KAFKAS TIP BİLİMLERİ DERGİSİ

Kafkas Journal of Medical Sciences

ISSN: 2146-2631
eISSN: 2587-053X

Cilt / Volume **14**
Sayı / Issue **3**
Aralık / Dec **2024**

ISSN: 2146-2631
eISSN: 2587-053X

KAFKAS
TIP BİLİMLERİ DERGİSİ

Kafkas Journal of Medical Sciences

Kafkas J Med Sci

Bu dergi Kafkas Üniversitesi Tıp Fakültesi'nin akademik yayın organıdır.
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Endekslenme *(Indexed in)*

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Yayın Hizmetleri *(Publication Service)*

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Ziya Gökalp Cad. 30/31, Kızılay - Ankara
Tel: (312) 431 30 62
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Baskı *(Printing)*
Miki Matbaacılık Ltd. Şti.
Matbaacılar Sitesi, 560 Sk. No:27, İvedik - Ankara
Tel: (312) 395 21 28

Baskı Tarihi *(Printing Date)*: **30 Aralık 2024**

Danışma Kurulu *(Advisory Board)*

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Rapid Antigen Test in the Diagnosis of COVID-19

COVID-19 Tanısında Hızlı Antijen Testi

Feray Ferda Senol[1], Altay Babacan[2], Mete Ozcan[3], Esra Timurkaan[4], Yuksel Akaya[5], Ozlem Aytac[1], Pinar Oner[1], Zulal Asci Toraman[6]

[1]Elazig Fethi Sekin City Hospital Microbiology, Elazig; 2Ufuk University Faculty of Medicine, Department of Pediatric Hematology Oncology, Ankara; [3]Firat University Faculty of Medicine, Department of Biophysics; [4]Elazig Fethi Sekin Hospital Internal Medicine, Elazig; [5]Health Sciences University Hamidiye Faculty of Medicine, Department of Microbiology, Istanbul; [6]Firat University Faculty of Medicine, Department of Microbiology, Elazig, Türkiye

ABSTRACT

Aim: *The COVID-19 pandemic has shown the importance of laboratory-based diagnosis in the control of infectious diseases. Early and accurate diagnosis of COVID-19 is crucial for limiting the spread of infection and determining the treatment to be applied to patients. Our study compared the results of real-time polymerase chain reaction (RT-PCR) and rapid antigen tests to diagnose COVID-19.*

Material and Method: *Nasal and throat swab samples sent from 500 patients to our laboratory were studied by RT-PCR method with Multiplex RT-qPCR Diagnostic Kit 1000 rxn (CORONEX, Türkiye) and by Abbot COVID-19 Ag Rapid Test Device (Germany) immunochromotographic method by the recommendations of the manufacturer companies. The patient's Demographic information in the study was taken from the hospital information automation system.*

Results: *Of the 500 patients participating in the study, 202 (40.4%) were women, and 298 (59.6%) were men. While 57 patients (11.4%) were detected positively by the RT-PCR method, 54(10.4%) were detected positively by a rapid antigen test method. Of the 57 patients found positive by the RT-PCR method, 8 (14%) were negative by a rapid antigen test method. Of the 54 patients found positive by the rapid antigen test method, 5 (9.25%) were negative by the RT-PCR method. According to the RT-PCR method, the sensitivity of the rapid antigen test test was 90.74%, and the specificity was 98.21%.*

Conclusion: *Although the RT-PCR test is the gold standard method for COVID-19 detection, rapid antigen tests may be useful as a screening test or as a supportive test in diagnosis during periods of intense virus activity or epidemic situations.*

Key words: *COVID-19 diagnosis; RT-PCR; rapid antigen test*

ÖZET

Amaç: *COVID-19 pandemisi, bulaşıcı hastalıkların kontrolünde laboratuvara dayalı tanının ne kadar önemli olduğunu göstermiştir. COVID-19'un erken ve doğru tanısı hastalara uygulanacak tedavinin yanı sıra enfeksiyonun yayılımını sınırlanması için de çok büyük önem arz eder. Çalışmamızda, COVID-19 tanısı için Gerçek zamanlı polimeraz zincir reaksiyonu (RT-PCR) ve Hızlı antijen test sonuçlarının karşılaştırılması amaçlandı.*

Materyal ve Metot: *Laboratuvarımıza, 500 hastadan gönderilen nazal ve boğaz sürüntü örnekleri Multipleks RT-qPCR Tanı Kiti 1000 rxn (CORONEX, Türkiye) ile RT-PCR yöntemiyle ve Abbot COVID-19 Ag Rapid Test Device (Germany) immünokromotografik yöntemiyle üretici firmaların önerileri doğrultusunda çalışıldı. Çalışmaya katılan hastalara ait demografik bilgiler hastane bilgi otomasyon sisteminden alındı.*

Bulgular: *Çalışmaya katılan 500 hastanın 202'si (%40,4) kadın 298'i (%59,6) erkekti. Hastaların 57'si (%11,4) RT-PCR yöntemiyle pozitif tespit edilirken, Hızlı antijen test yöntemiyle hastaların 54'ü (%10,4) pozitif olarak tespit edildi. RT-PCR yöntemiyle pozitif tespit edilen 57 hastanın 8'i (%14) Hızlı antijen test yöntemiyle negatif olarak tespit edildi. Hızlı antijen test yöntemiyle pozitif tespit edilen 54 hastanın 5'i (%9,25) RT-PCR yöntemiyle negatif olarak tespit edildi. RT-PCR yöntemine göre Hızlı antijen test testinin duyarlılığı (sensitivity) %90,74, özgüllüğü (specificity) %98,21 olarak tespit edildi.*

Sonuç: *COVID-19 tespitinde RT-PCR testi altın standart yöntem olmakla birlikte Hızlı antijen testleri, virüs aktivitesinin yoğun olduğu dönem veya salgın durumlarında tanıda tarama testi olarak ya da destekleyici test olarak kullanımının faydalı olabileceği sonucuna varıldı.*

Anahtar kelimeler: *COVID-19 tanı; RT-PCR; hızlı antijen test*

İletişim/Contact: *Feray Ferda Senol, Elazig Fethi Sekin City Hospital Microbiology, Elazig, Türkiye* • ***Tel:*** *0505 776 84 76* •
E-mail: *drferdasenol@yahoo.com* • ***Geliş/Received:*** *06.11.2023* • ***Kabul/Accepted:*** *15.08.2024*

ORCID: *Feray Ferda Şenol: 0000-0003-4705-5757* • *Altay Babacan: 0000-0002-1508-1056* • *Mete Özcan: 0000-0002-5551-4880* • *Esra Timurkaan: 0000-0001-5654-7070* • *Yüksel Akkaya: 0000-0002-3167-8055* • *Özlem Aytaç: 0000-0002-3305-6284* • *Pınar Öner: 0000-0001-9592-5986* • *Zülal Aşçı Toraman: 0000-0001-5202-8564*

Introduction

On Sunday, December 31, 2019, the World Health Organization (WHO) reported that deaths due to acute respiratory failure and pneumonia, the cause of which could not be determined, were observed in the fish and live animal market in Wuhan, Hubei province of China, and that a new virus[1] may have caused this situation. On 30 January 2020, WHO declared COVID-19 an "International public health emergency." With COVID-19 patients being seen intensively in 113 countries, COVID-19 was declared a pandemic on March 11, 2020.

SARS-CoV-2 is an enveloped virus containing a single-stranded positive-stranded RNA genome within the family Coronaviridae. As a result of genome studies, they have reported that it is a member of the betacoronavirus family, including MERS-CoV and SARS-COV, which have caused outbreaks in different countries in the past two decades. As a genomic structure, SARS-CoV-2 was 79% similar to SARS-CoV and 50% similar to MERS-CoV[2]. There are four structural proteins belonging to the virus, M (membrane protein), E (envelope protein), S (spike protein), N (nucleocapsid protein). Among these proteins, the S protein plays a very important role, especially in the attachment of the virus to the host and its entry into the cell[3].

Transmission routes for SARS-CoV-2, as in other coronaviruses, are indicated to develop after inhalation of virus-containing particles and direct or indirect contact with the oral mucosa, conjunctiva, and nasal tract. The primary target receptor points for the host are Angiotensin-converting enzyme 2 (ACE2) receptors located in the oropharynx and upper respiratory tract, which are human respiratory epithelial cells. It may also be involved in SARS-CoV-2 transmission in our gastrointestinal tract[4,5].

Accurate and timely diagnosis of COVID-19 is necessary for new patients to be identified and for the pandemic process to be followed[6]. Microbiologically, three basic methods that are routinely used for the diagnosis of COVID-19 are at the forefront. Of these methods, molecular tests are based on replicating the nucleic acid belonging to the virus and are very important in diagnosing acute infections. Secondly, antigen tests help diagnose early infection periods when it is impossible to perform molecular tests and virus antigens are present in high quantities[7]. Thirdly, antibody tests are methods that can contribute to the diagnosis of encountering the infection at a later stage of the infection or determining whether an immune response has developed[8].

The RT-PCR method is the most preferred test for diagnosing COVID-19 and is considered the gold standard. The RNA extraction process can be performed from oropharyngeal and nasopharyngeal upper respiratory tract swab samples and lower respiratory tract samples such as bronchoalveolar lavage and sputum. It has been stated that the results of the samples taken from the nasopharynx are two times better than those from the oropharynx. The SARS-CoV-2 RNA has been isolated in blood, urine and fecal samples[9]. However, it was found that the reliability of these samples was less than that of respiratory tract samples. In collecting upper respiratory tract samples, taking them within a few days from the onset of complaints is recommended. The RT-PCR test is known not to detect other viruses by cross-reaction other than SARS-CoV-2, so its originality is quite high. Although a clear rate cannot be given for sensitivity, it is stated that it is between 63–78%[9]. The SARS-CoV-2 rapid antigen test test sensitivity is between 0% and 94%. The sensitivity of the SARS-CoV-2 rapid antigen test tests is Dec. To diagnose SARS-CoV-2, WHO recommends a minimum sensitivity of 80% and specificity of 97% for rapid diagnostic tests that can be used in patients with symptoms compatible with COVID-19[10].

The study aims to compare RT-PCR and rapid antigen tests used in diagnosing COVID-19 and to determine their diagnostic performance.

Materials and Methods

Five hundred samples of COVID-19 suspects, 230 women and 270 men, were included in this study between March 25, 2022, and March 30, 2022. The lowest age of the people was 20, the highest was 53, and the average was 36.2±3.29. The study was done with a nose and throat sample on a single swab. Samples For the RT-PCR test, a Multiplex RT-qPCR Diagnostic Kit [1000 rxn] was used with 15 µl extract and 5 µl patient sample for a total of 20 µl by the manufacturer's recommendations. Human RNaseP (Ribonuclease P) genes were targeted with SARS-CoV-2-specific 'ORFLAB'and' N'genes. For amplification, it has been worked with CFX96 (Biorad, USA) in the test analysis. Ct significance ≤35 values have been accepted as positive. For the rapid antigen test, the COVID-19 Ag Rapid Test (Abbot, Germany) was performed using the immunochromatographic method per the manufacturer' s recommendations. The operations were carried out in the biosafety cabinet. In the case of the color change of the test band, the result was evaluated as positive.

Statistical Analysis

Receiver operating characteristic (ROC) was used to identify the optimal rapid antigen test cut-off level and determine its sensitivity and specificity for COVID-19. In addition, the area under the ROC curve (AUC) was used to assess the diagnostic performance of rapid antigen testing in COVID-19. Finally, the Hosmer-Lemeshow fit test was conducted to determine the agreement between observed and model-predicted proportions of COVID-19. All statistical calculations were performed using the IBM Statistical Package for Social Sciences (SPSS) program version 21.0 commercial software (IBM Inc, Chicago, II, USA).

Results

Of the 500 patients in this study, 202 (47.6%) were women, 298 (52.4%) were men, and the average age was 48. Fifty seven patients (11%) tested positive, and 443 (88.6%) tested negative with the RT-PCR method. Fifty four out of 500 patients have tested negative, and 446 out of them have tested positive with the rapid antigen test method. 8 patients who have tested positive with RT-PCR have been negative with the rapid antigen test method. Meanwhile, 5 patients who tested negative with RT-PCR tested positive with rapid antigen test method (Table 1).

Table 1. *The sensitivity and specificity of the RAT method according to the RT-PCR method*

Gender	Number of patients	RT PCR(+)	RAT(+)	Sensitivity	Specificity
Male	298	35	33	%87.88	%97.74
Female	202	22	21	%95.24	%98.90
TOTAL	500	57	54	%90.74	%98.21

The obtained results indicated that the rapid antigen test successfully discriminates COVID-19 patients from healthy controls (AUC=0.994, 95% CI: 0.983 to 0, 999) and exhibited acceptable discriminative ability (sensitivity=0.9074; specificity=0.9778) at the optimal cut-off value of 35.00 ng/ml (p <0.001) as shown in Figure 1. The obtained results indicated that the rapid antigen test successfully discriminated -19 patients from healthy controls (AUC=0.994, 95% CI: 0.983 to 0, 999) and exhibited acceptable discriminative ability (sensitivity=0.9074; specificity=0.9778) at the optimal cut-off value of 35.00 ng/ml (p <0.001) as shown in Figure 1.

The obtained results in male patients indicated that the rapid antigen test successfully discriminated COVID-19 patients from healthy controls (AUC=0.994, 95% CI: 0.983 to 0, 999) and exhibited acceptable discriminative ability (sensitivity=0.9074; specificity=0.9778) at the optimal cut-off value of 35.00 ng/ml (p <0.001) as shown in Figure 2.

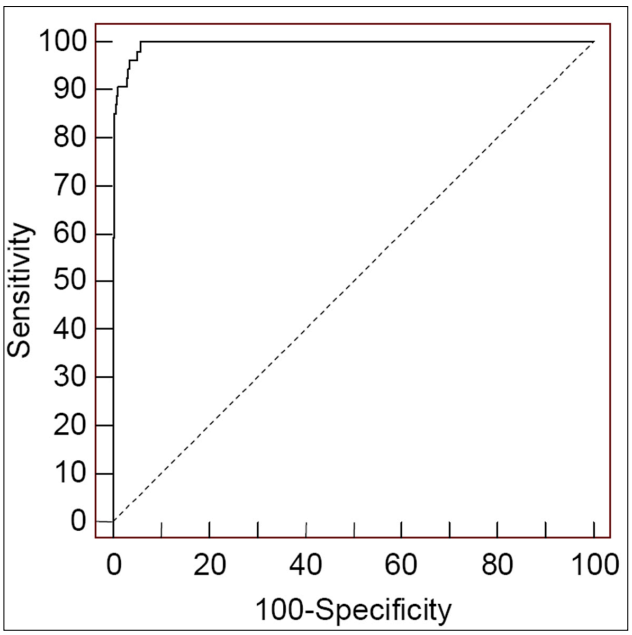

Figure 1. *ROC analysis of RAT tests in male and female patients.*

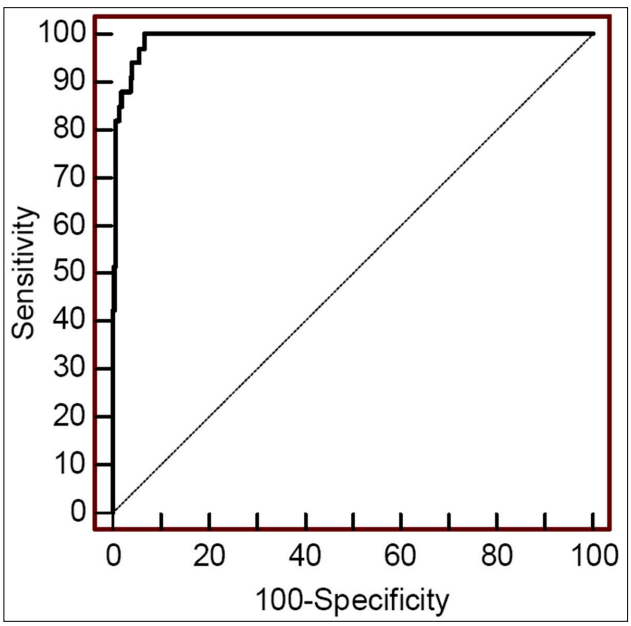

Figure 2. *ROC analysis of RAT tests in male patients.*

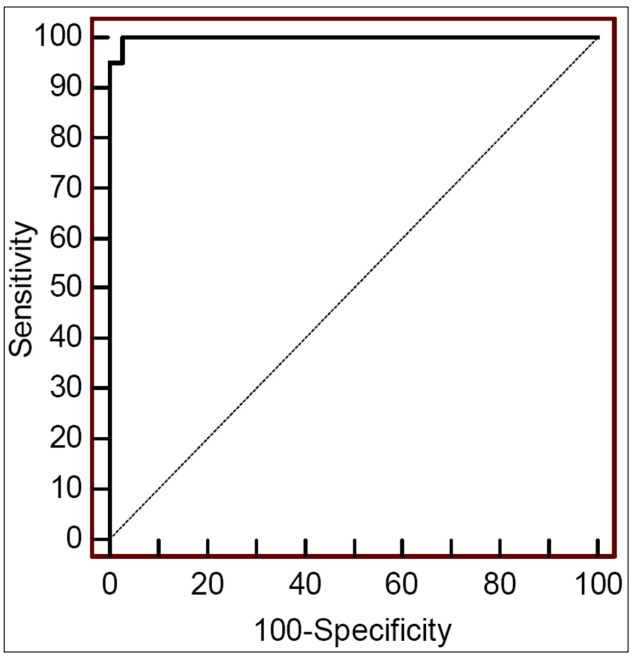

Figure 3. *ROC analysis of the RAT tests in female patients.*

The obtained results in female patients indicated that the rapid antigen test successfully discriminated -19 patients from healthy controls (AUC=0.994, 95% CI: 0.983 to 0, 999) and exhibited acceptable discriminative ability (sensitivity=0.9074; specificity=0.9778) at the optimal cut-off value of 35.00 ng/ml ($p < 0.001$) as shown in Figure 3.

Discussion

Pneumonia caused by COVID-19 needs to be differentiated because it can be confused with viral pneumonias such as influenza, adenovirus, respiratory syncytial virus, and pneumonia caused by mycoplasma. Respiratory tract infections caused by COVID-19 can be seen simultaneously with other viral respiratory tract infections, and no specific clinical difference distinguishes them from each other[11].

RT-PCR test is accepted as the gold standard in the diagnosis of COVID-19. However, regardless of the method used, the sensitivity and specificity of RT-PCR tests are less than 100%. Reasons such as sampling and transfer, virus dynamics in the person, extraction of RNA, and RT-PCR method may cause false negative results. The sensitivity of the RT-PCR method is estimated to be 70% and specificity 95%. However, there is no ideal test for comparison[12,13]. Chaimayo C et al. stated that 60 respiratory samples out of 454 were positive, and 394 were negative with RT-PCR. When the same samples worked with rapid SARS-CoV-2 antigen test, compared to RT-PCR, the sensitivity rate was 98.3%, and the specificity rate was 98.7%[14]. Torres I. et al. found the sensitivity with the CLINITEST rapid antigen test as 80.2%, specificity of 100% in symptomatic patients with suspected COVID-19 and 60.0% specificity, 100% in asymptomatic close contacts of COVID-19 patients[15]. Diao B et al., after detecting 80%.1 of the 251 patients tested positive for -19 with RT-PCR, these patients were studied with Fluorescent Immunochromatography (FIC) also compared to RT-PCR the sensitivity rate was 75.6%, the specific rate was 80.5%[16]. Nasopharyngeal swabs of 50 suspected patients for SARS-CoV-2 have been studied with Cori's coronavirus disease 2019 Ag Respi-Strip, RT-PCR Allplex 2019n-CoV tests and 11 out of these patients tested negative with both of these tests. Meanwhile, 27 of them tested negative with rapid antigen tests. In 39 patient samples that tested positive with RT-PCR, the average Ct rate was 22.78; in 12 patient samples that tested positive for both RT-PCR and rapid antigen test, the average Ct rate was 17.37. Compared to RT-PCR, the rapid antigen test's sensitivity rate was 30.77%, the specificity rate was 100%, and the antigen test had better performance with a high viral load. In contrast, with the lower viral load, it has been detected that it missed positivity[17]. In another study, 75 samples tested positive for SARS-CoV-2 RT-PCR, and 75 samples tested negative for SARS-CoV-2 RT-PCR have been researched for sensitivity and specificity for SARS-CoV-2 rapid antigen test. According to Ct rates of rapid antigen test sensitivity, It has been detected that in <25 is 100%, in 25–30 is 95%, in 30–35 is 44%.8 and in ≥35 is 22%.2; specificity of Ct rates was 96%. Eleven patients who have been detected positive with RT-PCR also detected negative with indicate rapid antigen test[18]. By Chiu RYT et al., these 11 patient's Ct rate was 32.56±4.59, and this rate increase of the Ct caused false negativity[19]. In the study by Jaaskelainen AE. et al.; Sofia (Quidel), Standard Q (SD Biosensor) and Panbio (Abbott) (three rapid tests) specificity rate was 100%, and the sensitivity rate for Pambio rapid antigen test was 87%, for Standard Q was 89% and for Sofia rapid antigen test was 92% compared to those which had a Ct rate below 25.)[20]. In our study, we found the sensitivity of the rapid antigen test kit to be 87.88%, the specificity as 97.74% in male patients, the sensitivity of the rapid antigen test kit as 95.24% and the specificity as 98.90% in female patients, and the sensitivity of the rapid antigen test kit as 90.74% and the specificity as 98.21% in all patients.

There was no significant difference between genders for rapid antigen tests. (p>0, 005)

As a result, it has been determined that the use of rapid antigen tests, which can be applied at the bedside, as an alternative to RT-PCR tests, can be beneficial in the diagnosis of COVID-19 due to reasons such as RT-PCR tests working in laboratory conditions, sample transfer, experienced personnel, high cost, working with the device, and evaluation of the result by the physician. However, as it is a qualitative test, it should be kept in mind that when false negative or false positive results of the test are considered, a negative result cannot exclude the possibility of COVID-19. For these reasons, the patient's clinical symptoms should be evaluated, and the diagnosis should be confirmed by RT-PCR test when necessary.

Statement of Ethics

Approval was obtained from the Firat University Faculty of Medicine/Türkiye Ethics Committee (Decision No: 2022/07–36 Date: 26.05.2022).

Conflict of Interest Statement

The authors declared no conflict of interest related to the article.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Retrospective Analysis of Patients Admitted to Physical Medicine and Rehabilitation Outpatient Clinics with Osteoarticular Findings and Diagnosed with Brucellosis

Fiziksel Tıp ve Rehabilitasyon Polikliniklerine Osteoartiküler Bulgularla Başvurarak Bruselloz Tanısı Alan Hastaların Retrospektif Analizi

Esra Sahingoz Bakirci[1], Gulseren Demir Karakilic[2], Ferda Surel[3], Tugba Alisik[4]

[1]Physical Medicine and Rehabilitation, Yozgat Bozok University Faculty of Medicine; [2]Physical Medicine and Rehabilitation, Yozgat City Hospital, Yozgat; [3]Physical Medicine and Rehabilitation, Ankara Bilkent City Hospital, Ankara; [4]Physical Medicine and Rehabilitation, AIBU Izzet Baysal Physical Therapy and Rehabilitation Training and Research Hospital, Bolu, Türkiye

ABSTRACT

Aim: *In endemic regions, we aimed to consider Brucella infection in the differential diagnosis of musculoskeletal manifestations and to emphasize the diversity of presentation.*

Material and Method: *Our study has a retrospective design. Between 01.01.2020 and 01.01.2024, patients who applied to the physical therapy and rehabilitation department of a second-stage public hospital located in a region where brucella infection is endemic and were diagnosed with brucellosis with osteoarticular findings were included in our study. Demographic data, complaints at presentation, musculoskeletal examination findings, Brucella serology tests, liver function tests, complete blood counts, sedimentation rate (ESR) and C-reactive protein (CRP) levels, and related musculoskeletal imaging data were recorded.*

Results: *47 patients were enrolled in the study. 68.1% (32/47) of the patients were male, while 31.9% (15/47) were female. The average age of the patients was 44.43±15.47 years. Joint pain and low back pain were the predominant symptoms observed in the majority of cases; the most common types of involvement were arthralgia (46.8%), sacroiliitis (19.1%), spondylodiscitis (14.9%) and monoarthritis (14.9%). We found elevated CRP in 48.93%, elevated ESR in 27.65%, leukocytosis in 14.89%, leukopenia in 8.51%, anemia in 31.91%, elevated ALT in 12.76% and elevated AST in 10.63%.*

Conclusion: *Brucellosis may present with various musculoskeletal findings. Considering Brucella infection in the differential diagnosis is crucial for preventing potential complications through prompt diagnosis and treatment in patients presenting with musculoskeletal symptoms, particularly in endemic areas.*

Key words: *arthritis; brucella; sacroiliitis; spondylodiscitis*

ÖZET

Amaç: *Brusella enfeksiyonunun endemik olduğu bölgelerde, osteoartiküler bulgular değerlendirilirken ayırıcı tanıda Brusella enfeksiyonunun da akılda tutulması gerekliliği ve prezentasyon çeşitliliğini vurgulamak amaçlandı.*

Materyal ve Metot: *Çalışmamız retrospektif bir tasarıma sahiptir. 01.01.2020 ile 01.01.2024 tarihleri arasında Brusella enfeksiyonunun endemik olduğu bir bölgede yer alan ikinci basamak bir devlet hastanesinin fizik tedavi ve rehabilitasyon bölümüne osteoartiküler bulgular ile başvurup bruselloz teşhisi alan hastalar çalışmamıza dâhil edildi. Olguların demografik verileri, başvuru şikâyetleri, kas-iskelet sistemi muayene bulguları, Brusella seroloji testleri, tam kan sayımları, sedimantasyon (ESH), C-reaktif protein (CRP) değerleri, karaciğer fonksiyon testleri ve ilgili kas-iskelet sistemi görüntüleme verileri kaydedildi.*

Bulgular: *Çalışmaya 47 bruselloz olgusu dâhil edildi. Hastaların %68,1'i (32/47) erkek, %31,9'u (15/47) kadındı. Hastaların yaş ortalaması 44,43±15,47 idi. En sık görülen semptomlar eklem ve bel ağrısı; en sık tutulum tipleri ise artralji (%46,8), sakroileit (%19,1), spondilodiskit (%14,9) ve monoartritti (%14,9). Hastaların %48,93'ünde CRP yüksekliği, %27,65'inde ESH yüksekliği, %14,89'unda lökositoz, %8,51'inde lökopeni, %31,91'inde anemi, %12,76'sında ALT yüksekliği, %10,63'ünde AST yüksekliği saptadık.*

Sonuç: *Bruselloz çeşitli kas-iskelet sistemi bulguları ile prezente olabilmektedir. Özellikle Brusella enfeksiyonunun sık görüldüğü bölgelerde kas-iskelet sistemine ait bulguları olan hastaları değerlendirirken ayırıcı tanıda brusellozun akılda tutulması erken teşhis ve tedavi ile olası komplikasyonları engelleyebilmek adına faydalı olacaktır.*

Anahtar kelimeler: *artrit; brusella; sakroileit; spondilodiskit*

İletişim/Contact: *Esra Sahingoz Bakirci, Physical Medicine and Rehabilitation, Yozgat Bozok University Faculty of Medicine, Yozgat, Türkiye.* • ***Tel:*** *0530 784 33 70* • ***E-mail:*** *dresrasahingoz@gmail.com* • ***Geliş/Received:*** *15.02.2024* • ***Kabul/Accepted:*** *30.05.2024*

ORCID: *Esra Şahingöz Bakırcı: 0000-0002-4610-0440 • Gülseren Demir Karakılıç: 0000-0003-1292-0835 • Ferda Surel: 0000-0003-3748-3109 • Tuğba Alışık: 0000-0003-1856-9513*

Introduction

Brucellosis is a zoonotic disease caused by gram-negative coccobacilli bacteria of the genus Brucella. Humans can contract it through inhalation, skin contact, or by consuming unpasteurized dairy products[1,2]. Brucellosis commonly affects the young and middle-aged population, with less frequency observed in children and geriatric patients[3]. Brucellosis is endemic in our country[4]. According to the analyses of the Public Health General Directorate of the Ministry of Health of the Republic of Türkiye, the incidence of brucellosis in the province of Yozgat, where our hospital is located, is above the national average in Türkiye[5].

Brucellosis can affect various systems with diverse and nonspecific clinical manifestations. The most common symptoms are fever, weakness, fatigue, loss of appetite, muscle-joint pain, headache, lymphadenopathy, digestive system symptoms, weight loss and night sweats[6]. This wide-ranging clinical presentation poses diagnostic challenges; some studies suggest that the initial diagnosis is incorrect in one-third of patients[7]. It is reported that the musculoskeletal system is involved in brucellosis, with a frequency between 10 and 85%. The most commonly affected musculoskeletal areas are the sacroiliac, spinal and peripheral joints. Osteomyelitis, discitis, bursitis and tenosynovitis are less common types of involvement. Musculoskeletal involvement can be seen in patients with acute, subacute or chronic brucellosis[8,9].

Brucellosis may present with a wide variety of musculoskeletal system findings. Physical medicine and rehabilitation (PMR) outpatient clinics are among the most common referral points for patients with musculoskeletal complaints. With this study, we emphasize that brucellosis infection should be considered in the differential diagnosis when evaluating musculoskeletal system findings in regions where brucellosis is endemic and to highlight the diversity of presentations.

Material and Method

Our study has a retrospective design. Patients who applied to Yozgat City Hospital PMR outpatient clinics between 01.01.2020 and 01.01.2024 and were diagnosed with brucellosis with osteoarticular findings were included in the study. The diagnosis of Brucella infection was based on clinical findings and a Brucella Coombs test titer above 1/160. The age, gender, presenting complaints, musculoskeletal system examination findings, Brucella serology tests, liver function tests, complete blood counts, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and relevant musculoskeletal imaging data were recorded for the cases included in our study. Erythrocyte sedimentation rate >20 mm/hour was considered elevated ESR; CRP >0.8 mg/dl was considered elevated CRP; aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values above 40 IU/ml were considered elevated. Hemoglobin levels below 12 g/dl were considered anemia in women and below 13 g/dl in men; a leukocyte count below 4000/mm^3 was leukopenia; a leukocytosis above 10000/mm^3 was leukocytosis; a platelet count <150000/mm^3 was thrombocytopenia, and a platelet count >450000/mm^3 was thrombocytosis.

The Ankara Bilkent City Hospital Ethics Committee approved our study (date: 10.01.2024, number: E2-24-6082).

Statistical Analysis

Data were analyzed using the IBM Statistical Package for Social Sciences (SPSS) program version 25.0 (IBM Inc., Armonk, NY, USA). The normality of numerical data distribution was examined using the Shapiro-Wilk test. Continuous variables with normal distribution were presented as mean and standard deviation, and those without normal distribution were presented as median and interquartile range (IQR; 25 th-75th percentiles), while qualitative data were expressed as frequency and percentages. Binary logistic regression analysis was performed to investigate possible variables that may be associated with the presence of arthritis and sacroiliitis/spondylitis. The confidence interval is 95%, and the accepted margin of error is 5%. The p-value is considered significant at $p<0.05$.

Results

Our study included 47 patients diagnosed with brucellosis with osteoarticular findings. Of the patients, 68.1% (32/47) were male, and 31.9% (15/47) were female. The mean age of the patients was 44.43±15.47 years. The mean age was 48.7±17.0 and 42.4±14.5 in females and males, respectively; no significant difference was found between females and males (p=0.194). The most common symptoms were joint and lower back pain; the most common types of involvement were arthralgia, sacroileitis, monoarthritis and spondylodiscitis. Of the patients with sacroileitis, 5 had unilateral, and 4 had bilateral involvement. Among the patients with monoarthritis, 4 had knee arthritis, 2 had ankle arthritis, and 1 had hip arthritis. Hip arthritis was detected in a 10-year-old girl, the only

Table 1. *Analysis of patients according to age, gender, symptoms and musculoskeletal system involvement type*

	N/%	Mean ± SD
Age		44.43±15.47
Female		48.7±17.0
Male		42.4±14.5
Gender		
Female	15 (31.9)	
Male	32 (68.1)	
Symptoms		
Joint pain	33 (70.2)	
Back pain	10 (21.3)	
Chest pain	1 (2.1)	
Weakness/Tiredness	1 (2.1)	
Neck pain	1 (2.1)	
Joint swelling	1 (2.1)	
Musculoskeletal involvement		
Polyarthralgia	12 (25.5)	
Monoarthralgia	10 (21.3)	
Sacroiliitis	9 (19.1)	
Monoarthritis	7 (14.9)	
Spondylodiscitis	7 (14.9)	
Polyarthritis	1 (2.1)	
Synovitis	1 (2.1)	

SD: Standard deviation

Table 2. *Analysis of patients' biochemical data*

	Median (IQR; 25%-%75)	N (%)
Brucella Coombs titer	640.00 (2240.00)	
Sedimentation (mm/hour)	18.00 (27.00)	
• Elevated sedimentation		13 (27.65)
C-Reactive Protein (mg/dl)	1.28 (3.04)	
• Elevated C-Reactive Protein		23 (48.93)
Leukocyte count (mm^3)	7400 (2400)	
• Leukocytosis		7 (14.89)
• Leukopenia		4 (8.51)
Neutrophil count (mm^3)	4400 (2150)	
Lymphocyte count (mm^3)	2100 (1200)	
Platelet count (mm^3)	256000 (95500)	
• Thrombocytopenia		1 (2.12)
• Thrombocytosis		-
Hemoglobin (mg/dl)	13.6 (1.75)	
• Anemia		15 (31.91)
ALT (IU/ml)	20 (15)	
• Elevated ALT		6 (12.76)
AST (IU/ml)	21 (9.5)	
• Elevated AST		5 (10.63)

IQR: interquartile range; ALT: alanine aminotransferase; AST: aspartate aminotransferase

child in the patient group. There was no accompanying osteomyelitis in the arthritis cases. Of the patients with spondylodiscitis, 5 had lumbar involvement, and 2 had thoracic involvement; there were no patients with cervical involvement. There were no accompanying abscesses or neurologic complications in cases of spondylodiscitis. Table 1 summarizes the age, gender, predominant symptoms, and musculoskeletal involvement types of the patients. We found elevated CRP in 48.93%, elevated ESR in 27.65%, leukocytosis in 14.89%, leukopenia in 8.51%, anemia in 31.91%, elevated ALT in 12.76% and elevated AST in 10.63%. Biochemical analyzes of the patients are presented in Table 2.

In binary logistic regression analysis, no significant correlation was found between age, gender, laboratory parameters, and the presence of arthritis and sacroiliitis/spondylodiscitis.

Discussion

The Central Anatolia region, where our hospital and our study population are located, has a high incidence of brucellosis[10]. Brucellosis can present with multisystem involvement and various nonspecific clinical findings. The musculoskeletal system is one of the systems that is frequently affected and should be kept in mind[8]. Therefore, brucellosis patients with musculoskeletal symptoms often visit PMR outpatient clinics.

Our patient group mainly consisted of male and young adult patients, which is in line with other studies[11,12]. The predominance of male patients may be related to the fact that men engaged in animal husbandry are more exposed to this infection. The most common involvement in our patient group was nonspecific arthralgia; however, radiologically, sacroileitis, spondylodiscitis and arthritis were prominent. In the study of Geyik et al.[12] where they evaluated osteoarticular involvement in brucellosis patients, sacroiliitis, peripheral joint involvement and spondylodiscitis were the most common involvement types, respectively. In another study conducted in our country, the types of involvement are as follows: sacroiliitis, peripheral joint involvement, synovitis and spondylodiscitis[11]. The results of these studies are in line with our data. Brucella sacroileitis is mostly seen in adults and may be unilateral or bilateral. Patients may present with severe pain in the sacral region. It is asymptomatic in 24% of patients. Sacroiliitis may be accompanied by osteomyelitis and abscess formation in the iliac muscle. Magnetic resonance imaging (MRI) has a high sensitivity in the diagnosis[13]. Figure 1 shows the MRI of a patient with sacroiliitis.

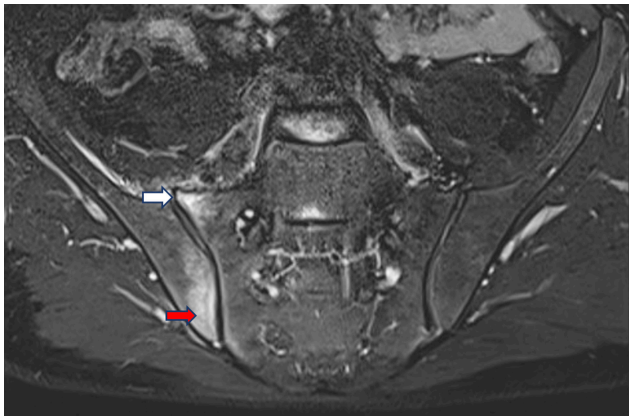

Figure 1. *Sacroiliac joint magnetic resonance image from a 38-year-old man with Brucellar sacroiliitis. Coronal T2-weighted fatsat image shows significant signal increase inferior to the iliac bone (red arrow) and superior to the sacral bone (white arrow) in the right sacroiliac joint.*

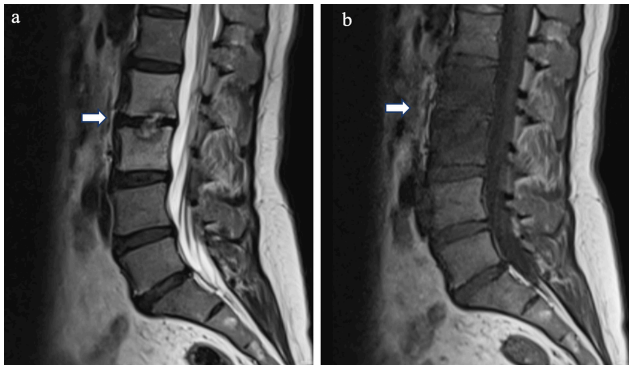

Figure 2. *Lumbar magnetic resonance images from a 47-year-old woman with Brucellar L2-L3 spondylodiscitis. T2-weighted sagittal image shows hyperintense signal intensities in L2-L3 vertebral bodies and the intervertebral disc (white arrow) (a). T1-weighted sagittal image shows homogeneous hypointensity in L2-L3 vertebral bodies and the intervertebral disc (white arrow) (b).*

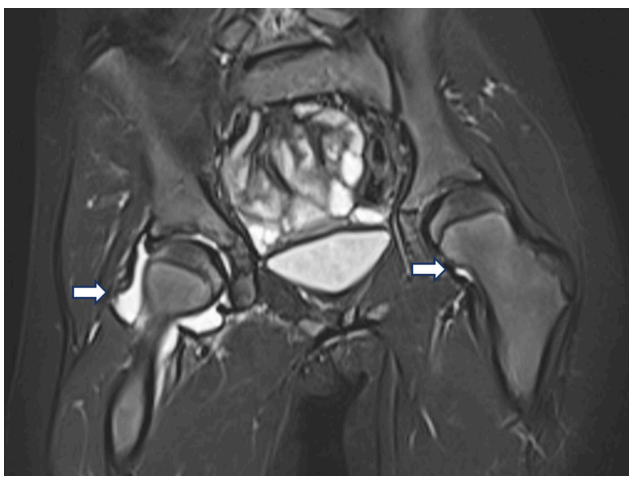

Figure 3. *Bilateral hip magnetic resonance image from a 10-year-old girl with prominent bilateral hip arthritis on the right. T2-weighted coronal image shows hyperintense areas that indicate joint effusion (white arrows).*

Spinal joints are frequently affected; the most common types of spinal involvement are spondylitis and spondylodiscitis. While patients generally complain of low back pain, radicular findings may also accompany them[8,14]. Brucella spondylodiscitis seen in our study was mostly involved in the lumbar region, followed by the thoracic region, while we did not have any cases showing cervical involvement. In the literature, similar to our data, the frequency of spondylodiscitis involvement was reported as lumbar, thoracic and cervical regions[11,15]. Although the L4-L5 segment is frequently affected, focal or multifocal involvement can be observed[7,16]. Paravertebral abscess and neurological complications may develop in cases of spondylodiscitis[17]. Due to the relatively low number of cases, we did not find any cases with paravertebral abscess, neurologic complications or involvement of the cervical region in our cases. Figure 2 shows MRI scans of a patient with spondylodiscitis.

Peripheral arthritis is a common osteoarticular involvement. The incidence of Brucella-induced arthritis ranges from 3% to 77% in various series. It may show monoarticular, oligoarticular or polyarticular distribution and may be in septic or reactive form. The peripheral joints most commonly affected by brucellosis include the knee, hip, and ankles. Shoulders, wrists, elbows, interphalangeal and sternoclavicular joints may be affected less frequently[16]. In our data, the knee joint was the most commonly affected joint, parallel to the literature. The most common type of osteoarticular involvement in children is monoarthritis. Hip and knee joints are frequently involved, and there may be accompanying osteomyelitis of the adjacent bone[13]. Among our cases, hip arthritis was present in only one pediatric patient. Difficulty in the diagnosis and treatment process can lead to serious complications such as femoral head dislocation and necrosis in hip arthritis[18]. It is important to remember it in the differential diagnosis, especially in endemic regions, to prevent possible complications and permanent disabilities. The MRI of the case in which hip arthritis was detected is shown in Figure 3.

Our data did not detect a significant correlation between laboratory parameters and the presence of arthritis, sacroiliitis, and spondylodiscitis. There were series in which acute phase reactants were found to be higher in patients with osteoarticular involvement than in those without osteoarticular involvement[11,19–21]. We found elevated CRP in 48.93% of our patients, elevated ESR in 27.65%, and anemia in 31.91%. Türkoğlu-Yilmaz et al.[22] found

elevated CRP in 91.5% and elevated ESR in 48.3% of Brucella cases. In the retrospective publications of Kiş et al.[23] CRP elevation was 39.6%; elevated ESR and anemia were detected in 32.4% and 37.8% of patients, respectively. In another study conducted in our city, CRP elevation was found in 83.3%, ESR elevation in 40%, and anemia in 51.6% of the patients[19]. These differences are due to the inclusion of only patients with osteoarticular findings in our study. It may be due to not knowing whether the patients are acute, subacute or chronic. While the analysis of osteoarticular findings in a region where Brucella infection is endemic is the strength of our study, the relatively low number of cases and the absence of a control group in the analysis of biochemical parameters are the weaknesses of our study. Another limitation is the lack of knowledge of Brucella species, which may impact clinical findings, prognosis, and treatment.

Conclusion

Physical medicine and rehabilitation outpatient clinics are typically the first point of contact for patients with musculoskeletal complaints. Brucellosis often affects the musculoskeletal system and can present with various findings. Brucella infection should be considered in the differential diagnosis to prevent potential complications through early diagnosis and treatment, especially in regions where brucellosis is endemic.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Comparison of Starion Vessel Sealing System with Conventional Technique and Harmonic Focus Ultrasonic Scalpel in Total Thyroidectom

Total Tiroidektomide Starion Damar Mühürleme Sisteminin Konvansiyonel Teknik ve Harmonik Odaklı Ultrasonik Cihaz ile Karşılaştırılması

Kagan Gokce[1], Nuriye Ozder[2], Omer Faik Ersoy[2], Coskun Polat[1]

[1]Istanbul Okan University, School of Medicine, Department of General Surgery, Istanbul; [2]Karabuk University, Faculty of Medicine, Department of General Surgery, Karabuk, Türkiye

ABSTRACT

Aim: *This study compares the use of Starion Tissue Welding SystemTM (STWS) in patients who underwent total thyroidectomy due to Multinodular Goiter (MNG) with the conventional technique and Harmonic Focus Ultrasonic ScalpelTM (HFUS) in terms of operation time, bleeding during and after the operation, and complications.*

Materials and Methods: *Between June 2013 and August 2014, 60 patients who underwent a total thyroidectomy due to MNG were retrospectively analyzed. The patients were divided into three groups according to the surgical method. Each group consisted of 20 patients. In the operations, ligation/coagulation and cutting procedures were performed to middle thyroid veins, upper and lower pole arteries/veins of the thyroid gland using conventional clamp and-tie technique in Group-A, STWS in Group-B, and HFUS in Group-C. Groups were compared regarding the operation time, the amount of bleeding during and after the operation, and the complications encountered.*

Results: *Operation times were established as 63.25±28.66 minutes in Group-A, 42.60±20.14 minutes in Group-B, and 49.60±9.17 minutes in Group-C ($p<0.05$). Perioperative bleeding was 93.50±56.05 ml in Group-A, 25.60±15.21 ml in Group-B, and 62.50±23.31 ml in Group-C ($p<0.05$). Postoperative blood drainage was 124.50±153.09 ml, 8.75±11.68 ml, and 35.75±25.91 ml, respectively ($p<0.05$). Regarding hemostasis, while two patients in Group-A required hemostasis within the first 24 hours, there was no need for hemostasis in Group-B and Group-C ($p=0.12$). The blood calcium levels measured on the first postoperative day were found to be 8.39±0.56 mg/dl in Group-A, 8.34±1.14 mg/dl in Group-B, 8.37±0.78 mg/dl in Group-C ($p=0.86$). Blood parathormone levels measured on the first postoperative day were 52.72±35.85 pg/dl, 34.77±20.0 pg/dl, and 56.41±36.56 pg/dl, respectively ($p=0.09$).*

Conclusion: *STWS can be used safely in thyroid surgery because it reduces bleeding during and after surgery, shortens the operation time, and has low complication rates.*

Key words: *thyroidectomy; tissue welding system; ultrasonic device; clamp and tie*

ÖZET

Amaç: *Bu çalışmada Multinodüler Guatr (MNG) nedeniyle total tiroidektomi yapılan hastalarda Starion Tissue Welding SystemTM (STWS) kullanımının, konvansiyonel teknik ve Harmonik Odaklı Ultrasonik ScalpelTM (HFUS) kullanımı ile operasyon süresi, ameliyat sırasında ve sonrasındaki kanama miktarı ile ameliyat sonrası komplikasyonlar açısından karşılaştırılması amaçlanmaktadır.*

Materyal ve Metot: *Haziran 2013 ile Ağustos 2014 tarihleri arasında MNG nedeniyle total tiroidektomi yapılan 60 hasta retrospektif olarak incelendi. Hastalar uygulanan cerrahi yönteme göre üç gruba ayrıldı. Her grup 20 hastadan oluşuyordu. Operasyonlarda Grup-A'da konvansiyonel klemp bağlama tekniği, Grup-B'de STWS, Grup-C'de HFUS kullanılarak orta tiroid damarlarına, tiroid bezinin üst ve alt kutup arterlerine/venlerine ligasyon/koagülasyon ve kesme işlemleri uygulandı. Gruplar operasyon süresi, operasyon sırasında ve sonrasında kanama miktarı ve karşılaşılan komplikasyonlar açısından karşılaştırıldı.*

Bulgular: *Operasyon süreleri Grup-A'da 63,25±28,66 dakika, Grup-B'de 42,60±20,14 dakika, Grup-C'de 49,60±9,17 dakika olarak belirlendi ($p<0,05$). Perioperatif kanama Grup-A'da 93,50±56,05 ml, Grup-B'de 25,60±15,21 ml, Grup-C'de 62,50±23,31 ml idi ($p<0,05$). Ameliyat sonrası kan drenajı sırasıyla 124,50±153,09 ml, 8,75±11,68 ml ve 35,75±25,91 ml idi ($p<0,05$). Hemostaz açısından Grup-A'daki iki hastada ilk 24 saat içinde hemostaz ihtiyacı duyulurken, Grup-B ve Grup-C'de hemostaz ihtiyacı olmadı ($p=0,12$). Ameliyat sonrası 1. günde ölçülen kan kalsiyum düzeyleri Grup-A'da 8,39±0,56 mg/dl, Grup-B'de 8,34±1,14 mg/dl, Grup-C'de 8,37±0,78 mg/dl olarak tespit edildi ($p=0,86$). Ameliyat sonrası 1. Günde ölçülen kan parathormon düzeyi sırasıyla 52,72±35,85 pg/dl, 34,77±20,0 pg/dl ve 56,41±36,56 pg/dl idi ($p=0,09$).*

Sonuç: *Tiroid cerrahisinde STWS, ameliyat sırasında ve sonrasında kanama miktarının azalması, ameliyat süresinin kısalması ve komplikasyon oranlarının düşük olması nedeniyle güvenle kullanılabilir.*

Anahtar kelimeler: *tiroidektomi; doku mühürleme sistemi; ultrasonik cihaz; klemp-bağlama*

İletişim/Contact: *Kagan Gokce, Icmeler Mahallesi, Aydinli Yolu Caddesi, Okan Universitesi Tip Fakultesi Arastirma ve Uygulama Hastanesi, Genel Cerrahi Anabilim Dali, No: 2, 34947 Tuzla, Istanbul, Türkiye* • ***Tel:*** *0532 581 05 90* • ***E-mail:*** *kgngkc@hotmail.com*
Geliş/Received: *25.02.2024* • ***Kabul/Accepted:*** *20.10.2024*

ORCID: *Kağan Gökçe: 0000-0003-4712-0512* • *Nuriye Özder: 0009-0004-6282-4643* • *Ömer Faik Ersoy: 0000-0003-1027-0349* • *Coşkun Polat: 0000-0001-6968-6926*

Introduction

Thyroidectomy has been performed quite frequently since the day it was defined. The frequency of thyroidectomy operations is higher in endemic areas for thyroid gland diseases, especially in the Black Sea Region of Türkiye. The morbidity of thyroid surgeries is very low, and the possibility of mortality is rare. Complications such as Recurrent Laryngeal Nerve (RLN), Superior Laryngeal Nerve (SLN), and parathyroid gland damage that may occur during thyroid surgeries can adversely affect the patient's life. Due to the high rates of bleeding, infection, and sepsis, as well as the morbidity and mortality rates of around 40%, this type of surgery was only frequently performed in general surgery clinics at the time of Theodore Kocher. Towards the end of the nineteenth century, innovative surgical techniques developed with Kocher's introduction of antisepsis, enucleation, and ligation techniques. With the standardization of vascular control in thyroid surgery, a significant reduction in mortality has been achieved. The importance of demonstrating the vascular structures and visualizing the anatomical structures has been understood by surgical standardization[1].

Hemostasis is conventionally achieved in thyroid surgery with clamps and sutures, tissue adhesives, or hemostatic clips. However, energy-based surgical instruments (ESIs), which started to be used in the 2000 s, coagulate vascular structures and can cut simultaneously. Coagulated vascular structures can be safely coagulated and cut without ligation. Although ESIs in use are unsuitable for large-scale arteries and veins, they can coagulate all arteries and veins encountered in thyroid surgery. In thyroid surgery, it has been shown that they shorten the operation time and reduce the risks and complications of surgery[2–4]. Hemostasis can be achieved in thyroidectomy operations with Harmonic Focus Ultrasonic Scalpel™ (Ethicon Inc., Cincinnati, OH, USA) (HFUS) and the bipolar vessel ligation system: LigaSure™ (Valleylab, Boulder, Colo, USA) (Ligasure), which have been used recently. Even minor bleeding during thyroid surgery can obscure the visualization of structures such as the RLN and parathyroid glands and cause damage to them. Therefore, performing thyroid surgery in a bloodless environment is very important. ESIs such as HFUS and Ligasure are successful in hemostasis; they reduce the amount of bleeding during the operation and the risk of hematoma that may develop in the postoperative period. They significantly reduce the operation time and the hospitalization in the postoperative period. They allow the thyroidectomy incision to be made smaller. They can also be used in endoscopic thyroidectomy[3,5].

The study, which compared the use of traditional surgical techniques with HFUS, reported that the use of HFUS shortens the operation time by 15–20% and is cost-effective compared to the traditional surgical technique. Although the costs of ESIs are high, they have reported that the increased cost due to the use of ESIs is balanced due to the shortened time of surgery and hospitalization[6]. ESIs work by producing various types of energy; they have coagulation and cutting abilities (e.g., ultrasound, radiofrequency, tissue welding). Starion Tissue Welding System™ (Starion Instruments, Sunnyvale, CA, USA) (STWS) produces less smoke when coagulating the tissue and has been used safely and effectively for hemorrhoidectomy in clinical practice. The safety and effectiveness of STWS in total thyroidectomy operations were studied in 2010 for Total Thyroidectomy (TT) operations. Starion Tissue Welding System™ was used for the first time and compared to the conventional surgical method for TT. It has been demonstrated that STWS can easily perform the sealing and cutting process, shorten the operation time, be used for open and minimally invasive video-assisted thyroidectomy, provide faster recovery, and be used safely regarding complications[7].

In our clinic, located in an endemic region for thyroid diseases, various ESIs or traditional methods were used in TT surgeries, depending on the financial means provided by the patients and their health insurance. In our study, we aimed to compare the results of using three different methods, STWS, clamp-and-tie technique, and HFUS, in terms of the operation time, the amount of bleeding during and after the operation, and the complications that developed.

Materials and Methods

Patients who underwent TT surgery in the General Surgery Clinic of Karabuk University Medical Faculty Hospital between June 2013 and August 2014 were included in the study. Patients with a histopathological diagnosis of malignant, toxic multinodular goiter and giant goiter who had undergone unilateral surgery, secondary surgery, and additional neck dissection were determined as exclusion criteria, and only Benign Multinodular Goiter (BMNG) patients (n=60) were retrospectively analyzed and included in the study. All of the patients were female patients. The patients

were divided into three groups according to the ESIs and clamp-and-tie techniques used in the surgery. Each group consisted of 20 patients. The same surgical team performed all operations. Ligation/coagulation and cutting procedures were performed using the clamp-and-tie technique (n=20) in Group-A, STWS (n=20) in Group-B, and HFUS (n=20) in Group-C. The patients were evaluated by examining the records of the operation time, the amount of bleeding during the operation, and the clinical findings of serum calcium, parathormone values, hoarseness, and hypocalcemia in terms of early RLN and parathyroid gland damage within the first 24 hours after the operation. Aspirative drainage amounts and re-hemostasis needs were examined in the postoperative period. By establishing these criteria, three groups were compared.

All patients were operated under general anesthesia with endotracheal intubation. A standard Kocher's Necklace Incision of 5–6 cm in length was made in all patients, and a TT operation was performed. In the operations performed, the skin, subcutaneous tissue, and platysma were passed using a scalpel and monopolar electrocautery. In each group, the midline was dissected with monopolar electrocautery. After the thyroid tissue is exposed, the middle thyroid veins, upper and lower pole arteries/veins of the thyroid gland were ligated/sealed and cut using the conventional clamp-and-tie technique in Group-A, STWS in Group-B, and HFUS in Group-C. Berry's ligament, which is in close anatomical proximity to the RLN, was separated from the surrounding tissues by dissecting using a dissector and ligatures in Group-A, using dissector and group-specific ESIs in the other two groups. An aspirative drain was placed in the thyroid lodge after bleeding control as a standard in each operation. The amount of bleeding during the operation was measured. Blood calcium and parathormone levels were measured on the first postoperative day. The amount of bleeding in the drain was recorded 24 hours after the operation, and the drain was removed. The patients were evaluated by physical examination and checked for Chvostek's or Trousseau's signs and dysphonia.

Statistical Analysis

Analyzes were performed using IBM Statistical Package for Social Sciences (SPSS) program version 10 (IBM, Inc. Chicago, IL, USA). In comparisons between groups, the test of differences between two independent groups was used to compare parametric data, and the chi-square test was used to compare non-parametric data. Statistically ($p<0.05$) values were considered significant.

Results

The mean age of Group-A was 52.85±12.12 years, Group-B was 52.75±13.09 years, and Group-C was 51.0±12.94 years. There was no statistically significant difference between the groups regarding mean age ($p=0.78$). The diameter of the largest nodule in the thyroid gland is 3.07±1.34 millimeters (mm) in Group-A, 3.1±1.42 mm in Group-B, and 2.95±1.3 mm in Group-C. Other nodule numbers and sizes were not taken into account. No statistically significant difference was found between the groups ($p>0.05$) (Table 1). When the mean operation times were evaluated, it was found that Group-A was 63.25±28.66 minutes (min), Group-B was 42.60±20.14 minutes, and Group-C was 49.60±9.17 minutes. A statistically significant difference was found between the mean operation times ($p<0.05$). When the groups were compared among themselves, it was observed that the shortest time was obtained in the STWS group ($p<0.017$) (Table 1). In terms of perioperative and postoperative bleeding, the amount of perioperative bleeding was 93.50±56.05 milliliter (ml) in Group-A, 25.60±15.21 ml in Group-B, 62.50±23.31 ml in Group-C ($p<0.05$). In contrast, postoperative bleeding was 124.50±153.09 ml, 8.75±11.68 ml, and 35.75±25.91 ml, respectively. A statistically significant difference was found between the groups ($p<0.05$). When the groups were compared, it was observed that the least amount of bleeding was observed in the STWS group ($p<0.017$) (Table 1) (Tables 2a and 2b). Regarding hemostasis, while two patients in Group-A required hemostasis within the first 24 hours, hemostasis was not needed in Group-B and C. However, no statistically significant difference was found between the groups ($p=0.12$) (Table 3). When evaluated in terms of blood calcium and parathormone results measured on the first postoperative day, calcium values were 8.39±0.56 milligrams/deciliter (mg/dl) in Group-A, 8.34±1.14 mg/dl in Group-B and 8.37±0.78 mg/dl in Group-C ($p=0.86$). Parathormone levels were found to be 52.72±35.85 picograms/deciliter (pg/dl), 34.77±20.0 pg/dl, and 56.41±36.56 pg/dl, respectively ($p=0.09$). There was no statistically significant difference in both parameters ($p>0.05$) (Table 1).

Table 1. *Results of patients who underwent bilateral total thyroidectomy*

	Device	Number	Average	Standard deviation	P value
Age (years)	Classic	20	52.85	12.12	0.78
	Thermal welding	20	52.75	13.09	
	Harmonic	20	51.00	12.94	
Operation time (minute)	Classic	20	63.25	28.66	<0.05
	Thermal welding	20	42.60	20.14	
	Harmonic	20	49.60	9.17	
Nodule diameter (millimeter)	Classic	20	3.07	1.34	>0.05
	Thermal welding	20	3.1	1.42	
	Harmonic	20	2.95	1.36	
Peroperative bleeding (milliliter)	Classic	20	93.50	56.05	<0.05
	Thermal welding	20	25.60	15.21	
	Harmonic	20	62.50	23.31	
Postoperative bleeding (milliliter)	Classic	20	124.50	153.09	<0.05
	Thermal welding	20	8.75	11.68	
	Harmonic	20	35.75	25.91	
Calcium (milligram per deciliter)	Classic	20	8.39	0.56	0.86
	Thermal welding	20	8.34	1.14	
	Harmonic	20	8.37	0.78	
Parathormon levels (picogram per decilitre)	Classic	20	52.72	35.85	0.09
	Thermal welding	20	34.77	20.0	
	Harmonic	20	56.41	36.56	

Table 2a. *Evaluation of the conventional method and the HFUS system among themselves*

	Device	P value	Bonferroni ratio
Operation time (minute)	Classic	0.16	p>0.017
	Harmonic		
Perioperative bleeding (milliliter)	Classic	0.42	p>0.017
	Harmonic		
Postoperative bleeding (milliliter)	Classic	0.26	
	Harmonic		

Table 2b. *Evaluation of HFUS system and STWS among themselves*

	Device	P value	Bonferroni ratio
Operation time (min)	Thermal welding	0.002	p<0.017
	Harmonic		
Perioperative bleeding (ml)	Thermal welding	0.001	p<0.017
	Harmonic		
Postoperative bleeding (ml)	Thermal welding	0.002	
	Harmonic		

Table 3. *Evaluation for complications (p>0.05)*

	Device	Number	Present	%	p-value
Hoarseness	Classic	20	0	0	p=0.59
	Thermal welding	20	1	5	
	Harmonic	20	1	5	
Hemostasis	Classic	20	2	10	p=0.12
	Thermal welding	20	0	0	
	Harmonic	20	0	0	

In terms of complications, transient hoarseness was not observed in Group-A but in one patient each in Group-B and C. These patients were evaluated with laryngoscopy when dysphonia was detected and unilateral partial paralysis was detected. These two patients were followed up clinically and by laryngoscopy, and it was found that dysphonia was resolved in less than 6 months of their follow-up. Permanent hoarseness did not develop in any patient. No clinical evidence of SLN injury or wound infection was found in any group. No statistically significant difference was found in complications (p=0.59) (Table 3).

Discussion

Total thyroidectomy is the most frequently performed surgery in the field of endocrine surgery. Thyroid surgeries are performed more frequently, especially in endemic regions such as our country. The morbidity after TT surgery is quite low, and mortality is not predicted under normal circumstances[8]. Thyroid tissue is a very rich blood supply organ and is prone to bleeding during surgery. To avoid complications of thyroidectomy operations, anatomical formations must be seen clearly. For this purpose, the fact that the operation area is quite dry and bloodless will facilitate visualization. The upper and lower pole vessels can be dissected, ligated, and cut to prevent bleeding during surgery. Traditionally, sutures and clips can be used for this, but ligatures and clips can slip out of place and cause bleeding. Using ESIs reduces this possibility and does not leave foreign objects, such as ligatures and clips, in the surgical field. There is a concern for thermal damage in these devices, especially in dissections performed close to the nerves, but studies show that the devices can be used safely without causing thermal damage[7,9]. One of the important complications that can be fatal after thyroidectomy is bleeding. Although the bleeding rate after thyroidectomy performed by experienced surgeons is reported to be approximately 1%, there are also publications reporting a bleeding rate of over 4%[10–12].

While most of the bleeding occurs in the first 6 hours after thyroidectomy, only 10% may occur after 24 hours. Bleeding after 7 days has also been reported in the literature in a few cases. It is important whether the bleeding is life-threatening or not. The majority of early bleeding is life-threatening and requires immediate surgical intervention. Bleeding occurring in the late period may not always be life-threatening and can be followed conservatively[13,14]. Hemostasis is, therefore, very important. As conventional methods of hemostasis, suturing and electrocautery are common. However, high lateral tissue damage due to electrocautery use may also cause damage to the surrounding vital tissues. In addition, burns may occur due to the patient's exposure to electricity. Recently, it has been shown that ESIs can be used safely in open and endoscopic thyroid surgery. The fact that hemostasis is one of the most important parts of the operation in thyroid surgery and that hemostasis is a time-consuming procedure has increased the use of ESIs in thyroid surgery[9,15].

A study used STWS for TT operations and compared it with the conventional method. In the study, it has been detected that using STWS shortens the operation time and provides a faster recovery opportunity by reducing the possibility of hypocalcemia in the postoperative period. However, to show the extent of the thermal damage in the surrounding tissue, they examined the part of the superior thyroid artery sealed with STWS and the surrounding tissue histopathologically by staining with hematoxylin and eosin, and they detected collateral damage of no more than 0.5 mm in the surrounding tissue. This shows that minimal thermal damage occurs and can be used safely[7].

Harmonic Focus Ultrasonic Scalpel™ often cuts with 55,500 Hz vibration. During this vibration, microlevel heat is generated and causes the denaturation of proteins. In the veins, both the closing and cutting process takes place. The heat generated is less than 60°C, and it is stated that the thermal heat damage is less than 1.5 mm. Starion Tissue Welding System™ is a simple resistive heater wire powered by low voltage direct current. The STWS is not a bipolar device, as no electrical current flows through the tissue between the tips of the STWS forceps. The active part of the device is the heating element, which consists of nickel chrome resistance and a thermally insulated back part. This layer prevents the heating effect of the nickel chrome wire from spreading to other parts of the device. Coagulation and cutting co-occur. The device closes both ends of a cut vessel. Due to the heat conduction in the nickel chrome wire, the cut area is larger than the diameter of the wire. The heat in the tissue is sufficient to cut the tissue by direct vaporization. This temperature varies between 300–400°C. Moving away from the wire's center, the temperature drops to 100°C, which is the ideal value for coagulating and bonding the tissues with protein denaturation[16].

In a study in which they compared the use of HFUS and conventional techniques in TTs for thyroid cancer and Basedow's disease, the average bleeding amount during the operation was 40 ml in the HFUS group. In comparison, it was 125 ml in the conventional method group, and the postoperative drainage amount was 120 ml and 175.5 ml, respectively. The study showed that using HFUS resulted in a statistically significant reduction in bleeding and total drainage during surgery[17]. In another study, in which they compared TT surgeries performed using HFUS and LigaSure and their results, it was found that both ESIs provided effective and safe dissection of all vessels and tissues. There was no significant difference in postoperative morbidity[18].

Many studies have stated that there is less bleeding in the use of HFUS and LigaSure compared to conventional methods. However, more studies need to be done on using STWS in thyroid surgery. In our study, hemostasis was required in patients who used the conventional method. The least amount of bleeding is in the thermal welding system. For this reason, we see that STWS can be used in thyroid surgery without the need to suture the upper and lower pole arteries/veins, and it is very effective in reducing the amount of bleeding and total drainage.

Compared to the conventional technique, the most important advantage of using HFUS is shortening the operation time. It has been revealed that an average reduction of 35.8 minutes in TT operations performed by an experienced surgeon using HFUS compared to TT surgeries performed with conventional technique[19]. Another study reported a time saving of 40 minutes for bilateral total thyroidectomy and 30 minutes for lobectomy[3]. However, using HS reduces the operation time by 18% on average[20]. In our study, the shortest operation times were obtained with STWS compared to the conventional method and the use of HFUS. This result is due to the shorter waiting time of the system during coagulation and cutting. Other advantages of using STWS are that it causes 5 mm or less thermal damage to the surrounding tissue and no vibration in contact with metallic surgical instruments. In addition, the device's ergonomics provide a more comfortable and controlled use[7].

As a result of the dissection performed during thyroidectomy operations, the blood supply to the parathyroid glands is often temporarily impaired. When operations performed due to MNG or thyroid cancer are compared, hypocalcemia is more common in operations performed due to cancer. When total thyroidectomy and thyroid lobectomy operations are compared, the probability of developing hypocalcemia is lower in lobectomies[21]. Nowadays, total thyroidectomy is replaced by thyroid lobectomy in selected cases of thyroid cancer. After this type of limited surgery, the probability of developing hypocalcemia decreases, and hospital stays also shorten. Therefore, when comparing the energy devices used in thyroidectomy operations in terms of hypocalcemia, it is important whether the surgery is performed for benign or malignant reasons[22].

Conclusion

In our study, it is notable that using STWS in TT surgeries reduces the amount of bleeding during and after the surgery, shortens the operation time, and has low complication rates. The shortening of the operation time when STWS is used is due to the ability of the STWS device to perform both cutting and coagulation simultaneously. The fact that blood Ca and Parathormone values measured postoperatively are not low indicates that using STWS is safe regarding parathyroid damage that may occur during surgery. In our study, dissection around the Berry Ligament was performed with STWS and HFUS. No permanent nerve damage was observed, indicating that dissecting around the nerve tissue with ESIs is safe. In addition, when compared to other ESIs, STWS is a simpler and more practical system that can work with a universal power supply. We believe that STWS, like other ESIs, is a safe alternative for TT surgeries.

Statement of Ethics

The approval for this study was obtained from Istanbul Okan University, Ethics Committee, dated October 18, 2023, and numbered 169/32.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Effects of 3,3'-Diindolylmethane on Rat Kidney Tissue

3,3'-Diindolylmethane'ın Sıçan Böbrek Dokusu Üzerindeki Etkileri

Secil Nazife Parlak[1], Seda Yakut[2], Habibe Gundogdu[3], Tuba Demirci[3]

[1]Department of Histology and Embryology, Faculty of Medicine, Agri Ibrahim Cecen University; [2]Department of Histology and Embryology, Faculty of Veterinary, Burdur Mehmet Akif Ersoy University, Burdur; [3]Department of Histology and Embryology, Faculty of Medicine, Ataturk University, Erzurum, Türkiye

ABSTRACT

Aim: *3,3'-diindolylmethane (DIM) is an important digestive product of indole-3 carbinol (I3C) obtained from the Brassica family (broccoli, cauliflower, cabbage, etc.) of vegetables. 3,3'-diindolylmethane is a substrate with potent immune modulatory activity and antitumor, antiviral, and anti-angiogenic effects. This study aimed to evaluate the effects of DIM on rat kidney tissue using histopathologic methods.*

Material and Method: *In the study, 36 males, 16-week-old, and 220–260 gr Wistar albino adult rats were used. Rats were divided into four equal groups: The control group received only corn oil by oral gavage. The other experimental groups received three different doses of DIM dissolved in corn oil, 10 mg/kg DIM (DIM 10 group), 50 mg/kg DIM (DIM 50 group), and 100 mg/kg DIM (DIM-100 group, were administered via oral gavage. Oral gavages were applied to experimental groups for 53 days. At the end of the experiment, all rats were euthanized, and the kidney tissues were dissected. For histopathological examination, the kidney tissue samples were stained with hematoxylin-eosin and Masson–trichrome.*

Results: *Our investigation revealed that the use of DIM at different doses for 53 days caused dose-dependent histopathological changes, including apoptotic to necrotic changes, interstitial inflammation to fibrotic connective tissue changes, and cast formations starting from the Henle loops and spreading to the renal tubules.*

Conclusion: *These histopathological changes could have occurred due to a DIM-mediated increase in reactive oxygen species (ROS). Further biochemical, molecular, and ultrastructural studies are needed to clarify these findings.*

Key words: *3,3'-diindolylmethane; indole-3 carbinol; kidney; reactive oxygen species (ROS)*

ÖZET

Amaç: *3,3'-diindolilmetan (DIM), Brassica ailesi (brokoli, karnabahar, lahana vb.) sebzelerinden elde edilen indol-3-karbinolün (I3C) önemli bir sindirim ürünüdür. DIM, güçlü bağışıklık modülatör aktiviteye sahip bir substrat olup antitümör, antiviral ve anti-anjiyogenik etkilere sahiptir. Bu çalışmada, DIM'in sıçan böbrek dokusu üzerindeki etkilerinin histopatolojik yöntemlerle değerlendirilmesi amaçlanmıştır.*

Materyal ve Metot: *Çalışmada 16 haftalık, 220–260 gr ağırlığında toplam 36 erkek Wistar albino erişkin sıçan kullanılmıştır. Sıçanlar dört eşit gruba ayrılmıştır: Kontrol grubuna sadece oral yolla mısır yağı verilmiştir. Diğer deney gruplarına mısır yağı içinde çözünmüş üç farklı dozda DIM uygulanmıştır: 10 mg/kg DIM (DIM 10 grubu), 50 mg/kg DIM (DIM 50 grubu) ve 100 mg/kg DIM (DIM 100 grubu). Oral uygulamalar deney gruplarına 53 gün boyunca uygulanmıştır. Deney sonunda tüm sıçanlar ötanazi edilmiş ve böbrek dokuları çıkarılmıştır. Histopatolojik inceleme için böbrek doku örnekleri hematoksilen-eozin ve Masson-trikrom ile boyanmıştır.*

Bulgular: *Çalışmamız, 53 gün boyunca farklı dozlarda DIM kullanımının, doz bağımlı histopatolojik değişikliklere neden olduğunu ortaya koymuştur. Bu değişiklikler, apoptotik ve nekrotik değişimlerden, interstisyel enflamasyondan fibrotik bağ dokusu değişimlerine ve Henle kıvrımlarından başlayarak renal tübüllere yayılan kast oluşumlarını içermektedir.*

Sonuç: *Bu histopatolojik değişiklikler, DIM'in reaktif oksijen türlerini (ROS) artırması sonucu ortaya çıkmış olabilir. Bu bulguları aydınlatmak için ileri düzey biyokimyasal, moleküler ve ultrastrüktürel çalışmalara ihtiyaç duyulmaktadır.*

Anahtar kelimeler: *3,3'-diindolilmetan; böbrek; reaktif oksijen türleri (ROS)*

İletişim/Contact: *Department of Histology and Embryology, Faculty of Medicine, Agri Ibrahim Cecen University, Agri, Türkiye* • ***Tel:*** *0505 800 18 16* • ***E-mail:*** *seeparlak@gmail.com.tr* • ***Geliş/Received:*** *13.03.2024* • ***Kabul/Accepted:*** *16.05.2024*

ORCID: *Seçil Nazife Parlak: 0000-0003-2008-9332 • Seda Yakut: 0000-0003-1673-5661 • Habibe Gündoğdu: 0000-0002-6151-4078 • Tuba Demirci: 0000-0002-8814-9648*

Kafkas J Med Sci 2024; 14(3):224–230
doi: 10.5505/kjms.2024.95770

Introduction

Vegetables from the Cruciferous (Brassicaceae) family contain considerable amounts of a bioactive phytochemical agent with inhibitory and therapeutic potential for tumorigenesis[1]. Broccoli, cabbage, and other cruciferous family members contain the bioactive compound indole-3-carbinol (I3C). I3C is chemically converted from the bioactive compound to the main condensation product, 3.3'-diindolylmethane (DIM), in the aqueous and acidic in vivo gastric environment[2].

3,3'-diindolylmethane is an agent that has antiviral, anti-angiogenic, and antitumor effects[3–6]. Its different pleiotropic effects on cancer cells are shown in many studies[3,7,8]. 3,3'-diindolylmethane functions by inhibiting survival signals on cells and activating multiple death pathways simultaneously. It has been reported that women who consume high levels of cruciferous vegetables have a reduced risk of cervical, endometrial, and breast cancers[5,9,10]. 3,3'-diindolylmethane causes the disruption of cell proliferation in prostate, colon, breast, cervical, and pancreatic cancers that is mediated by stimulating multiple signaling pathways. These signaling pathways inhibit tumor cell migration, invasion, and metastasis and trigger apoptotic cell death[11–14]. 3,3'-diindolylmethane promotes apoptosis by stimulating caspase-3 activity and changing the ratio of Bcl-2/Bax expression[8]. On the other hand, DIM provides a strong immunomodulatory effect by triggering the interferon-gamma signaling pathway, which promotes interferon-gamma and cytokine production[2,6,11,15]. With this effect, DIM plays a chemotherapeutic role in viral diseases such as rotavirus-induced gastroenteritis, respiratory syncytial virus, and HPV infection[16]. Rouse et al., in their experimental study, demonstrated the ameliorative effect of DIM against autoimmune encephalomyelitis, with apoptosis caused by the activation of T cells[17]. Some studies have shown that DIM can attenuate acute liver failure by regulating microRNAs to target IRAK4 and suppress Toll-like receptor signaling, highlighting its potential to protect against liver damage[18]. Furthermore, DIM has been explored for its neuroprotective properties, promoting the formation of brain-derived neurotrophic factor (BDNF) and antioxidant enzymes via the TrkB/Akt pathway activation, which can protect against oxidative stress-induced apoptosis in neuronal cells[19]. This suggests that DIM may have applications in neuroprotection and potentially in neurodegenerative diseases.

On the other hand, there are concerns regarding the use of DIM. Some studies have suggested a possible association between DIM, pulmonary embolism, and deep venous thrombosis[20]. Moreover, studies have linked DIM to potential negative effects on estrogen and androgen physiology, while its effects on cancer risk remain unclear[21]. While DIM shows promise in various health aspects, it is crucial to consider potential negative effects, indicating the need for further investigation.

In various contexts, 3.3'-diindolylmethane (DIM) has been linked to increased reactive oxygen species (ROS). 3,3'-diindolylmethane has been shown to increase the release of ROS from mitochondria, which causes p21 to be increased in human breast cancer cells[22]. Additionally, murine peritoneal macrophage cultures have linked DIM to promoting ROS production, indicating its potential to stimulate immune function through ROS modulation[6]. Furthermore, by affecting lipid ROS levels, DIM induces ferroptosis in gastric cancer cells, suggesting a role in ROS-mediated cell death mechanisms[23]. Our research aimed to examine the impact of long-term use of diindolylmethane (DIM) on the cellular structure of rat kidney tissues.

Material and Method

Experimental Protocol

The study was approved by the Committee for Institutional Animal Care and Use of Bingöl University Local Board of Ethics (Decision no: 125915/2023).

Thirty-six male, 16 weeks old, and weighing 220–260g Wistar albino rats were used in the study. The animals were obtained from Bingöl University Medical Experimental Research and Application Centre (Bingöl, Türkiye). The rats were housed at 40–60% humidity, at 24°C, with a 12 h light-dark cycle, and at standard laboratory conditions with available pellet chow (Bayramoğlu Food Co., Erzurum, Türkiye) and ad libitum water. Rats were divided into four equal groups. The control group received only corn oil (Oruçoğlu Co., Afyonkarahisar, Türkiye), which was given by oral gavage. The DIM 10 group received 10 mg/kg DIM (Sigma-Aldrich Co., St. Louis, MO, USA), DIM 50 received 50 mg/kg DIM, and DIM 100 received 100 mg/kg DIM that dissolved in corn oil by oral gavage[16].

Experimental groups received DIM for 53 days. At the end of the experiment, all rats were euthanized with sevoflurane inhalation anesthesia, and the kidney tissues were removed for histopathologic examinations.

Histopathological Analysis

Tissue Processing

We first fix the kidney tissue samples in an immersion solution (10% formalin) for 72 hours to stabilize cellular proteins and structures. Later, we dehydrated the kidney tissues with increasing alcohol concentrations to ensure dehydration and prevent shrinkage. We then passed the samples through the Xylol series for clearing and transparency. Finally, we passed the samples through the paraffin series and embedded them in paraffin wax (Agar, Cambridge, UK). The embedded tissue blocks were sectioned at 4 µm thickness using a microtome (Leica RM2125RT).

Hematoxylin-eosin Staining

We deparaffinized the tissue sections in a series of xylene, then rehydrated them using descending alcohol concentrations. We immersed the sections in Mayer's hematoxylin solution for five minutes. We rinsed the tissue sections and immersed them in a weak acid-alcohol solution for one to two seconds to remove excess dye. Next, we immersed the tissue sections in the eosin Y solution for one minute. After staining with eosin and rinsing, we dehydrated the sections by gradually increasing the alcohol concentration to eliminate excess water. Later, we cleared the tissue sections in xylene to make them transparent and suitable for mounting. We mounted the dehydrated and cleared sections onto slides using entellan, a mounting medium. We placed a coverslip over the sections. We then examined the tissue sections under a light microscope (Nikon Eclipse i50, Tokyo, Japan).

Masson-trichome Staining

Like H&E staining, paraffin-embedded tissue sections were deparaffinized in xylene and rehydrated through descending alcohol concentrations. Sections were first stained with Weigert's iron hematoxylin solution for eight minutes. After rinsing, we differentiated excess hematoxylin dye in an acid-alcohol solution for one to two seconds. We rinsed the tissue sections and stained them with Biebrich Scarlet-Acid Fuchsin solution. Afterward, we treated the sections with a phosphomolybdic acid solution for five minutes to eliminate the excess acid dye from the tissue. Finally, the sections were stained with an aniline blue solution. We dehydrated the tissue sections using an increasing alcohol concentration series, cleared them in xylene, mounted them onto glass slides, and cover-slipped them using a mounting medium. We then examined the tissue sections under a light microscope (Nikon Eclipse i50, Tokyo, Japan).

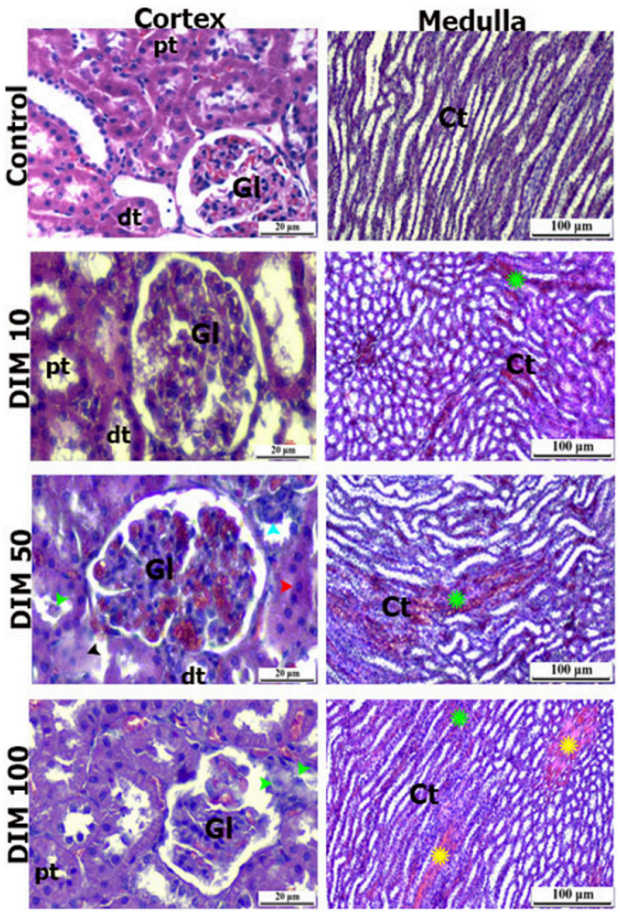

Figure 1. *Hematoxylin and eosin staining of kidney tissues of all groups,* ***Gl:*** *glomerulus,* ***dt:*** *distal tubule,* ***pt:*** *proximal tubule,* ***ct:*** *collecting tubules,* ***green star:*** *congestion in collecting tubules,* ***yellow star:*** *cast formation in Henle loops,* ***black arrowhead:*** *cellular swelling (finding of necrosis),* ***green arrowhead:*** *nuclear fragmentation (finding of apoptosis),* ***blue arrowhead:*** *a group of tubuler cells is experiencing cell and nuclear shrinkage (finding of apoptosis),* ***red arrowhead:*** *intense eosinophilic staining (finding of apoptosis).*

Results

Histopathological Results

In the histopathologic examinations, the kidney tissues of the control group had normal histological structures (Figures 1 and 2). 3,3'-diindolylmethane 10 group showed some destruction of the normal architecture of kidneys. There was mild glomerular and

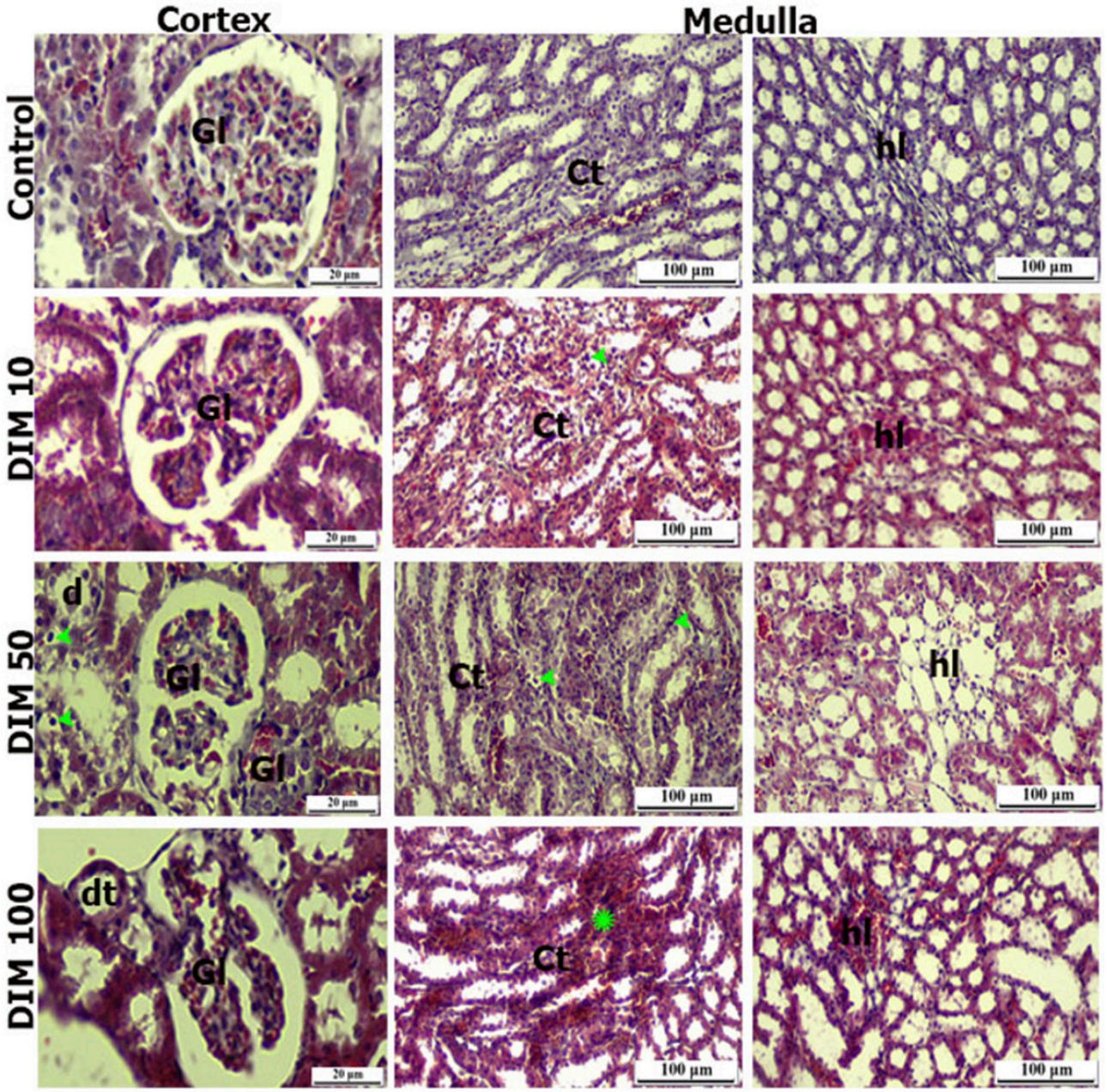

Figure 2. *Masson's trichrome staining of kidney tissues of all groups,* ***Gl:*** *glomerulus,* ***dt:*** *distal tubule,* ***ct:*** *collecting tubules,* ***hl:*** *Henle loop,* ***green arrowhead:*** *inflammatory cell,* ***green star:*** *necrotic and fibrotic change.*

tubular damage, interstitial inflammation, cast formation of Henle loops, and congestion in rat kidneys. Besides this, apoptotic changes in renal tubular cells and glomeruli were remarkable (Figures 1 and 2). In kidney tissues of the DIM 50 group, there was moderate glomerular and tubular damage, interstitial inflammation, cast formation of Henle loops, and congestion. Different from the DIM 10 group, there was swelling in renal tubular cells and dilatations in Henle loops (Figures 1 and 2). Kidney tissues of the DIM 100 group had a large amount of destruction of the normal architecture of kidneys. This group had extensive glomerular and tubular damage, interstitial inflammation, cast formation of Henle loops, and congestion. In addition to DIM 10 and 50 group findings, cast formation was seen in renal tubulars. Moreover, interstitial fibrous tissue formation and necrosis were significant in this group (Figures 1 and 2).

Discussion

3.3'-Diindolylmethane (DIM) is a compound found in cruciferous vegetables, known for its potential health benefits and cancer prevention[24]. It may also help in immune system support, anti-inflammatory properties, and detoxification[24–27]. However, more research is needed to understand its mechanisms of action and potential benefits fully. This study aimed to investigate the effects of DIM on rat kidney tissues at the cellular level.

The findings of studies related to DIM and kidneys argue that DIM has protective and healing effects on the kidney[28–31]. However, our study findings contradict the previous studies. Therefore, it is necessary to clearly explain the reason or reasons for which our findings are based.

Our study findings showed that DIM causes adverse changes in kidney tissue, from dose-dependent apoptosis to necrosis. So, what could be the reason for the study findings we obtained, while some studies contradict our findings and argue that DIM has protective and healing effects on the kidney? What can trigger apoptosis and necrosis at the tissue and cell level? Studies have demonstrated that DIM inhibits the growth and invasion of cancer cells, induces apoptosis, and suppresses inflammatory responses[32]. Roh et al.[33] suggested that DIM induced apoptosis in testicular tissue. Roh et al.[33] found that 3.3′-diindolylmethane causes immunotoxicity in neonatal mice by inducing apoptosis in splenocytes. Goldberg et al. discovered that modified versions of 3.3′-diindolylmethane (DIM) with a ring substitution cause programmed cell death and necrosis in both androgen-dependent and androgen-independent prostate cancer cells[34]. Ye et al. reported that 3.3'-diindolylmethane potentiates tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis of gastric cancer cells. Reactive oxygen species (ROS) can affect cell fate by promoting apoptosis. Simon et al.[35] showed that ROS significantly induces apoptosis under physiological and pathological conditions. Gach et al.[36] suggested that ROS promotes the initiation of apoptosis. Higuchi et al.[37] reported that caspase 3-like protease could induce ROS to cause both apoptosis and necrosis. Furthermore, Morgan et al.[38] suggested that necrotic cell death depended on the ROS. Xue et al.[6,39] showed increased malondialdehyde (MDA) levels mediated by DIM, suggesting that DIM induces ROS to increase MDA subsequently. Tripathi et al.[40] reported that ROS could affect cell fate by triggering T cells for apoptosis. The dose-dependent apoptotic and necrotic changes in our findings may be due to oxidative stress caused by increased DIM-mediated ROS.

Our study showed that DIM causes dose-dependent damage, including changes from interstitial inflammation to fibrotic connective tissue. In contrast to our findings, Xia et al.[29] reported that DIM reduced kidney damage. They detected decreased numbers of vimentin, α-SMA, fibronectin, and collagen I-positive cells, whereas they also reported increased numbers of E-cadherin-positive cells from immunohistochemical examinations. This study suggests that DIM treatment reduced interstitial fibrosis by suppressing local fibroblast activation. Martínez-Klimova et al.[41] reported that DIM prevented epithelial-to-mesenchymal transition in their study. Apart from these two studies, no others in the literature show if DIM causes interstitial inflammation and fibrosis in the kidney tissue.

In our study, a dose-dependent cast formation was observed in the renal tissue of DIM application, starting from the Henle loops and progressing to the renal tubules. Leibelt et al.[39] reported that numerous large hyaline casts were found in the kidney tubules of the DIM treated group, in their study. This single study examined only one negative DIM effect on kidney tissues in the literature. Our study supports the findings of Leibelt et al.[39] but different from Leibelt et al.[39], hyaline cast formations are also seen in the Henle loops and begin first in the Henle loops in low doses and progress to the renal tubules with increasing DIM dose. Furthermore, our study showed many histopathological changes that were negative effects of DIM treatment.

Conclusion

A wealth of research in the literature explores DIM's protective and advantageous effects on different types of tissues. Nevertheless, our study findings indicate that long-term use of DIM significantly increases the induction of histopathological alterations. The histological alterations in rat kidney tissue may be attributed to increased reactive oxygen species (ROS) mediated by DIM. To gain a deeper understanding, additional investigations involving biochemical, molecular, and ultrastructural analyses are required.

Declaration of Interest

The author declares that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

Self-funded.

Authors' Contributions

Study concept and design: SNP. Creation of experimental groups and experimental practices: S. N. P, SYY, H. G. Histopathological Analyzes: S. N. P, SYY, H. G, TD. Evaluating the results and writing the manuscript: S. N. P, SYY, H. G, TD.

Ethical Approval

Study approval was obtained from the Committee for Institutional Animal Care and Use of Bingöl University Local Board of Ethics (Decision no: 125915 on 05 October 2023).

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Determining the Impact of the Covid-19 Pandemic on Depression, Anxiety and Stress Levels in Cancer Patients

Covid-19 Pandemisinin Kanser Hastalarında Depresyon Kaygı ve Stres Durumlarına Etkisinin Belirlenmesi

Neriman Yukselturk Simsek[1], Ayten Demir[2]

[1]Gulhane Training and Research Hospital; [2]Ankara University, Faculty of Nursing, Ankara, Türkiye

ABSTRACT

Aim: *This study aims to determine the level of anxiety, depression and stress symptoms in patients receiving cancer treatment and monitoring during the coronavirus disease 2019 (COVID-19) pandemic.*

Methods: *This cross-sectional study was conducted among patients who applied to the oncology outpatient clinic between June 15 and July 15, 2021, and met the inclusion criteria. The impact of COVID-19 and its association with patient descriptive characteristics were examined using a 13-question survey. The relationship with stress, anxiety, and depression was evaluated using the Depression, Anxiety, and Stress Scale (DASS-21). Number, percentage, chi-square, and independent samples t-tests were performed in the statistical analysis stage of the study.*

Results: *It was observed that most of the individuals who participated in the study were between 44 and 56 years old, and there was no statistically significant relationship between their gender, employment status, change in the treatment plan, difficulty in getting to the hospital, disease stage and whether they received active treatment (p>0.05). It was determined that patients who were afraid of contracting COVID-19 from hospital staff during the outbreak (68.60%) said that their treatment plan had changed due to COVID-19 (58.97%) did not receive active treatment. It was observed that there was no statistically significant difference in the anxiety, depression and stress symptom levels of individuals receiving active treatment according to demographic characteristics (p>0.05).*

Conclusion: *During the coronavirus pandemic, individuals who were anxious about the progression of the disease were found to have higher levels of anxiety, depression and stress. Based on this information, it is recommended that patients' perspectives be included in treatment guidelines for the COVID-19 pandemic.*

Key words: *cancer; COVID-19; anxiety; depression; stress*

ÖZET

Amaç: *Bu çalışmanın amacı, kanser tedavisi ve takibi gören hastaların koranavirüs pandemisi (COVID-19) sürecinde anksiyete, depresyon ve stres belirti düzeylerini belirlemektir.*

Yöntem: *Bu kesitsel çalışma, 15 Haziran -15 Temmuz 2021 tarihleri arasında onkoloji polikliniğine başvuran ve dâhil edilme kriterlerini sağlayan 226 hasta ile yapıldı. COVID-19'un etkisi ve hastaların tanıtıcı özellikleri ile olan ilişkileri 13 maddelik anket sorusu ile incelendi. Katılımcıların anksiyete, depresyon ve stres belirti düzeyleri Depresyon, Anksiyete ve Stres Ölçeği (DASS-21) ile değerlendirildi. Çalışmanın istatistiksel analiz aşamasında sayı, yüzde, ki-kare ve bağımsız gruplar t testi uygulandı.*

Bulgular: *Araştırmaya katılan bireylerin çoğunlukla 44–56 yaş aralığında olduğu, cinsiyet, çalışma durumu, tedavi planının değişme durumu, hastaneye ulaşmada zorluk ve hastalık evresi ile aktif tedavi alma durumları arasında istatistiksel olarak anlamlı bir ilişki olmadığı görüldü (p>0,05). Salgın sürecinde hastane personelinden COVID-19 kapma korkusu yaşayan (%68,60) ve COVID-19'un tedavi planını değiştirdiğini (%58,97) belirten hastaların çoğunlukla aktif tedavi almadığı belirlendi. Aktif tedavi alan bireylerin ise anksiyete, depresyon ve stres belirti düzeylerinde demografik özelliklere göre istatistiksel olarak anlamlı bir farklılık olmadığı görüldü. (p>0,05).*

Sonuç: *Coronavirüs salgını sırasında hastalığın ilerlemesi konusunda endişeli olan bireylerin anksiyete, depresyon ve stres düzeylerinin daha yüksek olduğu görüldü. Bu bilgiler doğrultusunda COVID-19 pandemisindeki tedavi kılavuzlarına hastaların bakış açılarının da eklenmesi önerilir.*

Anahtar kelimeler: *kanser; COVID-19; endişe; depresyon; stres*

İletişim/Contact: *Neriman Yukselturk Simsek, Gulhane Egitim ve Arastirma Hastanesi, General Dr. Tevfik Saglam Cd., 06010 Etlik, Ankara, Türkiye* • ***Tel:*** *0536 322 65 41* • ***E-mail:*** *nyukselturk007@hotmail.com* • ***Geliş/Received:*** *16.03.2024* • ***Kabul/Accepted:*** *12.10.2024*

ORCID: *Neriman Yükseltürk Şimşek: 0000-0002-4611-6661* • *Ayten Demir: 0000-0002-5677-2347*

Introduction

The Coronavirus Pandemic 2019 (COVID-19) has led to significant changes in healthcare services worldwide[1]. Current evidence suggests that severe illness following COVID-19 infection may occur in older people, those with suppressed immune systems, and those with chronic diseases. Therefore, it is known that cancer patients, especially those undergoing systemic cancer treatments, are more susceptible to COVID-19 infection[2–3]. A study found that cancer patients have higher rates of intensive care unit admissions and COVID-19-related mortality compared to other patients[4]. This is thought to be due to the need for immunosuppressive treatment or more frequent clinical follow-up of patients on active treatment[3]. Considering these factors, both healthcare workers and patients face uncertainties. During this period, delays in chronic disease care and treatment have also been reported[1,3]. As a result, the pandemic poses significant challenges for those undergoing anti-cancer treatment[5–6].

In addition, cancer patients have additional psychological challenges in coping with a cancer diagnosis during the pandemic. This situation has shown that patients experience problems related to anxiety, depression, and stress[7–8]. Furthermore, treatment delays due to the pandemic, prevention of face-to-face clinic visits, or increased uncertainty with restrictions in daily life have also led to various psychological disorders such as anxiety, depression, and panic disorder[9–10].

Cancer is a group of diseases whose diagnosis, treatment, follow-up and consequences need to be considered in great detail. Therefore, international communities and committees have developed guidelines for oncologists. However, these guidelines are based solely on expert opinions. Similarly, there is currently no scientific evidence regarding the impact of COVID-19 on oncological care and the opinions of cancer patients[11].

Changes in cancer care due to the pandemic, uncertainties such as treatment delays, have contributed to fears experienced by cancer patients[12–14]. Healthcare is not limited to controlling and saving the lives of patients infected with COVID-19. It must also provide medical care for patients with other health issues unrelated to COVID-19. During the pandemic, there are limited studies on the impact of COVID-19 and patients' opinions on treatment and its effect on stress, anxiety and depression. Analyzing the negative effects of the pandemic from the patients' perspective is crucial for guiding healthcare professionals and finding solutions to problems. This unique situation will allow the evaluation of the additional psychological state caused by the COVID-19 pandemic in cancer patients.

This study aimed to analyze the impact of the COVID-19 pandemic on patients' views on cancer treatment and monitoring during the COVID-19 pandemic on their levels of stress, anxiety, and depression.

Research Questions:

This research will be undertaken to answer the following questions:

1. Is there a relationship between individuals' active treatment status and demographic data?
2. Is there a relationship between individuals' perspectives on the COVID-19 pandemic and their active treatment status?
3. Is there a difference between individuals' anxiety, depression and stress symptom levels according to their active treatment status?

Materials and Methods

Study Design and Participants

The population of this cross-sectional study consisted of patients admitted to the oncology outpatient clinic of Gülhane Training and Research Hospital between June 15, 2021, and July 15, 2021. Cross-sectional studies are a research method that aims to examine the current status of a group at a specific time period. In this study, purposive sampling was selected. 284 patients were admitted to the oncology outpatient clinic during the specified period. These were patients already diagnosed with cancer and are following up on their treatment or check-up visits at the outpatient clinic during the period of the COVID-19 pandemic. If the same patient had multiple hospital admissions within the study dates, they were included only once. Four patients who did not meet the inclusion criteria, 32 patients who had repeat examinations, and 22 patients who did not agree to participate were excluded from the study, resulting in a total of 226 patients included in the study.

Inclusion Criteria:

- had a diagnosis of cancer,
- age of 18–80 years,
- were able to read, write, and understand Turkish

Exclusion Criteria:

- severe cognitive impairment,
- severe psychiatric disorder,
- unable to speak Turkish

Ethics Committee Approval

Gülhane Training and Research Hospital of the University of Health Sciences Clinical Research Ethics Committee (26.05.2021) approved the conduct of the study with protocol decision 2021/31. Gülhane Training and Research Hospital Medical Specialization Education Board, with the decision number E-50487468–131 and Ministry of Health scientific research permit form number 2020–07–14T12_51_00, allowed the study to be carried out. After providing the necessary information about the study, informed consent was obtained from participating patients.

Data Collection

The research data were collected using the COVID-19 Impact Assessment Questionnaire and the Depression Anxiety Stress Scale (DASS-21).

The researcher collected the study's data using a face-to-face interview technique. Brief information about the study was given to cancer patients who applied to the Oncology Outpatient Clinic of Gülhane Training and Research Hospital between June 15 and July 15, 2021. Administering the data collection forms took approximately 20 minutes.

COVID-19 Impact Assessment Questionnaire

To assess the psychological impact of the pandemic on cancer patients, a 13-item questionnaire form was created based on literature[2,4,12,14]. The questionnaire consisted of two parts. The first part included five items related to patients' demographic characteristics: age, gender, employment status, type of illness, and disease stage. The second part consisted of eight items related to the impact of COVID-19, including experiencing difficulties in accessing the hospital during the COVID-19 outbreak, concerns about the progression of the disease, fear of contracting COVID-19 from patients or hospital staff, the impact of the pandemic on seeking treatment or follow-up at the hospital, changes in treatment plans due to the pandemic and how they were changed, and the impact of income loss due to the pandemic. Patients were asked to answer the questions using "yes," "no," and "multiple-choice" options.

Depression Anxiety Stress Scale (DASS-21)

The Depression Anxiety Stress Scale (DASS) was developed by Lovibond and Lovibond[15]. The validity and reliability study of the short form of the scale was conducted by Yilmaz, Boz, and Arslan[16]. The scale is designed to measure symptoms of depression, anxiety and stress and consists of 21 items. The score range of 0–9 on the scale indicates normal depression, 0–7 indicates normal anxiety, and 0–14 indicates normal stress. 7 items measure each dimension of depression, stress, and anxiety. The responses in the scale are coded as 0 "never," 1 "sometimes," 2 "often," and 3 "always." Items 1, 3, 10, 13, 15, 19, 20 are related to anxiety, items 2, 6, 8, 11, 14, 16, 21 are related to depression, and items 4, 5, 7, 9, 12, 17, 18 are related to stress.

Descriptive statistics and Cronbach's Alpha reliability coefficient results obtained from the total and sub-dimensions of the Depression, Anxiety and Stress Scale for the individuals who participated in the study are as follows. The mean scores for the anxiety subscale were 1.16, for the depression subscale were 1.47, for the stress subscale were 1.33, and for the total DASS scores were 1.32. The skewness and kurtosis coefficients of the individuals' depression, anxiety, and stress scale subscales and total scores were within the ±2 range, indicating that the data was suitable for univariate normal distribution[17]. On the other hand, Cronbach's Alpha coefficients for the depression, anxiety, and stress scale subscales and overall scores were 0.626, 0.681, 0.719, and 0.838, respectively.

Statistical Analysis

Statistical analyses were performed with Statistical Package for Social Sciences (IBM Statistical Package for Social Sciences (SPSS) program version) software, 20.0 version. During the statistical analysis stage of the study, chi-square association tests, descriptive statistics, and mean comparison tests were performed. In the first stage, chi-square tests were used to compare

the frequencies of the variables obtained by census in terms of groups of a variable. In the second stage, descriptive statistics values and Cronbach's alpha reliability coefficient results were presented based on the general and subscale scores of the scale used in the study. The descriptive statistics included mean (M), standard deviation (SD), minimum (Min), maximum (Max), skewness (Skew), and kurtosis (Kurt) values. At this stage, the skewness and kurtosis coefficients of the scores were examined, and it was found that these coefficients were within the ±2 range. According to this finding, the measurement scores were suitable for univariate normal distribution. For the measurement scores that were suitable for univariate normal distribution, independent samples t-tests were conducted to compare two independent groups, and ANOVA tests were conducted to compare three or more independent groups. Tukey test was used for multiple comparisons of the groups found to be significant in the ANOVA test[17–18].

Results

This study involved 226 patients. The ratio of males to females was 132:94, and the mean age was 51 (range, 28–74). Table 1 provides the basic demographic characteristics and active treatment status of the patients included in the study.

Table 2 presents the experiences of the patients included in the study with the COVID-19 pandemic and their active treatment status. The majority of individuals who experienced difficulty in reaching the hospital during the COVID-19 outbreak (60.71%) were concerned about the progression of their illness (69.48%), feared contracting COVID-19 from other patients or hospital staff (68.60%) and indicated that they had changed their treatment plans (58.97%) mostly did not receive active treatment. It was determined that most of the individuals (76.87%) whose admission to the hospital for treatment or follow-up was not affected during the COVID-19 pandemic did not receive active treatment.

Table 1. *Relationship between individuals' demographic characteristics and active treatment status*

Variable	Receiving Active Treatment n (%)	Not Receiving Active Treatment n (%)	Sum n (%)	p
Age groups				
18–30 years	3(42.86)	4(57.14)	7(3.10)	0.344[F]
31–43 years	15(39.47)	23(60.53)	38(16.81)	
44–56 years	31(30.39)	71(69.61)	102(45.13)	
57–69 years	17(23.61)	55(76.39)	72(31.86)	
70–82 years	1(14.29)	6(85.71)	7(3.10)	
Gender				
Male	35(26.52)	97(73.48)	132(58.41)	0.283[P]
Female	32(34.04)	62(65.96)	94(41.59)	
Employment status				
Yes	32(30.19)	74(69.81)	106(46.90)	0.982[P]
None	35(29.17)	85(70.83)	120(53.10)	
Disease stage				
1	5(26.32)	14(73.68)	19(8.41)	0.965[F]
2	44(30.56)	100(69.44)	144(63.72)	
3	18(29.03)	44(70.97)	62(27.43)	
4	0(0.00)	1(100.00)	1(0.44)	
Cancer Type				
Lung	8(24.24)	25(75.75)	33(14.6)	0.973[F]
Breast	5(15.16)	28(84.85)	33(14.6)	
Colon	5(23.81)	16(76.19)	21(9.3)	
Hematologic cancers	12(38.7)	19(61.29)	31(13.7)	
Urogenital cancers	7(18.91)	30(81.08)	37(16.4)	
Other cancers	20(28.16)	51(71.83)	71(31.42)	

F: Fisher's test; Y: Yates correction; P: Pearson Chi-square test

Table 2. *Relationship between individuals' perspectives on the COVID-19 pandemic and their status of receiving active treatment*

Variable	Receiving Active Treatment n (%)	Not Receiving Active Treatment n (%)	Sum n (%)	p
Experiencing any difficulty in accessing the hospital during the COVID-19 outbreak				
Yes	11(39.29)	17(60.71)	28(12.39)	0.331[Y]
None	56(28.28)	142(71.72)	198(87.61)	
Concern about disease progression during the COVID-19 outbreak				
Yes	65(30.52)	148(69.48)	213(94.25)	0.354[F]
None	2(15.38)	11(84.62)	13(5.75)	
Fear of contracting COVID-19 infection from other patients or hospital staff				
Yes	65(31.40)	142(68.60)	207(91.59)	0.100[Y]
None	2(10.53)	17(89.47)	19(8.41)	
Impact of the COVID-19 period on seeking treatment or follow-up at the hospital				
Same	31(23.13)	103(76.87)	134(59.29)	0.001[P]
Less	15(27.78)	39(72.22)	54(23.89)	
More	21(55.26)	17(44.74)	38(16.81)	
Effect of the COVID-19 outbreak on changing treatment plans				
Yes	16(41.03)	23(58.97)	39(17.26)	0.129[Y]
None	51(27.27)	136(72.73)	187(82.74)	
Type of change in treatment plan				
Surgery postponed	1(16.67)	5(83.33)	6(15.38)	0.052[F]
Chemotherapy postponed	12(57.14)	9(42.86)	21(53.85)	
Radiotherapy postponed	0(0.00)	5(100.00)	5(12.82)	
Chemotherapy ended earlier than planned	2(28.57)	5(71.43)	7(17.95)	
Consideration of factors contributing to the decision to change treatment plans				
Concern about COVID-19 exposure risk	9(50.00)	9(50.00)	18(46.15)	0.291[F]
Hospital/clinic rules related to COVID-19	1(14.29)	6(85.71)	7(17.95)	
Transportation concern	5(35.71)	9(64.29)	14(35.90)	
Loss of job/income due to COVID-19				
Yes	10(45.45)	12(54.55)	22(9.73)	0.143[Y]
None	57(27.94)	147(72.06)	204(90.27)	

F: Fisher's test; Y: Yates correction; P: Pearson Chi-square test

Table 3 shows the results of the comparison of individuals' depression levels based on their active treatment status, demographic characteristics, and perspectives on the COVID-19 pandemic. A statistically significant difference was found in the depression levels of individuals receiving and not receiving active treatment, based on their concerns about the progression of the disease during the COVID-19 pandemic, changing their treatment plan and their employment status ($p<0.05$).

Table 4 displays the results of the comparison of individuals' anxiety levels based on their active treatment status, demographic characteristics, and perspectives on the pandemic. It was found that there was a statistically significant difference in the anxiety levels of individuals who were not receiving active treatment compared to their anxiety about the progression of the disease during the COVID-19 pandemic ($p<0.05$).

Table 5 compares individuals' stress levels based on their active treatment status, demographic characteristics, and perspectives on the pandemic. It was determined that the stress levels of individuals not receiving active treatment were statistically significant regarding whether they were admitted to the hospital for treatment or follow-up during this period ($p<0.05$).

Table 3. *Comparison of individuals' depression levels based on their status of receiving active treatment, demographic characteristics, and perspectives on the pandemic*

Variable	Receiving Active Treatment	Not Receiving Active Treatment	General
Age groups			
18–30 years	1.71±0.14	1.39±0.54	1.53±0.43
31–43 years	1.64±0.30	1.57±0.43	1.59±0.38
44–56 years	1.47±0.41	1.46±0.43	1.46±0.42
57–69 years	1.48±0.39	1.41±0.43	1.43±0.42
70–82 years	1.71±0.00	1.21±0.31	1.29±0.34
Test Request	-	1043	1472
p	-[N]	0.387[A]	0.212[A]
Gender			
Male	1.52±0.38	1.41±0.45	1.44±0.43
Female	1.52±0.37	1.52±0.38	1.52±0.37
Test Request	0001	-1.65	-1512
p	0.999[T]	0.101[T]	0.132[T]
Employment status			
Yes	1.59±0.36	1.53±0.43	1.55±0.41
None	1.46±0.38	1.38±0.41	1.40±0.40
Test Request	1513	2178	2657
p	0.135[T]	0.031[T]	0.008[T]
Disease stage			
1	1.54±0.37	1.27±0.41	1.34±0.41
2	1.51±0.39	1.47±0.43	1.48±0.42
3	1.56±0.35	1.45±0.41	1.48±0.40
4	-	1.71±0.00	1.71±0.00
Test Request	-	-	-
p	-[N]	-[N]	-[N]
Experiencing any difficulty in accessing the hospital during the COVID-19 outbreak			
Yes	1.43±0.40	1.27±0.52	1.33±0.48
None	1.54±0.37	1.47±0.41	1.49±0.40
Test Request	0912	-1865	1928
p	0.365[T]	0.064[T]	0.055[T]
Concern about disease progression during the COVID-19 outbreak			
Yes	1.54±0.37	1.47±0.41	1.49±0.40
None	1.07±0.10	1.10±0.48	1.10±0.44
Test Request	-	2855	3443
p	-[N]	0.005[T]	0.001[T]
Fear of contracting COVID-19 infection from other patients or hospital staff			
Yes	1.53±0.37	1.46±0.41	1.48±0.40
None	1.14±0.20	1.37±0.52	1.35±0.50
Test Request	-	0815	1389
p	-[N]	0.416[T]	0.166[T]
Impact of the COVID-19 period on seeking treatment or follow-up at the hospital			
Same	1.52±0.40	1.50±0.43	1.50±0.42
Less	1.53±0.40	1.40±0.42	1.44±0.41
More	1.52±0.32	1.25±0.35	1.40±0.35
Test Request	0009	2837	1195
p	0.991[A]	0.062[A]	0.305[A]
Effect of the COVID-19 outbreak on changing treatment plans			
Yes	1.52±0.40	1.43±0.44	1.47±0.42
None	1.52±0.37	1.45±0.42	1.47±0.41
Test Request	-0095	-0251	-0095
p	0.924T	0.802T	0.924T
How the treatment plan changed			
Surgery postponed	1.43±0.00	1.57±0.23	1.55±0.21
Chemotherapy postponed	1.46±0.38	1.11±0.50	1.31±0.46
Radiotherapy postponed	-	1.54±0.51	1.54±0.51
Chemotherapy ended earlier than planned	2.07±0.10	1.63±0.13	1.76±0.24
Test Request	-	2549	2300
p	-[N]	0.085[A]	0.094[A]
Consideration of factors contributing to the decision to change treatment plans			
Concern about COVID-19 exposure risk	1.76±0.30	1.56±0.39	1.66±0.36[A]
Hospital/clinic rules related to COVID-19	1.00±0.00	1.62±0.12[A]	1.53±0.26
Transportation concern	1.26±0.29	1.11±0.50[B]	1.16±0.43[B]
Test Request	-	3995	7168
p	-[N]	0.034[A]	0.002[A]
Loss of your job/income or primary source of income due to COVID-19			
Yes	1.44±0.43	1.45±0.44	1.45±0.42
None	1.54±0.36	1.45±0.43	1.47±0.41
Test Request	0729	-0027	-0274
p	0.469[T]	0.979[T]	0.784[T]

A-B: No difference between groups with the same letter; T: Independent samples t-test; A: ANOVA test; N: The test result could not be calculated due to insufficient observations.

Table 4. *Comparison of individuals' anxiety levels based on their status of receiving active treatment, demographic characteristics, and perspectives on the pandemic*

Variable	Receiving Active Treatment	Not Receiving Active Treatment	General
Age groups			
18–30 years	1.43±0.14	0.79±0.36	1.06±0.44
31–43 years	1.30±0.36	1.18±0.40	1.23±0.39
44–56 years	1.14±0.37	1.15±0.38	1.15±0.37
57–69 years	1.24±0.32	1.13±0.30	1.15±0.31
70–82 years	1.86±0.00	1.02±0.41	1.14±0.49
Test Request	-	1226	0474
p	-[N]	0.302[A]	0.755[A]
Gender			
Male	1.20±0.32	1.11±0.35	1.14±0.34
Female	1.25±0.40	1.17±0.38	1.19±0.39
Test Request	-0468	-0964	-1196
p	0.641[T]	0.337[T]	0.233[T]
Employment status			
Yes	1.23±0.34	1.14±0.39	1.17±0.38
None	1.22±0.38	1.13±0.33	1.15±0.35
Test Request	0082	0231	0256
p	0.935[T]	0.818[T]	0.798[T]
Disease stage			
1	1.23±0.24	1.03±0.35	1.08±0.33
2	1.19±0.31	1.16±0.36	1.17±0.34
3	1.32±0.49	1.11±0.36	1.17±0.41
4	-	1.14±0.00	1.14±0.00
Test Request	-	-	-
p	-	-	-
Experiencing any difficulty in accessing the hospital during the COVID-19 outbreak			
Yes	1.38±0.41	1.08±0.46	1.19±0.46
None	1.19±0.35	1.14±0.35	1.16±0.35
Test Request	1557	-0707	0521
p	0.124[T]	0.481[T]	0.603[T]
Concern about disease progression during the COVID-19 outbreak			
Yes	1.22±0.36	1.16±0.34	1.18±0.35
None	1.21±0.30	0.77±0.41	0.84±0.42
Test Request	-	3656	3429
p	-	<0.001[T]	0.001[T]
Fear of contracting COVID-19 infection from other patients or hospital staff			
Yes	1.23±0.36	1.14±0.35	1.17±0.36
None	1.00±0.00	1.08±0.44	1.08±0.41
Test Request	-	0605	1078
p	-	0.546[T]	0.282[T]
Impact of the COVID-19 period on seeking treatment or follow-up at the hospital			
Same	1.17±0.35	1.15±0.36	1.16±0.36
Less	1.25±0.39	1.09±0.37	1.13±0.38
More	1.29±0.35	1.13±0.34	1.22±0.35
Test Request	0813	0460	0659
p	0.448[A]	0.632[A]	0.518[A]
Effect of the COVID-19 outbreak on changing treatment plans			
Yes	1.27±0.38	1.15±0.35	1.20±0.36
None	1.21±0.36	1.13±0.36	1.15±0.36
Test Request	0708	0219	0708
p	0.480[T]	0.827[T]	0.480[T]
How the treatment plan changed			
Surgery postponed	1.14±0.00	1.09±0.26	1.10±0.23
Chemotherapy postponed	1.27±0.43	0.98±0.44	1.15±0.45
Radiotherapy postponed	-	1.43±0.27	1.43±0.27
Chemotherapy ended earlier than planned	1.21±0.10	1.17±0.16	1.18±0.14
Test Request	-	2000	0923
p	-	0.146[A]	0.440[A]
Consideration of factors contributing to the decision to change treatment plans			
Concern about COVID-19 exposure risk	1.29±0.32	1.29±0.29	1.29±0.29
Hospital/clinic rules related to COVID-19	0.57±0.00	1.14±0.22	1.06±0.30
Transportation concern	1.34±0.44	0.98±0.44	1.11±0.46
Test Request	-	1772	1395
p	-	0.194[A]	0.261[A]
Loss of your job/income or primary source of income due to COVID-19			
Yes	1.13±0.20	0.98±0.26	1.05±0.24
None	1.24±0.38	1.15±0.36	1.17±0.37
Test Request	0907	-1.59	-1581
p	0.368[T]	0.114[T]	0.115[T]

A-B: No difference between groups with the same letter; T: Independent samples t-test; A: ANOVA test; N: The test result could not be calculated due to insufficient observations.

Table 5. *Comparison of individuals' stress levels based on their status of receiving active treatment, demographic characteristics, and perspectives on the pandemic*

Variable	Receiving Active Treatment	Not Receiving Active Treatment	General
Age groups			
18–30 years	1.71±0.62	1.04±0.61	1.33±0.67
31–43 years	1.37±0.47	1.25±0.35	1.30±0.40
44–56 years	1.30±0.49	1.32±0.41	1.32±0.43
57–69 years	1.33±0.49	1.37±0.46	1.36±0.47
70–82 years	1.86±0.00	1.21±0.50	1.31±0.52
Test Request	-	0893	0163
p	-[N]	0.470[A]	0.957[A]
Gender			
Male	1.38±0.47	1.31±0.42	1.33±0.44
Female	1.32±0.51	1.34±0.43	1.33±0.46
Test Request	0559	-0500	-0100
p	0.578[T]	0.618[T]	0.921[T]
Employment status			
Yes	1.41±0.51	1.36±0.38	1.37±0.42
None	1.30±0.47	1.28±0.46	1.29±0.46
Test Request	0877	1138	1448
p	0.384[T]	0.257[T]	0.149[T]
Disease stage			
1	1.20±0.48	1.19±0.38	1.20±0.40
2	1.34±0.50	1.32±0.42	1.32±0.45
3	1.43±0.45	1.36±0.45	1.38±0.45
4	-	1.29±0.00	1.29±0.00
Test Request	-	-	-
p	-[N]	-[N]	-[N]
Experiencing any difficulty in accessing the hospital during the COVID-19 outbreak			
Yes	1.23±0.47	1.20±0.45	1.21±0.45
None	1.38±0.49	1.33±0.42	1.35±0.44
Test Request	-0882	-1212	-1470
p	0.381[T]	0.227[T]	0.143[T]
Concern about disease progression during the COVID-19 outbreak			
Yes	1.38±0.47	1.35±0.42	1.36±0.43
None	0.57±0.40	0.96±0.41	0.90±0.42
Test Request	-	2966	3686
p	-	0.003[T]	<0.001[T]
Fear of contracting Corona infection from other patients or hospital staff			
Yes	1.36±0.49	1.33±0.43	1.34±0.45
None	1.21±0.51	1.23±0.38	1.23±0.38
Test Request	-	0952	1066
p	-[N]	0.343[T]	0.288[T]
Impact of the COVID-19 period on seeking treatment or follow-up at the hospital			
Same	1.38±0.47	1.37±0.41[A]	1.37±0.42
Less	1.34±0.37	1.29±0.45	1.30±0.43
More	1.31±0.59	1.09±0.42[B]	1.21±0.53
Test Request	0009	3299	2005
p	0.991[A]	0.040[A]	0.137[A]
Effect of the COVID-19 outbreak on changing treatment plans			
Yes	1.28±0.44	1.36±0.49	1.33±0.46
None	1.38±0.50	1.31±0.42	1.33±0.44
Test Request	-0051	0491	-0051
p	0.959[T]	0.624[T]	0.959[T]
How the treatment plan changed			
Surgery postponed	1.43±0.00	1.40±0.27	1.40±0.25
Chemotherapy postponed	1.26±0.33	1.06±0.55	1.18±0.44
Radiotherapy postponed	-	1.69±0.43	1.69±0.43
Chemotherapy ended earlier than planned	1.79±0.51	1.43±0.39	1.53±0.42
Test Request	-	2195	2842
p	-[N]	0.120[A]	0.052[A]
Consideration of factors contributing to the decision to change treatment plans			
Concern about COVID-10 exposure risk	1.49±0.37	1.57±0.46	1.53±0.41[A]
Hospital/clinic rules related to COVID-19	1.14±0.00	1.40±0.17	1.37±0.18
Transportation concern	1.11±0.27	1.06±0.55	1.08±0.46[B]
Test Request	-	2970	5031
p	-[N]	0.073[A]	0.012[A]
Loss of your job/income or primary source of income due to COVID-19			
Yes	1.23±0.43	1.36±0.48	1.30±0.45
None	1.37±0.49	1.32±0.42	1.33±0.44
Test Request	-0870	0314	-0340
p	0.388[T]	0.754[T]	0.734[T]

A-B: No difference between groups with the same letter; T: Independent samples t-test; A: ANOVA test; N: The test result could not be calculated due to insufficient observations.

Discussion

In this study, the perspectives of cancer patients on COVID-19 were evaluated along with their levels of anxiety, depression, and stress. The study found that patients not receiving active treatment were more concerned about the progression of the disease and afraid of contracting COVID-19 during the pandemic.

COVID-19 has rapidly spread globally, affecting numerous communities. In addition to its high mortality and morbidity rates, strict isolation measures and quarantines have created serious fear and anxiety in society. Those with chronic illnesses, especially cancer patients, have been particularly affected by the COVID-19 pandemic[9].

During the pandemic, ensuring the safe delivery of cancer treatments was a priority at the institution where the research was conducted. The main goal was to minimize patients' exposure to COVID-19 without compromising the effectiveness of treatment. Treatment plans for patients were determined by oncologists, considering factors such as the patient's disease status, treatment goals, age, and comorbidities. In-hospital admissions for treatment were limited to reduce the risk of hospital-acquired infections, thereby managing expected capacity issues. While most patients receiving active treatment continued their treatments without changes, appointments for patients not receiving active treatment were postponed for a certain period to reduce the risk of infection. Our study observed that most individuals who experienced difficulties in reaching the hospital during the COVID-19 outbreak feared COVID-19. The fear of disease progression can explain this concern. Given the capacity issues and priority to triaging patients receiving active treatment, this fear is reasonable.

In our study, the impact of patients' demographic characteristics on anxiety, stress, and depression levels was not found to be statistically significant. However, some studies have shown that women report more anxiety, stress, and fear compared to men with cancer[19,20]. Kang et al.[21] demonstrated in their study that COVID-19 has a greater psychological impact on young people, suggesting that personal and psychological risk factors may play a role. The predominance of male participants, mostly in the middle-aged group in our study, supported these findings. In our research, it was observed that the majority of individuals who lost their job/income or primary source of income due to COVID-19 did not receive active treatment. In contrast, a study by Romito et al.[20] reported that only 5% of patients experienced financial difficulties. In some regions of Türkiye, men tend to be the main earners in their families. This situation may have further increased the concerns of individuals who did not receive active treatment, as they faced not only the disease but also the loss of their income source during the COVID-19 pandemic, affecting them socially and economically.

In our study, a statistically significant difference was found in the depression levels during the COVID-19 pandemic ($p<0.05$). In light of these findings, the depression levels of individuals who were concerned about the progression of the disease during the COVID-19 pandemic were higher than those of individuals who were not concerned. The stress levels of individuals concerned about the progression of the disease during the COVID-19 pandemic were also higher compared to those not concerned. Additionally, individuals who decided to change their treatment plan due to hospital/clinic rules related to COVID-19 had higher levels of depression, stress, and anxiety. Consistent with our study, Qian et al.[22] showed in their study that more than half of cancer patients experienced anxiety, depression, or fear related to COVID-19. In a study by Wang et al.[23] it was found that 62% of cancer patients experienced anxiety related to the pandemic, which was one-third higher than the general population. In another study, depressive symptoms were reported in 31% of cancer patients[20]. In a similar study by Gebbia et al.[24] the needs and fears of 446 cancer patients were assessed via text messages, revealing significant fear and negative thoughts among the patients. In another study conducted in the Netherlands, many patients reported concerns about the impact of the COVID-19 pandemic on their follow-up and treatment[11]. These studies support the data from our study, showing that those concerned about the progression of the disease had higher depression scores. This could be due to the lack of face-to-face communication with staff in oncology departments, the risk of receiving less information about treatment side effects and cancer symptoms, and concerns about interruptions in antineoplastic treatments due to reduced outpatient visits. This situation could also be explained by the burden of cancer and the need to cope with the challenges brought by the pandemic.

In this study, the impact of the pandemic on the stress, anxiety, and depression levels of cancer patients was evaluated. At the end of the study, it was observed that cancer patients who had previously received treatment but were currently not undergoing active treatment were worried

about the progression of their disease. This may be due to their knowledge of a poor prognosis and their concern that their disease might worsen further during the COVID-19 pandemic. Additionally, patients who were more informed about chemotherapy or other treatments may have been more concerned about COVID-19 infection due to receiving explanations about the immunosuppressive effects of oncological treatments and the possibilities of complications resulting from COVID-19.

Conclusion

In conclusion, the COVID-19 pandemic has had inevitable consequences for health systems. This study showed that most patients are generally quite worried about their oncological treatment or follow-up. In this study, it was found that there was a statistically significant difference between the depression, stress and anxiety levels of all patients and their concerns about the progression of the disease ($p<0.05$). These findings suggest that existing guidelines on treating oncology patients and/or delaying or deferring treatment should be discussed in detail and customized according to disease condition, stage and treatment goal. They also suggested that patients should benefit from enhanced psycho-oncological support during a prolonged outbreak.

Limitations and Recommendation

The study's potential limitation is that it did not include detailed questions about patients' clinical data and cancer histories. Data were collected based on patient self-report. One of the most important limitations of the study is the lack of a healthy control group. Additionally, the fact that the study was conducted only in one hospital and the relatively small sample size are other limitations.

Future research should identify additional factors that contribute to heightened stress, anxiety, and depression levels among cancer patients and how these factors may vary with vaccination status, social media and other important determinants of health.

Ethics Committee Approval

Gülhane Training and Research Hospital of the University of Health Sciences Clinical Research Ethics Committee (26.05.2021) approved the conduct of the study with protocol decision 2021/31. After the necessary information about the study was provided, informed consent was obtained from the patients participating in the study.

Conflict of Interest

No conflict of interest was declared by the authors.

Author Contributions

Concept – NYŞ, AD; Supervision – AD; Materials – NYŞ; Data Collection and/or Processing – NYŞ; Analysis and/ or Interpretation – AD; Writing –NYŞ.

Peer-review

Externally peer-reviewed.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Investigation of the Effects of Formaldehyde Exposure on BDNF and FGF23 Levels in Kidney Tissues of Rats

Formaldehit Maruziyetinin Sıçanların Böbrek Dokularında BDNF ve FGF23 Düzeyleri Üzerine Etkilerinin Araştırılması

Feyza Aksu, Ramazan Fazıl Akkoç, Ahmet Kavaklı

Department of Anatomy, Faculty of Medicine, Fırat University, Elazıg, Türkiye

ABSTRACT

Aim: *Formaldehyde (FA) is a highly toxic chemical agent that can easily turn into gas at room temperature. It has a wide range of uses. Anatomists, pathologists, and histologists are frequently exposed to FA due to their areas of practice. Brain-derived neurotrophic factor is a protein secreted from the central nervous system and peripheral tissues that support the differentiation and growth of newly formed nerve cells and synapses. It is proven to be expressed in renal glomeruli. FGF23 is a bone-derived hormone that regulates phosphate and calcium homeostasis. It is a critical component of the abnormal mineral metabolism that complicates chronic kidney disease. This study investigates the effect of FA exposure on 3BDNF and FGF23 levels in rat kidney tissue.*

Material and Methods: *The study included 14 male Spraque-Dawley rats (7 animals in each group) aged between 8 and 10 weeks. During the experiment, the rats in the control group were exposed to natural atmospheric air in a glass bell jar. The rats in the FA group inhaled 10 ppm 8 h/day FA in a glass bell jar 5 days a week (excluding Saturdays and Sundays) for 4 weeks. At the end of the experiment, the rats were decapitated, and their kidneys were removed. Kidney tissues were homogenized and tested using the ELISA method.*

Results: *It was observed that BDNF levels significantly decreased and FGF23 levels significantly increased in the kidney tissues of FA-exposed rats compared to the control group.*

Conclusions: *This study first demonstrated the effect of exposure to FA on FGF23 and BDNF levels in rat kidney tissue. This study will contribute to future research on oxidants that increase FGF23 and BDNF expression or antioxidants that target their reduction. Accordingly, we consider it will also contribute to public health.*

Key words: *formaldehyde exposure; FGF23; BDNF; kidney; rat*

ÖZET

Amaç: *Formaldehid (FA) oda sıcaklığında gaz haline rahatlıkla geçebilen, geniş kullanım alanı olan, oldukça zehirli kimyasal bir ajandır. Anatomist, patolog ve histologlar işi gereği FA'ya devamlı maruz kalmaktadır. BDNF, hem merkezi sinir sistemi hem de periferik dokulardan salgılanan, yeni oluşan sinir hücresi ve sinapsların farklılaşmasını, büyümesini destekleyen; böbrek glomerüllerinde eksprese edildiği gösterilmiş bir proteindir. FGF23, fosfat ve kalsiyum homeostazisini düzenleyen kemik kaynaklı bir hormondur. Kronik böbrek hastalığını karmaşık hale getiren anormal mineral metabolizmasının kritik bir bileşeni olarak ortaya çıkmıştır. Bu çalışmanın amacı FA maruziyetinin sıçan böbrek dokusunda BDNF ve FGF23 düzeylerini nasıl etkilendiğini araştırılmasıdır.*

Gereç ve Yöntem: *Çalışmada Spraque-Dawley cinsi 8–10 haftalık 14 adet (her grupta yedi hayvan) erkek sıçan kullanıldı. Kontrol grubundaki sıçanlar deney süresince cam fanus içerisinde normal atmosfer havasına maruz bırakıldı. Formaldehid grubundaki sıçanlar deney süresince cam fanus içerisinde solunum yolu ile haftada beş gün (cumartesi, pazar hariç) dört hafta 10 ppm sekiz saat/gün FA'ya maruz bırakıldı. Deney sonunda hayvanlar dekapite edilip böbrekleri alındı. Böbrek dokuları homojenize edilip elisa yöntemi ile çalışıldı.*

Bulgular: *FA'ya maruz kalan sıçanların böbrek dokularında BDNF düzeylerinin kontrol grubuna kıyasla önemli ölçüde düştüğü bulunurken, FGF23 düzeylerinin ise kontrol grubuna göre istatistiksel olarak anlamlı arttığı tespit edildi.*

Sonuç: *Bu çalışmada FA maruziyetinin sıçan böbrek dokusunda FGF23 ve BDNF düzeylerine etkisi ilk kez gösterilmiştir. Bu çalışmanın, ileride yapılacak FGF23 ve BDNF ekspresyonunu artıran oksidan veya azaltılmasını hedef alan antioksidanların araştırılmasına fayda sağlayacağı kanaatindeyiz. Böylelikle halk sağlığına katkıda bulunacağı kanaatindeyiz.*

Anahtar kelimeler: *formaldehid maruziyeti; FGF23; BDNF; böbrek; rat*

İletişim/Contact: *Feyza Aksu, Department of Anatomy, Faculty of Medicine, Fırat University, Elazıg, Türkiye* • ***Tel:*** *0536 720 55 02* • ***E-mail:*** *feyza-aksu@hotmail.com* • ***Geliş/Received:*** *18.03.2024* • ***Kabul/Accepted:*** *08.07.2024*

ORCID: *Feyza Aksu: 0000-0001-8595-0010* • *Ramazan Fazıl Akkoç: 0000-0002-0559-8932* • *Ahmet Kavaklı: 0000-0003-0754-8901*

Kafkas J Med Sci 2024; 14(3):242–245
doi: 10.5505/kjms.2024.10576

Introduction

Formaldehyde (FA), a common metabolite found in all mammals, is a colorless gas highly soluble in water, which has a pungent odor and is an irritant in pure form. FA is an essential chemical with broad commercial use. It has acute and chronic effects on humans. FA is used as a tissue protector and bactericide in medicine. Occupational exposure applies to individuals and users at manufacturers of products containing FA[1,2]. FA is known to have adverse effects on the skin, eyes, liver, kidneys, and the nervous, reproductive, digestive, and respiratory systems[3,4].

The brain-derived neurotrophic factor (BDNF) is a protein secreted by the central nervous system and peripheral tissues and belongs to the neurotrophin family. It supports the differentiation and growth of newly formed nerve cells and synapses while maintaining the viability of existing neurons. It was first isolated from pig brain tissue in 1982 and detected in human blood in 1995. Brain-derived neurotrophic factor protein is reported in the brain areas where learning, memory, and high-level thinking functions are performed, namely the hippocampus, cortex, and prefrontal cortex[5]. Brain-derived neurotrophic factor is also peripherally synthesized by vascular endothelial cells, platelets, leukocytes, monocytes, and T and B lymphocytes. In addition, BDNF mRNA expression has been reported in the kidney, heart, lung, bladder, large vessels, spleen, visceral epithelial cells, and smooth muscle cells[6]. By immunofluorescent staining, BDNF is found to be expressed in glomeruli of normal human kidney sections, especially in podocytes and parietal epithelial cells, to a lesser extent. Brain-derived neurotrophic factor is suggested to serve as a new potential biomarker for glomerular kidney injury[7]. Studies have also revealed that BDNF has an anti-apoptotic effect in the kidney[8,9].

The fibroblast growth factor 23 (FGF23) gene encodes a 32 kDa glycoprotein of 251 amino acids secreted by osteocytes and bone marrow stromal cells. Unlike other autocrine FGFs, it circulates freely as an endocrine hormone due to the absence of heparin-binding motifs[10]. The FGF23 signaling in target organs is mediated by binding to FGF receptors (FGFRs), prominently expressed in the kidney, parathyroid gland, bones, and other tissues[11]. FGF23 is a bone-derived endocrine hormone that regulates phosphate and calcium homeostasis. FGF23 is a central regulator of normal mineral metabolism, is resistant to vitamin D, and causes hypophosphatemic rickets syndromes. It is also a critical component of the abnormal mineral metabolism that complicates chronic kidney disease[12,13]. Studies have reported a gradual increase in FGF23 levels in chronic kidney disease[14,15].

No study was found in the recent literature review to investigate the effects of FA exposure on BDNF and FGF23 levels in kidney tissue. This study aimed to investigate these effects in rat kidney tissue.

Material and Method

This study was initiated after obtaining approval from the Firat University Local Ethics Committee for Animal Experiments (2024/01–04). The study included 14 male Spraque-Dawley rats (7 in the control group and 7 in the FA-treated group) aged between 8 and 10 weeks. The rats were housed in a 100×50×20 cm glass bell jar during the 4-week experiment. Control group: The rats in this group were exposed to natural atmospheric air in a glass bell jar during the experiment. Formaldehid group: The rats in this group inhaled 10 ppm 8 h/day FA in a glass bell jar 5 days a week (excluding Saturdays and Sundays) for 4 weeks during the experiment[16]. At the end of the experiment, the rats were decapitated, and their kidneys were removed. Renal tissue supernatants were prepared by harvesting 100 mg of fresh kidney tissue from each tissue and homogenized in phosphate buffer[17]. Homogenates were centrifuged at 4°C for 5 minutes, and the supernatants were transferred to Eppendorf tubes. In the renal tissue supernatants, the BDNF and FGF23 levels were determined by the ELISA method using rat BDNF and FGF23 ELISA kits supplied by Sunred Biological Technology Co. Ltd. (Shanghai, CHINA) as specified in the manufacturer's catalog (catalog no; BDNF: 201–11–0477, FGF23:201–11–0171) and per the study procedures.

Statistical Analysis

All values were expressed as mean ± standard error. The conformity with the normal distribution was examined through Shapiro Wilk test. Statistical evaluation was performed using the independent sample t-test. For all analyses, $P < 0.05$ was considered to be statistically significant. The IBM Statistical Package for Social Sciences (SPSS) program version 22.0 for Windows (licensed by Firat University, Elazig, Türkiye), was used for data analysis.

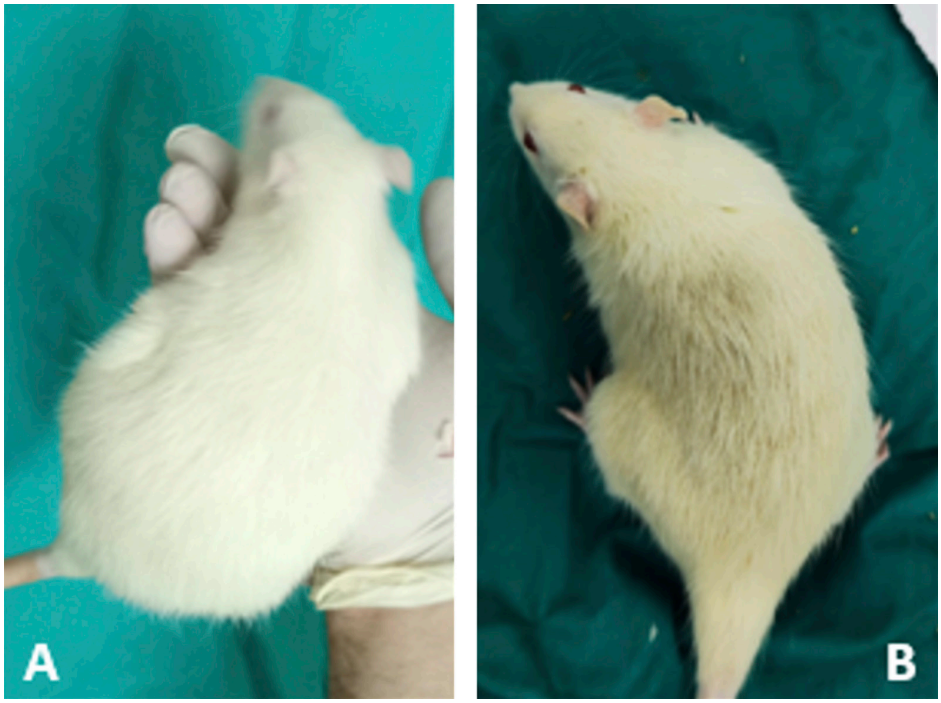

Figure 1. *(A) Control group rat with white fur. (B) FA group rat with yellow fur.*

Results

The FA exposure value measured at different hours during the study period was 10.20±0.22 ppm. During the four-week study, the hairs of the rats in the control group remained white, while yellowing was observed in the hairs of the rats in the FA group (Figure 1). In addition, the rats in the FA group exhibited slower movements and more frequent nose cleaning, blinking, and licking.

The BDNF levels were significantly decreased, while FGF23 levels were significantly increased in the kidney tissues of the rats exposed to FA compared to the control group ($p<0.05$) (Table 1).

Table 1. *Kidney tissue FGF23 and BDNF levels*

	FGF23 (pg/ml) median ± standard error	BDNF (ng/ml) median ± standard error
FA group	209.3± 63.57*	1.07± 0.08**
Control group	123.1± 33.83	1.45± 0.12

* When compared with the control group p=0.003
** When compared with the control group p=0.032

Discussion

In the body, most of the BDNF production occurs in the nervous system. Brain-derived neurotrophic factor is also secreted from the kidneys. Studies have provided evidence that BDNF may be a potential marker in patients with chronic kidney disease. In patients with chronic kidney disease, BDNF may be another marker associated with insulin resistance, sarcopenia, depression, oxidative stress, and inflammation[18]. A previous study reported that BDNF expression was suppressed by cisplatin in normal rat kidneys[13]. Ge et al.[19] investigated the effect of FA toxicity on brain BDNF levels and reported that exposure to FA caused a statistically significant decrease in brain BDNF levels. Similar to the study conducted by Ge et al., exposure to FA significantly decreased BDNF levels in kidney tissue in the present study. The literature review revealed no other study investigating the effect of exposure to FA on BDNF levels in kidney tissue.

FGF23, first discovered in the mouse brain, is an endocrine hormone secreted by bone and stimulates phosphate excretion in the kidneys. In the literature, increased levels of FGF23 were reported in chronic kidney disease, leading to left ventricular hypertrophy and autosomal dominant hypophosphatemic rickets[20,21]. Previous studies reported that FGF23 levels increased as the glomerular filtration rate decreased in children and adults with chronic kidney disease[14,22]. In the present study, exposure to FA led to a statistically

significant increase in FGF23 levels in kidney tissue. No other study was found in the literature on the effect of exposure to FA on FGF23 levels in kidney tissue.

Conclusion

The literature review revealed no study investigating the effect of exposure to FA on FGF23 and BDNF levels in kidney tissue. This study's data showed that exposure to FA decreased BDNF levels and increased FGF23 expression in kidney tissue. This study will contribute to future research on oxidants that increase FGF23 and BDNF expression or antioxidants that target their reduction.

Acknowledgment

We would like to thank Firat University Department of Biostatistics and Medical Informatics faculty member Assoc. Dr. Mehmet Onur Kaya.

Financial Disclosure

The work was supported by the Firat University (2024/01–04).

Conflicts of Interests

The authors report no conflicts of interest.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Assessment of the Diagnostic and Prognostic Value of the Serum Netrin-1 Level in Patients Presenting the Emergency Department with Sepsis

Acil Servise Sepsis Nedeniyle Başvuran Hastalarda Serum Netrin 1 Düzeyinin Tanı ve Prognoz Üzerine Etkisi

Mustafa Onder Gonen[1], Basar Cander[2], Ramazan Koylu[3], Huseyin Mutlu[4], Ramiz Yazici[5], Oznur Koylu[6]

[1]Department of Emergency Medicine, Meram State Hospital, Konya; [2]Department of Emergency Medicine, Bezmialem Vakif University, Istanbul; [3]Department of Emergency Medicine, Konya City Hospital, Konya; [4]Department of Emergency Medicine, Medical School, Aksaray University, Aksaray; [5]Department of Emergency Medicine, Kanuni Sultan Suleyman Training and Research Hospital, Istanbul; [6]Department of Biochemistry, Konya City Hospital, Konya, Türkiye

ABSTRACT

Aim: *Sepsis affects approximately 50 million people across the world each year and causes 11 million deaths. Many biomarkers have been investigated for the early diagnosis of sepsis and patient responses to infection and treatment, as well as to help clinicians predict risk and plan treatment. Netrin-1 plays an important role by directing the migration of neutrophils, particularly monocytes, in inflamed tissue. This study aimed to determine whether Netrin-1 was an effective marker in the diagnosis, treatment follow-up, and prognosis evaluation of patients with sepsis and septic shock.*

Material and Method: *This observational and prospective study was conducted at the emergency department with 121 individuals over 18, including 71 patients diagnosed with sepsis and 50 healthy volunteers. The patients were further evaluated in two subgroups: sepsis and septic shock. Blood samples were taken from the patient and control groups at the time of presentation and on the third day. Netrin-1 levels were examined in both groups.*

Results: *The netrin-1 levels of the patients with sepsis and septic shock upon presentation to the hospital were significantly higher than the control group (p<0.0001). However, there was no significant difference between the netrin-1 levels measured at the time of presentation and on the third day in the sepsis and septic shock groups (p: 0.0522 and 0.0786, respectively). Neither the presentation nor the third-day netrin-1 level had a statistically significant correlation with mortality (p=0.075 and 0.254, respectively).*

Conclusion: *Our results showed that netrin-1 was an effective biomarker for diagnosing sepsis and septic shock. However, it was not a risk factor for mortality or clinical risk scores in patients with these conditions.*

Key words: *netrin-1; sepsis; emergency department*

ÖZET

Amaç: *Sepsis; dünya çapında yıllık yaklaşık 50 milyon kişiyi etkilemekte ve 11 milyon kişinin ölümüne neden olmaktadır. Sepsisin erken tanısı, hastanın enfeksiyona tepkisini, tedavinin yanıtını ve klinisyenin hasta riskini tahmin etmesine ve tedavinin planlanmasına yardımcı olmak için birçok biyobelirteç araştırılmıştır. Netrin-1, nötrofillerin ve özellikle de iltihaplı dokudaki monositlerin göçünü yönlendirerek önemli bir rol oynar. Bu çalışmada sepsis ve septik şoklu hastalarda Netrin –1'in tanıda, tedavi takibinde ve prognozu göstermede etkili bir belirteç olup olmadığını belirlemeyi amaçladık.*

Materyal ve Metod: *Bu gözlemsel ve prospektif çalışma, acil serviste 18 yaş üstü 71 sepsis tanısı konan hasta ve 50 gönüllü olmak üzere 121 hasta ile yapılmıştır. Hastalar sepsis ve septik çok olarak ikiye ayrıldı. Hasta ve kontrol grubundan ilk başvuruda ve 3. gün kan örnekleri alındı. Her iki grupta da Netrin-1 düzeyleri çalışıldı.*

Bulgular: *Sepsis ve septik şoklu hastaların hastaneye geliş netrin-1 düzeyleri kontrol grubuna göre anlamlı olarak yüksek tespit edildi (p<0,0001). Ancak sepsis ve septik şoklu hastaların acil servise geliş ve 3. gün netrin –1 düzeyleri arasında anlamlı fark yoktu (sırasıyla 0,0522 ve 0,0786) Hem acil servise başvuru Netrin-1 düzeyinin hem de 3. gün netrin-1 düzeyinin istatistiksel olarak mortalite ile ilişkisi yoktu. (p sırasıyla: 0,075, 0,254)*

Sonuçlar: *Sonuçlarımız Netrin-1 'in sepsis ve septik şok tanısında etkili bir biyobelirteç olduğunu göstermektedir. Ancak netrin –1'in sepsis ve septik şok tanılı hastalarda mortalitenin bir gösterisi ve klinik risk skorlamaları için risk faktörü olmadığını gösterdik.*

Anahtar kelimeler: *netrin-1; sepsis; acil servis*

İletişim/Contact: *Huseyin Mutlu, Department of Emergency Medicine, Medical School, Aksaray University, Aksaray, Türkiye* • ***Tel:*** *0 505 349 32 63* • ***E-mail:*** *hmutlu70@hotmail.com* • ***Geliş/Received:*** *23.03.2024* • ***Kabul/Accepted:*** *08.05.2024*

ORCID: *Mustafa Önder Gönen: 0000-0002-6059-4387 • Başar Cander: 0000-0002-3308-5843 • Ramazan Köylü: 0000-0002-7685-8340 • Hüseyin Mutlu: 0000-0002-1930-3293 • Ramiz Yazıcı: 0000-0001-9210-914x • Öznur Köylü: 0000-0002-6888-6309*

Kafkas J Med Sci 2024; 14(3):246–251
doi: 10.5505/kjms.2024.28566

Introduction

Sepsis is defined as life-threatening organ dysfunction caused by an irregular host response to infection[1]. Sepsis affects approximately 50 million individuals worldwide each year and causes 11 million deaths[2–3]. Therefore, it has become one of the leading causes of critical illness and mortality[3]. The World Health Organization emphasizes sepsis as a health priority due to the growing incidence of this condition every year and its significant societal and economic consequences[3–6]. Despite significant advances in understanding the pathophysiology, making a diagnosis, and providing supportive treatment options for sepsis, the mortality rates associated with sepsis and septic shock in emergency departments and intensive care units remain very high[4–8]. Relevant guidelines recommend using scoring systems, such as the acute physiology and chronic health evaluation (APACHE)-II and the sequential organ failure assessment (SOFA) for the early diagnosis of sepsis and septic shock and predicting mortality[5–9]. Prompt identification and implementation of suitable therapy within the initial hours following the onset of sepsis enhance treatment outcomes[6–9]. Numerous biomarkers have been investigated for the early diagnosis of sepsis and patients' responses to infection and treatment, as well as to help clinicians predict risk and plan treatment[6–13].

Netrins are laminin-like proteins that direct axonal migration and neuronal growth in the central nervous system[14–16]. Netrin-1 has been found to possess chemoattractive or chemo-repulsive properties in various biological processes other than neuronal developmen[14–18]. In addition, netrin-1 has been shown to play an important role in cell adhesion, cell migration, proliferation, and cell survival in tissues[16–21]. In this context, netrin-1 has been reported to regulate organogenesis, angiogenesis, tumorigenesis, and inflammation[18–22]. As in neuronal development, netrin-1 plays an important role in the inflammation process by directing the migration of neutrophils and, in particular, monocytes in inflamed tissue[20–25]. Due to these characteristics of netrin-1, the level of this protein is likely to change infective conditions, such as sepsis and septic shock. Therefore, the current study aimed to determine whether netrin-1 was an effective marker for the diagnosis, treatment follow-up, and prognosis evaluation of patients with sepsis and septic shock.

Material and Method

Study Design and Participants

This observational and prospective study was conducted at the emergency medicine clinic of our hospital from October 2013 to March 2014 after receiving approval from the local ethics committee (approval number: 2013/24). Seventy-one patients with sepsis and 50 volunteers without any disease were included in the study. The control group included people over 18 without acute or chronic disease who came to the hospital for routine screening. Volunteer patients who met the criteria for sepsis or septic shock at the emergency department and were over the age of 18 were included in the sample. The classification of sepsis and septic shock was made according to the most recent definitions (Sepsis-3)[1.] Demographic characteristics, medical history, vital signs, laboratory findings, length of hospital stay, and mortality status were recorded for the patients admitted to the intensive care unit or inpatient ward from the emergency department. The Glasgow Coma Scale (GCS), APACHE II[22], and SOFA[21] scores were calculated to determine the severity of organ dysfunction and clinical condition. Patients with acute and/or chronic kidney disease, a history of cerebrovascular accident, neurological disease, liver failure, or malignancy, pregnant and breastfeeding women, and patients who died or were discharged within 72 hours after presenting to the emergency department were excluded from the study. All patients were treated according to the recommendations of the sepsis guidelines[1].

Laboratory Analysis

To evaluate netrin-1 levels, blood samples were taken within 15 minutes following presentation to the emergency department (day 1) and placed in tubes containing ethylenediaminetetraacetic acid on the third day after presentation. After centrifugation at 2.800 rpm for 20 min at 4°C, plasma samples were placed in Eppendorf tubes and stored at -80°C until analysis. Netrin-1 levels were measured in duplicate assays using enzyme-linked immunosorbent assay kits (MyBioSource MBS044526; MyBioSource, Inc., San Diego, California), and the results were expressed in picograms (pg) per mg.

The netrin-1 levels of the patients were compared according to routinely checked infection markers evaluated on the first and third days, clinical (GCS, APACHE-II, and SOFA) scores, treatment efficacy, and mortality status.

Statistical Analysis

The data obtained from the study were given as median (interquartile range) and mean ± standard deviation values. P values of <0.05 were considered statistically significant. Statistical analyses were undertaken using descriptive statistics, and non-parametric data were compared between groups using the Kruskal-Wallis and Mann-Whitney U tests. The data were transferred to electronic media and analyzed using the IBM Statistical Package for Social Sciences (SPSS) program version 15.0 software package. Numerical data were compared according to the outcome. Multivariate logistic regression analysis revealed the relationship between different parameters and outcomes. Sepsis findings and numerical parameters were evaluated using the Mann-Whitney U test, and the t-test was conducted.

Results

The study included 121 individuals: 71 (58.7%) were patients with sepsis or septic shock, and 50 (41.3%) were healthy controls. Male patients constituted 61.9% (44) of the case group and 56% of the control group. The mean age was 72±22 years for the case group and 67±21 years for the control group. In the evaluation of the case group, 27 (38%) patients were found to have sepsis, while 44 (62%) had septic shock. Table 1 shows the distribution of the demographic data for the case and control groups.

The distribution of the netrin-1 levels of the case and control groups is presented in Figure 1. The netrin-1 levels evaluated at the time of presentation to the hospital were significantly higher among the patients with sepsis and septic shock than the control group (p <0.0001). However, there was no significant difference between the presentation and third-day netrin-1 levels of the patients with sepsis and septic shock (p: 0.0522 and 0.0786, respectively). The laboratory values of the case and control groups are given in Table 2.

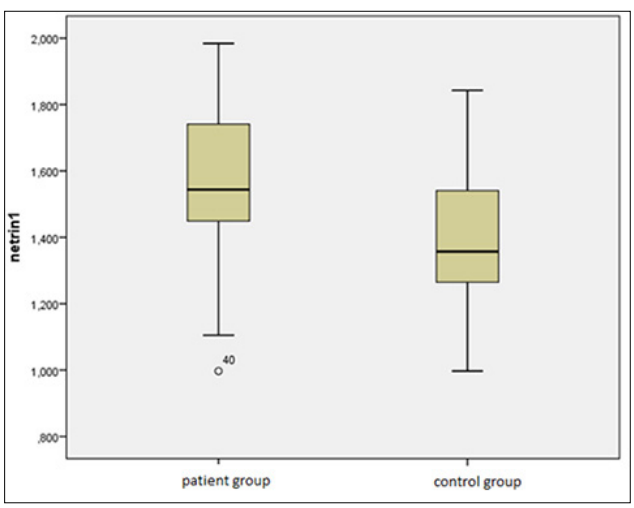

Figure 1. *Distribution of netrin-1 levels in patient and control groups.*

Table 1. *Clinical and demographic data of the sample*

Variables	Control group (n=50)	Case group (n=71)		P-value
Diagnosis [c]		Sepsis (n=27)	Septic shock (n=44)	
Age (years)	67 (21)	71±18	73±20	0.128
Male sex	28 (56%)	18 (25.3%)	26 (36.6%)	0.094
APACHE-II score (at presentation)		19.75±5.81	22.17±7.81	
SOFA (at presentation)		8.22±3.55	9.86±2.69	
GCS score (at presentation)		14.9		
Diabetes mellitus		8 (19.5%)	64 (32.7%)	0.096
Hypertension		13 (31.7%)	28 (14.3%)	0.007*
Coronary artery disease		32 (78.0%)	112 (57.1%)	0.013*
Cerebrovascular accident		11 (26.8%)	68 (34.7%)	0.331
Source of infection: abdomen[c]		11 (43.9%)	24 (25.5%)	0.018*
Source of infection: lungs[c]		10 (61.0%)	21 (25.0%)	<0.001*
Source of infection: soft tissue[c]		3 (61.0%)	2 (25.0%)	<0.001*
Survival status: survival[c]		20	15	<0.001*
Survival status: mortality[c]		1	29	
Treatment: mechanical ventilation[c]		2	22	0.004*
Type of infection: bacterial Gram-negative[c]		12	25	<0.001*
Type of infection: bacterial, Gram-positive[c]		8	17	0.007*
Type of infection: fungal[c]		4	8	0.246
Type of infection: unknown[c]		7	5	0.006*
Mixed infection (2 or more pathogens) [c]		8	9	<0.001*

[a]Mean ± standard deviation, [b]Median (first-third quartile), [c]Number (percentage) of patients. APACHE-II, acute physiology and chronic health evaluation-II; SOFA, sequential organ failure assessment, GCS: Glasgow Coma Scale.

Table 2. *Comparison of the first-day and third-day values of selected parameters between the study groups*

Parameters (evaluation time)	Control (C)	Sepsis (A)	Septic shock (B)	p (C vs. A)	p (C vs. B)	p (A vs. B)	Global p (ranked ANOVA)
Netrin-1 (pg/mL) (day 1)	1.36±0.19	1.57±0.19	1.61±0.28	**<0.0001**	**<0.0001**	0.0522	**<0.0001**
Netrin-1 (pg/mL) (day 3)		1.60±0.19	1.63±0.29			0.0786	
CRP (mg/L)	12.54±9.8	78.12±59.6	158±78.6	**<0.0001**	**<0.0001**	<0.0001	
PCT (µg/L)	0.04±0.01	3.9 (1.9–14.2)	20.9 (7.9–44.2)	**<0.0001**	**<0.0001**	<0.0001	
Lactate (pg/mL)	0.54±0.26	4.1 (3.9–9.1)	8.1 (3.9–19.1)	**<0.0001**	**<0.0001**	<0.0001	

ANOVA, analysis of variance, PCT, procalcitonin; CRP, C-reactive protein. Statistically significant values (p <0.05) are shown in bold.

Table 3. *Evaluation of parameters related to survival status*

	Survival status		
Parameters (evaluation time)	Survival	Mortality	P
Netrin-1 (pg/mL) (day 1)	1.57±0.19	1.61±0.28	0.075
Netrin-1 (pg/mL) (day 3)	1.57±0.19	1.61±0.28	0.254
CRP (mg/L) (day 1)	78.12±59.6	158±78.6	<0.001
PCT (µg/L) (day 1)	3.9 (1.9–14.2)	20.9 (7.9–44.2)	<0.001
Lactate (pg/mL) (day 1)	4.1 (3.9–9.1)	8. 1(3.9–19.1)	<0.001
APACHE-II score (at presentation)	17.45±5.91	23.47±7.94	<0.001
SOFA score (at presentation)	7.72±3.15	9.97±3.49	0.0098
GCS score (at presentation)	14.9(12–15)	13.8(7–15)	0.032

PCT, procalcitonin; CRP, C-reactive protein, APACHE II, acute physiology and chronic health evaluation-II; SOFA, sequential organ failure assessment, GCS: Glasgow Coma Scale. Statistically significant values (p <0.05) are shown in bold.

While the procalcitonin (PCT), C-reactive protein (CRP), and lactate levels and the APACHE II, SOFA, and GCS scores were associated with mortality in the presence of sepsis, neither the presentation nor the third-day netrin-1 level had a statistically significant relationship with mortality (p=0.075 and 0.254, respectively). The relationship of the remaining laboratory values and scoring systems with mortality is shown in Table 3. There was no correlation between netrin-1 levels and the APACHE II, SOFA, or GCS scores.

Discussion

Sepsis, a prevalent condition accompanied by life-threatening organ dysfunction as a result of irregular host response to infection, is responsible for high mortality and morbidity rates as well as decreased quality of life[4,13,28]. In this serious condition, biomarkers guide the diagnosis of infection, prognosis determination, treatment response, and/or clinicians' risk prediction[10–13,26]. Prominent biomarkers used in sepsis include PCT and CRP. In addition, the existing guidelines on the management of sepsis also mention that sepsis biomarkers can complement clinical assessment, and many researchers have investigated novel biomarkers in sepsis[13,27]. Therefore, in the current study, we investigated whether netrin-1 was an effective biomarker in predicting diagnosis, response to treatment, and prognosis in patients with sepsis. To our knowledge, this is the first study in the literature to explore the relationship between netrin-1 and sepsis.

This study revealed high netrin-1 levels in patients with sepsis and septic shock when they presented to the emergency department. However, these patients' netrin-1 levels did not increase in the following days. In addition, neither the presentation nor the third-day netrin-1 level had any significant relationship with mortality. There was also no significant correlation between netrin-1 and clinical scores, such as APACHE II and SOFA.

In the study conducted by Hwang et al. in 29618 intensive care patients, Apache and Sofa scores were significantly higher in those who died compared to those who did not die[28].

Likewise, Kramer et al. found that Apache score was an indicator of mortality in a study conducted on 5000

patients in 13 hospitals, and similar to these studies, we found that Apache and Sofa were an indicator of mortality[29]. Procalcitonin and CRP are the most frequently studied biomarkers in sepsis. They are considered potential sepsis biomarkers and are commonly used in antibiotic selection and evaluation of treatment in critically ill patients[6–12]. They have also been reported as an indicator of mortality[6–10]. In our study, we found that PCT and CRP are strong predictors of mortality in patients with sepsis.

In the pathogenesis of sepsis, there appears to be a complex response that combines pro-inflammatory and anti-inflammatory properties and occurs with impaired homeostasis[2–4,30]. During the initial hyperinflammatory response, organ dysfunction and hypoperfusion develop due to the migration of neutrophils and monocytes to the inflamed tissue and the intense release of inflammatory mediators[4–8,31,32]. Ly et al. reported that netrin-1 could modulate the migration of monocytes and leukocytes into and out of inflamed tissue[23]. In our study, the patients' high levels of netrin-1 at the time of presentation to the emergency department suggest that netrin-1 may have played a role in the hyperinflammatory response that occurred in the first period of sepsis.

Lui et al. found that the expression of netrin-1 and its receptor UNC5B decreased in rats with sepsis through an anti-inflammatory effect[33] In a study conducted by Tui et al. with 150 septic patients, the netrin-1 levels of the patients with sepsis who developed acute renal failure increased within the first 24 hours and then returned to normal[34]. Similarly, in the current study, we observed that netrin-1 increased through the pro-inflammatory effect in the early period of sepsis. Still, this increase was limited in the later period due to the anti-inflammatory effect.

Our study has some limitations. First, it was a single-center pilot study. Second, the relatively small number of patients in the sample may have caused a higher rate of B error. Therefore, the study's findings need to be confirmed with a larger case series. Lastly, netrin-1 levels were only measured in plasma, and further investigations should be undertaken to evaluate the infected tissue and urine levels of this protein.

Conclusions

Our results showed that netrin-1 was an effective biomarker for diagnosing sepsis and septic shock. However, netrin-1 was not a risk factor for mortality or clinical risk scores in these patients. Given the complex nature of sepsis, further studies are needed to investigate the combination of netrin-1 and other biomarkers reflecting different biological pathways in terms of their ability to predict early diagnosis and prognosis.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Investigation of the Protective Effect of Thymoquinone on the Livers of the Obese Rats Induced by a High-Fat Diet

Yüksek Yağlı Diyetle Oluşturulan Obez Sıçanların Karaciğerleri Üzerinde Timokinonun Koruyucu Etkisinin Araştırılması

Isinsu Alkan[1], Elfide Gizem Bakirhan[2], Nur Hande Tufek[3], Gulay Arslan[4], Berrin Zuhal Altunkaynak[5], Suleyman Kaplan[4]

[1]Department of Basic Science, Faculty of Dentistry, Nevsehir Haci Bektas Veli University, Nevsehir; [2]Department of Histology and Embryology, Faculty of Medicine, Adiyaman University, Adiyaman; [3]In-vitro Fertilization Center, Samsun Medical Park Hospital; [4]Department of Histology and Embryology, Faculty of Medicine, Ondokuz Mayis University, Samsun; [5]Department of Histology and Embryology, Faculty of Medicine, Okan University, Istanbul, Türkiye

ABSTRACT

Aim: *Obesity, which reduces the quality of life and forms the basis of many diseases, is caused by the imbalance between calories consumed and calories expended. The increase in calories consumed and fat accumulation in the body also cause many health problems. Obesity-related fatty liver and liver damage are one of these diseases. Fatty liver causes deterioration of functions and, accordingly, changes in homeostasis. Thymoquinone, an antioxidant, is preferred for liver damage. This study aimed to examine the protective effect of thymoquinone on liver damage caused by obesity.*

Materials and Methods: *In our study, 24 adult male Wistar albino rats were randomly divided into four groups (n: 6). Non-obese control (NOC) and non-obese thymoquinone (NTQ) groups were fed with standard chow; obese control (OC), obese-thymoquinone (OTQ) groups were fed with high-fat diet (40% of calories from fat) for 15 weeks. All treatment groups were given 10 mg/kg thymoquinone i. p. for six weeks. After the experimental study, the subjects were placed under intracardiac perfusion, and liver tissues were removed. Liver samples were subjected to tissue processing and cut into five µm thick sections for stereological and histopathological analyses. The obtained samples were stained with hematoxylin-eosin. The preparations were examined under a camera microscope, liver and sinusoid volumes were analyzed using the Cavalieri method, and hepatocyte counts were analyzed using the physical dissector method.*

Results: *In the volumetric analyses, an increase was observed in both total liver and sinusoid volumes in the OC group compared to the NOC group. Again, in both volume analyses, it was observed that the volumes in the OTQ groups decreased compared to the OC groups. In the examination of the hepatocyte count, an increase was observed in the OC group compared to the NOC group, while it was observed that it decreased in the OTQ groups compared to the OC groups. In addition, it was observed that the number of hepatocytes in the NTQ group increased significantly compared to the NOC group. When histopathological analyses were examined, ballooned hepatocytes were selected in the OC group, while this ballooning was not observed in the OTQ group.*

Conclusion: *Considering the analyses and the data obtained, thymoquinone may have a protective effect on obesity-induced liver damage. In addition, more comprehensive analyses should examine the pathways through which this effect progresses.*

Key words: *thymoquinone; liver; obesity; hepatoprotective effect; stereology*

ÖZET

Amaç: *Yaşam kalitesini düşüren ve birçok farklı hastalığın temelini oluşturan obezite alınan kalori ile harcanan kalori arasındaki dengenin bozulmasından kaynaklanmaktadır. Alınan kalorinin artması ve vücutta yağ birikimin gözlenmesi beraberinde birçok farklısağlık sorununu da ortaya çıkarmaktadır. Obeziteye bağlı karaciğer yağlanması ve karaciğer hasarı bu hastalıklardan biridir. Karaciğer yağlanması fonksiyonların bozulmasına ve buna bağlı olarak homestazın değişmesine sebebiyet vermektedir. Bir antioksidan olan thymoquinone karaciğer hasarlarında tercih edilmektedir. Bu çalışmada obeziteye bağlı olarak oluşan karaciğer hasarında thymoquinone'nun protektif etkisi incelenmesi amaçlanmıştır.*

Materyal ve Metot: *Çalışmamızda 24 adet yetişkin erkek Wistar albino sıçan rastgele dört gruba (n: 6) ayrıldı. Non-obez kontrol (NOC) ve Non-obez timokinon (NTQ) grubu standart yem ile beslenirken; obez kontrol (OC), obez-timokinon (OTQ) grupları15 hafta boyunca yüksek yağlı diyet (kalorinin %40'ı yağdan) ile beslendi. Tüm tedavi gruplarına altı hafta boyunca i.p. 10 mg/kg timokinon verildi. Deneysel çalışmadan sonra denekler intrakardiyak perfüzyona alındı ve karaciğer dokuları çıkarıldı. Karaciğer örnekleri doku işlemine tabi tutuldu ve stereolojik ve histopatolojik analizler için 5µm kalınlığında kesildi. Elde edilen örnekler hematoksilen-eozin ile boyandı. Preparatlar kameralı mikroskop altında incelendi ve karaciğer ve sinüzoid hacmi Cavaileri metodu ile hepatosit sayısı ise fiziksel disektör metodu ile analiz edildi.*

Bulgular: *Yapılan hacimsel analizlerde hem total karaciğer hem de sinüzoid hacimlerinde OC grubunda NOC grubuna göre artış izlendi. Yine her iki hacim analizinde de hacimlerin OTQ gruplarında OC gruplarına oranla azaldığı görüldü. Hepatosit sayısının incelenmesinde ise OC grubunda NOC grubuna oranla artış izlenirken; OTQ gruplarında OC gruplarına oranla azaldığı görüldü. Bunların yanı sıra NTQ grubundaki hepatosit sayısınında NOC grubuna göre önemli derecede arttığı izlendi. Histopatolojik analizler incelendiğinde ise OC grubunda balonlaşmış hepatositler seçilirken, OTQ grubunda bu balonlaşmaya rastlanamadı.*

Sonuç: *Yapılan analizler ve elde edilen veriler dikkate alındığında timokinonun obezite kaynaklı karaciğer hasarında protektif etkiye sahip olabileceği düşünülmektedir. Bunun yanı sıra bu etkinin hangi yolaklar üzerinden ilerlediği daha kapsamlı analizler ile incelenmelidir.*

Anahtar kelimeler: *timokinon; karaciğer; obezite; hepatoprotektif etki; stereoloji*

İletişim/Contact: *Isinsu Alkan, Department of Basic Science, Faculty of Dentistry, Nevsehir Haci Bektas Veli University, Nevsehir, Türkiye* • ***Tel:*** *0554 617 42 89* • ***E-mail:*** *isinsuaydin@gmail.com* • ***Geliş/Received:*** *19.04.2024* • ***Kabul/Accepted:*** *22.11.2024*

ORCID: *Işınsu Alkan: 0000-0002-9819-7766 • Elfide Gizem Bakırhan: 0000-0002-7401-2317 • Hande Tüfek: 0000-0002-5481-0610 • Gülay Arslan: 0000-0002-2981-2462 • Berrin Zuhal Altunkaynak: 0000-0001-7513-0094 • Süleyman Kaplan: 0000-0003-1477-5002*

Introduction

Obesity is an excessive or abnormal accumulation of body fat in adipose tissue that leads to health problems. It is measured by body mass index (BMI) percentile[1]. A BMI above 30 is considered obese[2]. Obesity is an epidemiologic problem with overweight people worldwide. It may cause significant health risks such as cardiovascular problems, diabetes, hyperlipidemia and hypertension, and metabolic syndrome, and it complicates many medical conditions, including critical diseases[3,4]. The most common change observed with increased fat in obesity is liver steatosis. In liver steatosis, changes in the liver, such as minimal liver fat infiltration, hepatocyte ballooning with or without fibrosis, and inflammatory cell infiltration, are observed[5,6]. The inflammatory process occurring in the liver creates changes in oxidative stress homeostasis. Antioxidants tolerate the effects of the free radicals formed. In addition to the body's antioxidant metabolism, antioxidants are taken with different natural compounds. While treating obesity-induced liver damage can be reduced in many ways, antioxidants are one of the most preferred treatments[7–10]. Thymoquinone is an antioxidant that is often preferred in liver damage. Nigella sativa, also known as Black cumin and from the Ranunculaceae family, contains thymoquinone. *Nigella sativa* is a herbaceous plant located in the Middle East[10,11]. *Nigella sativa* is used frequently, especially in the Middle East, India, and Iran. The thymoquinone (TQ) contained in black cumin has anti-inflammatory and antioxidant effects[10,11]. TQ can be effective in reversing the toxicity caused by obesity with both its antioxidant and hepatoprotective effects[12]. In addition, researchers reported TQ's anti-inflammatory, anti-histaminic, and immuno-modulatory effects, which prevent membrane lipid peroxidation in hepatocytes[13]. Studies about the usage of TQ in liver fibrosis have shown that TQ helps reduce the damage caused in the liver by reducing oxidative stress parameters[11]. Likewise, TQ treatment was performed in mice with liver damage by tamoxifen, and it was shown that it significantly inhibits glutathione depletion and lipid peroxidation accumulation in tamoxifen-induced hepatic toxicity. In addition, it has been reported that it normalizes the activity of superoxide dismutase and improves the damage to the liver histopathology[14].

This study aimed to investigate the histopathological changes in the livers of rats fed high-fat diets to cause obesity and the possible effects of thymoquinone, which has antioxidant activity, on these changes.

Materials and Methods

Animals and Experimental Procedure

The Ethical permissions for this study were obtained from the Experimental Animal Studies Ethics Committee of Ondokuz Mayis University (HADYEK/35, 27.04.2011). Twenty-four adult male Wistar albino rats were randomly divided into four groups: non-obese control (NC group), obese control (OC group), non-obese thymoquinone (NTQ group) and obese-thymoquinone (OTQ group) groups. All groups had six rats. The non-obese control and non-obese thymoquinone groups received a conventional diet for 15 weeks, but the obese control and obese-thymoquinone groups were fed a high-fat diet (40% calories from fat). The rats weighing 300–350 grams were considered obese. After the ninth week, the TQ group received an intraperitoneal injection of 10 mg/kg thymoquinone (Sigma-Aldrich, catalog no: 274666) dissolved in 0.04 ml saline in addition to their diet for six weeks per day. The rats were kept in a conventional cage with a 12-hour light/12-hour dark cycle, 50% humidity, and temperatures between 20 and 24°C with ad libitum feeding.

Tissue Procedures and Analysis

After the experimental processes, the rats were administered intramuscular ketamine and prilocaine hydrochloride (0.5 ml/300 g ketamine and 0.25 mg/100 g prilocaine hydrochloride; Sigma Chemical Comp., St. Louis, MO, USA). Then, the livers were removed and placed in a formaldehyde solution for fixation. After that, liver tissues processed with graded alcohol and xylene were embedded in a paraffin block and cut with 5 μm thickness by rotary microtome (Leica RM 2135, Germany). Sections were stained with hematoxylin-eosin for histopathological and stereological analyses.

Stereological Methods

This study used stereological methods to determine the total liver volume, sinusoidal volume, and total number of hepatocytes. The Cavalieri methods with point counting grids were used to estimate the volumes of the liver and sinusoids. The physical disector was used to determine the total number of hepatocytes.

Volume Estimation with the Cavalieri Principle

5 μm sections of liver tissue stained with hematoxylin-eosin were used for the volume analysis. Volume analyses were estimated using the Cavalier method in the

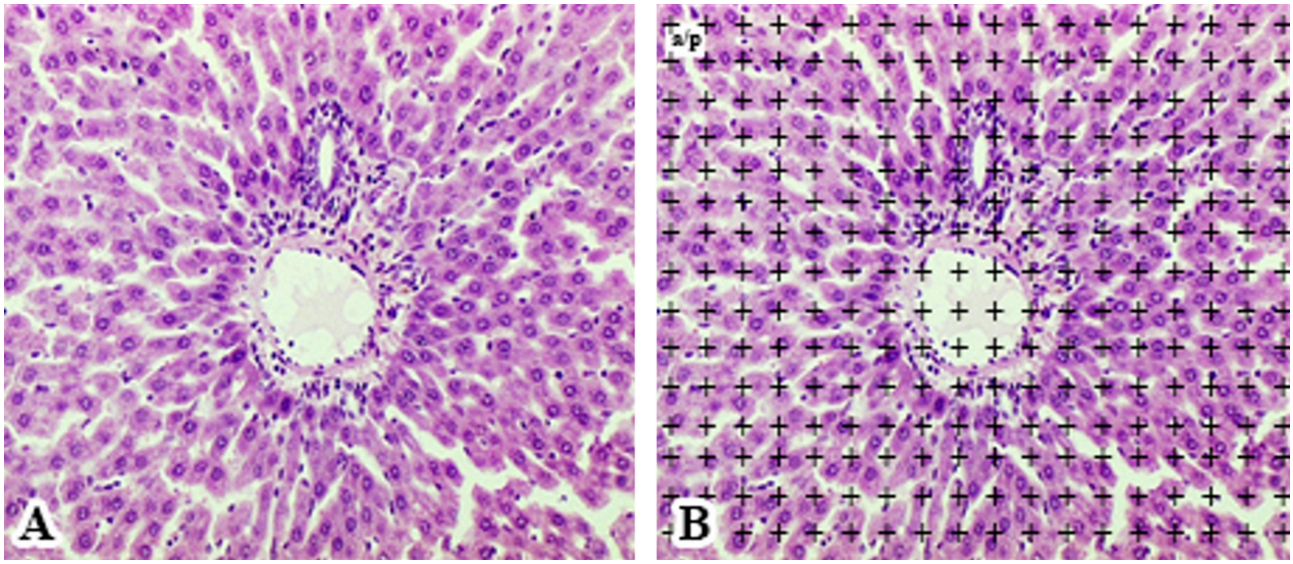

Figure 1. *A liver section and a superimposed dot-counting grid*

ImageJ program25. The liver tissue images were taken with a microscope with a camera attachment (Olympus BH-2, Tokyo, Japan). A 9000 μm 2 point counting grid for the sinusoid and the total volume were used for estimation (Figure 1).

The point-counting grid was randomly fixed on the image, and the points that hit each area were counted (Figure 1). The volume was estimated by the formula that was given below:

Volume: $t \times a/p \times \sum P$

In this equation, "t" stands for the section's thickness, "a/p" for each point's area on the point-counting grid, and "∑P" for the total number of points that hit the area of interest[15].

Estimation of Hepatocytes Number

Disector pairs were taken from the liver by systematic random sampling. And 15–20 section pairs were obtained from each animals. All liver images were taken with a microscope (Olympus BH-2, Tokyo, Japan). Hepatocyte nucleoli were accepted as dissector particles. Particles observed in the reference section but not in the look-up section were counted, and then the process was repeated by changing the sections to increase the particle count. In the unbiased counting frame placed on the sections, the particles in the countable areas were included in the count for the disector method[26]. The mean numerical density of hepatocytes (N_V (HC)) per mm^3 was estimated using the following formula:

$Nv\,(HC)=\sum Q^{-}(HC) / t\,(af)$

In this equation, "ΣQ-(HC)" stands for the total number of disector particles, "t" for section thickness, and "af" for the area of the unbiased counting frame.

Statistical Analyses

Data were statistically analyzed using the IBM Statistical Package for Social Sciences (SPSS) program version 21.0 (IBM; Chicago, USA). One-way ANOVA and Tukey tests were used to determine significant differences between groups. In contrast, the Shapiro-Wilk test was performed to determine if the data had a normal distribution. In the analysis, values with $p<0.05$ were deemed statistically significant, while those with $p<0.01$ were considered statistically highly significant.

Result

The Total Volume of Liver

Total liver volumes were estimated using the Cavalieri principle, and the results are summarized in Table 1 and Figure 2.

The liver volume was 7.61 mm^3 in the OC group and 6.507 mm^3 in the NOC group. There were significant differences between the NOC and OC groups. The liver volume in the OC group was higher than in the NOC group ($p\leq0.05$). The volume of the liver was 5.37 mm^3 in the OTQ group. The volume of the OTQ group was lower than the OC group, and the volume of the liver decreased in the NQT and OTQ groups compared to the NOC group.

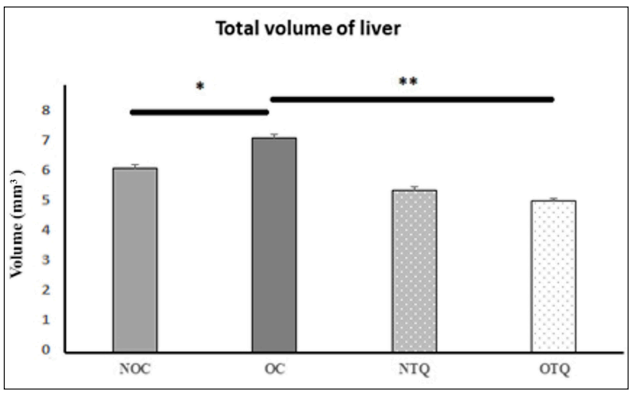

Figure 2. *The total volumes of the liver are shown. (**NOC:** non-obese control group; **OC:** obese control group; **NTQ:** non-obese thymoquinone group; **OTQ:** obese thymoquinone group; ***:** Significant differences; ****:** High significant differences)*

Table 1. *The average, CV and SEM values (mm³) of the total liver volume*

	NOC	OC	NTQ	OTQ
Average	6.507	7.61[a]	5.72	5.37[b]
CV	0.04	0.03	0.02	0.02
SEM	0.11	0.11	0.07	0.05

NOC: non-obese control group; OC: obese control group; NTQ: non-obese thymoquoinone group; OTQ: obese thymoquoinone group; CV, coefficient of variation; SEM, standard error mean; a: Significant differences between NOC and OC groups; b: Significant differences between OC and OTQ groups.

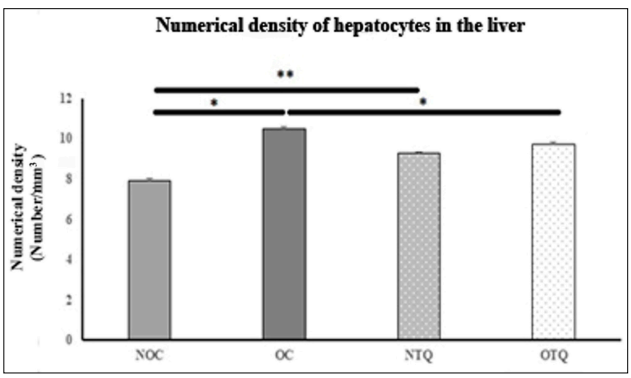

Figure 3. *The numerical density of hepatocytes is shown. (**NOC:** non-obese control group; **OC:** obese control group; **NTQ:** non-obese thymoquinone group; **OTQ:** obese thymoquinone group; ***:** Significant differences; ****:** High significant differences)*

Table 2. *Average numerical density of the hepatocyte (number/mm³) in the liver*

	NOC	OC	NTQ	OTQ
Average	7.97	10.58[a]	9.27[b]	9.62[c]
CV	0.02	0.03	0.02	0.02
SEM	0.13	0.23	0.08	0.09

NOC: non-obese control group; OC: obese control group; NTQ: non-obese thymoquoinone group; OTQ: obese thymoquoinone group; CV, coefficient of variation; SEM, standard error mean; a: Significant differences between NOC and OC groups; b: Significant differences between OC and OTQ groups.

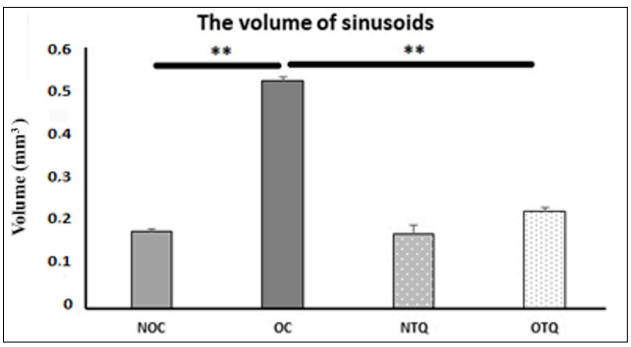

Figure 4. *The volumes of sinusoids are shown. (**NOC:** non-obese control group; **OC:** obese control group; **NTQ:** non-obese thymoquinone group; **OTQ:** obese thymoquinone group; ***:** Significant differences; ****:** High significant differences)*

Table 3. *The average, CV and SEM values (mm³) of the sinusoid volume*

	NOC	OC	NTQ	OTQ
Avarage	0.17	0.53[a]	0.19	0.22[b]
CV	0.05	0.02	0.02	0.07
SEM	0.004	0.005	0.013	0.007

NOC: non-obese control group; OC: obese control group; NTQ: non-obese thymoquoinone group; OTQ: obese thymoquoinone group; CV, coefficient of variation; SEM, standard error mean; a: Significant differences between NOC and OC groups; b: Significant differences between OC and OTQ groups.

The Number of Hepatocytes

The physical disector estimated the number of hepatocytes, and the results are given in Table 2 and Figure 3.

The numerical density of hepatocytes was 10.58 (number/mm³) in the OC group and 7.97(number/mm³) in the NOC group. Compared to the NOC group, the OC group had a higher numerical density of hepatocytes ($p \leq 0.05$). The numerical density of the OTQ group was 9.62(number/mm³), and the differences between OC and OTQ were quite noticeable. The density of the hepatocytes in the NTQ group was 9.27 (number/mm³). Compared to the NOC group, the NTQ group had a higher numerical density of hepatocytes ($p \leq 0.05$). Additionally, the OTQ group's hepatocyte density was lower than that of the OC group ($p \leq 0.05$).

The Volume of Sinusoids

The volume of sinusoids was estimated using the Cavalieri principal, and the results are summarized in Table 3 and Figure 4.

There were significant differences between the NOC and OC groups. The sinusoid volume in the OC group was 0.53 mm3, and the sinusoid volume in the NOC

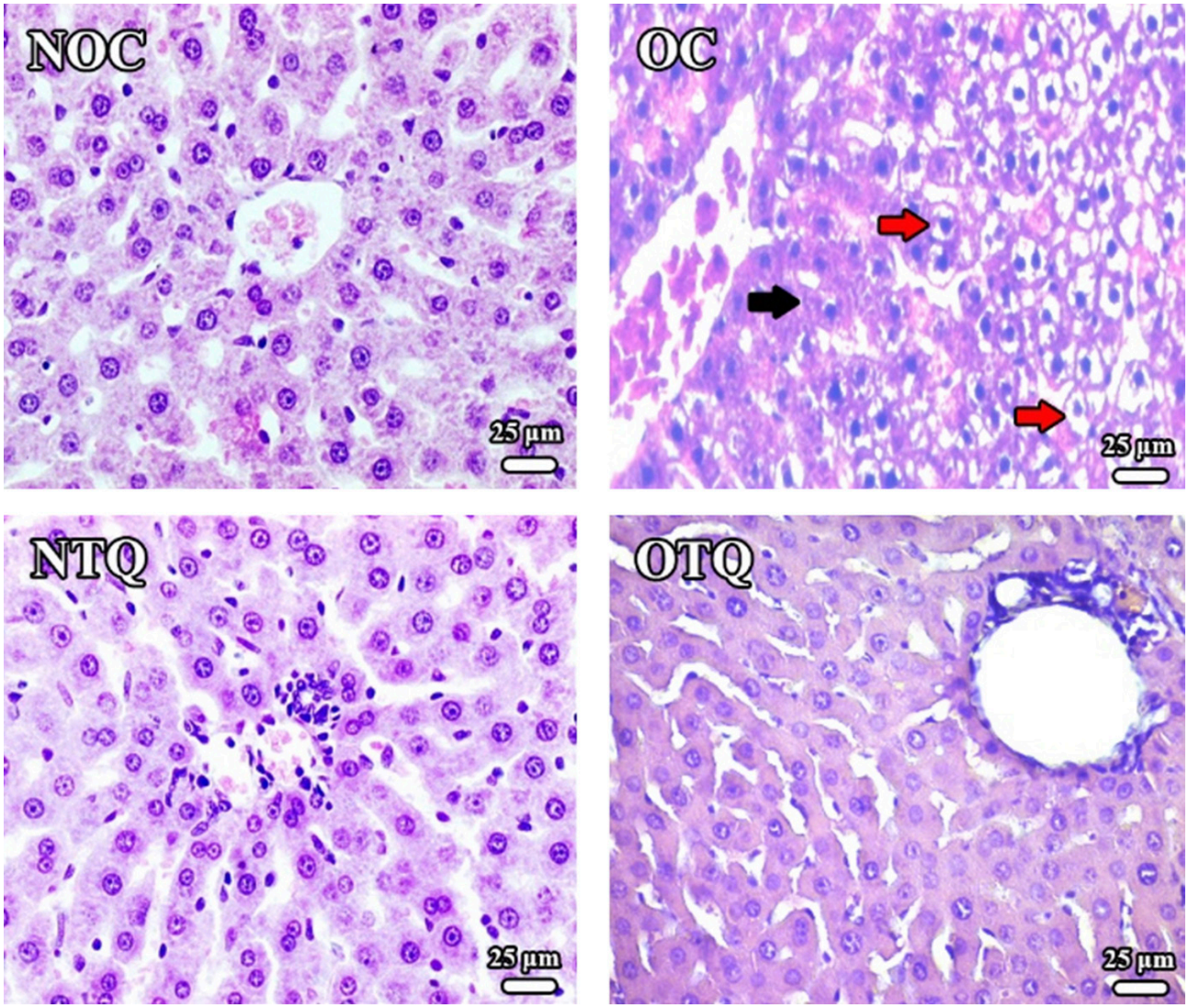

Figure 5. *Liver tissues of all groups are seen.* ***Red color arrow:*** *Hypertrophic hepatocytes with impaired nuclei,* ***black arrow:*** *Hepatocyte with unclear cell borders. Stained with hematoxylin and eosin (****NOC:*** *non-obese control group;* ***OC:*** *obese control group;* ***NTQ:*** *non-obese thymoquinone group;* ***OTQ:*** *obese thymoquinone group)*

groups was 0.17 mm^3. The volume in the OC group was higher than in the NOC group ($p \leq 0.01$). The volume of the OTQ group was lower than the OC group. The volume of the OTQ group was 0.22 mm^3.

Histological Results

In the light microscopic examination of the liver tissues, it was observed that the control group had a healthy morphology. The nuclei of hepatocytes were round and centrally located, hepatocytes arranged in cords. In the OC group, hepatocyte cords were observed as scattered, while hypertrophic hepatocytes with impaired nuclear borders and pathological appearance were remarkable (Figure 5). In the treatment groups treated with thymoquinone, hepatocytes had a healthy appearance, clear nuclear borders, and prominent nucleoli (Figure 5). Among the cords of regularly monitored hepatocytes, sinusoids, central vein, and portal areas had clear borders and healthy vascular structures (Figure 5).

Discussion

Obesity is characterized by fat accumulation in the body if the calories consumed and the calories expended are unbalanced. It is a multifactorial disease with a very high prevalence[4]. While fat accumulation is concentrated in certain parts of the body, the accumulation of adipose tissue on specific organs leads to

functional losses. Increasing fat may cause many metabolic diseases such as insulin resistance, fatty liver, and cardiovascular disease[4,16]. Obesity causes balloon-like patterns in hepatocytes called balloon degeneration, fatty degeneration in liver tissue, and decreased metabolic functions[15,17].Fatty liver has been caused by fat accumulation in hepatocytes, and over time, the inflammatory process in which immune cells play a role begins. If the treatment is not carried out correctly, the disease becomes complicated by hepatic fibrosis[18]. Obesity-induced fattening occurs physiologically and biochemically with changes in oxidative stress parameters and the liver's morphological changes. For this reason, regulation of oxidative stress parameters will minimize morphological and physiological changes resulting from fat accumulation. Antioxidant systems regulate oxidative stress parameters. Antioxidants affect free oxygen radicals and cause stress levels to decrease. Considering the changes in the liver, thymoquinone is thought to be the most suitable antioxidant for the liver. Thymoquinone has both hepatoprotective and antioxidant effects. For this reason, it is used as a therapeutic agent in many cultures. This study aimed to examine the possible impact of the mentioned properties of thymoquinone on the liver damaged by obesity.

There are many different studies examining the effects of obesity on liver tissue[19,20]. Studies have shown that obese individuals have impaired liver function and that the changes in the liver examined by ultrasound are 4.6 times higher in obese individuals compared to healthy individuals[19]. In this study, the effects of obesity on the liver were examined both stereologically and histopathologically. In the examinations, it was seen that in the non-obese control group, hepatocytes were arranged in the form of chords, they had healthy morphology, and the borders and nuclei of the hepatocytes were distinct. In the obese control group, it was noticed that the cord arrangement of the hepatocytes was disrupted, lipid accumulation was observed inside them, and their borders were irregular. This result is similar to the results in the literature. In a study conducted on 30 Wistar Albino rats in 2014, it was reported that fibrosis and ballooning in hepatocytes were observed in the livers of rats fed a high-fat diet[21]. In another study, fat accumulation was observed in the hepatocytes of rats fed a high-fat diet[22]. The fat accumulation observed histopathologically was also examined stereologically. In the stereological analyses, increases were observed in total liver and sinusoid volumes. In a study conducted in 2019, liver and sinusoid volumes of rats fed a high-fat diet were examined using the Cavalieri method, and an increase was observed in the group fed a fatty diet[15]. This is an expected result considering the fatty liver, ballooning, and inflammation processes that occur with obesity. Likewise, an increase in the number of hepatocytes was observed. The irregularity of the cords can explain this result.

Fatty liver due to obesity has many different pathophysiologies and occurs due to different factors. Oxidative stress, one of these factors, increases considerably in the case of obesity. Antioxidant mechanisms are mechanisms that reduce oxidative stress and protect organ physiology. Liver damage caused by increased fat in obesity can be treated with antioxidants. Thymoquinone is one of the most frequently preferred antioxidants and is the essential oil of Nigella sativa. It has anti-diabetic, antioxidant, hepatoprotective, neuro and nephroprotective, anti-tumor, and anti-mutagenic effects[23]. Many studies in the literature show positive effects of TQ on the liver[11,24,25]. The effects of TQ on energy metabolism, glucose and lipid mechanisms, and oxidative stress in mitochondria provide regulation of organ functions[13]. In a study using fifty male Wistar rats, liver damage was performed by creating a lipopolysaccharide-induced inflammation model and treated with TQ. It was shown that fibrosis in the liver was decreased with TQ and returned to a healthy state[11]. TQ was used for the treatment in the study on the damage of fatty liver disease and steatohepatitis in liver tissue with hyperthyroidism. It was shown that an intralobular inflammatory reaction is observed associated with significant increases in the density of resident hepatic macrophages, activated hepatic stellate cells, and alpha-smooth muscle actin observed with hyperthyroidism in disease groups; decreased damage in the livers was observed after treatment with TQ, and the inflammatory effect was reduced[26]. All these effects of TQ are thought to occur by reducing oxidative stress parameters. Reducing free radicals that cause tissue damage will also change the formation and appearance of the inflammatory response. A study showed that liver damage due to diabetes mellitus is eliminated with TQ on the morphological change and oxidative stress enzymes[25]. Another study showed that TQ treated AST, ALP levels, and oxidative stress parameters in rats with diazinon exposure[27]. In this study, stereological and histopathological analyses were performed to investigate the possible effects of TQ on the liver tissues of rats, and an obesity model was created. Stereological analyses showed changes in total liver and sinusoid

volumes. When the total liver and sinusoid volumes were examined, the thymoquinone-treated obese group had significantly lower volumes than the obese control group. These results support thymoquinone's antioxidant and hepatoprotective effects by positively affecting the liver. In the analysis of hepatocyte density, it was observed that the density decreased in the obese group that received thymoquinone injection compared to the obese group. These results suggest that TQ causes a decrease in total liver volume by regulating the sinusoidal volume. Considering the anti-lipidemic property of thymoquinone, it can be suggested that obesity in the sinusoid capillaries reduces the sinusoid volume by decreasing the increased lipid accumulation[28]. The histopathological analyses in this study show that TQ groups showed decreased ballooning appearance, degeneration, and fibrosis and that the livers had a healthy pattern compared to the obese control group. The regularity of hepatic cords and the absence of ballooning patterns, which we observed in the histopathological results, also support our stereological findings and show that TQ may improve liver damage through many mechanisms.

When all these results are considered, thymoquinone may have a protective effect. This effect of TQ is obtained by reducing oxidative stress. However, the study has limitations because oxidative stress parameters were not analyzed within the scope of the study, and the mechanisms were not investigated with different analysis methods. More analysis is needed to explain the mechanism of action.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Is There an Association between First Trimester 25-Hydroxy Vitamin D Levels and Gestational Diabetes Mellitus?

Birinci Trimester 25-Hidroksi D Vitamini Düzeyleri ile Gestasyonel Diabetes Mellitus Arasında İlişki Var mı?

Samet Kirat

Kafkas University Faculty of Medicine, Academic Department of Obstetrics and Gynecology, Kars, Türkiye

ABSTRACT

Aim: *Pregnant women have high Vitamin D deficiency rates. Some studies have suggested that vitamin D deficiency is associated with gestational diabetes mellitus (GDM). This study aimed to investigate the association between first trimester 25-hydroxy vitamin D levels and the risk of developing GDM.*

Material and Method: *A retrospective analysis was conducted on 409 pregnant women aged 18–50 years with singleton pregnancies who had their 25(OH) vitamin D levels checked in the first trimester and underwent an oral glucose tolerance test (OGTT) between 24–28 weeks of gestation. Demographic data, 25(OH) vitamin D levels, and OGTT results were recorded. Gestational diabetes mellitus was diagnosed according to the criteria of the International Association of Diabetes and Pregnancy Study Groups (IADPSG) criteria. Vitamin D levels were categorized into deficiency (<20 ng/ml), insufficiency (20–30 ng/ml), and adequacy (>30 ng/ml).*

Results: *The median age of the participants was 33 years, and 24.6% (n=101) had GDM. The median 25(OH) vitamin D level was 18 ng/ml, and deficiency was found in 57.2% (n=234), insufficiency in 34.2% (n=140), and adequate level in 8.6% (n=35). The median age (32 vs 34 years; p=0.008), smoking rate (18.8% vs. 8.4%; p=0.007), and median 25(OH) vitamin D level (10(4–30) ng/ml vs 20 (3–151) ng/ml; p<0.001) were significantly lower in patients with GDM than in those without GDM. 25(OH) vitamin D <12 ng/ml (aOR: 0.086, 95% C. I.: 0.51–0.144, p<0.001) and 25(OH) vitamin D <20 ng/ml (aOR: 0.073, 95% C. I.: 0.034–0.155, p<0.001) were strongly associated with GDM in logistic regression analysis.*

Conclusion: *The risk of GDM can be minimized by regular vitamin D supplementation at prophylactic doses before pregnancy.*

Key words: *vitamin D deficiency; gestasyonel diabetes mellitus; oral glucose tolerance test*

ÖZET

Amaç: *Gebe kadınlarda yüksek oranda D Vitamini eksikliği görülmektedir. Bazı çalışmalar D vitamini eksikliğinin gestasyonel diabetes mellitus (GDM) ile ilişkili olduğunu öne sürmektedir. Bu çalışmanın amacı, ilk trimester 25-hidroksi D vitamini düzeyleri ile GDM gelişme riski arasındaki ilişkiyi araştırmaktır.*

Materyal – Metod: *İlk trimesterde 25(OH) D vitamini düzeylerini kontrol ettiren ve 24–28. gebelik haftaları arasında oral glukoz tolerans testi (OGTT) yapılan 18–50 yaş arası tekil gebeliği olan 409 gebe kadın üzerinde retrospektif bir analiz yapılmıştır. Demografik veriler, 25(OH) D vitamini düzeyleri ve OGTT sonuçları da kaydedilmiştir. Gestasyonel diyabetes mellitus tanısı Uluslararası Diyabet ve Gebelik Çalışma Grupları Birliği (IADPSG) kriterlerine göre konulmuştur. D vitamini düzeyleri eksiklik (<20 ng/ml), yetersizlik (20–30 ng/ml) ve yeterlilik (>30 ng/ml) olarak kategorize edilmiştir.*

Bulgular: *Katılımcıların ortanca yaşı 33'tür ve %24,6'sında (n=101) GDM vardır. Toplam kohortun 25(OH) D vitamini düzeyi ortancası 18 ng/ml olup, %57,2'sinde (n=234) eksiklik, %34,2'sinde (n=140) yetersizlik ve %8,6'sında (n=35) yeterli düzey saptanmıştır. GDM'si olanlarda yaş ortancası (32 yaş ~ 34 yaş; p=0,008), sigara içme oranı (%18,8 ~ %8,4; p=0,007) ve 25(OH) D vitamini düzeyi ortancası (10(4–30) ng/ml ~ 20 (3–151) ng/ml; p<0,001) GDM'si olmayanlara göre anlamlı derecede düşüktü. 25(OH) D vitamini <12 ng/ml (aOR: 0,086, %95 C. I.: 0,51–0,144, p<0,001) ve 25(OH) D vitamini <20 ng/ml (aOR: 0,073, %95 C. I.: 0,034–0,155, p<0,001) lojistik regresyon analizinde GDM ile güçlü bir şekilde ilişkili bulunmuştur.*

Sonuç: *Folik asit takviyesine benzer şekilde, GDM riski de gebelikten önce başlanan profilaktik dozda düzenli D vitamini takviyesi ile en aza indirilebilir.*

Anahtar kelimeler: *D vitamini eksikliği, gestasyonel diabetes mellitus, oral glukoz tolerans testi*

İletişim/Contact: *Samer Kirat, Department of Gynecology and Obstetrics, Kafkas University Faculty of Medicine, Kars, Türkiye* • ***Tel:*** *0555 261 83 08* • ***E-mail:*** *sametkirat1989@hotmail.com* • ***Geliş/Received:*** *29.05.2024* • ***Kabul/Accepted:*** *03.09.2024*

ORCID: *Samet Kırat: 0000-0001-7262-4320*

Introduction

Cholecalciferol (vitamin D3) synthesized from cholesterol in the skin under the influence of sunlight, and ergocalciferol (vitamin D2) absorbed from the intestines through dietary products are transported to the liver by vitamin D-binding protein (DBP) and converted first into 25 hydroxy vitamin D and then into its active form, 1.25-dihydroxy vitamin D, in the kidneys[1]. Vitamin D is involved in hormone secretion, regulation of immune function and regulation of cell proliferation and differentiation. Deficiency is also associated with obesity, diabetes, hypertension, cancer, infections, and cardiovascular diseases[2].

The global consensus recommendation panel on the prevention and management of rickets defines vitamin D levels as follows: deficiency is indicated by levels below 30 nmol/L (<12 ng/mL), insufficiency by levels between 30–50 nmol/L (12–20 ng/mL), and adequacy by levels above 50 nmol/L (>20 ng/mL). These thresholds have been widely accepted as a reference for evaluating vitamin D status[3]. During pregnancy, the daily requirement for vitamin D increases owing to the mother's and developing fetus' s additional needs. However, despite its critical role in maternal and fetal health, particularly in bone and skeletal development, studies report that vitamin D deficiency is highly prevalent among pregnant women, ranging from 18% to 84% in different populations[4].

According to the American Diabetes Association (ADA), Gestational Diabetes Mellitus (GDM) is defined as carbohydrate intolerance of varying degrees, characterized by dysfunction of β-cells and insulin resistance that begins or is first recognized during pregnancy[5]. Women with GDM are at an increased risk for cesarean delivery, type 2 DM, hypoglycemia, congenital anomalies, fetal macrosomia, shoulder dystocia, and antenatal death. Furthermore, GDM can lead to long-term metabolic complications in offspring, such as childhood obesity and impaired glucose tolerance. Early diagnosis and appropriate management are crucial for minimizing these maternal and fetal risks and improving pregnancy outcomes[6].

The development of GDM is associated with well-defined risk factors such as advanced maternal age, family history of diabetes, ethnicity, and obesity. Recent studies have suggested that vitamin D deficiency may constitute a risk factor for GDM by increasing insulin resistance and causing hyperinsulinemia[7]. In this study, we aimed to evaluate the relationship between 25(OH) vitamin D levels measured in the first trimester and the development of GDM and to contribute to the literature on the potential role of vitamin D in the pathophysiology of GDM.

Materials and Methods

Patients and Data Collection

Between January 2019 and December 2023, 409 pregnant women aged 18–50 with singleton pregnancies who were admitted to the Department of Obstetrics and Gynecology at Kafkas University had their 25(OH) vitamin D levels measured during the first trimester and underwent an oral glucose tolerance test (OGTT) between 24 and 28 weeks of gestation were included in this study.

Pregnant women with a history of multiple pregnancies, additional systemic diseases before pregnancy, those who did not undergo OGTT for various reasons, and those who did not have 25(OH) vitamin D levels in the first trimester were excluded from the study.

Demographic data, including age, gravidity, parity, number of abortions, smoking history, year of delivery, week of delivery, birth weight, and neonatal intensive care unit (NICU) hospitalization indication, were obtained retrospectively from medical records. 25(OH) Vitamin D levels and OGTT results were also recorded.

Terms and Definitions

In the 75 g oral glucose loading test, GDM is diagnosed if fasting blood glucose is ≥92 mg/dl, 1st hour is ≥180 mg/dl, 2nd hour is ≥153 mg/dl, and at least two values are higher[8]. For 25(OH) vitamin D levels, <20 ng/ml (50 nmol/L) was accepted as deficiency, 20–30 ng/ml (50–75 nmol/L) as insufficient, and >30 ng/ml (75 nmol/L) as adequacy[9].

Ethics Committee Approval

This study was approved by the Non-Interventional Clinical Research Ethics Committee of the Kafkas University Faculty of Medicine (30/04/2024, 80576354–050–9). This study complied with the recommendations of the Declaration of Helsinki for human biomedical research.

Statistical Analysis

The Windows SPSS program (version 24.0) was used for statistical analyses. The Kolmogorov-Smirnov test

evaluated whether the continuous variables were normally distributed. The data showing abnormal distribution as a result of the test are expressed as median (minimum-maximum), and the data showing normal distribution are expressed as mean ± standard deviation. Categorical variables are presented as numbers (n) and percentages (%). The chi-square test was used to compare pregnant women with and without GDM. Since it is a 2×2 table, the Pearson chi-square test was used if the theoretical minimum frequency was >25, the Yates chi-square test between 5 and 25, Fisher's exact test was used if it was <5, and the p result was written. The Kruskal-Wallis test was used to make comparisons according to 25(OH) vitamin D levels. Statistical significance was set at p<0.05. Prism software (version 8, GraphPad Software, San Diego, California, USA) was used for data analysis and graphs.

Results

Demographic, 25(OH) Vitamin D level and newborn data

The median age of the 409 pregnant women was 33 (19–50). Median gravida was 2 (1–8), median parity was 1 (0–5), and median abortion was 0 (0–4). Among the women, 11 % (n=45) smoked during pregnancy. A double screening test was performed in 46% (n=188) of the patients, triple screening tests in 5.6% (n=23), and detailed ultrasonography (USG) in 8.3% (n=34). The median 25(OH) vitamin D level was 18 (3–151) ng/ml. The 25(OH) vitamin D level was <20 ng/ml in 57.2% (n=234) of pregnant women, 20–30 ng/ml in 34.2% (n=140), and >30 ng/ml in 8.6% (n=35). GDM was present in 24.6 % (n=101) of women.

Of the babies born to these pregnant women, 48.2 % (n=197) were female and 51.8% (n=212) were male. In total, 9.5% (n=39) of the babies were born at <37 weeks and 90.5% (n=370) at 37–42 weeks. Birth weight was 1500–2500 grams in 5.4% (n=22), 2500–4000 grams in 92.4% (n=378) and >4000 grams in 2.2% (n=9). The median 1st minute APGAR score was 8 (2–9), and the median 5th minute APGAR score was 9 (7–10). Among the infants, 8.8 % (n=36) were admitted to the neonatal intensive care unit (NICU) with a diagnosis of transient tachypnea of the newborn (TTN), 2.2% (n=9) sepsis, and 0.7% (n=3) respiratory distress syndrome (RDS). Detailed data are presented in Table 1.

Table 1. *Demographic, 25(OH) Vitamin D level and newborn data*

	Total Cohort (n=409)
Age (years) (Median (Min-Max))	33 (19–50)
Smoking history (n, %)	45 (11%)
Number of Gravida (Median (Min-Max))	2 (1–8)
Number of Parities (Median (Min-Max))	1 (0–5)
Number of Abortions (Median (Min-Max))	0 (0–4)
Screening Tests	
Double screening test (n, %)	188 (46%)
Triple screening test (n, %)	23 (5%.6)
Detailed ultrasonography (USG) (n, %)	34 (8%.3)
Gestational diabetes mellitus (GDM) (n, %)	101 (24%.6)
25(OH) Vitamin D level (Median (Min-Max))	18 (3–151)
<20 ng/ml (n, %)	234 (57%.2)
20–30 ng/ml (n, %)	140 (34%.2)
>30 ng/ml (n, %)	35 (8%.6)
Baby Sex (n, %)	
Female	197 (48%.2)
Male	212 (51%.8)
Birth Week (n, %)	
<37 weeks	39 (9%.5)
37–42 weeks	370 (90%.5)
>42 weeks	0 (0%)
Infant Birth Weight (n, %)	
1500–2500 gram	22 (5%.4)
2500–4000 gram	378 (92%.4)
>4000 gram	9 (2%.2)
1st minute APGAR score (Median (Min-Max))	8 (2–9)
5th minute APGAR score (Median (Min-Max))	9 (7–10)
Neonatal Intensive Care Unit (NICU)	
Transient Tachypnea of the Newborn (TTN) (n, %)	36 (8%.8)
Sepsis (n, %)	9 (2%.2)
Respiratory Distress Syndrome (RDS) (n, %)	3 (0%.7)

Comparison of pregnant women with and without GDM

The median age of patients with GDM was 32 years (19–44), and the median age of patients without GDM was 34 years (22–50) (p=0.008). The smoking rate during pregnancy was significantly higher in those with GDM than in those without GDM (n=19, 28.8% vs. n=26, 8.4%; p=0.007). The number of gravida and abortions were similar in both groups, but parity was significantly higher in those without GDM than in those with GDM (n=1 (0–5) vs. n=1 (0–3); p=0.05). The rates of the double screening test (n=58, 57.4% vs. n=130, 42.2%; p=0.008) and detailed USG (n=16, 15.8% vs. n=18, 5.8%; p=0.003) were significantly higher in those with GDM than in those without GDM.

The median 25(OH) vitamin D level was 10 (4–30) ng/ml in patients with GDM and 20 (3–151) ng/ml in those without GDM (p<0.001). Vitamin 25(OH) D <12 ng/ml (n=71, 70.3% vs. n=52, 16.9%; p<0.001)

and vitamin 25(OH) D <20 ng/ml (n=93, 92.1% vs. n=141, 45.8%; p<0.001) were significantly higher in patients with GDM compared to those without GDM. 25(OH) vitamin D levels of 20–30 ng/ml (n=132, 42.9% vs. n=8, 7.9%; p<0.001) and 25(OH) vitamin D levels of >30 ng/ml (n=35, 11.4% vs. n=0, 0%; p<0.001) were significantly in those without GDM higher than in those with GDM. 25(OH) vitamin D <12 ng/ml (aOR: 0.086, 95% C. I.: 0.51–0.144, p<0.001) and 25(OH) vitamin D <20 ng/ml (aOR: 0.073, 95% C. I.: 0.034–0.155, p<0.001) were strongly associated with GDM in logistic regression analysis. The distribution of 25(OH) vitamin D levels in the patients with and without GDM is shown in Figure 1.

NICU admissions with a diagnosis of TTN were significantly higher in patients with GDM than in those without GDM (n=15, 14.9% vs. n=21, 6.8%; p=0.023). Table 2 provides detailed data.

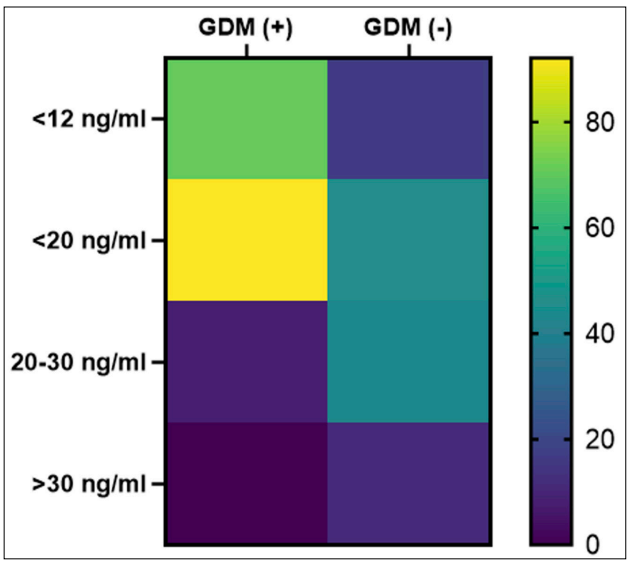

Figure 1. *25(OH) vitamin D levels in patients with and without GDM.*

Table 2. *Comparison of pregnant women with and without GDM*

	Those with GDM (n=101)	Those without GDM (n=308)	p
Age (years) (Median (Min-Max))	32 (19–44)	34 (22–50)	0.008
Smoking history (n, %)	19 (18%.8)	26 (8%.4)	0.007
Number of Gravida (Median (Min-Max))	2 (1–6)	2 (1–8)	0.266
Number of Parities (Median (Min-Max))	1 (0–3)	1 (0–5)	0.050
Number of Abortions (Median (Min-Max))	0 (0–3)	0 (0–4)	0.116
Screening Tests			
Double screening test (n, %)	58 (57%.4)	130 (42%.2)	0.008
Triple screening test (n, %)	8 (7%.9)	15 (4%.9)	0.365
Detailed ultrasonography (USG) (n, %)	16 (15%.8)	18 (5%.8)	0.003
25(OH) Vitamin D level (Median (Min-Max))	10 (4–30)	20 (3–151)	<0.001
<12 ng/ml (n, %)	71 (70%.3)	52 (16%.9)	<0.001
<20 ng/ml (n, %)	93 (92%.1)	141 (45%.8)	<0.001
20–30 ng/ml (n, %)	8 (7%.9)	132 (42%.9)	<0.001
>30 ng/ml (n, %)	0 (0%)	35 (11%.4)	<0.001
Baby Sex (n, %)			
Female	52 (51%.5)	145 (47%.1)	0.442
Male	49 (48%.5)	163 (52%.9)	0.442
Birth Week (n, %)			
<37 weeks	8 (7%.9)	31 (10%.1)	0.659
37–42 weeks	93 (92%.1)	277 (89%.9)	0.659
>42 weeks	0 (0%)	0 (0%)	0.659
Infant Birth Weight (n, %)			
1500–2500 gram	6 (5%.9)	16 (5%.2)	0.360
2500–4000 gram	91 (90%.1)	287 (93%.2)	0.360
>4000 gram	4 (4%)	5 (1%.6)	0.360
1st minute APGAR score (Median (Min-Max))	8 (5–9)	7 (2–9)	0.006
5th minute APGAR score (Median (Min-Max))	9 (7–10)	9 (7–10)	0.776
Neonatal Intensive Care Unit (NICU)			
Transient Tachypnea of the Newborn (TTN) (n, %)	15 (14%.9)	21 (6%.8)	0.023
Sepsis (n, %)	3 (3%)	6 (1%.9)	0.695
Respiratory Distress Syndrome (RDS) (n, %)	1 (1%)	2 (0%.6)	0.728

Table 3. *Comparison of groups according to 25(OH) vitamin D level*

	25(OH) D <20 ng/ml (n=234)	25(OH) D 20–30 ng/ml (n=140)	25(OH) D >30 ng/ml (n=35)	p value
Age (years) (Mean ± SD)	33.36±5.26	33.56±5.48	35.49±5.51	0.096
Smoking history (n, %)	27 (11%.5)	16 (11%.4)	2 (5%.7)	0.579
Number of Gravida (Median (Min-Max))	2 (1–8)	2 (1–7)	3 (1–5)	0.085
Number of Parities (Median (Min-Max))	1 (0–4)	1 (0–5)	1 (0–4)	0.300
Number of Abortions (Median (Min-Max))	0 (0–4)	0 (0–4)	0 (0–3)	0.470
Screening Tests				
Double screening test (n, %)	117 (50%)	49 (35%)	22 (62%.9)	0.002
Triple screening test (n, %)	15 (6%.4)	5 (3%.6)	3 (8%.6)	0.376
Detailed ultrasonography (USG) (n, %)	20 (8%.5)	11 (7%.9)	3 (8%.6)	0.971
Gestational diabetes mellitus (GDM)	93 (39%.7)	8 (5%.7)	0 (0%)	<0.001
Baby Sex (n, %)				
Female	114 (48%.7)	63 (45%)	20 (57%.1)	0.423
Male	120 (51%.3)	77 (55%)	15 (42%.9)	0.423
Birth Week (n, %)				
<37 weeks	20 (8%.5)	17 (12%.1)	2 (5%.7)	0.375
37–42 weeks	214 (91%.5)	123 (87%.9)	33 (94%.3)	0.375
Infant Birth Weight (n, %)				
1500–2500 gram	16 (6%.8)	4 (2%.9)	2 (5%.7)	0.441
2500–4000 gram	213 (91%)	132 (94%.3)	33 (94%.3)	0.441
>4000 gram	5 (2%.1)	4 (2%.9)	0 (0%)	0.441
1st minute APGAR score (Median (Min-Max))	8 (2–9)	7 (5–9)	8 (3–9)	0.828
5th minute APGAR score (Median (Min-Max))	9 (7–10)	9 (8–10)	9 (8–10)	0.550
Neonatal Intensive Care Unit (NICU)				
Transient Tachypnea of the Newborn (TTN) (n, %)	24 (10%.3)	7 (5%)	5 (14%.3)	0.108
Sepsis (n, %)	5 (2%.1)	3 (2%.1)	1 (2%.9)	0.962
Respiratory Distress Syndrome (RDS) (n, %)	2 (0%.9)	1 (0%.7)	0 (0%)	0.858

Comparison of groups according to 25(OH) vitamin D level

Age, number of gravida, parity, number of abortions, smoking rate, rate of screening tests, infant sex, infant birth week, infant birth weight, 1st and 5th minute APGAR scores, NICU hospitalization rates with TTN, sepsis, and RDS were similar in all three groups. However, the number of GDM patients was significantly higher in those with 25(OH) vitamin D <20 ng/ml than in those with 25(OH) vitamin D 20–30 ng/ml and 25(OH) vitamin D >30 ng/ml (n=93, 39.7% vs. n=8, 5.7% vs. n=0, 0%; p=0.002). Detailed data are presented in Table 3.

Discussion

Our study analyzed demographic data, 25(OH) vitamin D levels, and neonatal data of women with and without GDM. 25(OH) vitamin D levels were significantly lower in pregnant women with GDM than in those without GDM (10[4–30] ng/mL vs 20 [3–151] ng/mL; p<0.001). It was found that 92.1% of pregnant women with GDM and 45.8% of those without GDM had 25(OH) vitamin D levels <20 ng/mL (p<0.001). In addition, the GDM rate was 70.3% in pregnant women with 25(OH) vitamin D levels <12 ng/mL. Multivariate logistic regression analysis revealed that 25(OH) vitamin D levels <12 ng/mL (aOR: 0.086, 95% CI: 0.051–0.144) and <20 ng/mL (aOR: 0.073, 95% CI: 0.034–0.155, p<0.001) were strongly associated with GDM.

Human and animal studies have shown that vitamin D deficiency can lead to marked changes in insulin concentration and sensitivity[10]. Vitamin D plays a critical role in glucose metabolism and may have direct effects on insulin resistance and beta cell dysfunction via its receptor and indirect effects through calcium homeostasis regulation[11]. Alterations in calcium levels have been reported to influence insulin secretion and may impair pancreatic beta cell function[12]. These mechanisms collectively suggest that vitamin D deficiency may adversely affect glucose metabolism and the insulin response. These findings underline the importance

of vitamin D in metabolic processes and its potential role in metabolic disorders, such as diabetes[13].

Studies in healthy individuals have shown an inverse relationship between serum 25(OH) vitamin D levels, glucose concentration and insulin resistance[14–17]. However, studies evaluating the relationship between vitamin D levels in pregnancy, glucose metabolism, and GDM present conflicting findings. In studies conducted in Türkiye, low 25(OH) vitamin D levels during pregnancy were significantly associated with GDM risk[18]. In the study by Zhang et al., 25(OH) vitamin D levels measured at 16 weeks of gestation were compared between 24–28 weeks of gestation in pregnant women with GDM and healthy women; vitamin D levels were significantly lower in pregnant women with GDM. These findings suggest that maternal vitamin D deficiency detected in early pregnancy may be associated with the development of GDM[19].

Maghbooli et al. found that pregnant women with severe vitamin D deficiency (<12.5 nmol/L=5 ng/mL) were significantly more likely to be diagnosed with GDM[20]. Similarly, in a prospective cohort study of 655 pregnant women, low 25(OH) vitamin D levels in the first trimester were strongly correlated with the incidence of GDM between 24–28 weeks of gestation[21]. In a case-control study by Soheilykhah et al., 25(OH) vitamin D deficiency was significantly higher in patients with GDM (83.3% vs. 71.2%; p=0.007), which increased the risk of GDM 2.02 times (95% CI: 0.88–4.6)[22]. In a meta-analysis of 12 studies evaluating the association between GDM and vitamin D deficiency, it was reported that the risk of GDM was 1.38 times (95% CI: 1.12–1.70) higher when maternal 25(OH) vitamin D levels were <50 nmol/L and 1.55 times (95% CI: 1.21–1.98) higher when levels were <75 nmol/L[23].

However, some studies do not support this relationship. In a case-control study by Baker et al., first trimester 25(OH) vitamin D deficiency (<50 nmol/L=20 ng/mL) was found to be similarly low among pregnant women with and without GDM (8.3% vs. 6%; p=0.95)[24]. Similarly, in a study by Park et al. on Korean pregnant women, no significant association was found between vitamin D levels in the first trimester and GDM risk[25]. These conflicting findings suggest that further comprehensive and standardized studies are needed better to understand the association between vitamin D deficiency and GDM[26].

Studies have also been conducted to determine whether vitamin D supplementation during the antenatal period prevents GDM development. Similar rates of GDM development were found in the two groups (n=257) that received 2000 IU and 4000 IU vitamin D supplements at 12–16 weeks of gestation[22,27]. In a randomized controlled trial in which low-dose (400 IU/day) and high-dose (5000 IU/day) vitamin D supplements were administered to two groups with low 25(OH) vitamin D levels before the 20th week of gestation and the effects on glucose metabolism were evaluated, the OGTT results at 26–28 weeks of gestation were found to be similar in both groups[28]. It has been reported that high-dose vitamin D treatment has no protective effect against GDM but significantly increases neonatal vitamin D levels[28].

This study had some limitations. Its retrospective design, the fact that it was conducted in only one center, and the fact that extrinsic factors (e.g., sun exposure and diet) could not be assessed may have limited the extent of the results. However, the strengths of the study are also noteworthy. In particular, the fact that it was based on a large sample group increases the generalizability of the findings. In addition, the categorical classification of vitamin D levels and detailed statistical analyses significantly strengthened the reliability and scientific value of the results.

Conclusion

This study revealed that low vitamin D levels during pregnancy are strongly associated with the development of GDM. In particular, severe deficiency levels of <12 ng/mL significantly increased the risk of GDM. These findings emphasize the impact of vitamin D deficiency on metabolic processes and its potential importance in pregnancy. In the future, larger prospective and multicenter studies will contribute to a better understanding of the relationship between vitamin D deficiency and GDM. Furthermore, examining the effect of different doses and durations of vitamin D supplementation on GDM may provide more specific guidance for clinical practice. Like folic acid supplementation, we believe the likelihood of GDM can be minimized with regular prophylactic vitamin D supplementation starting from the pre-pregnancy period, especially in areas with low sunlight, such as Eastern Anatolia.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Investigating the Impact of Psychosocial Problems in Parents Following an Earthquake on the Psychosocial Issues in Their Children and Their Relationship with Their Children

Deprem Sonrası Ebeveynlerde Görülen Psikososyal Problemlerin Çocuklarındaki Psikososyal Sorunlar ve Ebeveynlerin Çocuklarıyla İlişkileri Üzerine Etkisi

Gizem Kerimoglu Yildiz[1], Mustafa Yildiz[2], Aysu Gulmez[2], Munire Batmaz[2], Merve Deprem[2]

[1]Division of Pediatric Nursing; [2]Nursing Department, Faculty of Health Science, Mustafa Kemal University, Hatay, Türkiye

ABSTRACT

Aim: *This study aimed to determine the psychosocial status of parents and children one year after the earthquakes that affected 11 provinces in our country on February 6, 2023, and to identify the effects of parents' psychosocial problems on their health perceptions, the occurrence of psychosocial symptoms in their children, and parent-child communication.*

Material and Method: *The study had a cross-sectional descriptive design, and the sample included 204 individuals who volunteered to participate via online social media platforms between November 2023 and February 2024 and met the inclusion criteria. The data were collected using the "Parent and child information form" developed by the researchers based on the literature, the "Health Perception Scale," the "Beck Anxiety Inventory," the "Beck Depression Inventory," the "Parent-Child Relationship Scale," and the "Pediatric Symptom Checklist (PSC-17)" for children aged 6–16. Completing the data collection instruments took approximately 15–20 minutes.*

Results: *It was determined that 45% of parents had mild anxiety, while 35% had mild depression. Only 1% of children had a PSC-17 score of 12 or higher. There was no significant difference in communication with their children based on the parents' anxiety and depression levels (p>0.05). Children of parents experiencing anxiety and depression after the earthquake had statistically significant psychosocial problems. Parents' health perception scores varied according to their depression levels. Advanced analysis indicated that parents with moderate and severe depression scores had lower health perception scores compared to parents with low and mild depression scores (p: 0.02; p: 0.012).*

Conclusion: *Even one year after the earthquake, parents continued to experience anxiety and depression, and this situation affected the occurrence of psychosocial problems in their children. Additionally, parents with high levels of depression had lower health perception scores. These results demonstrate the continued need for psychosocial support for parents after an earthquake.*

Key words: *anxiety; depression; earthquake; health perception; parents; parent-child relation*

ÖZET

Amaç: *Bu çalışma, 6 Şubat 2023'te ülkemizde 11 ili etkileyen depremlerden bir yıl sonra ebeveynlerin ve çocukların psikososyal durumlarını belirlemeyi ve ebeveynlerin psikososyal problemlerinin sağlık algıları, çocuklarında psikososyal semptomların görülmesi ve ebeveyn-çocuk iletişimi üzerindeki etkilerini tespit etmeyi amaçlamaktadır.*

Materyal ve Metot: *Kesitsel ve tanımlayıcı tasarıma sahip bu çalışmanın örneklemi, Kasım 2023 ile Şubat 2024 tarihleri arasında çevrimiçi sosyal medya platformları aracılığıyla çalışmaya katılmaya gönüllü olan ve dâhil edilme kriterlerine uyan toplam 204 bireyden oluştu. Veriler, araştırmacılar tarafından literatür temel alınarak geliştirilen "Ebeveyn ve çocuk bilgi formu", "Sağlık Algısı Ölçeği", "Beck Anksiyete Envanteri", "Beck Depresyon Envanteri", "Ebeveyn-Çocuk İlişkisi Ölçeği" ve 6–16 yaş arası çocuklar için "Pediatrik Semptom Kontrol Listesi (PSC-17)" kullanılarak toplandı. Veri toplama araçlarını tamamlamak yaklaşık 15–20 dakika sürdü.*

Bulgular: *Ebeveynlerin %45'inde hafif anksiyete, %35'inde ise hafif depresyon olduğu belirlendi. Çocukların sadece %1'inde PSC-17 skoru 12 veya üzerinde bulundu. Ebeveynlerin anksiyete ve depresyon seviyelerine göre çocuklarıyla iletişimlerinde anlamlı bir fark bulunmadı (p>0,05). Deprem sonrası anksiyete ve depresyon yaşayan ebeveynlerin çocuklarında istatistiksel olarak anlamlı psikososyal problemler görüldü. Ebeveynlerin sağlık algısı puanları, depresyon seviyelerine göre değişiklik gösterdi. İleri analiz, orta ve şiddetli depresyon puanına sahip ebeveynlerin sağlık algısı puanlarının, düşük ve hafif depresyon puanına sahip ebeveynlere kıyasla daha düşük olduğunu gösterdi (p: 0,02; p: 0,012).*

Sonuç: *Depremden bir yıl sonra bile ebeveynler anksiyete ve depresyon yaşamaya devam etmekte ve bu durum, çocuklarında psikososyal problemler görülmesini etkilemektedir. Ayrıca, yüksek depresyon seviyesine sahip ebeveynlerin sağlık algısı puanları daha düşük bulunmuştur. Bu sonuçlar, deprem sonrası ebeveynlerin psikososyal desteğe ihtiyaç duyduklarını göstermektedir.*

Anahtar kelimeler: *anksiyete; depresyon; deprem; sağlık algısı; ebeveynler; ebeveyn-çocuk ilişkisi*

İletişim/Contact: *Gizem Kerimoglu Yildiz, Nursing Department, Faculty of Health Science, Mustafa Kemal University, Hatay, Türkiye* • ***Tel:*** *0537 865 94 76* • ***E-mail:*** *gizemkrm@gmail.com* • ***Geliş/Received:*** *14.06.2024* • ***Kabul/Accepted:*** *21.10.2024*

ORCID: *Gizem Kerimoğlu Yıldız: 0000-0002-6850-7233 • Mustafa Yıldız: 0009-0008-2872-3020 • Aysu Gülmez: 0009-0007-4550-2329 • Münire Batmaz: 0009-0009-6341-1513 • Merve Deprem: 0009-0002-3541-4214*

Introduction

Disasters cause physical, social, economic, and cultural losses for individuals, disrupt or halt perceived life, and can be either human-made or natural[1]. Unpredictable natural events, whose impact areas and levels of severity cannot be precisely known and that result in significant loss of life and property, are referred to as "natural disasters"[2]. Among the most frequently encountered natural disasters are earthquakes. Due to its geological position, Turkiye is in a high seismic activity zone and is classified as a first-degree earthquake region[3]. In recent years, various earthquakes of different magnitudes have occurred, and in February 2023, two earthquakes with magnitude 7.8 and 7.6 affected 11 of our provinces, resulting in substantial loss of life and property[4].

Natural disasters such as earthquakes are traumatic experiences that can cause acute stress due to the risk of death and the potential for harm to oneself or loved ones. Additionally, they can lead to ongoing stress due to housing and economic, social, and emotional issues. Consequently, individuals may be psychosocially affected and experience conditions such as post-traumatic stress disorder (PTSD), anxiety, and depression[5,6]. Therefore, monitoring and supporting survivors' psychosocial well-being after disasters like earthquakes is crucial. Studies have shown that if appropriate support is not provided to individuals affected by disasters, long-term and increasing psychological symptoms may develop[7–9]. Conversely, when proper support is given, individuals can more easily adjust to their loss, adapt to their environment, and return to their everyday lives[10].

It is noted that traumatic events affect not only the individuals directly involved but also those with whom they interact[11]. Therefore, supporting parents, who are the primary caregivers of children, is particularly important after traumatic experiences. Nelson et al.[12] stated that the effects and behavioral patterns passed from parents to children can occur in various forms. They explain the transmission of negative emotions from parents to children with the spillover hypothesis, which conveys the same intensity of emotion. Conversely, the compensatory hypothesis explains how parents protect their children from adverse effects. This emotional transmission can also occur in one parent's stress affecting the other parent, which affects communication with the child, known as the crossover hypothesis[9,12]. Numerous studies have investigated the effects of various disasters on children's mental health, demonstrating the significant impact of parental approaches on children[13–16]. For example, a study conducted in Australia after Cyclone Larry found that children from dysfunctional families exhibited more behavioral problems three months post-cyclone[17].

Perceived health status is a subjective, multidimensional, and robust indicator that enables individuals to determine their overall health condition biologically, mentally, and socially. Health perception can vary according to individuals' living conditions, life expectations, and perspectives[18,19]. Although subjective, health perception is also considered a psychosocial health indicator. It is noted that the psychosocial dimension increasingly plays a significant role in determining health and illness perceptions[20,21]. However, no studies have been found in the literature evaluating the impact of health perception on psychosocial problems that may develop after an earthquake. Additionally, there are no studies assessing parents' health perceptions and the occurrence of psychosocial problems after disasters or the impact of these factors on their children's psychosocial conditions and parent-child relationships. Therefore, this study addresses two critical issues. Firstly, it aimed to uncover how the earthquake affected parents and their children and identify psychosocial adversities. It sought to determine how these conditions affect parents' health perceptions and communication levels with their children.

Materials and Method

Study type and aim

This cross-sectional descriptive study aimed to determine the psychosocial status of parents and children one year after the earthquakes that affected 11 provinces in our country on February 6, 2023, and to identify the effects of parents' psychosocial problems on their health perceptions, the occurrence of psychosocial symptoms in their children, and parent-child communication. In line with this aim, the following research questions have been formulated. – What is the prevalence of anxiety and depression in parents and the level of occurrence of psychosocial problems in children one year after the earthquake? – Is there a difference between the anxiety and depression experienced by parents and their health perceptions after the earthquake? – Is there a difference between the anxiety and depression experienced by parents and their communication with their children after the earthquake? – Is there a difference between the anxiety and depression experienced by parents and the psychosocial problems of their children after the earthquake?

The population and sample

The study population comprised all parents in the 11 provinces affected by the February 6 earthquake. The study sample included all individuals who volunteered to participate via online social media platforms between November 2023 and February 2024 and met the inclusion criteria. During this period, 204 parents were reached without a sampling selection method. A post-power analysis was conducted to determine if this sample size was sufficient for the power of data analysis. According to the power analysis based on the Beck Anxiety Inventory, with an alpha error rate of 0.05 and an effect size of 0.38 for 204 parents, the study's power was 95%. The sample included parents with at least one child aged 6–16 who lived in the earthquake region, knew Turkish and agreed to participate in the study.

Data collection

The data collection form was prepared using Google Forms and distributed to participants via social media. In the initial phase of the data collection form, participants were informed that the Personal Data Protection Law would not share their data. The exact page also included information about the study's purpose, the fact that participation was entirely voluntary and that participants could stop filling out the form at any stage of the study. After reading all this information and consenting to participate in the study, participants were directed to pages containing the data collection instruments. Completing the data collection instruments took approximately 15–20 minutes.

Instruments

The data were collected using the "Parent and child information form" developed by the researchers based on the literature, the "Health perception scale," the "Beck anxiety inventory," the "Beck depression inventory," the "Parent-child relationship scale," and the "Pediatric symptom checklist (PSC-17)" for children aged 6–16.

Parent and child information form: This form, prepared by the researchers, consists of 26 questions to determine the demographic data of parents and children and the parents' situations related to the earthquake. The form is divided into two sections. The first section includes seven questions related to the sociodemographic data of the parents (age, gender, marital status, occupation, education, etc.) and eight questions regarding their location, living, and health conditions post-earthquake. The second section comprises 11 questions aimed at gathering information about the children. This section includes questions about the age and gender of the youngest child aged 6–16 and their psychological and social impact from the earthquake.

Beck anxiety inventory (BAI): Developed to measure the frequency of anxiety symptoms experienced by individuals, [22] the Turkish adaptation of the BAI was conducted by Ulusoy, Şahin, and Erkmen[23]. The BAI consists of 21 items. It is a four-point Likert-type scale, rated from zero (not at all) to three (severely). The total score can range from 0 to 63, with scores of 0–9 indicating minimal anxiety; 10–18, mild anxiety; 19–29, moderate anxiety; and 30–63, severe anxiety. A higher score on the scale indicates a higher level of anxiety. The Cronbach's alpha coefficient of the scale was determined to be 0.93[23]. In this study, the Cronbach's alpha coefficient was found to be 0.95 and was used to assess the anxiety levels of parents following the earthquake.

Beck depression inventory (BDI): Developed by Beck and colleagues to measure the severity of individuals' depressive complaints[24]. The inventory is a four-point Likert-type scale consisting of 21 items. It is scored between zero (not at all) and three (severely), with total scores ranging from 0 to 63. A higher score on the scale indicates a higher severity of depression. The inventory includes items related to emotions (2 items), behaviors (2 items), cognitive state (10 items), interpersonal symptoms (1 item), and physical symptoms (6 items). Scores are interpreted as follows: 0–9 indicating minimal depression, 10–18 mild depression, 19–29 moderate depression, and 30–63 severe depression. The reliability and validity of the scale were assessed by Hisli in 1989, with a Cronbach's alpha value calculated at 0.74[25]. In this study, the Cronbach's alpha coefficient was determined to be 0.901, and it was used to assess the depression levels of parents following the earthquake.

Pediatric symptom checklist (PSC-17): This (PSC-17) scale allows parents to evaluate their children's behaviors. It is used for the early diagnosis of psychosocial problems in children aged 6–16[26]. The Turkish validity and reliability of the scale have been established, indicating it can be used with Turkish parents[27]. This scale includes 17 items related to children's emotions and behaviors. It is a three-point Likert-type scale where 0=Not true/Never, 1=Sometimes or somewhat accurate, and 2=Very often true. Parents complete the scale based on their child's situation or the past six months.

All items on the scale are positive. The maximum score obtainable is 34, and the minimum score is 0. A total score of 12 or higher indicates the presence of a psychosocial problem, and such children need further evaluation for a definitive diagnosis[27]. In the original study, the internal consistency coefficient for the subdimensions of the scale was determined to be $\alpha \geq 0.79$[26]. In this study, Cronbach's alpha internal consistency coefficient was determined to be 0.773. The scale was used to identify the psychosocial and behavioral problems observed by parents in their children post-earthquake. The total score obtained from the scale was compared with the parents' levels of anxiety and depression to evaluate whether the psychosocial problems experienced by parents affected their children's psychosocial status.

Parent-child relationship scale (PCRS): The scale was developed by Aytaç et al.[28] (2018) to evaluate the relationship between parents and children. The scale consists of a total of 15 items and two subscales. The first subscale assesses the positive parent-child relationship, including warmth, interest, and sensitivity. The second subscale evaluates the negative parent-child relationship, including conflict, punishment, and negative emotions. It is a five-point Likert-type scale, with scores ranging from 1 to 5. Each subscale is evaluated separately. High scores on the first subscale indicate an increase in the positive relationship between the parent and child. The internal consistency coefficient for this subscale is 0.71. High scores on the second subscale indicate an increase in the negative relationship[28]. The internal consistency coefficient for this subscale is 0.74. In this study, the total Cronbach's alpha internal consistency coefficient of the scale was found to be 0.907.

Health perception scale (HPS): The scale was developed by Diamond et al.[29] (2007) to evaluate individuals' perceptions of health. The adaptation of the scale into Turkish was conducted by Kadioğlu and Yildiz[30] (2012). It is a five-point Likert-type scale comprising 15 items and four sub-dimensions. These sub-dimensions are "Control Center," "Self-Awareness," "Certainty," and "Importance of Health." The items are scored between 1 and 5, where 1=strongly disagree, 2=disagree, 3=neutral, 4=agree, and 5=strongly agree. Items 2, 3, 4, 6, 7, 8, 12, 13, and 15 are negatively phrased and reverse-scored. The total score on the scale can range from a minimum of 15 to a maximum of 75. The Cronbach's alpha values for the subgroups of the scale range from 0.82 to 0.91, with the total Cronbach's alpha coefficient being 0.70 or higher[30]. In this study, the Health Perception Scale assesses individuals' health perceptions post-earthquake and determines if it impacts their psychosocial issues.

Ethical Considerations

The study received ethical approval from the University's Social and Humanities Ethics Committee (26.10.2023/09/02). An informed consent form was provided before parents filled out the online questionnaire. Only after they indicated their acceptance by marking the relevant field were they able to view the study questions. Parents were informed that their participation was voluntary, that no personal data would be recorded, and that they could withdraw from the study at any stage. The study adhered to the Declaration of Helsinki guidelines.

Data Analysis

The findings obtained in the study were evaluated using IBM Statistical Package for Social Sciences (SPSS) program version 26 (IBM Inc, Chicago, II, USA) for statistical analyses. The normality of the distribution of variables was assessed with the Shapiro-Wilk test. Descriptive statistical methods such as mean/median, standard deviation, and frequency calculations were used to evaluate the study data. The Mann-Whitney U test, a non-parametric test, was used to assess the scores obtained from the scales to determine the difference between the two means. In comparison, the Kruskal-Wallis test was used to evaluate more than two means. The Mann-Whitney U test was used for post hoc analyses to determine the source of the differences between groups. The level of significance was planned to be set at $p < 0.05$.

Results

A total of 204 parents participated in the study between November 2023 and February 2024.

When examining the characteristics of the parents who participated in the study, most were mothers (70.6%) aged 35–44 (46.6%). Most parents resided in Hatay province (80.9%), and 59.3% had lost at least one relative due to the earthquake. More than half of the parents reported experiencing disruptions in accessing health services post-earthquake (57.8%), primarily due to damaged hospitals (47.1%). Half of the parents

(50.5%) had relocated to a different city after the earthquake, while those who did not relocate mainly stayed in tents (22.1%). It was found that only 8.3% of parents received psychological support following the earthquake (Table 1).

Table 1. *General characteristics of the parents*

Characteristics of the parents		n	%
Gender	Male	60	29.4
	Female	144	70.6
Age	18–24	8	3.9
	25–34	56	27.5
	35–44	95	46.6
	45–54	38	18.6
	55–64	7	3.4
Education	Primary	28	13.7
	Secondary	19	9.3
	High school	47	23.0
	University	90	44.1
	Postgraduate	20	9.8
Marital status	Married	185	90.7
	Single	19	9.3
Chronic disease	Yes	27	13.2
	No	177	86.8
Working status	Employed	115	56.4
	Unemployed	89	43.7
City of residence	Hatay	165	80.9
	Adana	12	5.9
	Gaziantep	10	4.9
	Şanlıurfa	8	3.9
	Adıyaman	5	2.5
	Kahramanmaraş	2	1.0
	Malatya	2	1.0
Loss of relatives	Yes	121	59.3
	No	83	40.7
Degree of proximity to the deceased	1. degree	6	4.9
	2. degree	25	20.7
	3. degree	71	58.7
	Friend	19	15.7
Receiving health services post-earthquake	Yes	86	42.2
	No	118	57.8
Reasons for not receiving health services*	Hospitals being damaged	96	47.1
	Lack of transportation	53	26.0
	Lack of supplies	53	26.0
	Lack of healthcare workers	42	20.6
Relocation post-earthquake	Yes	101	50.5
	No	103	49.5
Post-earthquake accommodation*	In a tent	45	22.1
	In their own home	25	12.3
	At a relative's house	25	12.3
	In a rented house	14	6.9
	In a container	6	2.9
Duration of leaving the city post-earthquake	Within the first week	68	33.3
	After the first week	26	12.7
	After the first month	6	3.0
Receiving psychological support post-earthquake	Yes	17	8.3
	No	187	91.7

Table 2 presents information about the children who participated in the study. According to the data, 55.9% of the children were male, and 92.2% had no chronic illnesses. Only 11.8% of parents reported receiving psychological support for their children after the earthquake, with psychologists being the most consulted professionals (7.8%). When asked how their children reflected their emotional changes post-earthquake, most parents indicated that their children were afraid of being alone (57.4%) and overly reactive to sounds (41.2%). Nevertheless, 61.8% of parents reported that their children adapted well post-earthquake. Additionally, 49.5% of parents rated their children's communication with them as "good," and 45.1% rated their children's communication with friends as "good."

Table 2. *Information about the children*

Characteristics of the children		n	%
Gender	Male	114	55.9
	Female	90	44.1
Chronic disease	Yes	16	7.8
	No	188	92.2
Receiving health services post-earthquake	Yes	111	54.4
	No	93	45.6
Reasons for not receiving health services *	Hospitals being damaged	73	35.8
	Lack of transportation	45	22.1
	Lack of supplies	39	19.1
	Lack of healthcare workers	38	18.6
Receiving psychological support for a child after an earthquake	Yes	24	11.8
	No	180	88.2
Source of support*	Psychotherapist	16	7.8
	Psychiatrist	6	2.9
	Pedagog	6	2.9
	Teacher	4	2.0
	Pediatrist (Physician)	2	1.0
Children's ways of expressing emotional changes post-earthquake*	Fear of being alone	117	57.4
	Overreacting to sounds	84	41.2
	Talking	68	33.3
	Aggression	45	22.1
	Did not react	38	18.6
	Playing games	35	17.2
	Crying	32	15.7
Adaptation status post-earthquake	Yes	126	61.8
	No	78	38.2
Parent-child communication post-earthquake	Very good	33	16.2
	Good	101	49.5
	Moderate	58	28.4
	Bad	8	3.9
	Very bad	4	2.0
Peer communication post-earthquake	Very good	31	15.2
	Good	92	45.1
	Moderate	71	34.8
	Bad	6	2.9
	Very bad	4	2.0

*Multiple answers.

Table 3. *Anxiety and depression status of parents and behavioral problems in children*

Scales		N	%	Mean ± SD (Min-Max)
BAI	Minimal anxiety	60	29.4	19.78±14.62
	Mild anxiety	46	22.5	(0–61)
	Moderate anxiety	48	23.5	
	Severe anxiety	50	24.5	
BDI	Minimal depression Mild depression	61	29.9	15.35±9.76
	Moderate depression Severe depression	73	35.8	(0–48)
		53	25.9	
		17	8.3	
PSC 17	Under 12	202	99	1.83±2.35
	12 and above	2	1	(0–17)

BAI: Beck anxiety inventory; BDI: Beck depression inventory; PSC 17: Pediatric symptom checklist.

Table 4. *Parent-child relations, psychosocial problems in children, and health perception scores according to parental anxiety and depression*

BAI	PCRS Total Mean ± SD (Mean Rank)	Positive Mean ± SD (Mean Rank)	Negative Mean ± SD (Mean Rank)	PSC 17 Mean ± SD (Mean Rank)	HPS Mean ± SD (Mean Rank)
Minimal anxiety	55.71±10.28 (101.03)	36.81±7.59 (107.96)	14.43±4.23 (96.84)	1.25±1.39[a] (89.35)	48.10±3.57 (116.83)
Mild anxiety	55.67±9.56 (100.99)	36.36±7.07 (101.97)	14.82±3.74 (100.66)	1.10±1.46[b] (82.49)	46.80±4.38 (99.79)
Moderate anxiety	54.83±8.90 (93.91)	35.97±6.62 (95.54)	14.25±3.95 (92.24)	2.35±2.13[c] (121.10)	46.75±4.74 (102.22)
Severe anxiety	57.08±9.89 (113.90)	36.44±7.28 (103.12)	16.18±3.78 (120.83)	2.72±3.49[d] (118.83)	45.98±4.66 (88.06)
Test*; p	2.955; 0.399	1.193; 0.755	6.918; 0.075	17.825 0.001**	6.669; 0.083
BDI					
Minimal depression	55.50±10.26 (98.79)	36.32±7.52 (103.10)	14.80±4.36 (102.09)	1.78±2.70[x] (96.57)	47.95±3.82[m] (116.07)
Mild depression	55.72±9.97 (105.36)	36.52±7.46 (104.73)	14.64±3.69 (99.14)	1.36±1.34[y] (95.65)	47.68±3.70[n] (109.31)
Moderate depression	56.90±7.60 (105.98)	37.20±5.79 (105.15)	15.09±3.71 (104.96)	2.16±2.86[z] (108.18)	45.41±4.97[p] (82.75)
Severe depression	54.11±12.23 (92.71)	33.94±8.11 (82.53)	15.82±4.86 (110.74)	3±2.26[t] (135.47)	45.23±5.26[r] (86.18)
Test*; p	1.067; 0.785	2.170; 0.538	0.668; 0.881	7.816; 0.049**	11.500; 0.009**

BAI: Beck anxiety inventory; BDI: Beck depression inventory; PCRS: Parent-child relationship scale; PSC 17: Pediatric symptom checklist.
*Kruskal-Wallis Test; ** p>0.05
a<c 0.004; a<d 0.007; b<c 0.001; b<d 0.002; x<t 0.020; y<t 0.006; m>n 0.002; n>p 0.012

Table 3 illustrates the depression and anxiety statuses of parents one year after the earthquake. Accordingly, it was determined that 45% of parents had mild anxiety, while 35% had mild depression. It was observed that only 1% of children had a PSC-17 score of 12 or higher.

Table 4 illustrates the relationship between parents' anxiety and depression levels post-earthquake and their communication with their children, it was found that there was no significant difference in communication with their children based on the parents' anxiety and depression levels (p>0.05). However, when examining the presence of psychosocial problems in children, it was found that children of parents experiencing anxiety and depression after the earthquake had statistically significant psychosocial problems. Advanced analysis to determine the source of this difference revealed that the PSC-17 scores of children of parents with mild anxiety were statistically significantly lower than those of children of

parents with moderate and high anxiety levels (moderate anxiety p: 0.046; high anxiety p: 0.004). Similarly, children of parents with high depression scores had statistically significantly higher PSC-17 scores than those of children of parents with low and moderate depression scores (moderate depression p: 0.02; high depression p: 0.006). When examining parents' health perceptions based on their anxiety and depression levels, it was found that parents' health perception scores varied according to their depression levels. Advanced analysis indicated that parents with moderate and severe depression scores had lower health perception scores compared to parents with low and mild depression scores (p: 0.02; p: 0.012).

Discussion

Experiencing a natural disaster like an earthquake can profoundly impact society, leading to mental health problems such as stress, anxiety, and depression[31]. This study aimed to uncover how the earthquake affected parents and their children, identify the existing psychosocial adversities, and determine how these conditions influenced parents' health perceptions and communication levels with their children. Research indicates that mental health problems such as anxiety, depression, and post-traumatic stress disorder (PTSD) can increase in individuals after natural disasters like earthquakes. Thapa et al.[32] (2018) assessed anxiety, depression, and PTSD among 198 individuals following the 2015 earthquake in Nepal and found that 20% of participants exhibited significant anxiety symptoms, while 8% displayed considerable depression symptoms. A systematic review investigating the prevalence of depression and PTSD in mothers after natural disasters found that 38% of mothers experienced depression post-earthquake[33]. Similarly, Xi et al.[34] (2020) evaluated the prevalence of mental health problems such as anxiety and depression three months after the 2017 Jiuzhaigou earthquake and reported an anxiety prevalence rate of 53.8% and a depression prevalence rate of 69.6%. A study conducted six months after the 2005 Pakistan earthquake with 361 participants found anxiety and depression prevalence rates of 77.3% and 70.9%, respectively. In the current study, it was determined that 28.9% of parents had high anxiety, and 8.3% had high depression. These findings not only align with existing literature but also indicate that anxiety and depression remain prominent one year after the earthquake. However, because the levels of anxiety and depression in parents before and immediately after the earthquake were not known, comparisons with post-earthquake levels could not be made. This complicates the interpretation of whether there were differences in parents' anxiety and depression levels before or after the earthquake.

Natural disasters significantly impact not only parents but also children. Exposure to earthquakes is well-known to lead to an increased prevalence of psychological disorders in children and adolescents, including post-traumatic stress disorder (PTSD), depressive disorder, substance abuse, anxiety, and somatization disorders[35–37]. Emotional and behavioral difficulties (e.g., fears, depressive mood, and behavioral problems) affecting daily life (e.g., peer relationships, academic performance) are significant for children and adolescents. Recent research has observed that earthquakes can delay emotion processing,[38] coping strategies,[39,40] and the psychosocial functions of children and adolescents[41,42]. In this study, parents assessed their children's emotional changes. When asked to express their observations on how their children reflected their emotional changes post-earthquake, most parents reported being afraid of being alone (57.4%) and were overly reactive to sounds (41.2%). Nevertheless, parents stated that their children adapted post-earthquake (61.8%), and their communication with both parents (49.5%) and friends (45.1%) was "good." When the occurrence of behavioral problems in children was evaluated using the PSC-17 scale, it was found that 99% of children scored below 12, indicating no behavioral issues. This is a positive indicator for children in the earthquake-affected region. However, despite these results being obtained through a scale, they are based solely on parents' observations, lacking children's self-assessments, making the evidence insufficient to draw definitive conclusions.

Experiencing stress during childhood is associated with an increased risk of emotional and behavioral problems, which may be linked to psychopathology later in life[43,44]. Extremely stressful experiences, such as living in war zones and being exposed to natural disasters, have been associated with these changes[45–47]. Under normal circumstances, it has been indicated that stress experienced by parents for various reasons is related to developmental problems in their children[48–50]. A study conducted two years after the 2012 earthquake in Italy involved 682 children aged 9–14 and aimed to identify predictors of post-traumatic stress disorder (PTSD) and emotional and behavioral problems in children and adolescents. Three significant factors affecting emotional and behavioral problems were identified: the number of traumatic events experienced,

the severity of exposure to the event, and parental psychopathology[37]. During Hurricane Katrina, a study examining the relationship between anxiety symptoms in children aged 6–17 before and after the hurricane found that perceived parental styles of acceptance and over-control affected anxiety levels[14]. Additionally, dysfunctional parental attitudes have been reported to be associated with poor mental health in children[15]. A study conducted three years after the magnitude nine earthquake in eastern Japan showed that parenting styles influenced behavioral problems among children post-earthquake[16]. In this study, when examining the occurrence of psychosocial problems in children based on parents' anxiety and depression levels, it was found that children of parents who experienced anxiety and depression after the earthquake had a statistically significant higher incidence of psychosocial problems. Specifically, children of parents with mild anxiety levels had lower PSC-17 scores compared to children of other parents. Conversely, children of parents with high depression scores had higher PSC-17 scores compared to children of parents with low and moderate depression scores. This result is consistent with the literature and shows that children are affected by the negative mental processes of their parents.

How health is perceived affects an individual's acquisition and maintenance of healthy living behaviors and thoughts about their health status[51]. Accordingly, those with a high health perception are expected to exhibit more health-protective and health-enhancing behaviors. However, considering that health is defined as a state of complete physical and mental well-being,[52] it is crucial that health perception is related to physical and psychological health. The literature also indirectly explains the relationship between health perception and mental problems. Studies have shown that individuals with mental issues such as depression are affected in their health utilization[53,54]. Individuals with high levels of depression may perceive their health negatively. As a result of depression, their health perception can deteriorate, affecting all areas of life and leading to withdrawal from even daily activities[55]. When examining the health perceptions of parents based on their anxiety and depression levels, it was found that parents with moderate and high depression scores also had lower health perception scores compared to other parents (low depression p: 0.029; and mild depression p: 0.012). The differences observed in this study between levels of depression and health perception can be considered indicative of the amount of the need for psychosocial support.

Limitations of the Study

This study is significant in examining parents' mental problems post-earthquake and their impact on their relationships with their children, the occurrence of behavioral problems in children, and parents' health perceptions. However, an important limitation of the study is the lack of knowledge about the anxiety and depression levels of parents before the earthquake. As a result, comparisons with post-earthquake anxiety and depression levels could not be made. Additionally, not knowing the initial levels of anxiety and depression immediately after the earthquake makes it difficult to interpret whether there has been a reduction in these levels among parents. Another limitation is that the behavioral problems in children were assessed based on proxy reports from parents. None of these children were medically evaluated for behavioral issues.

Conclusion and Recommendations

This study aimed to identify parents' psychosocial problems and evaluate their impact on their health perception, relationships with their children, and the presence of psychosocial symptoms in their children. The findings indicate that even one year after the earthquake, parents continued to experience anxiety and depression, albeit at mild levels, and this situation affected the occurrence of psychosocial problems in their children. Additionally, parents with high levels of depression had lower health perception scores. These results demonstrate the continued need for psychosocial support for parents after an earthquake. Psychosocially supported parents are more likely to raise children without psychosocial problems, who, in turn, will become adults without psychosocial issues in the future.

Considering that the physical and psychosocial health of parents, who constitute a significant portion of the population, has crucial impacts on both community health and their children's health, nurses must fulfill their roles in health protection and education. During this process, nurses should promote, protect, and instill proper health behaviors in individuals, families, and the community. Nurses need to be aware of the needs of each family member, the smallest unit of society. Nurses should be supportive in helping individuals cope with potential and existing problems. To support parents and help them cope with psychosocial issues, nurses must first be aware of the existing or possible symptoms.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Relationship Between Waist-to-Neck Circumference Ratio and Coronary Artery Calcium Score

Bel Boyun Çevresi Oranı ve Koroner Kalsiyum Skoru Arasındaki İlişki

Sidar Siyar Aydin[1], Selim Aydemir[2], Onur Altinkaya[2], Murat Ozmen[2], Emrah Aksakal[2], Muhammed Cuneyt Seker[1], Oguzhan Birdal[1]

[1]Department of Cardiology, Ataturk University, Faculty of Medicine; [2]Department of Cardiology, University of Health Sciences, Erzurum City Hospital, Erzurum, Turkiye

ABSTRACT

Aim: *Coronary artery calcium score (CACS) is the leading parameter for detecting subclinical atherosclerosis. The waist-to-neck circumference ratio (WNR) is advantageous in determining visceral fat levels associated with atherosclerosis. In light of these findings, our study aimed to investigate the relationship between CACS and WNR.*

Material and Method: *We conducted a cross-sectional study at a single center as part of our research. The study consists of 362 patients who visited the cardiology outpatient clinic from March to May 2024. They underwent multidetector coronary computed tomography and had their CACS calculated. After obtaining written consent from the patients, past disease histories, waist circumference, neck circumference, blood pressure, and laboratory parameters were recorded. Waist-to-neck circumference ratio was calculated. Patients were divided into two groups: CACS=0 and CACS >0.*

Results: *Of all the patients, 70.7% (256 patients) had a CACS score of 0, with the remaining 29.3% (106 patients) having a CACS score greater than. The mean age in the group with CACS >0 was 52.85±8.79 years, and the mean age was statistically significantly higher (p<0.001). Waist-to-neck circumference ratio was statistically significantly higher in the group with CACS >0 (p<0.001). In logistic regression analyses, WNR emerged as a significant independent predictor of detecting the presence of CACS (p=0.001). The Receiver operating characteristic curve (ROC) analysis was performed to evaluate the ability of WNR to predict the presence of CACS, the area under the curve was determined as 0.665 (0.604–725), the cut-off was 2.75, the sensitivity was 67.9%, and the specificity was 67.2% (p<0.001).*

Conclusion: *Our study found a significant relationship between WNR and the presence of CACS.*

Key words: *anthropometric measurements; atherosclerosis; waist-neck circumference ratio; coronary artery calcium score*

ÖZET

Amaç: *Koroner arter kalsiyum skoru (KAKS) subklinik ateroskleroz tespiti için en önde gelen parametredir. Bel boyun çevresi oranı (BBÇO) içerdiği parametreler sebebiyle visseral yağlanmayı tahmin etmek için kullanılabilir. Visseral yağlanma ateroskleroz ile ilişkilidir. Bu bulgular ışığında çalışmamız KAKS ile BBÇO arasındaki ilişkiyi araştırmayı amaçlamıştır.*

Materyal ve Metod: *Çalışmamız tek merkez kesitsel çalışma olarak tasarlanmıştır. Mart-Mayıs 2024 tarihleri arasında kardiyojoji polikliniğine başvuran multidedektör koroner bilgisayarlı tomografi çekilen ve KAKS hesaplanmış 362 hastayı kapsamaktadır. Hastalardan yazılı onam alındıktan sonra geçmiş hastalık öyküleri, bel çevresi, boyun çevresi, kan basıncı ve laboratuvar parametreleri kaydedildi. Bel boyun çevresi oranı hesaplandı. Hastalar KAKS=0 ve KAKS >0 olan olarak iki gruba ayrıldı.*

Bulgular: *Hastaların %70,7'sinde (256 hasta) KAKS=0 iken, %29,3'ünde (106 hasta) KAKS >0 olarak değerlendirildi. Koroner arter kalsiyum skoru >0 olan grupta yaş ortalaması 52,85±8,79 yıldı ve bu grupta yaş ortalaması istatistiksel olarak anlamlı daha yüksekti (p<0,001). Bel boyun çevresi oranı KAKS >0 olan grupta istatistiksel olarak anlamlı daha yüksekti (p<0,001). Yapılan tek değişkenli ve çok değişkenli lojistik regresyon analizinde BBÇO'nun KAKS varlığı tespitinde bağımsız bir öngördürücü olduğu bulundu (p=0,001). BBÇO'nun KAKS varlığını tahmin etme yeteneğini değerlendirmek için yapılan ROC analizinde eğri altında kalan alan 0.665 (0,604–725), cut-off 2,75, sensitivite %67,9 ve spesifite %67,2 olarak belirlendi (p<0,001).*

Sonuç: *Çalışmamız BBÇO ve KAKS varlığını arasında anlamlı bir ilişki olduğunu göstermiştir.*

Anahtar Kelimeler: *antropometrik ölçümler; ateroskleroz; bel boyun çevresi oranı; koroner arter kalsiyum skoru*

İletişim/Contact: *Sidar Siyar Aydin, Department of Cardiology, Ataturk University, Faculty of Medicine, Cat Yolu Cad., 25240 Yakutiye, Erzurum, Türkiye* • ***Tel:*** *0553 617 66 52* • ***E-mail:*** *s.siyaraydin@gmail.com* • ***Geliş/Received:*** *18.06.2024* • ***Kabul/Accepted:*** *05.09.2024*

ORCID: *Sidar Şiyar Aydın, 0000-0002-8204-1505* • *Selim Aydemir, 0000-0001-6654-2521* • *Onur Altınkaya, 0000-0002-2178-6140* • *Murat Özmen, 0000-0002-6237-1398* • *Emrah Aksakal, 0000-0001-5765-4281* • *Muhammed Cüneyt Şeker, 0009-0008-6467-959X* • *Oğuzhan Birdal, 0000-0002-0667-2516*

Introduction

Cardiovascular diseases are one of the leading causes of death worldwide. Atherosclerosis is one of the most significant causes of cardiovascular diseases. It is crucial to detect and treat atherosclerosis early. One of the main non-invasive tests used to detect atherosclerosis is multidetector coronary tomography (MDCT) and coronary artery calcium score (CACS) calculated via MDCT[1]. Coronary artery calcium score is a good tool for detecting atherosclerosis and assessing the risk of developing adverse cardiovascular events[2]. However, the disadvantages of the examination are the allergic reaction caused by the contrast material applied during MDCT imaging, the risk of contrast nephropathy that may develop afterward, and the expense of the examination[3]. These examination disadvantages emphasize the need for alternative diagnostic tools to detect the presence of atherosclerosis and CACS.

Anthropometric measurements like waist circumference (WC), neck circumference (NC), and body mass index (BMI) can help in detecting visceral obesity[4]. Recent studies suggest that the fat tissue in the upper body may produce inflammatory cytokines, leading to insulin resistance and a higher risk of cardiovascular disease. The fact that visceral obesity is associated with atherosclerosis and major adverse cardiac events makes this situation important[5]. The waist-to-neck circumference ratio (WNR) is a possible marker for sleep apnea in children, connected to body fat distribution and obesity[6]. Considering the association of upper body fat distribution with cardiovascular disease risk and obesity, our study aimed to evaluate the relationship between WNR and CACS.

Materials and Methods

Study Design and Patient Selection

The format of this study was a cross-sectional, single-center study. This study included 362 patients who applied to the cardiology outpatient clinic of our center between March and May 2024 and underwent MDCT during non-invasive tests performed to evaluate chest pain of cardiac origin. We obtained written consent from the admitted patients and recorded their previous history, systolic and diastolic blood pressures, age, gender information, hemogram, biochemical parameters, and WC, NC, and BMI information. Coronary artery calcium score was obtained and recorded from MDCT reports. Waist-to-neck circumference ratio was calculated by dividing WC by NC. Excluded from the study were patients with coronary artery disease, malignancies, chronic inflammatory diseases, Cushing's syndrome, those who had undergone bariatric surgery, individuals with goiter and spine anomalies affecting neck circumference, and patients with severe liver and kidney failure. This study was performed by the Declaration of Helsinki and with the approval of the local ethics committee.

Measurement of Laboratory Parameters

Venous blood was collected from participants following an 8–12 hour fast. Using electrical impedance, hemoglobin, hematocrit, platelet, and white blood cell counts were determined. Biochemical parameters were analyzed using standard laboratory methods (Beckman Coulter LH 780, Miami, FL, ABD). Total cholesterol, triglyceride, and high-density lipoprotein levels were determined utilizing the Beckman Coulter AU5800 analytical platform. The calculation of low-density lipoprotein levels involved the Friedewald equation.

Blood Pressure and Anthropometric Measurements

The blood pressure was measured using a sphygmomanometer. There was an assurance that no cigarettes or caffeine had been consumed 30 minutes before the measurement. The cardiologist took the measurement following a minimum 5-minute rest period. Individuals without a hypertension (HT) diagnosis had their blood pressure measured in both arms, with the highest reading recorded.

Weight and height values were documented to compute BMI. The scale is in kilograms for weight and centimeters for height. Body mass index equals weight divided by height squared.

For NC measurement, the patient was seated upright and facing forward. Female patients had their midpoint measured between the upper neck and upper sternum, while male patients had their measurement taken below the laryngeal prominence.

For WC measurement, the patient was standing upright with the patient's legs shoulder-width apart, and the measurement was based on the midpoint between the lowest rib and the spina iliaca anterior superior.

Coronary Artery Calcium Score Measurement

The CACS was determined using an MDCT scan and calculated using the modified Agatston protocol 7. The same radiology specialist examined the MDCT results. Coronary artery calcium score progression is recognized when the CACS shows a measurement greater than zero.

Statistical Analysis

Numbers and percentages were used to represent categorical data. Nonparametric data was analyzed using the χ^2 test. We examined the normality of all variables by conducting the Kolmogorov–Smirnov test and examined the homogeneity of variances with the Levene test before conducting significance tests. T-tests for independent groups were used to analyze data that showed a normal distribution and homogeneity. The Mann-Whitney U test was used to evaluate differences between parameters that did not show normal distribution between two groups, while comparisons between more than two groups were performed by analysis of variance or Kruskal-Wallis tests. The relationships between quantitative variables were evaluated using the Spearman correlation test. Univariate and multivariate logistic regression was performed to analyze the defined risk factors for CACS progression and determine independent risk factors. All analyses were performed using the IBM Statistical Package for Social Sciences (SPSS) program version 23.0 (IBM Corp., NY, USA) statistical package. For all statistical analyses, the significance level was deemed to be two-sided, $p < 0.05$.

Results

Our study included 362 patients who applied to our center's cardiology outpatient clinic and underwent MDCT between March and May 2024. Patients were categorized into two groups based on their coronary artery calcium score (CACS): one group had a CACS of 0, while the other group had a CACS >0 (there is CACS progression). Of all the patients, 70.7% (256 patients) had a CACS score of 0, with the remaining 29.3% (106 patients) having a CACS score greater than. Although there were more female participants in the group with CACS >0, there was no statistically significant difference in terms of gender between the groups. The mean age in the group with CACS >0 was 52.85±8.79 years, and the mean age was statistically significantly higher (p<0.001). In the group where CACS >0 occurred, the prevalence of HT was 37.7% (40 patients), compared to 25% (64 patients) in the group with CACS=0 (p=0.015). Platelet levels were significantly lower in the group with CACS >0 (p=0.008). Glucose, alanine aminotransferase (ALT), and creatinine were statistically significantly higher in the group with CACS >0 (p=0.03, p<0.001, and p=0.008, respectively). BMI, weight, WC, Systolic blood pressure (SBP), and WNR were statistically significantly higher in the group with CACS >0 (p<0.001, p<0.001, p<0.001, p=0.013, and p<0.001, respectively). Review Table 1 for the details.

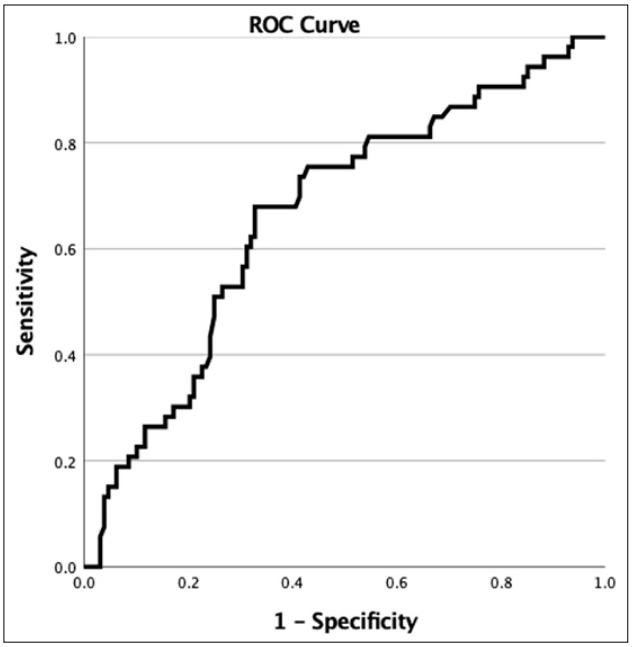

Figure 1. *Receiver operating characteristic curve (ROC) analysis.*

A univariate and multivariate logistic regression analysis was performed to identify independent factors associated with detecting the presence of CACS. Age, platelet count, ALT levels, and WNR were all independent predictors of the presence of CACS (p=0.001, p=0.004, p=0.002, and p=0.001, respectively). The results of the regression analysis are detailed in Table 2.

Receiver operating characteristic curve analysis was performed to evaluate the ability of WNR to predict the presence of CACS. As a result of the analysis, the area under the curve (AUC) was determined as 0.665 (0.604–725), the cut-off was 2.75, the sensitivity was 67.9%, and the specificity was 67.2% (p<0.001) (Figure 1).

Table 1. *Baseline demographic characteristics*

Variables	Coronary calcium score=0 (n=256)	Coronary calcium score >0 (n=106)	P value
Gender, male (%)	110 (43)	40 (37.7)	0.358
Age (years)	46.48±11.67	52.85±8.79	**<0.001**
Height (cm)	167.14±8.59	167.26±9.74	0.466
BMI (kg/m^2)	28.40±5.41	30.13±5.11	**<0.001**
Weight (kg)	79.24±15.39	84.02±14.23	**<0.001**
NC (cm)	35.46±3.86	36.22±3.46	0.281
WC (cm)	95.5±13.4	104.35±13.90	**<0.001**
CVE, n (%)	4 (1.6)	4 (3.8)	0.193
HF, n (%)	6 (2.3)	0 (0.0)	0.112
COPD, n (%)	6 (2.3)	4 (3.8)	0.450
HT, n (%)	64 (25)	40 (37.7)	**0.015**
DM, n (%)	22 (8.6)	8 (7.5)	0.742
SBP (mmHg)	128.49±16.73	133.34±19.25	**0.013**
DBP (mmHg)	74.96±10.22	77.75±12.29	0.055
WBC (10^3/μL)	9.22 (7.8–10.91)	8.84 (7.16–11.05)	0.226
Hemoglobin (g/dL)	15.46±1.69	15.79±1.51	0.269
Platelet (10^3/μL)	296 (242–348)	265 (236–338)	**0.008**
CRP (mg/L)	3 (1.07–7.84)	4.40 (1.72–10)	0.199
Glucose (mg/dL)	92 (85–97)	94 (89–98)	0.03
ALT (U/L)	27 (19–37)	33 (22–43)	**<0.001**
Total Cholesterol (mg/dL)	187.62±40.28	194.84±63.10	0.701
HDL (mg/dL)	44.32±11.53	43.17±10.08	0.319
LDL (mg/dL)	129 (98–148)	126 (92–150)	0.626
Triglyceride (mg/dL)	167 (95–237)	171(86–265)	0.563
Creatinine (mg/dL)	0.88±0.19	0.92±0.18	**0.008**
HbA1C (%)	5.7 (5.38–6.15)	5.8 (5.35–6.28)	0.924
Calcium (mg/dl)	9.52±0.65	9.6±0.52	0.341
WNR	2.70±0.35	2.88±0.34	**<0.001**

BMI: body mass index; NC: neck circumference; WC: waist circumference; CVE: cerebrovascular event; HF: heart failure; COPD: chronic obstructive pulmonary disease; HT: hypertension; DM: diabetes mellitus; SBP: systolic blood pressure; DBP: diastolic blood pressure; WBC: white blood cells; CRP: C-reactive protein; ALT: alanine aminotransferase; HDL: high-density lipoprotein; LDL: low-density lipoprotein; WNR: waist-to-neck circumference ratio.

Table 2. *Univariate and multivariate logistic regression analysis*

Variables	Univariate OR (95% CI)	P value	Multivariate OR (95% CI)	P value
Age	1.057 (1.033–1.081)	**<0.001**	1.043 (1.017–1.071)	**0.001**
BMI	1.061 (1.017–1.108)	**0.006**	1.005 (0.951–1.063)	0.852
SBP	1.016 (1.003–1.029)	**0.018**	1.005 (0.990–1.021)	0.508
Platelet	0.996 (0.993–0.999)	**0.004**	0.995 (0.992–0.998)	**0.004**
Glucose	1.000 (0.996–1.003)	0.918	0.998 (0.993–1.002)	0.357
ALT	1.021 (1.009–1.033)	**<0.001**	1.022 (1.008–1.036)	**0.002**
Creatinine	4.184 (1.294–13.530)	**0.017**	2.326 (0.636–8.501)	0.202
WNR	4.003 (2.101–7.626)	**<0.001**	4.054 (1.744–9.424)	**0.001**

BMI: body mass index; SBP: systolic blood pressure; ALT: alanine aminotransferase; WNR: waist-to-neck circumference ratio; OR: odds ratio; CI: confidence interval.

Discussion

Our study found a significant relationship between WNR and the presence of CACS. Waist-to-neck circumference ratio is a simple parameter to utilize, and to our knowledge, our study is the first to evaluate this relationship.

Subclinical atherosclerosis is a condition that can cause individuals to experience adverse cardiovascular events[8]. Detection and early treatment of this condition may contribute positively to the patient's morbidity and mortality[9]. Multidetector coronary tomography and CACS are crucial diagnostic tools for identifying subclinical coronary atherosclerosis. Not only is CACS related to adverse cardiovascular outcomes, but it also shows a strong association with visceral adiposity[10]. Additionally, adverse cardiovascular events were associated with CACS and visceral adiposity[11,12]. Waist circumference, NC, and BMI have previously been used to detect visceral adiposity[13,14]. Implementing alternative, non-invasive anthropometric measurements in assessing subclinical atherosclerosis could be advantageous, particularly in light of the radiation exposure, contrast material complications, lengthy procedure time, and cost of using MDCT for CACS calculation. With this feature, our study emphasized the importance of anthropometric measurements.

Body mass index indicates general obesity. However, it may cause abdominal obesity to be overlooked. While general and abdominal obesity are typically correlated, some individuals have a normal BMI but high WC measurements[15,16]. Waist circumference alone may be an indicator of coronary artery disease. Some studies have even defined it as a better parameter than BMI in detecting coronary artery disease. Because even if the individual has a normal BMI, a higher waist circumference still poses a risk for cardiovascular diseases[17]. Recent advancements in imaging techniques have led to investigations on the detection of subcutaneous, intra-abdominal, and visceral adipose tissues and their association with cardiovascular diseases[18]. There is a hypothesis that inflammatory cytokines released from particular fatty areas may be involved in the pathogenesis of atherosclerotic heart disease [5]. This indicates that WC could be a more dependable indicator than BMI when evaluating the likelihood of cardiovascular disease. Our study revealed that patients with CACS >0 had significantly higher WC, similar to the literature.

In recent studies, NC is closely associated with diseases such as metabolic syndrome and metabolic parameters such as BMI, blood lipid panel, and fasting blood glucose[19,20]. However, there are conflicting findings regarding the relationship of NC with cardiovascular diseases and CACS. In a study conducted in China on elderly patients with acute coronary syndrome, there was a relationship between NC and angiographically measured CACS[21]. On the other hand, a multicenter study from 2016 revealed a relationship between NC and carotid artery intima-media thickness but did not find any association with CACS[22]. In our study, NC did not show a statistically significant difference in the groups with CACS=0 and CACS >0. This situation was similar to some studies in the literature.

Waist-to-neck circumference ratio may be a valuable and easily applicable potential parameter because it represents WC and NC, which can indicate visceral obesity. In our study, both groups were similar in terms of gender representation. Gender plays a role in determining the limits of WC in metabolic syndrome. However, in our study, determining the WNR cut-off value as a single value for both genders may provide clinical convenience.

In our study, low platelet levels, advanced age, and high ALT levels were also statistically and significantly associated with CACS. Our risk of atherosclerotic heart disease increases with aging. There is also a positive correlation between age and CACS. The reason for this could be the advancement of atherosclerosis, heightened inflammation, and deterioration of the vascular system as one ages[23]. In our study, age was statistically significantly associated with CACS, consistent with the literature. Alanine aminotransferase is a parameter that shows hepatic functions. A study revealed a connection between increased ALT levels and atherosclerosis, CACS, and hepatic steatosis, whether liver disease was present or not. The association between hepatic steatosis and atherosclerosis explains this situation. Also, there may be a connection between inflammation and oxidative stress related to elevated ALT levels and the presence of CACS[24]. To our knowledge, there is limited data in the literature investigating the relationship between platelet count and CACS. However, in a study examining the relationship between mean platelet volume and CACS, patients with CACS showed lower platelet levels[25]. Once again, this might be because of the impact of oxidative stress and inflammatory mediators on blood cells, like platelets.

Our study had several limitations. Although it was designed cross-sectionally, the number of patients included was relatively small. The patients included in the study were relatively young. The patients' metabolic parameters, such as blood pressure and lipid panel, were closer to normal. Future studies with a larger patient sample size could enhance the significance of this issue even more.

Conclusion

Our study found a significant relationship between WNR and the presence of CACS, which plays a crucial role in detecting subclinical atherosclerosis. Given the high expenses, lengthy procedure, and potential risks associated with radiation and contrast in MDCT, employing a cost-effective and straightforward measure like WNR to estimate CACS could offer clinical benefits.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest regarding this article's research, authorship, and/or publication.

Funding

The author (s) received no financial support for this article's research, authorship, and/or publication.

Informed consent

Informed consent was obtained from all participants in the study.

Ethical Approval

This study was approved by the Atatürk University Ethical Committee (Protocol Number 29.03.2024-B. 30.2.ATA.0.01.00/199).

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Do Pediatric Patients with Familial Mediterranean Fever who have Phenotypically Predominant Arthritis, Arthralgia, and Myalgia Reflect a More Serious Underlying Disease than Those with Phenotypically Predominant Abdominal Pain?

Tekrarlayan Artrit, Artralji, Miyalji Fenotipinin Ön Planda Olduğu Ailevi Akdeniz Ateşi Olan Çocuklar Peritoneal Fenotipin Ön Planda Olduğu Çocuklara Göre Farklı Özellikler Gösteriyor mu?

Tugba Nur Kutlu Beseren[1], Muferet Erguven[2], Sefer Ustebay[3]

[1]Department of Pediatrics, Kafkas University School of Medicine, Kars; [2]Department of Pediatric Rheumatology, Duzce University School of Medicine, Duzce; [3]Department of Pediatrics, Bandirma 17 Eylul University School of Medicine, Bandirma, Türkiye

ABSTRACT

Aim: *The study aimed to determine the presence of different demographic, familial, clinical, laboratory, and genotypic characteristics between children with Familial Mediterranean Fever (FMF) with recurrent arthritis, arthralgia, and myalgia phenotype and children with FMF with recurrent abdominal attacks phenotype.*

Material and Method: *The study included patients who were admitted to the Pediatric Rheumatology Outpatient Clinic of the Kafkas University Research Hospital before 2020, who were diagnosed with FMF and followed up for at least 1 year, and who were diagnosed with clinically recurrent arthritis, arthralgia, myalgia, and recurrent abdominal pain. Patients were divided into two groups: 151 patients with recurrent arthritis, arthralgia, and myalgia with FMF phenotypic features (Group 1) and 102 patients with recurrent diffuse, incomplete peritoneal involvement with predominant FMF phenotypic features (Group 2). The demographic and familial characteristics, age at diagnosis, and other clinical, laboratory, and genetic features of these 2 groups were compared, and the differences were statistically evaluated.*

Results: *While 60.6% of the patients in Group 1 and 39.4% in Group 2 were female, 58.7% of the patients in Group 1 and 41.3% in Group 2 were male. No significant statistical difference was found between the two groups. When the groups were compared regarding genetic mutations, M694V homozygotes were observed in 17.2% of Group 1 and 8.8% of Group 2. There was a statistically significant difference between Group 1 and Group 2 in M694V homozygotes ($p<0.05$)*

Conclusion: *Patients with FMF with predominant recurrent arthritis, arthralgia, and myalgia tended to be diagnosed at an older age and exhibited more frequent symptoms such as pallor and nausea compared to patients with FMF who predominantly had peritoneal involvement. Furthermore, pleural involvement was frequently observed, and the co-occurrence with the homozygous M694V mutation was genotypically high.*

Key words: *familial Mediterranean fever; arthritis; arthralgia; myalgia; involvement*

ÖZET

Amaç: *Tekrarlayan artrit, artralji, miyalji fenotipinin ön planda olduğu Ailevi Akdeniz Ateşi (AAA) olan çocuklar ile tekrarlayan yaygın ve inkomplet abdominal atakların ön planda olduğu AAA'li çocuklar arasında farklı demografik, ailesel, klinik, laboratuvar, genotipik özelliklerin olup olmadığını değerlendirmekti.*

Materyal ve Metod: *Kafkas Üniversitesi Araştırma Hastanesi Çocuk Romatoloji polikliniğine2020 yılından önce başvuran AAA tanısı alıp en az bir yıl takip ettiğimiz hastalarımız arasından klinik olarak tekrarlayan artrit, artralji, miyaljisinin ön planda olduğu hastalar ile tekrarlayan karın ağrısının ön planda olduğu hastalar çalışma kapsamına alındı. Hastalar tekrarlayan artrit, artralji, myalji tutulumun var olduğu AAA TİPİ fenotipik özelliği öncelikli gösteren 151 hasta (Grup1) ve tekrarlayan yaygın, inkomplet peritoneal tutulumun var olduğu AAA tipi fenotipik özelliğini baskın olarak gösteren 102 (Grup2) hasta olarak iki gruba ayrıldı. Bu 2 grubun demografik, ailesel özellikleri, tanıdaki gecikme yaşı diğer klinik, laboratuvar ve genetik özellikleri karşılaştırılarak farklar istatistiksel olarak değerlendirildi.*

Bulgular: *Grup 1'de %60,6 oranında kız, Grup 2'de %39,4 oranında kız var iken; Grup 1'de %58,7 oranında erkek, Grup 2'de %41,3'ü erkek idi. iki grup arasında anlamlı bir istatistiksel fark saptanmadı. Genetik mutasyon açısından gruplar karşılaştırıldığında; . M694V homozigot Grup 1 %17,2 oranında görülmekteyken, Grup 2'de %8,8 oranında görülmekteydi. Grup 1 ve Grup 2 M694V homozigot istatistiksel olarak anlamlı farklılık göstermişti ($p<0,05$).*

Sonuç: *Tekrarlayan artrit, artralji, miyalji tutulumunun baskın olduğu AAAli hastalarda peritoneal tutulumun baskın olduğu AAAli hastalara göre tanı yaşında gecikme ile birlikte daha büyük tanı yaşı, solukluk, mide bulantısı gibi semptomların daha sık olduğu görüldü. Bu da fenotipik olarak artrit, artralji, myaljinin ağırlıklı olduğu AAA olan hastalar sanılanın aksine fenotipinde karın ağrısının ağırlıklı olduğu gruba göre altta yatan çok daha ciddi kliniği bize göstermekte idi.*

Anahtar Kelimeler: *ailevi Akdeniz ateşi; artrit; artralji; miyalji; tutulum*

İletişim/Contact: *Tugba Nur Kutlu Beseren, Department of Pediatrics, Kafkas University School of Medicine, Kars, Türkiye* • ***Tel:*** *0539 374 75 35* • ***E-mail:*** *tnkutlu@yahoo.com.tr* • ***Geliş/Received:*** *11.07.2024* • ***Kabul/Accepted:*** *08.11.2024*

ORCID: *Tuğba Nur Kutlu Beşeren: 0000-0003-4583-0860 • Müferet Ergüven: 0000-0002-3255-1208 • Sefer Üstebay: 0000-0003-1507-5921*

Introduction

Familial Mediterranean Fever (FMF) is a recurrent, autosomal recessive disease characterized by serous membrane inflammation. It results in abdominal, chest, and joint pain accompanied by fever. FMF is common in populations of Eastern Mediterranean origin, especially Jews, Turks, Armenians, and Arabs. The prevalence of the disease is 1/1000, and the carrier rate is as high as 1/5[1].

Two separate groups cloned the gene causing FMF in 1997. The International FMF Consortium and the French FMF Consortium showed that the gene encoding a 781-amino-acid protein called pyrin/marenostrin on the short arm of chromosome 16, 16p13.3, is associated with FMF[2].

The gene involved in the disease is called MEFV (MEditerraneaneanFeVer), identified in 1997, consisting of 10 exons, 3505 nucleotides, 785 amino acids, and encoding a protein called pyrin[3]. Pyrin is a component of the inflammatory complex that drives active interleukin-1β production within the cell. Pyrininmutant forms cause uncontrolled interleukin-1β production and increased inflammatory response[2]. Today, over 200 mutations have been identified in this gene[2]. The most common mutations are M694V, M680I, M694I, and V726A[4]. The most common mutations in Turkish FMF patients were reported as M694V and M680I[1].

M694V genotype exhibits the most severe disease course. Due to the autosomal recessive inheritance pattern of FMF, the disease is more common in individuals born of consanguineous marriages[5]. Although familial Mediterranean fever is diagnosed with elevated clinical and acute phase markers, the demonstration of genetic mutations is helpful in the definitive diagnosis. TellHashomer criteria, Livneh criteria, and, since 2009, the criteria set out by Yalcinkaya et al. have been widely used to diagnose the disease[6]. The disease is characterized by fever and pain attacks caused by inflammation in body parts, including the abdomen, chest, and joints.

Abdominal pain attacks are the most common clinical manifestation of Familial Mediterranean Fever after fever. The most common cardinal findings other than fever in FMF are presentation with abdominal pain and joint and muscle involvement. Attacks with recurrent abdominal pain (diffuse, incomplete peritoneal attacks) are intense, and FMF is the primary clinical diagnosis in the differential diagnosis of diseases with abdominal pain. However, in children with arthritis, arthralgia, and myalgia, the diagnosis of FMF is often delayed because FMF is not considered in the differential diagnosis.

Chest pain in Familial Mediterranean Fever occurs due to pleuritis or pericarditis. During attacks, pleural effusion and atelectasis can rarely be observed on chest radiography. In patients homozygous for M694V, pleural involvement was reported to be more frequent than in patients with other mutations[7].

Regarding this objective, the differences between children with FMF with and without recurrent arthritis, arthralgia, and myalgia involvement (recurrent diffuse, incomplete abdominal attacks) were compared statistically.

Materials and Methods

The study was planned retrospectively on children with recurrent arthritis, arthralgia, and myalgia in phenotype I FMF and without recurrent arthritis, arthralgia, and myalgia in phenotype II FMF (with predominant recurrent abdominal pain) who were admitted to the Pediatric Outpatient Clinic and Pediatric Rheumatology Outpatient Clinic of the Kafkas University Research Hospital until 2020 and diagnosed with FMF based on Tel Hashomer or Yalcinkaya-Ozen criteria.

The inclusion criteria for this study are as follows:

1. Children aged 2–18 years diagnosed with FMF based on Tel-Hashomer or Yalcinkaya-Ozen criteria with well-recorded clinical records,
2. Children with FMF with recurrent arthritis, arthralgia, myalgia (at least 2 times in 1 year), and recurrent abdominal pain (at least 2 times in 1 year) who were diagnosed in our clinic,
3. Children with FMF were analyzed for genetic mutations,
4. Children with FMF without comorbidities such as chronic metabolic disease or malnutrition.

The ethical approval for our study was obtained from the Ethics Committee of Kafkas University Faculty of Medicine, with the number 80576354–050–99/276, concluding that it complied with the ethics committee guidelines. Patients in the Pediatric Rheumatology outpatient clinic were grouped as phenotype with recurrent arthritis, arthralgia, myalgia (PHENOTYPE I) and without (PHENOTYPE II) (with a predominant phenotype of recurrent abdominal pain), and these groups were evaluated by dividing them into specific forms. Demographic, familial, clinical, and laboratory characteristics and genetic mutation analyses were recorded in these forms.

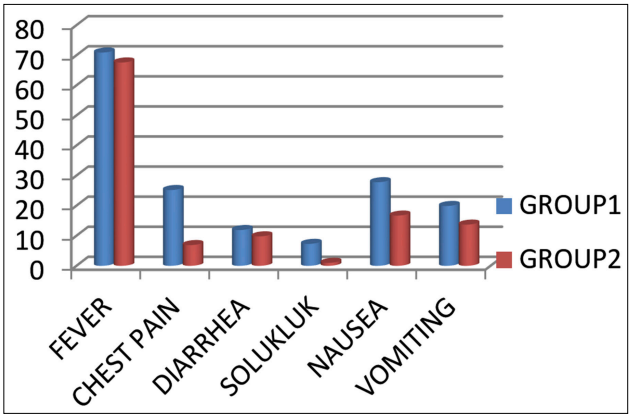

Figure 1. *Distribution of clinical results between groups (%)*

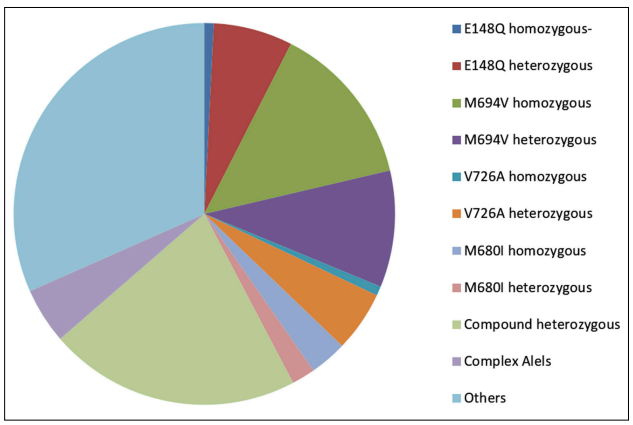

Figure 2. *Distribution of genetic mutations in patients (%)*

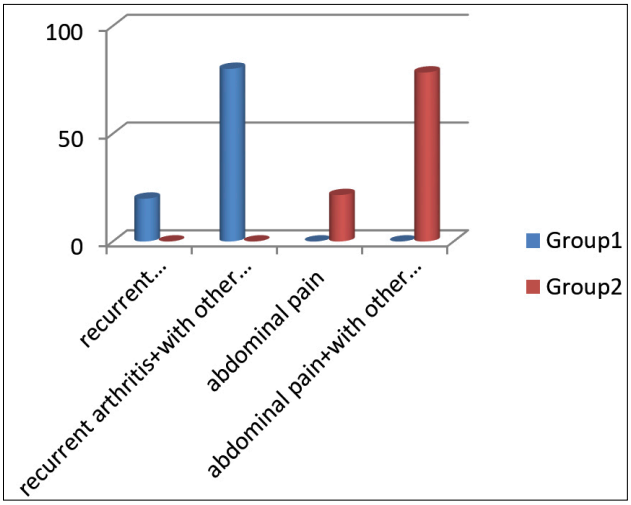

Figure 3. *Distribution of clinical findings between groups.*

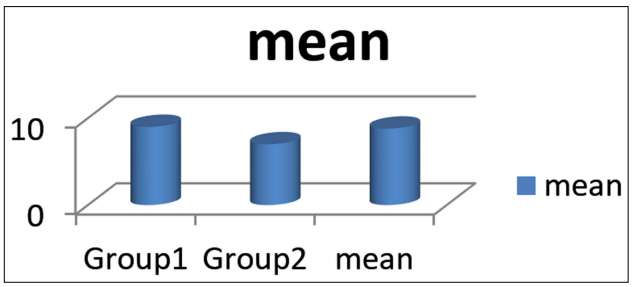

Figure 4. *Age distribution and mean values between groups.*

Statistical Analysis

Statistical analysis IBM Statistical Package for Social Sciences (SPSS) program version 20.0 software was used to evaluate the data, and the conformity of the variables to normal distribution was determined using the Kolmogorov-Smirnov test. In two independent groups, if the numerical variables met the normal distribution condition, the Student test was performed; if they did not meet the normal distribution condition, the Mann-Whitney U test was performed. The chi-square test was used to compare categorical variables between independent groups. The statistical significance level was accepted as $p<0.05$.

Results

The mean age of 253 patients in our study was 8.8±4.31 years. The mean age of the patients was 9±4.2 years in Group 1 and 7±4.16 years in Group 2. A statistically significant difference was observed between Group 1 and Group 2, with Group 1 having a higher mean age than Group 2 ($p<0.05$). 51.6% of our patients in Group 1 had a family history of FMF, and 59.7% in Group 2 had a family history of FMF ($p>0.05$). In Group 1, 73.5% of patients had 2 or more attacks per year, while 26.5% had 2 attacks per year ($p>0.05$). In Group 2, 65.7% of patients had 2 or more attacks per year, while 34.3% had 2 attacks per year ($p>0.05$). The most common complaint at presentation in our overall FMF patients was fever. This was followed by arthritis-arthralgia, myalgia (except febrile myalgia) (59.6%), abdominal pain (40.3%), nausea (23.3%), chest pain (17.7%), vomiting (17.3%), diarrhea (11.06%) and pallor (4.74%), respectively. While 70.9% of the patients in Group 1 presented with fever, this rate was 67.6% in Group 2 ($p>0.05$). In Group 1, 25.2% of the patients presented with chest pain (pleural involvement) compared to 6.9% of the patients in Group 2 ($p<0.05$). 7.3% of the patients in Group 1 and 1% in Group 2 presented with pallor ($p <0.05$). 27.8% of the patients in Group 1 and 16.7% in Group 2 presented with a complaint of nausea ($p <0.05$). 11.9% of patients

Table 1. *Distribution of age groups and comparison of mean values between groups*

Age Groups (years)		n	%	Mean± Std. deviation	P value
Under 4 years old	Group 1	15	9.9	2.93±1.09	p>0.05
	Group 2	29	28.4	2.86±1.15	
5-9 years old	Group 1	63	41.7	7.09±1.31	
	Group 2	45	44.1	6.91±1.44	
10-14 years old	Group 1	48	31.8	12.2±1.42	
	Group 2	19	18.6	11.4±1.57	
15 years old and above	Group 1	25	16.6	15.8±15.7	
	Group 2	9	8.8	15.7±0.83	

in Group 1 and 9.8% in Group 2 presented with diarrhea (p>0.05). Nephritic/nephrotic proteinuria was observed in 4.74% of our FMF patients. This rate was 5.3% in Group 1 and 3.9% in Group 2 (p>0.05).

The distribution of children according to age groups is as follows: Under 4 years old, 9.9% in Group 1 and 28.4% in Group 2; 5–9 years old, 41.7% in Group 1 and 44.1% in Group 2; 10–14 years old, 31.8% in Group 1 and 18.6% in Group 2; 15 years old and above, 16.6% in Group 1 and 8.8% in Group 2.

Regarding genetic mutations, E148Q homozygote was observed in 1.3% of Group 1, and M694V homozygote was observed in 17.2% of Group 1 and 8.8% of Group 2. When patients without mutation were compared, this rate was 10.5% in Group 1 and 15.6% in Group 2.

Genetic mutations were not statistically significant when compared for either group (p>0.05).

Of the patients included in our study, 13.8% were M694V homozygous, 9.8% were M694V heterozygous, 3.1% were M680I homozygous, 1.97%were M680I heterozygous, 0.79% were V726A homozygous, 5.1% were V726A heterozygous, and 12.6% were mutation negative.

Discussion

The distribution of the children included in our study according to age groups was as follows: 18.5% under the age of 5, 41.5% between the ages of 5 and 10, 26.4% between the ages of 10 and 15, and 13.4% aged 15 years and older. The distribution of children according to age groups is as follows: Under 4 years old, 9.9% in Group 1 and 28.4% in Group 2; 5–9 years old, 41.7% in Group 1 and 44.1% in Group 2; 10–14 years old, 31.8% in Group 1 and 18.6% in Group 2; 15 years old and above, 16.6% in Group 1 and 8.8% in Group 2. The average age at diagnosis in the literature is between 12 and 13 years, which is almost identical in both groups in our study and consistent with the literature. Presentation with recurrent abdominal pain is more common in the younger age group, especially patients under 4 years of age. Joint and muscle involvement is less common in this age group than in the 10–14 age group and children older than 15. However, the inability of young children to adequately express arthralgia and myalgia should be taken into account.

The female-to-male ratio in the study conducted by the Turkish FMF study group 1 was 1:1.4, while in the study conducted by Majeed et al., in 47 pediatric patients with FMF of Arab origin, it was 1.1:1[8].

In the Sonmezgoz et al.[9] study, the female-to-male ratio was 1:1.07, while in the study conducted by Çağlar et al., it was 1:1.31 5. In the study conducted by Soylemezoglu et al., the female-to-male ratio was found to be 1.16:1 [10]. In our study, this ratio was 1:1.21, whereas it was 1.04:1 for Group 1 and 1:1.04 for Group 2.

Erguven et al. found that the age of delay in diagnosis was 3.5 years in their series of 120 pediatric patients[11]. In the study conducted by Sonmezgoz et al.[9], similarly, this period was found to be 3.5 years. The time until diagnosis was 30.4±29.2 months in the patients included in our study. The time to diagnosis in Group 1 and Group 2 was 24±31.3 months and 12±24.14 months, respectively. There was a statistically significant difference between Group 1 and Group 2; duration time was higher in Group 1 than in Group 2 (p<0.05). Patients in Group 1 were diagnosed later than patients in Group 2 because they presented with complaints of arthritis and arthralgia rather than abdominal pain at the time of diagnosis. This shows that many physicians may miss the diagnosis of FMF and delay the diagnosis

when patients present with joint symptoms. They are likely to be diagnosed with different rheumatic diseases. It suggests that physicians lack knowledge and experience about joint involvement in patients with FMF.

In Group I, only cases with 3 or more musculoskeletal involvement per year were included in the study, primarily in cases with recurrent arthritis, arthralgia, and myalgia. Due to the nature of our case selection, the number of Group I cases with arthritis, arthralgia, and myalgia may not be compatible with the literature, as our study plan included all phenotype I and phenotype II FMF cases with 2 or more attacks in 1 year. These cases were on colchicine treatment. In addition, only arthritis and arthralgia were evaluated in most literature studies. Since arthritis, arthralgia, and myalgia (except febrile myalgia) were assessed together in our study, the number and percentage of arthritis, arthralgia, and myalgia cases were higher than in the literature. Also, due to the nature of the group selection, the exact percentage of other clinical signs and symptoms in all FMF cases may differ from the literature.

Regarding the distribution of the number of attacks in each group, 73.5% of the patients in Group 1 had 2 or more attacks per year, while 26.5% had 2 attacks per year. In group 2, 65.7% of patients had 2 or more attacks per year, while 34.3% had 2 attacks per year. No statistically significant relationship was observed when both groups were compared regarding the number of attacks ($p>0.05$).

Regarding the clinical findings, 70.9% of the patients in Group 1 and 67.6% in Group 2 presented with fever; no statistical significance was determined in fever complaints ($p>0.05$). Regarding presentation with chest pain (pleural and pericardial involvement), 25.2% of patients in Group 1 and 6.9% of patients in Group 2 presented with chest pain, which was statistically significant ($p<0.05$). This is further complicated because pleural involvement is more common in arthritis, arthralgia, and myalgia than abdominal pain. We could not find this relationship in the literature. It should be studied with a larger number of patients.

Regarding the complaint of pallor, 7.3% of the patients in Group 1 and 1% in Group 2 presented with pallor, which was found to be statistically significant ($p<0.05$). When it was investigated, it was found that the pallor was not due to any underlying chronic additional disease or anemia, and it was considered to be due to pleural and pericardial involvement and nausea, which were significantly detected in patients in Group 1. This suggests that patients with arthritis, arthralgia, and myalgia are more severely affected clinically, contrary to what is assumed. 27.8% of patients in Group 1 and 16.7% in Group 2 presented with nausea, which was statistically significant ($p<0.05$). This shows that patients with predominant arthritis, arthralgia, and myalgia also had mild gastrointestinal involvement, albeit not very severe.

Of the patients, 13.8% were M694V homozygous, 9.8% were M694V heterozygous, 3.1% were M680I homozygous, 1.97% were M680I heterozygous, 0.79% were V726A homozygous, 5.1% were V726A heterozygous, and 12.6% were mutation negative. When the groups were compared regarding genetic mutations, E148Q homozygote was observed in 1.3% and E148Q heterozygote in 5.2% in Group 1, while these rates were 0% and 8.8% in Group 2, respectively. M694V homozygotes were observed in 17.2% in Group 1 and 8.8% in Group 2.

In the literature, M694V mutation was correlated with a higher incidence of arthritis and erysipelas-like erythema[12]. In the study of Berdeli et al., M694V mutation was associated with the development of amyloidosis, febrile attacks, and joint findings[13]. Similarly, in our study, M694V was more common in patients with arthritis.

In conclusion, the late diagnosis of patients with arthritis, arthralgia, myalgia, and myalgia, as well as the fact that pleural involvement and M694 homozygous mutation are more common in these patients, indicate a more severe disease course compared to cases with predominant peritoneal involvement.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Code Blue Application and Results in Our Hospital: a 5-Year Single-Center Analysis

Hastanemizin Mavi Kod Uygulama ve Sonuçları: Beş Yıllık Tek Merkez Analizi

Kamuran Uluc[1], Murat Sahin[2], Mustafa Bilgehan Ayik[2], Soner Kina[3]

[1]Department of Intensive Care, Mus State Hospital; [2]Department of Anesthesiology and Reanimation, Mus State Hospital, Mus; [3]Department of Anesthesiology and Reanimation, Kafkas University Faculty of Medicine, Kars, Türkiye

ABSTRACT

Aim: *The code blue (CB) system is used in hospitals to provide a rapid and effective response in situations requiring emergency medical intervention. We aim to evaluate CB calls in our hospital and raise awareness retrospectively.*

Materials and Methods: *CB forms related to calls received via the CB system at Muş State Hospital between 01.05.2019 and 01.05.2024 were retrospectively reviewed and recorded.*

Results: *In our study, there were a total of 295 CB calls for patients. The average age of the patients was 65.36±8.89 years, and 124 (42%) were female. Of the 295 calls, 137 (46.4%) were made during working hours, and 158 (53.6%) were made outside. The difference between the number of CB calls made during and outside working hours was not statistically significant (p=0.433). The average response time to CB calls was 1.80±0.87 minutes, with no significant difference in response times between working hours and outside of working hours (p=0.471). The average duration of CPR performed on patients was 30.4±12.7 minutes. Incorrect CB calls were identified in a total of 45 cases. Of these calls, 16 (35.5%) were made during working hours, and 29 (64.5%) were made outside of working hours, with the incorrect CB calls being significantly higher outside of working hours (p=0.019). Among the departments and units where CB calls were made, the highest number of calls came from the Internal Medicine Department (16.27%). This was followed by the Angio Unit (11.86%) and the Pulmonology Department (9.49%). The most common probable diagnosis for CB calls was cardiac arrest, with a total of 98 cases (33.22%) related to this diagnosis. This was followed by respiratory depression (23.39%) and low oxygen saturation (15.25%).*

Conclusion: *The rapid and well-trained response of the team attending CB calls increases patients' chances of survival. Regular in-hospital training and drills are important to reduce the rates of incorrect CB calls.*

Key words: *code blue; response time; cardiopulmonary resuscitation; incorrect code blue; in-hospital training*

ÖZET

Amaç: *Mavi kod, hastanelerde acil tıbbi müdahale gerektiren durumlarda hızlı ve etkili bir yanıt sağlamak amacıyla kullanılan bir sistemdir. Amacımız hastanemizdeki mavi kod çağrılarını retrospektif olarak değerlendirmek ve farkındalık oluşturmaktır.*

Materyal ve Metod: *01.05.2019–01.05.2024 tarihleri arasında Muş Devlet Hastanesi'nde mavi kod sistemiyle alınan çağrılara ait mavi kod formları retrospektif olarak incelenip kaydedilmiştir.*

Bulgular: *Çalışmamızda toplam 295 hastaya ait mavi kod çağrısı mevcuttur. Hastaların yaş ortalaması 65,36±8,89 yıl olup hastaların 124'ü (%42) kadındı. İki yüz doksan beş çağrının 137'si (%46,4) mesai saatleri içinde, 158'i (%53,6) ise mesai saatleri dışında yapılmıştır. Mesai saatleri içinde ve dışında yapılan mavi kod çağrıları arasındaki fark istatistiksel olarak anlamlı deildi (p=0,433). Mavi kod çağrılarına ulaşma süresi ortalama 1,80±0,87 dakika olup, mesai saatleri içinde ve dışında bu süreler arasında anlamlı bir fark yoktur (p=0,471). Hastalara yapılan CPR süresi ortalama 30,4±12,7 dakikadır. Yanlış mavi kod çağrıları, toplam 45 çağrıda tespit edilmiştir. Bu çağrıların 16'sı (%35,5) mesai saatleri içinde, 29'u (%64,5) ise mesai saatleri dışında yapılmış olup, mesai saatleri dışındaki yanlış mavi kod çağrıları anlamlı derecede fazla bulundu (p=0,019). Mavi kod çağrılarının yapıldığı servis ve üniteler arasında en yüksek çağrı sayısı, Dâhiliye Servisi'nden (%16,27) yapılmıştır. Bunu sırasıyla Anjiyo Ünitesi (%11,86) ve Göğüs Hastalıkları Servisi (%9,49) takip etmiştir. Mavi kod çağrılarının en yaygın olası tanısı kardiyak arrest olup, toplam 98 vaka (%33,22) bu tanı ile ilişkilendirilmiştir. Bunu solunum depresyonu (%23,39) ve oksijen saturasyonu düşüklüğü (%15,25) takip etmiştir.*

Sonuç: *Mavi kod çağrılarına giden ekibin hızlı ve eğitimli olması hastaların hayatta kalma şansını artırmaktadır. Yanlış mavi kod oranlarının azaltılması için düzenli olarak hastane içi eğitimler ve tatbikatların yapılması önemlidir.*

Anahtar kelimeler: *mavi kod; ulaşma süresi; kardiyopulmoner resüsitasyon; yanlış mavi kod; hastane içi eğitimler*

İletişim/Contact: *Kamuran Uluc, Department of Intensive Care, Mus State Hospital, Mus, Turkiye* • ***Tel:*** *0507 786 74 34* • ***E-mail:*** *kamuranuluc@hotmail.com* • ***Geliş/Received:*** *27.07.2024* • ***Kabul/Accepted:*** *25.09.2024*

ORCID: *Kamuran Uluç: 0000-0001-6128-0462* • *Murat Şahin: 0000-0002-3314-0187* • *Mustafa Bilgehan Ayık: 0009-0003-6229-9933* • *Soner Kına: 0000-0003-3774-9632*

Introduction

Code blue (CB) is a hospital emergency call and response system that ensures professional teams respond swiftly to patients requiring immediate medical intervention[1,2]. The CB system was first implemented in the United States in 2000. In international colored code applications, blue is universally used for CB[3]. Approximately 200.000 in-hospital cardiac arrests occur annually in the United States. Despite the poor prognosis, the survival rate varies between 11% and 35% among hospitals[4,5].

The Turkish Ministry of Health initiated a professional CB system in 2008 following the establishment of quality standards. The use of CB in hospitals became mandatory with the regulations published in 2009 and the "Patient and Employee Safety Regulation" enacted in 2011. The Ministry of Health designated "2222" as the phone system for CB calls[6,7]. If implemented effectively and understood by the entire CB team, the CB system allows rapid identification and intervention in cases of in-hospital cardiopulmonary arrest, helping to reduce mortality and morbidity[1,2].

This study aims to retrospectively evaluate CB incidents in a secondary care state hospital and raise awareness about CB.

Materials and Methods

This retrospective study was approved by the non-interventional ethics committee of Kafkas University Medical Faculty (No: 2024/05/463/36) and conducted following the Helsinki Declaration. Data from CB calls at Muş State Hospital between 01.05.2019 and 01.05.2024 were recorded. Muş State Hospital is a secondary care state hospital with 445 beds, including 30 tertiary intensive care beds. Patients with complete data attended via CB calls were included in the study, while those with incomplete data were excluded.

Data recorded included patient age, gender, the unit or ward where the call was made, whether the call was during or outside working hours, the CB team's response time, the possible reason for the CB, duration of cardiopulmonary resuscitation (CPR), outcome of CPR, and the post-CB process (death, transfer to intensive care, admission to the emergency department for observation, referral to another center, follow-up, and treatment at the scene). Information was collected from CB forms and the hospital information system.

The response time was defined as the duration between the CB call and the team's arrival. According to the Utstein model, in-hospital cardiac arrest is defined as a patient not requiring basic or advanced life support[8]. The CB team in our hospital comprises an anesthesiologist, an anesthesia technician, and a security officer, with the team led, coordinated, and supervised by a specialist doctor. Upon receiving a CB notification, the team proceeds to the scene with an emergency response bag. The CB ends when the team reaches the scene. After evaluating and intervening with the patient, the team fills out the CB form.

Call times were classified as within working hours (weekdays 08:00–16:00) and outside working hours (weekdays 16:00–08:00 and weekends). Official holidays and public holidays were also considered outside working hours.

Statistical Analysis

Numerical variables are presented as mean ± standard deviation. Frequency and percentage values were used to describe categorical variables. The statistical significance of differences between mean values was calculated using Student's t-test. Fisher exact test or chi-square test was used to analyze incidence data. A p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. Calculations were performed using IBM Statistical Package for Social Sciences (SPSS) program version 22 software (IBM Inc., Chicago, IL, USA).

Results

A total of 295 CB calls were reviewed. The mean age of the patients was 65.36±8.89 years, with no significant difference in age distribution between calls made during and outside working hours (p=0.362). Gender distribution included 124 (42%) female and 171 (58%) male patients, with no significant difference between genders (p=0.643). Of the 295 calls, 137 (46.4%) were made during working hours, and 158 (53.6%) were outside working hours, with no significant difference between them (p=0.433) (Table 1).

The average response time for CB calls was 1.80±0.87 minutes, with no significant difference between working and nonworking hours (p=0.471). The average CPR duration was 30.4±12.7 minutes. There were 45 false CB calls, 16 (35.5%) during working hours and 29 (64.5%) outside working hours, with a significant increase in false calls outside working hours (p=0.019) (Table 1).

Table 1. *Comparison of demographic characteristics of patients and CB calls during and outside working hours*

	Total patients	Working hours	Outside working hours	P-value
Number of patients n (%)	295(100)	137(46.4)	158(53.6)	0.433
Age (years) mean ± SD	65.36±8.89	65.02±7.23	64.15±8.14	0.362
Gender				
Female n (%)	124(42)	59(43)	65(41.1)	0.643
Male n (%)	171(58)	78(57)	93(58.9)	
Response time (minutes) mean ± SD	1.80±0.87	1.73±0.86	1.81±0.73	0.471
CPR duration (minutes) mean ± SD	30.4±12.7	30.5±12.2	30.2±16.9	0.684
Incorrect CB n (%)	45(100)	16(35.5)	29(64.5)	0.019
Post CB process				
Exitus n (%)	106(35.9)	43	63	0.023
Admitted to ICU n (%)	76(25.7)	33	43	0.014
Transferred to other centers n (%)	22(7.5)	10	12	0.745
Admitted to ER for observation n (%)	26(8.8)	12	14	0.543
Treatment and follow-up on site n (%)	65(22.1)	30	35	0.456

n: number of patients; %: percentage; SD: standard deviation; CB: code blue; CPR: cardiopulmonary resuscitation; ICU: intensive care unit; ER: emergency room; p<0.05 was considered statistically significant.

Following CB calls, 106 patients (35.9%) died, with 43 during working hours and 63 outside working hours (p=0.023). Seventy-six patients were transferred to intensive care, with 33 during working hours and 43 outside working hours (p=0.014). The number of patients referred to another center was 22, with no significant difference between working and outside working hours (p=0.745). The number of patients admitted for observation in the emergency department was 26, with no significant difference between working and outside working hours (p=0.543). The number of patients treated and monitored at the scene was 65, with a similar distribution between working and outside working hours (p=0.456) (Table 1).

The Internal Medicine Department received the highest number of CB calls (16.27%), followed by the Angio Unit (11.86%) and the Pulmonary Diseases Department (9.49%). The Interventional Radiology Unit received the lowest number of calls (1.02%), and the General Surgery Outpatient Clinic received the lowest number of calls (1.02%) (Table 2).

The most common probable diagnosis for CB calls was cardiac arrest, with a total of 98 cases (33.22%). This was followed by respiratory depression (23.39%) and low oxygen saturation (15.25%). Less common diagnoses included asthma attacks (1.02%) and conversion disorders (1.02%) (Table 3).

Table 2. *Department and units where CB calls are issued*

	Total Calls n (%)	Correct Calls	Incorrect Calls
Palliative care unit	18(6.10)	16	2
Hemodialysis unit	21(7.12)	19	2
Angio unit	35(11.86)	31	4
Phlebotomy unit	9(3.05)	8	1
Endoscopy unit	13(4.41)	11	2
Chemotherapy unit	15(5.08)	13	2
Internal medicine department	48(16.27)	44	4
Pulmonology department	28(9.49)	25	3
General surgery outpatient clinic	3(1.02)	2	1
General surgery department	7(2.37)	5	2
Internal medicine outpatient clinic	7(2.37)	5	2
Obstetrics and delivery room	10(3.39)	8	2
Orthopedics department	10(3.39)	8	2
Cardiology department	9(3.05)	7	2
Ophthalmology outpatient clinic	4(1.36)	3	1
Infectious diseases department	6(2.03)	5	1
Plastic surgery department	8(2.71)	6	2
Interventional radiology unit	3(1.02)	2	1
COVID-19 department	18(6.10)	14	4
ENT department	4(1.36)	3	1
Pediatric ICU/ department	8(2.71)	6	2
Urology department	6(2.03)	5	1
Physical therapy department	5(1.69)	4	1

n: number of patients, %: percentage, CB: code blue, ENT: ear, nose and throat, ICU: intensive care unit.

Table 3. *Possible diagnoses of CB calls*

	n (%)
Cardiac arrest	98(33.22)
Respiratory depression	69(23.39)
Low oxygen saturation	45(15.25)
Hypotension	27(9.15)
Syncope	18(6.10)
Aspiration	14(4.75)
Epileptic seizure	9(3.05)
Anaphylaxis	5(1.69)
Hypoglycemia	4(1.36)
Asthma attack	3(1.02)
Conversion	3(1.02)

n: number of patients, %: percentage, CB: code blue.

Discussion

In-hospital cardiac arrests are one of the leading causes of high morbidity and mortality. Cardiac arrest occurs in one to five out of every 1.000 patients, leading to an in-hospital mortality rate of approximately 80%[9,10]. Despite this high death rate, there has been no significant improvement in in-hospital survival rates over the past few decades[11,12]. A study conducted in Korea on 958 patients with in-hospital cardiac arrest found that 28% of these patients were discharged alive[13]. This once again emphasizes the need for rapid detection and intervention in cases of in-hospital cardiac arrest.

In the study by Senem et al.[14], 46.8% of CB calls were for women, and the average age of patients was 48.8±21.06 years. Another study reported that 38% of calls were for women, with an average age of 64.25±20.6 years[15]. In another study, 44% of calls were for women, with an average age of 75.14±12.86 years[8]. A different study found that 33.3% of calls were for women, with an average age of 56.06 years[16]. In line with the literature, 42% of CB calls in our study were for women, with an average age of 65.36±8.89 years. The lower arrest rates in women may be related to the less frequent occurrence of coronary problems such as myocardial infarction and angina pectoris in women[17]. These findings suggest that lower rates of cardiac arrest in women are associated with a possible prevalence of coronary disease, but further investigation is warranted.

In-hospital cardiac arrests are common, and some CB calls can be false alarms. In the study by Betül et al.[18], 80 out of 419 CB calls were false alarms. Another study reported 74 false calls out of 694 CB calls[16]. A study found that 381 out of 1.035 CB calls were false alarms[15]. An analysis of four years of CB calls found that false alarms ranged from 4% to 31%[19]. Our study had 45 (15.25%) false CB calls. To reduce these false alarm rates, periodic CB training for all hospital staff can help create more aware personnel, thus reducing the rate. This can also increase motivation within the CB team and improve patient survival in other CB incidents.

Cardiopulmonary arrest is the cessation of respiration or circulation. The CB team must reach the call point within 3 minutes to start CPR. This duration is critical for patients experiencing arrest, as delayed intervention increases the death rate and worsens neurological damage[20,21]. The response time of the CB team to the call is crucial for mortality and morbidity. In the study by Müge et al.[8], this duration was 1.97±0.72 minutes. Another study found the average response time to be 1.85±0.45 minutes for outpatient cases and 2.10±0.55 minutes for inpatient cases[1]. A different study reported it as 108.83±42.83 seconds[14]. In line with the literature, our study found an average response time of 1.80±0.87 minutes. The rapid arrival of the team to the scene is critical in reducing mortality rates, and continuous training can contribute to shortening this time.

The hospital units where CB calls are made differ in various studies. In the study by Müge et al.[8], 33% of calls were from the palliative care unit, 24% from the internal medicine department, and 16% from the pulmonology department. Another study reported that 62% of calls were from inpatient units and 25% from outpatient clinics[14]. In another study, 21% of calls were from the orthopedic department, followed by 20% from the general surgery department [1]. A different study reported the highest number of calls from the palliative care unit, followed by the internal medicine department[19]. This situation shows a need for more emergency interventions in different hospital wards and that training and supervision in these units should be increased.

One of the most critical factors affecting mortality and morbidity in patients after cardiac arrest is the response time to CPR[13,22]. Studies have shown that mortality increases if CPR duration exceeds 10 minutes, while survival rates increase with CPR durations of less than 10 minutes[23]. Shin et al.[13] reported CPR durations of 26–30 minutes, Möhnle et al.[23] reported 17–20 minutes, and Vinay et al.[24] reported 12–19 minutes. In studies conducted in Türkiye, Özlem et al.[25] found an average CPR duration of 27 minutes (minimum: 10,

maximum: 50). Selçuk et al.[19] reported average CPR durations ranging from 22.1 to 28.6 minutes over four years. Our study found an average CPR duration of 30.4±12.7 minutes.

Various studies found that the rates of CB calls made outside working hours were 66.82%, 26.2%, 54%, 62.7%, 62.22%, and 52.5%[8,14–16,25,26]. In line with the literature, our study found this rate to be 53.6%.

In the study by Senem et al.[14], following CB intervention, 64.9% of patients were admitted for observation in the emergency department, and 35.1% were transferred to the intensive care unit. Another study reported that 41.2% of patients were admitted to the intensive care unit, 42.5% were declared deceased in the intervened unit, 15.8% received treatment in the unit, and 0.3% underwent emergency surgery[16]. A different study reported that 8.4% of patients were admitted to the intensive care unit, 39.1% were declared deceased, 41% were referred to another center, 9.7% were admitted for observation in the emergency department, and 0.42% underwent emergency surgery[26]. Another study found that 44% of patients were declared deceased, 39% were admitted to the intensive care unit, and 3% continued treatment in the intervened unit[8]. In our study, 35.9% of patients were declared deceased, and 25.7% were admitted to the intensive care unit.

Limitations

Our study has some limitations, including its retrospective nature, the inclusion of single-center data, and incomplete or missing data. Multicenter, prospective studies with a larger number of patients can be planned.

Conclusion

Code blue data serves as an indicator of hospital quality assessment. Code blue organization is critical for the survival of patients requiring advanced life support within the hospital. Accurate recording of all interventions the CB team performs is important for future studies and quality standards. Periodic training and drills with all hospital staff can reduce the rate of false CB calls in our study.

Conflict of Interest

There are no disclosed conflicts of interest for the authors.

Compliance with Ethical Statement

The non-interventional ethics committee of Kafkas university medical faculty gave its approval for the study to be conducted (Decision No: 2024/05/463/36).

Financial Support

There was no institutional or organizational funding for the study.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Investigation of Autonomic Dysfunction Following COVID-19: the Role of Heart Rate Recovery Indices

COVID-19 Sonrası Otonomik Disfonksiyonun Araştırılması: Kalp Hızı İyileşmesi Parametrelerinin Rolü

Metin Coksevim[1], Mustafa Yenercag[2]

[1]Ondokuz Mayis University School of Medicine Cardiology Department; [2]Samsun Training and Research Hospital Department of Cardiology, Samsun, Türkiye

ABSTRACT

Aim: *COVID-19, caused by SARS-CoV-2, has been linked to long-term complications known as "long COVID," including autonomic dysfunction. Heart rate recovery (HRR) following exercise is a widely used marker for assessing autonomic function. This study evaluated HRR in Long-Term COVID patients and its association with inflammatory markers.*

Materials and Methods: *This retrospective study included 152 participants: 76 Long-Covid patients and 76 controls. Heart rate recovery was measured at 1, 2, 3, 4, and 5 minutes post-exercise using the Bruce protocol. Baseline demographic data and inflammatory markers, including CRP, D-dimer, and ferritin, were collected. Correlation analysis was performed between HRR and these markers.*

Results: *Long-Covid patients had significantly elevated levels of CRP (14.6±2.3 mg/L vs. 3.3±2.5 mg/L), D-dimer (128.2±42.1 µg/ml vs. 21.1±9.7 µg/ml), and ferritin (277.5±146.8 ng/ml vs. 112.3±78.2 ng/ml) compared to controls (p <0.001). HRR_1 and HRR_2 were significantly reduced in post-COVID patients (22.4±7.4 vs. 29.0±8.0; 31.5±17.4 vs. 40.2±10.9; p <0.001). Heart rate recovery at 3, 4, and 5 minutes was also markedly lower. A moderate negative correlation was found between HRR_2 and CRP (rs=-0.537), D-dimer (rs=-0.459), and ferritin (rs=-0.461) (p <0.001).*

Conclusion: *Long-Covid patients exhibited impaired HRR, indicating autonomic dysfunction, which correlated with elevated inflammatory markers. These findings highlight the importance of HRR as a marker for autonomic imbalance in Long-Covid, suggesting a need for further investigation into therapeutic strategies for autonomic dysfunction.*

Key words: *long COVID; heart rate recovery (HRR); autonomic dysfunction; inflammatory markers*

ÖZET

Amaç: *SARS-CoV-2'nin neden olduğu COVID-19,"Geç-Covid" olarak bilinen ve otonomik disfonksiyon da dâhil olmak üzere uzun vadede çeşitli komplikasyonlarla ilişkilendirilmiştir. Egzersiz sonrası kalp hızı iyileşmesi (HRR), otonom fonksiyonun değerlendirilmesinde yaygın olarak kullanılan bir göstergedir. Bu çalışma, Geç-Covid hastalarında HRR'yi ve bu iyileşmenin enflamatuvar belirteçlerle olan ilişkisini değerlendirmeyi amaçlamaktadır.*

Materyal ve Metod: *Bu retrospektif çalışmaya 76 Geç-Covid hastası ve 76 kontrol olmak üzere toplam 152 katılımcı dâhil edilmiştir. HRR, Bruce protokolü kullanılarak egzersiz sonrası bir, iki, üç, dört ve beşinci dakikalarda ölçülmüştür. Katılımcıların demografik verileri ve CRP, D-dimer ve ferritin gibi enflamatuvar belirteçler toplanmıştır. Kalp hızı iyileşmesi ile bu belirteçler arasında korelasyon analizi yapılmıştır.*

Bulgular: *Geç-Covid hastalarında CRP (14,6±2,3 mg/L vs. 3,3±2,5 mg/L), D-dimer (128,2±42,1 µg/ml vs. 21,1±9,7 µg/ml) ve ferritin (277,5±146,8 ng/ml vs. 112,3±78,2 ng/ml) seviyeleri kontrol grubuna kıyasla anlamlı derecede yüksekti (p <0,001). Geç-Covid hastalarında HRR_1 ve HRR_2 değerleri anlamlı şekilde düşüktü (22,4±7,4 vs. 29,0±8,0; 31,5±17,4 vs. 40,2±10,9; p <0,001). Kalp hızı iyileşmesi üç, dört ve beşinci dakikalarda da belirgin şekilde düşüktü. HRR_2 ile CRP (rs=-0,537), D-dimer (rs=-0,459) ve ferritin (rs=-0,461) arasında orta düzeyde negatif korelasyon bulundu (p <0,001).*

Sonuç: *Geç-Covid hastalarında otonom disfonksiyonu gösteren bozulmuş HRR gözlemlendi ve bu, yüksek enflamatuvar belirteçlerle ilişkiliydi. Bu bulgular, HRR'nin Geç-Covid'de otonom dengesizlik için bir belirteç olarak önemini vurgulamakta olup, otonom disfonksiyon için terapötik stratejilerin daha fazla araştırılmasının gerekliliğini ortaya koymaktadır.*

Anahtar kelimeler: *geç-covid; kalp hızı iyileşmesi (HRR); otonom disfonksiyon; enflamatuvar belirteçler*

İletişim/Contact: *Metin Coksevim, Samsun Ondokuz Mayis University School of Medicine Cardiology Department, 25270, Atakum, Samsun, Türkiye* • ***Tel:*** *0537 704 20 22* • ***E-mail:*** *metincoksevim@gmail.com* • ***Geliş/Received:*** *19.09.2024* • ***Kabul/Accepted:*** *21.10.2024*

ORCID: *Metin Çoksevim: 0000-0001-6907-6941* • *Mustafa Yenerçağ: 0000-0002-0933-7852*

Kafkas J Med Sci 2024; 14(3):296–303
doi: 10.5505/kjms.2024.70962

Introduction

The novel coronavirus disease (COVID-19), caused by the new type of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), first emerged in Wuhan-China, and was declared a global pandemic by the World Health Organization on March 11, 2020. Since then, COVID-19 has led to significant morbidity and mortality worldwide[1]. Clinical manifestations during the acute phase of the infection vary widely, from mild upper respiratory symptoms to severe viral pneumonia that may result in multi-organ failure[2]. In a subset of patients who survive the acute phase, various symptoms persist or reemerge over an extended period. Symptoms that cannot be attributed to any other cause within the 4–12 weeks following COVID-19 infection are referred to as *"post-acute," "new,"* or *"ongoing"* COVID-19, while those lasting beyond 12 weeks are termed *"chronic," "long,"* or *"post-COVID"*[3]. Long COVID patients often report a range of dysautonomic symptoms, including palpitations, fatigue, and dizziness, but the exact mechanisms underlying these symptoms remain unclear[4]. Despite this uncertainty, the incidence of Long-Covid continues to rise, with profound impacts on both individual health and socioeconomic costs[5].

Heart rate recovery (HRR) following exercise, defined as the difference between the peak heart rate (HR) and the HR at various intervals during recovery, is a widely recognized marker of autonomic function[6,7]. Since dysautonomia is thought to play a role in Long-Covid, HRR could serve as a valuable marker for autonomic dysfunction in these patients[3,8]. Given that autonomic dysfunction is linked to adverse cardiovascular outcomes, assessing HRR in Long-Covid patients could provide valuable insights into their prolonged symptoms and potential cardiovascular risks[9,10].

Nevertheless, the available data on dysautonomia is limited, and no previous study has focused on HRR in the Long-Term COVID-19 population. This study aims to fill this gap by evaluating HRR in Long-Term COVID-19 patients, providing new insights into the autonomic dysregulation that may contribute to persistent symptoms. Understanding these mechanisms is essential for improving patient care and developing targeted rehabilitation strategies to address the long-term cardiovascular risks posed by COVID-19.

Material and Methods

Study Population

This retrospective study included patients diagnosed with COVID-19 via Polymerase Chain Reaction (PCR) testing between November 1, 2020, and January 1, 2021. All were treated on an outpatient basis without hospitalization and presented to the cardiology clinic at least 12 weeks after their positive test, following an asymptomatic period. These patients underwent exercise tests after reporting symptoms such as exertional dyspnea, palpitations, and/or atypical chest pain.

The control group consisted of individuals who underwent exercise testing before the pandemic (before December 2019) to exclude the possibility of asymptomatic or undiagnosed COVID-19 infection.

Exclusion criteria included patients with overt cardiovascular disease (e.g., coronary artery disease, arrhythmia, hypertension, left ventricular hypertrophy, or moderate to severe valvular heart disease), severe renal insufficiency (eGFR <50 ml/dak/1.73 m^2), morbid obesity, diabetes mellitus or obstructive sleep apnea. Patients who had experienced severe COVID-19 infections requiring intensive care or high-flow oxygen therapy or who were taking medications known to affect autonomic nervous system function (e.g., beta-blockers, inhaled beta-mimetics, atropine, glycosides, selective serotonin reuptake inhibitors, angiotensin-converting enzyme inhibitors), were also excluded. In addition, the study did not include patients who did not reach at least 85% of their age– and gender-predicted maximum heart rate during the exercise test.

The study included 152 participants who met the inclusion criteria and divided them into the 'COVID-19 (+) Group' and the age– and gender-matched 'Control Group'. Demographic data and biochemical, hematological, and inflammatory parameters were obtained from local databases. Figure 1 presents a study flowchart.

The study protocol adhered to the ethical principles outlined in the 1975 Declaration of Helsinki, and ethical approval was obtained from the local ethics committee.

Assessment of Heart Rate Recovery

All participants underwent an exercise stress test based on the Bruce protocol. The test was terminated once participants reached at least 85% of the age- and

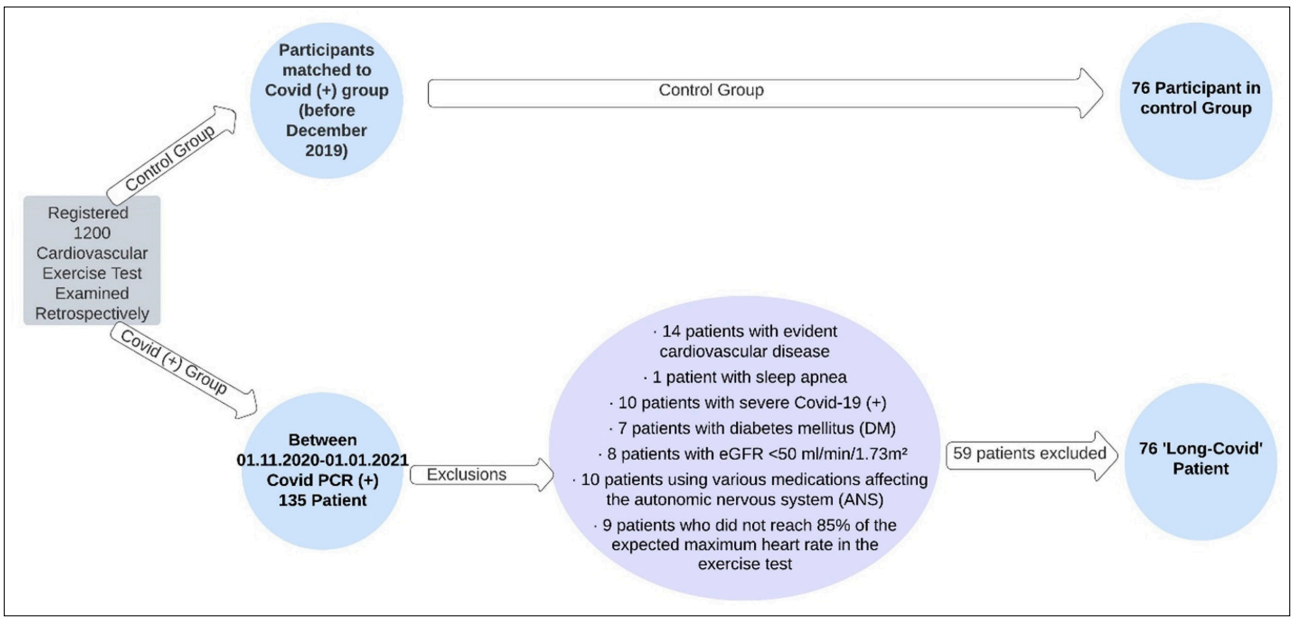

Figure 1. *Flowchart of the study population.*

gender-predicted maximum heart rate. After reaching peak exercise, participants immediately dismounted the treadmill and rested supine.

Heart rate measurements were taken at baseline, peak exercise, and recovery periods at 1, 2, 3, 4, and 5 minutes of the post-exercise period. Baseline (resting), peak exercise, and post-exercise blood pressure values were also recorded.

Definitions

COVID-19 Severity:

Mild COVID-19: Defined according to the World Health Organization (WHO) Guidelines as patients who were followed up on an outpatient basis, did not require hospitalization, and exhibited symptoms such as fever, muscle/joint pain, cough, or sore throat, without respiratory distress (respiratory rate <24/min, oxygen saturation on room air [SpO2] >93%)[11].

Long Covid: Signs and symptoms that continue or develop after acute COVID-19. It includes both ongoing symptomatic COVID-19 (from 4 to 12 weeks) and post-COVID-19 syndrome (12 weeks or more). In this document, we used '***Long-Covid***' as signs and symptoms continue beyond the acute phase of COVID-19, in line with the definition by NICE and the NIH (who refer to it as post-Acute Sequelae of SARS-CoV-2 infection or PASC)[3,12].

HRR: HRR was defined as the peak heart rate minus heart rate at each recovery period (HR_{1-5}) after exercise[6].

HRR_1: Peak HR – Maximal HR at 1st minute of the recovery period.

HRR_2: Peak HR – Maximal HR at 2nd minute of the recovery period.

HRR_5: Peak HR – Maximal HR at the 5th minute of the recovery period.

Proportions of Heart Rate Recovery (PHRR): (HRR_2/ Resting pulse rate) × 100[13]

Systolic Blood Pressure (SBP) Change: SBP rise=Peak SBP – baseline (standing) SBP

Diastolic Blood Pressure Change: DBP rise=Peak DBP – baseline (standing) DBP

Exaggerated Blood Pressure response: The exaggerated BP response is peak exercise SBP ≥210 mmHg[14].

Statistical analysis

IBM Statistical Package for Social Sciences (SPSS) program version 22.0 for Windows (IBM Inc., Chicago, IL, USA) was used for all statistical analyses. The Kolmogorov-Smirnov test was performed to assess the normality of the distribution. Normally distributed quantitative variables were expressed as mean ± standard deviation and categorical variables as numbers and percentages. Differences between groups were evaluated

Table 1. *Baseline characteristics and laboratory findings of the study population*

Parameters	COVID-19 Patients (n=76)	Control Group (n=76)	p value
Age, years	41.3±12.9	42.1±11.7	0.170
Male, n	44	37	0.255
HT, n	18	22	0.361
DM, n	9	12	0.221
Smoking, n	14	12	0.330
BMI, kg/m^2	25.2±1.7	24.9±1.7	0.403
WBC, 10^3 µL	7.9±4.3	7.0±3.9	0.069
Neutrophil, 10^3 µL	4.4±2.3	4.0±1.2	0.072
Hemoglobin, g/dL	13.6±0.8	13.7±0.6	0.195
Creatinin, mg/dl	0.9±0.09	1±0.15	0.392
Sodium, mEq/L	137±1.8	135.9±2	0.161
Potassium, mmol/L	4.4±0.3	4.2±0.3	0.406
ALT, IU/L	27.9±8.6	26.7±9.7	0.212
AST, IU/L	29.5±8.4	28.2±15	0.302
Total cholesterol, mg/dl	181±36	183±35.6	0.385
Triglyceride, mg/dl	139±36.8	134.6±36.1	0.081
HDL-C, mg/dl	36.1±8.9	37.1±7.6	0.182
LDL-C, mg/dl	118.7±26.1	117.1±27.21	0.291
Ferritin, ng/ml	277.5±146.8	112.3±78.2	<0.001
D-dimer, µg/ml	128.2±42.1	21.1±9.7	<0.001
CRP, mg/L	14.6±2.3	3.3±2.5	<0.001

Data are given as mean ± SD or n (%).
COVID-19:coronavirus disease 2019; DM: diabetes mellitus; HT: hypertension; BMI: body mass index; WBC: white blood cell; ALT: alanine aminotransferase; AST: aspartate aminotransferase; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; CRP: C-reactive protein.

using the Student's t-test for normally distributed data and the Mann-Whitney U test for non-normally distributed data. Categorical variables were compared using Chi-square tests. Spearman's correlation analysis examined the relationship between CRP, D-dimer, ferritin, and HRR_2. The strength of the correlations was interpreted as follows: 0–0.25 (weak), 0.26–0.50 (moderate), 0.51–0.75 (strong), and 0.76–1.00 (very strong). Statistical significance was defined as a p-value of <0.05.

Results

Table 1 summarizes the study groups' baseline clinical characteristics and laboratory parameters. The mean age of the participants was 41.6±11.9 years, and 53.3% were male. No significant differences regarding baseline demographic characteristics were observed between the COVID-19 and the control groups.

In the laboratory findings, the COVID-19 group exhibited significantly higher levels of D-dimer (128.2±42.1 µg/ml vs. 21.1±9.7 µg/ml; p <0.001), ferritin (277.5±146.8 ng/ml vs. 112.3±78.2 ng/ml; p <0.001), and C-reactive protein (CRP) (14.6±2.3 mg/L vs. 3.3±2.5 mg/L; p <0.001) compared to the control group. There were no significant differences between the groups in other laboratory parameters (p>0.05).

Table 2 presents the results of the exercise stress test and echocardiography. No significant differences were found between the groups in echocardiographic findings (p>0.05). Similarly, baseline and maximal heart rates, systolic and diastolic blood pressures, exercise duration, and metabolic equivalents achieved during the exercise stress test were comparable between the groups.

However, HRR indices in the COVID-19 group were significantly lower than those in the control group. The first-minute (HRR_1) and second-minute (HRR_2) HRR were markedly reduced in the COVID-19 group compared to the control group (22.4±7.4 vs. 29.0±8.0; p <0.001, and 31.5±17.4 vs. 40.2±10.9; p <0.001, respectively). Similarly, HRR indices at the third, fourth, and fifth minutes of the recovery period were significantly lower in the COVID-19 group (p <0.001 for all comparisons) (Figure 2).

Table 2. *Comparison of variables from echocardiography and exercise stress tests of COVID-19*

Coronavirus disease 2019 patients and controls			
Parameters	COVID-19 Patients (n=76)	Control Group (n=76)	p value
Echocardiographic findings			
LVEF (%)	65.3±6.7	64.9±6.5	0.790
LVEDD, mm	47.7±5.0	48.0±4.3	0.710
LVESD, mm	30.4±4.5	30.9±3.6	0.522
IVSD, mm	9.1±1.2	9.2±1.3	0.661
LVPWD, mm	8.4±1.3	8.3±1.0	0.436
PASP, mm Hg	26.3±5.1	25.3±5.6	0.372
Left atrial size, mm	31.2±3.8	30.4±4.0	0.350
Exercise stress test findings			
Exercise time, min	8.7±2.7	9.1±2.1	0.112
Baseline heart rate, beats/min	81±14.4	79.8±13.9	0.201
Maximal heart rate, beats/min	169.7±17.9	171.4±12.0	0.083
Maximal, METS	10.2±2.9	10.9±2.5	0.102
Baseline systolic BP, mm Hg	127.2±17.8	123.5±15.5	0.128
Baseline diastolic BP, mm Hg	78.3±10.3	76.6±11.5	0.142
Peak systolic BP, mm Hg	167.4 ±29.8	170.3±23.1	0.104
Peak diastolic BP, mm Hg	74.4±13.1	75.4±11.3	0.268
HRR_1	22.4±7.4	29.0±8.0	<0.001
HRR_2	31.5±17.4	40.2±10.9	<0.001
HRR_3	45.5±16.2	51.1±9.8	<0.001
HRR_4	53.2±13.4	59.3±11.7	<0.001
HRR_5	60±15.3	65.5±10.9	<0.001

Data are given as mean ± SD or n (%)
COVID-19: coronavirus disease 2019; Bpm: beats per minute; LVEF: left ventricular ejection fraction; LVEDD: left ventricular end-diastolic diameter; LVESD: left ventricular end-systolic diameter; IVSD: interventricular septum diameter; LVPWD: left ventricular posterior wall diameter; LVEF: left ventricular ejection fraction; BP: blood pressure; METS: metabolic equivalents (1 MET=3.5 ml/kg/min of oxygen consumption); HRR: heart rate recovery.

A significant negative correlation was found between CRP levels (rs=-0.537, p <0.001), D-dimer levels (rs=-0.459, p <0.001), ferritin levels (rs=-0.461, p <0.001), and HRR_2 in patients with COVID-19.

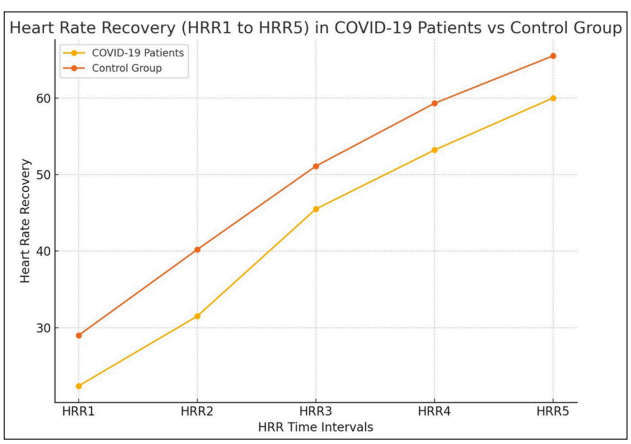

Figure 2. *Heart rate recovery indices difference between COVID-19 patients and the control group over the recovery intervals.*

Discussion

This study highlights the importance of assessing and monitoring autonomic nervous system-related symptoms as potential long-term complications of COVID-19. The main findings of the study are as follows: patients classified as 'Long-Covid,' who presented with various symptoms after the recovery from acute COVID-19, had significantly elevated levels of CRP, D-dimer, and ferritin. Furthermore, all heart rate recovery indices (HRR_{1-5}) measured during the exercise stress test were significantly lower in the COVID-19 group compared to the control group. Correlation analysis revealed a moderate negative correlation between HRR and infection-related markers (CRP, D-dimer, and ferritin).

The World Health Organization estimated in 2023 that millions of individuals could be affected by Long-Covid conditions and urged countries to take it seriously by investing in research, recovery, and

rehabilitation[15]. The typical Long-Covid syndrome includes symptoms like shortness of breath, palpitations, dizziness, and syncope, particularly when standing (orthostatic intolerance)[16]. Pathophysiologically, this is thought to result from either sympathetic activation due to a cytokine storm or direct viral damage leading to autonomic dysfunction[3,8,16]. In this context, dysautonomia is recognized as a component of Long-Covid syndrome, often manifesting as alterations in heart rate regulation. While many studies have evaluated early-phase orthostatic intolerance syndromes such as orthostatic hypotension (OH), vasovagal syncope (VVS), and postural orthostatic tachycardia syndrome (POTS), the long-term effects remain unclear[8].

Exercise testing is a valuable tool for evaluating individual exercise performance and understanding the various physiological processes that may become dysfunctional following COVID-19[16]. It can also evaluate autonomic nervous system function based on heart rate response to exercise. Several studies have explored this in COVID-19 patients. Clavario et al. reported that patients with more severe COVID-19 (defined by a percent-predicted peak oxygen uptake [%pVO_2] of <85%) exhibited lower peak heart rates at three months of post-infection period[17]. Jimeno-Almazán et al. demonstrated that even patients with mild COVID-19 could develop chronotropic incompetence and other autonomic disorders, which may contribute to long-term exercise intolerance after the resolution of acute infection[18]. Similarly, Ladlow et al. found significant evidence of dysautonomia in the same patient population using cardiopulmonary exercise testing and heart rate variability (HRV) assessments[19].

Asarcikli et al. evaluated HRV parameters using rhythm Holter monitoring in post-COVID patients during the 12–26 week period after infection. They found that HRV parameters were increased in COVID patients compared to controls, indicating heightened parasympathetic tone, although no significant changes were noted in the sympathetic system[2]. Another study by Yüksel et al. found that psoriasis patients had significantly reduced HRR_{1-5} parameters, demonstrating autonomic dysfunction in this patient group[20]. Huckstep et al. reported lower HRR_{1-2} in young adults born preterm, highlighting the potential long-term effects of early-life conditions on autonomic function[21]. Additionally, Dewar et al. showed that reduced HRR in patients with cardiovascular disease is associated with cardiovascular and all-cause mortality[22].

Recent studies have comprehensively evaluated patients who have recovered from COVID-19 based on their long-term symptomatic status. In a study by Karakayali et al., electrocardiographic parameters related to ventricular depolarization and repolarization were examined in symptomatic and asymptomatic patients post-COVID period[23]. The study revealed that fragmented QRS was significantly more prevalent in symptomatic individuals, suggesting its potential role as a predictor of cardiac symptoms such as palpitations and chest discomfort. A notable difference was observed in the Tpeak-Tend interval, which was significantly lower in symptomatic patients[23]. In another study by Karakayali et al., valuable insights into autonomic dysfunction in post-COVID patients were provided[24]. The study found no significant difference in autonomic function between symptomatic and asymptomatic individuals; however, symptomatic patients exhibited a higher burden of silent ischemia, characterized by increased ST depression and HRV measurements[24]. These findings indicate a complex relationship between cardiac symptoms and autonomic nervous system function in the post-COVID period and highlight the need for further research into the impact of COVID-19 on autonomic dysfunction.

In our study, long-term COVID patients exhibited significantly lower HRR_{1-5} than the control group. Furthermore, HRR_2 showed a significant negative correlation with disease markers such as CRP, D-dimer, and ferritin. These findings underscore the importance of dysautonomia, as indicated by impaired HRR, in the post-COVID period. Impaired HRR may serve as a marker of autonomic imbalance, which could contribute to the persistent symptoms experienced by long-term COVID-19 patients.

Limitations

This study has several limitations that should be considered when interpreting the results. First, its retrospective design may introduce selection bias, as it only includes patients who presented to the cardiology clinic with symptoms after their COVID-19 infection. The lack of longitudinal follow-up also limits the ability to determine the long-term effects of autonomic dysfunction in long-term COVID patients.

Second, the sample size was relatively small, with only 76 patients in the COVID-19 (+) group meeting the inclusion criteria, which may reduce the generalizability of the findings.

Finally, exercise stress testing to assess autonomic function might not capture all aspects of autonomic dysregulation, particularly in those with subtle or non-exercise-related dysfunctions. Further studies with larger, more diverse populations and extended follow-up periods are needed to validate these findings and explore the full spectrum of autonomic dysfunction in post-COVID patients.

Conclusion

This study highlights the significance of assessing and monitoring autonomic nervous system-related symptoms as potential long-term complications of COVID-19. The main findings indicate that patients categorized as 'Long-Covid' demonstrated significantly higher CRP, d-dimer, and ferritin levels. Furthermore, all HRR parameters (HHR_{1-5}) were notably lower in the Covid-19 (+) group. A moderate negative correlation was observed between HRR and disease-related parameters (CRP, d-dimer, and ferritin), suggesting a relationship between impaired autonomic function and inflammation markers in Long-Covid conditions. The findings of this study emphasize the importance of HRR as a marker of autonomic dysfunction in Long-Covid patients, highlighting the need for further research to explore the long-term impacts and develop appropriate therapeutic strategies.

Acknowledgments

We extend our gratitude to the resident physicians of the Cardiology Department at Ondokuz Mayis University Faculty of Medicine for their assistance during the data collection phase. We also wish to thank Dr. Önder Aydemir, M. D., for his contributions to the statistical analysis of our study.

Disclosures

Conflict of interest: No financial or other relationship might be perceived as leading to a conflict of interest.

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

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

The Importance of Micronutrient Deficiency in the Etiology of Anemia in the First Trimester of Pregnancy: a Cross-Sectional Study

Gebeliğin İlk Trimesterinde Anemi Etiyolojisinde Mikrobesin Eksikliğinin Önemi: Kesitsel Bir Çalışma

Samet Kirat

Kafkas University Faculty of Medicine, Academic Department of Obstetrics and Gynecology, Kars, Türkiye

ABSTRACT

Aim: *Anemia is a common pregnancy complication, with iron, folate, and vitamin B12 deficiencies being the most frequent causes. This study aimed to evaluate the role of micronutrient deficiencies in the etiology of anemia during the first trimester of pregnancy.*

Material and Method: *Between January 2023 and January 2024, 271 pregnant women aged 18–50 years who presented to the Obstetrics and Gynecology Outpatient Clinic of a tertiary university hospital were included in this study. Demographic data and hemoglobin, serum iron, total iron binding capacity (TIBC), ferritin, folate, and vitamin B12 levels were obtained retrospectively from medical records. According to the Centers for Disease Control and Prevention (CDC), 1st trimester hemoglobin <11 g/dl and hematocrit <33% were considered as anemia, ferritin <15 ng/ml as iron deficiency, serum folate <3 ng/ml, and vitamin B12 <200 pg/ml as a deficiency.*

Results: *In total, 107 (39.5%) pregnant women had anemia. Pregnant women with and without anemia were similar in terms of age, gravidity, parity, abortion, gestational week, infant birth weight, APGAR scores at 1 and 5 min, folate levels, and vitamin B12 deficiency levels. Those with anemia had significantly lower serum iron (p=0.006) and ferritin levels (p<0.001), and higher TIBC levels (p<0.001) than those without anemia. Ferritin was <15 ng/ml in 61.7% (n=66) of those with anemia (p<0.001). Vitamin B12 deficiency was present in one of the three pregnant women with anemia. There were only four pregnant women with folate deficiency, and none of them had anemia.*

Conclusion: *Providing adequate micronutrient support before and during pregnancy prevents anemia. Therefore, pregnancy follow-up protocols should emphasize regular screening for micronutrient deficiencies.*

Key words: *anemia; iron deficiency; ferritin; folate; vitamin B12 deficiency*

ÖZET

Amaç: *Anemi, gebelikte sık görülen bir komplikasyondur ve en sık anemi sebepleri demir, folat ve B12 vitamini eksiklikleridir. Bu çalışmanın amacı, gebeliğin ilk üç ayında anemi etiyolojisinde bu mikro besin eksikliklerinin rolünü değerlendirmektir.*

Materyal – Metod: *Çalışmaya Ocak 2023 ile Ocak 2024 arasında üçüncü basamak bir üniversite hastanesi Kadın Hastalıkları ve Doğum Polikliniği'ne başvuran 18–50 yaş arası 271 gebe dâhil edildi. Hastalara ait demografik veriler ve hemoglobin, serum demiri, total demir bağlama kapasitesi (TDBK), ferritin, folat ve B12 vitamini düzeyleri retrospektif olarak tıbbi kayıtlardan elde edildi. Hastalık Kontrol ve Önleme Merkezlerine (CDC) göre, 1. trimester hemoglobin <11 g/dl ve hematokrit <%33 anemi, ferritin <15 ng/ml demir eksikliği, serum folat <3 ng/ml ve vitamin B12 <200 pg/ml olması eksiklik düzeyi olarak kabul edildi.*

Bulgular: *Anemisi olan 107 (%39,5) gebe kadın vardı. Anemisi olan ve anemisi olmayan gebeler yaş, gravida, parite, abortus, doğum haftası, bebek doğum ağırlığı, 1. ve 5. dakikadaki APGAR skorları, folat düzeyleri ve B12 vitamini eksikliği düzeyi açısından benzerdi. Anemisi olanların serum demir (p=0,006) ve ferritin düzeyleri (p<0,001) anemisi olmayanlara göre anlamlı olarak daha düşük, TDBK düzeyleri daha yüksekti (p<0,001). Anemisi olanların %61,7'sinde (n=66) ferritin <15 ng/ml idi (p<0,001). Anemisi olan her üç gebeden birinde B12 vitamini eksikliği mevcuttu. Folat eksikliği olan sadece dört gebe vardı ancak hiçbirinin anemisi yoktu.*

Sonuç: *Gebelik öncesi ve sırasında yeterli mikro besin desteği sağlanması, anemiyi önlemek için kritik öneme sahiptir. Bu nedenle, gebelik takip protokollerinde mikro besin eksikliklerinin düzenli taranmasına daha fazla önem verilmelidir.*

Anahtar kelimeler: *anemi; demir eksikliği; ferritin; folat; vitamin B12 eksikliği*

İletişim/Contact: *Samet Kirat, Department of Gynecology and Obstetrics, Kafkas University Faculty of Medicine, Kars, Türkiye* •
Tel: *0555 261 83 08* • ***E-mail:*** *sametkirat1989@hotmail.com* • ***Geliş/Received:*** *16.10.2024* • ***Kabul/Accepted:*** *30.10.2024*

ORCID: *Samet Kırat: 0000-0001-7262-4320*

Introduction

Anemia is one of the most common complications of pregnancy and can have serious consequences on both maternal and fetal health. According to World Health Organization (WHO) data, the prevalence of anemia during pregnancy is approximately 36% worldwide, and this rate is higher, especially in low- and middle-income countries[1]. Anemia during pregnancy has been associated with significant complications, including maternal mortality, premature delivery, low birth weight, and intrauterine growth retardation. Anemia not only directly affects maternal health but may also have permanent adverse effects on fetal development[2].

The most common causes of anemia during pregnancy include iron, folic acid, and vitamin B12 deficiencies[3]. Iron deficiency is the most common cause of anemia in pregnancy due to increased blood volume, and this need increases even more with placental development and fetal growth in the first trimester[4]. These deficiencies are more common when appropriate nutrition and prenatal care are inadequate[5].

Iron deficiency constitutes 50–75% of anemia during pregnancy and may have serious consequences for both the mother and fetus when left untreated[6]. Inadequate iron intake may lead to problems such as fatigue, increased risk of infection, and complications during labor for the mother. In fetuses, the risk of intrauterine growth retardation and premature birth increases. Folate and vitamin B12 deficiencies also play important roles in developing anemia. Folic acid deficiency may lead to severe developmental problems, such as neural tube defects in the fetus, while vitamin B12 deficiency may lead to megaloblastic anemia[7]. Low socioeconomic conditions and malnutrition increase the prevalence of these deficiencies; therefore, prenatal care should be strengthened in at-risk groups[8].

In this study, we aimed to evaluate the role of iron, folate, and vitamin B12 deficiencies in the etiology of anemia in the first trimester of pregnancy and to contribute to the development of more effective prevention strategies to reduce the risk of anemia in pregnancy by early diagnosis of micronutrient deficiencies.

Materials and Methods

Patients and Data Collection

This study included 271 pregnant women aged 18–50 who presented to the Obstetrics and Gynecology Outpatient Clinic of a tertiary university hospital between January 2023 and January 2024.

Medical records were retrospectively accessed for demographic data, including age, gravida, parity, abortion, comorbidity, and medication. The first-trimester hemoglobin, iron, total iron binding capacity (TIBC), ferritin, folate, and vitamin B12 levels were also recorded.

Pregnant women with unavailable 1 st-trimester hemoglobin, serum iron, TIBC, ferritin, folate, and vitamin B12 levels in the medical records, those with additional systemic diseases, smokers during pregnancy, those with obstetric vaginal bleeding during pregnancy, and those using any iron and/or vitamin preparation were excluded. In addition, 58 pregnant women with additional systemic diseases and 19 pregnant women taking medications were excluded.

Terms and Definitions

According to the Centers for Disease Control and Prevention (CDC), 1 st-trimester hemoglobin <11 g/dl and hematocrit <33% anemia[9], ferritin <15 ng/ml[9], serum folate <3 ng/ml[10] and vitamin B12 <200 pg/ml[11] are considered deficiency levels.

Ethics Committee Approval

This study was approved by the Non-Interventional Clinical Research Ethics Committee of Kafkas University Faculty of Medicine (01/10/2024, 80576354–050–99/535) and complied with the recommendations of the Declaration of Helsinki for human biomedical research.

Statistical Analysis

The Windows SPSS program (version 24.0) was used for statistical analyses. When appropriate, categorical variables are presented as numbers with corresponding percentages and were assessed using the chi-square test or Fisher's exact test. Continuous variables were expressed as median (minimum-maximum) or mean ± standard deviation using the Kolmogorov-Smirnov test and assessed using Student's t-test or Mann-Whitney U test. Spearman correlation analysis determined the

relationship between hemoglobin levels and serum iron, ferritin, folate, and vitamin B12 levels. Statistical significance was set at p <0.05. Prism software (version 8, GraphPad Software, San Diego, California, USA) was used for the analysis and graphical data.

Results

Demographic and laboratory data of the total cohort

The mean age of the 271 pregnant women in the first trimester was 33.31±5.43 years. Median gravida was 2 (1–8), median parity was 1 (0–6), and median abortion was 0 (0–3). 15.9% (n=43) were preterm, 84.1% (n=228) were term; 5.2% (n=14) were <2500 grams, 93% (n=252) were 2500–4000 grams and 1.8% (n=5) were >4000 grams. The median hemoglobin level was 11.90 (6.6–16.1), and 39.5% (n=107) had a hemoglobin level <11 g/dl. The median serum iron was 71 (12–278) μg/dL, median TIBC was 321 (25–754) μmol/L, and median ferritin was 15 (2–199) ng/ml, respectively. Ferritin levels were <15 ng/ml in 47.2% (n=128) of pregnant women. The median folate level was 8.20 (1.91–24.8) ng/ml, and four pregnant women (1.5%) had folate levels <3 ng/ml. The median vitamin B12 levels were 240 (12–1146) pg/ml, and 33.6% (n=91) had vitamin B12 levels <200 pg/ml. Detailed data are presented in Table 1.

Table 1. *Demographic and laboratory data of the total cohort*

	Total cohort (n=271)
Age (mean ± SD)	33.31±5.43
Gravida (median (min-max))	2 (1–8)
Parity (median (min-max))	1 (0–6)
Abortion (median (min-max))	0 (0–3)
Birth week (n, %)	
<37 weeks	43 (15%.9)
37–42 weeks	228 (84%.1)
>42 weeks	0 (0%)
Infant birth weight (n, %)	
<2500 gram	14 (5%.2)
2500–4000 gram	252 (93%)
>4000 gram	5 (1%.8)
1st minute APGAR score (median (min-max))	8 (2–10)
5th minute APGAR score (median (min-max))	9 (8–10)
Hemoglobin (median (min-max))	11.90 (6.6–16.1)
Hemoglobin <11 g/dl (n, %)	107 (39%.5)
Serum iron (median (min-max))	71 (12–278)
TIBC (median (min-max))	321 (25–754)
Ferritin (median (min-max))	15 (2–199)
Ferritin <15 ng/ml (n, %)	128 (47%.2)
Folate (median (min-max))	8.20 (1.91–24.8)
Folate <3 ng/dl (n, %)	4 (1%.5)
Vitamin B12 (median (min-max))	240 (12–1146)
Vitamin B12 <200 pg/dl (n, %)	91 (33%.6)

TIBC: total iron binding capacity.

Comparison of pregnant women with and without anemia

There were 107 pregnant women with anemia and 164 pregnant women without anemia. Pregnant women with and without anemia were similar in terms of age, gravidity, parity and abortion, gestational week, infant birth weight, APGAR scores at 1 and 5 min, folate levels, and B12 deficiency levels. Those with anemia had significantly lower serum iron (58(12–278) μg/dL vs 76 (13–227) μg/dL; p=0.006) and ferritin levels (10(2–199) ng/ml vs 18 (2–184) ng/ml; p<0.001) and higher TIBC levels (351(25–754) μmol/L vs 301 (103–635) μmol/L; p<0.001). Ferritin was <15 ng/ml in 61.7% (n=66) of those with anemia and 37.8% (n=62) of those without anemia and was significantly lower in those with anemia compared to those without anemia (p<0.001). Vitamin B12 levels were significantly lower in patients with anemia than in those without (225(78–663) pg/ml vs 249.5 (12–1146) pg/ml; p=0.020). Among the pregnant women with anemia, 39.2% (n=42) had only iron deficiency, 0.9% (n=1) had only vitamin B12 deficiency, and 24.2% (n=26) had both iron and vitamin B12 deficiency (Figure 1). Detailed data are presented in Table 2.

Comparison of pregnant women according to ferritin level

Age, gravidity, parity, abortion, gestational week, infant birth weight, and APGAR scores at 1 and 5 min were similar according to ferritin levels. Hemoglobin was <11 g/dl in 51.6% (n=66) of those with ferritin <15 ng/ml and 28.7% (n=41) of those with ferritin >15 ng/ml (p<0.001). Median folate (7.45 (1.91–24.2) ng/ml vs 9 (2.6–24.8) ng/ml; p=0.018) and vitamin B12 (225(78–519) pg/ml vs 246 (12–1146) pg/ml; p=0.007) levels were significantly lower in patients with ferritin <15 ng/ml than in those with ferritin >15 ng/ml. Vitamin B12 deficiency was present in 40.6% (n=52) of patients with ferritin levels <15 ng/ml and 27.3% (n=39) of patients with ferritin levels >15 ng/ml (p=0.020).

Table 2. *Comparison of pregnant women with and without anemia*

	Pregnant women with anemia (n=107)	Pregnant women without anemia (n=164)	p
Age (mean ± SD)	33.71±5.90	33.05±5.11	0.333
Gravida (median (min-max))	2 (1–8)	2 (1–8)	0.079
Parity (median (min-max))	1 (0–6)	0 (0–5)	0.090
Abortion (median (min-max))	0 (0–3)	0 (0–3)	0.282
Birth week (n, %)			
<37 weeks	15 (14%)	28 (17%.1)	0.615
37–42 weeks	92 (86%)	136 (82%.9)	0.615
>42 weeks	0 (0%)	0 (0%)	*
Infant birth weight (n, %)			
<2500 gram	6 (5%.6)	8 (4%.9)	1.0
2500–4000 gram	98 (91%.6)	154 (93%.9)	0.627
>4000 gram	3 (2%.8)	2 (1%.2)	0.386
1st minute APGAR score (median (min-max))	8 (7–9)	8 (2–10)	0.723
5th minute APGAR score (median (min-max))	9 (8–10)	9 (8–10)	0.279
Hemoglobin (median (min-max))	10.3 (6.6–11.0)	12.8 (11.1–16.1)	<0.001
Serum iron (median (min-max))	58 (12–278)	76 (13–227)	0.006
TIBC (median (min-max))	351 (25–754)	301 (103–635)	<0.001
Ferritin (median (min-max))	10 (2–199)	18 (2–184)	<0.001
Ferritin <15 ng/ml (n, %)	66 (61%.7)	62 (37%.8)	<0.001
Folate (median (min-max))	7.84 (4–24.8)	8.44 (1.91–24.2)	0.452
Folate <3 ng/dl (n, %)	0 (0%)	4 (2%.4)	0.156
Vitamin B12 (median (min-max))	225 (78–663)	249.5 (12–1146)	0.020
Vitamin B12 <200 pg/dl (n, %)	39 (36%.4)	52 (31%.7)	0.419

TIBC: total iron binding capacity.

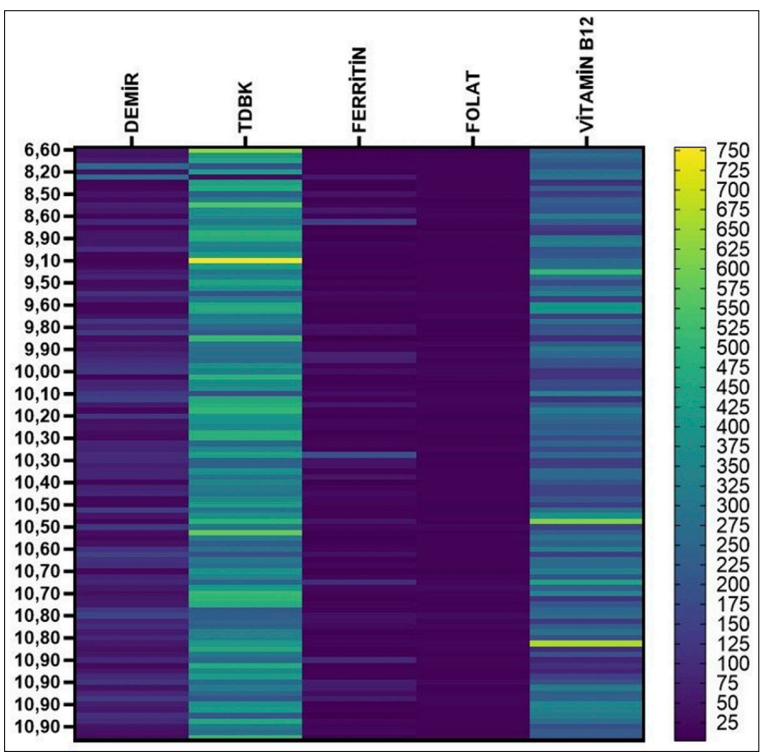

Figure 1. *The relationship between hemoglobin levels and iron, iron-binding capacity, folate and vitamin B12 in pregnant women with anemia.*

Comparison of pregnant women with and without vitamin B12 deficiency

Pregnant women with and without vitamin B12 deficiency were statistically similar in age, gravidity, abortion, gestational week, infant birth weight, APGAR scores at 1 and 5 min, hemoglobin, serum iron, TIBC, ferritin, and folate levels. The ferritin level <15 ng/ml was significantly higher in those with vitamin B12 deficiency than in those without vitamin B12 deficiency (n=52 (57.1%) vs n=76 (42.2%); p=0.020). Parity count was significantly higher in patients with vitamin B12 deficiency than in those without vitamin B12 deficiency (1(0–6) vs 0 (0–5); p=0.036).

Comparison of pregnant women with and without folate deficiency

The mean age of the four pregnant women with folate deficiency was statistically significantly lower than those without folate deficiency (26.75±2.63 years vs 33.41±5.41 years; p=0.015). Gravida, parity, gestational week, birth weight, infant birth weight, 1st and 5th minute APGAR scores, hemoglobin, serum iron, TIBC, ferritin, and vitamin B12 levels were similar in folate-deficient and non-folate-deficient women. The number of abortions was significantly higher in patients without folate deficiency than in those with folate deficiency (0(0–3) vs 1 (0–1); p=0.029).

Spearman Correlation Analysis Results

Spearman correlation analysis revealed a statistically significant positive correlation between hemoglobin levels and vitamin B12 (r=0.167, p=0.006), serum iron (r=0.167, p=0.006), and ferritin (r=0.216, p<0.001).

Discussion

This study examined the contribution of micronutrient deficiencies, including iron, vitamin B12, and folic acid, to the etiology of anemia in the first trimester of pregnancy. Anemia was detected in 40% of the pregnant population evaluated in our study, and iron deficiency was the most common etiology. In addition, 30% of pregnant women with anemia have low vitamin B12 levels. Folic acid deficiency was present in only four pregnant women, none of whom had anemia.

The prevalence of 1st trimester anemia during pregnancy varies among countries. It has been reported to be 27.8% in Türkiye[12], 18.2% in the USA[13], 15.9% in Sri Lanka[13], 10.6% in Jordan[14] and 31.8% in Ethiopia[15]. A systematic review by the WHO shows that it varies between 24–44% in low-income and middle-income countries[16]. In our study, the prevalence of anemia in the first trimester was 40%. The higher prevalence of anemia compared to the literature may be because the region where the study was conducted was rural and economically less developed, with malnutrition and limited access to health services.

In young pregnancies, it may be difficult for the body to meet the increased iron demand owing to ongoing growth and development. However, factors such as decreased body iron stores or chronic diseases may increase the risk of anemia in older pregnant women[17]. It has been reported that the prevalence of anemia is lower in pregnant women between the ages of 18–35 years and increases in pregnant women aged >35 years, which has negative effects on maternal complications and neonatal outcomes[17,18]. In our study, pregnant women aged <20 years and those aged >35 years were compared regarding anemia, but no significant difference was found (p=0.976). As socioeconomic status, dietary habits, access to health services, and pregnancy follow-up were similar in both age groups, factors other than age may have significantly affected the prevalence of anemia.

Each pregnancy depletes maternal iron stores, and the risk of anemia may increase during the subsequent pregnancy. Studies have shown that high gravida and parity trigger the development of anemia during pregnancy. A study conducted in Ethiopia found that the risk of anemia was higher in women who gave birth at intervals of less than two years[15]. In a study conducted in Türkiye, the anemia rate was 42.3% in women who had four or more live births[12]. In Ghana, it was shown that the risk of anemia was significantly increased in women who had many births compared to those who had fewer births[16]. In our study, we could not conclude that gravida, parity, or number of abortions were associated with anemia. The fact that gravida, parity, and number of abortions were not found to be associated with anemia in our study may have weakened the relationship because of insufficient sample size, different diagnostic criteria for anemia, or the effect of regional nutrition and health conditions.

Smoking can impair iron absorption by causing oxidative stress, reduce the oxygen-carrying capacity of hemoglobin, and lead to malnutrition, which can interfere with iron absorption and micronutrients, such as folic acid, increasing the risk of anemia. Several studies

have shown that smoking increases the risk of anemia during pregnancy. In a study conducted in India, anemia was found in 72% of pregnant smokers and was associated with iron deficiency[19]. In another study, the prevalence of anemia in smoking women was 27.6%[15]. To obtain more reliable results related to micronutrient deficiency in the etiology of anemia, pregnant women who smoked were excluded from our study.

The prevalence of anemia in women with chronic diseases in the first trimester of pregnancy is quite high, ranging up to 40% in women with chronic kidney disease[18], around 25% in women with thyroid disorders[20], 30–50% in autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis[18], and 30–60% in chronic infections such as diabetes and HIV[18,20]. These systemic diseases decrease erythropoietin production and cause anemia by disrupting iron metabolism. In our study, to obtain more reliable results regarding micronutrient deficiency in the etiology of anemia, pregnant women with systemic diseases and taking medication were not included.

Iron deficiency is the primary cause of anemia in the first trimester of pregnancy due to increased blood volume, and fetal growth demands that low iron stores cannot meet[13]. This condition is prevalent in areas with malnutrition, intestinal parasites, and a low socioeconomic status[18]. Also, heightened pregnancy inflammation disrupts iron metabolism and contributes to iron deficiency anemia[19]. The reported prevalence rates are 18.2% in the USA[13], 16.64% in Türkiye[21], 19.4% in China[13], 72% in India[19], and 27.6% in Ethiopia[15]. A systematic review and meta-analysis found that iron supplementation in the first trimester reduced the prevalence of anemia and improved pregnancy outcomes, highlighting the importance of early detection and management of iron deficiency[16]. Our study identified iron deficiency in three out of five pregnant women with anemia, likely due to nutritional deficiencies in the economically underdeveloped rural study area.

Vitamin B12 deficiency is recognized as one of the main causes of anemia during pregnancy. Vitamin B12 plays a vital role in DNA synthesis and erythrocyte maturation. Its deficiency leads to megaloblastic anemia, impaired erythropoiesis, and reduced oxygen transport capacity. This deficiency decreases maternal energy levels and negatively affects the neurological development of the fetus[13,15]. Therefore, early dietary adjustments and micronutrient supplements play key roles in protecting maternal and fetal health[15]. Studies reported that vitamin B12 deficiency was 74% in India[15], 12.4% in the UK[13], 46.5% in Ethiopia[18], 33% in Türkiye[13] and 22.2% in Brazil[13]. These rates vary depending on geographical factors and dietary habits. According to data in the literature, vitamin B12 deficiency was found in one out of every three pregnant women with anemia.

Folic acid is essential for DNA synthesis, cell division, and fetal development, with increased demand during pregnancy due to increased cell division, particularly during placental and fetal growth. Insufficient folic acid can hinder erythrocyte maturation, causing megaloblastic anemia and fetal developmental issues such as neural tube defects. Consequently, the WHO recommends a daily supplement of 400 μg folic acid for all pregnant women to prevent anemia and neural tube defects[22,23]. This deficiency is more prevalent in patients with malnutrition and low socioeconomic status. Studies have reported varying anemia rates among pregnant women with folic acid deficiency: 44% in India[24], 23%[22], 28.9%[23] in Ethiopia, and 25%[25] in Nepal. In a domestic study, the prevalence of anemia during pregnancy was 27.1%, primarily due to folic acid deficiency[12]. The fact that only four pregnant women with folic acid deficiency were identified in our study and anemia was not observed in these women may be due to the insufficient sample size, prevalence of folic acid supplementation practices in the region, or predominance of other causes of anemia, which may have led to the inability to determine the role of folic acid deficiency in the etiology of anemia.

The most important limitation of our study was its retrospective and single-center design. This may limit the generalizability of the findings. In addition, although most people in the region where the study was conducted were engaged in animal husbandry as a source of livelihood, anemia was detected in two out of every five pregnant women. The most common causes of anemia were iron and vitamin B12 deficiencies. The strength of our study is that to examine the role of micronutrient deficiencies in the etiology of anemia, we selected the cohort only from healthy pregnant women who were not on medication and had no additional systemic diseases. Thus, our findings provide more reliable conclusions regarding the association between anemia and micronutrient deficiencies.

Conclusion

Adequate micronutrient supplementation before and during pregnancy is critical for preventing anemia development. In particular, micronutrient deficiencies such as iron, folic acid, and vitamin B12 adversely affect maternal health during pregnancy and threaten healthy fetal development. Therefore, the early recognition of these deficiencies and appropriate supplementation will contribute to maternal health status and favorable birth outcomes. Thus, assessing micronutrient deficiencies during pregnancy and integrating interventions for these deficiencies into pregnancy follow-up protocols should be considered effective approaches for maternal and fetal health.

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ARAŞTIRMA MAKALESİ / *RESEARCH ARTICLE*

Computer-Based Characterization of Specific Cystic Renal Masses of Bosniac Classification with Traditional Machine Learning and Modified Deep Learning Methods

Geleneksel Makine Öğrenme ve Özelleştirilmiş Derin Öğrenme Yöntemleri ile Bosniak Sınıflandırmasına Ait Özel Kistik Böbrek Kitlelerinin Bilgisayar Tabanlı Karakterizasyonu

Mehmet Ezer[1], Ali Berkan Ural[2], Canver Onal[3]

[1]Department of Urology; [2]Department of Electrical Electronics Engineering, Circuit and Systems, Biomedical, Kafkas University, Kars, Türkiye; [3]Department of Radiology, Faculty of Medicine;

ABSTRACT

Aim: *This study aims to develop a novel artificial intelligence-based pre-diagnosis system for classifying cystic renal masses (CRM) according to the Bosniak classification. The objective is to distinguish between the five diagnostic stages of the Bosniak classification using traditional machine learning (ML) and deep learning (DL) techniques.*

Material and Method: *A total of 20 contrast-enhanced CT images were collected for each of the five Bosniak stages (I, II, IIF, III, IV), verified by a uro-oncologist and radiologist. Additional image variations were generated using the Keras image processing library during the data augmentation phase, resulting in 600 images per stage. This process included operations such as brightness and contrast modification, image rotation, noise addition, and flipping. These augmented images were then used to train both ML and DL models. The k-Nearest Neighbors (kNN) algorithm was applied for the ML approach, while modified Convolutional Neural Networks (CNN) and VGG-16 models were used for the DL approach. Model performances were evaluated using receiver operating characteristic (ROC) analysis and area under the curve (AUC) metrics.*

Results: *The kNN algorithm accurately classified the Bosniak stages with an AUC of 0.854. The VGG-16 model demonstrated superior performance, with an AUC of 0.978, achieving higher classification accuracy than the kNN model.*

Conclusion: *The computerized Bosniak classification system based on CT images effectively differentiates between the five Bosniak stages. This system, utilizing both ML and DL models, has the potential to enhance the pre-diagnosis of CRM in clinical settings and can also effectively exclude other types of renal masses.*

Key words: *renal mass; Bosniak classification; machine learning; feature extraction; deep learning*

ÖZET

Amaç: *Bu çalışma, Bosniak sınıflamasına göre kistik renal kitleleri sınıflandıran yapay zeka tabanlı yeni bir ön tanı sistemi geliştirmeyi amaçlamaktadır. Amacımız, Bosniak sınıflamasındaki beş tanısal aşamayı (I, II, IIF, III, IV) geleneksel makine öğrenimi (ML) ve derin öğrenme (DL) teknikleri kullanarak ayırt etmektir.*

Materyal – Metod: *Her bir Bosniak aşaması (I, II, IIF, III, IV) için üro-onkolog ve radyolog tarafından doğrulanmış toplam 20 kontrastlı BT görüntüsü toplandı. Veri çoğaltma aşamasında, Keras görüntü işleme kütüphanesi kullanılarak ek görüntü varyasyonları üretildi ve her aşama için 600 görüntü elde edildi. Bu süreçte parlaklık ve kontrast ayarı, görüntü döndürme, gürültü ekleme ve çevirme gibi işlemler yapıldı. Bu çoğaltılmış görüntüler, hem Makine Öğrenmesi hem de DÖ modellerinin eğitiminde kullanıldı. Makine Öğrenmesi yaklaşımı için k-En Yakın Komşu (kNN) algoritması uygulanırken, Derin Öğrenme yaklaşımı için değiştirilmiş Konvolüsyonel Sinir Ağları (KSA) ve VGG-16 modelleri kullanıldı. Model performansları, ROC (alıcı işletim karakteristiği) analizi ve eğri altındaki alan (EAA) metrikleri ile değerlendirildi.*

Bulgular: *kNN algoritması, Bosniak tiplerini doğru sınıflandırmada 0.854 AUC değerine ulaştı. Karşılaştırmalı olarak, VGG-16 modeli 0.978 AUC ile üstün performans gösterdi ve kNN modelinden daha yüksek sınıflandırma doğruluğu sağladı.*

Sonuç: *BT görüntüleri baz alınarak geliştirilen bilgisayarlı Bosniak sınıflama sistemi, Bosniak aşamaları arasında etkili bir şekilde ayrım yapmaktadır. Makine Öğrenmesi ve DÖ modellerini kullanan bu sistem, klinik ortamlarda kistik renal kitlelerin ön tanısını geliştirme potansiyeline sahip olup, diğer böbrek kitleleri türlerini dışlamak için de etkili olabilir.*

Anahtar kelimeler: *renal kitle; Bosniak sınıflaması; Makine Öğrenmesi; özellik çıkarımı; derin öğrenme*

İletişim/Contact: *Mehmet Ezer, Kafkas University Medical School, Department of Urology, Kars, Türkiye* • ***Tel:*** *0474 225 21 09* • ***E-mail:*** *mehmetezer@gmail.com* • ***Geliş/Received:*** *01.11.2024* • ***Kabul/Accepted:*** *18.11.2024*

ORCID: *Mehmet Ezer: 0000-0003-4422-6768* • *Canver Önal: 0000-0001-6797-2574* • *Ali Berkan Ural: 0000-0001-5176-9280*

Introduction

Cystic renal lesions are fluid-filled sacs in the kidneys and are formations that exhibit a wide range of different characteristics[1]. According to research, the incidence of cystic renal lesions in individuals over the age of 50 can be as high as 50%. The incidence of these lesions increases with age, and they are generally benign[2]. However, careful evaluation is necessary because some cystic renal lesions may have malignant potential[3].

The basic methods used to diagnose cystic renal lesions include ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI)[4]. Ultrasonography is a widely used, non-invasive, and low-cost method. However, when detailed evaluation of the internal structure and septa of the lesions is required, advanced imaging methods such as CT and MRI should be used[5]. These methods are more effective in determining whether the lesions contain solid components, whether there is calcification, and whether wall thickness[6].

The most common classification system used to evaluate cystic renal lesions is Bosniak staging[7]. Bosniak classification helps determine the risk of malignancy based on the radiological features of the lesions. Bosniak stage I and II lesions are generally considered benign, while Bosniak stage III and IV lesions have a higher risk of malignancy[8]. Therefore, Bosniak III and IV lesions may require surgical excision or close follow-up. However, Bosniak stage IIF presents a more complex picture[9]. Lesions in this stage are between Bosniak II and III and usually require observation[10]. Although the malignant potential of these lesions is low, regular follow-up is recommended when a definitive distinction cannot be made. The Bosniak IIF classification is a crucial category within the Bosniak classification system, which is used to assess renal cysts based on their imaging characteristics in CT scans. Bosniak IIF specifically pertains to complex cysts that demonstrate some atypical features, such as a few thin septa or a slightly increased attenuation, which suggests the potential for benign but atypical behavior.

Traditional evaluation of these cysts often relies on the subjective interpretation of radiologists, which can lead to inconsistencies in diagnosis. However, integrating image processing and deep learning techniques presents a transformative approach to enhance diagnostic accuracy. By utilizing convolutional neural networks (CNNs), these advanced technologies can automatically analyze imaging data, extracting relevant features and patterns that may be imperceptible to the human eye. Training deep learning models on large, annotated datasets allows for the development of robust algorithms to distinguish between Bosniak II and IIF cysts with high precision. Image preprocessing techniques, such as normalization and augmentation, further optimize the quality of the input data, ensuring that the models are trained effectively. This automated approach not only aims to reduce interobserver variability but also enhances the overall efficiency of the diagnostic process, ultimately leading to better patient management and treatment outcomes. As research in this field progresses, the synergy between radiology and artificial intelligence continues to pave the way for improved clinical practices in evaluating renal lesions.

The Bosniak classification is an important tool in determining the malignant potential of lesions, but clinicians should always adopt a multidisciplinary approach and make individualized decisions on a patient basis[11]. In this process, accurate diagnostic methods and meticulous evaluation of the findings are important for patient health and treatment outcomes.

Machine learning (ML) methods that analyze and convert data into quantitative data can give clinicians valuable information and achieve an optimized and delivered model to characterize specific tumors and CRMs[12]. Moreover, with the improvement of AI models for clinical pre-diagnosis CAD systems, Deep Learning (DL) models have gained importance in the pre-diagnosis clinical area. For this reason, DL models can be used to characterize and diagnose CRMs, especially Bosniak classification, and more successful results can be obtained in detail[13].

This study aims to develop a computerized pre-diagnosis system based on the Bosniak classification of cystic renal masses (CRM). The study aims to analyze data obtained from contrast-enhanced CT images using machine learning (ML) and deep learning (DL) models. By accurately and rapidly distinguishing the five diagnostic stages of the Bosniak classification, this system aims to provide AI-assisted support in the clinical diagnosis process, reduce interpretation variability among radiologists and clinicians, and contribute to achieving more objective results in the decision-making process.

Materials and Methods

Data Acquisition (Dataset) and Radiological Assessment

This retrospective study obtained ethical approval, and the local institutional review board waived the requirement for informed consent (approval number: 80576354–050–99/458). Our database consisted of 20 raw CT images per case stage (I, II, IIF, III, IV), and these CT examinations were performed with the renal mass analyses protocol.

An uro-oncologist and a radiologist re-evaluated the contrast-enhanced abdominal CT images of patients diagnosed with cystic renal masses (CRM) at the University Hospital. They classified them according to the Bosniak staging. Twenty CT images were selected for each Bosniak stage, for a total of one hundred images, which were taken for analysis.

Spectrogram images were mainly created and used for DL models in the data augmentation phase. Keras image processing library, such as *Imagedatagenerator*, was mainly used to increase the number and variability of images in the collected database. The augmentation operations were a randomly developed factor value, brightness modification, sharpness modification, image rotation, adding Gaussian, adding salt & pepper noise, adding speckle noise, contrast modification, image translation, zooming in an image with the segments, and flipping images. At the end of these processes, in addition to the 20 original images for each Bosniak type, 580 new images were generated through augmentation, resulting in 600 images per stage being prepared for the examination phase.

Proposed System

Our customized detection CAD system for Bosniak classification from CTs consisted of important modules detailed in figure (Figure 1).

Image Acquisition

The initial step involved uploading the test image data into the system. CT image sets in DICOM (. dcm) format were uploaded to the system at this stage. The uploaded 2D images were also rescaled to a specific resolution of 240×240 μm. This study was conducted using interface-supported tools and developed within the MATLAB 2020 and 2024 environments. The figure shows five types of images classified according to the Bosniak classification as examples (Figure 2).

Image Processing

In the pre-processing part of this study, user-defined uploaded Bosniak CT images were first converted to jpeg format. Then, the pre-processing step was performed for the next step. Images were resized to 255×255 um. In the next step, images are analyzed and converted to gray-level images. Then, these images were filtered with a 3×3 median filter to remove noise and possible artifacts from the images[14]. Due to possible insufficient contrast of the images, the contrast was adjusted. For this case,

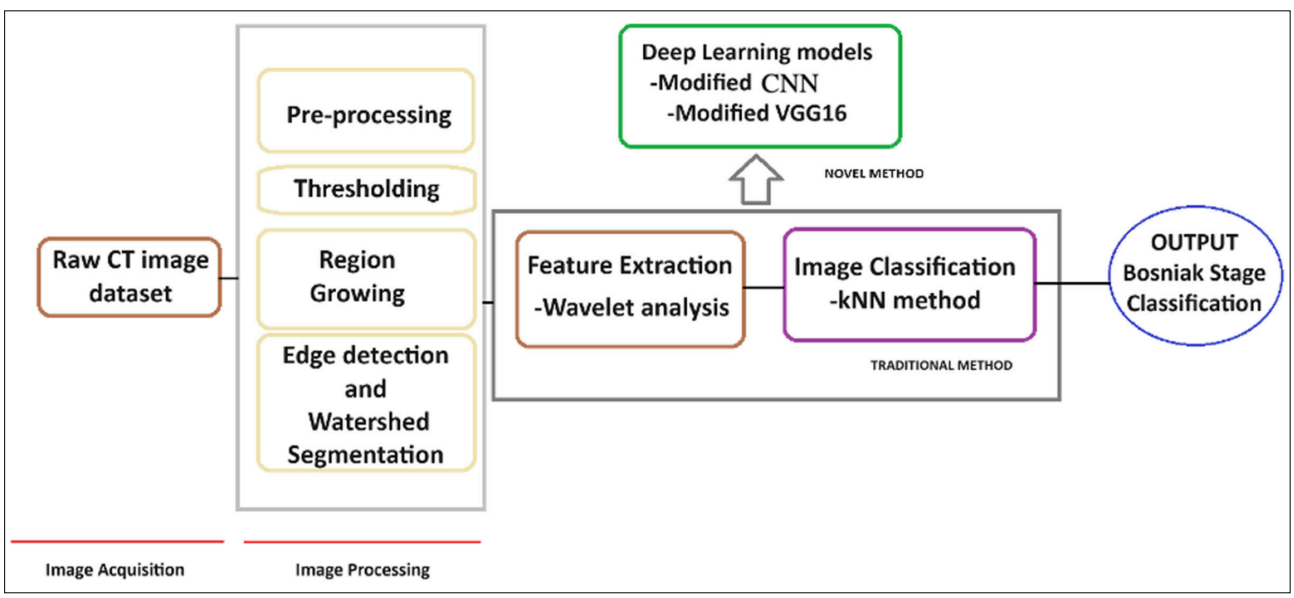

Figure 1. *Flowchart of the proposed system.*

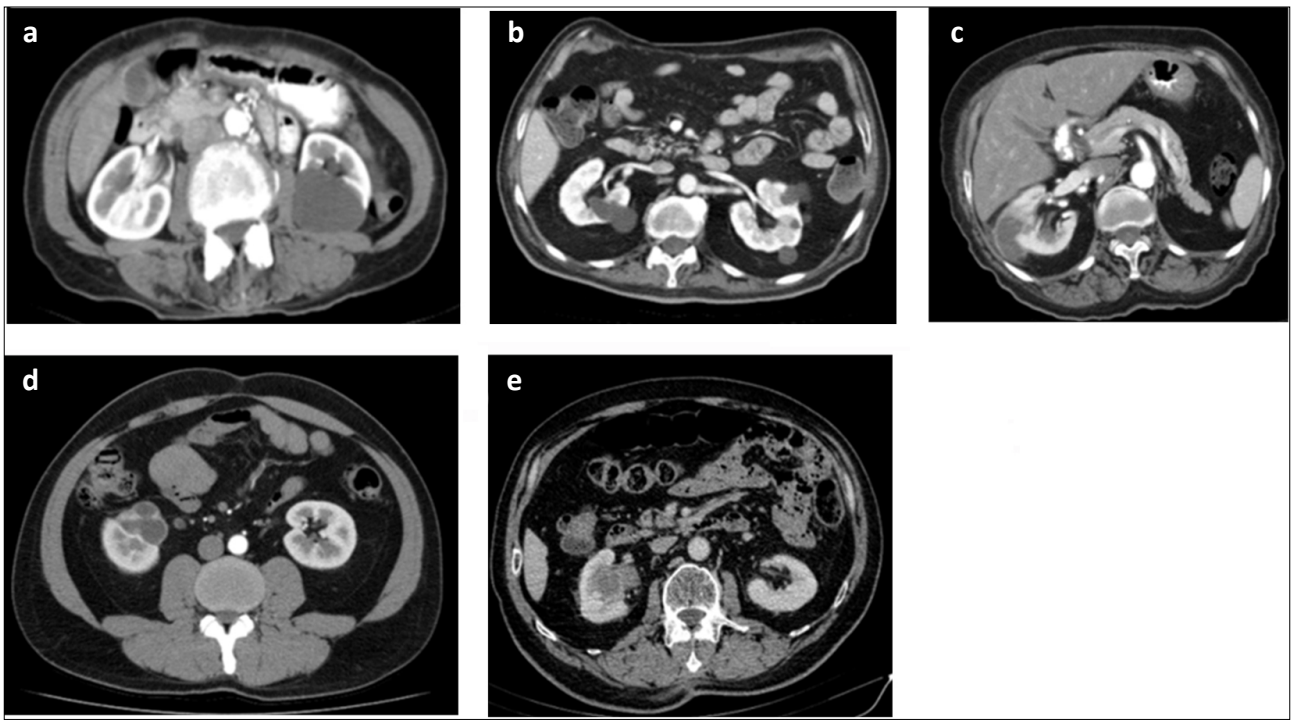

Figure 2. *Representative computed tomography images from the dataset used for evaluation, showing an example for each subtype of the Bosniak classification: Bosniak Type I (a), Type II (b), Type IIF (c), Type III (d), and Type IV (e).*

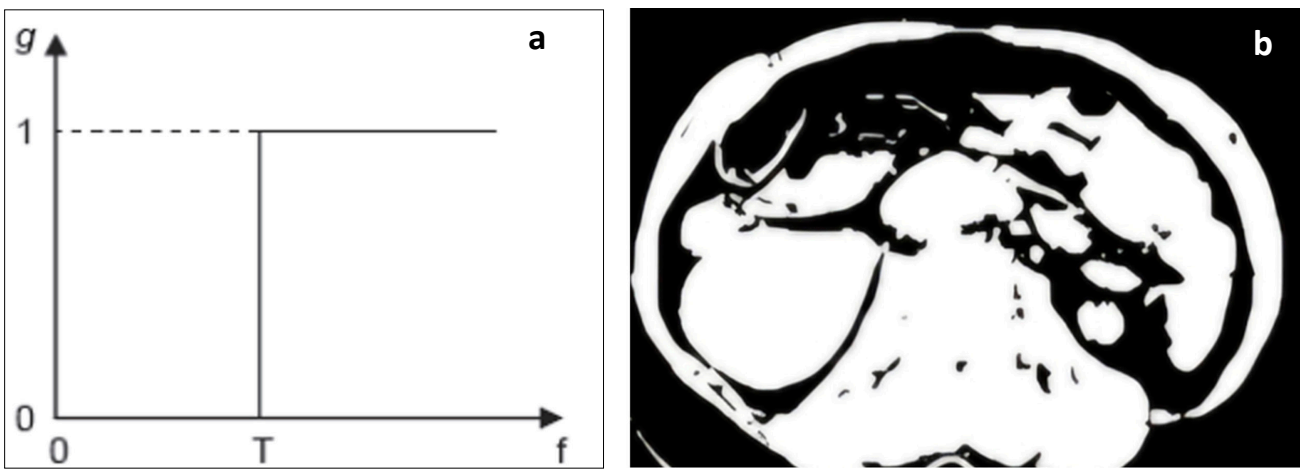

Figure 3. *Thresholding Step: (a) Single value threshold and graphical representation of the threshold for a specified value; (b) Results obtained from the thresholding process in MATLAB.*

an improved cubic curve contrast enhancement method was used to increase the texture, and images became clearer[15].

The other step was Thresholding. This section showed that abnormal areas in radiological images became white after a certain threshold, other parts of the image were eliminated, and the black color tone was highlighted[16]. Then, the highlighted images most likely indicated the region and regions included in the mass. The process continues to the next stage, which is the interpretation of the regions. For this stage, a hybrid method combining Gray level and Otsu thresholding methods was used and applied to the images. Contrast was the image's darkest parameter and could be defined as the difference between the brightest area[17]. Traditionally, thresholding was a simple way to perform the section to set a range of brightness values in the original image. Then, the pixels within the range as foreground were selected, and all other areas were to

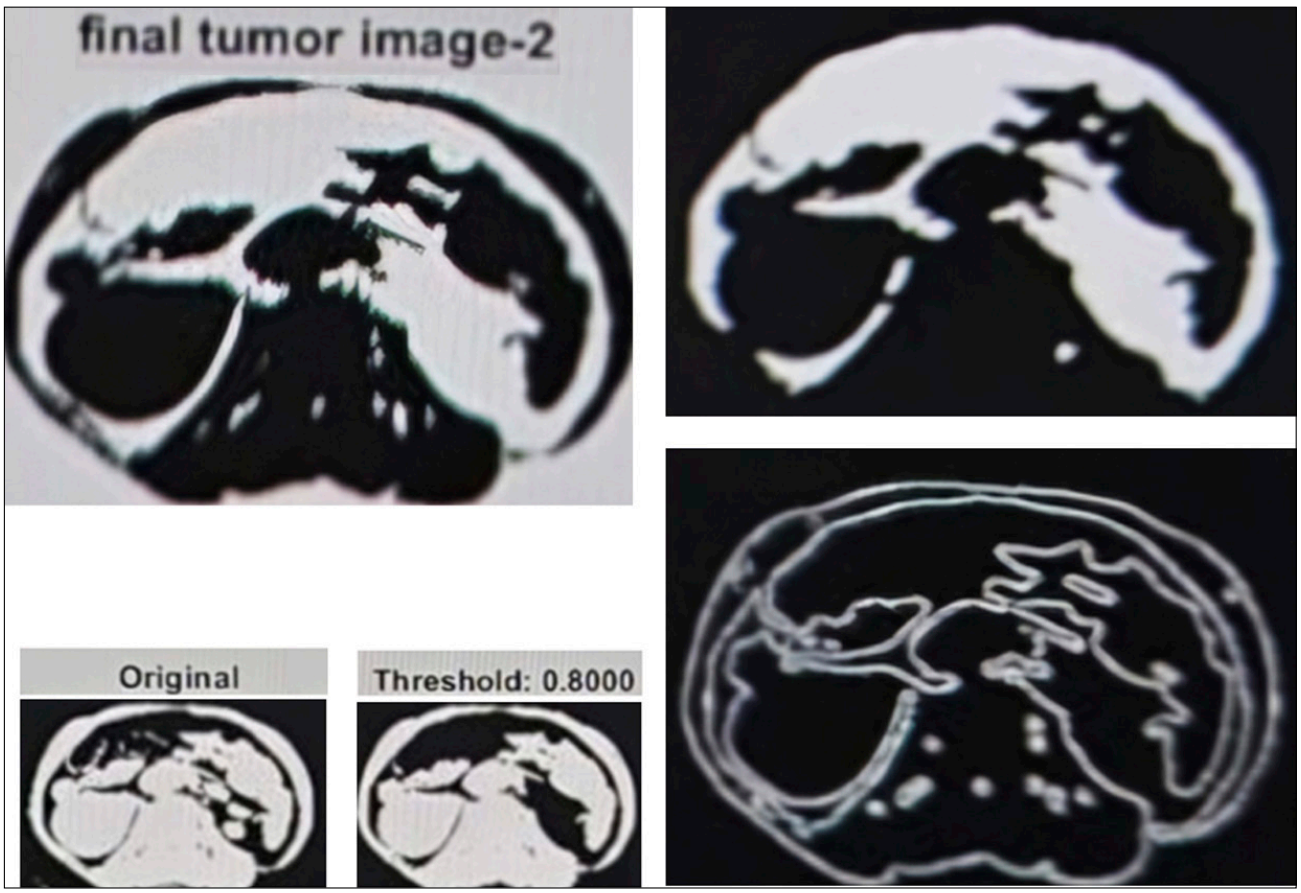

Figure 4. *Region-growing results in MATLAB*

reject pixels in the background. The figure shows the thresholding process, including the single value threshold and a graphical representation (Figure 3a), with the process results presented in MATLAB (Figure 3b).

The other step was Region Growing. In this step, Sobel edge detection and marker-controlled Watershed segmentation methods were chosen and used, and a hybrid Region growing process was achieved. For this step, images obtained were given in MATLAB in figure (Figure 4). The pseudocode of the hybrid region growing method was given in detail in figure (Figure 5).

Feature Extraction and Traditional Classification with ML Methods

In determining and interpreting abnormal areas, feature extraction was performed first on the mass areas[18]. This step included medical and image processing areas (Contrast, Correlation, Homogeneity, Variance, Energy, Entropy, External Curvature, Mean, Standard Deviation, etc.) that were obtained as numerical data for regions, and the analyzing process was achieved. The 14 features mentioned in the figure are given in detail (Figure 6).

In the classification via Machine Learning (ML) methods stage, k Nearest Neighbor (kNN) was a classification method used in data mining. This method performed the classification process according to the class of the nearest neighbor as much as the k value given. In the kNN method, the distance of the test data to the training data was obtained by the Euclidean method[19]. In the classification phase with kNN mentioned above, 20 and 730 CT images per Bosniak class were used and 14 features were obtained totally and these matrixes were saved to an Excel file for fedding into the ML model for classification and interpretation.

Transfer Learning-DL Approaches

In the experimental part, a transfer learning approach was performed to analyze and evaluate the performance metrics of the common ML and modified DL deep models on the Bosniak CT dataset. In this phase, feature extraction was not needed for the DL part, and the spatial characteristics of EEG data were extracted using the CNN network[20]. The developed CNN model algorithm was formulated and given below.

```
%region growing
    im=im2double(BW);
    T=0.8;
    [r,c]=size(im);
    A=zeros(r,c); % segmented mask
    F=[]; % frontier list
    subplot(1,2,1);
    imshow(im);
    title('Original');
    imwrite(im,'processedimage.jpg')
    s=uint16(ginput(1));    % get the click coordinates
    s=[s(2),s(1)];      % [row,col]
    A(s(1),s(2))=1;
    F=[F;s];
    while(~isempty(F)) % if frontier is empty
        n=neighbours(F(1,1),F(1,2),r,c);   % 4 neighbourhood
        for i=1:size(n,1)
            if(abs(im(F(1,1),F(1,2))-im(n(i,1),n(i,2)))<T && A(n(i,1),n(i,2))~=1)
                A(n(i,1),n(i,2))=1;
                F=[F;n(i,1),n(i,2)];
            end
        end
        F(1,:)=[];
    end
    subplot(1,2,2);
    imshow(A);
    title(sprintf('Threshold: %0.4f',T));
```

```
    sobel edge detection
    W1 = edge(A,'Canny');
    igure,imshow(BW1)
    mshowpair(BW1,BW,'montage')
    %marker controlled watershed seg.
    fm=A;
    e = strel('disk', 2);
    top = imtophat(afm, se);
    bot = imbothat(afm, se);
    igure, imshow(Itop, []), title('top-hat image');

    igure, imshow(Ibot, []), title('bottom-hat image');

    =imadd(Itop, afm);
    hos X
    hos Ibot
    bot=im2double(Ibot);

    enhance = imsubtract(X, Ibot);
    igure, imshow(Ienhance), title('final tumor image')
    image enhancement
    ec = imcomplement(Ienhance);
    igure, imshow(Iec), title('final tumor complement image-1');

    emin = imextendedmin(Iec,1);
    impose = imimposemin(Iec, Iemin);
    igure, imshow(Iemin), title('extended minima image');
    igure, imshow(Iimpose), title('final processed image-2');
```

Figure 5. *Pseudocodes of hybrid region growing method.*

Algorithm: Convolutional Neural Network (CNN)

1. Input: Number of samples, channels

2. $a_b^l = \sum_{c \in r} a_c^{l-1} \otimes K_{bc}^l + d_b^l$

3. The features belonging to the signals were down sampled in an average small neighborhood for obtaining new features after the convolution process. Indeed, the pooling process was achieved via the formula given below.

$$F = f(a_b^l = \sum_{c \in r} a_c^{l-1} \otimes K_{bc}^l + d_b^l) =$$

$$f(x_c^l \text{down}(a_c^{l-1}) + d_b^l)$$

4. The output was the first fully connected layer, and this could be obtained by weighting the input via the given formula

$$u^I = w^I a^{I-1} + d^I$$

For the equations given above,

```
seg_img=Iec;
% Extract features using DWT
x = double(seg_img);
m = size(seg_img,1);
n = size(seg_img,2);
signal1 = seg_img(:,:);

[cA1,cH1,cV1,cD1] = dwt2(signal1,'db4');
[cA2,cH2,cV2,cD2] = dwt2(cA1,'db4');
[cA3,cH3,cV3,cD3] = dwt2(cA2,'db4');

DWT_feat = [cA3,cH3,cV3,cD3];
G = pca(DWT_feat);

[g] = graycomatrix(G);
%stats = graycoprops(g,{'contrast','homogeneity','correlation','Energy'});
stats = graycoprops(g,'Contrast Correlation Energy Homogeneity');

Contrast = stats.Contrast;
%sprintf('Contrast is: %g%%',Contrast)

Correlation = stats.Correlation;

Energy = stats.Energy;

Homogeneity = stats.Homogeneity;

Mean = mean2(G);

Standard_Deviation = std2(G);
```

```
Entropy = entropy(G);

RMS = mean2(rms(G));

Variance = mean2(var(double(G)));

a = sum(double(G(:)));
Smoothness = 1-(1/(1+a));

Kurtosis = kurtosis(double(G(:)));

Skewness = skewness(double(G(:)));

% Inverse Difference Movement
m = size(G,1);
n = size(G,2);
in_diff = 0;
for i = 1:m
    for j = 1:n
        temp = G(i,j)./(1+(i-j).^2);
        in_diff = in_diff+temp;
    end
end
IDM = double(in_diff);
%white area calculation
nWhite_pixels=sum(Iec(:));
[rows columns depth]=size(Iec);
percentage_of_white_area=(nWhite_pixels/(rows*columns))*100;
disp(percentage of white area);
```

Figure 6. *Pseudocodes of Feature Extraction process and the obtained features.*

a_b^l: the mth channel activation value,

a_c^{l-1}: the mth channel output,

f(·): the activation function,

p: the selected feature sets of input,

K_{bc}^l: a convolution function,

d_b^l: the bias value,

x_c^l: the offset coefficient,

x_c^l, d_b^l: the bias coefficients,

a_b: the activation value of the fully connected layer,

w^ι : the weight of the fully connected layer.

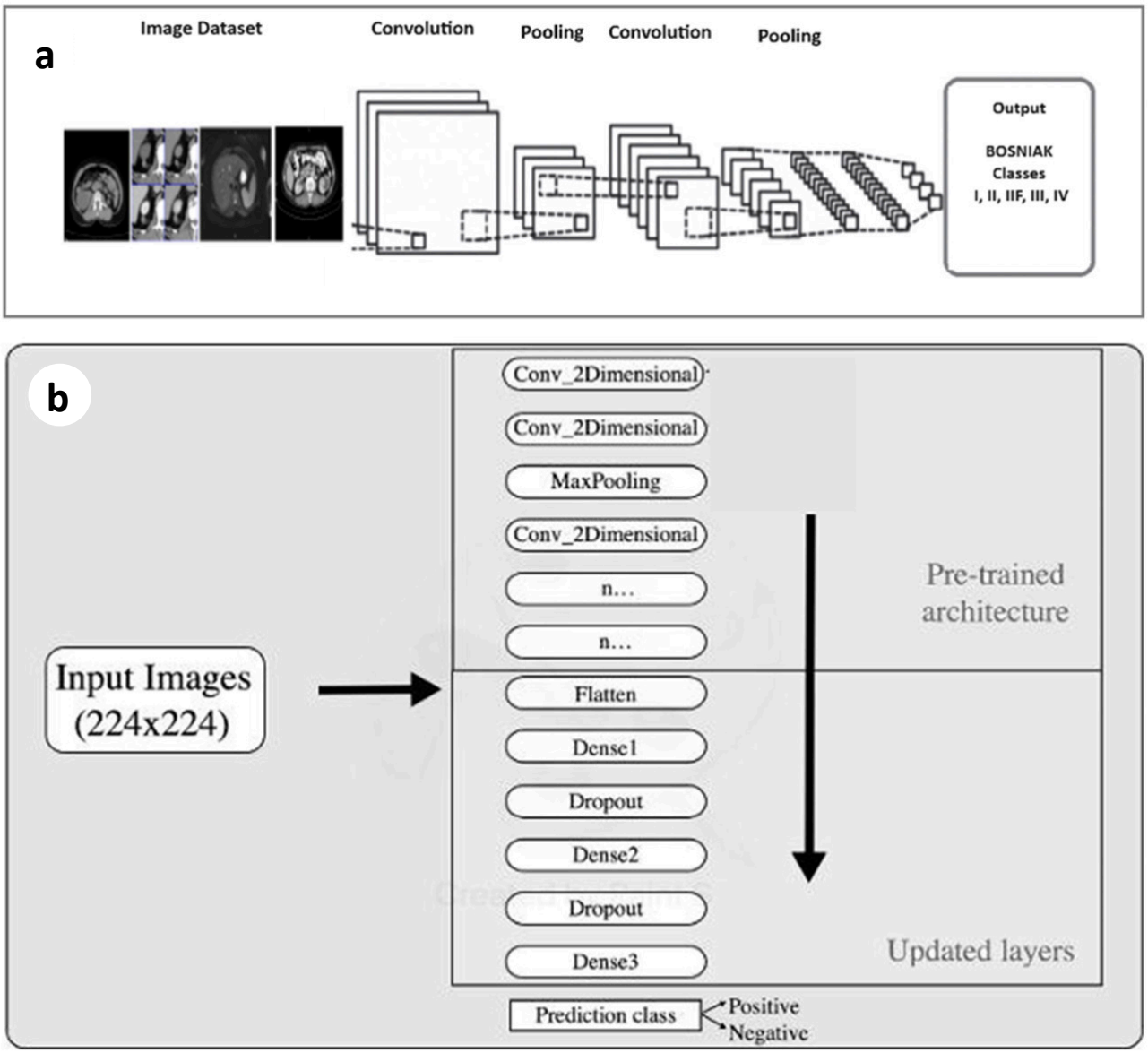

Figure 7. *The architectures of the models used in the study are shown: CNN model architecture (a) and VGG16 modified model architecture (b).*

For this model, the parts of the fundamental model were the pre-trained phase, up-to-date layer and estimation class. Using MATLAB environment, the CNN model has eight constant layers; other parts could be improvable. For the fully connected phase, Artificial Neural Networks (ANNs) were performed in detail. According to the total model, 17 Convolutional Neural Network (CNN) layers were used[21]. After filtering, a Maximum Pooling layer and dense and drop-out layers gathered this layer. In the figure, the CNN model was given in detail (Figure 7).

In addition, 10-fold cross-validation was chosen to eliminate the influence of the selected training and test data for the model evaluation. The equations are given below,

m: the number of samples,

$\hat{y}_n$**:** the predicted value, y_n: the real value.

$$CV_e = 0.1 \text{ x } \sum_{k=1}^{10} e_k$$

$$e_k = \frac{1}{m} x \ \sum_{l=1}^{m} ((\hat{y}_n - y_n)^2$$

Results

The experimental part of the study was achieved via MATLAB 2020 and 2024 versions with an 11 th Generation Intel brand i7–11900 processor, 2.500Ghz, 64GB DDR4 memory, and 1024GB SD storage capacity in detail. Indeed, these images were then anonymized

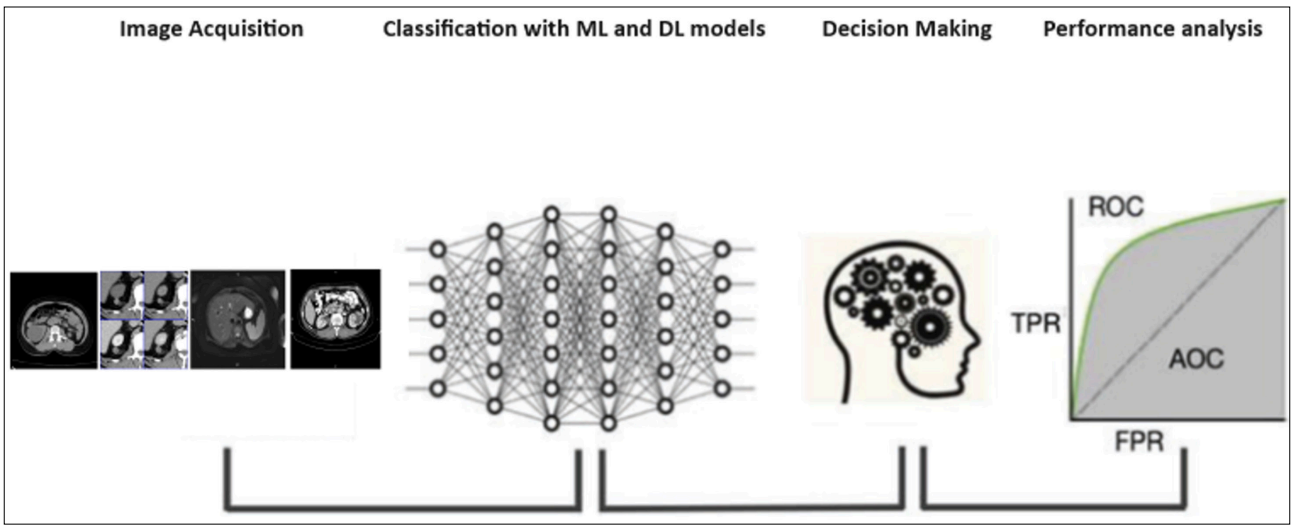

Figure 8. *Performance evaluation of the proposed system.*

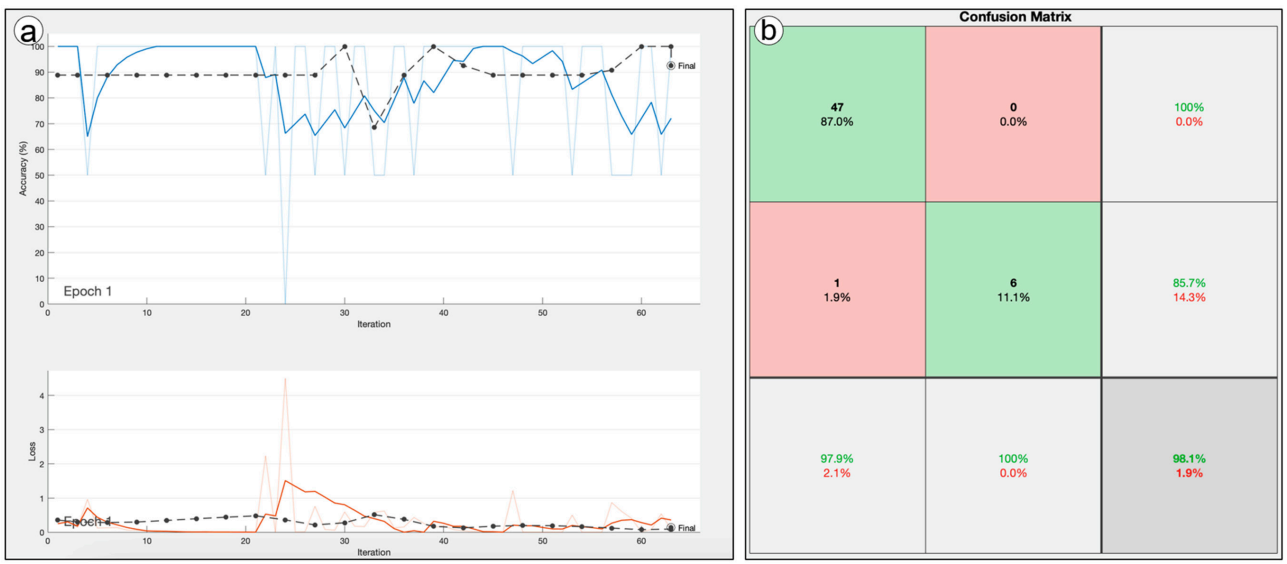

Figure 9. *Obtained accuracy and loss results for the CNN model (a); Confusion matrix for the CNN model (b).*

and preprocessed through signal labeling using the MATLAB tool. The performance calculation procedure of the whole system is given in Figure (Figure 8).

CT images were processed as the first step in the experimental part, and Feature Extraction was performed. With the wavelet method, features were obtained, and these feature matrix (gray matrix) was classified with the common Machine Learning kNN algorithm. According to the results, the classification accuracy was obtained with the 85% for Bosniak classification for kNN classifier.

Then, the next part was classification with DL models and modified CNN and VGG16 models were used in the classification stage. According to the figure, the ROC AUC curve and confusion matrix for the CNN model were given in detail (Figure 9). The CNN model has achieved more complex classification results with 98% with better capabilities. In Figure, the accuracy results for the confusion matrices of VGG16 for 10-fold cross-validation were given in detail (Figure 10).

According to the confusion matrices above, the last model (VGG16) approach reduced the misclassifications. According to the models, the best accuracy was obtained from the VGG16 deep model compared to the CNN model.

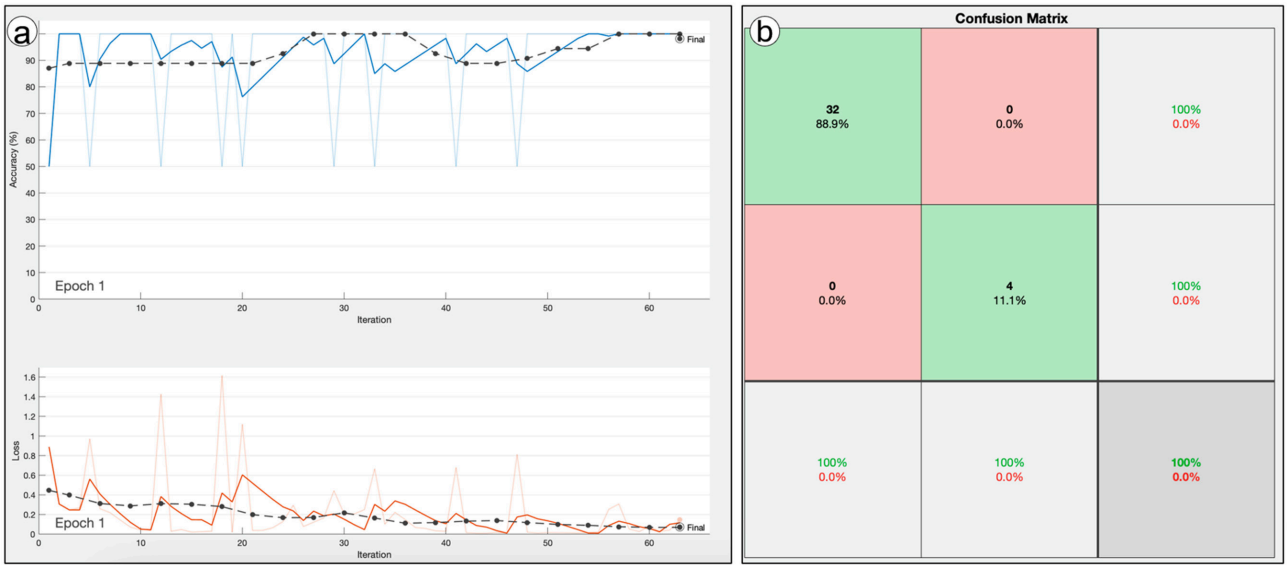

Figure 10. *Obtained accuracy and loss results for the VGG16 model (a); Confusion matrix for the VGG16 model (b).*

Discussion

In this study, we hypothesized that an improved version of CT-based machine learning and modified deep learning algorithms could be used to classify cystic renal masses more successfully in the Bosniak Classification System. Our results showed significant differences in some texture parameters and features, including mean, deviation, entropy, kurtosis and skewness. Entropy represents the total irregularity in the gray-level intensities of a lesion or mass.

According to the ML classification, the accuracy result was obtained as 85% for the kNN algorithm. Indeed, according to the classification with modified DL models, the accuracy result was obtained for the CNN model, and the accuracy result was obtained as 98% for the VGG16 model. When we analyzed all the results, the highest and best results were obtained from the VGG16 classifier and then the kNN classifiers. Indeed, every training and classification results were different from each other for detecting the mental disease case.

Even while our suggested model performed well in classification on datasets with significant imbalances or few samples, it was still far from perfect and contained flaws. For instance, the training and labeling of the photos for the sample preparation in our suggested model required significant processing power, and the training speed was comparatively slow. As a result, we will provide more image labeling and samples in our future work to improve the detection quality, as it has been demonstrated that some diseases are still not correctly diagnosed because of inadequate data sources. All models used in the study could be successfully used for different cases or CT types to help clinicians via CAD pre-diagnosis systems.

Limitations

One of the main limitations of this study is the limited number of patients available for each Bosniak stage. Particularly for the rarer subtypes, such as Bosniak type III and IV, we had a limited number of patients in our dataset. Thus, we aimed to utilize the maximum number of patients from the available data pool. To partially address this limitation, synthetic data were generated through image augmentation, a technique frequently employed in machine learning systems. However, when compared to raw data from real patients, the clinical validity of these augmented images remains a topic of debate. Despite this, the study provides a valuable contribution by offering a different approach compared to similar studies in the literature. Utilizing larger datasets in future research will enhance the accuracy and reliability of the model and strengthen the generalizability of the findings.

Additionally, the artificial intelligence models used in this study are based solely on imaging data. Factors such as the patient's symptoms, laboratory results, and overall condition should also be considered in clinical decision-making. Therefore, adopting a more comprehensive and multidisciplinary approach will not only improve the model's accuracy but also facilitate its integration into clinical practice.

Conclusion

In this study, the computerized AI-based version of Bosniak classification was mainly achieved. Indeed, these were performed by the common ML method and the trend DL model. According to the ML classification, the accuracy result was obtained as 85% for the kNN algorithm. Indeed, according to the classification with modified DL models, the accuracy result was obtained as for CNN model and the accuracy result was obtained as 98% for VGG16 model. Diagnosing renal cysts at a curable stage has become important and gained importance in the medical area. Also, it was important to reduce the overuse, overdiagnosis procedures, etc. Indeed, the Bosniak classification was generally used to distinguish benign from malignant masses. The CT-based Bosniak classification system can accurately diversify the five diagnosis types, and this system can be achieved with other CRM types. It has good performance metrics, which may facilitate treatment decision-making and is less affected by interobserver disagreements.

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OLGU SUNUMU / *CASE REPORT*

Urrets-Zavalia Syndrome After Penetrating Keratoplasty

Penetran Keratoplasti Sonrası Urrets-Zavalia Sendromu

Mustafa Yildirim[1], Sadullah Keles[2], Betul Gokcek Barutcigil[3]

[1]Department of Ophthalmology, Ataturk University Scholl of Medicine, Erzurum; [2]Department of Ophthalmology, Kecioren Dunyagoz Hospital, 06310, Ankara; [3]Department of Ophthalmology, Health Sciences University, Erzurum Regional Health Application and Research Center, Erzurum, Türkiye

ABSTRACT

Urrets-Zavalia syndrome (UZS) was first described in 1963 as a case of atrophic dilated pupil accompanied by secondary glaucoma, which developed after penetrating keratoplasty (PKP) using mydriatics in patients with keratoconus. Urrets-Zavalia syndrome is a surgical complication in which the pupil remains fixed and dilated after an intraocular surgical intervention. Although it is thought to be especially related to penetrating keratoplasty, it can also be seen after other intraocular surgical procedures (Trabeculectomy, deep anterior lamellar keratoplasty, cataract, goniotomy). The most important risk factor is iris ischemia due to increased intraocular pressure during or after surgery. The study aimed to present the management of Urrets-Zavalia Syndrome, which developed after penetrating keratoplasty in two patients with left eye corneal granular dystrophy and left eye keratoconus.

Key words: *penetrating keratoplasty; Urrest-Zavalia syndrome; keratoconus; corneal dystrophy*

ÖZET

Urrets-Zavalia sendromu (UZS) ilk olarak 1963 yılında keratokonuslu hastalarda midriyatikler kullanılarak yapılan penetran keratoplasti (PKP) sonrası gelişen, sekonder glokomun eşlik ettiği atrofik genişlemiş pupilla olgusu olarak tanımlanmıştır. Urrets-Zavalia sendromu, göz içi cerrahi girişim sonrası pupillanın sabit ve geniş kalmasıyla oluşan bir cerrahi komplikasyondur. Özellikle penetran keratoplasti ile ilişkili olduğu düşünülmekle birlikte diğer göz içi cerrahi girişimlerden sonra da (Trabekülektomi, derin anterior lameller keratoplasti, katarakt, goniotomi) görülebilmektedir. En önemli risk faktörü, ameliyat sırasında veya sonrasında göz içi basıncının artmasına bağlı olarak oluşan iris iskemisidir. Çalışmanın amacı, sol göz kornea granüler distrofisi ve sol göz keratokonusu olan iki hastada penetran keratoplasti sonrası gelişen Urrets-Zavalia Sendromu'nun yönetimini sunmaktır.

Anahtar kelimeler: *penetran keratoplasti, Urrets-Zavalia sendromu, keratokonus, kornea distrofisi*

Introduction

Urrets zavalia syndrome (UZS) was first defined in 1963 as a syndrome in which a dilated pupil is formed with iris atrophy following penetrating keratoplasty[1]. Although this condition was initially thought to occur only in keratoconus patients, it can also be seen after penetrating keratoplasty (PKP), deep anterior lamellar keratoplasty (DALK), descemet stripping endothelial keratoplasty (DSEK), goniotomy, laser iridoplasty, iatrogenic mydriasis and implantation of phakic intraocular lenses[2].

Other features of this syndrome include ectropion uvea, pigment dispersion, iris atrophy, anterior subcapsular lens opacities, and secondary glaucoma. The incidence of urrets-zavalia syndrome after keratoplasty has been reported to be between 2.2% and 17.7%[1].

The etiology of UZS has not been fully elucidated. Pupillary block caused by intraoperative air or gas tamponed is thought to cause increased intraocular pressure and secondary ischemia in the iris, so the fixed dilated pupil can be seen[3]. Intraoperative or postoperative strong mydriatic use, intraoperative iris trauma, parasympathetic nerve injury, argon laser peripheral iridoplasty, and iris-claw phakic intraocular lens implantation may cause the development of UZS[1,3–5].

İletişim/Contact: *Mustafa Yildirim, Department of Ophthalmology, Ataturk University School of Medicine, Erzurum, Türkiye* • ***Tel:*** *0536 243 11 87* • ***E-mail:*** *mustafa.yildirim@atauni.edu.tr* • ***Geliş/Received:*** *12.03.2024* • ***Kabul/Accepted:*** *27.08.2024*

ORCID: *Mustafa Yıldırım: 0000-0002-2706-3592 • Sadullah Keleş: 0000-0002-8568-4899 • Betül Gökçek: 0000-0002-4263-6512*

Trauma to the iris caused by trephines or scissors can result in a fixed dilated pupil[6]. While some surgeons think that the pressure behind the lens during PKP may cause permanent damage to the iris and pupil due to the lens being pushed forward[7], some surgeons think this is not the case[8].

Many studies have reported that fixed and dilated pupil development mostly develops on the penetrating keratoplasty background[1,2,4,7,9]. The reason why this situation occurs more frequently after PKP compared to other operations has not been fully explained. It is thought that the application technique of the surgery performed may cause this. It is thought that iris ischemia may occur in PKP, as iris vessels may be more prone to compression in the area of the incision margin of the host cornea[4].

Since the etiology of UZS is not clearly known, it is not possible to prevent its development. It is evaluated that preoperative use of mannitol, ensuring good anterior chamber stabilization during surgery, avoiding iris trauma, complete clearance of viscoelastic substances, and control of postoperative eye pressure can prevent the development of UZS.

Case 1

A 15-year-old male patient was examined for vision loss in both eyes. In the ophthalmological examination, bilateral vision was evaluated as CF 5 meters. His intraocular pressure was in the normal range. In the slit lamp examination, he had bilateral granular dystrophy in the cornea. Keratoplasty was planned for the patient due to corneal dystrophy. Penetrating keratoplasty was performed on the patient's left eye. On the first day after surgery, we observed that he had a fixed dilated pupil in the left eye. No other abnormalities were detected on examination. The patient was discharged with topical droplets: steroid, antibiotic, and autologous serum. In the following visit, visual acuity in the left eye reached 1/10. Intraocular pressure was normal. The slit lamp examination of the anterior segment was normal, and the number of endothelial cells was 1450 mm^2. In the follow-up visit after 2 months of operation, the fixed dilated pupil on the left eye persisted, and posterior synechiae developed. In the consecutive control examinations, fixed pupil dilatation and posterior synechiae remained. The patient who developed an increase in lens opacity in the 6th month was followed up for 1 year. After 1 year from the keratoplasty, the patient underwent cataract surgery, combined lens extraction and intraocular lens implantation into the capsule. In addition, pupilloplasty surgery is performed for fixed dilated pupils. After the procedures, the visual acuity in the left eye was 2/10, and intraocular pressure was 14 mmHg. The graft was clear, and sutures in the iris were intact. After the cataract surgery, the endothelial cell count was 1280 mm^2. Preoperative and postoperative anterior segment photographs of the patient are shared in the Figure 1.

Case 2

A 25-year-old female patient was examined with the complaint of visual impairment in the right eye. In the ophthalmological examination, the visual acuity was CF 5 meters, and intraocular pressure was 11 mmHg in the right eye. In the slit lamp examination, there was keratoconus in both eyes. Penetrating keratoplasty was performed with the diagnosis of left keratoconus. During the procedure, the hypotonic iris was noticeable. On the first day after surgery, we observed that she had a fixed dilated pupil in the left eye. No other abnormalities were detected on examination. Topical pilocarpine 2% was given to the patient to relieve the mydriasis. The patient was discharged with topical droplets: steroid, antibiotic, and autologous serum. In the first month's visit after penetrating keratoplasty, the visual acuity was CF 5 meters, and IOP was normal. The graft was clear, 10.0 nylon sutures were

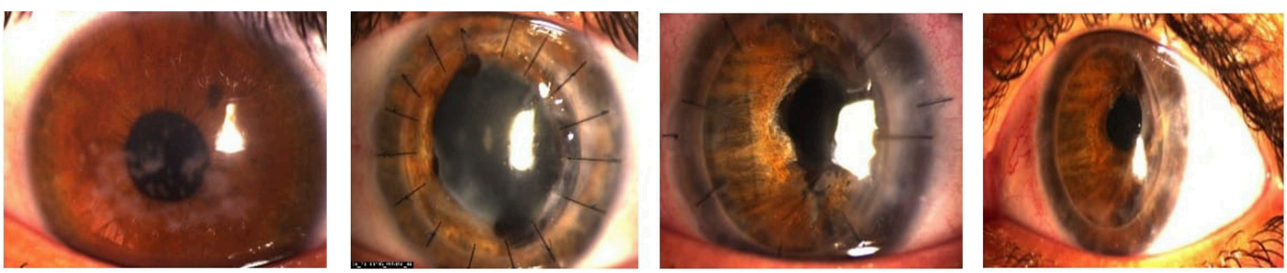

Figure 1. *Preoperative, postoperative 6th month, postoperative cataract and pupilloplasty surgery, the last situation.*

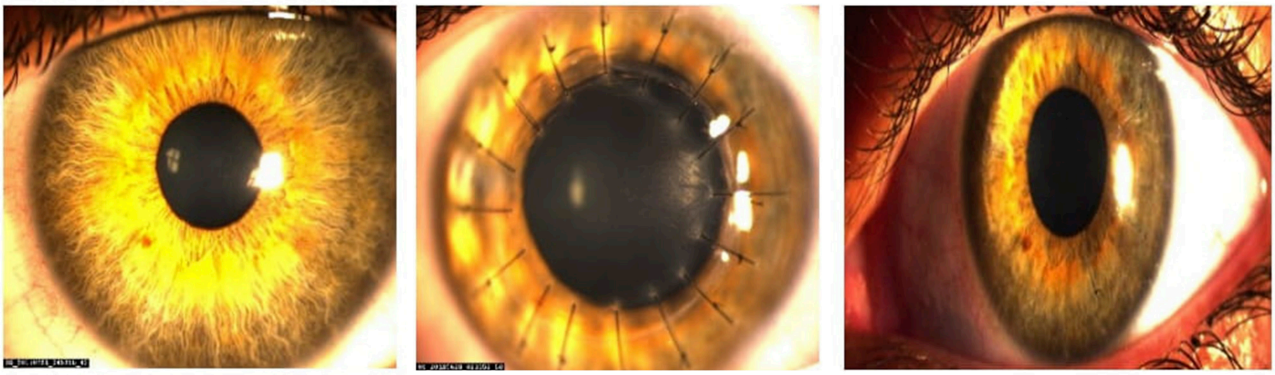

Figure 2. *Preoperative, postoperative 1st day, the last situation.*

intact, and the other structures were normal in the anterior segment evaluation. Apart from this, no additional examination findings were observed, such as posterior synechiae or increased intraocular pressure. Pupil diameter decreased in response to topical pilocarpine treatment. After the 1 year post-op visit, the visual acuity in the left eye was 2/10, and the IOP was 16 mmHg. In the slit lamp examination, the graft was clear, and the pupil size was normal. Other structures were evaluated as normal. The number of endothelial cells was 1750 mm^2. Preoperative and postoperative anterior segment photographs of the patient are shared in the Figure 2. Informed consent forms were obtained from both cases.

Discussion

Although UZS was initially thought to develop only after penetrating keratoplasty in keratoconus patients, it can also be seen after deep anterior lamellar keratoplasty, descement membrane endothelial keratoplasty, goniotomy, laser iridoplasty, iatrogenic mydriasis and phakic intraocular lens implantation[2]. Considering that most of the cases in which UZS developed were phakic, it can be better understood why the cases mostly developed after keratoplasty[10]. Although intraoperative or postoperative intraocular pressure elevation has been confirmed in almost all cases, no clear way to control this situation has been demonstrated[11]. Iridectomy and using hyperosmolar agents have been noted as preventive measures against UZS[2,12]. The frequency of surgical interventions has been reported as 51.8% in PKP, 18.1% in DALK, 8.2% in DSAEK, 8.2% in cataract, 1.9% in trabeculectomy and 0.9% after goniotomy[13,14]. In the cases in which UZS was reported, 42.5% was accompanied by keratoconus, 23.7% stromal dystrophy, 9.4% Fuch's dystrophy, 8.5% plateau-iris syndrome, 8.5% senile cataract, 1.9% primary open-angle glaucoma, 1.9% high myopia, 0.9% congenital myopia[14].

Various mechanisms have been proposed for the etiology of UZS. Urrets-Zavali suggested that the use of atropine during surgery causes the iris to contact the peripheral cornea, leading to peripheral anterior synechiae and secondary glaucoma1. However, a study by Geyer et al. in 1991 concluded that the occurrence of UZS after keratoplasty was unrelated to atropine[15].

Sharif and Casey reviewed data from 100 patients who underwent PKP for keratoconus[16]. They concluded that the incidence of UZS decreased from 4% to 1.5% after a preoperative intravenous injection of 20% mannitol. They noted that the hypertonic solution effectively reduced vitreous volume and compressed the iris.

Although Urrets-Zavalia syndrome is a well-defined clinical entity, it is not considered a preventable condition because its predisposing factors have not been demonstrated. When it develops, there is no specific treatment. However, miotic agents and pupillary reconstruction are used as treatment alternatives. Fixed dilated pupils, which appeared immediately after penetrating keratoplasty, were observed in the patients included in the study. In one of the patients, cataract surgery was performed due to lens opacity, and pupilloplasty was performed in the same session. Another patient's response to pilocarpine was considerable.

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