. This study aimed to examine the histopathological results in patients undergoing surgical excision for hemorrhoidal disease, anal fistula, and anal fissure, determine the incidence of malignancy or premalignant lesions, and evaluate the results in terms of a selective approach that could replace routine examination.

## MATERIALS AND METHODS

Data of patients who underwent surgery with a diagnosis of benign anorectal disease between January 2013 and December 2023 in the Department of General Surgery, Gazi University Faculty of Medicine, were retrospectively screened using the hospital computer operating system and patient file archives. The inclusion criteria for the study were as follows: 1) preoperative diagnosis of benign anorectal disease, 2) age 18 and older, and 3) postoperative histopathological examination. The exclusion criteria for the study were as follows: 1) incomplete patient archive data. The demographic characteristics (age and gender), surgical procedures (hemorrhoidectomy, fissurectomy, fistulectomy), and histopathological results (benign, malignant, or infectious) of patients meeting the study criteria were evaluated.

All procedures performed in this study are in accordance with the ethical standards of the institutional and/or national research committee and the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. This study was approved by the Gazi University Local Ethics Committee (approval number: E-77082166-604.01.02-839857, date: 02.01.2024).

### Statistical Analysis

All data were transferred to a computer environment, and SPSS 20.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Relevant variables were analyzed using descriptive statistics. Categorical measurements were reported as numbers and percentages, whereas continuous measurements were reported as mean  $\pm$  standard deviation and range.

## RESULTS

A total of 830 patients underwent surgery for benign anorectal diseases. Among them, 482 patients met the study criteria and constituted the final analysis study group (Figure 1). Of these patients, 329 (68.3%) were male and 153 (31.7%) were female. The median age was 48 with a mean age of 48.8 years (range, 18-84). Upon examination of the surgical procedures, fissurectomy was performed in 58 patients (12%), hemorrhoidectomy in 230 patients (47.7%), and fistulectomy in 194 patients (40.2%). Histopathological examination revealed benign findings in all patients. No malignancy was detected in any patient; however, human papilloma virus (HPV)-related lesions were diagnosed in 10 patients (2.1%) (Table 1). Of the patients with HPV-related lesions, 3 were males and 7 were females.

## DISCUSSION

Advancements in medical science and healthcare services have led to a global increase in life expectancy. As a natural consequence, there is a demographic shift toward an aging population, and epidemiologically, the types of diseases and causes of death are also changing. In addition to these developments, healthcare expenditures in all countries are increasing at a faster rate than per capita national income growth, prompting policymakers to seek

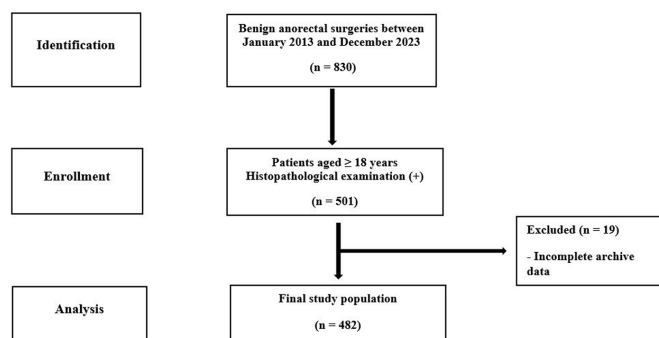


Figure 1. Sample collection scheme.

Table 1. Demographics, surgical procedure, and histopathological results

Characteristics	
Age (year)	Median 48, mean 48.8 $\pm$ 13.1 (range, 18-84)
<b>Gender</b>	
Female	329 (68.3%)
Male	153 (31.7%)
<b>Surgical procedure</b>	
Fissurectomy	58 (12%)
Fistulectomy	194 (40.2%)
Hemorrhoidectomy	230 (47.7%)
<b>Histopathology</b>	
Benign	482 (100%)
HPV-related lesion*	10 (2.1%)
Malign	0

\*HPV: Human papilloma virus.

ways to control healthcare spending (2). In contemporary times, controlling costs in healthcare services is particularly emphasized, especially in terms of ensuring the effective utilization of healthcare expenditures (1). While research has consistently revealed that a significant percentage, varying between 25% and 40%, of laboratory tests are deemed unnecessary, the demand for such tests continues to rise. This increase can be attributed to various factors, including the desire for thorough diagnostic evaluations, peer and commercial pressures, patient expectations, and apprehensions regarding legal consequences (4).

It is noteworthy that a significant portion of the cost of routine histopathological examination is attributed to the most commonly submitted specimens (1). Therefore, there are studies in the literature questioning the routine pathological examination in common surgical procedures in general surgery, such as hernia repair, appendectomy, and cholecystectomy (1,3-6). In a study conducted by the Royal College of Pathologists in the United Kingdom, the workload associated with gastrointestinal specimens lacking clinical value or having limited value was investigated, and a list of diseases for which histopathological examination is not recommended was provided (4). Another study examining the effectiveness of routine histopathology following appendectomy, cholecystectomy, and hemorrhoidectomy concluded that routine examination of hemorrhoidectomy specimens is unnecessary, whereas examination limited to cases over 60 years old and cases with empyema is sufficient for the gallbladder and appendix (5). However, there are also studies advocating for the continued routine examination of cholecystectomy specimens, albeit at a low level, because of their potential to provide diagnoses of invasive cancer and other conditions that may impact postoperative monitoring (6).

A significant portion of our clinical practice comprises surgeries for benign anorectal diseases. Among proctologic surgeries, hemorrhoidectomy, anal fistula, and fissure surgeries are the most common. Given that these procedures are often performed as outpatient or office-based procedures to reduce costs, the necessity of histopathological examination is being questioned. There is no clarity or consensus on this issue in the literature. In a study examining 914 hemorrhoidectomy specimens, it was reported that histopathological examination for hemorrhoidal disease is unnecessary (5). However, in a large series involving 10,532 cases of hemorrhoidectomy and anal fistula excision, unexpected pathologic malignancy was detected at a rate of 0.16%. This study particularly emphasizes that being aged 60 years is a risk factor. The fourfold increase in the frequency of incidental malignancy in individuals aged 60 years is remarkable (7). One of the largest series in the literature, based on a retrospective analysis of 21,257 cases of hemorrhoidectomy, reported an unexpected malignancy in only one patient. This result emerges as a significant argument in favor of selective histopathological examination instead of routine practice. In our literature review, no cases of unexpected malignancy were encountered following anal fissure surgery. In our study, none of the patients were diagnosed with an unexpected malignancy. Therefore, we cannot identify a specific patient group for whom selective examination might be recommended instead of routine examination. We do not support routine examination in surgeries performed with a preoperative diagnosis of benign anorectal disease; however, we cannot ignore the necessity of a selective approach. It is evident

that extensive studies are needed to determine which age group or characteristics of patients would benefit from the selective approach.

An interesting finding of our study was the diagnosis of HPV-related lesions in 10 patients (2.1%). None of these patients had suspicion of anal intraepithelial neoplasia (AIN) or HPV-related lesions during preoperative clinical evaluation. Of the patients with HPV-related lesions, 3 were males and 7 were females. However, due to the retrospective nature of our study, we did not have access to the clinical histories of these patients to determine their association with HPV. In a prospective study examining the prevalence of AIN in macroscopically normal hemorrhoidectomy and fissurectomy specimens, a rate of 3.2% AIN was reported. This study, encompassing 2997 procedures, demonstrated that the prevalence of subclinical AIN in macroscopically normal hemorrhoidectomy and fissurectomy specimens is significant (3.2% overall, 2.5% high-grade disease). Our findings are similar to and supportive of the findings of this study. AIN is a premalignant lesion of squamous cell carcinoma. Screening tests for AIN and human papillomavirus vaccine are important for high-risk patients, including those who are positive for human immunodeficiency virus and men who have sex with men (9). It seems imperative not to compromise on routine histopathological examination for these two high-risk groups.

### **Study Limitations**

The most significant limitation of our study is its retrospective design and the restricted nature of the patient population. The formation of a final population with histopathological examination conducted in 482 out of 830 patients from the initial population actually indicates that surgeons did not request histopathological examination for every patient. Here, it appears that a selective or perhaps even random approach was adopted without an objective selection criterion.

### **CONCLUSION**

In conclusion, routine histopathological examination after benign anorectal surgeries may not be necessary for patients without a history of HPV-related risk factors. However, standardized and large-scale studies are needed to identify the patient population suitable for a selective approach. These studies can help establish clear criteria for which patients should undergo routine histopathological examination, contributing to more efficient resource usage in clinical practice.

### **Ethics**

**Ethics Committee Approval:** This study was approved by the Gazi University Local Ethics Committee (approval number: E-77082166-604.01.02-839857, date: 02.01.2024).

**Informed Consent:** Retrospective study.

### **Authorship Contributions**

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

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

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## REFERENCES

1. Matthyssens LE, Ziol M, Barrat C, Champault GG. Routine surgical pathology in general surgery. *Br J Surg* 2006; 93: 362-8.
2. Hayran O. Can Preventive Services Help to Reduce Health Care Expenditures? *Journal of BSHR* 2019; 3: 170-5.
3. Swank HA, Eshuis EJ, Ubbink DT, Bemelman WA. Is routine histopathological examination of appendectomy specimens useful? A systematic review of the literature. *Colorectal Dis* 2011; 13: 1214-21.
4. Cross SS, Stone JL. Proactive management of histopathology workloads: analysis of the UK Royal College of Pathologists' recommendations on specimens of limited or no clinical value on the workload of a teaching hospital gastrointestinal pathology service. *J Clin Pathol* 2002; 55: 850-2.
5. Lohsiriwat V, Vongjirad A, Lohsiriwat D. Value of routine histopathologic examination of three common surgical specimens: appendix, gallbladder, and hemorrhoid. *World J Surg* 2009; 33: 2189-93.
6. Kozan R, Özyaydin S, Bayhan H, Leventoğlu S, Karamercan A, Anadol AZ, et al. Routine Histopathological Examination of the Specimen After Laparoscopic Cholecystectomy: Can We Be Brave Enough to Give Up? *Turk J Gastroenterol* 2021; 32: 218-24.
7. He X, Huang J, Yao J, Chen Z, Lian L, Li S, et al. Routine histopathologic examination of "benign" anal lesions: is it necessary? *Surg Today* 2015; 45: 416-21.
8. Cataldo PA, MacKeigan JM. The necessity of routine pathologic evaluation of hemorrhoidectomy specimens. *Surg Gynecol Obstet* 1992; 174: 302-4.
9. Sakamoto T, Akiyama S, Narasaka T, Suzuki H, Sekine S, Saito Y, et al. Anal Intraepithelial Neoplasia: Precursor of Anal Squamous Cell Carcinoma. *J Anus Rectum Colon* 2022; 6: 92-9.



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## Surgical Treatment of the Oroantral Fistula in a Patient with Myasthenia Gravis: Clinical Considerations and Anesthetic Management

### Miyastenia Gravisli Bir Hastada Oroantral Fistülün Cerrahi Tedavisi: Klinik ve Anestezi Yönetimi

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#### ABSTRACT

Myasthenia gravis (MG) is an autoimmune neuromuscular disease characterized by muscle weakness and fatigue. MG affects neuromuscular junctions and is characterized by the development of antibodies immunoglobulin G against postsynaptic acetylcholine receptors cause disease. Definitive diagnosis is made by electromyogram. Women are more affected than men. Fluctuating weakness and easy fatigability of skeletal muscles are observed when the limbs are involved. The weakness may involve a single muscle group or may be generalized. The muscle group is the ocular muscles and often causes diplopia and ptosis. On this rare occasion, careful perioperative anesthetic management is required to avoid life-threatening complications in both intraoperative and postoperative periods. In this case report, a 47-year-old female patient with MG requiring oroantral fistula treatment is described. Anesthetic management was performed under local anesthesia using appropriate doses, and the postoperative patient follow-up was uneventful.

**Keywords:** Anesthesia, dental treatment, myasthenia gravis

#### ÖZ

Miyastenia gravis (MG), kas zayıflığı ve yorgunluğu ile karakterize otoimmün nöromusküler bir hastalıktır. MG, nöromusküler kavşağı etkiler ve postsinaptik asetilkolin reseptörlerine karşı antikolların immünoglobulin G gelişmesiyle karakterizedir. Kesin tanı elektromiyogram ile konur. Kadınlar erkeklerden daha fazla etkilenir. Uzunlar tutulduğu zaman iskelet kaslarında zayıflık ve kolay yorulma gözlenir. Kaslardaki zayıflık, tek bir kas grubunu içerebilir veya generalize olabilir. Göz kasları etkilendiğinde sıklıkla diplopi ve pitozise neden olur. MG nadir görülen bir durumdur, hem intraoperatif hem de postoperatif dönemlerde hayatı tehdit eden komplikasyonlardan kaçınmak için dikkatli perioperatif anestezi değerlendirilmesi yapılmalıdır. Bu olgu sunumunda MG'li 47 yaşında bir kadın hastanın oroantral fistül tedavisi anlatılmaktadır. Anestezi yönetimi uygun dozda lokal anestezi altında yapıldı ve postoperatif hasta takibi sorunsuz bir şekilde gerçekleştirildi.

**Anahtar Sözcükler:** Anestezi, diş tedavisi, miyastenia gravis

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## INTRODUCTION

Myasthenia gravis (MG) is a chronic autoimmune neuromuscular disorder characterized by a decrease in the nicotinic acetylcholine receptor of the post-synaptic membrane of the neuromuscular junction, leading to muscle fatigue and weakness (1). Oropharyngeal and facial muscle weakness is frequent at the onset of the disease; therefore, dentists are often the first healthcare workers to encounter patients with MG (2).

Management of MG is a challenging issue for oral and maxillofacial surgeons. In dentistry, routine dental procedures for MG patients are difficult and special considerations are required (3).

In the present case report, the surgical management of an oroantral fistula in a 47-year-old female patient with MG under local anesthesia is described. The clinical features and treatment of MG have also been discussed along with the use of medications, perioperative considerations, and anesthetic management (4).

## CASE REPORT

A 47-year-old female patient was referred to the oral and maxillofacial surgery department for an oroantral fistula in the left maxillary molar region. Her medical history included MG and thymectomy. The medications included azathioprine and pyridostigmine. During pre-operative examination, the patient had muscular weakness such as ptosis, diplopia, dysphagia, decreased ability to swallow, and fatigue. During clinical examination, an oroantral fistula in the left maxillary molar region was observed. Radiological examination

with panoramic radiography and cone beam computed tomography revealed perforation of the floor of the left maxillary sinus. Based on the clinical and radiographic findings, a diagnosis of oroantral fistula was made (Figure 1). The treatment plan included double-layered closure of the oroantral fistula with a buccal fat pad and oral mucosa.

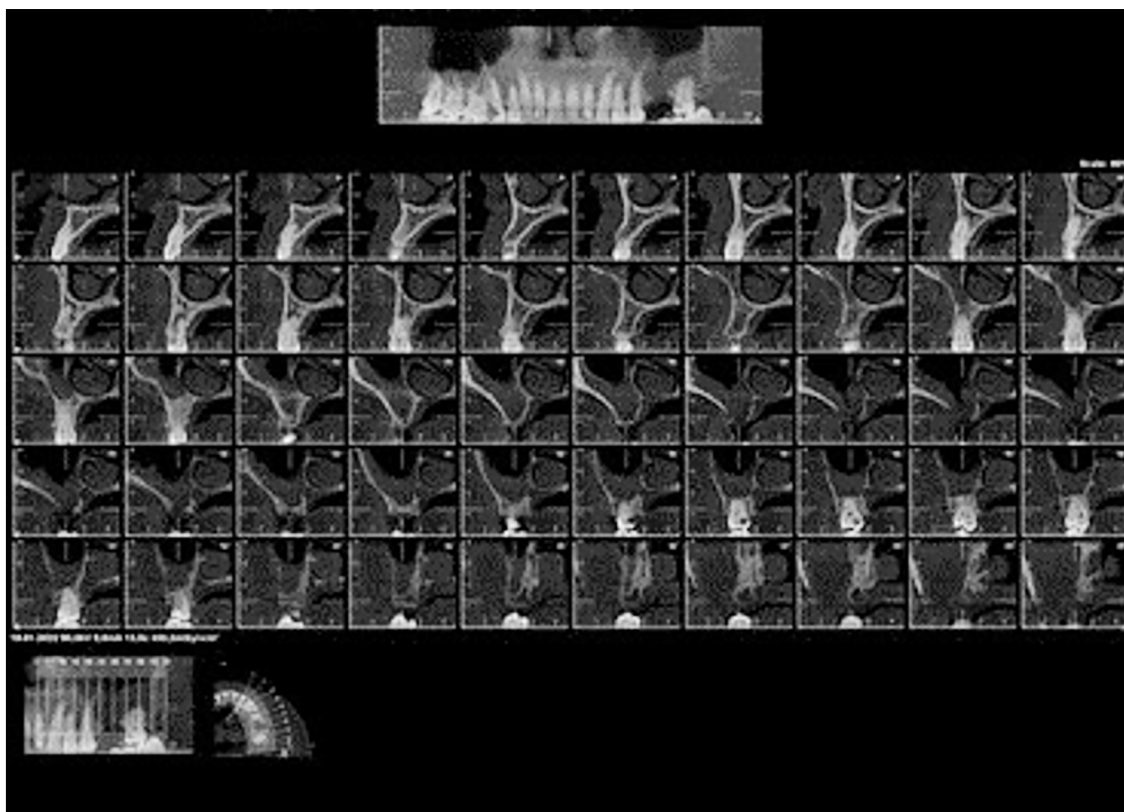
### a. Anesthetic Management

Anesthetic and postoperative medical management were planned according to the consultation with the patient's neurologist. The operation was performed under local anesthesia. The patient was ordered to take her medications 1.5 h before surgery. Non-invasive blood pressure, heart rate, and peripheral oxygen saturation were monitored and recorded 5 min apart.

Posterior superior alveolar anesthesia and local infiltration anesthesia were performed using 2 carpules containing 2% lidocaine buffered with 1:100,000 epinephrine.

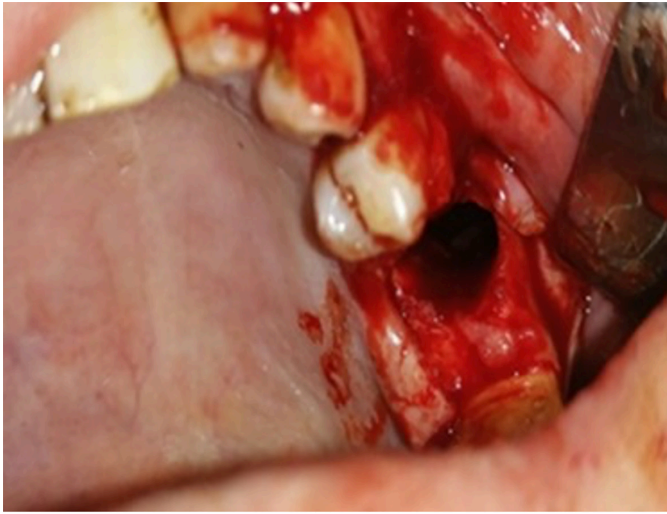
### b. Surgical Management

A trapezoidal mucoperiosteal flap was created using a sulcular incision along the alveolar ridge. The oroantral fistula was explored, and debridement of the maxillary sinus was performed by cadwell luc approach (Figure 2) Considering the anatomical position of the papilla parotidea, a 1 cm vertical incision was made posterior to the zygomatic buttress. Buccal extension of Bichat's fat pad was explored by blunt dissection through the buccinator muscle. The necessary amount of Bichat's fat pad was mobilized with light pressure to completely cover the oroantral fistula entirely (Figure 3) (5,6). The

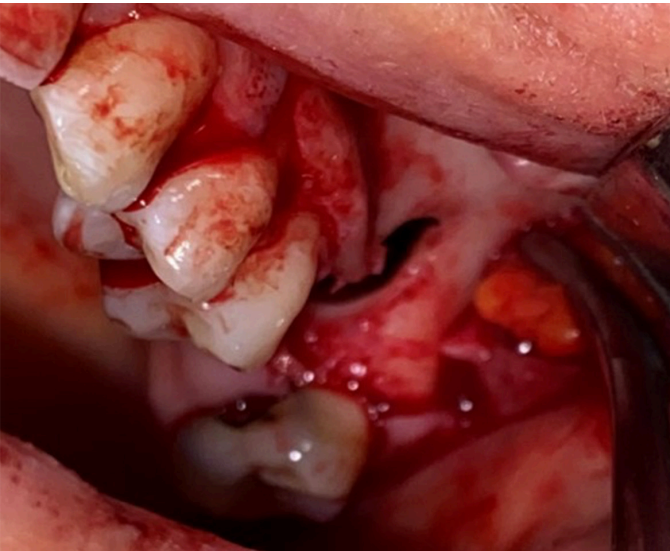


**Figure 1.** CBCT imaging showing the oroantral fistula and sinus pathology.

CBCT: Cone-beam computed tomography.



**Figure 2.** Intraoperative view of the oroantral fistula.



**Figure 3.** Bichat adipose tissue was used to close the entire defect.

mucoperiosteal flap was sutured with 4.0 resorbable suture without tension (Figure 4).

Amoxicillin 500 mg (Devamox, Deva, İstanbul, Türkiye) and paracetamol 500 mg (Parol, Atabay, İstanbul, Türkiye) were prescribed twice daily for 7 days. During the postoperative period, the patient was followed up in the recovery room, and blood pressure, oxygen saturation, and heart rate were monitored for 2 h. The postoperative recovery period was uneventful, and no anesthetic complications were observed.

## DISCUSSION

MG is an autoimmune disorder characterized by skeletal muscle weakness and fatigue (3). Antibody-mediated autoimmune attack directed toward the acetylcholine receptors may cause muscular weakness and fatigue (7). The disease is most frequently observed in the muscles of the eyes, face, neck, and shoulders (3). The first peak for women occurs in the second and third decades, whereas the peak in men is observed in the fifth and sixth decades (7).



**Figure 4.** The mucoperiosteal flap was sutured without tension.

Myasthenic crisis is a result of severe involvement of the respiratory muscles and may lead to a life-threatening respiratory collapse, which may necessitate mechanical ventilation (3). Infections, surgical procedures, and emotional stress may be the precipitants of myasthenic crisis (1). In case of elective surgery, the time period when the patient is on minimal medication and in a stable phase should be selected (8). In this case, the medical history revealed that the patient had been stable for 4 years.

Dental treatment is a stressful procedure because of the fear of pain, local anesthesia, and the dental setting. Stress may trigger a myasthenic crisis and should be taken into consideration before the surgical procedures (7). Morning appointments with a short duration should be preferred to minimize muscle weakness (9). Patients with MG also have greater muscular strength in the morning. Oral anticholinesterase agents should be administered 1.5 h before the surgical operation. Therefore, the effect of medications can be maximized during the treatment session (7,9). In this case report, the patient was recommended to take her medications 1.5 h before the procedure and scheduled for the morning appointment.

The commonly prescribed drugs and anesthetics used for dental procedures have a potential risk of inducing muscle weakness and fatigue, thereby leading to respiratory (8). Dental treatments may be performed under local anesthesia (9). Ester-type local anesthetics are contraindicated, whereas amide local anesthetics can be safely applied (4,9). Ester-type local anesthetics are primarily inactivated by plasma cholinesterases and have decreased efficacy in MG patients on AChE treatment (10). Lidocaine, including 1:100,000 epinephrine, is advantageous for maximizing anesthesia efficiency at the surgical site, while minimizing the total anesthetic dose (4). Local infiltration, intraligamentary, and intrapulpal injections may help to reduce the doses of local anesthetics in comparison with regional blocks (3). Bilateral inferior alveolar nerve blocks may cause swallowing difficulties and must be avoided in MG patients (3). General anesthesia is also preferred for specific conditions. Local anesthesia (using amide local analgesics in minimal dosage) is preferred to general anesthesia (9). Anti-cholinesterase agents may increase the effect of succinylcholine and inhibit the effect of

non-depolarizing neuromuscular blocking drugs (11). Sensitivity to non-depolarizing agents has been reported in patients with minimal effected patients. Intermediate and short-acting non-depolarizing agents should be preferentially (12). Volatile anesthetics enhance the effects of non-depolarizing agents; therefore, it is important to be aware of their impact on patients with MG (13). In patients with MG, barbiturates and propofol can be used for general anesthesia without unwanted effects. Opioid analgesics in therapeutic concentrations do not interfere with neuromuscular transmission; however, central respiratory depression may appear when opioids are (12). Considering these factors, we preferred to perform the surgical treatment under local anesthesia instead of general anesthesia.

The oroantral communication is an unnatural ostium-mucosal connection between the maxillary sinus and oral cavity (5). In the literature, the technique of closing the oroantral fistula with a Bichat fat pad has been described as a successful technique (5,6). The advantages of using a buccal fat pad include easy access, minimal dissection, excellent blood supply, reduced donor site morbidity, low risk of infection, short operation time, and minimal scar formation (6). Based on these advantages, Bichat's fat pad was preferred for the treatment of the oroantral fistula in a patient with MG.

MG is a rare autoimmune disorder that presents challenges for oral and maxillofacial surgeons. Therefore, close monitoring of vital signs increases the chance of early diagnosis of complications that may occur in the patient. Moreover, to avoid complications that may be caused by the disease, it is essential to understand the nature of the disease and make the right choice of medication.

### **Ethics**

**Informed Consent:** It was obtained.

### **Authorship Contributions**

Concept: T.K., Design: E.B.Y., Supervision: E.B.Y., Resources: Y.K., Materials: N.Ş., Data Collection or Processing: T.K., Analysis or Interpretation: Y.K., Literature Search: T.K., Writing: E.B.Y., Critical review: Y.K.

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

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### **REFERENCES**

1. Blichfeldt-Lauridsen L, Hansen BD. Anesthesia and myasthenia gravis. *Acta Anaesthesiol Scand* 2012; 56: 17-22.
2. Tamburrini A, Tacconi F, Barlattani A, Mineo TC. An update on myasthenia gravis, challenging disease for the dental profession. *J Oral Sci* 2015; 57: 161-8.
3. Patil PM, Singh G, Patil SP. Dentistry and the myasthenia gravis patient: a review of the current state of the art. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2012; 114: e1-8.
4. Patton LL, Howard JF Jr. Myasthenia gravis: dental treatment considerations. *Spec Care Dentist* 1997; 17: 25-32.
5. Arce K. Buccal fat pad in maxillary reconstruction. *Atlas Oral Maxillofac Surg Clin North Am* 2007; 15: 23-32.
6. Alonso-González R, Peñarrocha-Diago M, Peñarrocha-Oltra D, Aloy-Prósper A, Camacho-Alonso F, Peñarrocha-Diago M. Closure of oroantral communications with Bichat's buccal fat pad. Level of patient satisfaction. *J Clin Exp Dent* 2015; 7: e28-33.
7. Yarom N, Barnea E, Nissan J, Gorsky M. Dental management of patients with myasthenia gravis: a literature review. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2005; 100: 158-63.
8. Collins S, Roberts H, Hewer I. Anesthesia and Perioperative Considerations for Patients with Myasthenia Gravis. *AANA J* 2020; 88: 485-91.
9. Lotia S, Randall C, Dawson LJ, Longman LP. Dental management of the myasthenic patient. *Dent Update* 2004; 31: 237-42.
10. Prabhu SS, Khan SA, Doudnikoff AL, Reebye UN. Anesthetic considerations for a patient with myasthenia gravis undergoing deep sedation in an outpatient oral surgery setting. *J Dent Anesth Pain Med* 2019; 19: 67-72.
11. Dillon FX. Anesthesia issues in the perioperative management of myasthenia gravis. *Semin Neurol* 2004; 24: 83-94.
12. Jain PK, Kalra P, Sharma M, Joshi A. Anaesthetic Management of a patient with Myasthenia Gravis and Small Bowel Intussusception for Jejunum-Ileal Anastomoses. *The Indian Anaesthetists* 2015; 16: 1-4.
13. Postevka E. Anesthetic implications of myasthenia gravis: a case report. *AANA J* 2013; 81: 386-8.

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## A Rare Prenatal Case: Greig Cephalopolysyndactyly Syndrome

### Nadir Bir Prenatal Olgu: Greig Sefalopolisindaktili Sendromu

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#### ABSTRACT

Greig cephalopolysyndactyly syndrome (GCPS) is a rare genetic disorder characterized by macrocephaly, prominent forehead, hypertelorism, preaxial and/or postaxial polydactyly, and cutaneous syndactyly. Mutations that cause haploinsufficiency in the zinc finger protein family member 3 (*GLI3*) gene, which is located on the short (p) arm p14 region of chromosome 7 (Chr.7), have been associated with this syndrome. Here, a case of prenatal GCPS with haploinsufficiency of the *GLI3* gene is presented. A 32-year-old woman, in her 21<sup>st</sup> week of the first pregnancy, was referred to our center for cytogenetic analysis of amniotic fluid because of the detection of polyhydramnios, polydactyly, aortic stenosis, and the absence of vesica biliaris visualization on fetal ultrasound. Chromosome analysis was terminated with an interstitial short arm (p12-p15.1) deletion of chromosome 7 consisting of the *GLI3* gene region (7p14). The presence of the short arm terminal region of the relevant chromosome was confirmed by fluorescence *in situ* hybridization analysis. Array comparative genomic hybridization technique verified the breakpoint regions and revealed a 17.4 Mb deletion covering the *GLI3* gene. To date, reported prenatal cases with GCPS syndrome are very rare. Here we present a case of GCPS syndrome diagnosed in the prenatal period due to a *de novo* unbalanced chromosomal rearrangement.

**Keywords:** Chromosome 7, *GLI3* gene, greig cephalopolysyndactyly syndrome, deletion, polydactyly, prenatal

#### ÖZ

Nadir bir genetik hastalık olan greig sefalopolisindaktili sendromu (GCPS) makrosefali, belirgin alın, hipertelorizm, preaksiyel ve/veya postaksiyel polidaktili ve kutanöz sindaktili ile karakterizedir. Bu sendrom 7. kromozomun kısa kolunda (7p14 bölgesinde) yer alan çinko parmak gen ailesi 3 (*GLI3*) geninin haplo yetersizliğine neden olan mutasyonları ile ilişkilidir. Burada *GLI3* geninin haplo yetersizliği ile ilişkili bir prenatal GCPS olgusu sunulmaktadır. Fetal ultrasonografide polihidroamniyöz, polidaktili ve aort stenozu saptanması ve safra kesesinin izlenmemesi nedeniyle 32 yaşındaki kadının 21 haftalık ilk gebeliğine ait amniyon mayi sitogenetik analiz için merkezimize refere edilmiştir. Kromozom analizi 7. kromozomun *GLI3* gen bölgesini de (p14) içeren kısa kolunun (p12-p15.1) interstisyel delesyonu olarak sonuçlanmıştır. Floresan *in situ* hibridizasyon analizi, ilgili kromozomun kısa kol telomer bölgesinin korunduğunu göstermiştir. Karşılaştırmalı genomik hibridizasyon tekniği detaylı olarak kırık bölgelerini ve *GLI3* genini de içeren 17,4 mb'lik delesyonu teyit etmiştir. Bugüne kadar GCPS sendromlu prenatal olgu literatürde oldukça nadirdir. Burada *de novo* dengesiz kromozomal yeniden düzenlenmeye bağlı prenatal dönemde GCPS sendromu tanısı almış bir olgu sunulmaktadır.

**Keywords:** *GLI3* geni, greig sefalopolisindaktili sendromu, delesyon, kromozom 7, polidaktili, prenatal

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## INTRODUCTION

Greig cephalopolysyndactyly syndrome [(GCPS), MIM #175700] is a disorder caused by mutations in the zinc finger gene family 3 (*GLI3* gene), which is crucial for limb, brain, and craniofacial development. It follows an autosomal dominant inheritance pattern. The prevalence of this syndrome is approximately 1-9/1,000,000 (1). The *GLI3* gene is located on the short arm of chromosome 7 (Chr.7-p14 band). The prominent clinical findings are macrocephaly, prominent forehead, hypertelorism, preaxial and/or postaxial polydactyly, and cutaneous syndactyly. In cases with multiple congenital anomalies, it is often assessed as a sequential gene syndrome (2,3).

The *GLI3* gene encodes a pivotal transcription factor essential for embryonic development. It also controls downstream targets of the sonic hedgehog pathway, which regulates cell proliferation and differentiation (4). Approximately 80% of GCPS cases linked to *GLI3* gene haploinsufficiency result from single nucleotide changes, whereas approximately 20% stem from copy number alterations (5).

The vast majority of the reported cases are postnatal with variable expression. Here, the presented case is a rare case of prenatal GCPS due to a *de novo* unbalanced chromosomal rearrangement belonging to the interstitial deletion of chromosome 7, covering the *GLI3* gene region.

## CASE REPORT

At 32 years of age, a primigravida at 21-week of gestation underwent amniocentesis because of abnormal ultrasonographic (USG) findings. USG revealed polydactyly in the feet, polyhydramnios, and aortic valve stenosis, whereas the vesica biliaris could not be visualized. The parents are non-consanguineous.

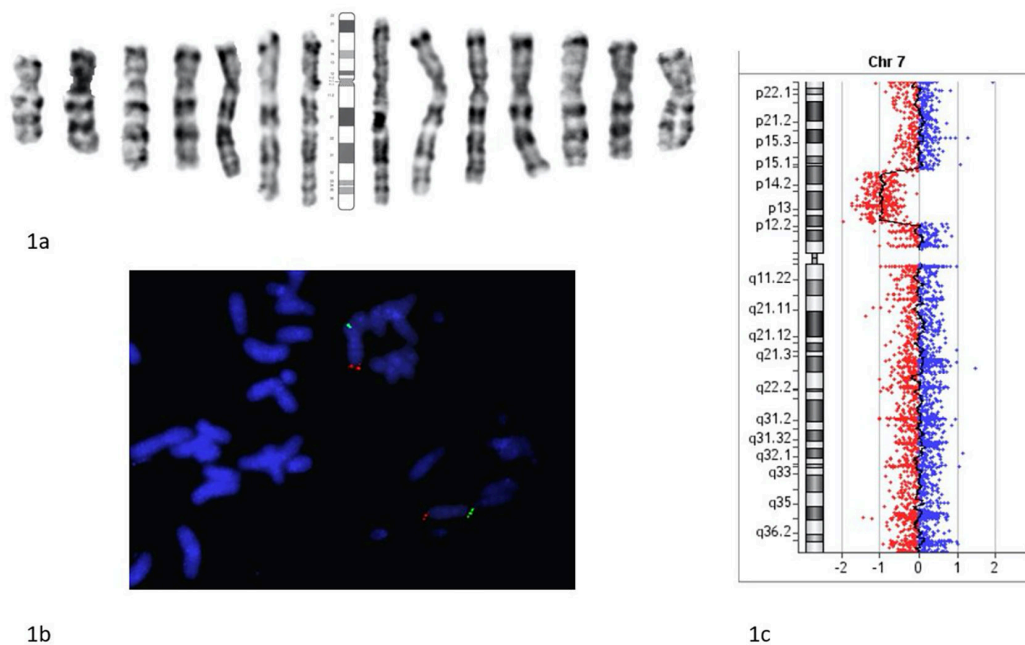
Interphase fluorescent *in situ* hybridization (I-FISH) analysis for common euploidies (Chr.13, 18, 21, and gonosomes) of uncultured amniocytes showed a normal hybridization pattern. Cytogenetic analysis from long-term tissue cultures revealed an interstitial deletion on the short arm of Chr.7, and metaphase FISH analysis confirmed the presence of telomeric regions [(46,XX,del(7)(7pter→7p15.1::7p12→7qter)] (Figure 1a, b).

Array comparative genomic hybridization (array-CGH) (Human Genome G3 SurePrint 8x60K ISCA Array; Agilent Technologies, Santa Clara, California) verified the deletion [17.4 megabase (Mb) deletion], including the *GLI3* gene located on the 7p12.3-14.3 (hg19:7:30307418-47765428) (Figure 1c). Cytogenetic analysis was also carried out on the parents, and their karyotypes were normal. The detected unbalanced chromosomal rearrangement was considered a *de novo*.

After unbiased genetic counseling was given in terms of GCPS, the family decided to continue the pregnancy. Because of fetal cardiac arrest, the mother had a stillbirth at 25 weeks of gestation. Prominent forehead, biparietal narrowing, hypertelorism, flat and wide nasal bridge, broad thumbs in the hands and feet, and preaxial polydactyly in the foot were observed in the clinical examination of the exitus fetus, all consistent with GCPS (Figure 2). Written consent was obtained from the family.

## DISCUSSION

GCPS is primarily caused by single nucleotide or copy number changes in the *GLI3* gene. Rarely, chromosomal alteration within the deletion of related genes can cause GCPS syndrome, as in the present case. Considering other allelic disorders of the *GLI3* gene, such as Pallister Hall syndrome (midaxial/postaxial polydactyly,



**Figure 1.** Comprehensive analysis of the fetus; partial karyotype, metaphase FISH image, and array-CGH image. (a) Partial karyotypes of deleted Chr.7 (left side) and normal Chr.7 (right side). (b) Metaphase FISH images displaying Chr.7 pter and qter regions (green and red signals respectively). (c) Array-CGH image showing the breakpoint region 7p12.3-14.3, revealing a 17.4 Mb deletion on chromosome 7 (Human Genome G3 SurePrint 8x60K ISCA array).

FISH: Fluorescent *in situ* hybridization, CGH: Comparative genomic hybridization.



**Figure 2.** Clinical findings of the exitus fetus were a prominent forehead, biparietal narrowing, hypertelorism, flat and wide nasal bridge, broad thumbs in the hands and feet, and preaxial polydactyly in the foot.

asymptomatic bifid epiglottis, imperforate anus, and hypothalamic hamartoma), evaluation of the individual's genetic and phenotypic characteristics pattern is essential (6). Fortunately, genetic and phenotypic correlations are well documented and can be used to diagnose each condition more accurately (5,7,8). Similar to the present case, large deletions of the *GLI3* gene are more commonly associated with GCPS than with Pallister Hall syndrome.

Array-CGH analysis of this case revealed a 17.4-Mb deletion, resulting in the loss of genes such as *POU6F2*, *GCK*, *TBX20*, *CDK13*, *CAMK2B*, and *GARS*, besides the *GLI3* gene. *POU6F2* gene deletion may increase the risk of Wilms tumor, whereas the deletion of the *GCK* gene may lead to MODY (9,10). The family was informed that intellectual influence was unpredictable because of the involvement of other genes. The deletion interval of the fetus was interpreted on the DECIPHER database, and most of the dysmorphic signs were common, except for the absence of vesica biliaris.

During pregnancy, polydactyly can be diagnosed earlier, whereas macrocephaly and hypertelorism can mainly be detected in the third trimester. Fetal USG plus genetic testing make prenatal diagnosis feasible, allowing early diagnosis and appropriate management of the newborn (8). It allows the potential medical and surgical interventions that the newborn may need to follow birth. Beyond that, diagnosis is also essential for the family's subsequent pregnancies. If chromosomal alteration is familial, counseling should be given regarding preimplantation genetic diagnosis and invasive prenatal testing. Even if the parental karyotype analysis is normal and considered *de novo*, the risk of recurrence exists, albeit low, because of possible changes that cannot be detected by conventional cytogenetic analysis, such as gonadal mosaicism and familial micro-inversions.

In sum, this case holds significance as it was prenatally diagnosed on the basis of suspicious fetal ultrasound findings and subsequently confirmed through genetic testing. GCPS syndrome can be

challenging to diagnose prenatally (11). As in this case, families should be briefed on the condition, its potential causes, and prenatal diagnosis and management options.

#### **Ethics**

**Informed Consent:** The parent of the case signed the written informed consent form.

#### **Authorship Contributions**

Surgical and Medical Practices: T.N., P.T.C., Concept: T.H., G.K., M.Y.K., Design: T.H., G.K., M.Y.K., Data Collection or Processing: T.H., M.Y.K., Analysis or Interpretation: T.H., G.K., M.Y.K., Literature Search: T.H., Writing: T.H., M.Y.K.

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

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#### **REFERENCES**

1. Biesecker LG. The Greig cephalopolysyndactyly syndrome. *Orphanet J Rare Dis* 2008; 3: 10.
2. Johnston JJ, Olivos-Glander I, Turner J, Aleck K, Bird LM, Mehta L, et al. Clinical and molecular delineation of the Greig cephalopolysyndactyly contiguous gene deletion syndrome and its distinction from acrocallosal syndrome. *Am J Med Genet A* 2003; 123A: 236-42.
3. Schwarzbraun T, Windpassinger C, Ofner L, Vincent JB, Cheung J, Scherer SW, et al. Genomic analysis of five chromosome 7p deletion patients with Greig cephalopolysyndactyly syndrome (GCPS). *Eur J Med Genet* 2006; 49: 338-45.
4. Ruiz i Altaba A. Gli proteins encode context-dependent positive and negative functions: implications for development and disease. *Development* 1999; 126: 3205-16.
5. Biesecker LG, Johnston JJ. Greig Cephalopolysyndactyly Syndrome. 2001 Jul 9 [updated 2024 Feb 15]. In: Adam MP, Feldman J, Mirzaa

- GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024.
6. Biesecker LG. GLI3-Related Pallister-Hall Syndrome. 2000 May 25 [updated 2024 Feb 22]. In: Adam MP, Feldman J, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2024.
  7. Jamsheer A, Sowińska A, Trzeciak T, Jamsheer-Bratkowska M, Geppert A, Latos-Bieleńska A. Expanded mutational spectrum of the GLI3 gene substantiates genotype-phenotype correlations. *J Appl Genet* 2012; 53: 415-22.
  8. Garcia-Rodriguez R, Rodriguez-Rodriguez R, Garcia-Delgado R, Romero-Requejo A, Medina-Castellano M, Garcia Cruz L, et al. Prenatal diagnosis of Greig Cephalopolysyndactyly Syndrome. When to suspect it. *J Matern Fetal Neonatal Med* 2022; 35: 2162-5.
  9. Perotti D, De Vecchi G, Testi MA, Lualdi E, Modena P, Mondini P, et al. Germline mutations of the POU6F2 gene in Wilms tumors with loss of heterozygosity on chromosome 7p14. *Hum Mutat* 2004; 24: 400-7.
  10. Schulz S, Volleth M, Muschke P, Wieland I, Wieacker P. Greig cephalopolysyndactyly (GCPS) contiguous gene syndrome in a boy with a 14 Mb deletion in region 7p13-14 caused by a paternal balanced insertion (5; 7). *Appl Clin Genet* 2008; 1: 19-22.
  11. Aslanger AD, Sarac Sivriköz T, Kalayci T, Basaran S, Uyguner O. Fetal hand anomalies: 18 cases diagnosed between 2020-2022 from a single tertiary care center. *Experimed* 2022; 12: 149-54.



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## A General Overview of Mesenchymal Stem Cell Therapies in Drug- and Chemical-Induced Liver Injury Models

İlaç ve Kimyasal Kaynaklı Karaciğer Hasarı Modellerinde Mezenkimal Kök Hücre Tedavi Uygulamalarına Genel Bir Bakış

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### ABSTRACT

Liver injury is commonly seen in the population depending on the drug and chemical usage. Different groups of drugs and chemicals lead to different pathogeneses in the liver, such as necrosis, fibrosis, or inflammation. Although the liver has a high regenerative capability, drug- and chemical-induced liver injury may result in organ failure. Because of the limitations of liver transplantation, therapy methods alternative to organ transplantation still need to be studied. Owing to their differentiation and regeneration abilities, mesenchymal stem cells (MSCs) have recently drawn attention as potential therapeutic agents. In this review, we focused on the effects of human MSCs derived from adipose tissue, bone marrow, umbilical cord, placenta, and amniotic membrane on drug- and chemical-induced liver injury models. Recent studies have reported recovery by the application of human MSCs from different sources. Although MSC therapy leads to amelioration in liver function, researchers still try to improve therapeutic efficacy. To this end, different modifications and application modalities of MSCs were investigated in drug-induced liver injury models. To understand the molecular mechanisms of MSCs' effects on liver injury, animal studies are required to drive research perspectives for future progress.

**Keywords:** Drugs and chemicals, liver injury, liver regeneration, mesenchymal stem cells

### ÖZ

İlaç ve kimyasal kullanımına bağlı olarak toplumda karaciğer hasarını yaygın olarak görmektedir. Farklı ilaç ve kimyasal grupları karaciğerde nekroz, fibrozis veya enflamasyon gibi farklı patogenezlere yol açmaktadır. Karaciğer yüksek bir yenilenme kapasitesine sahip olmasına rağmen ilaç ve kimyasalların neden olduğu karaciğer hasarı organ yetmezliği ile sonuçlanabilmektedir. Karaciğer naklinde karşılaşılan zorluklar nedeniyle organ nakline alternatif tedavi yöntemlerinin daha fazla araştırılması gerekmektedir. Farklılaşma ve rejenerasyon kapasiteleri nedeniyle mezenkimal kök hücreler (MKH) son zamanlarda potansiyel terapötik ajanlar olarak dikkat çekmektedir. Bu derlemede, yağ dokusu, kemik iliği, göbek kordonu, plasenta ve amniyotik membrandan elde edilen insan MKH'lerin ilaç ve kimyasal kaynaklı karaciğer hasarı modelleri üzerindeki etkilerine odaklanılmıştır. Güncel çalışmalar, farklı dokulardan elde edilen insan MKH'lerinin uygulanmasıyla iyileşmenin görüldüğünü bildirmektedir. MKH tedavisi, karaciğer fonksiyonlarında iyileşme sağlasa da, araştırmacılar hala MKH'lerin terapötik etkinliklerini artırmaya yönelik çalışmalarını sürdürmektedir. Bu amaçla ilaca bağlı karaciğer hasarı modellerinde MKH'lerin farklı modifikasyonları ve uygulama yöntemleri araştırılmıştır. MKH'lerin karaciğer hasarı üzerindeki etkilerinin moleküler mekanizmalarını anlamak ve gelecekteki çalışmalara yönelik araştırma perspektifine yön vermek amacıyla hayvan çalışmaları gerekmektedir.

**Anahtar Sözcükler:** İlaçlar ve kimyasallar, karaciğer hasarı, karaciğer rejenerasyonu, mezenkimal kök hücreler

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## INTRODUCTION

The liver is an organ prone to drug- and chemical-induced damage because it metabolizes many xenobiotics. Drug-induced liver injury may be categorized as either idiosyncratic or intrinsic depending on individual factors or the dose of drug, respectively (1). Mitochondrial damage, protein adducts formed by reactive metabolites, endoplasmic reticulum stress, and apoptosis contribute to the pathological molecular basis of drug-induced liver injury (2). Different types of drugs may cause acute hepatotoxicity. Use of drugs such as rifampicin, sulfonamide, isoniazid, and disulfiram may lead to acute hepatic damage characterized by hepatocyte degeneration, apoptotic bodies, and small necrotic foci in all zones. Drugs such as diphenylhydantoin and diaminodiphenyl-sulfone induce lymphocyte and eosinophil infiltration, and cause necrosis in the portal area of the liver. Although drugs such as halothane, acetaminophen (APAP), and ketoconazole cause hepatocyte necrosis and also disruption of microcirculation specifically in the centrilobular area (zone 3), hepatocyte necrosis may become more severe in some cases and may be seen also in periportal area (zone 1). Fibrosis may be mild or absent in the histopathology of hepatotoxicity induced by such

drugs. In addition, these drugs can cause hepatocyte loss, resulting in liver damage characterized by extensive necrosis (3). Although the liver is an organ with a high regeneration capacity, there are many conditions leading to liver failure due to necrosis and fibrosis in acute or chronic processes. These conditions include infections such as viral hepatitis and chemical or biological factors such as drugs and toxic substances. Liver transplantation, the most effective treatment for liver failure, is a challenging approach because of transplant rejection, limited number of donors (4), and side effects resulting from immunosuppression (5). Therefore, experimental and clinical studies reviewing regenerative therapeutic approaches provide new insights into treatment strategies alternative to transplantation. In this paper, we reviewed experimental studies investigating the effects of human MSCs obtained from different sources, including adipose tissue, bone marrow, umbilical cord, placenta, and amniotic membrane, on drug- and chemical-induced liver injury (Figure 1, Table 1). We limited our literature review to the key words “drug-induced liver injury” and the name of the mesenchymal stem cell (MSC) types and looked into the Web of Science and PubMed databases.

**Table 1.** Effects of hMSCs and their derivatives on drug- and chemical-induced liver injury

MSCs/source	Drug/chemical agent	Action principle	Outputs	
Human adipose tissue-derived mesenchymal stem cells	Human adipose-derived MSCs (6)	CCI4	Paracrine signaling	- Decreased ALT levels - Increased PCNA expression - Improvement in histopathological alterations
	Fox2-overexpressing hAD-MSCs (7)	TAA	Paracrine signaling	- No change in ALT and AST levels - Improvement in necrosis - Recovery in ALB and BIL levels
	Lipid-conjugated heparin-coated hAD-MSCs (8)	APAP	Greater engraftment with coating	- Reduction in ALT and AST levels - Decreased CYP2E1 expression - Increased HGF expression
	HLC with 3D-AHAM scaffold (9)	CCI4	Long-term and improved incorporation	- Reduction in the ALT level - Improvement in histopathological alterations
	Secretome (10)	AMI	Paracrine signaling	- Reduced necrosis - Improved inflammation - Decreased $\alpha$ SMA expression and apoptosis - Enhanced proliferation
Human placenta-derived mesenchymal stem cells	Human placenta-derived MSCs (11)	CCI4	Anti-fibrotic effect	- Improvement in the biochemical parameters - Decreased Col I and $\alpha$ SMA expressions
	Human placenta-derived MSCs (12)	TAA	Anti-inflammatory and antioxidant effects	- Decreased TNF $\alpha$ and IL6 levels - Increased HO1 expression and SOD1 and CAT activities
	Human placenta-derived MSCs (13)	CCI4	Autophagy-related signaling pathways	- Increased Beclin1, LC3 II, ATG7, Cyc A, and Cyc E expressions
Human Amniotic-derived Mesenchymal Stem Cells	Hepatocyte progenitor-like cells (14)	CCI4	Paracrine effect and hepatogenic differentiation	- Decreased ALT and AST levels - Reduced TNF $\alpha$ and IL2 levels - Increased IL-10 level
	CD34 <sup>+</sup> and CD34 <sup>-</sup> hAM-MSCs (15)	TAA	Anti-fibrotic effect	- Decreased ALT and AST levels - Reduced col I, $\alpha$ SMA and TGF $\beta$ expressions

Table 1. Continued

MSCs/source		Drug/chemical agent	Action principle	Outputs
Human umbilical cord-derived mesenchymal stem cells	Human umbilical cord-derived MSCs (16)	CCI4	Hepatogenic differentiation	- Decreased ALT and AST levels - Reduced apoptosis - Increased proliferation
	Exosomes (17)	CCI4	Antioxidant and anti-inflammatory effects of GPX1	- Decreased 8-OHdG expression - Reduced apoptosis and oxidative stress
	MSC-derived miR-455-3p-enriched exosomes (18)	CCI4	Anti-inflammatory effect	- Decreased ALT and AST levels - Reduced G-CSF, IL6, IL17, MCP-1, and IP-10 levels - Improvement in histopathological alterations
	Preconditioning of MSCs (19)	CCI4	Anti-inflammatory effect	- Decreased MCP-1, TNF- $\alpha$ , IL-6, and CXCL1 levels
Human bone marrow-derived mesenchymal stem cells	Human bone marrow-derived MSCs (20)	CCI4	Paracrine effect	- Reduced necrosis and fibrosis
	Human bone marrow-derived MSCs (21)	CCI4	Anti-mutagenic effect	- Decreased chromosomal aberrations and DNA fragmentation

8-OHdG: 8-hydroxy-2'-deoxyguanosine,  $\alpha$ SMA:  $\alpha$  smooth muscle actin, ALB: Albumin, ALT: Alanine aminotransferase, AMI: Amiodarone, APAP: Acetaminophen, AST: Aspartate aminotransferase, BIL: Bilirubin, CCI4: Carbon tetrachloride, Col I: Collagen I, CYP2E1: Cytochrome 2E1, G-CSF: Granulocyte colony stimulating factor, HGF: Hepatocyte growth factor, HO1: Heme oxygenase-1, IL: Interleukin, IP-10: Interferon gamma-induced protein 10, MCP-1: Monocyte chemoattractant protein-1, MSCs: Mesenchymal stem cells, PCNA: Proliferating cell nuclear antigen, TAA: Thioacetamide, TGF $\beta$ : Transforming growth factor beta.

## 2. Possible Therapeutic Use of Human MSCs in Drug- and Chemical-Induced Hepatic Injury

Animals used to establish liver injury models in studies investigating cellular treatments include mice, rats, guinea pigs, and cats (22). APAP, carbon tetrachloride (CCI4), thioacetamide (TAA), acetylsalicylic acid, cocaine, chemotherapy, amoxicillin-clavulanate, cephalosporins, isoniazid, and bromobenzene are the common agents used to form drug- and chemical-induced liver injury models (22-24).

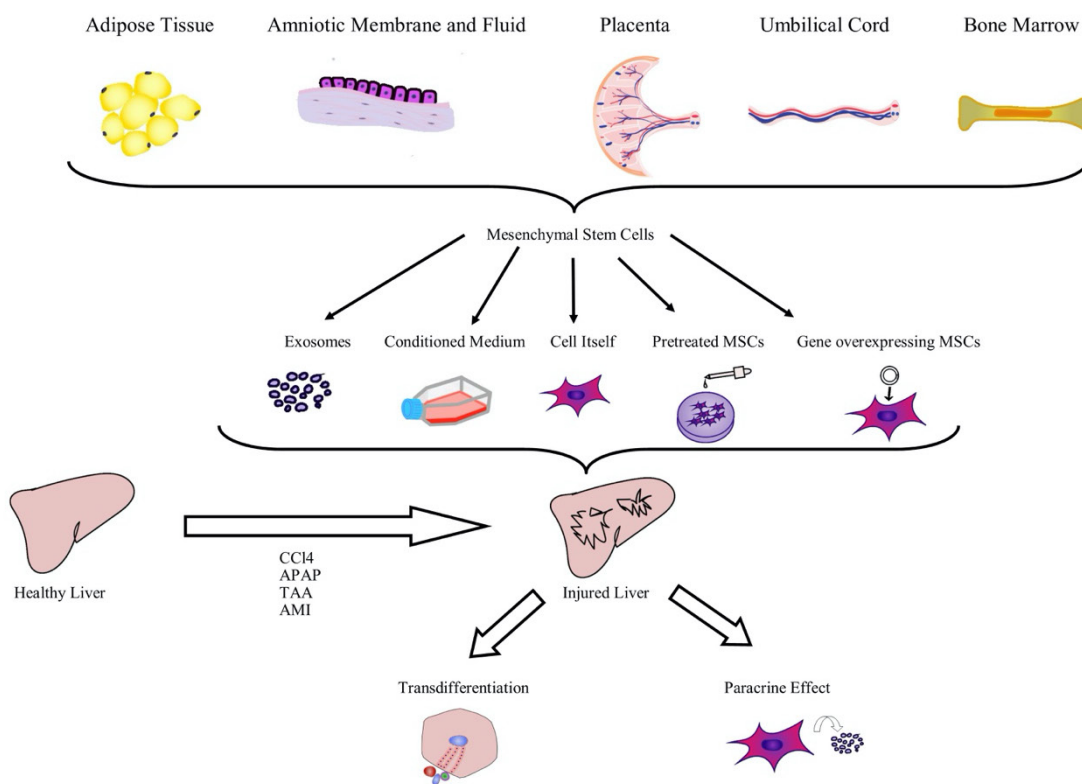
Different perinatal and adult tissue sources such as amniotic fluid, amniotic membrane, umbilical cord blood and stroma, placenta, bone marrow, and adipose tissue serve as MSC sources (25). MSCs, resembling fibroblasts morphologically, express CD105, CD73, and CD90 surface markers, whereas they lack CD14, CD34, CD45, CD11B, CD79 $\alpha$ , CD19, and human leukocyte antigen-DR isotype (HLA-DR) (26). In addition to *in vitro* differentiation into adipocytes, osteoblasts, and chondrocytes, studies have revealed that MSCs are able to differentiate into various cell lineages including endothelial cells, smooth muscle cells, cardiomyocyte, hepatocytes, pancreatic  $\beta$ -cells and neuronal cells, and *in vitro* differentiation of MSCs requires different culture conditions in accordance with their tissue of origin (27-28). Furthermore, it was revealed that MSCs, which can differentiate into endoderm-derived hepatocytes *in vitro*, exhibit hepatocyte-specific protein expression as well as hepatocyte functions such as glucose production and storage (29,30).

One of the reasons underlying the use of MSCs in cellular therapy for tissue damage is that when they are administered through blood circulation, these cells tend to migrate to the injured area and contribute to the healing process by different mechanisms (31). Owing to their high proliferative capacity and multipotency, MSCs are frequently used in studies on tissue regeneration because they can directly differentiate into the parenchymal cells of the tissue to

be transplanted and transform into stromal components to support the parenchyma (32). For instance, in cases of liver injury, some transplanted MSCs turn into hepatocytes, whereas others contribute to liver regeneration by providing revascularization through vascular endothelial growth factor (VEGF) regulation (33). Studies have shown that the contribution of these cells to the healing process is not limited to the differentiation into tissue components; they also display paracrine effects through the substances they secrete (7,34). MSCs participate in regeneration by secreting soluble proteins, nucleic acids, lipids, and exosomes. Notably, exosomes are of particular importance for research on cell-free treatment approaches (35). MSCs secrete various paracrine substances, including proangiogenic and immunoregulatory factors, factors regulating the extracellular matrix, and factors that induce the migration and proliferation of MSCs (36). Hence, in case of tissue injury, MSCs are able to regulate angiogenesis and support regeneration with paracrine effects with chemokines that they secrete, such as fibroblast growth factor, insulin-like growth factor, hepatocyte growth factor (HGF), and VEGF (31). Regulation of angiogenesis, attenuation of fibrosis with factors they excrete (11,26), prevention of the resident cells from undergoing apoptosis, and regulation of the pro-inflammatory and anti-inflammatory cell balance (37) are some of their regulatory actions contributing to the tissue regeneration process (38).

### 2.1. Adipose Tissue-Derived MSCs in Drug- and Chemical-Induced Hepatic Injury Models

Besides medical treatments and organ transplantation, there are also MSC therapy approaches in drugs or chemical-induced liver injury in clinical practice (31,39), and experimental studies are needed to bring new insights into clinical research. In one of the experimental studies, Gad et al. (40) revealed a recovery by the administration of an adipose tissue-derived MSC (AD-MSC) acute



**Figure 1.** Schematic representation of mesenchymal stem cells and their products or different modification strategies used for treating drug- and chemical-induced liver injury.

AMI: Amiodarone, APAP: Acetaminophen, CCl<sub>4</sub>: Carbon tetrachloride, TAA: Thioacetamide, MSCs: Mesenchymal stem cells.

hepatotoxicity model. In addition, Saidi et al. (6) compared the effects of different doses of AD-MSC on liver regeneration in which they administered either  $1 \times 10^6$  and  $2 \times 10^6$  human AD-MSC (hAD-MSC) to mice with CCl<sub>4</sub>-induced acute liver failure. A significant decrease in alanine aminotransferase (ALT) levels, attenuation in congestion, vacuolization, and necrosis, and enhancement in PCNA expression were reported at both doses. Although it was suggested that hAD-MSC transplantation has a therapeutic effect by supporting regeneration and preventing necrosis, it was also pointed out that the recovery was more prominent in the animals given  $2 \times 10^6$  of cells (6).

Further experimental research is also conducted to improve the therapeutic outcomes and to overcome the limitations. Thus, substances obtained from cells and manipulations such as preconditioning were also investigated. In a study, Chae et al. (7) analyzed the differences that may occur as a result of gene overexpression in MSCs. When they examined both the *in vitro* and *in vivo* differentiation of hAD-MSCs into hepatogenic cells, markers indicating hepatic differentiation, such as alpha fetoprotein (AFP), cytokeratin 18 (CK18), dipeptidyl peptidase 4 (CD26), connexin 32 mRNA levels, and cytochrome 450 (CYP450) expression, were shown to be elevated in cells that highly express forkhead box protein a2 (Foxa2), a major transcription factor for liver trans-differentiation. Furthermore, when the hAD-MSCs overexpressing Foxa2 were subcutaneously transplanted into nude mice with liver injury, there

was a decrease in hepatic necrosis and an improvement in albumin (ALB) and bilirubin (BIL) levels, whereas there was no change in ALT and aspartate aminotransferase (AST) values. In accordance with the conditioned medium (CM) analysis, researchers suggested that cells overexpressing Foxa2 displayed their therapeutic effects with paracrine signaling (7).

In another study, the effects of culture medium-related conditions on cell transformation were investigated. Yin et al. (41) examined the therapeutic effects and *in vitro* and *in vivo* differentiation of hAD-MSCs into hepatocytes. As a result, they demonstrated that AD-MSCs incubated with serum-medium much notably exhibited hepatocyte-specific features such as polygonal morphology and glycogen accumulation. When evaluated functionally, these cells were revealed to exhibit an appropriate morphological transformation in serum-free medium, also expressed ALB and AFP, and could store glycogen. They observed that the addition of trichostatin A (TSA), a histone deacetylase inhibitor, to the differentiation protocol stimulated hepatocyte maturation of these cells, resulting in an increase in ALB synthesis and a gradual decrease in AFP synthesis. Moreover, while LDL uptake was not carried out by the cells that were not exposed to TSA, the emergence of this function by TSA application manifested the effectiveness of TSA in inducing differentiation. In the continuation of the study, they stated that hAD-MSC application improved liver function by decreasing serum levels of ALT, AST, and direct BIL during the acute phase of

CCl4-induced liver injury; however, it did not produce a difference in histopathological evaluation. Liver sections labeled with a human-specific ALB antibody demonstrated that the transplanted cells were engrafted and functional; however, they did not exhibit hepatocyte morphology (41). Although the contribution of hAD-MSCs to the regeneration of injured liver through paracrine effects or direct transformation into hepatocytes is not yet clear, Yin et al. (41) demonstrated the potential of these cells to differentiate into mature hepatocyte-like cells (HLC) *in vivo* and *in vitro*.

In a study, lipid-conjugated heparin coating for the liver-targeted delivery of MSCs was used to enhance the outcomes. Hwang et al. (8) analyzed the therapeutic efficacy of hAD-MSC coated with lipid-conjugated heparin in a mice model of acute liver failure induced by APAP administration. Hepatoma (HepG2) cells were exposed to APAP and then cultured with either hAD-MSC-CM or CM harvested from lipid-conjugated heparin-coated hAD-MSC culture (Lip-hep/hAD-MSC-CM). They obtained the result demonstrating that both CMs improved the viability and functionality of injured HepG2 cells compared to the regular medium. In the next step of this investigation, Hwang et al. (8) administered hAD-MSC and Lip-hep/hAD-MSC to mice with liver injury induced by APAP. They achieved better outcomes by Lip-hep/hAD-MSC administration compared to hAD-MSC only administration, that a better recovery at ALT and AST levels were accompanied by an increment in HGF expression and a reduction in the level of CYP2E1. Moreover, they observed that cells coated with lipid-conjugated heparin displayed a greater degree of engraftment into the injured liver (8).

MSC transplantation is a therapeutic approach that is advantageous from many aspects; however, insufficient engraftment of transplanted cells remains an important issue that reduces its efficacy. To overcome this point, Yuan et al. (9) constructed a graft that was a combination of acellular human amniotic membrane (AhAM) and HLCs derived from hAD-MSCs (hAD-MSC-HLCs). They attempted to explore the effects of this graft on the fate of transplanted cells. hAD-MSC-HLCs cultured either on two-dimensional-AHAM (2D-AHAM) or on Col I were compared with freshly differentiated hAD-MSC-HLCs with respect to *in vitro* differentiation both morphologically and functionally. Morphology and CYP and ALB expressions were found to be better with the use of hAD-MSC-HLCs cultured on 2D-AHAM, rather than with the use of Col I or freshly differentiated hAD-MSC-HLCs. Furthermore, when three-dimensional AHAM (3D-AHAM) was examined, the best results were achieved among all these modalities. 3D-AHAM yielded higher ALB and urea concentrations and greater CYP activities than 2D-AHAM and freshly differentiated hAD-MSC-HLCs. In the next step, researchers evaluated the *in vivo* fate of 3D-AHAM application with and without hAD-MSC-HLCs in CCl4-induced damage. They observed a decrease in areas of inflammation, congestion, and hemorrhage and an improvement in serum ALT levels in animals transplanted with hAD-MSC-HLC-3D-AHAM compared with animals transplanted with only 3D-AHAM. They revealed that combination with 3D-AHAM may provide complete and long-term integration of transplanted hAD-MSC-HLC to the host liver (9).

Along with approaches involving modifications, preconditioning, or pretreatments of cells to be transplanted, the effects of other cellular components of the host on the engraftment and differentiation

of transplanted cells became a considerable point to investigate. In this regard, Hong et al. (42) analyzed the effects of Kupffer cell activity on liver damage. First, they established a liver injury model using 2-acetylaminofluorene, and they treated the injured animals with glycine in order to prevent the resident macrophages, the Kupffer cells, to generate an inflammatory reaction. When they administered glycine alone, they suppressed the secretion of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) without any alteration in the number of Kupffer cells. They significantly reduced both the levels of TNF- $\alpha$  and the number of Kupffer cells with the combined delivery of glycine and hAD-MSCs, without having any toxic side effects. Kupffer cell inhibition by means of glycine application yielded an enhancement in both engraftment of transplanted cells and transdifferentiation of them into hepatocytes (42).

Huang et al. (10) evaluated the effects of the secretome of hAD-MSCs on drug-induced liver injury by two different drugs, tamoxifen and amiodarone (AMI). Two different hepatocyte cell lines (HEPG2 and HepaRG) with different expression levels of CYP enzymes were examined in the *in vitro* part of the study. Treatment with the secretome of hAD-MSCs increased proliferation and a decrease in apoptosis compared with non-secretome-treated cells. While the ROS (reactive oxygen species) production decreased by hAD-MSC secretome treatment in both cell lines, it was shown that TNF- $\alpha$ , interleukin-6 (IL-6), and iNOS expressions increased in only the HepaRG cell line due to its higher levels of CYP enzyme expression compared to HEPG2 cells. *In vivo* protective effects of the secretome of hAD-MSCs were examined in a high-fat diet mouse model with AMI-induced liver injury, since mice with normal diet were not affected by AMI. hAD-MSC secretome administration alleviated injury by decreasing steatosis, necrosis, and infiltration of T-lymphocytes and macrophages. Moreover, secretome treatment reversed the damage by decreasing fibrosis and apoptosis and increasing proliferation, as indicated by PCNA expression in AMI-induced liver injury (10).

## 2.2. Placenta-Derived MSCs in Drug- and Chemical-Induced Hepatic Injury Models

MSCs of fetal origin display differentiation potency on a scale between embryonic and adult MSCs. They have more advantageous than adult MSCs in terms of their wider range of differentiation potency and lower immunogenicity. Although their differentiation potency remains lower than that of embryonic stem cells, they do not lead to tumors as in embryonic stem cells. As one of the MSCs of fetal origin, placenta-derived MSCs have been examined in many studies, including those on liver injury and regeneration (43). Lee et al. (11) examined the effects of chorionic plate-derived MSC (CP-MSC) obtained from term placenta on CCl4-induced liver injury in rats. Homing of transplanted CP-MSCs and their transdifferentiation into hepatocytes were verified by the expression of human-specific nuclei and CK18 and CK19 hepatocyte markers. In addition, the reduction of CCl4-induced fibrosis in the CP-MSC-transplanted animals was demonstrated by the decrease in Col I and  $\alpha$ SMA expressions, in addition to Masson's trichrome staining. In addition to morphological regeneration, improvement in hepatic functions in the transplanted animals was also revealed by biochemical parameters, including blood levels of glutamate-oxaloacetate transaminase, glutamate-pyruvate transaminase, and total BIL. Thus, it has been suggested that CP-MSC transplantation, owing to

its anti-fibrotic effect, is a new treatment alternative in cases of liver injury (11). The anti-inflammatory effect of CP-MSc transplantation in addition to anti-fibrotic effect was also demonstrated in one of the current studies, in which Na et al. (12) generated a liver injury model by the administration of TAA. CP-MSc transplantation resulted in a decrease in pro-inflammatory factors such as IL-6 and TNF- $\alpha$ , whereas an increase in IL-10 levels was found. Antioxidant effects of CP-MScs were also introduced in this study; CP-MSc transplantation led to an increase in the expression of heme oxygenase-1, superoxide dismutase 1, and catalase. In addition, CP-MSc transplantation resulted in increased regenerative capacity, as revealed by elevated ALB and nicotinamide adenine dinucleotide phosphatase (NADPH) oxidase 4 (NOX4) expression (12).

In a similar study, Jung et al. (13) revealed the pathways involved in the regenerative effects of CP-MSc transplantation in liver injury. The anti-inflammatory effect of CP-MScs was demonstrated by decreased leukocyte infiltration and elevated IL-10 expression. Additionally, it was underlined that hypoxia-inducible factor 1- $\alpha$  expression, which is involved in survival-related signal transmission, and its transition to the nucleus was higher in the CP-MSc-transplanted animals. B-cell lymphoma 2 (Bcl2) and Bax (Bcl-2-associated X protein) expressions were found to be higher in the CP-MSc-transplanted animals, whereas cleaved PARP [poly (ADP-ribose) polymerase] and caspase 3/7 activities were lower than that of the non-transplanted animals, indicating the regulatory effects of CP-MScs on apoptosis. In addition, it has been shown that autophagy-related factors (such as PI3K class III, Beclin1, ATG7, ATG5-12 and LC3 II) and proteins involved in proliferation (such as PI3Kp110 $\alpha$ , Smad2/3, cyclin A, cyclin E, and PTTG1) were overexpressed because of CP-MSc administration in cases of CCl4-induced liver damage. Furthermore, Ki67 (+) cell number and protein levels related to liver regeneration (such as IL-6, gp130, SCF, ABCG1, ABCG2) were elevated in the animals transplanted with CP-MSc. As a result, CP-MSc transplantation appears to contribute to liver regeneration through autophagy-related pathways (13).

### 2.3. Amniotic Membrane- and Fluid-Derived MSCs in Drug- and Chemical-Induced Hepatic Injury Models

Similar to other MSCs of fetal origin, MSCs derived from the amniotic fluid or amniotic membrane display superiority to adult MSCs in terms of potency and do not stimulate a rejection response owing to their low immunogenicity, that they do not express HLA-DR (44-46). Zagoura et al. (14) examined the therapeutic efficacy of human amniotic fluid-derived MSCs (hAF-MScs) and their derivatives at different differentiation states on CCl4-induced hepatic injury. Although *in vitro* studies showed that HLCs differentiated from hAF-MScs tended to resemble liver cells with respect to their morphology, functions, and cell markers, they failed to engraft injured liver and improve its function. Therefore, only hAF-MScs and hepatocyte progenitor-like cells differentiated from hAF-MScs (HPLC) were preferred to be compared in terms of their therapeutic effects. HPLC significantly decreased AST and ALT levels compared with undifferentiated hAF-MScs, whereas their effects on the improvement of ALB secretion and the survival of the injured animals were comparable. As for the modulatory effects on inflammation, HPLC was revealed to display more prominent

effects on the modulation of the inflammatory response, that HPLC apparently elevated IL-10 levels while decreasing the TNF- $\alpha$  and IL-2. Results of CM analysis, and moreover the improvement provided by the intrahepatic administration of these CMs suggest that the therapeutic effects of cell transplantation are due to not only *in situ* differentiation but also to the paracrine effects of these cells and the substances they secrete (14).

A previous study by Lee et al. (15) presented the effects of CD34(+) and CD34(-) human amniotic membrane-derived MSCs (hAM-MScs) on TAA-induced liver injury. *In vitro* experiments indicated that CD34(+) hAM-MScs exhibited much more potential in terms of differentiation into neurogenic, cardiomyogenic, and hepatic lineages in comparison to CD34(-) hAM-MScs. In the TAA-induced injury model, both CD34(+) and CD34(-) hAM-MScs led to a better reduction in fibrosis than AD-MScs, as demonstrated by collagen deposition and the amount of hydroxyproline. In addition, the expression of genes related to fibrosis [e.g. col I,  $\alpha$ SMA, transforming growth factor beta (TGF- $\beta$ )] reduced in hAM-MSc-transplanted animals in contrast to the AD-MSc-administered group. Both CD34(+) and CD34(-) hAM-MScs decreased the ALT and AST levels significantly; however, only CD34(+) hAM-MSc transplantation resulted in a drastic increase in ALB amount. After 3 weeks of transplantation, they observed the PKH26-labelled cells, indicating the engraftment of hAM-MScs, near the portal tract and fibrotic regions in the injured liver. Because of the study, they suggested that CD34(+) hAM-MScs provided recovery in TAA-induced liver injury in comparison to CD34(-) hAM-MScs. In addition, they disclosed that the reason for the insufficient recovery effects of AD-MSc could be related to its administration route (15).

### 2.4. Umbilical Cord-Derived MSCs in Drug- and Chemical-Induced Hepatic Injury Models

Similar to other fetal MSCs, human umbilical cord MSCs (hUC-MScs) are harvested from a type of tissue that is routinely discarded during delivery; thus, they do not require an invasive procedure to be harvested. Therefore, UC-MScs serve as a reasonable and safe MSC source for autogenic and allogenic transplantation because of their differentiation potency and low immunogenicity. Yan et al. (16) transplanted hUC-MScs to rats injured by CCl4 and revealed an improvement in histopathology and biochemical parameters (ALT and AST) in a time-dependent manner. Moreover, transplantation of hUC-MScs induced proliferation and decreased apoptosis in CCl4-induced liver injury compared with non-transplanted animals. They demonstrated that transplanted cells were sequestered in both kidney and lung but also successfully engrafted into the liver, revealed using human 17 $\alpha$  gene expression. In addition, the cells engrafted into the liver started to express AFP and CK18, indicating hepatogenic differentiation (16). To reveal the effects of the microenvironment on cell differentiation and functionality, Xue et al. (47) investigated the changes in hUC-MSc culture exposed to liver homogenate supernatant. They reported that cultured cells started to express the hepatocyte markers AFP, CK18, and tryptophan 2,3-dioxygenase (TPH2). Furthermore, it was revealed that these cells released ALB and urea and CYP3A activity. The number of PKH26-labeled hUC-MScs was increased in CCl4-induced liver injury compared with the control, which may indicate homing and the potential of pathotropic migratory properties of hUC-MScs.

Results indicated a time-dependent increase in the expression of AFP, CK18, TPH2, and ALB in the liver of rats administered hUC-MSCs following CCl<sub>4</sub>-induced injury. In addition, they showed the improving effects of directly administered hUC-MSCs on biochemical and histopathological alterations (47).

Yan et al. (17) compared the different administration routes and dose-dependent efficiency of exosomes derived from hUC-MSC (hUC-MSC-Ex). They administered hUC-MSC-Ex through either the tail vein or oral gavage at determined doses of 8, 16, 32 mg/kg. Although there was not a significant difference between the efficiency with respect to routes, there was a significant difference between the survival rate in terms of different doses, with the best one achieved at 32 mg/kg. They revealed that all tried doses led to improvement in biochemical analysis; however, recovery in terms of histology was achieved at both 16 and 32 mg/kg doses, indicating the poor effect of 8 mg/kg application. To demonstrate the effects of hUC-MSC-Ex on antioxidant and apoptotic processes, they performed 2'-7'-dichlorofluorescein diacetate (DCF-DA), 8-hydroxy-2'-deoxyguanosine (8-OHdgG), malondialdehyde, and terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) assays. The results showed that oxidative stress and proinflammatory cytokines were decreased by hUC-MSC-Ex therapy. In addition, TUNEL assay results revealed a decreased apoptotic rate of hepatocytes in hUC-MSC-Ex-administered animals. Because of further *in vitro* studies of the research in question, they showed that hUC-MSC-Ex reduced apoptosis by increasing Bcl2 expression and decreasing cleaved Caspase3 expression, as well as Annexin V and Hoechst 33342 staining. To determine the molecular basis of the effects of hUC-MSC-Ex, they investigated the glutathione peroxidase 1 (GPX1) activity in CCl<sub>4</sub>-injured L02 cells treated with hUC-MSC-Ex. They found that the activity levels of GPX1 in injured cells treated with hUC-MSC-Ex increased compared with injured cells treated with human lung fibroblast (HFL-1)-derived exosomes (HFL-Ex). Knockdown of GPX1 resulted in the reversal of all the *in vitro* and *in vivo* therapeutic effects of hUC-MSC-Ex, proving that the therapeutic mechanisms were under the control of the GPX1 enzyme (17).

In one of their previous studies in which they induced liver injury by toxins (LPS and  $\alpha$ -amatoxin), Guo et al. (48) suggested that MSCs reduce injury by suppressing cytokine storm through IL-6 reduction. Based on this conclusion, Shao et al. (18) hypothesized that exosomes derived from IL-6-preconditioned hUC-MSCs may attenuate liver injury. Exposure to IL-6 resulted in both an increase in concentration and a change in the composition of exosomes. According to sequencing results, Mir-455-3p, which is related to IL-6 signaling pathways, was upregulated within the exosomes after IL-6 preconditioning. TargetScan and miRanda analyses revealed that a possible target of Mir-455-3p was the PIK3r1 gene. Transfection of macrophages with Mir-455-3p decreased PIK3r1 expression; therefore, they suggested that Mir-455-3p interrupted macrophage activation in a PIK3r1-dependent manner. When they injected Mir-455-3p into animals with either endotoxin or chemically induced liver injury, they found that Mir-455-3p delivery decreased the levels of inflammatory cytokines and improved liver function in both models (18).

De Witte et al. (19) proposed that preconditioning of hUC-MSCs with cytokines, growth factors, and cell culture conditions enhanced their

immunosuppressive potential and decreased their immunogenicity. For this purpose, hUC-MSCs were treated with inflammatory factors [interferon gamma (IFN- $\gamma$ ), IFN- $\beta$ , TGF- $\beta$ , TNF- $\alpha$ ], vitamins (vitamin D3, vitamin B6, retinoic acid), and serum starvation. The *in vitro* results of experiments revealed that pretreatment of hUC-MSCs with IFN- $\gamma$  and multiple cytokine cocktail (MC) led to hUC-MSCs decreasing T-cell proliferation. In addition, IFN- $\gamma$ , MC, and TGF- $\beta$  treatment inhibited natural killer cell lysis. When pretreated hUC-MSCs were intravenously administered to mice with CCl<sub>4</sub>-induced liver injury, no differences were observed between the experimental groups with respect to the immunomodulatory effects of hUC-MSCs or ALT levels. They suggested that the reason for failure was the distribution of hUC-MSCs because most of the hUC-MSCs were entrapped in the lung. Therefore, in the following step, the effect of pretreated hUC-MSCs on inflammation was analyzed in an *ex vivo* liver tissue model established by LPS exposure. *Ex vivo* experiment results showed that MC pretreatment decreased the expression of inflammatory genes, including monocyte chemoattractant protein-1, TNF- $\alpha$  and interferon gamma-induced protein 10. Based on these findings, they suggested that intrahepatic delivery of hUC-MSCs pretreated with multiple cytokine combinations would be advantageous in ameliorating liver injury (19).

### 2.5. Bone Marrow-Derived MSCs in Drug- and Chemical-Induced Hepatic Injury Models

Miryounesi et al. (20) compared the therapeutic effects of single-dose and repeated administration of human bone marrow-derived MSCs (hBM-MSCs). The same total number of cells ( $3 \times 10^6$  cells) was transplanted to mice with CCl<sub>4</sub>-induced liver injury, either as a single dose or as dividing in three equal doses at intervals of one week. Repeated delivery of the cells yielded more effective engraftment of the cells. Animals with repeated administration displayed significantly less liver fibrosis than those with single dose administration. When transdifferentiation of the implanted cells was investigated with the demonstration of human-specific ALB expression, no positivity was observed. Therefore, recovery in the injured liver is attributed to the paracrine effects of engrafted cells rather than their direct transdifferentiation into liver cells (20).

In another study, Aithal et al. (21) combined hBM-MSC transplantation with silymarin, which is known to display hepatoprotective effects, and compared the efficacy with their individual effects on liver injury induced by CCl<sub>4</sub>. Better results were achieved with either single high-dose hBM-MSCs or combined therapy in terms of plasma HGF levels. High-dose hBM-MSC application showed a significant antimutagenic effect, which was further amplified with the addition of silymarin, as revealed by chromosomal aberration assay. The pathways related to the antimutagenic effects of hBM-MSCs were not still fully understood but might be related to their antioxidant abilities (21).

## CONCLUSION

Over the years, MSCs have been the subject of hundreds of investigations. In preclinical research, MSCs have been investigated to treat different pathological conditions such as cancer, cardiovascular, and neurodegenerative diseases (25,49-51). In the earlier periods of these investigations, basic issues such as the type of MSCs, optimal cell doses and delivery routes, engraftment, and

use of cell-itself or its *in vitro* transdifferentiate derivatives were assessed. Later, researchers focused on the microenvironment that actually predominated the *in vivo* behavioral pattern of the cells. Therefore, many preconditioning methods have been attempted to enhance the *in vivo* transdifferentiation of transplanted cells. Among the research on drug- and chemical-induced liver injury, many preconditioning methods and modalities for targeted delivery or graft construction have been investigated to overcome inadequate engraftment and enhance the *in vivo* transdifferentiation of transplanted cells. Further studies may be conducted to compare the efficiency of these methods in combination with different MSC types.

Many studies have revealed that MSCs reduce fibrosis, regulate inflammation, and decrease the apoptosis of host cells, rather than contributing to the healing process by direct transdifferentiation. Thus, the paracrine effects of MSCs in addition to their differentiation potency became the center of research interest. In this regard, cell products such as exosomes or secreted harvested from MSCs were investigated in terms of their effects on injury. There are also studies demonstrating the paracrine effects of MSCs on drug-induced liver injury models (52,53). Among the MSC types, hCP-MSCs were directly applied to animals without any modifications. Different studies have emphasized the anti-inflammatory and antioxidant effects of hCP-MSCs; however, little is known about the secretory or exosome derived from these cells to show the paracrine effects. In contrast to hCP-MSCs, hUC-MSCs and their exosomes and preconditioned hUC-MSCs with cytokines or vitamins were analyzed in a study. The results revealed that these modifications of hUC-MSCs increased potential therapeutic effects compared with cell-only administration. In addition, umbilical cord-derived MSCs have a higher proliferation rate and a greater diversity of paracrine secretions than other MSCs of fetal origin (54). This may explain why researchers are interested in hUC-MSCs.

There are limited number of experimental studies using hBM-MSCs. This approach may depend on many factors such as the invasiveness of MSC isolation and rejection response in allogeneic transplantation. Thus, most experimental studies utilize animal BM-MSCs. Because we focused on the use of only human-derived MSCs, critical inference was made throughout the limited studies reviewed in this paper.

Animal models play a crucial role in pre-clinical studies. Prior to clinical application, it is essential to have information about the physiopathological mechanisms underlying liver injury. We observed that mostly CCl<sub>4</sub>, TAA, APAP, and AMI were used to mimic drug- and chemical-induced liver injury. However, different drugs may induce liver injury through different molecular mechanisms, such as inducing immune system activation (55). Considering this, there are still few studies exploring the effects of MSCs in damage induced by different drugs such as chemotherapeutics. Because adverse effects of chemotherapy are crucial in clinical practice, studies on the effects of MSCs in chemotherapy-induced liver injury may reveal whether MSCs, which are known for their immunomodulatory effects, can be used as a supportive treatment to the chemotherapeutic agent (25). Therefore, the effects of MSC cells on chemotherapeutic-induced liver injury must be investigated *in vivo*, as well.

In conclusion, to increase the efficacy of stem cell-based therapies and to find new modalities alternative to liver transplantation in clinical practice, treatment strategies based on detailed analyses of products derived from MSCs are required.

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### Ethics

### Authorship Contributions

Concept: M.Ş.C., S.Ö., Z.Y., Design: M.Ş.C., S.Ö., Z.Y., Analysis or Interpretation: M.Ş.C., S.Ö., Z.Y., Literature Search: M.Ş.C., Writing: M.Ş.C., S.Ö., Z.Y.

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### REFERENCES

- Chalasanı N, Björnsson E. Risk factors for idiosyncratic drug-induced liver injury. *Gastroenterology* 2010; 138: 2246-59.
- Fernandez-Checa JC, Bagnaninchi P, Ye H, Sancho-Bru P, Falcon-Perez JM, Royo F, et al. Advanced preclinical models for evaluation of drug-induced liver injury - consensus statement by the European Drug-Induced Liver Injury Network [PRO-EURO-DILI-NET]. *J Hepatol* 2021; 75: 935-59.
- Goodman ZD. Phenotypes and Pathology of Drug-Induced Liver Disease. *Clin Liver Dis* 2017; 21: 89-101.
- Perera MT, Mirza DF, Elias E. Liver transplantation: Issues for the next 20 years. *J Gastroenterol Hepatol* 2009; 24(Suppl 3): S124-31.
- Toti L, Manzia TM, Sensi B, Blasi F, Baiocchi L, Lenci I, et al. Towards tolerance in liver transplantation. *Best Pract Res Clin Gastroenterol* 2021; 54-55: 101770.
- Saidi R, Rajeshkumar R, Sharifabrizi A, Zimmerman A, Walter O. Human Adipose-Derived Mesenchymal Stem Cells Promote Liver Regeneration. *J Invest Surg* 2015; 28: 303-8.
- Chae YJ, Jun DW, Lee JS, Saeed WK, Kang HT, Jang K, et al. The Use of Foxa2-Overexpressing Adipose Tissue-Derived Stem Cells in a Scaffold System Attenuates Acute Liver Injury. *Gut Liver* 2019; 13: 450-60.
- Hwang Y, Kim JC, Tae G. Significantly enhanced recovery of acute liver failure by liver targeted delivery of stem cells via heparin functionalization. *Biomaterials* 2019; 209: 67-78.
- Yuan J, Li W, Huang J, Guo X, Li X, Lu X, et al. Transplantation of human adipose stem cell-derived hepatocyte-like cells with restricted localization to liver using acellular amniotic membrane. *Stem Cell Res Ther* 2015; 6: 217.
- Huang YL, De Gregorio C, Silva V, Elorza ÁA, Léniz P, Aliaga-Tobar V, et al. Administration of Secretome Derived from Human Mesenchymal Stem Cells Induces Hepatoprotective Effects in Models of Idiosyncratic Drug-Induced Liver Injury Caused by Amiodarone or Tamoxifen. *Cells* 2023; 12: 636.
- Lee MJ, Jung J, Na KH, Moon JS, Lee HJ, Kim JH, et al. Anti-fibrotic effect of chorionic plate-derived mesenchymal stem cells isolated



- from human placenta in a rat model of CCl<sub>4</sub>-injured liver: potential application to the treatment of hepatic diseases. *J Cell Biochem* 2010; 111: 1453-63.
12. Na J, Song J, Kim HH, Seok J, Kim JY, Jun JH, et al. Human placenta-derived mesenchymal stem cells trigger repair system in TAA-injured rat model via antioxidant effect. *Aging* 2020; 13: 61-76.
  13. Jung J, Choi JH, Lee Y, Park JW, Oh IH, Hwang SG, et al. Human placenta-derived mesenchymal stem cells promote hepatic regeneration in CCl<sub>4</sub>-injured rat liver model via increased autophagic mechanism. *Stem Cells* 2013; 31: 1584-96.
  14. Zagoura DS, Roubelakis MG, Bitsika V, Trohatou O, Pappa KI, Kapelouzou A, et al. Therapeutic potential of a distinct population of human amniotic fluid mesenchymal stem cells and their secreted molecules in mice with acute hepatic failure. *Gut* 2012; 61: 894-906.
  15. Lee PH, Tu CT, Hsiao CC, Tsai MS, Ho CM, Cheng NC, et al. Antifibrotic Activity of Human Placental Amnion Membrane-Derived CD34+ Mesenchymal Stem/Progenitor Cell Transplantation in Mice With Thioacetamide-Induced Liver Injury. *Stem Cells Transl Med* 2016; 5: 1473-84.
  16. Yan Y, Xu W, Qian H, Si Y, Zhu W, Cao H, et al. Mesenchymal stem cells from human umbilical cords ameliorate mouse hepatic injury in vivo. *Liver Int* 2009; 29: 356-65.
  17. Yan Y, Jiang W, Tan Y, Zou S, Zhang H, Mao F, et al. hucMSC Exosome-Derived GPX1 Is Required for the Recovery of Hepatic Oxidant Injury. *Mol Ther* 2017; 25: 465-79.
  18. Shao M, Xu Q, Wu Z, Chen Y, Shu Y, Cao X, et al. Exosomes derived from human umbilical cord mesenchymal stem cells ameliorate IL-6-induced acute liver injury through miR-455-3p. *Stem Cell Res Ther* 2020; 11: 37.
  19. de Witte SFH, Merino AM, Franquesa M, Strini T, van Zoggel JAA, Korevaar SS, et al. Cytokine treatment optimises the immunotherapeutic effects of umbilical cord-derived MSC for treatment of inflammatory liver disease. *Stem Cell Res Ther* 2017; 8: 140.
  20. Miryounesi M, Piryaei A, Pournasr B, Aghdami N, Baharvand H. Repeated versus single transplantation of mesenchymal stem cells in carbon tetrachloride-induced liver injury in mice. *Cell Biol Int* 2013; 37: 340-7.
  21. Aithal AP, Bairy LK, Seetharam RN, Rao MK. Human bone marrow-derived mesenchymal stromal cells in combination with silymarin regulate hepatocyte growth factor expression and genotoxicity in carbon tetrachloride induced hepatotoxicity in Wistar rats. *J Cell Biochem* 2019; 120: 13026-36.
  22. McGill MR, Jaeschke H. Animal models of drug-induced liver injury. *Biochim Biophys Acta Mol Basis Dis* 2019; 1865: 1031-9.
  23. Hoofnagle JH, Björnsson ES. Drug-Induced Liver Injury - Types and Phenotypes. *N Engl J Med* 2019; 381: 264-73.
  24. Hefler J, Marfil-Garza BA, Pawlick RL, Freed DH, Karvellas CJ, Bigam DL, et al. Preclinical models of acute liver failure: a comprehensive review. *PeerJ* 2021; 9: e12579.
  25. Bolontrade M, García M. Mesenchymal Stromal Cells as Tumor Stromal Modulators. Elsevier Science, 2016.
  26. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; 8: 315-7.
  27. Meirelles Lda S, Nardi NB. Murine marrow-derived mesenchymal stem cell: isolation, in vitro expansion, and characterization. *Br J Haematol* 2003; 123: 702-11.
  28. Yoo JU, Johnstone B. The role of osteochondral progenitor cells in fracture repair. *Clin Orthop Relat Res* 1998: S73-81.
  29. Schwartz RE, Reyes M, Koodie L, Jiang Y, Blackstad M, Lund T, et al. Multipotent adult progenitor cells from bone marrow differentiate into functional hepatocyte-like cells. *J Clin Invest* 2002; 109: 1291-302.
  30. Seo MJ, Suh SY, Bae YC, Jung JS. Differentiation of human adipose stromal cells into hepatic lineage in vitro and in vivo. *Biochem Biophys Res Commun* 2005; 328: 258-64.
  31. Han Y, Yang J, Fang J, Zhou Y, Candi E, Wang J, et al. The secretion profile of mesenchymal stem cells and potential applications in treating human diseases. *Signal Transduct Target Ther* 2022; 7: 92.
  32. Adas G, Koc B, Adas M, Duruksu G, Subasi C, Kemik O, et al. Effects of mesenchymal stem cells and VEGF on liver regeneration following major resection. *Langenbecks Arch Surg* 2016; 401: 725-40.
  33. Hu C, Li L. Improvement of mesenchymal stromal cells and their derivatives for treating acute liver failure. *J Mol Med (Berl)* 2019; 97: 1065-84.
  34. Kinnaird T, Stabile E, Burnett MS, Lee CW, Barr S, Fuchs S, et al. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. *Circ Res* 2004; 94: 678-85.
  35. Zhang L, Ma XJ, Fei YY, Han HT, Xu J, Cheng L, et al. Stem cell therapy in liver regeneration: Focus on mesenchymal stem cells and induced pluripotent stem cells. *Pharmacol Ther* 2022; 232: 108004.
  36. Can A. Kök Hücre Biyolojisi, Türleri ve Tedavide Kullanımları. Akademisyen Yayınevi, 2018.
  37. Li M, Lv Y, Chen F, Wang X, Zhu J, Li H, et al. Co-stimulation of LPAR1 and S1PR1/3 increases the transplantation efficacy of human mesenchymal stem cells in drug-induced and alcoholic liver diseases. *Stem Cell Res Ther* 2018; 9: 161.
  38. Vasanthan J, Gurusamy N, Rajasingh S, Sigamani V, Kirankumar S, Thomas EL, et al. Role of Human Mesenchymal Stem Cells in Regenerative Therapy. *Cells* 2020; 10: 54.
  39. Ezquer F, Huang YL, Ezquer M. New Perspectives to Improve Mesenchymal Stem Cell Therapies for Drug-Induced Liver Injury. *Int J Mol Sci* 2022; 23: 2669.
  40. Gad AM, Hassan WA, Fikry EM. Significant curative functions of the mesenchymal stem cells on methotrexate-induced kidney and liver injuries in rats. *J Biochem Mol Toxicol* 2017; 31.
  41. Yin L, Zhu Y, Yang J, Ni Y, Zhou Z, Chen Y, et al. Adipose tissue-derived mesenchymal stem cells differentiated into hepatocyte-like cells in vivo and in vitro. *Mol Med Rep* 2015; 11: 1722-32.
  42. Hong IH, Han SY, Ki MR, Moon YM, Park JK, You SY, et al. Inhibition of kupffer cell activity improves transplantation of human adipose-derived stem cells and liver functions. *Cell Transplant* 2013; 22: 447-59.
  43. Saleh M, Taher M, Sohrabpour AA, Vaezi AA, Nasiri Toosi M, Kaviani M, et al. Perspective of placenta derived mesenchymal stem cells in acute liver failure. *Cell Biosci* 2020; 10: 71.
  44. De Coppi P, Bartsch Jr G, Siddiqui MM, Xu T, Santos CC, Perin L, et al. Isolation of amniotic stem cell lines with potential for therapy. *Nat Biotechnol* 2007; 25: 100-6.
  45. In 't Anker PS, Scherjon SA, Kleijburg-van der Keur C, de Groot-Swings GM, Claas FH, Fibbe WE, et al. Isolation of mesenchymal stem cells of fetal or maternal origin from human placenta. *Stem Cells* 2004; 22: 1338-45.
  46. Can A, Yigman Z. Mesenchymal Stem/Stromal Cells From Neonatal Tissues. Bolontrade MF, Garcia MG, (editors). Mesenchymal Stromal Cells as Tumor Stromal Modulators, 2017.
  47. Xue G, Han X, Ma X, Wu H, Qin Y, Liu J, et al. Effect of Microenvironment on Differentiation of Human Umbilical Cord Mesenchymal Stem

- Cells into Hepatocytes In Vitro and In Vivo. *Biomed Res Int* 2016; 2016: 8916534.
48. Guo G, Zhuang X, Xu Q, Wu Z, Zhu Y, Zhou Y, et al. Peripheral infusion of human umbilical cord mesenchymal stem cells rescues acute liver failure lethality in monkeys. *Stem Cell Res Ther* 2019; 10: 84.
49. Yigman Z, Ozdemir ED, Turan NN, Ulus AT, Can A. Umbilical cord mesenchymal stromal cells engraft and transdifferentiate into cardiomyocyte-like cells following acute myocardial ischemia\*. *Acta Histochem* 2020; 122: 151578.
50. Chen HX, Liang FC, Gu P, Xu BL, Xu HJ, Wang WT, et al. Exosomes derived from mesenchymal stem cells repair a Parkinson's disease model by inducing autophagy. *Cell Death Dis* 2020; 11: 288.
51. Dezawa M, Hoshino M, Ide C. Treatment of neurodegenerative diseases using adult bone marrow stromal cell-derived neurons. *Expert Opin Biol Ther* 2005; 5: 427-35.
52. Ohara M, Ohnishi S, Hosono H, Yamamoto K, Yuyama K, Nakamura H, et al. Extracellular Vesicles from Amnion-Derived Mesenchymal Stem Cells Ameliorate Hepatic Inflammation and Fibrosis in Rats. *Stem Cells Int* 2018; 2018: 3212643.
53. Jiang W, Tan Y, Cai M, Zhao T, Mao F, Zhang X, et al. Human Umbilical Cord MSC-Derived Exosomes Suppress the Development of CCl4-Induced Liver Injury through Antioxidant Effect. *Stem Cells Int* 2018; 2018: 6079642.
54. Wu M, Zhang R, Zou Q, Chen Y, Zhou M, Li X, et al. Comparison of the Biological Characteristics of Mesenchymal Stem Cells Derived from the Human Placenta and Umbilical Cord. *Scientific Reports* 2018; 8: 5014.
55. Yuan L, Kaplowitz N. Mechanisms of drug-induced liver injury. *Clin Liver Dis* 2013; 17: 507-18.



## Rare Old Distal Carpal Row Dislocation: Wait, do not Reduce, or Operate

### Nadir Eski Distal Karpal Sıra Çıkığı: Bekleyin, Azaltmayın veya Çalıştırmayın

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#### ABSTRACT

Carpal dislocations most frequently result from high-energy axial loading of the forearm with the wrist extended and can easily be overlooked. In this case, we conclude that stable, chronic dislocations with patient adaptation did not require any reduction or operation.

**Keywords:** Distal carpal row dislocation, carpal bones

Post-traumatic carpal dislocations are rare entities that most frequently result from high-energy axial loading of the forearm with the wrist extended and can easily be overlooked (1,2). Several variants of carpal dislocations exist. The most commonly observed cases involve lunate wrist trauma (3). If clinicians do not employ a high index of suspicion, they readily miss the diagnosis of carpal dislocations in the emergency department. These injuries can result in persistent pain and stiffness if left untreated. X-rays are the imaging modality of choice in early post-traumatic situations, where carpal dislocations frequently appear with only mild anomalies. If there is a difference between clinical and radiological findings, a computed tomography (CT) scan should be used as a problem-solving tool. The eight carpal bones form a complex structure (two horizontal rows) that enables the wrist to move in three dimensions. To maintain wrist stability, the proximal row is an intercalated section between the radius and the distal carpal row (trapezium, trapezoid, capitate, and hamate) (4). Treatment in the acute setting should always include closure reduction and immobilization to relieve pressure on nearby structures. K-wire may be used in conjunction with immobilization to provide the wrist with more stability after reduction. This was the previously recommended treatment. However, new research has

#### ÖZ

Karpal çıkıklar çoğunlukla el bileği ekstansiyondayken önkolun yüksek enerjili aksiyal yüklenmesinden kaynaklanır ve kolayca gözden kaçabilir. Bu durumda stabil, kronik, hasta adaptasyonu ile ortaya çıkan çıkıkların herhangi bir redüksiyon veya operasyon gerektirmediği sonucuna vardık.

**Anahtar Sözcükler:** Distal karpal sıra çıkığı, karpal kemikler

revealed a high incidence of recurrent instability and arthritis. Open reduction is now the accepted standard approach because it has produced superior results for most injuries compared with closed reduction (2).

We report a case of complex carpal injury in a 46-year-old policeman involving dislocation of the entire distal carpal row. He presented to the emergency department with a complaint of tolerable right wrist pain. He allegedly fell on his extended right hand while trying to catch a thief, at which point he started feeling mildly tolerable pain at the wrist joint. What brought him to the emergency department was not pain, but he recalls that the exact mechanism of trauma happened ten years ago while he tried to catch a thief. At that time, he complained of mild pain but never sought medical advice. After a recent trauma, he decided to get a doctor's opinion regarding both traumas. On examination, the right hand showed a bit of extension limitation with mild bulging during hand extension and a pain score of 3/10. A right-hand X-ray was requested and revealed overlapped carpal bones, as shown in Figure 1. A CT scan was requested to obtain more details regarding the carpal bones. Figure 2 shows a CT scan that confirmed total distal carpal row dislocation. Both carpal bone

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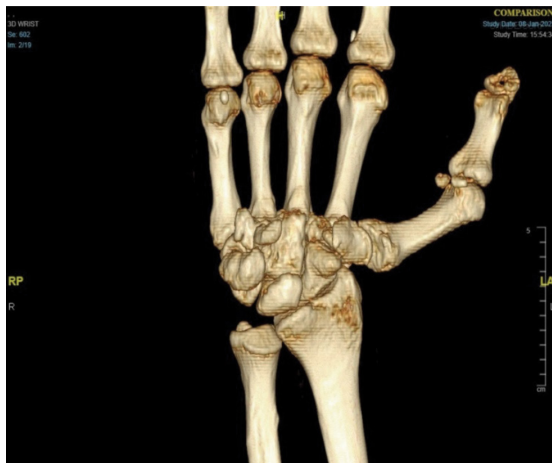


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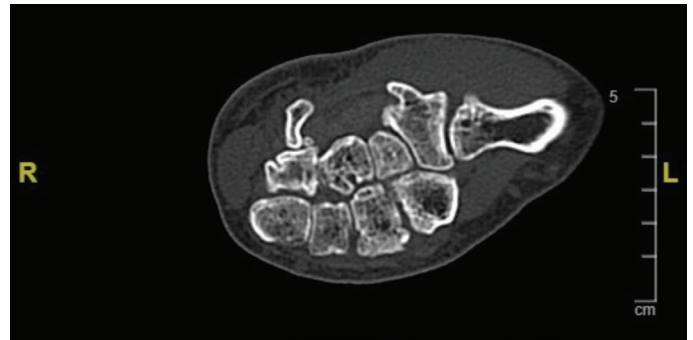


**Figure 1.** Right dorsal view X-ray showing carpal bones with abnormal anatomical alignment.



**Figure 2.** Right hand 3D volar view CT showing overlapped proximal and distal carpal bones.

CT: Computed tomography.



**Figure 3.** Cross-sectional CT at the level of the right carpal bones clearly showing overlapping.

CT: Computed tomography.

rows were shown to be on top of each other in cross-sectional CT at the carpal bone level, representing the distal row, which overlapped the proximal row, as shown in Figure 3.

In this case, we can emphasize that stable, chronic dislocations with patient adaptation did not require any reduction or operation and were not considered emergencies that required urgent reduction or manipulation. In conclusion, even if the patient presented to the emergency department with a trauma of the exact mechanism without new findings, we may choose to wait rather than reduce or operate.

### **Ethics**

**Informed Consent:** It was obtained.

### **Authorship Contributions**

Surgical and Medical Practices: M.M.M.A.C., A.N.S., W.A.W.S., Concept: M.M.M.A.C., A.N.S., W.A.W.S., Design: M.M.M.A.C., A.N.S., W.A.W.S., Data Collection or Processing: M.M.M.A.C., A.N.S., W.A.W.S., Analysis or Interpretation: M.M.M.A.C., A.N.S., W.A.W.S., Literature Search: M.M.M.A.C., A.N.S., W.A.W.S., Writing: M.M.M.A.C., A.N.S., W.A.W.S.

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### **REFERENCES**

1. Lefere M, Dallaudière B, Omoumi P, Cyteval C, Larbi A. Rare carpometacarpal dislocations. *Orthop Traumatol Surg Res* 2016; 102: 813-6.
2. Steinmetz G, Corning E, Hulse T, Fitzgerald C, Holy F, Boydston S, et al. Carpometacarpal Fracture-Dislocations: A Retrospective Review of Injury Characteristics and Radiographic Outcomes. *Hand (N Y)* 2021; 16: 362-7.
3. Clarke D, Franklin S, Mullings S, Jones KJWIMJ. Chronic Lunate Dislocation: An Unusual Cause of Carpal Tunnel Syndrome: Avoiding Diagnostic Pitfalls and Synopsis of Management Options 2021; 69: 452-6.
4. Frane N, Goldenberg W. Perilunate Dislocation: StatPearls Publishing. Treasure Island (FL), 2022.