e-ISSN: 2147-2092

GAZI MEDICAL JOURNAL

2024
July
Volume 35 • Issue 3

galenos yayınevi

medicaljournal.gazi.edu.tr

GAZI MEDICAL JOURNAL

medicaljournal.gazi.edu.tr

Editorial Team

GAZI MEDICAL JOURNAL

medicaljournal.gazi.edu.tr

Please refer to the journal's webpage (https://medicaljournal.gazi.edu.tr/) for "About the Journal" and "Submissions".

The editorial and publication process of Gazi Medical Journal are shaped in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing.

Gazi Medical Journal is indexed in Emerging Sources Citation Index, Scopus, Directory of Open Access Journals, EuroPub, Islamic World Science Citation Center, ABCD Index.

The online published articles are freely available on the public internet.

Owner: Musa Yıldız on Behalf of Gazi University

Responsible Manager: Mehmet Ali Ergün

Publisher Contact
Address: Molla Gürani Mah. Kaçamak Sk. No: 21/1 34093 İstanbul, Türkiye
Phone: +90 (539) 307 32 03

E-mail: info@galenos.com.tr/yayin@galenos.com.tr
Web: www.galenos.com.tr
Publisher Certificate Number: 14521

Publication Date: July 2024
e-ISSN: 2147-2092
International scientific journal published quarterly.

GAZI MEDICAL JOURNAL

medicaljournal.gazi.edu.tr

CONTENTS

Original Investigations - Özgün Araştırmalar

GAZI MEDICAL JOURNAL

medicaljournal.gazi.edu.tr

CONTENTS

GAZI MEDICAL JOURNAL

medicaljournal.gazi.edu.tr

CONTENTS

Literature Review With Cases - Vakalarla Literatür İncelemesi

GMJ 2024;35:224-227

DOI: http://dx.doi.org/10.12996/gmj.2024.2972

Receptor Status Differences in Prognosis for Breast Cancer

Meme Kanseri Prognozunda Reseptör Durumu Farklılıkları

Yelda Deligöz Bildacı[1], Deniz Yamaç[2], Uğur Coşkun[3]

[1]Department of Internal Diseases, Dokuz Eylül University Faculty of Medicine, İzmir, Türkiye
[2]Güven Hospital, Ankara, Türkiye
[3]Department of Internal Diseases, Gazi University Faculty of Medicine, Ankara, Türkiye

ABSTRACT

Objective: Breast cancer is a type of cancer that originates in breast tissue cells. It is the most common cancer type in the world after lung cancer. The prognosis of the disease mostly depends on the type and stage of cancer. One of the worst prognoses is seen in a specific type called Triple-negative breast cancer (TNBC), which represents not having any of the three most recognized receptors, namely estrogen, progesterone, and c-erb2 receptors. Our objective was to determine the difference in overall and disease-free survival for breast cancer types categorized by receptor status.

Methods: This is a retrospective matched case-control study with breast cancer patients of two types. A total of 102 patients were divided equally into having TNBC of 51 patients in one arm and triple-positive breast cancer (TPBC) of 51 patients in the other arm. Analyses were run for disease prognostic values and patients' demographic values.

Results: Disease free survival were 63±10.6 months and 93.2±4.9 months in the fifth year for the TNBC and TPBC groups, respectively. (p=0.004) Overall survival was significantly different as 73.9±7.3 months for TNBC and 97.7±2.3 months for TPBC (p=0.002).

Conclusion: TNBC prognosis is worse than that of other breast cancer types. The most important reason is being unable to use hormonal treatment because of the receptor status, and a disease-specific targeted treatment could not have been developed so far. Therefore, it is necessary to identify new molecular targets and develop treatments for them.

Keywords: Breast cancer, estrogen receptor, progesterone receptor, HER2

ÖZ

Amaç: Meme kanseri, meme dokusu hücrelerinden kaynaklanan bir kanser türüdür. Dünyada akciğer kanserinden sonra en sık görülen kanser türüdür. Hastalığın prognozu çoğunlukla kanserin türüne ve evresine bağlıdır. En kötü prognozlardan biri, Triple-negatif meme kanseri (TNBC) adı verilen spesifik bir türde görülür; bu, en çok tanınan üç reseptörden (östrojen, progesteron ve c-erb2 reseptörleri) herhangi birinin bulunmadığını temsil eder. Amacımız, reseptör durumuna göre kategorize edilen meme kanseri türleri için genel ve hastalıksız sağkalımdaki farkı belirlemekti.

Yöntemler: Bu, iki tipteki meme kanseri hastalarıyla yapılan retrospektif, eşleştirilmiş bir olgu kontrol çalışmasıdır. Toplam 102 hasta, bir koldaki 51 hastanın TNBC'ye ve diğer koldaki 51 hastanın Triple-pozitif meme kanserine (TPBC) sahip olması şeklinde eşit olarak bölünmüştür. Analizler hastalığın prognostik değerleri ve hastaların demografik değerleri için yapıldı.

Bulgular: Hastalıksız sağkalım beşinci yılda TNBC ve TPBC grupları için sırasıyla 63±10,6 ay ve 93,2±4,9 ay idi (p=0,004). Genel sağkalım süresi TNBC için 73,9±7,3 ay, TPBC için 97,7±2,3 ay olarak anlamlı farklılık gösterdi (p=0,002).

Sonuç: TNBC prognozu diğer meme kanseri türlerine göre daha kötüdür. En önemli nedeni reseptör durumu nedeniyle hormonal tedavinin uygulanamaması ve bugüne kadar hastalığa özgü hedefe yönelik bir tedavinin geliştirilememiş olmasıdır. Bu nedenle yeni moleküler hedeflerin belirlenmesi ve bunlara yönelik tedavilerin geliştirilmesi gerekmektedir.

Anahtar Sözcükler: Meme kanseri, östrojen reseptörü, progesteron reseptörü, HER2

Address for Correspondence/Yazışma Adresi: Yelda Deligöz Bildacı, MD, Department of Internal Diseases, Gazi University Faculty of Medicine, Ankara, Türkiye
E-mail / E-posta: yeldadeligoz@gmail.com
ORCID ID: orcid.org/0000-0001-9888-995X

Received/Geliş Tarihi: 20.01.2021
Accepted/Kabul Tarihi: 25.01.2024

INTRODUCTION

Breast cancer is the most common type of cancer among women in Türkiye (1). Prognosis varies because of the biological diversity of the disease, individual differences in the course of the disease, and as a result, the applied treatments differ from each other. Estrogen receptors (ER) are a group of proteins responding to 17β-estradiol with two subtypes as α and β. ERα has been shown to be overexpressed in breast cancer cells (2). Similar to ER, the progesterone receptor (PR) has two isoform, PR-A and PR-B, which reside inside the cells responding to progesterone hormone with steroid structure (3). All cells, including breast epithelial cells, carry two copies of the epithelial growth factor receptor-2 gene (also known as the *HER2* or *c-erbB2* gene). It has been shown that 20-25% of breast cancers carry multiple copies of this gene. HER2-overexpressing tumors are prone to early metastasis and have a poor prognosis (4).

Triple-negative breast cancers (TNBC) are tumors that are negative for estrogen and PRs and HER2. About 10-20% of breast cancer cases are in the TNBC subtype (5). In comparison with hormonal receptor-expressing breast cancer, prognosis is relatively worse and overall survival time. This is because they have a tendency toward visceral metastasis compared with receptor-positive subtypes (6-8). Another reason for the poor prognosis of TNBC is that there is no precise, targeted, proven efficient treatment modality. While there are many agents currently used in cases of ER- and PR-positive breast cancer, new molecules need to be identified in TNBC.

In this study, which was conducted in a single oncology department, our aim was to compare the overall survival parameters of two groups which were consisting of TNBC patients and triple-positive breast cancer patients.

MATERIALS AND METHODS

This study was approved by the Gazi University Clinical Research Ethics Committee (approval number: 254, date: 29.06.2011).

This retrospective matched case-control study was conducted in the Gazi University Faculty of Medicine, Department of Internal Disease with cases followed up at the oncology outpatient clinic from 2001 to 2011. Each study group included 51 patients. Group A comprised TNBC patients, and the other arm (group B) comprised ER, PR, and HER2-positive patients (TPBC). As indicated in the reference study, cases with receptor expression values below 10% for each receptor type and those exhibiting negative immunostaining for HER2 during the initial pathological evaluation were categorized as group A (9). Conversely, cases with receptor expression values exceeding 10% for ER and PR, along with positive immunostaining for HER2, were classified as group B.

The American Joint Committee on Cancer 2003 TNM classification and Scarf-Bloom-Richardson staging system were used for consecutively staging and grading disease. Nottingham Prognostic Index (NPI): 0.2 tumor size (biggest measured diameter) + nodal status [with negative axillary node (1), 1-3 positive node (2), ≥4 positive node (3)] + tumor grade; was used for numeration of prognostic differences for statistical purposes. NPI less than 3.4, between 3.4-5.4 and more than 5.4 were assumed as good, moderate, and bad prognosis, respectively.

Patients were treated according to the up-to-date guidelines for adjuvant, neo-adjuvant, targeted therapy, and radiation therapy according to their initial stages. Patients with metastatic disease and recurrent disease were put on palliative chemotherapy for further treatments.

Statistical Analysis

SPSS program (Released 2008. SPSS Statistics for Windows, version 17.0. Chicago) was used for statistical analyses. Chi-square, Kaplan-Meier survival analysis, and log-rank test were used when appropriate.

RESULTS

Demographics and histopathology: The median age of 102 patients' median age were found as 54.0±12.9 without any significant difference between the two groups (55.1±13.9 and 54.4±12.0 consecutively for group A and B, p=0.772). The characteristics of patients are compared in Table 1.

When histological types were examined, 92.2% (n= 94) of the cases were infiltrative ductal carcinoma, 1% (n=1) were papillary, 1% (n=1) spindle, 2% (n=2) were medullary, 2% (n=2) were mucinous, 1% (n=1) were lobular, and 1% (n=1) were mixed carcinoma. Spindle and medullary breast cancer cases were found to be TNBC, and all papillary, lobular, and mixed types were TPBC.

The mean follow-up time of the cases was 40.97±25.22 months (range; 2-117 months). It was found that 21.6% (n=11) of TNBC cases were diagnosed at the metastatic stage (two of them were diagnosed on initial staging), while 3.9% (n=2) of TPBC cases were detected with metastasis during follow-up. The incidence of metastatic disease progression in group A was higher than that in group B (p=0.008).

Table 1. Characteristic properties of patients

		TNBC-group A	TPBC-group B	Difference (p)
Age (mean)		55.1±13.9	54.4±12.0	0.772
Gender	Female	49	49	NS
	Male	2	2	NS
Menopausal status	Premenopausal	23	23	NS
	Post menopause	26	26	NS
Initial NPI status	Good	4	13	**0.04**
	Moderate	29	25	
	Bad	18	13	
Initial staging	Stage 1	3	14	0.071
	Stage 2	38	26	
	Stage 3	8	11	
	Stage 4	2	0	
Tumor grade (mean)		2.71±0.50	2.2±0.66	**0.000**

Significant differences are marked in bold. NS: Not significant, TNBC: Triple-negative breast cancer, TPBC: Triple-positive breast cancer, NPI: Nottingham Prognostic Index.

When NPI scores were taken into consideration, for the TNBC group, 1 out of 4 patients scored as having a good prognosis, 7 out of 29 patients scored as having a moderate prognosis, and 3 out of 18 patients scored as having a bad prognosis came up with disease recurrence. On the other hand, for the TPBC group, 0 out of 13 patients scored as having a good prognosis, 1 out of 24 patients scored as having a moderate prognosis, and 1 out of 13 patients scored as having a bad prognosis came up with disease recurrence. In comparison of NPI status in connection with disease recurrence, no significant correlation was found between groups A and B (p=0.675 and p=0.60, respectively).

It was observed that 81.8% of recurrences developed in the first 3 years. Disease-free survival rates were 95.9±2.9 months, 86.7±5.1 months, 80.7±6.3 months, 70.9±8.5 and 63±10.6 respectively for the first 5 years of the TNBC group. On the other hand, for TPBC patients, the 5-year disease-free survival was 93.2±4.9 months. When both groups were compared, disease-free survival was observed to be lower in patients with TNBC (Figure 1) (p=0.004).

The overall survival of group A is 94.0±13.4 months, 81.1±5.7 months, 78.6±6.1 months, and 73.9±7.3 months for the first, second, third, and fourth years consecutively. There were deaths recorded for 3 patients in the 1st year, 6 patients in the 2nd year, 1 patient in the 3rd year, and 1 patient in the 4th year. The overall survival of group B patients for 5 years was 97.7±2.3 months. Only one patient lost her life in the 18th month of follow-up. Overall survival of TNBC was significantly lower than that of TPBC (p=0.002) (Figure 2).

DISCUSSION

Breast cancer is a heterogeneous and complex disease about biological behavior, response to treatment, and prognosis. This prognostic information for each individual patient is based on the analysis of biological markers in the primary tumor, including ER, PGR, HER-2 NEU, and Ki67 (10) together with age, tumor size, histological grade, and lymph node involvement. As mentioned before in this article, TPBCs derive benefit from hormonal therapy and targeted therapy, while to target TNBC patients, there are limited therapeutic options. Perou et al. (11) also subdivided TNBC immunohistochemically as basal-like breast cancer, which is mostly studied in the TNBC group. The reason for TNBC to be foregrounded compared with other breast cancer subtypes is that it has a worse prognosis and targeted treatments can not be applied in this breast cancer subtype as a factor contributing to its poor prognosis (11).

Results from numerous studies have shown that TNBC is characterized by a high morphological and core-cytoplasm ratio (12,13). In some observational studies, TNBC was reported to be diagnosed in a younger patient population with an advanced stage of disease and predominantly higher tumor grade (14,15). In our study, we found no significant difference between the two groups according to age. However, the findings matched those of Jack et al. (14) TNBC is more likely diagnosed with advanced stage (p=0.071) and predominantly with higher tumor grade (p=0.001).

Many studies have reported that TNBC patients have poor prognoses compared with other breast cancer subtypes using The NPI, which has also been used for studies conducted later (16). Although our results showed a difference between initial NPI scores, we could not show a significant difference between NPI prognostic criteria scores and disease recurrence for both groups. A possible explanation for this could be the short follow-up period with a lower number of patients.

In a study by Carey et al. (17), it was found that disease-free survival and overall survival of TNBC cases were lower than those of other breast cancer groups. In addition, this patient group had recurrences that were observed mostly in the first 3 years, compared to later periods seen in TPBC patients (17). We observed similar findings as significantly lower disease-free survival in TNBC patients compared with TPBC patients. In addition, it was determined that 81.8% of the relapses observed in group A developed in the first 3 years in accordance with the literature. We also found that overall survival was lower in the TNBC group than in the TPBC group.

Study Limitations

The limitations, including its retrospective design, short follow-up, and lesser number of patients.

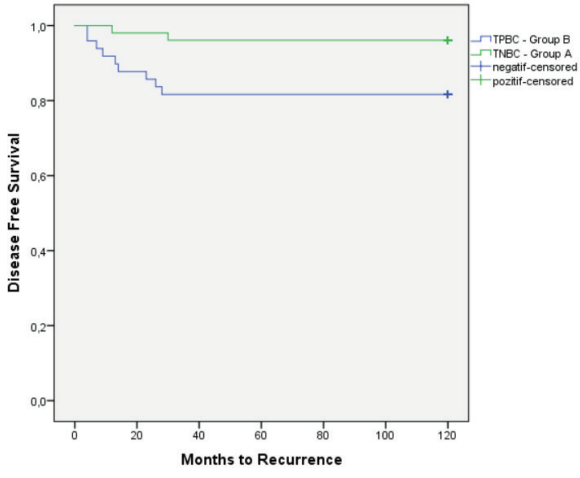


Figure 1. Disease-free survival of the groups.

TNBC: Triple-negative breast cancer, TPBC: Triple-positive breast cancer.

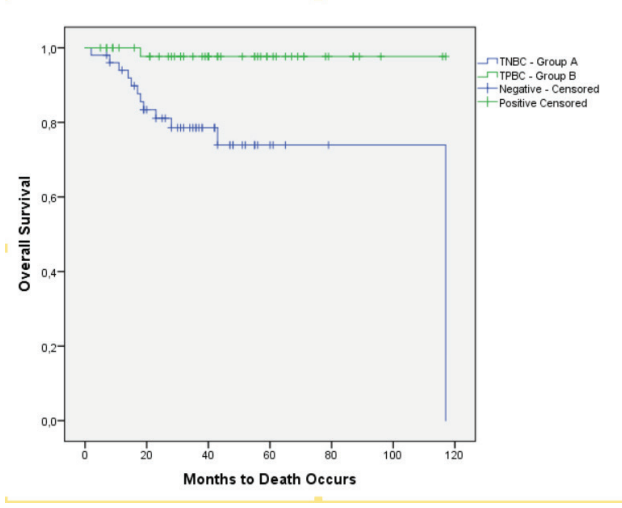


Figure 2. Overall survival analysis of both groups.

TNBC: Triple-negative breast cancer, TPBC: Triple-positive breast cancer.

CONCLUSION

Even with the limitations, including its retrospective design, short follow-up, and lesser number of patients, our results are in accordance with those of published literature and point toward the aggressive nature of TNBC as well as superior outcome of TPBC patients.

Therefore, it is necessary to identify new molecular targets and develop treatments for them.

Ethics

Ethics Committee Approval: This study was approved by the Gazi University Clinical Research Ethics Committee (approval number: 254, date: 29.06.2011).

Informed Consent: Retrospective study.

Authorship Contributions

Surgical and Medical Practices: D.Y., U.C., Concept: Y.D.B., Design: Y.D.B., U.C., Data Collection or Processing: Y.D.B., D.Y., U.C., Analysis or Interpretation: Y.D.B., D.Y., Literature Search: Y.D.B., Writing: Y.D.B.

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

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

REFERENCES

1. Cancer Statistics. In: Health RoTMo, editor. 2017.
2. Deroo BJ, Korach KS. Estrogen receptors and human disease. J Clin Invest. 2006; 116: 561-70.
3. Kastner P, Krust A, Turcotte B, Stropp U, Tora L, Gronemeyer H, et al. Two distinct estrogen-regulated promoters generate transcripts encoding the two functionally different human progesterone receptor forms A and B. EMBO J. 1990; 9: 1603-14.
4. Casciato DA TM. Breast Cancer. Manual of Clinical Oncology 6th ed. Lippincott Williams & Wilkins; 2009:237-65.
5. Cheang MC, Voduc D, Bajdik C, Leung S, McKinney S, Chia SK, et al. Basal-like breast cancer defined by five biomarkers has superior prognostic value than triple-negative phenotype. Clin Cancer Res. 2008; 14: 1368-76.
6. Liedtke C, Mazouni C, Hess KR, André F, Tordai A, Mejia JA, et al. Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. J Clin Oncol. 2008; 26: 1275-81.
7. Nielsen TO, Hsu FD, Jensen K, Cheang M, Karaca G, Hu Z, et al. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res. 2004; 10: 5367-74.
8. Sorlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci U S A. 2003; 100: 8418-23.
9. Yi M, Huo L, Koenig KB, Mittendorf EA, Meric-Bernstam F, Kuerer HM, et al. Which threshold for ER positivity? a retrospective study based on 9639 patients. Ann Oncol. 2014; 25: 1004-11.
10. Nolè F, Crivellari D, Mattioli R, Pinotti G, Foa P, Verri E, et al. Phase II study of an all-oral combination of vinorelbine with capecitabine in patients with metastatic breast cancer. Cancer Chemother Pharmacol. 2009; 64: 673-80.
11. Perou CM, Sørlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, et al. Molecular portraits of human breast tumours. Nature. 2000; 406: 747-52.
12. Livasy CA, Karaca G, Nanda R, Tretiakova MS, Olopade OI, Moore DT, et al. Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. Mod Pathol. 2006; 19: 264-71.
13. Fulford LG, Easton DF, Reis-Filho JS, Sofronis A, Gillett CE, Lakhani SR, et al. Specific morphological features predictive for the basal phenotype in grade 3 invasive ductal carcinoma of breast. Histopathology. 2006; 49: 22-34.
14. Jack RH, Davies EA, Renshaw C, Tutt A, Grocock MJ, Coupland VH, et al. Differences in breast cancer hormone receptor status in ethnic groups: a London population. Eur J Cancer. 2013; 49: 696-702.
15. Sajid MT, Ahmed M, Azhar M, Mustafa QU, Shukr I, Ahmed M, et al. Age-related frequency of triple negative breast cancer in women. J Coll Physicians Surg Pak. 2014; 24: 400-3.
16. Lee AH, Ellis IO. The Nottingham prognostic index for invasive carcinoma of the breast. Pathol Oncol Res. 2008; 14: 113-5.
17. Carey LA, Perou CM, Livasy CA, Dressler LG, Cowan D, Conway K, et al. Race, breast cancer subtypes, and survival in the Carolina Breast Cancer Study. JAMA. 2006; 295: 2492-502.

GMJ 2024;35:228-235

DOI: http://dx.doi.org/10.12996/gmj.2023.3813

Comparison of Chest Computed Tomography Findings in Patients with H1N1 and Coronavirus Disease-2019 Pneumonia

H1N1 ve Koronavirüs Hastalığı-2019 Pnömonisi Olan Hastalarda Akciğer Bilgisayarlı Tomografi Bulgularının Karşılaştırılması

Sadullah Şimşek[1], Cihan Akgül Özmen[2]

[1]Department of Radiology, Nusaybin State Hospital, Mardin, Türkiye
[2]Department of Radiology, Dicle University Faculty of Medicine, Diyarbakır, Türkiye

ABSTRACT

Objective: In this study, we compared the differences between high-resolution computed tomography (CT) features of two types of viral pneumonia associated with H1N1 virus and coronavirus disease-2019 (COVID-19).

Methods: A total of 25 patients with H1N1 pneumonia were compared with 150 patients with COVID-19 pneumonia. The findings were analyzed by IBM SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, N.Y., USA). CT findings were compared between groups by chi-square test, and scale variables were compared using the t-test. Finally, significant findings for the H1N1 or COVID-19 groups were evaluated by logistic regression analysis.

Results: The median age of COVID-19 pneumonia patients was 54.6±15.9 years, and the median age of H1N1 pneumonia patients was 46.2±12.4 years (p<0.01). H1N1 pneumonia patients were younger than COVID-19 pneumonia patients. Based on the distribution pattern, diffuse pattern, peripheral pattern, and bronchopulmonary infiltration were observed more frequently in COVID-19 pneumonia (46.0%, 80.7%, 49.3%, respectively, p<0.05). Mediastinal lymphadenopathy and pleural effusion were observed more frequently in patients with H1N1 pneumonia (24% and 36%, respectively, p<0.05). Logistic regression analysis demonstrated that advanced age and a diffuse-peripheral pattern indicated COVID-19 (p=0.031, p=0.029).

Conclusion: Most of the lesions caused by COVID-19 pneumonia are located in the peripheral region and near the pleura, whereas those caused by influenza virus pneumonia are far from the peripheral region.

Keywords: COVID-19, pneumonia, CT, influenza virus, coronavirus

ÖZ

Amaç: Bu çalışmada H1N1 virüsü ve koronavirüs hastalığı-2019 (COVID-19) ile ilişkili iki tip viral pnömoninin yüksek çözünürlüklü bilgisayarlı tomografi (BT) özellikleri arasındaki farkları karşılaştırmayı amaçladık.

Yöntemler: H1N1 pnömonisi olan toplam 25 hasta, COVID-19 pnömonisi olan 150 hasta ile karşılaştırıldı. Bulgular, IBM SPSS Statistics for Windows, sürüm 22 (IBM Corp., Armonk, N.Y., ABD) tarafından analiz edildi. BT bulguları gruplar arasında ki-kare testi ile ölçek değişkenleri ise t-testi kullanılarak karşılaştırıldı. Son olarak; H1N1 veya COVID-19 grupları için anlamlı olan bulgular lojistik regresyon analizi ile değerlendirildi.

Bulgular: COVID-19 pnömoni hastalarının medyan yaşı 54,6±15,9 ve H1N1 pnömoni hastalarının medyan yaşı 46,2±12,4 idi (p<0,01). H1N1 pnömoni hastaları, COVID-19 pnömoni hastalarından daha gençti. Dağılım paternine göre, COVID-19 pnömonisinde yaygın patern, periferik patern ve bronkopulmoner infiltrasyon daha sık görülür (sırasıyla, %46,0, %80,7, %49,3, p<0,05). H1N1 pnömonisinde mediastinal lenfadenopati ve plevral efüzyon daha sık gözlendi (sırasıyla, %24 ve %36; p<0,05). Lojistik regresyon analizi, ileri yaş ve yaygın periferik paternin COVID-19 ile anlamlı ilişkisini destekledi (p=0,031 ve p=0,029).

Sonuç: COVID-19 pnömonisinin neden olduğu lezyonların çoğu periferik bölgede ve plevraya yakın yerleşimlidir ve influenza virüsü pnömonisinin neden olduğu lezyonlar periferik bölgeden uzaktır.

Anahtar Sözcükler: COVID-19, pnömoni, BT, influenza virüs, koronavirüs

Address for Correspondence/Yazışma Adresi: Sadullah Şimşek, MD, Department of Radiology, Nusaybin State Hospital, Mardin, Türkiye
E-mail / E-posta: sadullahsimsek@gmail.com
ORCID ID: orcid.org/0000-0002-8322-7475

Received/Geliş Tarihi: 18.02.2023
Accepted/Kabul Tarihi: 02.06.2023

INTRODUCTION

A novel coronavirus known as coronavirus disease-2019 (COVID-19) was identified in lower respiratory tract samples from multiple patients in China, and this virus emerged during an epidemic caused by severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) (1). The World Health Organization (WHO) classified this outbreak as an international public health emergency on January 30, 2020, and declared it a global pandemic on March 11, 2020 (2). Therefore, early detection, diagnosis, and isolation of COVID-19 patients are very important. The reverse transcriptase-polymerase chain reaction (RT-PCR) is the reference test used to confirm the diagnosis of COVID-19 infection. Recently, a large number of false-negative RT-PCR results have been reported (3). These results make diagnosis very difficult. Therefore, computed tomography (CT) of the chest is one of the best tools for primary diagnosis, estimation of disease severity, and monitoring of treatment. CT Manifestations are variable and may present as ground glass opacities (GGO), consolidation or combinations, crazy paving signs, interlobular septal thickening, air bronchogram, fibrous strictures, pleural effusions, and mediastinal lymphadenopathy. Most lesions are located in the peripheral subpleural region, mainly in the posterior or inferior lobe (4).

In spring 2009, several cases of human-to-human transmission by a subtype known as H1N1 were documented. By the end of May 2009, 41 countries had reported 11,000 cases and 85 deaths (5). Consequently, a level 6 pandemic was declared by the WHO. Clinical symptoms are mild in the majority of patients, with a flu-like illness (6). However, a small percentage of patients with H1N1 viral pneumonia may develop acute respiratory distress syndrome or severe cardiopulmonary failure. CT is an important tool for primary diagnosis, evaluation of complications that may develop after treatment, and determination of the degree of pulmonary disease (7). The radiologic findings reported were unilateral or bilateral GGO, either associated with focal or multifocal areas of consolidation or not. GGO and areas of consolidation have a predominantly bronchovascular and subpleural distribution, similar to organizing pneumonia (8,9). Recognition and analysis of these well-defined signs or patterns of involvement in chest CT are important because they can be treated at an early stage of the disease. In this study, we compared the differences between the high-resolution CT features of two types of viral pneumonia associated with H1N1 virus and COVID-19.

MATERIALS AND METHODS

The study was designed retrospectively. The study protocol was approved by the Mardin Provincial Health Directorate Scientific Study Board (approval number: E-37201737-949). Data search was performed to identify H1N1 and COVID-19 patients using the hospital's electronic archive system. For the identification of H1N1 virus infection, a specific period was set up from December 2010 to February 2013. Finally, 25 patients with PCR-confirmed H1N1 virus infection with chest CT were included in the study. In addition, the archive was scanned for patients with COVID-19 pneumonia between 1 July and 1 August 2020, and 150 patients were randomly selected COVID-19 patients with positive PCR results were included in the study. Patients with decompensated heart failure and lung malignancy were excluded from the study. Chest CT scans were obtained and re-evaluated using the hospital's Picture Archiving and Communication Systems.

CT images were obtained using 64-multidetector computed tomography (MDCT) scanners Brilliance Circular Edition (Philips) and 256-MDCT Somatom Drive (Siemens Healthineers), with the use of standard tube voltage and tube current settings. While in the supine position, patients underwent CT without IV contrast medium but with suspended end-inspiration. After contiguous 8 or 10 mm chest CT sections were obtained, images were reconstructed with a slice thickness of 1.00 mm. All images were photographed at window levels appropriate for the lung parenchyma (width, 1200 HU; level, -600 HU) and mediastinum (width, 360 HU; level, 55 HU).

CT images were re-evaluated sequentially and independently by two radiologists with 10 and 15 years of experience. CT findings were recorded as present or absent on images with a slice thickness of 1.00 mm. The imaging findings that we evaluated included ground-glass opacity, consolidation, involved lung lobes(which?), subpleural line, crazy paving sign, diffuse distribution (involved five lobes), peripheral or bronchovascular distribution, bronchial wall thickening, air bronchogram, halo sign, nodular appearance lymphadenopathy (short-axis diameter >1 cm), and pleural effusion (10,11).

Statistical Analysis

The findings were analyzed by IBM SPSS Statistics for Windows, version 22 (IBM Corp., Armonk, N.Y., USA). CT findings were compared between groups by chi-square rest, and scale variables were compared using the t-test. Finally, significant findings for the H1N1 or COVID-19 groups were evaluated by logistic regression analysis.

RESULTS

A total of 25 patients with H1N1 pneumonia were compared with 150 patients with COVID-19 pneumonia. The median age of COVID-19 pneumonia patients was 54.6±15.9 years, and the median age of H1N1 pneumonia patients was 46.2±12.4 years ($p<0.01$). H1N1 pneumonia patients were younger than COVID-19 pneumonia patients. Findings in all patients are listed in Table 1. The lower lobes were most commonly affected in both lungs. The most frequently observed finding was GGO areas (85.7%). The least frequently observed finding was pleural effusion (10.9%). Of all patients, 110 (62.9%) were male and 65 (37.1%) were female. The distribution between gender and pulmonary findings is shown in Table 2. There was no association between gender and the distribution of pulmonary findings ($p>0.05$). Comparisons of the CT characteristics of H1N1 pneumonia and COVID-19 pneumonia are shown in Table 3. GGO (Figure 1) and consolidation (Figure 2) were present in both H1N1 pneumonia and COVID-19 pneumonia, and no significant difference was observed ($p=0.794$ and $p=0.119$, respectively). The most common site of involvement in COVID-19 and H1N1 pneumonia is the lower lobe of the lung. Based on the distribution pattern, diffuse pattern, peripheral pattern, and bronchopulmonary infiltration were observed more frequently in COVID-19 pneumonia (46.0%, 80.7%, 49.3%, respectively, $p<0.05$). Mediastinal lymphadenopathy and pleural effusion (Figure 3) were observed more frequently in patients with H1N1 pneumonia (24% and 36%, respectively, $p<0.05$). Nodular involvement, crazy paving sign (Figure 4), GGO,

Table 1. Overall summary of the findings

		n	%
Gender	Male	110	62.9%
	Female	65	37.1%
Right upper lobe		111	63.4%
Right middle lobe		112	64.0%
Right lower lobe		146	83.4%
Left upper lobe		112	64.0%
Left lower lobe		151	86.3%
Ground-glass opacities		150	85.7%
Consolidation		115	65.7%
Diffuse pattern		71	40.6%
Peripheral pattern		136	77.7%
Bronchovascular distribution		81	46.3%
Crazy paving sign		54	30.9%
Nodular consolidation		50	28.6%
Air bronchogram		58	33.1%
Halo sign		37	21.1%
Bronchial thickening		25	14.3%
Pleural effusion		19	10.9%
Mediatinal lymphadenopathy		22	12.6%
Subpleural line		56	32.0%
Study groups	COVID-19	150	85.7%
	H1N1	25	14.3%

COVID-19: Coronavirus disease-2019.

consolidation, subpleural line, bronchial wall thickening, halo sign, and air bronchogram did not differ between patients with H1N1 pneumonia and patients with COVID-19 pneumonia (p>0.05). According to logistic regression analysis, advanced age and the presence of a diffuse-peripheral pattern indicated COVID-19. The rate of pleural effusions and mediastinal lymphadenopathy is higher with H1N1 involvement. The distinguishing characteristics for COVID-19 and H1N1 pneumonia according to multivariate analysis are shown in Table 4.

DISCUSSION

The first cases of COVID-19 pneumonia occurred in December 2019 in Wuhan, China. This virus is transmitted from person to person and has caused a pandemic. Because viral pneumonia can be caused by SARS-CoV-2 and H1N1 virus infections, we sought to determine whether there were differences in pneumonia caused by these viruses. The results of the present study showed that mediastinal lymphadenopathy and pleural effusion were more common in patients with H1N1 pneumonia, whereas peripheral and diffuse patterns (involving five lobes) were more common in patients with COVID-19 pneumonia. There were no significant differences in other CT findings. Among the findings of this study, we show that the mean age of COVID-19 pneumonia patients is younger than that of H1N1 pneumonia patients. This finding is consistent with previous studies (11-14).

In our study, peripherally located GGO was observed in 80.7% of patients with COVID-19 pneumonia. Similarly, Song et al. (15) reported a rate of 86% and Xu et al. (16) reported peripheral GGO with a rate of 96.4% in the literature.

Table 2. Distribution of findings according to gender

		Gender				p
		Male		Female		
		n	%	n	%	
Ground-glass opacities	No	13	11.8%	12	18.5%	0.225
	Yes	97	88.2%	53	81.5%	
Consolidation	No	38	34.5%	22	33.8%	0.925
	Yes	72	65.5%	43	66.2%	
Right upper lobe	No involvement	43	39.1%	21	32.3%	0.368
	Involvement	67	60.9%	44	67.7%	
Right middle lobe	No involvement	40	36.4%	23	35.4%	0.896
	Involvement	70	63.6%	42	64.6%	
Right lower lobe	No involvement	19	17.3%	10	15.4%	0.745
	Involvement	91	82.7%	55	84.6%	
Left upper lobe	No involvement	42	38.2%	21	32.3%	0.434
	Involvement	68	61.8%	44	67.7%	
Left lower lobe	No involvement	18	16.4%	6	9.2%	0.185
	Involvement	92	83.6%	59	90.8%	
Diffuse pattern	No	66	60.0%	38	58.5%	0.841
	Yes	44	40.0%	27	41.5%	

Table 2. Continued

		Gender				p
		Male		Female		
		n	%	n	%	
Peripheral pattern	No	27	24.5%	12	18.5%	0.350
	Yes	83	75.5%	53	81.5%	
Bronchovascular distribution	No	60	54.5%	34	52.3%	0.774
	Yes	50	45.5%	31	47.7%	
Crazy paving sign	No	72	65.5%	49	75.4%	0.169
	Yes	38	34.5%	16	24.6%	
Nodular consolidation	No	79	71.8%	46	70.8%	0.882
	Yes	31	28.2%	19	29.2%	
Air bronchogram	No	76	69.1%	41	63.1%	0.414
	Yes	34	30.9%	24	36.9%	
Halo sign	No	87	79.1%	51	78.5%	0.992
	Yes	23	20.9%	14	21.5%	
Bronchial thickening	No	91	82.7%	59	90.8%	0.142
	Yes	19	17.3%	6	9.2%	
Pleural effusion	No	99	90.0%	57	87.7%	0.635
	Yes	11	10.0%	8	12.3%	
Mediatinal lymphadenopathy	No	96	87.3%	57	87.7%	0.936
	Yes	14	12.7%	8	12.3%	
Subpleural line	No	71	64.5%	48	73.8%	0.203
	Yes	39	35.5%	17	26.2%	
Study groups	COVID-19	93	84.5%	57	87.7%	0.565
	H1N1	17	15.5%	8	12.3%	

COVID-19: Coronavirus disease-2019.

Table 3. Distribution of findings according to study groups

		Study groups				p
		COVID-19		H1N1		
		n	%	n	%	
Gender	Male	93	62.0%	17	68.0%	0.565
	Female	57	38.0%	8	32.0%	
Ground-glass opacities	No	21	14.0%	4	16.0%	0.794
	Yes	129	86.0%	21	84.0%	
Consolidation	No	48	32.0%	12	48.0%	0.119
	Yes	102	68.0%	13	52.0%	
Right upper lobe	No involvement	57	38.0%	7	28.0%	0.336
	Involvement	93	62.0%	18	72.0%	
Right middle lobe	No involvement	54	36.0%	9	36.0%	1.000
	Involvement	96	64.0%	16	64.0%	
Right lower lobe	No involvement	23	15.3%	6	24.0%	0.381
	Involvement	127	84.7%	19	76.0%	
Left upper lobe	No involvement	57	38.0%	6	24.0%	0.177
	Involvement	93	62.0%	19	76.0%	

Table 3. Continued

		Study groups				p
		COVID-19		H1N1		
		n	%	n	%	
Left lower lobe	No involvement	18	12.0%	5	20.0%	0.119
	Involvement	132	88.0%	20	80.0%	
Diffuse pattern (involved five lobes)	No	81	54.0%	23	92.0%	<0.001
	Yes	69	46.0%	2	8.0%	
Peripheral pattern	No	29	19.3%	10	40.0%	0.022
	Yes	121	80.7%	15	60.0%	
Bronchovascular distribution	No	76	50.7%	18	72.0%	0.048
	Yes	74	49.3%	7	28.0%	
Crazy paving sign	No	103	68.7%	18	80.0%	0.643
	Yes	47	31.3%	7	20.0%	
Nodular consolidation	No	108	72.0%	20	72.0%	0.738
	Yes	42	28.0%	5	28.0%	
Air bronchogram	No	102	68.0%	15	60.0%	0.431
	Yes	48	32.0%	10	40.0%	
Halo sign	No	118	78.7%	20	80.0%	0.880
	Yes	32	21.3%	5	20.0%	
Bronchial thickening	No	131	87.3%	19	76.0%	0.134
	Yes	19	12.7%	6	24.0%	
Pleural effusion	No	137	91.3%	19	76.0%	0.034
	Yes	13	8.7%	6	24.0%	
Mediatinal lymphadenopathy	No	137	91.3%	16	64.0%	0.001
	Yes	13	8.7%	9	36.0%	
Subpleural line	No	103	68.7%	20	80.0%	0.643
	Yes	47	31.3%	5	20.0%	

COVID-19: Coronavirus disease-2019.

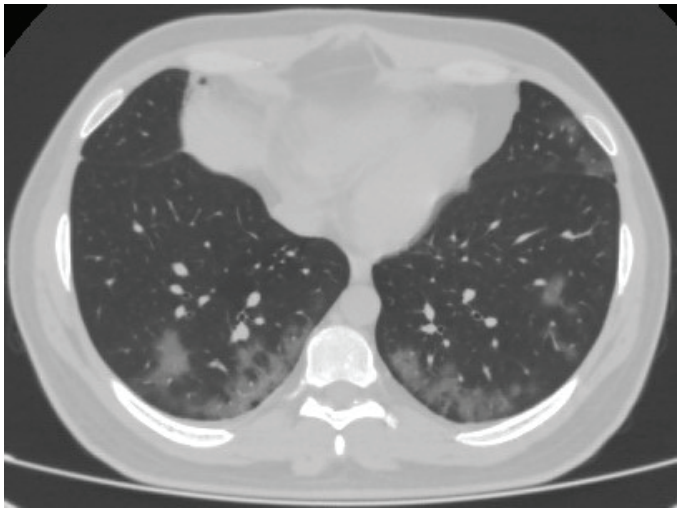


Figure 1. GGO consistent with peripherally located COVID-19 pneumonia is found in both lower lobes of the lungs.

GGO: Ground glass opacities, COVID-19: Coronavirus disease-2019.

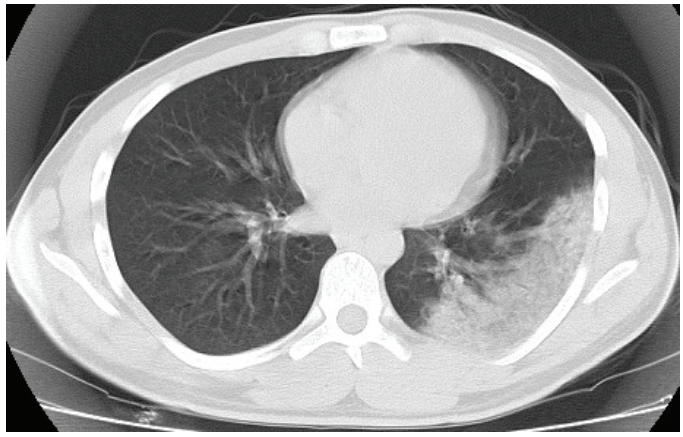


Figure 2. A large peripherally located consolidation area is observed in the lower lobe of the left lung.

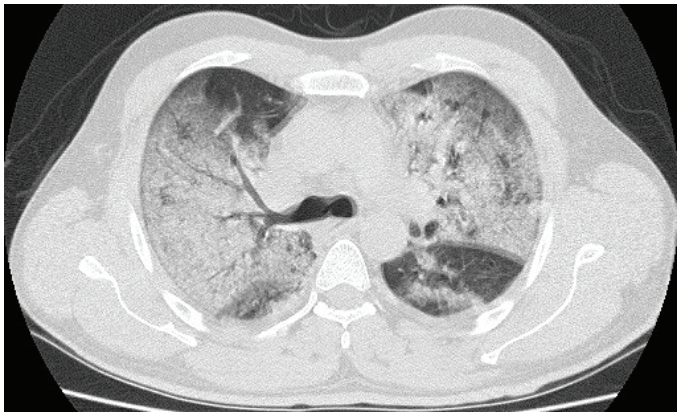


Figure 3. Diffuse GGO consolidation areas consistent with H1N1 pneumonia are observed in the upper lobes of both lungs. Effusion is present in both pleural spaces.

GGO: Ground glass opacities.

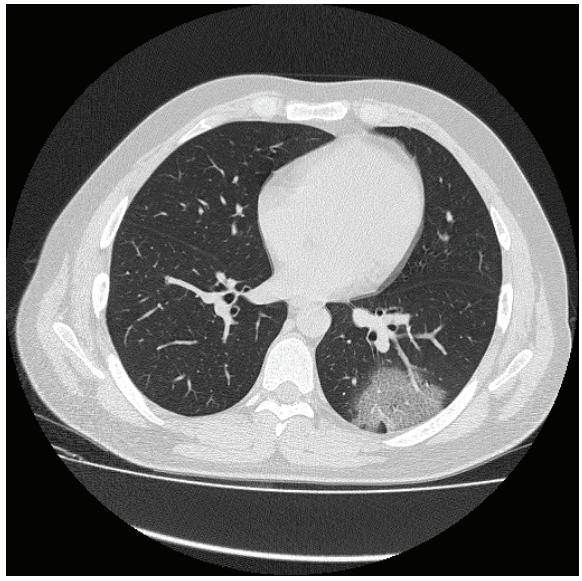


Figure 4. A patient diagnosed with COVID-19 pneumonia in the lower lobe of the left lung with a peripheral localized crazy paving sign.

COVID-19: Coronavirus disease-2019.

Table 4. Distinguishing features of COVID-19 and H1N1 pneumonia according to multivariate analysis. Advanced age and the presence of diffuse and peripheral patterns indicate that COVID-19, pleural effusion, and hilar lymphadenopathy are higher in H1N1 involvement

	B	Sig.	Exp(B)	95% CI for Exp(B)	
				Lower	Upper
Age	-0.041	0.031	0.960	0.925	0.996
Diffuse pattern	-1,764	0.029	0.171	0.035	0.834
Peripheral pattern	-1,260	0.026	0.284	0.094	0.860
Bronchovascular distribution	-0.821	0.147	0.440	0.145	1,336
Pleural effusion	0.949	0.201	2,582	0.603	11,055
Mediastinal lymphadenopathy	2,071	0.002	7,933	2,193	28,702
Constant	0.817	0.461	2,263		

Sig.: Significance, COVID-19: Coronavirus disease-2019, CI: Confidence interval.

In the present study, we found a diffuse distribution pattern (involvement of five lung lobes) more frequently in COVID-19 pneumonia than in H1N1 pneumonia. Kuang et al. (11) and Liu et al. (17) reported that the diffuse distribution pattern was more common in H1N1 pneumonia. Similarly, the diffuse distribution pattern was generally reported in the literature as a more frequent finding for H1N1 pneumonia. The reason why we found the opposite result in our study can be explained by the fact that our hospital is a tertiary hospital and patients with severe COVID-19 pneumonia in our region are followed up in our hospital.

Consolidation and GGO are common in both types of pneumonia. Consolidation has been reported as a second dominant feature in COVID-19 patients within a few days of disease onset. The presence of consolidation may indicate an increase in disease severity (18). In our present study, GGO was observed in 86% and consolidation in 68% of patients with COVID-19 pneumonia, whereas GGO was observed in 84% and consolidation in 52% of patients with H1N1 pneumonia. No difference was found between pneumonia in consolidation and GGO (0.119 and 0.794, respectively). Yin et al. (10) obtained similar results in their study.

Which lobe is more commonly involved and do the involved lobes help in diagnosis? We compared involvement in the five lobes of the lung and found no significant results. However, diffuse patterns and peripheral distribution patterns are more common in COVID-19 pneumonia. In the study by Kuang et al. (11), no association was found between the lobes involved, but peripheral and diffuse distributions were found to be significant in relation to COVID-19 pneumonia. This is in agreement with the results of our current study. Mediastinal lymphadenopathy and pleural effusion are relatively rare findings in COVID-19 pneumonia (19). These findings are also observed in relatively low numbers of H1N1 pneumonia. However, the findings of mediastinal lymphadenopathy and pleural effusion were significantly higher in H1N1 pneumonia than in COVID-19 pneumonia. In a study by Valente et al. (20), 14.3% of patients with H1N1 pneumonia had mediastinal lymphadenopathy ($p<0.001$), and Onigbinde et al. (21) also emphasized this finding in his study.

In some studies reviewed, the incidence of lymphadenopathy was low in patients with COVID-19 pneumonia. In one study, lymphadenopathy was observed in 2.7% of COVID-19 patients and in 10.0% of non-COVID-19 patients (p<0.001) (22). However, the presence of lymphadenopathy is considered an important risk factor for severe COVID-19 pneumonia and is probably due to bacterial superinfection (23). In our study, pleural effusion was a rare finding in COVID-19 pneumonia, and similar findings have been reported in previous studies (21,24). In Yin et al. (10), the frequency of pleural effusion was increased in H1N1 pneumonia compared with COVID-19 pneumonia (p=0.002). Our results were consistent with those reported in the literature.

The results show that mediastinal lymphadenopathy and pleural effusion are more common in patients with H1N1 pneumonia, whereas diffuse peripheral distribution is more common in patients with COVID-19 pneumonia. Other CT features, including nodular appearance, crazy paving sign, GGO, consolidation, subpleural line, bronchial wall thickening, halo sign, and air bronchogram, did not differ between patients with H1N1 pneumonia and those with COVID-19 pneumonia.

Crazy paving sign refers to the presence of ground-glass opacities with superimposed interlobular septal thickening and intralobular septal thickening on chest CT. It is a non-specific finding that can occur in many diseases. Recently, a study found that the crazy paving sign occurs in the third week after the onset of symptoms and indicates an advanced stage of the disease (23). Studies have indicated that it increases in COVID-19 pneumonia (10,11,25). However, no significant difference was found in our study (p=0.643). In the study of Lin et al. (1), patients with H1N1 pneumonia and COVID-19 pneumonia were compared, and no significant difference was found in terms of crazy paving sign (p=1.000). In the proportional analysis of our patients, 31.3% of patients with COVID-19 pneumonia and 20% of patients with H1N1 pneumonia showed a crazy paving sign in favor of COVID-19 pneumonia. We believe that the lack of statistical significance in our study is due to the small number of H1N1 pneumonia patients.

Study Limitations

This study has some limitations. Our study was retrospective. This is because the time periods during which COVID-19 pneumonia and H1N1 pneumonia occurred after the SARS-CoV-2 epidemic were different. The number of H1N1 pneumonia cases included was relatively small. The cases may have represented different stages of the disease, resulting in differences in the appearance of radiological findings. Because this study was retrospective, it may be necessary to consider that individuals with H1N1 pneumonia and COVID-19 pneumonia could have additional infections or coinfections, which could lead to differences in imaging between individuals.

CONCLUSION

We found that most of the lesions caused by COVID-19 pneumonia were located in the peripheral region and near the pleura, whereas the diffuse distribution (five-lobe involvement) was randomly distributed and the lesions caused by influenza virus pneumonia were located far from the pleura. Mediastinal lymphadenopathy and pleural effusion were more prominent in influenza virus pneumonia than in COVID-19 pneumonia. These symptoms should be considered in the differential diagnosis of COVID-19 or H1N1 pneumonia when evaluating patients using chest CT.

Ethics

Ethics Committee Approval: The study protocol was approved by the Mardin Provincial Health Directorate Scientific Study Board (approval number: E-37201737-949).

Informed Consent: Retrospective study.

Authorship Contributions

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

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

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

REFERENCES

1. Lin L, Fu G, Chen S, Tao J, Qian A, Yang Y, et al. CT manifestations of coronavirus disease (COVID-19) pneumonia and influenza virus pneumonia: A comparative study. Am J Roentgenol. 2021; 216: 71-9.
2. Eslambolchi A, Maliglig A, Gupta A, Gholamrezanezhad A. COVID-19 or non-COVID viral pneumonia: How to differentiate based on the radiologic findings? World J Radiol. 2020; 12: 289-301.
3. Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, et al. Correlation of Chest CT and RT-PCR Testing for Coronavirus Disease 2019 (COVID-19) in China: A Report of 1014 Cases. Radiology. 2020; 296: 32-40.
4. Fu F, Lou J, Xi D, Bai Y, Ma G, Zhao B, et al. Chest computed tomography findings of coronavirus disease 2019 (COVID-19) pneumonia. Eur Radiol 2020; 30: 5489-98.
5. Pandemic (H1N1) 2009 - update 96 [Internet]. [cited 2022 Aug 4]. Available from: https://www.who.int/emergencies/disease-outbreak-news/item/2010_04_16-en
6. Henzler T, Meyer M, Kalenka A, Alb M, Schmid-Bindert G, Bartling S, et al. Image Findings of Patients with H1N1 Virus Pneumonia and Acute Respiratory Failure. Acad Radiol. 2010; 17: 681-5.
7. Amorim VB, Rodrigues RS, Barreto MM, Zanetti G, Marchiori E. Computed tomography findings in patients with H1N1 influenza A infection. Radiol Bras. 2013; 46: 299-306.
8. Ajlan AM, Quiney B, Nicolaou S, Müller NL. Swine-origin influenza A (H1N1) viral infection: radiographic and CT findings. AJR Am J Roentgenol. 2009; 193: 1494-9.
9. Amorim VB, Rodrigues RS, Barreto MM, Zanetti G, Hochhegger B, Marchiori E. Influenza A (H1N1) pneumonia: HRCT findings. J Bras Pneumol. 2013; 39: 323-9.
10. Yin Z, Kang Z, Yang D, Ding S, Luo H, Xiao E. A Comparison of Clinical and Chest CT Findings in Patients with Influenza A (H1N1) Virus Infection and Coronavirus Disease (COVID-19). AJR Am J Roentgenol. 2020; 215: 1065-71.
11. Kuang PD, Wang C, Zheng HP, Ji WB, Gao YT, Cheng JM, et al. Comparison of the clinical and CT features between COVID-19 and H1N1 influenza pneumonia patients in Zhejiang, China. Eur Rev Med Pharmacol Sci. 2021; 25: 1135-45.
12. Han R, Huang L, Jiang H, Dong J, Peng H, Zhang D. Early Clinical and CT Manifestations of Coronavirus Disease 2019 (COVID-19) Pneumonia. AJR Am J Roentgenol. 2020; 215: 338-43.

13. Zhao W, Zhong Z, Xie X, Yu Q, Liu J. Relation between Chest CT Findings and Clinical Conditions of Coronavirus Disease (COVID-19) Pneumonia: A Multicenter Study. AJR Am J Roentgenol. 2020; 214: 1072-7.
14. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020; 395: 497-506.
15. Song F, Shi N, Shan F, Zhang Z, Shen J, Lu H, et al. Emerging 2019 Novel Coronavirus (2019-nCoV) Pneumonia. Radiology. 2020; 295: 210-7.
16. Xu YH, Dong JH, An WM, Lv XY, Yin XP, Zhang JZ, et al. Clinical and computed tomographic imaging features of novel coronavirus pneumonia caused by SARS-CoV-2. J Infect. 2020; 80: 394-400.
17. Liu M, Zeng W, Wen Y, Zheng Y, Lv F, Xiao K. COVID-19 pneumonia: CT findings of 122 patients and differentiation from influenza pneumonia. Eur Radiol. 2020; 30: 5463-9.
18. Ye Z, Zhang Y, Wang Y, Huang Z, Song B. Chest CT manifestations of new coronavirus disease 2019 (COVID-19): a pictorial review. Eur Radiol. 2020; 30: 4381-89.
19. Zheng Y, Wang L, Ben S. Meta-analysis of chest CT features of patients with COVID-19 pneumonia. J Med Virol. 2021; 93: 241-9.
20. Valente T, Lassandro F, Marino M, Squillante F, Aliperta M, Muto R. H1N1 pneumonia: our experience in 50 patients with a severe clinical course of novel swine-origin influenza A (H1N1) virus (S-OIV). Radiol Med. 2012; 117: 165-84.
21. Onigbinde SO, Ojo AS, Fleary L, Hage R. Chest Computed Tomography Findings in COVID-19 and Influenza: A Narrative Review. Biomed Res Int. 2020; 2020: 6928368.
22. Bai HX, Hsieh B, Xiong Z, Halsey K, Choi JW, Tran TML, et al. Performance of Radiologists in Differentiating COVID-19 from Non-COVID-19 Viral Pneumonia at Chest CT. Radiology. 2020; 296: 46-54.
23. Shi H, Han X, Jiang N, Cao Y, Alwalid O, Gu J, et al. Radiological findings from 81 patients with COVID-19 pneumonia in Wuhan, China: a descriptive study. Lancet Infect Dis. 2020; 20: 425-34.
24. Marchiori E, Zanetti G, Fontes CA, Santos ML, Valiante PM, Mano CM, et al. Influenza A (H1N1) virus-associated pneumonia: High-resolution computed tomography-pathologic correlation. Eur J Radiol. 2011; 80: 500-4.
25. Li Y, Xia L. Coronavirus Disease 2019 (COVID-19): Role of Chest CT in Diagnosis and Management. AJR Am J Roentgenol. 2020; 214: 1280-6.

GMJ 2024;35:236-242

DOI: http://dx.doi.org/10.12996/gmj.2023.3843

Depot Versus Oral Antipsychotics in Patients with Schizophrenia: Which is Better on Side Effects, Functional Improvement, and Life Satisfaction

Şizofreni Hastalarında Depo Antipsikotiklere Karşı Oral Antipsikotikler: Yan Etki, İşlevsel İyileşme ve Yaşam Doyumu Üzerinde Hangisi Daha İyi?

İlknur Kiraz Avcı[1], Mehmet Avcı[2], Buket Koparal[3]

[1]Clinic of Psychiatry, Community Mental Health Center, Rize State Hospital, Rize, Türkiye
[2]Department of Guidance and Psychological Counseling, Recep Tayyip Erdogan University Faculty of Medicine, Rize, Türkiye
[3]Department of Psychiatry, Gazi University Faculty of Medicine, Ankara, Türkiye

ABSTRACT

Objective: Schizophrenia is a chronic mental disorder that reduces quality of life, causes deterioration in social and occupational functioning, and requires continuous care for psychosocial rehabilitation. The aim of this study was to investigate functional improvement, life satisfaction, and drug side effects in patients with schizophrenia and schizoaffective disorder using depot antipsychotics, oral antipsychotics, or both.

Methods: The study included 162 patients with clinically stable schizophrenia and schizoaffective disorder who were regularly followed up at the Rize Community Mental Health Center for at least 1 year. The Satisfaction with Life Scale (SLS), Glasgow Antipsychotic Side-Effect Scale (GASS), and Functional Remission of General Schizophrenia (FROGS) scale were administered to patients with available sociodemographic and clinical data.

Results: There were significant differences between the groups with respect to age at onset, number of hospitalizations, and gender. The mean GASS score of patients using both depot and oral antipsychotics was significantly higher than that of those using only depot antipsychotics. There was no significant difference between the groups in terms of mean SLS, total FROGS scores, and sub-dimension FROGS scores.

Conclusion: It was concluded that it would be appropriate to prefer depot antipsychotics in the presence of poor prognostic factors such as medication non-adherence, frequent hospitalization, and lack of insight. There is a need for multicenter prospective studies with longer follow-up of patients for side effects, life satisfaction, quality of life, and functional improvement to achieve more significant results.

Keywords: Schizophrenia, side effects, depot antipsychotics, life satisfaction

ÖZ

Amaç: Şizofreni, yaşam kalitesini düşüren, sosyal ve mesleki işlevsellikte bozulmaya neden olan, psikososyal rehabilitasyon için sürekli bakım gerektiren kronik bir ruhsal bozukluktur. Bu çalışmanın amacı, sadece depo antipsikotikler, sadece oral antipsikotikler veya her ikisini birden kullanan şizofreni ve şizoaffektif bozukluk hastalarında fonksiyonel iyileşme, yaşam doyumu ve ilaç yan etkilerini araştırmaktır.

Yöntemler: Çalışmaya Rize Toplum Ruh Sağlığı Merkezi'nde en az bir yıldır düzenli olarak takip edilen, klinik olarak stabil şizofreni ve şizoaffektif bozukluk tanılı 162 hasta dahil edildi. Sosyo-demografik ve klinik verileri mevcut olan hastalara Yaşam Doyumu Ölçeği (SLS), Glasgow Antipsikotik Yan Etki Ölçeği (GASS) ve Genel Şizofrenide İşlevsel İyileşme Ölçeği (FROGS) uygulandı.

Bulgular: Gruplar arasında başlangıç yaşı, hastaneye yatış sayısı ve cinsiyet açısından anlamlı fark vardı. Hem depo hem de oral antipsikotik kullanan hastaların ortalama GASS skoru, sadece depo antipsikotik kullananlara göre anlamlı olarak yüksekti. SLS, FROGS toplam puanları ve FROGS alt boyut puan ortalamaları açısından gruplar arasında anlamlı fark yoktu.

Sonuç: İlaç uyumsuzluğu, sık hastaneye yatış ve içgörü eksikliği gibi kötü prognostik faktörlerin varlığında depo antipsikotiklerin tercih edilmesinin uygun olacağı kanısına varıldı. Daha anlamlı sonuçlar elde etmek için yan etkiler, yaşam memnuniyeti, yaşam kalitesi ve fonksiyonel iyileşme açısından hastaların daha uzun süre takip edildiği çok merkezli prospektif çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: Şizofreni, yan etki, depo antipsikotik, yaşam doyumu

Address for Correspondence/Yazışma Adresi: Buket Koparal, MD, Department of Psychiatry, Gazi University Faculty of Medicine, Ankara, Türkiye
E-mail / E-posta: bkt_svnc@hotmail.com
ORCID ID: orcid.org/0000-0003-1874-270X

Received/Geliş Tarihi: 31.03.2023
Accepted/Kabul Tarihi: 09.09.2023

INTRODUCTION

Schizophrenia is a chronic mental disorder characterized by positive symptoms, such as delusions and hallucinations, as well as negative symptoms, such as social withdrawal and cognitive impairment, creating a great burden on patients, their families, and society and leading to disabilities (1). Schizophrenia reduces the quality of life, causes deterioration in social and occupational functionality, and requires continuous care for psychosocial rehabilitation (2,3). Antipsychotic drugs used to alleviate acute symptoms and prevent long-term relapse are the mainstay of treatment for schizophrenia (4). Pharmacological treatment for schizophrenia should be individually tailored to the needs and preferences of the patient. In addition to ameliorating psychotic symptoms and preventing relapse, it should aim to improve the patient's psychosocial functionality level, independence, and quality of life, considering treatment adherence of the patient and drug side effects (5).

Various studies have found a medication non-adherence rate of 40-80% in patients with schizophrenia who are prone to forget the drug dose or deliberately discontinue the drug (6,7). The most common causes of medication non-adherence include poor insight, drug side effects, belief that the drug will worsen the disease, lack of motivation, and cognitive loss (6).

It is very important to avoid the high risk of relapse after the first psychotic episode of schizophrenia because subsequent episodes impair the quality of life of patients and harm their psychosocial functionality (3,8). It has been observed that patients with schizophrenia who regularly use their medications have low rates of emergency admission and hospitalization and that early intervention reduces long-term negative outcomes and preserves functionality (9).

Considering medication non-adherence, the use of depot antipsychotics for long-term control of psychotic symptoms is increasing, and studies comparing oral antipsychotics with depot forms have come to the fore (10-12). Recent studies have indicated lower non-adherence to treatment with long-acting antipsychotic depot injections (8,13). Studies have also investigated the attitudes of patients, their relatives, and healthcare professionals toward depot antipsychotics. Adequately informing patients about depot antipsychotics and establishing therapeutic cooperation between clinician and patient for drug selection appears to be important (8).

The aim of this study was to investigate functional improvement, life satisfaction, and drug side effects in patients with schizophrenia who were followed up at Rize Community Mental Health Center (CMHC) and were using only depot antipsychotics, oral antipsychotics, or both.

MATERIALS AND METHODS

Procedure and Participant

Before starting the research, the necessary ethical approval was obtained from the Human Research Ethics Committee of Rize Provincial Directorate of Health (approval number: E-64247179-799). Participants were informed about the study and signed consent forms were obtained. The study included 162 patients who were diagnosed with schizophrenia and schizoaffective disorder according to the DSM-5 diagnostic criteria and were followed up at the Rize CMHC for at least 1 year. The eligibility criteria were as follows: regular attendance at follow-up visits, regular use of medications, use of only oral antipsychotics, only depot antipsychotics, or both, clinical stability, and consent to participate in the study.

The study employed a comparative cross-sectional design to investigate functional improvement, life satisfaction, and drug side effects among patients with schizophrenia who were administered depot antipsychotics, oral antipsychotics, and both for at least 6 months. A comparative cross-sectional study design is a quantitative research approach that involves comparing data collected from two or more distinct groups (patients with schizophrenia using different types of antipsychotics) at a single point in time. The data for this study were collected between July 2021 and December 2021.

The clinical stability of participants was assessed using the Clinical Global Impression (CGI) scale, with a CGI score of 5 or less indicating clinical stability (14). Those with cognitive impairment, neurological diseases such as epilepsy, a history of head trauma, and mental retardation were excluded from the study.

Tools

A sociodemographic data form, Satisfaction with Life Scale (SLS), Glasgow Antipsychotic Side-Effect Scale (GASS), and Functional Remission of General Schizophrenia (FROGS) scale were administered to participants by the CMHC chief physician who followed up with them, namely the researcher.

Sociodemographic data form: The sociodemographic data form was prepared by the researcher to investigate sociodemographic characteristics such as age, gender, economic status, educational level, marital status, and employment status, as well as clinical characteristics including age of onset, disease duration, number of hospitalizations, suicide attempt, family history, etc.

Satisfaction with Life Scale: The scale consists of 5 items. The Turkish validity and reliability study was conducted by Dağlı and Baysal (15) in 2016, and the correlation coefficient of the scale was found to be 0.92.

Glasgow Antipsychotic Side-Effect Scale: The scale developed in order to evaluate the side effects of antipsychotic drugs consists of 22 items. The Turkish validity and reliability study of the scale was performed by Aslan et al. (16).

Functional Remission of General Schizophrenia: FROGS is a 5-point Likert-type scale consisting of 19 items to evaluate improvements in functionality independent of disease symptoms. The administration of the scale was carried out in the form of semi-structured interviews. The Turkish validity and reliability study of the scale was performed by Emiroğlu (17), who reported a reliability coefficient of 0.90 for the scale.

Statistical Analysis

The SPSS version 26 statistical software package was used for data analysis. One-Way ANOVA was used to determine whether there were differences between the GASS, SLS, and FROGS scores of participants who used only injectable drugs, only oral drugs, or both. Pearson correlation analysis was used to determine the correlations between these variables. Regression analysis was performed to determine to what extent GASS scores predicted SLS and FROGS scores. The level of statistical significance was set at 0.05 for all analyses.

RESULTS

The demographic information of the participants is given in detail in Table 1. When the groups were compared in terms of sociodemographic variables, a significant difference was found in terms of disease onset age [$F(2,159)=3.81$, $p<0.05$), number of hospitalizations [$F(2,159)=3.14$, $p<0.05$), and gender [$\chi^2(2,162)=7.89$, $p<0.05$). However, there was no significant difference between the groups according to other variables ($p>0.05$).

A One-Way ANOVA was performed to compare the effect of three different groups (long acting: depot, oral, combination) on SLS, GASS, and FROGS scores (Table 2). A One-Way ANOVA revealed a statistically significant difference in GASS scores between at least two groups [$F(2,159)=4.15$, $p<0.05$, $\eta^2=0.05$). Tukey's HSD test for multiple comparisons found that the mean value of GASS was significantly different between combination ($M=14.81$, standard deviation (SD)=7.93) and long-acting ($M=9.39$, $SD=5.23$). However, One-Way ANOVA revealed that there was not a statistically

Table 1. Comparison of sociodemographic and clinical data between groups

	Long acting (depot), (n=18)		Oral, (n=74)		Combination, (n=70)		p
	M	SD	M	SD	M	SD	
Age	45.67	12.66	44.01	11.90	43.60	9.96	0.784[a]
Disease onset age	27.89	10.45	22.92	6.45	22.86	7.07	0.024[a]
Disease duration	17.78	12.59	21.07	10.42	20.67	11.55	0.530[a]
Number of hospitalizations	1.44	1.69	2.76	3.47	3.69	4.03	0.046[a]
BMI	29.78	4.43	29.07	4.54	29.64	4.63	0.701[a]
	n	%	n	%	n	%	
Gender							
Female	8	44.4	16	21.6	10	14.3	0.019[b]
Male	10	55.6	58	78.4	60	85.7	
Marital status							
Single	9	50.0	48	64.9	44	62.9	0.451[b]
Married	6	33.3	21	28.4	16	22.9	
Divorced	3	16.7	5	6.8	10	14.3	
SES							
Low	2	11.1	14	18.9	11	15.7	0.772[c]
Middle	16	88.9	56	75.7	56	80.0	
High	0	0.0	4	5.4	3	4.3	
Job status							
Unemployed	13	72.2	41	55.4	29	41.4	0.073[b]
Employee	4	22.2	13	17.6	15	21.4	
Retired	1	5.6	20	27.0	26	37.1	
Psychiatric diagnosis							
Schizophrenia	18	100.0	70	94.6	63	90.0	0.280[c]
Schizoaffective	0	0.0	4	5.4	7	10.0	
Suicide attempt							
Yes	1	5.6	15	20.3	18	25.7	0.169[b]
No	17	94.4	59	79.7	52	74.3	
Psychiatric diagnosis in the family							
Yes	7	38.9	37	50.0	35	50.0	0.673[b]
No	11	61.1	37	50.0	35	50.0	
Other disease							
No	13	72.2	59	79.7	60	85.7	0.367[b]
Yes	5	27.8	15	20.3	10	14.3	

[a]: ANOVA, [b]: Chi square, [c]: Fisher's exact test, M: Mean, SD: Standard deviation, BMI: Body mass index.

significant difference in SLS and FROGS scores between the two groups (p>0.05).

Pearson correlation analysis was performed before performing hierarchical multiple regression analysis. The predictor variables that had significant relationships with the outcome variables were included in the regression analysis. Predictive variables (e.g., antidepressants and mood stabilizers) that did not have significant relationships with the outcome variables were not included in the regression model.

A hierarchical multiple regression was run to determine if the addition of age and GASS score improved the prediction of SLS scores (Table 3). First, age was entered in the model, which resulted in a beta coefficient of 0.11. Age accounted for approximately 0.07% of the variance in SLS [$F(1,160)=12.18$, $p<0.01$, $R^2=0.07$). The second step of the regression analysis involved entering GASS into the regression equation along with age. Age had a beta value of 0.11 and GASS had -0.22. Age and GASS for approximately 0.20% of the variance in SLS ($F(2,159)=20.30$, $p<0.001$, $R^2=0.20$).

DISCUSSION

This study compared patients with schizophrenia and schizoaffective disorder using only oral antipsychotics, only depot antipsychotics,

Table 2. One-Way ANOVA results

	Group	n	M	SD	F	p	η^2	Difference
GASS	Long acting[a]	18	9.39	5.23	4.15	0.018	0.05	c>a
	Oral[b]	74	12.41	7.92				
	Combination[c]	70	14.81	7.93				
SLS	Long acting	18	15.44	3.36	1.39	0.252	0.02	
	Oral	74	14.76	4.83				
	Combination	70	13.73	4.77				
FROGS	Long acting	18	58.50	16.07	1.01	0.367	0.01	
	Oral	74	56.57	13.26				
	Combination	70	54.21	12.29				
Social functioning	Long acting	18	19.56	5.58	1.42	0.245	0.02	
	Oral	74	18.76	5.00				
	Combination	70	17.69	4.70				
Health and treatment	Long acting	18	12.94	4.01	0.09	0.918	0.00	
	Oral	74	12.80	3.11				
	Combination	70	12.63	3.30				
Daily life	Long acting	18	20.44	5.49	1.11	0.333	0.01	
	Oral	74	19.85	4.31				
	Combination	70	19.01	3.97				
Occupational functionality	Long acting	18	5.56	2.12	1.00	0.372	0.01	
	Oral	74	5.16	1.98				
	Combination	70	4.89	1.76				

GASS: Glasgow Antipsychotic Side Effect Scale, SLS: The Satisfaction with Life Scale, FROGS: Functional Remission of General Schizophrenia Scale, M: Mean, SD: Standard deviation.

Table 3. Hierarchical multiple regression prediction of SLS from age and GASS

	B	SH	B	t	p	R^2
Model 1						
Constant	9.47	1.45		6.51	<0.001	0.07
Age	0.11	0.03	0.27	3.49	0.001	
Model 2						
Constant	12.21	1.45		8.41	<0.001	0.20
Age	0.11	0.03	0.27	3.85	<0.001	
GASS	-0.22	0.04	-0.36	-5.15	<0.001	

Outcome variable: SLS, Model 1 $F(1,160)=12.18$, $p<0.01$. Model 2 $F(2,159)=20.30$, $p<0.001$, SLS: Satisfaction with Life Scale, GASS: Glasgow Antipsychotic Side Effect Scale.

or both in terms of drug side effects, life satisfaction, and functional improvement. The analysis of the sociodemographic data revealed no significant difference between the groups with respect to age, marital status, employment status, perceived socio-economic level, suicide attempt, and family history of psychiatric illness. All three groups had higher body mass indexes than the normal ranges. The groups were similar with regard to comorbid diseases such as hypertension, obesity, diabetes, and hypercholesterolemia. This is consistent with previous reports showing an increased risk of cardiovascular and metabolic diseases in patients with schizophrenia (18). The comparison of the groups showed a significant difference in terms of age at onset, number of hospitalizations, and gender. The analyses revealed a higher number of hospitalizations in the group using both depot and oral antipsychotics compared with those using only depot antipsychotics, with an earlier age of onset. This may be related to the addition of depot antipsychotics to treatment in the presence of poor prognostic factors such as early onset, frequent hospitalization, and clinicians' tendency to use multiple antipsychotics (19).

This study showed that the mean GASS score of patients using both depot and oral antipsychotics was significantly higher than that of patients using only depot antipsychotics; however, there was no significant difference in regard to using only oral antipsychotics. The higher number of patients using two or three antipsychotics in combination in the group using only oral antipsychotics may have affected the results. Given these data, we concluded that the use of multiple antipsychotics increases the incidence of side effects regardless of the depot or oral form (1). It is known that more side effects can lead to medication non-adherence (6). Medication non-adherence appears to lead to a vicious cycle in the disease process by causing exacerbation of symptoms and hospitalization (20). Therefore, it should be noted that the use of multiple antipsychotics increases the incidence of side effects and adversely affects medication adherence rather than curing the disease.

This study demonstrated no significant difference between the groups with respect to mean SLS, total FROGS scores, and sub-dimension FROGS scores. Studies have shown low rates of long-term improvement both clinically and socially, although most of the patients initially respond well to antipsychotic treatment and especially positive symptoms are controlled (3,11). In particular, discontinuation of antipsychotic treatment leads to relapses, with an increased risk of self-harm or harming others, social isolation, reduced social and occupational functionality, loss of self-esteem, and decreased quality of life (3,21). This is associated with increased caregiver burden, frequent hospitalization, and increased healthcare costs (22). The absence of difference between our study groups in terms of SLS, FROGS total scores, and FROGS subscale scores, the regular use of the drugs by the patients regardless of the drug group, regular follow-up at the CMHC, and the relatively good therapeutic relationship established with the patients may have resulted in a certain level of functional improvement with the control of disease symptoms. Moreover, the long disease duration may have caused the patients to accept the disease and adapt to the treatment over the years. Providing psychoeducation about the disease process and regular drug use, observing and monitoring drug use, administering drugs once daily if necessary, reviewing the side effects of the patient experiencing discomfort due to drug side effects, and switching to depot antipsychotics can increase medication adherence and contribute to improving the quality of life and social functionality (3).

Depot antipsychotics developed to reduce medication non-adherence have some advantages and disadvantages over oral antipsychotics (22). Depot antipsychotics provide advantages such as maintaining stable blood levels of drugs, reducing the need for daily reminders to patients, avoiding gastrointestinal absorption problems and liver first-pass effects, and preventing accidental or intentional overdoses. Even if the patient misses an injection, a sudden drop in the blood level of an antipsychotic drug can be prevented, provided the clinician has sufficient time to intervene. Injections can also increase therapeutic interaction because they are administered by a healthcare professional at regular intervals (2,23). On the other hand, slower titration, a longer time to reach steady-state, and long-term cumulative side effects can be considered disadvantages of depot antipsychotics (24).

Although depot antipsychotics have been suggested to play an important role in increasing medication adherence, preventing relapses, and reducing hospitalizations in patients with schizophrenia (2), a study found that depot antipsychotics added to oral antipsychotic treatment did not provide any benefit in terms of treatment adherence (25). Furthermore, a recent study showed a higher rate of multiple antipsychotic use, longer length of hospital stay, and higher hospitalization frequency in patients with poorer treatment adherence (19). Because we did not investigate the effect of depot antipsychotics on treatment adherence in our study, it is not possible to speculate on this issue. However, our study showed a higher number of hospitalizations in patients using both depot and oral antipsychotics, suggesting that clinicians tended to add depot antipsychotics to oral treatment in cases of frequent hospitalization, medication non-adherence, and relapses.

Although depot antipsychotics are preferred when oral treatments fail or are inadequate, there are studies providing very robust evidence for the use of depot antipsychotics as first-line therapy (2,3,26). Some studies have stated that clinicians avoid using depot antipsychotics even in cases of medication non-adherence (27). A study reported that health professionals found depot antipsychotics stigmatizing, worried about serious side effects, considered the increasing cost, thought that patients would not accept depot antipsychotic treatment, and assumed that the therapeutic relationship would deteriorate (28). On the other hand, a review reported that 18-40% of patients preferred depot antipsychotics rather than regularly taking oral antipsychotics every day (29). In this regard, we believe that it is important to consider factors such as the clinical findings of the patient, frequency of hospitalization, age of onset, level of insight, and the patient's preference when selecting oral or depot drugs. Because this study demonstrated no significant difference between the groups with regard to life satisfaction and functional improvement, we suggest that depot antipsychotics be used only in case of medication non-adherence or based on the patient's preference, considering the requirement for administering depot antipsychotics by a healthcare professional and increasing cost. Similarly, a recent study comparing oral second-generation antipsychotics and the depot form of risperidone reported no difference between the two drug groups in terms of recovery and no advantage of the depot form in patients with early-stage

schizophrenia, recommending the use of depot antipsychotics only in cases of medication non-adherence and patient preference (29).

This study also showed that age and GASS total score were predictors of life satisfaction. The increase in age had a positive effect on life satisfaction, and high GASS scores were associated with lower life satisfaction. The study result showing that age was a predictor of life satisfaction may be related to the acceptance of the disease by the patients over the years. A higher number of side effects negatively affect the life satisfaction of patients. Therefore, avoiding the use of multiple antipsychotics is also essential for life satisfaction.

Study Limitations

The positive aspects of this study may include regular follow-up of the patients at the CMHC for at least 1-year, regular use of medications by them, a well-established therapeutic relationship with the treatment team, timely administration of injections, and clinical stability in terms of positive psychotic symptoms. The limitations of this study are the cross-sectional evaluation of patients by a single researcher, the inability to distinguish between first- and second-generation antipsychotics, and the absence of a sufficient sample size to compare the oral and depot forms of the drug with the same active ingredient.

CONCLUSION

This study demonstrated no difference between the groups with respect to life satisfaction and functional improvement, thereby suggesting the use of depot antipsychotics in the presence of poor prognostic factors such as medication non-adherence, frequent hospitalization, and lack of insight. Data regarding the effects of depot antipsychotics on functional improvement, quality of life, and life satisfaction and their use in cases of medication non-adherence and in early-stage patients are still limited and contradictory. There is a need for multicenter prospective studies focusing on early-stage patients with a longer follow-up period for side effects, life satisfaction, quality of life, and functional improvement to achieve more significant results.

Ethics

Ethics Committee Approval: Before starting the research, the necessary ethical approval was obtained from the Human Research Ethics Committee of Rize Provincial Directorate of Health (approval number: E-64247179-799).

Informed Consent: Participants were informed about the study and signed consent forms were obtained.

Authorship Contributions

Concept: İ.K.A., M.A., Design: İ.K.A., Supervision: B.K., Materials: İ.K.A., B.K., Analysis or Interpretation: M.A., B.K., Literature Search: İ.K.A., M.A., B.K., Writing: İ.K.A., B.K., Critical Review: B.K.

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

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

REFERENCES

1. National Collaborating Centre for Mental Health (UK). Psychosis and Schizophrenia in Adults: Treatment and Management: Updated Edition 2014. London: National Institute for Health and Care Excellence (UK); 2014.
2. Biagi E, Capuzzi E, Colmegna F, Mascarini A, Brambilla G, Ornaghi A, et al. Long-acting injectable antipsychotics in schizophrenia: literature review and practical perspective, with a focus on aripiprazole once-monthly. Adv Ther. 2017; 34: 1036-48.
3. Heres S, Lambert M, Vauth R. Treatment of early episode in patients with schizophrenia: the role of long acting antipsychotics. Eur Psychiatry. 2014; 29(Suppl 2): 1409-13.
4. Leucht S, Zhao J. Early improvement as a predictor of treatment response and remission in patients with schizophrenia: a pooled, post-hoc analysis from the asenapine development program. J Psychopharmacol. 2014; 28: 387-94.
5. Hasan A, Falkai P, Wobrock T, Lieberman J, Glenthoj B, Gattaz WF, et al. World Federation of Societies of Biological Psychiatry (WFSBP) guidelines for biological treatment of schizophrenia, part 2: update 2012 on the long-term treatment of schizophrenia and management of antipsychotic-induced side effects. World J Biol Psychiatry. 2013; 14: 2-44.
6. Dilbaz N, Karamustafalıoğlu O, Oral T, Önder E, Çelin M. Psikiyatri Polikliniğe Başvuran Şizofreni Hastalarında Tedaviye Uyumun ve Uyumu Etkileyen Faktörlerin Değerlendirilmesi. Klinik Psikofarmakoloji Bulteni. 2006; 16: 223-32.
7. Kahn RS, Fleischhacker WW, Boter H, Davidson M, Vergouwe Y, Keet IP, et al. Effectiveness of antipsychotic drugs in first-episode schizophrenia and schizophreniform disorder: an open randomised clinical trial. Lancet. 2008; 371: 1085-97.
8. Kirschner M, Theodoridou A, Fusar-Poli P, Kaiser S, Jäger M. Patients' and clinicians' attitude towards long-acting depot antipsychotics in subjects with a first episode of psychosis. Ther Adv Psychopharmacol. 2013; 3: 89-99.
9. Penttilä M, Jääskeläinen E, Hirvonen N, Isohanni M, Miettunen J. Duration of untreated psychosis as predictor of long-term outcome in schizophrenia: systematic review and meta-analysis. Br J Psychiatry. 2014; 205: 88-94.
10. Kane JM, Garcia-Ribera C. Clinical guideline recommendations for antipsychotic long-acting injections. Br J Psychiatry Suppl. 2009; 195: 63-7.
11. Olivares JM, Pinal B, Cinos C. Comparison of long-acting antipsychotic injection and oral antipsychotics in schizophrenia. Neuropsychiatry. 2011; 1: 275.
12. Weiden PJ, Schooler NR, Weedon JC, Elmouchtari A, Sunakawa A, Goldfinger SM. A randomized controlled trial of long-acting injectable risperidone vs continuation on oral atypical antipsychotics for first-episode schizophrenia patients: initial adherence outcome. J Clin Psychiatry. 2009; 70: 1397-406.
13. Nasrallah H. The case for long-acting antipsychotic agents in the post-CATIE era. Acta Psychiatr Scand. 2007; 115: 260-7.
14. Guy W. ECDEU assessment manual for psychopharmacology: US Department of Health, Education, and Welfare, Public Health Service; 1976.
15. Dağlı A, Baysal N. Adaptation of The Satisfaction with Life Scale Into Turkish: The Study of Validity and Reliability. Electronic Journal of Social Sciences. 2016; 15: 1250-62.

16. Aslan EA, Batmaz S, Yüncü ÖA, Sevil N, Erkek B. Glasgow antipsikotik yan etkilerini değerlendirme ölçeği'nin Türkçe versiyonunun geçerlilik ve güvenilirliği. Klinik Psikiyatri Dergisi. 2020; 23: 290-301.
17. Emiroğlu B. Şizofreni Hastalarında İşlevsel İyileşme Ölçeği›nin Türkçe versiyonunun geçerlilik ve güvenilirlik çalışması: Maltepe Üniversitesi, Sosyal Bilimler Enstitüsü; 2009.
18. Carrà G, Bartoli F, Carretta D, Crocamo C, Bozzetti A, Clerici M, et al. The prevalence of metabolic syndrome in people with severe mental illness: a mediation analysis. Soc Psychiatry Psychiatr Epidemiol. 2014; 49: 1739-46.
19. Çetin Ş, Şengül MCB, Özdel O. Antipsychotic polypharmacy: Retrospective evaluation of cases with psychotic disorder followed in outpatient clinic (tur). J Clin Psy. 2021; 24: 191-8.
20. Koparal B, Ünler M, Utku HÇ, Candansayar S. Revolving Door Phenomenon and Related Factors in Schizophrenia, Bipolar Affective Disorder and Other Psychotic Disorders. Psychiatr Danub. 2021; 33: 18-26.
21. Thornicroft G, Brohan E, Rose D, Sartorius N, Leese M; INDIGO Study Group. Global pattern of experienced and anticipated discrimination against people with schizophrenia: a cross-sectional survey. Lancet. 2009; 373: 408-15.
22. Kane JM. Improving patient outcomes in schizophrenia: achieving remission, preventing relapse, and measuring success. J Clin Psychiatry. 2013; 74: 18.
23. Kishimoto T, Nitta M, Borenstein M, Kane JM, Correll CU. Long-acting injectable versus oral antipsychotics in schizophrenia: a systematic review and meta-analysis of mirror-image studies. J Clin Psychiatry. 2013; 74: 957-65.
24. Sacchetti E, Grunze H, Leucht S, Vita A. Long-acting injection antipsychotic medications in the management of schizophrenia. Evidence-Based Psychiatric Care. 2015; 1: 27-36.
25. Eker SS, Akkaya C, Cangür Ş, Sarandöl A, Deniz G, Kırlı S. Oral antipsikotik kullanımına eklenmiş depo antipsikotik tedavisinin şizofreni hastalarının tedaviye uyumları üzerine etkisi. Klinik Psikofarmakoloji Bülteni. 2009; 19: 273-8.
26. Tiihonen J, Haukka J, Taylor M, Haddad PM, Patel MX, Korhonen P. A nationwide cohort study of oral and depot antipsychotics after first hospitalization for schizophrenia. Am J Psychiatry. 2011; 168: 603-9.
27. Hamann J, Kissling W, Heres S. Checking the plausibility of psychiatrists' arguments for not prescribing depot medication. Eur Neuropsychopharmacol. 2014; 24: 1506-10.
28. Besenius C, Clark-Carter D, Nolan P. Health professionals' attitudes to depot injection antipsychotic medication: a systematic review. J Psychiatr Ment Health Nurs. 2010; 17: 452-62.
29. Malla A, Chue P, Jordan G, Stip E, Koczerginski D, Milliken H, et al. An Exploratory, Open-Label, Randomized Trial Comparing Risperidone Long-Acting Injectable with Oral Antipsychotic Medication in the Treatment of Early Psychosis. Clin Schizophr Relat Psychoses. 2016; 9: 198-208.

Original Investigation | Özgün Araştırma

GMJ 2024;35:243-247

DOI: http://dx.doi.org/10.12996/gmj.2024.3846

Antiviral Activity of *Pistacia atlantica* subsp. Kurdica Extract Against Herpes Simplex Virus Type 1

Pistacia atlantica subsp.'nin Antiviral Etkinliği Herpes Simplex Virüsü Tip 1'e Karşı Kurdica Ekstraktı

Shahin Gavanji[1], Hojjat Baghshahi[2], Azizollah Bakhtari[3], Ali Mohamadi[4], Zahra Hamami Chamgordani[5], Mojtaba Khandan[2], Javaher Sinaei[4]

[1]Department of Biotechnology, University of Isfahan Faculty of Advanced Sciences and Technologies, Isfahan, Iran
[2]Barij Medicinal Plants Research Center, Kashan, Iran
[3]Department of Reproductive Biology, Shiraz University of Medical Sciences School of Advanced Medical Sciences and Technologies, Shiraz, Iran
[4]Institute of Traditional Medicine and Herbal Plants of Iran, Isfahan, Iran
[5]Department of Adult Health Nursing, Isfahan University of Medical Sciences Faculty of Nursing and Midwifery, Isfahan, Iran

ABSTRACT

Objective: Most research on the therapeutic effects of wild pistachio species has focused on bacteria and fungi. This study investigated the flavonoid and phenolic contents of different leaves and hulls of *Pistacia atlantica (P. atlantica)* subsp. Kurdica (*P. atlantica* subsp. Kurdica) extracts and their effect on herpes simplex virus 1 (HSV1).

Methods: In this study, aqueous, ethanolic, and methanolic extracts of the leaf and hull of *P. atlantica* subsp. Kurdica were prepared by the percolation method. The total phenolic and flavonoid contents in different extracts were measured by UV/Vis spectrophotometry using the Folin-Ciocâlteu reagent and the colorimetric method of aluminum chloride, respectively. On African green monkey kidney cells (VERO), the ethanolic extract of *P. atlantica* subsp. Kurdica was tested for toxicity. Furthermore, 50% cytotoxic and inhibitory concentrations (CC_{50} and IC_{50}) were identified.

Results: Compared with aqueous and methanolic extracts, the ethanolic extract of the leaf and hull of *P. atlantica* subsp. Kurdica had the highest phenolic and flavonoid concentrations. In addition, our study showed that CC_{50} values were 661.81 and 795.21 µg/mL. Also, IC_{50} values were 97.51 and 110.82 µg/mL for leaf and hull extracts, respectively. The selectivity index for the ethanolic leaf and hull extracts were 6.79 and 7.18, respectively.

Conclusion: Our results showed that *P. atlantica* subsp. Kurdica had an anti-HSV1 effect in a dose-dependent manner but at a higher dose than acyclovir. Ethanolic extract of *P. atlantica* subsp. Kurdica is probably a suitable herbal medicine with anti-herpetic effects.

Keywords: Antiviral, herbal medicine, pistachio, tradition medicine, VERO

ÖZ

Amaç: Yabani fıstık türlerinin tedavi edici etkileri üzerine yapılan araştırmaların çoğu bakteri ve mantarlara odaklanmıştır. Bu çalışmada, *Pistacia atlantica* (*P. atlantica*) subsp Kurdica (*P. atlantica* subsp. Kurdica) ekstraktlarının farklı yaprak ve kabuklarının flavonoid ve fenolik içerikleri ve bunların herpes simplex virüsü 1 (HSV1) üzerindeki etkileri araştırılmıştır.

Yöntemler: Bu çalışmada *P. atlantica* subsp. Kurdica'nın yaprak ve kabuğunun sulu, etanolik ve metanolik ekstraktları süzülme yöntemiyle hazırlandı. Farklı ekstraktlardaki toplam fenolik ve flavonoid içerikleri, sırasıyla; Folin-Ciocâlteu reaktifi ve alüminyum klorürün kolorimetrik yöntemi kullanılarak UV/Vis spektrofotometrisi ile ölçüldü. Afrika yeşil maymun böbrek hücrelerinde (VERO), *P. atlantica* subsp. Kurdica'nın etanolik ekstraktı toksisite açısından test edildi. Ayrıca %50 oranında sitotoksik ve inhibitör konsantrasyonlar (CC_{50} ve IC_{50}) belirlendi.

Bulgular: Sulu ve metanolik ekstraktlarla karşılaştırıldığında, *P. atlantica* subsp. Kurdica'nın yaprak ve kabuğunun etanolik ekstraktı en yüksek fenolik ve flavonoid konsantrasyonlarına sahipti. Ayrıca çalışmamızda CC_{50} değerlerinin 661,81 ve 795,21 µg/mL olduğu görüldü. Ayrıca yaprak ve kabuk ekstreleri için IC_{50} değerleri sırasıyla 97,51 ve 110,82 µg/mL olarak bulunmuştur. Etanollü yaprak ve kabuk ekstraktlarının seçicilik indeksleri sırasıyla 6,79 ve 7,18 idi.

Sonuç: Sonuçlarımız *P. atlantica* subsp. Kurdica'nın doza bağlı olarak ancak asiklovire göre daha yüksek dozda anti-HSV1 etkisine sahip olduğunu gösterdi. *P. atlantica* subsp. Kurdica'nın etanolik ekstraktı muhtemelen anti-herpetik etkileri olan uygun bir bitkisel ilaçtır.

Anahtar Sözcükler: Antiviral, bitkisel ilaç, fıstık, geleneksel tıp, VERO

Address for Correspondence/Yazışma Adresi: Hojjat Baghshahi, MD, Barij Medicinal Plants Research Center, Kashan, Iran
E-mail / E-posta: Baghshahi_h1989@yahoo.com
ORCID ID: orcid.org/0000-0003-0540-471X

Received/Geliş Tarihi: 04.04.2023
Accepted/Kabul Tarihi: 13.05.2024

INTRODUCTION

Herpes simplex virus 1 (HSV1) is a highly prevalent and contagious virus that can appear as permanent and latent infections with frequent outbreaks of oral lesions (1). Although there is currently no medication for the virus, antiviral drugs are frequently used to suppress and minimize outbreaks. Chemical antiviral drugs have caused the occurrence of allergies, side effects, and drug resistance, and finding new and effective treatments for the prevention and treatment of HSV outbreaks has been considered (2-5). It has been reported that approximately 65-80% of the world's population uses traditional medicine and medicinal plants as complementary medicine (6).

Pistacia atlantica (P. atlantica) subsp. Kurdica (*P. atlantica* subsp. Kurdica) is one of the subspecies of wild pistachio *P. atlantica* in the west (Zagros mountains) and less in the central and eastern regions of Iran (7,8). The leaves and fruits of this tree in folk medicine have many properties, including anti-atherogenic, blood sugar-lowering, liver protective, cell-protective, anti-genotoxic, anti-inflammatory, anti-ulcer, antipyretic, anti-fungal, anti-bacterial, anti-parasitic, Anti-mutagenic, antioxidant, and anti-cancer activity, as well as stimulant and diuretic (3,9,10). These pharmacological properties have been used in many diseases therapies (7,10).

Most studies have been conducted on the effects of this plant on bacteria and fungi (10-12), but there are very few reports on its antiviral effects (13). The current study determined the antiviral effects of *P. atlantica* subsp. Kurdica hull and leaf extracts on HSV1.

MATERIALS AND METHODS

Plant Collection and Extraction

Hulls and leaves of *P. atlantica* subsp. Kurdica were obtained from Ilam Province, Iran. Under sterile conditions, the samples were dried for 10 days in the shade. After that, a grinding machine ground them. Extraction was performed by the percolation method using different solvents such as ethanol, methanol 70%, and water. In brief, 50 g of plant powder was poured into the percolator and soaked in various solvents for 72 h. Then, the solid part was separated from the solvent part using Whatman paper with 11 µm pore sizes. The obtained extracts were then concentrated using a rotary evaporator under vacuum at a temperature of 40 °C. The extracts were kept in small sterile containers at a temperature of 4 °C in a dark place for further tests.

Determination of the Total Phenolic Content

The total phenolic content in samples of different hull and leaf extracts was measured by UV-Vis spectrophotometer using Folin-Ciocâlteu reagent (FCR). Briefly, 1 mg/mL of the various hull and leaf extracts were added to 0.5 mL of FCR and kept for 7 min at room temperature. Then, 0.4 mL of 7.5% sodium carbonate solution was added. After 1 h, the absorbance of different samples was read using a spectrophotometer at 765 nm. Gallic acid was used as a standard to draw the calibration curve. The total phenolic content was reported on the basis of mg of gallic acid equivalent/g of extract powder.

Determination of the Total Flavonoid Content

The aluminum chloride colorimetric assay was used to determine the total flavonoid content in the samples. Thus, 1 mg/mL of each extract was added to 0.1 mL of 10% aluminum chloride and 0.1 mL of 1 M potassium acetate. The solutions were placed at room temperature for 30 min. The total flavonoid content was measured on the basis of mg RE/g of extract powder using the absorbance of a spectrophotometer at 415 nm.

Cells and Viruses

African green monkey kidney (VERO) cells and HSV1 were obtained from the Traditional Medicine Research Institute, Isfahan, Iran. VERO cells were cultured in Eagle's minimum essential medium enriched with 10% newborn calf serum, 100 U/mL penicillin (Gibco), and 100 µg/mL streptomycin sulfate (Gibco).

Cytotoxicity Assay

The cytotoxic effects of different extracts were determined by a photometric technique using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) (14). In each well of the 96-well plate, 180 microliters of cell suspension (VERO cells) equal to $3x10^4$ µL cells were added. The extracts were added to the wells at different concentrations from 50 to 1000 µg/mL. The culture medium containing 0.5% dimethyl sulfoxide (DMSO) without plant extract was considered a negative control, whereas the antiviral drug acyclovir was considered a positive control. The plate was incubated for 48 h in an incubator with 5% CO_2 at 37 °C. Then 20 µL of MTT solution was added to the wells and incubated for 2 h. The 100 µL of DMSO was poured into the wells and mixed well to dissolve the formazan crystals. The absorbance was determined at 560 nm using an ELISA reader. The VERO cell survival percentage was estimated using the following equation, and for the positive and negative control groups, 100 was considered. A concentration of the tested compounds that reduces the cell survival rate by half was considered IC_{50}.

$$\text{VERO cell survival percentage} = \left[\frac{\text{Test compound OD} - \text{Blank OD}}{\text{Negative control OD} - \text{Blank OD}}\right] \times 100$$

Antiviral Activity

A plaque reduction test on VERO cells was used to determine the anti-HSV1 activity of the hull and leaf extracts. In each well of a 24-well plate, $400x10^3$ µL of VERO cells were incubated in 1 mL of Dulbecco's Modified Eagle Medium (DMEM) containing 3% inactivated FBS for 24 h. Then one µL of thawed viral suspension was added to each well. After 10 min, 20 µL of different concentrations of extracts were added to each well along with 2 mL of preheated DMEM medium with methylcellulose. In the negative and positive control groups, 20 µL DMSO diluted with deionized water and 20 µL acyclovir were added to the wells. The plates were incubated for 48 h and stained. Plaques were counted in each well, and the inhibition percentage of each extract concentration was determined using the following equation:

$$\text{Percentage of plaque inhibition} = \left[1 - \frac{(\text{number of plaque}) \text{ tested}}{\text{Number of control plaque}}\right] \times 100$$

Statistical Analysis

ANOVA was used to analyze the data using GraphPad Prism 6 software (GraphPad Software, La Jolla, CA, USA), and Tukey's multiple comparison tests were used to compare means. Differences with a $p<0.05$ were considered significant.

RESULTS

The phenolic and flavonoid contents of the ethanolic, methanolic, and aqueous extracts of the leaf and hull are shown in Figure 1. The present results showed that the phenolic and flavonoid contents were the highest in the ethanolic extract and the lowest in the aqueous extract.

Antiviral activity indices of ethanolic extracts of the leaves and hulls of *P. atlantica* subsp. Kurdica are shown in Table 1. The ethanolic extract of the hull had higher indices than the leaf. The antiviral activity of each extract was expressed as a selectivity index (SI), which was determined to be 7.18 and 6.79 for hull and leaf extracts, respectively (Table 1).

CC_{50} leaf: y=-0.2027x + 184.15,

CC_{50} hull: y=-0.1627x + 179.38,

IC_{50} leaf: y=1.5445x - 100.61,

IC_{50} hull: y=1.8442x - 154.37.

The survival rate of VERO cells at concentrations of 400 to 900 µg/mL for leaf and 400 to 1100 µg/mL for hull showed that the cell survival rate decreased with increasing dose (Figure 2). Ethanolic extracts of the leaves and hulls of *P. atlantica* subsp. Kurdica at the concentration of 400-500 and 400 µg/mL, respectively, caused the maximum survival rate, and at concentrations of 900 and 1100 µg/mL, respectively, the survival rate was equal to zero.

The anti-herpes activity of the ethanolic extracts of the leaf and hull has been shown in comparison with that of acyclovir (Figure 3). The results of this study stated that the concentration range of anti-herpes activity of leaf and hull extracts was from 60 to 130 and 80 to 140 µg/mL, respectively, which increased with the dose of this antiviral activity. In the positive control group (acyclovir), this range was 0.04 to 0.4 µg/mL.

Table 1. Assessment of CC_{50}, IC_{50}, and selectivity index in ethanolic extracts of the leaves and hulls of *P. atlantica* subsp. Kurdica

Extracts	CC_{50} (µg/mL)	R^2 (%)	IC_{50} (µg/mL)	R^2 (%)	CC_{50}/IC_{50} (SI)
Leaf	661.81	99.51	97.51	98.12	6.79
Hull	795.21	94.92	110.82	97.53	7.18

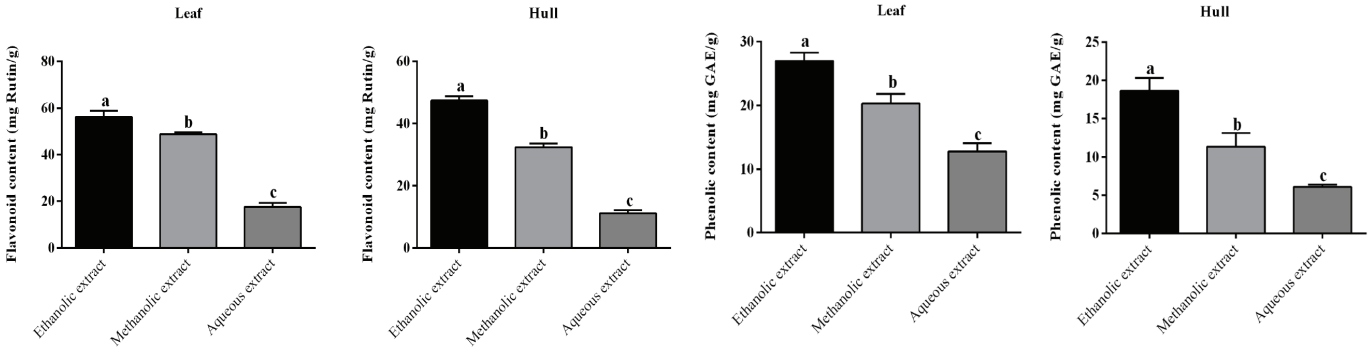


Figure 1. Phenolic and flavonoid contents of different leaf and hull extracts of *P. atlantica* subsp. Kurdica. Each value refers to the mean ± standard variation. [a-c]Different letters in each bar indicate significant differences at the 5% level.

GAE: Gallic acid equivalent.

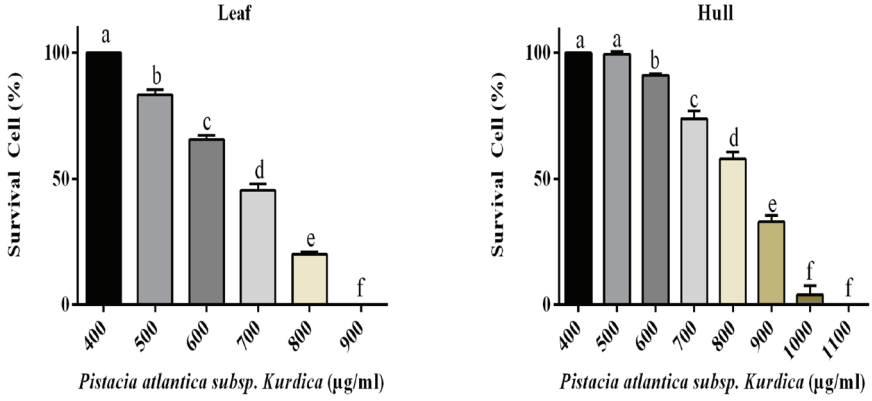


Figure 2. VERO cell survival at different concentrations of ethanolic extracts of leaf and hull of *P. atlantica* subsp. Kurdica. Each value refers to the mean ± standard variation. [a-f]Different letters in each bar indicate significant differences at the 5% level.

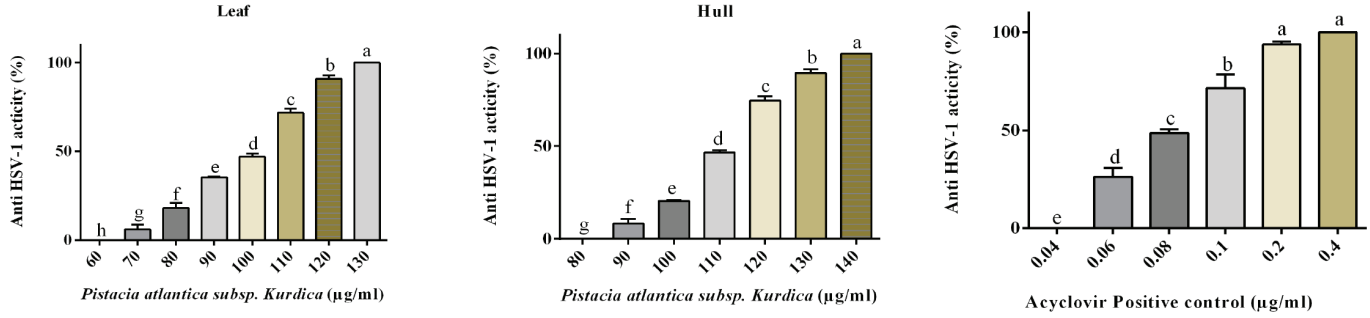


Figure 3. Antiherpes simplex virus-1 activity of ethanolic extracts of the leaves and hulls of *P. atlantica* subsp. Kurdica compared with acyclovir. Each value refers to the mean ± standard variation. [a-e]Different letters in each bar indicate significant differences at the 5% level.

HSV1: Herpes simplex virus 1.

DISCUSSION

According to reports, many traditional medicinal plants and herbs have potent antiviral activity (15). Some antiviral constituents include various structural classes such as flavonoids, tannins, alkaloids, terpenes, coumarins, proteins, peptides, polysaccharides, lignans, naphtho- and anthraquinones (16,17). The antiviral activity, and the antimicrobial and antifungal activities of pistachio, is related to phenolic and flavonoid compounds (18-20). The main phenolic compounds of the *Pistacia* genus include caffeic acid, catechin, eryodictiol-7-O-glucoside, gallic acid, protocatechuic acid, ellagic acid, ursolic acid, chlorogenic acid, ferulic acid, juglone, synaptic acid, and vanillic acid (13,18). However, there have been few studies on the antiviral effect of wild pistachio species (13). Our findings confirm the anti-HSV1 effect of *P. atlantica* subsp. Kurdica. Chlorogenic acid and caffeic acid have antiviral effects (21).

The antibacterial, antifungal, and antiviral effects of various sections of cultivated Vera pistachios were investigated by Ozçelik et al. (22). Their results showed that these extracts have significant antibacterial and antifungal effects. They also showed high antiviral effects against HSV and parainfluenza virus; therefore, its seed and kernel extracts showed higher antiviral effects than other plant organs (22). In addition, the antiviral effects of 75 plant species have been investigated against herpes virus, Sindbis virus, and poliovirus. Owing to its phenolic compounds, *Pistacia lentiscus* extract was found to have the greatest antiviral action against the herpes virus (23).

Our study showed that CC_{50} values were 661.81 and 795.21 µg/mL. Also, IC_{50} values were 97.51 and 110.82 µg/mL for leaf and hull extracts, respectively. The SI for the ethanolic leaf and hull extracts were 6.79 and 7.18, respectively. In line with our study, Karimi et al. (13) showed anti-adenovirus effects of ethanolic extract and n-butanol fraction of *P. atlantica* leaf. They stated that CC_{50}, IC_{50}, and SI on Hep-2 cells were 434.7, 16.37 µg/mL, and 26.5.

Musarra-Pizzo et al. (18) demonstrated that polyphenol-rich extracts of natural *Pistacia vera L.* shelled at 0.4, 0.6, and 0.8 mg/mL concentration reduced the expression of the viral proteins ICP8, UL42, and US11, and finally lead to minimize of viral DNA synthesis in VERO cells. They reported values of CC_{50}, IC_{50}, and SI equal to 1.2, 0.4, mg/mL, and 3. These differences may be caused by the pistachio species or the tested cells.

CONCLUSION

The results of this study showed that the ethanol extract of wild pistachio leaves and hull both have similar anti-HSV1 effects. These extracts are comparable to acyclovir at a higher dose. Further clinical research is required to confirm the therapeutic effects of anti-HSV1.

Ethics

Ethics Committee Approval: Ethic committee approval is not required.

Informed Consent: Informed consent approval is not required.

Author Contributions

Concept: S.G., H.B., Design: S.G., H.B., A.B., Analysis or Interpretation: A.B., Literature Search: A.M., Z.H.C., M.K., J.S., Writing: S.G., H.B.

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

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

REFERENCES

1. Bandara HMHN, Samaranayake LP. Viral, bacterial, and fungal infections of the oral mucosa: Types, incidence, predisposing factors, diagnostic algorithms, and management. Periodontol 2000. 2019; 80: 148-76.
2. Crimi S, Fiorillo L, Bianchi A, D'Amico C, Amoroso G, Gorassini F, et al. Herpes Virus, Oral Clinical Signs and QoL: Systematic Review of Recent Data. Viruses. 2019; 11: 463.
3. Rahman HS. Phytochemical analysis and antioxidant and anticancer activities of mastic gum resin from Pistacia atlantica subspecies kurdica. Onco Targets Ther. 2018; 11: 4559-72.
4. Ambika AP, Nair SN. Wound Healing Activity of Plants from the Convolvulaceae Family. Adv Wound Care (New Rochelle). 2019; 8: 28-37.
5. Mulat M, Pandita A, Khan F. Medicinal Plant Compounds for Combating the Multi-drug Resistant Pathogenic Bacteria: A Review. Curr Pharm Biotechnol. 2019; 20: 183-96.
6. Pan SY, Litscher G, Chan K, Yu ZL, Chen HQ, Ko KM. Traditional medicines in the world: where to go next? Evid Based Complement Alternat Med. 2014; 2014: 739895.
7. Hatamnia AA, Rostamzad A, Hosseini M, Abbaspour N, Darvishzadeh R, Malekzadeh P, et al. Antioxidant capacity and phenolic composition of leaves from 10 Bene (Pistacia atlantica subsp. kurdica) genotypes. Nat Prod Res. 2016; 30: 600-4.

8. Shahghobadi H, Shabanian N, Rahmani M-S, Khadivi A. Genetic characterization of Pistacia atlantica subsp. kurdica from northern Zagros forests in Iran. Trees. 2019; 33: 481-90.
9. Peksel A, Arisan-Atac I, Yanardag R. Evaluation of antioxidant and antiacetylcholinesterase activities of the extracts of Pistacia atlantica Desf. Leaves. J Food Biochem. 2010; 34: 451-76.
10. Ben Ahmed Z, Yousfi M, Viaene J, Dejaegher B, Demeyer K, Heyden YV. Four Pistacia atlantica subspecies (atlantica, cabulica, kurdica and mutica): A review of their botany, ethnobotany, phytochemistry and pharmacology. J Ethnopharmacol. 2021; 265: 113329.
11. Gerchman Y, Inbar M. Distinct antimicrobial activities in aphid galls on Pistacia atlantica. Plant Signal Behav. 2011; 6: 2008-12.
12. Azeez SH, Gaphor SM. Evaluation of Antibacterial Effect against Porphyromonas gingivalis and Biocompatibility of Essential Oil Extracted from the Gum of Pistacia atlantica Kurdica. Biomed Res Int. 2019; 2019: 9195361.
13. Karimi A, Moradi MT, Gafourian A. In vitro anti-adenovirus activity and antioxidant potential of Pistacia atlantica Desf. leaves. Res J Pharmacogn. 2020; 7: 53-60.
14. Batool S, Javaid S, Javed H, Laiba Asim, Shahid I, Khan M, et al. Addressing artifacts of colorimetric anticancer assays for plant-based drug development. Med Oncol. 2022; 39: 198.
15. Garber A, Barnard L, Pickrell C. Review of Whole Plant Extracts With Activity Against Herpes Simplex Viruses In Vitro and In Vivo. J Evid Based Integr Med. 2021; 26: 2515690X20978394.
16. Lozada-Ramírez JD, Ortega-Regules AE, Hernández LR, Anaya de Parrodi C. Spectroscopic and spectrometric applications for the identification of bioactive compounds from vegetal extracts. Appl Sci. 2021; 11: 3039
17. Liu AL, Du GH. Antiviral Properties of Phytochemicals. Dietary Phytochemicals and Microbes. 2012: 93–126.
18. Musarra-Pizzo M, Pennisi R, Ben-Amor I, Smeriglio A, Mandalari G, Sciortino MT. In Vitro Anti-HSV-1 Activity of Polyphenol-Rich Extracts and Pure Polyphenol Compounds Derived from Pistachios Kernels (Pistacia vera L.). Plants (Basel). 2020; 9: 267.
19. Mandalari G, Barreca D, Gervasi T, Roussell MA, Klein B, Feeney MJ, et al. Pistachio Nuts (Pistacia vera L.): Production, Nutrients, Bioactives and Novel Health Effects. Plants (Basel). 2021; 11: 18.
20. Rezaie M, Farhoosh R, Pham N, Quinn RJ, Iranshahi M. Dereplication of antioxidant compounds in Bene (Pistacia atlantica subsp. mutica) hull using a multiplex approach of HPLC-DAD, LC-MS and (1)H NMR techniques. J Pharm Biomed Anal. 2016; 117: 352-62.
21. Jassim SA, Naji MA. Novel antiviral agents: a medicinal plant perspective. J Appl Microbiol. 2003; 95: 412-27.
22. Ozçelik B, Aslan M, Orhan I, Karaoglu T. Antibacterial, antifungal, and antiviral activities of the lipophylic extracts of Pistacia vera. Microbiol Res. 2005; 160: 159-64.
23. Agut H, Bonnafous P, Gautheret-Dejean A. Human Herpesviruses 6A, 6B, and 7. Microbiol Spectr. 2016;4.

GMJ 2024;35:248-254

DOI: http://dx.doi.org/10.12996/gmj.2024.4210

The Effect of "Carvacrol" on Ischemia-Reperfusion Injury in the Skeletal Muscles of Rats

Ratlarda "Karvakrol"ün İskelet Kasında İskemi-Reperfüzyon Hasarı Üzerine Etkisi

Barış Mardin[1], Abdullah Özer[1], Başak Koçak[2], Ayşegül Küçük[3], Mustafa Hakan Zor[1], Şaban Cem Sezen[4], Mustafa Kavutçu[5], Mustafa Arslan[6], Gürsel Levent Oktar[1]

[1]Department of Cardiovascular Surgery, Gazi University Faculty of Medicine, Ankara, Türkiye
[2]Department of Cardiovascular Surgery, Sivas Numune Hospital, Sivas, Türkiye
[3]Department of Physiology, Kütahya Health Sciences University Faculty of Medicine, Kütahya, Türkiye
[4]Department of Histology and Embryology, Kırıkkale University Faculty of Medicine, Kırıkkale, Türkiye
[5]Department of Medical Biochemistry, Gazi University Faculty of Medicine, Ankara, Türkiye
[6]Department of Anesthesiology and Reanimation, Gazi University Faculty of Medicine, Ankara, Türkiye

ABSTRACT

Objective: Ischemia is characterized by an inadequate supply of nutrients and oxygen due to reduced blood circulation to specific tissues or organs. Reperfusion injury refers to the detrimental effects of abrupt exposure of the ischemic tissue to elevated oxygen levels and subsequent generation of reactive oxygen derivatives inside the tissue. In surgical procedures characterized by frequent occurrences of ischemia-reperfusion (I/R), such as transplant and cardiovascular surgery, it is crucial to prioritize the preservation of tissue integrity and mitigation of associated damage. In this study, we formulated a hypothesis suggesting that carvacrol (Car), an aromatic hydrocarbon present in some plant species, might decrease I/R injury by implication of its antioxidant, anti-apoptotic, and anti-inflammatory functions.

Methods: We planned our study with 18 rats, randomly divided into three groups: control group, I/R group, and I/R + Car group. In the control group, laparotomy was performed only, and blood and lower limb muscle tissue samples were taken. In the I/R group, after laparotomy, lower extremity ischemia is achieved for 60 min, and reperfusion is achieved for another 60 min. In I/R + Car, rats were administered 100 mg/kg Car via intraperitoneal injection 30 min after ischemia. Then, 60 min of ischemia and 60 min of reperfusion were achieved. Lower limb muscle samples were examined both histologically and biochemically. We evaluated the levels of malondialdehyde (MDA), glutathione-S transferase (GST), and catalase (CAT). In addition, serum ischemia-modified albumin (IMA) levels were measured.

Results: Our study showed that the findings of I/R injury decreased prominently in the I/R + Car group's samples. GST, MDA, CAT, and IMA levels were significantly higher in the I/R group than in the control

ÖZ

Amaç: İskemi, herhangi bir doku veya organa giden kan akımının azalması veya kesilmesi sonucunda ortaya çıkan besin ve oksijen yetersizliği durumudur. Reperfüzyon hasarı ise iskemiye uğramış bir yapının ani olarak yüksek oksijene maruz kalması ve ortaya çıkan reaktif oksijen türevlerinin dokuda oluşturduğu hasara verilen isimdir. Özellikle transplant cerrahisi, kalp ve damar cerrahisi gibi iskemi-reperfüzyon (İ/R) olaylarının sık görüldüğü cerrahilerde, dokuyu İ/R'den mümkün mertebe korumak, oluşan hasarı en aza indirmek gibi konular önem kazanmaktadır. Çalışmamızda bazı bitkilerde bulunan bir aromatik hidrokarbon olan karvakrol (Car) molekülünün, antioksidan, anti-apoptotik ve anti-enflamatuvar etkileri sayesinde İ/R hasarını önleyebileceği hipotezini kurguladık.

Yöntemler: 200-250 gram ağırlıktaki 18 adet Wistar albino rat rastgele olarak 3 eşit gruba ayrılıp, bu gruplardan birincisi kontrol, ikincisi İ/R, üçüncüsü İ/R + Car grubu olarak belirlendi. Kontrol grubunda sadece laparotomi yapıldı, kan ve alt ekstremite doku örnekleri alındı. İ/R grubunda laparotomi sonrası 60 dakika süreyle alt ekstremite iskemisi yaratıldı ve sonraki 60 dakika boyunca reperfüzyon sağlandı. İ/R + Car grubunda ise 100 mg/kg Car, iskemi yaratılmasından 30 dakika önce intraperitoneal olarak uygulandı. Akabinde 60 dakika iskemi ve 60 dakika reperfüzyon oluşturuldu. Ratların alt ekstremite kas dokusu örneklerinde malondialdehid (MDA), glutatyon S-tranferaz (GST) ve katalaz (CAT) düzeyleri ölçülüp, histopatolojik inceleme yapıldı. Kan örneklerinde ise iskemi modifiye albümin (İMA) düzeyleri ölçüldü.

Bulgular: İ/R + Car grubunun örneklerinde, İ/R hasarını gösteren bulguların istatistiksel olarak anlamlı şekilde azaldığı gösterilmiştir. GST, MDA, CAT ve İMA düzeylerinin; İ/R grubunda, kontrol ve İ/R +

Address for Correspondence/Yazışma Adresi: Başak Koçak, MD, Department of Cardiovascular Surgery, Sivas Numune Hospital, Sivas, Türkiye
E-mail / E-posta: drbasakkocak@gmail.com
ORCID ID: orcid.org/0000-0002-3767-0687

Received/Geliş Tarihi: 13.05.2024
Accepted/Kabul Tarihi: 12.06.2024

ABSTRACT

and I/R + Car groups. (p=0.024, p=0.010, p=0.030 and p<0.0001; respectively). Histopathological examination revealed no degeneration in the control group. Conversely, contraction, hypertrophy, nucleus degeneration, necrotic fibers, and hyalinization were observed in the I/R group. In the I/R + Car group, inflamed areas were less frequent than in the I/R group, and vascular dilatation of myofibre was noted.

Conclusion: This study demonstrates that the Car molecule protects against I/R injury. Therefore, we contend that more experimental cohort investigations are needed to examine the impact of the Car molecule on preventing I/R injury.

Keywords: Lower extremity, ischemia-reperfusion, carvacrol, malondialdehyde, catalase

ÖZ

Car gruplarına göre anlamlı düzeyde yüksek olduğu görüldü (sırasıyla; p=0,024, p=0,010, p=0,030 ve p<0,0001). Histopatolojik incelemede, kontrol grubunda yer alan kas liflerinde herhangi bir dejenerasyona rastlanmazken, İ/R grubunda miyofibrillerde kontraksiyon, bazı liflerde hipertrofi, nükleuslarda dejenerasyon, nekrotik miyofibriller, hyalinizasyon gibi çizgili kas hasarına dair bulgular izlenmiştir. İ/R + Car grubunda ise bu hasarlı alanların İ/R grubuna göre kısmen daha az olduğu ve miyofibrillerin içerisinde yer alan vasküler yapılarda dilatasyon olduğu gözlenmiştir.

Sonuç: Yaptığımız çalışmada Car molekülünün İ/R hasarını önlemeye yardımcı bir madde olduğu deneysel ve istatistiksel olarak ortaya konulmuştur. Karvakrole dair çeşitli organ ve dokular üzerinde yapılan bazı diğer çalışmalar incelendiğinde benzer sonuçlar alındığını görmekteyiz. Bu nedenle daha geniş deney gruplarında ve daha ileri düzey çalışmalar ile karvakrol molekülünün İ/R hasarını önlemeye yönelik etkisinin araştırılması gerektiğini düşünmekteyiz.

Anahtar Sözcükler: Alt ekstremite, iskemi-reperfüzyon, karvakrol, malondialdehid, katalaz

INTRODUCTION

Ischemia is the insufficiency of oxygen and nutrients in tissues caused by the cessation of blood flow. This condition may develop acutely because of several factors, including vasospasm, embolism, trauma, or chronic conditions such as atherosclerosis, tumor compression, and intimal hyperplasia. Ischemia leads to reduced oxidative metabolism processes in the affected cells. At the same time, alterations in mitochondria and cell membranes result in reversible or permanent cell damage. Furthermore, this condition evokes apoptosis in adjacent cells, leading to significant destruction at both organ and tissue levels (1).

Following the removal of ischemia, the reperfusion phase begins. Reperfusion is the whole of the cellular responses to the sudden increase in oxygen and nutrients that occurs when tissue is restricted from oxygen and nutrients and reaches blood support again.

Developing ischemia-reperfusion (I/R) injury to the skeletal muscle is a possible medical condition. Inadequate or no blood supply for muscle tissue causes ischemia. When blood supply is restored, reperfusion may unexpectedly increase the ratio of mortality and morbidity due to extended inflammation and necrotic and apoptotic events caused by reactive oxygen species (ROS) (2).

The primary target of ROS is polyunsaturated fatty acids in the cell membrane. The modifications that occur when ROS specifically targets these fatty acids are essential mechanisms responsible for cellular damage. Lipid peroxide radicals are produced by the interaction between fatty acids and ROS. Metals present in surroundings can catalyze processes involving lipid peroxides, forming breakdown components such as propanal, hexanal,4-hydroxynonenal, and malondialdehyde (MDA) (3).

Another parameter we measured in our manuscript, ischemia-modified albumin (IMA), which can be defined as a molecule related to injury, is increased in plasma after ischemia due to changes in the N-terminal structure. It is assumed that the fundamental reason for this change is increased hydroxyl in reperfusion (4).

glutathione-S transferase (GST) is an enzyme that removes some toxic compounds that cells encounter or make during metabolism by glucuronidation. This mechanism is especially crucial in tissues with high metabolic activity, such as the liver and kidney. Mitochondrial levels play a vital role in cells affected by I/R damage and can save the cell from death (5).

Catalase (CAT) facilitates the transformation of hydrogen peroxide (H_2O_2) molecules, which are present in peroxisomes in various tissues, particularly in the liver, and released due to the action of enzymes like xanthine oxidase and urate oxidase in these organelles, into water (H_2O) and oxygen (O_2) molecules. If tissue levels of CAT are insufficient after I/R injury, protection against the detrimental impacts of H_2O_2 will be difficult cellular damage.

Carvacrol (Car) is a monoterpenoid phenol compound present in different amounts in plants like red bergamot (monarda didyma), black cumin (nigella sativa), and corn lavender (lavandula multifida), particularly in thyme species (origanum spp.). It is also found in other aromatic compounds related to the terpene and phenol groups. It is primarily present in the oil of oregano (O. vulgare), thyme (T. vulgris), pepperwort (lepidium flavum), wild bergamot (citrus aurantium var. bergamia loisel), and other plants (6). The literature emphasizes its anti-oxidan (7), anti-inflamatory (8), anti-bacteriel (9), and pro-apoptotic (10) in a few cancer species and anti-apoptotic (11) in cells affected by environmental conditions.

In our study, we aimed to determine the effects of Car on I/R injury by measuring specific cell damage markers mentioned in rats.

MATERIALS AND METHODS

The experimental methods were appropriately implemented with the approval granted by our institution, the Gazi University Local Animal Care and Use Committee (approval number: E.184884, date: 27.12.2017). Car was obtained from Sigma-Aldrich in a sterile glass container at a concentration of >98% (product number: W224502-100G-K).

Study Design and Experimental Protocol

A total of 18 male Wistar albino rats, weighing 200-250 gr, were fed and kept at a temperature of 20-21 °C, considered a light cycle. Their food and water supplies were not interrupted until two hours before the experiment began. The rats were randomized to three groups (n=6); the Control group (C), the I/R group, and the I/R + Car group.

Before the surgical procedure, the rats were subjected to an intraperitoneal injection of 50 mg/kg ketamine and 10 mg/kg xylazine while in a supine position and exposed to a warm lamp. A dosage of 100 mg/kg of Car was administered to the I/R + Car group via intraperitoneal injection 30 minutes before laparotomy. Rats with aseptically prepared skin underwent midline laparotomy. In the I/R and I/R + Car groups, the aorta was carefully explored at the infrarenal level after the intestines were removed with wet gauze. A non-traumatic microvascular clamp was placed in the infrarenal abdominal aorta (IAA). The microvascular clamp in IAA was removed after 60 min, and reperfusion was achieved for 60 min. The absence of a pulse in the distal aorta throughout the clamping procedure confirmed aortic ischemia. In contrast, the recurrence of a pulse in the distal aorta after removing the clamp confirmed aortic reperfusion. In the control group, laparotomy was the sole procedure, and I/R was not performed. Saline was applied to the peritoneal cavity at the appropriate temperature to reduce heat and fluid loss from the abdomen during periods of I/R, and the abdominal incision was temporarily covered with wet gauze after the clamp was placed and removed from the IAA. In the final stage of the study, adequate anesthesia and analgesia were provided, tissue samples were collected, and the rats were euthanized via intracardiac blood collection.

Histopathological and Biochemical Analyses

Muscle tissue samples from the lower extremities were preserved in a 10% formaldehyde solution for histological analysis, and the presence of muscle atrophy-hypertrophy, degeneration, congestion, leukocyte cell infiltration, muscle nuclei-oval-pyknotic nucleus, fragmentation, hyalinization, and apoptosis were examined under a light microscope.

Lower extremity muscle tissue samples were taken for biochemical examination and stored at 80 °C, and the MDA, GST, and CAT levels were evaluated in the tissues using ELISA.

The blood samples were stored at +4 °C and then centrifuged. IMA levels were then determined by spectrophotometry.

Statistical Analysis

The data were analyzed using variance analysis in the Statistical Package for the Social Sciences (Chicago, IL, USA) 22.0 program for Windows statistical software. The Kruskal-Wallis test was used to evaluate biochemical and histological markers. The value of $p<0.05$ was considered statistically significant.

RESULTS

In GST, there was a significant difference among groups. (p=0.024). GST enzyme activity was remarkably higher in the I/R group than in the control and I/R + Car groups (p=0.009, p=0.046, respectively) (Table 1).

Similarly, we also found a notable discrepancy in CAT (p=0.030). CAT levels were significantly lower in the control and I/R + Car groups than in the I/R group (p=0.016, p=0.028, respectively) (Table 1).

Another important difference was in MDA (p=0.010). Similarly, MDA levels were significantly increased in the I/R group compared with the control and I/R + Car groups, as shown in Table 1 (p=0.003, p=0.033, respectively).

A significant difference was observed when the groups were compared on the basis of IMA levels (p<0.0001). In the I/R group, IMA levels were significantly higher than those in the control and I/R + Car groups, significantly (p<0.0001, p=0.001, respectively) (Table 1).

In Figures 1-6, normal lower extremity muscle tissue is shown. Histopathological examination revealed no degeneration in the control group. On the other hand, contraction, hypertrophy, nucleus degeneration, necrotic fibers, and hyalinization were prominently observed in the I/R group, as shown in Figure 7-12. In the I/R + Car group, these inflamed areas were subtle compared with the I/R group, and vascular dilatation of myofibre was also noted (Figure 13-18).

DISCUSSION

In the present study, we hypothesized that the parameters of cell damage, GST, MDA, IMA, and CAT, would decrease in the I/R + CAR group. Our results are in accordance with our hypothesis.

In the literature, IMA has proven to be used in assessing the efficacy of specific substances applied in clinical settings to prevent I/R injury (12). Regarding other parameters we measured, GST and MDA also had results similar to those of previous research (13,14).

Research on various age groups has indicated that they may have increased vulnerability to damage caused by I/R due to lower levels of CAT activity and quantity at an early age (15). It has also been reported that CAT replacement using biotechnology products, which increases intracellular CAT activity, can reduce cellular damage (16).

Based on the current understanding, there is a limitation of research examining the impact of Car on I/R injury in the skeletal muscles of

Table 1. Muscle tissue anti-oxidant enzyme activities and oxidant (MDA) levels [mean ± S.E.]

	Control group, (n=6)	I/R group, (n=6)	I/R + Car group, (n=6)	p**
GST (mIU/mg-protein)	0.14±0.02*	0.22±0.02	0.16±0.02*	0.024
CAT (IU/mg-protein)	520.33±53.23*	705.00±58.90	538.33±26.22*	0.030
MDA (nmol/mg-protein)	0.99±0.18*	1.75±0.14	1.23±0.14*	0.010
IMA (ΔA)	0.12±0.01*	0.18±0.01	0.14±0.01*	<0.0001

*P<0.05: Compared to group I/R, **Significance level with Kruskal-Wallis test p<0.05. MDA: Malondialdehyde, GST: Glutathione-S transferase, CAT: Catalase, IMA: Ischemia-modified albumin, S.E.: Standard error, I/R: Ischemia-reperfusion, Car: Carvacrol.

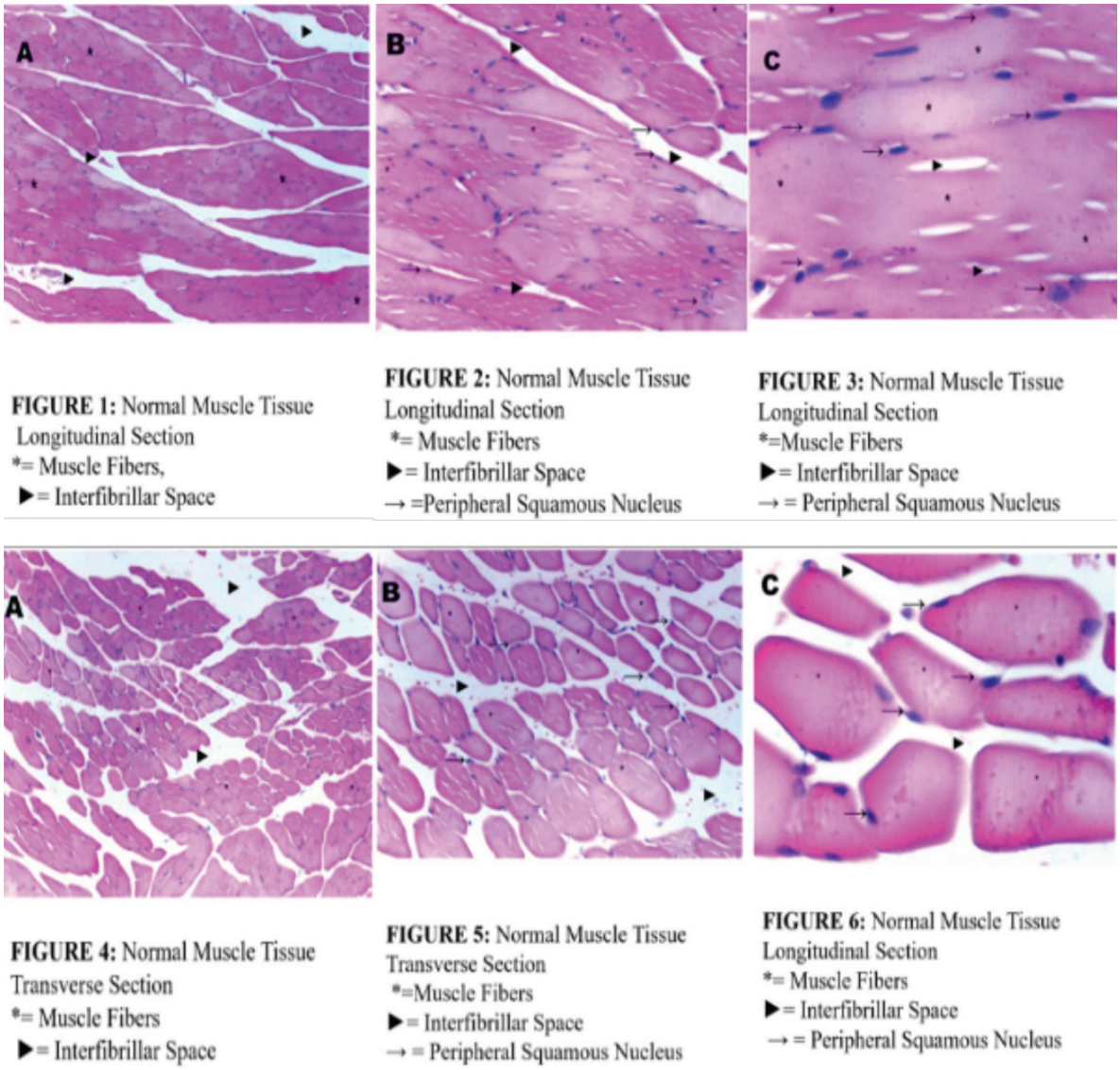


Figure 1-6. Lower extremity muscle tissue.

the lower extremities. However, additional research on Car's effects on other organs and tissues has revealed that outcomes compatible with our research have been achieved (1,17-19).

Metabolic and structural changes are observed in cells during the ischemia phase. When blood flow to tissue terminates, cellular oxidative phosphorylation declines, reducing the production of high-energy phosphates like adenosine 5'-triphosphate (ATP) and phosphocreatine. The release of energy reserves in cells inhibits the cell membrane's Na^+/K^+- ATPase pump. Subsequently, the concentrations of Na^+ and Ca^{+2} within the cells increased. Elevating the concentration of Ca^{+2} ions within the cell is detrimental to cell viability. At the same time, it triggers the activation of many enzymes, including phospholipase, protease, and endonuclease, which begin the cascade of events leading to apoptosis. During this period, the ion concentration of cells changes, relating to increased production of proinflammatory cytokines. At the same time, there is a decrease in the secretion of antioxidant enzymes. The immune system's antioxidant processes generally eliminate ROS from the surroundings, maintaining a balance inside the organism. However, because of earlier phases, during reperfusion, ROS are disseminated throughout the body via systemic circulation (20,21).

Despite the accumulation of data, the precise process of I/R injury still needs to be better understood. As mentioned before, I/R injury may be observed in numerous clinical situations. It is important to note that although these situations might have similar pathogenesis, essential differences exist in their treatment strategies regarding the primary condition. In reperfusion, it is vital to have a hedge to protect cells against I/R injury due to leukocyte migration and its inflammatory process. In some clinical circumstances, such as transplant surgery, immunosuppressive treatments are commonly used to suppress immune responses. However, immunosuppressive

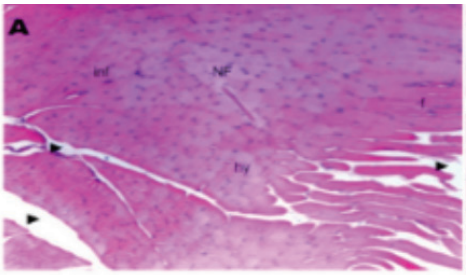


FIGURE 7: I/R Group Muscle Tissue
Longitudinal Section
►= Interfibrillar Space
NF= Necrotic Fibrils
hy= Hyalinization
f= Fragmantetion
inf= Infiltration

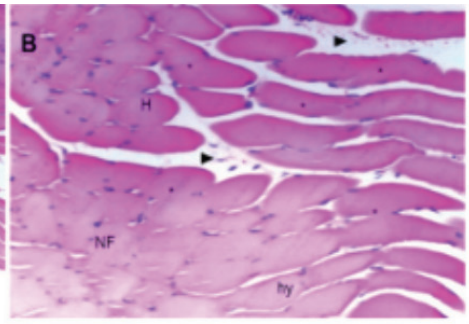


FIGURE 8: I/R Group Muscle Tissue
Longitudinal Section
►=Interfibrillar Space
NF=Necrotic Fibrils
hy= Hyalinization
H= Hypertrophy

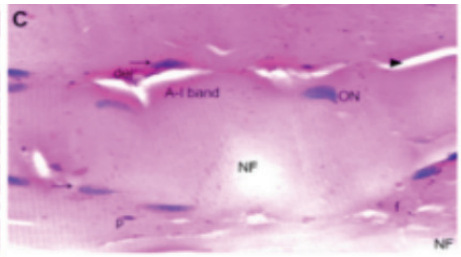


FIGURE 9: I/R Group Muscle Tissue
Longitudinal Section
►= Interfibrillar Space
NF= Necrotic Fibrils
f= Fragmentation
p= Picnotic Nucleus
ON= Oval Nucleus
dej= Degeneration
→ = Peripheral Squamous Nucleus
A-I Band= Sarcomer Bands

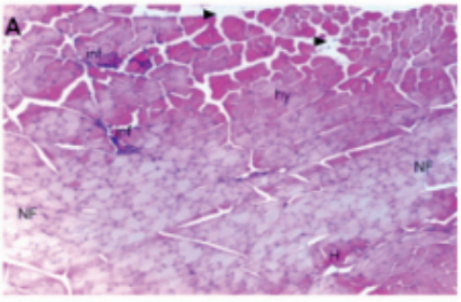


FIGURE 10: I/R Group Muscle Tissue
Transverse Section
►= Interfibrillar Space
NF= Necrotic Fibrils
H= Hypertrophy
dej= Degeneration
hy=Hyalinization
inf=Infiltration

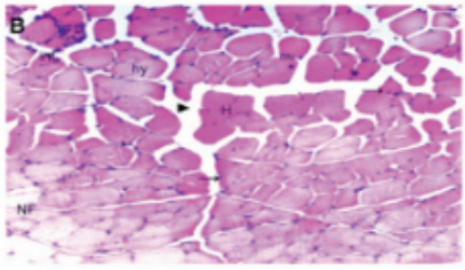


FIGURE 11: I/R Group Muscle Tissue
Transverse Section
*=Muscle Fibers
►= Interfibrillar Space
NF= Necrotic Fibrils
inf= Infiltration
ON= Oval Nucleus
H= Hypertrophic
hy=Hyalinization
→ =Peripheral Squamous Nucleus

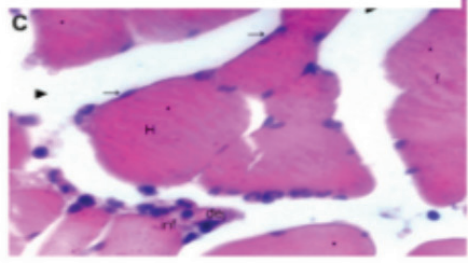


FIGURE 12: I/R Group Muscle Tissue
Transverse Section
*=Muscle fibers
►=Interfibrillar Space
f= Fragmentation
inf=Infiltration
ON= Oval Nucleus
dej= Degeneration
H= Hypertrophy
→ =Peripheral Squamous Nucleus

Figure 7-12. Lower extremity I/R group muscle tissue.
I/R: Ischemia-reperfusion.

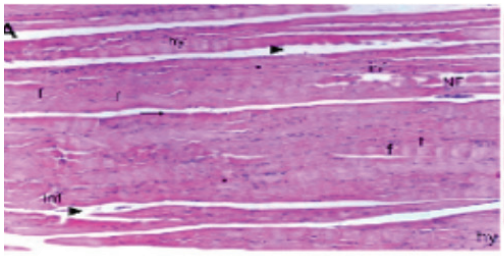


FIGURE 13: I/R+Car Group Muscle Tissue
Longitudinal Section
*=Muscle Fibers
► Interfibrillar Space
f= Fragmentation
inf Infiltration
NF=Necrotic Fibrils
hy Hyalinization
→ =Peripheral Squamous Nucleus

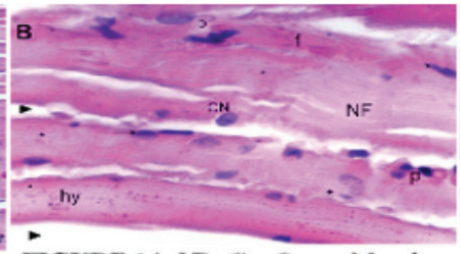


FIGURE 14: I/R−Car Group Muscle
Longitudinal Section
*=Muscle Fibers
►= Interfibrillar Space
f= Fragmentation
NF= Necrotic Fibrils
hy Hyalinization
ON=Oval Nucleus
p Picnotic Nucleus
H=Hypertrophy
→ = Peripheral Squamous Nucleus

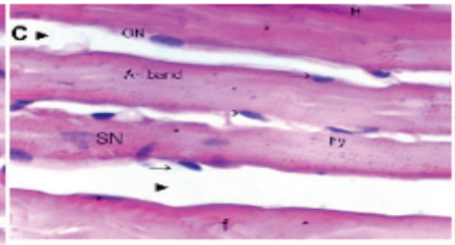


FIGURE 15: I/R+Car Group Muscle
Longitudinal Section
*=Muscle Fibers
► Interfibrillar Space
f= Fragmentation
hy= Hyalinization
ON= Oval Nucleus
H= Hypertrophy
→ A-I Band Sarcomer Bands.
SN(CN)= Central Nuclues

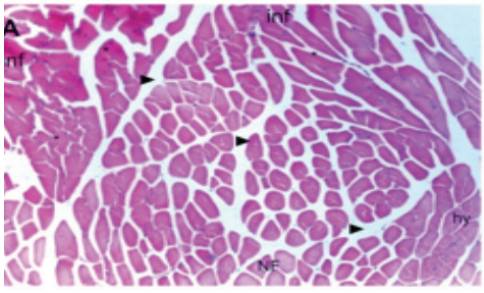


FIGURE 16: I/R+Car Group Muscle
Transverse Section
*= Muscle fibers
►= Interfibrillar Space
inf= Infiltration
NF= Necrotic Fibrils
hy= Hyalinization

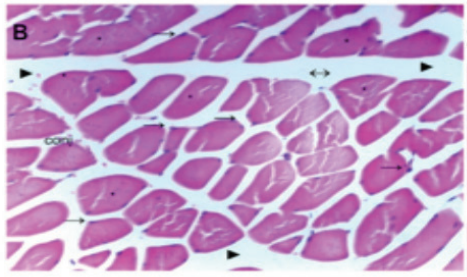


FIGURE 17: I/R+Car Group Muscle
Transverse Section
*=Muscle Fibers
►=Interfibrillar Space
H= Hypertrophy
→ = Peripheral Squamous Nucleus
conj=Conjestion
↔ =Vasodilatation

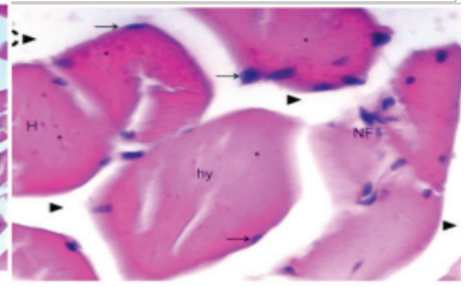


FIGURE 18: I/R+Car Group Muscle
Transverse Section
*= Muscle Fibers
►= Interfibrillar Space
NF=Necrotic Fibrils
hy= Hyalinization
H= Hypertrophy
→ = Peripheric Squamous Nucleus

Figure 13-18. Lower extremity I/R + Car group muscle tissue.

I/R: Ischemia-reperfusion, Car: Carvacrol.

medication is not suitable for cardiac or vascular surgery, and is not recommended at least. These major discrepancies might help to understand the reasons for the considerable research on various pharmacological agents that can be used for I/R injury. Previous studies have examined the impact of Car on I/R damage in various tissues, including myocardial tissue (22), cerebral tissue (23), gastric tissue (24), and liver tissue (25). They demonstrated that Car administration might have favorable outcomes in mitigating I/R injury. However, to the best of our knowledge, more studies are needed on this subject, specifically skeletal muscle.

CONCLUSION

In summary, after further research, particularly in humans, confirms the findings of our study, a detailed demonstration of Car's protective effects against I-R injury will be evident, leading to an expansion of its indications for use.

Ethics

Ethics Committee Approval: The experimental methods were appropriately implemented with the approval granted by our institution, the Gazi University Local Animal Care and Use Committee (approval number: E.184884, date: 27.12.2017).

Informed Consent: Patient approval has not been obtained as it is performed on animals.

Author Contributions

Concept: B.M., A.Ö., B.K., A.K., M.H.Z., Ş.C.S., M.K., M.A., G.L.O., Design: B.M., A.Ö., B.K., A.K., M.H.Z., Ş.C.S., M.K., M.A., G.L.O., Data Collection or Processing: B.M., A.Ö., B.K., A.K., M.H.Z., Ş.C.S., M.K., M.A., G.L.O., Analysis or Interpretation: B.M., A.Ö., B.K., A.K., M.H.Z., Ş.C.S., M.K., M.A., G.L.O., Literature Search: B.M., A.Ö., B.K., A.K., M.H.Z., Ş.C.S., M.K., M.A., G.L.O., Writing: B.M., A.Ö., B.K., A.K., M.H.Z., Ş.C.S., M.K., M.A., G.L.O.

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

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

REFERENCES

1. Sims NR, Muyderman H. Mitochondria, oxidative metabolism and cell death in stroke. Biochim Biophys Acta. 2010; 1802: 80-91.
2. Kılıç Y, Küçük A, Arslan M, Kirişçi M, Özer A, Mortaş T, et al. Assessment of the Effects of Quercetin on Lung Injury After Hind Limb Ischemia Reperfusion in Rats. Journal of Harran Medical Faculty. 2022; 19: 343-49.
3. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxidative Medicine and Cellular Longevity. 2014, p. 1-31.
4. Roy D, Quiles J, Gaze DC, Collinson P, Kaski JC, Baxter GF. Role of reactive oxygen species on the formation of the novel diagnostic marker ischaemia modified albumin. Heart. 2006; 92: 113-4.
5. Marí M, Morales A, Colell A, García-Ruiz C, Fernández-Checa JC. Mitochondrial Glutathione, a Key Survival Antioxidant. Antioxid Redox Signal. 2009; 11: 2685-700.
6. Gholami-Ahangaran M, Ahmadi-Dastgerdi A, Azizi S, Basiratpour A, Zokaei M, Derakhshan M. Thymol and carvacrol supplementation in poultry health and performance. Vet Med Sci. 2022; 8: 267-88.
7. El-Sayed el-SM, Mansour AM, Abdul-Hameed MS. Thymol and Carvacrol Prevent Doxorubicin-Induced Cardiotoxicity by Abrogation of Oxidative Stress, Inflammation, and Apoptosis in Rats. J Biochem Mol Toxicol. 2016; 30: 37-44.
8. Hotta M, Nakata R, Katsukawa M, Hori K, Takahashi S, Inoue H. Carvacrol, a component of thyme oil, activates PPARalpha and gamma and suppresses COX-2 expression. J Lipid Res. 2010; 51: 132-9.
9. Ultee A, Smid EJ. Influence of carvacrol on growth and toxin production by Bacillus cereus. Int J Food Microbiol. 2001; 64: 373-8.
10. Bhakkiyalakshmi E, Suganya N, Sireesh D, Krishnamurthi K, Saravana Devi S, Rajaguru P, et al. Carvacrol induces mitochondria-mediated apoptosis in HL-60 promyelocytic and Jurkat T lymphoma cells. Eur J Pharmacol. 2016; 772: 92-8.
11. Zeidán-Chuliá F, Gursoy M, de Oliveira BH, Gelain DP, Könönen E, Gursoy UK, et al. Focussed microarray analysis of apoptosis in periodontitis and its potential pharmacological targeting by carvacrol. Arch Oral Biol. 2014; 59: 461-9.
12. Karahan SC, Koramaz I, Altun G, Uçar U, Topbaş M, Menteşe A, et al. Ischemia-Modified Albumin Reduction after Coronary Bypass Surgery Is Associated with the Cardioprotective Efficacy of Cold-Blood Cardioplegia Enriched with N-Acetylcysteine: A Preliminary Study. Eur Surg Res. 2010; 44: 30-6.
13. Khalatbari Mohseni G, Hosseini SA, Majdinasab N, Cheraghian B. Effects of N-acetylcysteine on oxidative stress biomarkers, depression, and anxiety symptoms in patients with multiple sclerosis. Neuropsychopharmacol Rep. 2023; 43: 382-90.
14. Blogowski W, Dolegowska B, Pikula E, Gutowski P, Starzynska T. The effect of PGE administration on the activity of oxidative system in erythrocytes and platelets during ischemia reperfusion injury and on postoperative renal function in patients undergoing open abdominal aortic aneurysm reconstruction. J Biol Regul Homeost Agents.2012; 26: 429-38.
15. Cabigas EB, Ding G, Chen T, Saafir TB, Pendergrass KD, Wagner MB, et al. Age- and chamber-specific differences in oxidative stress after ischemic injury. Pediatr Cardiol. 2012; 33: 322-31.
16. Huang GQ, Wang JN, Tang JM, Zhang L, Zheng F, Yang JY, et al. The Combined Transduction of Copper, Zinc-Superoxide Dismutase and Catalase Mediated by Cell-Penetrating Peptide, PEP-1, to Protect Myocardium from Ischemia-Reperfusion Injury. J Transl Med. 2011; 9: 73.
17. Wassel CL, Loomba R, Ix JH, Allison MA, Denenberg JO, Criqui MH. Family history of peripheral artery disease is associated with prevalence and severity of peripheral artery disease: the San Diego population study. J Am Coll Cardiol. 2011; 58: 1386-92.
18. Valentine RJ, Guerra R, Stephan P, Scoggins E, Clagett GP, Cohen J. Family history is a major determinant of subclinical peripheral arterial disease in young adults. J Vasc Surg. 2004; 39: 351-6.
19. Ridker PM, Stampfer MJ, Rifai N. Novel risk factors for systemic atherosclerosis: a comparison of C-reactive protein, fibrinogen, homocysteine, lipoprotein(a), and standard cholesterol screening as predictors of peripheral arterial disease. JAMA. 2001; 285: 2481-5.
20. Lee Y, Gustafsson AB. Role of apoptosis in cardiovascular disease. Apoptosis. 2009; 14: 536-48.
21. Köksal Z, Kurtipek Ö, Arslan M, Dursun AD, Yığman Z, Özer A. (2023). Protective effects of hydrogen rich saline solution in rats with experimental myocardial ischemia reperfusion injury. Heliyon. 2023; 9: e22973.
22. Song X, Chen A, Liu Y, Wang XB, Zhou Y, Liu L, et al. Carvacrol pretreatment attenuates myocardial oxidative stress and apoptosis following myocardial ischemia-reperfusion in mice. Nan Fang Yi Ke Da Xue Xue Bao. 2013; 33: 1624-7.
23. Yu H, Zhang ZL, Chen J, Pei A, Hua F, Qian X, et al. Carvacrol, a Food-Additive, Provides Neuroprotection on Focal Cerebral Ischemia/Reperfusion Injury in Mice. PLoS One. 2012; 7: e33584.
24. Oliveira IS, da Silva FV, Viana AF, dos Santos MR, Quintans-Júnior LJ, Martins Mdo C, et al. Gastroprotective activity of carvacrol on experimentally induced gastric lesions in rodents. Naunyn Schmiedebergs Arch Pharmacol. 2012; 385: 899-908.
25. Canbek M, Uyanoglu M, Bayramoglu G, Senturk H, Erkasap N, Koken T, et al. Effects of carvacrol on defects of ischemia-reperfusion in the rat liver. Phytomedicine. 2008; 15: 447-52.

GMJ 2024;35:255-259

DOI: http://dx.doi.org/10.12996/gmj.2023.3891

Role of Inflammation Markers and Osteopontin in the Prediction of Fetal Stress

Fetal Distresde Osteopontin ve Enflamasyon Markerlarının Rolü

Betül Tokgöz Çakır, Hande Esra Koca, Elif Akkaş Yılmaz, Tuncay Küçüközkan

Department of Obstetrics and Gynecology, University of Health Sciences Türkiye, Dr. Sami Ulus Women's and Children's Health Training and Research Hospital, Ankara, Türkiye

ABSTRACT

Objective: To determine the possible association between acute fetal stress during labor and maternal blood levels of osteopontin (OPN), white blood cells, sedimentation, and C-reactive protein (CRP).

Methods: The study included women with a term pregnancy (37 weeks or more) who had a cesarean section during the active phase of labor. The study included 30 term pregnancies who underwent cesarean section for fetal distress and 30 pregnant women who underwent cesarean section for other indications (prior uterine surgery, head-pelvis incompatibility, large baby, non-progressing plot) as a control group. The levels of OPN and other inflammatory markers (leukocytes, sedimentation, CRP) in the maternal venous blood of 60 pregnant women were compared, and whether there was a significant association between the groups was investigated.

Results: OPN levels in the fetal stress group were higher than those in the control group, and the difference was statistically significant (p=0.008). There was no statistically significant difference in white blood cell, blood sedimentation, and CRP levels between the two groups (p>0.005).

Conclusion: Placental inflammation plays a role in the etiology of fetal stress, and OPN may be released because of fetal stress. The increase in OPN in maternal blood during labor may be an important marker for predicting fetal stress.

Keywords: CRP, C-section, fetal distress, leukocyte count, osteopontin

ÖZ

Amaç: Doğum sırasında akut fetal stres ile anne kanındaki osteopontin (OPN), beyaz kan hücreleri, sedimantasyon ve C-reaktif protein (CRP) seviyeleri arasındaki olası ilişkiyi belirlemektir.

Yöntemler: Çalışmaya term gebeliği (37 hafta ve üzeri) olan ve doğumun aktif fazında sezaryen olan kadınlar dahil edildi. Çalışmaya fetal distres nedeniyle sezaryen uygulanan 30 term gebelik ve diğer endikasyonlar (geçirilmiş uterin cerrahi, baş-pelvis uyumsuzluğu, büyük bebek, ilerlemeyen eylem) nedeniyle sezaryen yapılan 30 gebe kontrol grubu olarak alındı. Altmış gebenin maternal venöz kanında OPN ve diğer inflamatuar belirteçlerin (lökosit sayısı, sedimantasyon, CRP) düzeyleri karşılaştırıldı ve gruplar arasında anlamlı bir ilişki olup olmadığı araştırıldı.

Bulgular: Fetal stres grubunda OPN düzeyleri kontrol grubuna göre daha yüksekti ve aradaki fark istatistiksel olarak anlamlıydı (p=0,008). İki grup arasında lökosit sayısı, sedimentasyon ve CRP düzeylerinde istatistiksel olarak anlamlı fark yoktu (p>0,005).

Sonuç: Plasental inflamasyon fetal stresin etiyolojisinde rol oynar ve fetal stres nedeniyle OPN salınabilir. Doğum sırasında anne kanındaki OPN artışı, fetal stresi tahmin etmede önemli bir belirteç olabilir.

Anahtar Sözcükler: CRP, sezaryen, fetal distres, lökosit sayısı, osteopontin

Address for Correspondence/Yazışma Adresi: Betül Tokgöz Çakır, MD, Department of Obstetrics and Gynecology, University of Health Sciences Türkiye, Dr. Sami Ulus Women's and Children's Health Training and Research Hospital, Ankara, Türkiye
E-mail / E-posta: btltkgz@gmail.com
ORCID ID: orcid.org/0000-0003-0202-4981

Received/Geliş Tarihi: 22.05.2023
Accepted/Kabul Tarihi: 10.07.2023

INTRODUCTION

Fetal distress is a pathophysiologic condition in which fetal oxygen is not available in sufficient quantity (1). Fetal hypoxia can lead to permanent fetal damage or death if not corrected or if emergency delivery is not performed. When a clinical diagnosis of fetal distress is made, physicians aim for rapid delivery because they cannot clearly determine the severity of hypoxia (2). There are antepartum and intrapartum markers of fetal distress, such as abnormal fetal heart rate (recurrent late decelerations, wavy baseline bradycardia), decreased blood oxygen pressure (pO_2) in fetal blood, meconium staining of amniotic fluid, lactate increase in fetal scalp, and low pH (3-5). The diagnosis of postpartum fetal distress was based on low pH in cord blood, worsening Apgar score (6,7), and other parameters (8,9). The negative predictive value of the tests used for antepartum assessment (fetal movements, fetal heart rate, fetal respiration, and amniotic fluid) is 99.8%, whereas the positive predictive value ranges from 10% to 40% (6). This means that the fetus is not always under stress when the test is positive.

The body's first response to the immunological phase is the innate, non-specific response that precedes the specific immune response. The acute phase response is a systemic response to local or systemic disturbances of homeostasis due to infection, tissue damage, trauma or surgery, neoplastic growth, or immunologic disturbances (10). When tissue is damaged, the tissue itself triggers a series of responses. Pro-inflammatory cytokines are released, and the vascular system and inflammatory cells are activated. These reactions are in turn associated with the production of additional cytokines and other inflammatory mediators, which are distributed in the extracellular fluid and enter the bloodstream (11). The most important of these acute-phase reactors are the erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), fibrinogen, and ferritin. While ESR and CRP are most commonly used, fibrinogen and ferritin are rarely used (11).

Osteopontin (OPN) is a phosphorylated and glycosylated protein found in various biological fluids (12,13). It is found in the epithelial cells of the bile duct, pancreas, lung, sweat glands, placenta, gastrointestinal tract, urinary tract, breast, and reproductive system. Because OPN is released from the epithelium at the sites of contact between the embryo and uterus during pregnancy, it is believed to be able to communicate between the placenta and uterine tissue to support pregnancy (14,15).

The purpose of this study was to determine if there is a relationship between fetal stress, OPN, white blood cell (WBC) count, sedimentation, and CRP levels measured in maternal venous blood during active labor.

MATERIALS AND METHODS

This prospective cross-sectional study included pregnant women who underwent cesarean section for fetal distress and indications other than fetal distress. The study was conducted at Dr. Sami Ulus Women's and Children's Health Teaching and Research Hospital, Department of Obstetrics and Gynecology, Ankara, Türkiye, according to the principles of the Declaration of Helsinki. Institutional Review Board approval was obtained before the start of the study, and mothers provided signed informed consent [Ankara Etlik Zübeyde Hanım Gynecology Traning and Research Hospital Ethics Committee; (approval number: 2017/7, date: 13.09.2017)].

Sixty pregnant women with a gestational age of 37 weeks participated in the study. Of these, 30 were delivered by cesarean section when signs of fetal distress were present. The other 30 cases were pregnancies delivered by cesarean section for reasons other than fetal distress (previous uterine surgery, head-pelvis incompatibility, large baby, and breech presentation). Cesarean section was performed during active labor in all patients. Fetal distress was confirmed by fetal meconium staining, an APGAR score of 6, acidotic cord blood, or the need for neonatal intensive care.

The levels of OPN and other inflammatory markers (leukocytes, sedimentation, CRP) in the venous blood of pregnant women were compared, and whether there was a significant association between these markers and fetal distress was investigated. Women between the ages of 20 and 40 years who had a singleton pregnancy participated in the study. Pregnant women with obstetric complications (gestational diabetes, preeclampsia/eclampsia) or chronic systemic diseases (thyroid dysfunction, diabetes, hypertension, chronic kidney/heart disease) were excluded from the study. In total, 15 mL of venous blood from the patients was divided into three equal parts. Two venous blood samples were collected in EDTA tubes for the measurement of CRP and OPN, and one venous blood sample was collected for the measurement of blood sedimentation. Serum was separated by centrifugation and stored at -80 °C until analysis. Plasma concentrations of OPN and CRP were measured using ELISA. At the end of the ELISA study, the CRP and OPN levels of each serum sample were calculated using the optical density values of known calibrators.

Serum OPN levels in participants' samples were analyzed using the Elabsiciens Human OPN ELISA Kit from Atlas Biotechnology Laboratory. At the hospital, leukocyte dissection and laser and total leukocyte electrical impedance measurements were performed using the Beckman Coulter LH 780 automated blood counter. Western blotting was used to measure blood sedimentation. The birth dates of pregnant women (cesarean indication, week of gestation) were obtained from delivery room records, and the results were recorded by the researchers along with the values of OPN, CRP, leukocytes, and blood sedimentation.

Primary outcome measures, levels of maternal OPN, and other inflammatory markers will be the success rate in predicting fetal distress; secondary outcomes will be age, gestational week, and birth weight of women who underwent cesarean section for fetal distress and women who underwent cesarean section outside this indication.

Statistical Analysis

Statistical analysis was performed using SPSS (IBM SPSS Statistics 20). Frequency tables were used to interpret the results. Variables were examined using visual (histogram, probability plots) and analytic methods (Kolmogorov-Smirnov test/Shapiro-Wilk test) to determine whether or not they were normally distributed. Descriptive analyses were performed using means and standard deviations for normally distributed variables, and medians and quartiles (Q1-Q3) were used for numerical data that were not normally distributed. The Mann-Whitney U test and Student's t-test were performed to compare

parametric variables in the two groups with and without normal distribution, respectively. The levels of OPN and other inflammatory markers (leukocytes, sedimentation, CRP) in the venous blood of pregnant women were compared, and whether there was a significant association between these markers and fetal distress was investigated. The statistical significance level was set at 0.05 for all analyses.

RESULTS

A total of 60 pregnant women were included in the study. Thirty pregnant women underwent cesarean section with the indication of fetal distress and 30 pregnant women underwent cesarean section without the indication of fetal distress. Cesarean indications included 2 (6%) head-pelvis incompatibility, 4 (14%) non-progressive measures, 3 (10%) breech presentation, 3 (10%) large infants, and 18 (60%) old cesarean sections. The characteristics of the patients and their dates of birth are shown in Table 1. Between the two groups, the WBC count, blood sedimentation (p=0.07), and CRP (p=0.85) levels were not statistically significant (p>0.005). There was a significant difference in OPN levels between the two groups (p=0.006) (Table 2).

DISCUSSION

Currently, efforts are being made worldwide to reduce the number of births by cesarean section. Fetal distress and failure of labor to progress are the most common indications for cesarean section, unless the uterus has been previously operated on (16,17). Previous studies have identified several biomarkers to predict fetal distress, including fetal heart rate patterns, umbilical artery pH, and lactate levels. There are studies suggesting that fetal complications are more common in pregnant women with non-reactive NST results; therefore, NST should be routinely performed as a valuable diagnostic test in the prenatal period (18). In addition to NST, biophysical scores (fetal movements, fetal heart rate, fetal respiration, amniotic fluid, and NST), modified biophysical scores (amniotic fluid and NST), and various Doppler parameters (umbilical artery, fetal cerebral artery, ductus venosus) are used to assess fetal well-being. Numerous studies in the literature question the reliability of these tests. However, a test with high positive and negative predictive values that could be an alternative to these tests has not yet been found.

ESR, CRP, and WBC count are commonly used markers of inflammation. In this study, we measured maternal serum OPN levels in addition to these markers. We hypothesized that they might increase in maternal serum because of possible inflammation of the placenta during fetal stress. OPN plays an important role in inflammation, biomineralization, cell viability, and wound healing. In addition, OPN is also known to be released from the placenta. OPN is thought to increase inflammation in acute and chronic inflammatory diseases by contributing to the proliferation of macrophages and T cells in areas of inflammation (19).

In our study, we found that blood sedimentation, CRP, and WBC levels, which are known to increase during normal pregnancy (20,21), did not increase more in maternal blood during fetal distress. In their studies evaluating the diagnostic value of chorioamnionitis, Amirabi et al. (22) examined 71 pregnant women with early rupture of membranes for CRP, ESR, and WBC but emphasized that the diagnostic value of ESR was minimal. In contrast to these studies, Nowak et al. (23), in their work with 80 pregnant women, considered that CRP is the most reliable indicator of histological chorioamnionitis and that it may be able to diagnose intrauterine infection earlier than WBC or ESH. However, in our study, no significant association was found between CRP levels and fetal distress. These parameters, whose levels increased in chorioamnionitis, did not increase in acute fetal distress during the active phase. In contrast to chorioamnionitis, it can be assumed that fetal distress is not only of placental origin, but that both fetal and maternal causes contribute to this condition.

Numerous studies have been conducted in the literature using various biomarkers to predict fetal distress. Bligh et al. (24) found in their study of 438 pregnant women that low placental growth factor was associated with low birth weight, cesarean section due to fetal

Table 1. Patients' characteristics

Variable	Fetal distress (n=30)	Control group (n=30)	p-value
Maternal age median (Q1-Q3)	26 (25-30.2)	29.5 (24-33)	0.32
BMI (kg/m^2) median (Q1-Q3)	28.4 (26.3-31)	28.1 (26-29.9)	0.56
Birth week (week) median (Q1-Q3)	39.5 (38-41)	39 (38-39)	0.07
Birth weight (gram) (mean ± SD)	3,336±458	3,273±414	0.57

Data are presented as median (Q1-Q3) or mean ± standart deviation. A p-value of <0.05 indicates a significant difference. BMI: Body mass index, SD: Standard deviation.

Table 2. Examination of the values taken from maternal venous blood according to cesarean status

Variable (n=60)	Cesarean birth		p-value
	Fetal stres (n=30)	Control (n=30)	
Sedimentation (mm/h); median (Q1-Q3)	27 (20-40)	30 (24.7-48.5)	p=0.07
CRP (mg/dL); median (Q1-Q3)	8.6 (4.7-12.7)	7.9 (4.9-13.1)	p=0.85
WBC (mcL); (mean **±** SD)	14.3±4.1	12.6±4	p=0.11
OPN (ng/mL); median (Q1-Q3)	21.9 (20.8-23.3)	20.3 (18.3-22.3)	**p=0.006**

Data are presented as median (Q1-Q3) or mean **±** standart deviation. A p-value of <0.05 indicates a significant difference. Statistically significant p values are in bold. CRP: C-reactive protein, WBC: White blood cell, OPN: Osteopontin.

risk, and poor neonatal outcome. Knight et al. (25) distinguished between weeks of gestation and SLC9B1 methylation in venous blood samples from pregnant women and found that the fetal burden could be estimated. In our study, we found that OPN levels were elevated in the blood of pregnant women who underwent cesarean section for fetal distress before term. Considering the functions of OPN, this situation can be explained by the increased expression of OPN in the placenta during fetal distress.

Plasma OPN concentrations increase in patients with preeclampsia and endothelial damage (26). A study in mice has shown that OPN is required for the initiation of inflammation (27). OPN is known to play a role in biomineralization, inflammation, dystrophic calcification, wound healing, granulomatous formation, fibrosis, nitric oxide regulation, tumor metastasis, and maintenance of cell viability (28).

In their studies of human and mouse placentas, Gabinskaya et al. (29) demonstrated that OPN and fibronectin are expressed in functioning placental compartments. Thus, we can conclude that OPN expression increased in the functional placenta during acute fetal distress and was significantly higher in our study. However, an increase in other inflammatory markers (WBC, ESR, CRP) might be required for chronic inflammatory processes. Therefore, we hypothesized that although OPN levels were higher in the fetal distress group than in the control group, no significant changes were observed in other parameters.

OPN is critical to successful pregnancy. It was observed that the expression levels of natural decidual cells (dNK) and OPN were low, especially in women with repeated pregnancy loss (30). At the same time, OPN levels in cord blood were also measured, and OPN was found to play a physiological role in fetal growth and development (31).

Study Limitations

A limitation of the study is that only a few patients participated in the study, and a larger population is needed on this topic. Because of technical and financial difficulties, we could not measure OPN levels in cord blood. More accurate information can be obtained from OPN measurements from cord blood and maternal blood. We believe that further studies on the functions of OPN during pregnancy are needed.

In our study, we found that OPN was significantly higher in the venous blood of pregnant women who developed fetal distress than in the control group. We believe that OPN may be a useful biomarker for predicting fetal distress.

CONCLUSION

Placental inflammation plays a role in the etiology of fetal distress, and OPN may be released because of fetal distress. We hypothesize that the increase in OPN in maternal blood during labor may be an important marker for predicting fetal distress. Further research on this topic is needed because the mechanisms contributing to the increase in OPN during fetal distress are not yet known; therefore, this increase may be used in predicting fetal distress in the clinical setting.

Ethics

Ethics Committee Approval: Institutional Review Board approval was obtained before the start of the study [Ankara Etlik Zübeyde Hanım Gynecology Traning and Research Hospital Ethics Committee; (approval number: 2017/7, date: 13.09.2017)].

Informed Consent: Mothers provided signed informed consent.

Author Contributions

Concept: B.T.Ç., H.E.K., E.A.Y., T.K., Design: B.T.Ç., H.E.K., E.A.Y., T.K., Data Collection or Processing: B.T.Ç., H.E.K., E.A.Y., T.K., Analysis or Interpretation: B.T.Ç., H.E.K., E.A.Y., T.K., Literature Search: B.T.Ç., H.E.K., E.A.Y., T.K., Writing: B.T.Ç., H.E.K., E.A.Y., T.K.

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

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

REFERENCES

1. Harris AP, Witter FR. Fetal Distress. In: Datta S, Hepner DL, eds. Anesthetic and Obstetric Management of High-Risk Pregnancy. Springer. 2004: 491-503.
2. MacKenzie IZ, Cooke I. Prospective 12 month study of 30 minute decision to delivery intervals for “emergency” caesarean section. BMJ. 2001; 322: 1334-5.
3. Wiberg-Itzel E, Lipponer C, Norman M, Herbst A, Prebensen D, Hansson A, et al. Determination of pH or lactate in fetal scalp blood in management of intrapartum fetal distress: randomised controlled multicentre trial. BMJ. 2008; 336: 1284-7.
4. Steer PJ. Fetal distress. Current Obstetrics & Gynaecology. 2002; 12: 15-21.
5. Tharmaratnam S. Fetal distress. Baillieres Best Pract Res Clin Obstet Gynaecol. 2000; 14: 155-72.
6. Sykes GS, Molloy PM, Johnson P, Gu W, Ashworth F, Stirrat GM, Turnbull AC. Do Apgar scores indicate asphyxia? Lancet. 1982; 1: 494-6.
7. Abessolo FO, Ngou JP, Meye JF, Yangou JM, Lemamy GJ, Ngou-Milama EJ. Souffrance foetale: pouvoir informant des lactates et du statut antioxydant versus le score d’Apgar [Fetal distress: Information provided by lactate levels and antioxidant status, compared with the Apgar score]. Sante. 2009; 19: 15-9.
8. Datta S, Hepner DL, eds. Anesthetic and Obstetric Management of High-Risk Pregnancy. Springer. 2004.
9. Practice bulletin no. 145: antepartum fetal surveillance. Obstet Gynecol. 2014; 124: 182-92.
10. Gruys E, Toussaint MJ, Niewold TA, Koopmans SJ. Acute phase reaction and acute phase proteins. J Zhejiang Univ Sci B. 2005; 6: 1045-56.
11. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med. 1999; 340: 448-54.
12. Young MF, Kerr JM, Termine JD, Wewer UM, Wang MG, McBride OW, et al. cDNA cloning, mRNA distribution and heterogeneity, chromosomal location, and RFLP analysis of human osteopontin (OPN). Genomics. 1990; 7: 491-502.
13. Luedtke CC, McKee MD, Cyr DG, Gregory M, Kaartinen MT, Mui J, et al. Osteopontin expression and regulation in the testis, efferent ducts, and epididymis of rats during postnatal development through to adulthood. Biol Reprod. 2002; 66: 1437-48.
14. Johnson GA, Burghardt RC, Joyce MM, Spencer TE, Bazer FW, Gray CA, et al. Osteopontin is synthesized by uterine glands and a 45-kDa cleavage fragment is localized at the uterine-placental interface throughout ovine pregnancy. Biol Reprod. 2003; 69: 92-8.

15. Raouf S, Sheikhan F, Hassanpour S, Bani S, Torabi R, Shamsalizadeh N. Diagnostic value of non stress test in latent phase of labor and maternal and fetal outcomes. Glob J Health Sci. 2014; 7: 177-82.
16. Hussein AI, Kurtay S, Omar AA, Yusuf AA, Mohamud RYH. An Analysis of the Rate, Indications, and Associated Maternal Mortality for Cesarean Sections at a Tertiary Care Hospital, First Report from Somalia. Int J Womens Health. 2023; 15: 225-33.
17. Frappaolo AM, Logue TC, Goffman D, Nathan LM, Sheen JJ, Andrikopoulou M, et al. Cesarean Delivery Trends Among Patients at Low Risk for Cesarean Delivery in the US, 2000-2019. JAMA Netw Open. 2023; 6: e235428.
18. Kiettisanpipop P, Phupong V. Intrapartum and neonatal outcome of screening non-stress test (NST) compared with no screening NST in healthy women at 40-40 (+6) weeks of gestation. J Obstet Gynaecol Res. 2015; 41: 50-4.
19. Lund SA, Wilson CL, Raines EW, Tang J, Giachelli CM, Scatena M. Osteopontin mediates macrophage chemotaxis via α4 and α9 integrins and survival via the α4 integrin. J Cell Biochem. 2013; 114: 1194-202.
20. van den Broe NR, Letsky EA. Pregnancy and the erythrocyte sedimentation rate. BJOG. 2001; 108: 1164-7.
21. Hwang HS, Kwon JY, Kim MA, Park YW, Kim YH. Maternal serum highly sensitive C-reactive protein in normal pregnancy and pre-eclampsia. Int J Gynaecol Obstet. 2007; 98: 105-9.
22. Amirabi A, Naji S, Yekta Z, Sadeghi Y. Chorioamnionitis and diagnostic value of C-reactive protein, erythrocyte sedimentation rate and white blood cell count in its diagnosis among pregnant women with premature rupture of membranes. Pak J Biol Sci. 2012; 15: 454-8.
23. Nowak M, Oszukowski P, Szpakowski M, Malinowski A, Maciołek-Blewniewska G. Intrauterine infections. I. The role of C-reactive protein, white blood cell count and erythrocyte sedimentation rate in pregnant women in the detection of intrauterine infection after preliminary rupture of membranes. Ginekol Pol. 1998; 69: 615-22.
24. Bligh LN, Alsolai AA, Greer RM, Kumar S. Prelabor screening for intrapartum fetal compromise in low-risk pregnancies at term: cerebroplacental ratio and placental growth factor. Ultrasound Obstet Gynecol. 2018; 52: 750-6.
25. Knight AK, Conneely KN, Kilaru V, Cobb D, Payne JL, Meilman S, et al. SLC9B1 methylation predicts fetal intolerance of labor. Epigenetics. 2018; 13: 33-9.
26. Stenczer B, Rigó J Jr, Prohászka Z, Derzsy Z, Lázár L, Makó V, et al. Plasma osteopontin concentrations in preeclampsia - is there an association with endothelial injury? Clin Chem Lab Med. 2010; 48: 181-7.
27. Koh A, da Silva APB, Bansal AK, Bansal M, Sun C, Lee H, et al. Role of osteopontin in neutrophil function. Immunology. 2007; 122: 466-75.
28. Denhardt DT, Noda M. Osteopontin expression and function: Role in bone remodeling. J Cell Biochem. 1998; 72(Suppl 30-31): 92-102.
29. Gabinskaya T, Salafia CM, Gulle VE, Holzman IR, Weintraub AS. Gestational age-dependent extravillous cytotrophoblast osteopontin immunolocalization differentiates between normal and preeclamptic pregnancies. Am J Reprod Immunol. 1998; 40: 339-46.
30. Qu X, Yang M, Zhang W, Liang L, Yang Y, Zhang Y, Deng B, Gao W, Liu J, Yang Q, Kong B, Gong F. Osteopontin expression in human decidua is associated with decidual natural killer cells recruitment and regulated by progesterone. In Vivo. 2008; 22: 55-61.
31. Joung KE, Christou H, Park KH, Mantzoros CS. Cord blood levels of osteopontin as a phenotype marker of gestational age and neonatal morbidities. Obesity (Silver Spring). 2014; 22: 1317-24.

GMJ 2024;35:260-264

DOI: http://dx.doi.org/10.12996/gmj.2023.3892

Retrospective Assessment of Factors Affecting Carboxyhemoglobin Levels in Patients Undergoing Robotic Surgery

Robotik Cerrahi Uygulanan Hastalarda Karboksihemoglobin Düzeylerini Etkileyen Faktörlerin Retrospektif Olarak Değerlendirilmesi

Selin Erel[1], Özge Erkoçak[1], Ülgen Öztürk Toyran[2], Gözde İnan[1]

[1]Department of Anesthesiology and Reanimation, Gazi University Faculty of Medicine, Ankara, Türkiye
[2]Department of Anesthesiology and Reanimation, University of Health Sciences Türkiye, İstanbul Prof. Dr. Cemil Taşcıoğlu City Hospital, İstanbul, Türkiye

ABSTRACT

Objective: Electrocautery in laparoscopic surgeries converts abdominal carbon dioxide gas into carbon monoxide, which binds to hemoglobin with a higher affinity than oxygen and can impair oxygen transport and lead to hypoxia. This study aimed to assess changes in carboxyhemoglobin (COHb) levels and investigate the factors affecting COHb levels in patients undergoing robotic surgery.

Methods: Forty-two patients were included in this retrospective study. Patient demographics, anesthetic used, airway pressures, and COHb levels at different time points (baseline COHb-1st hour COHb-post-pneumoperitoneum COHb-postoperative care unit COHb-deltaCOHb) were considered. Age, gender, American Society of Anesthesiology (ASA) physical score, body mass index, smoking status, surgery type, anesthesia type, patient position, fresh gas flow (FGF), insufflation, and airway pressures, which may have an effect on COHb levels, were compared.

Results: No significant relationship was found between COHb levels at all measured time points and factors such as gender, ASA scores, surgery type, anesthesia type, position, FGF, insufflation pressure, and airway pressure. Smokers had significantly higher COHb levels than passive smokers, non-smokers, and ex-smokers at baseline (p=0.003), 1st hour (p=0.006), and post-pneumoperitoneum COHb (p=0.009) levels.

Conclusion: Long surgery time, use of different types of anesthetics, and low FGF does not increase the risk of elevated COHb levels. Hence, different anesthetic drugs and low FGF, regardless of the position of the patient or the length of the procedure, can be used in robotic surgery without increasing the risk.

Keywords: Robot-assisted surgery, carbon monoxide, carboxyhemoglobin, robotic surgery

ÖZ

Amaç: Laparoskopik ameliyatlarda elektrokoter, abdominal karbondioksit gazını, hemoglobine oksijenden daha yüksek afiniteyle bağlanan ve oksijen taşınmasını bozarak hipoksiye yol açabilen karbon monoksite dönüştürür. Bu çalışmada robotik cerrahi uygulanan hastalarda karboksihemoglobin (COHb) düzeyindeki değişikliklerin değerlendirilmesi ve COHb düzeyini etkileyen faktörlerin araştırılması amaçlandı.

Yöntemler: Bu retrospektif çalışmaya 42 hasta dahil edildi. Hasta demografik özellikleri, kullanılan anestezikler, hava yolu basınçları ve farklı zaman noktalarındaki COHb düzeyleri (başlangıç COHb-1. saat COHb-pnömoperiton sonrası COHb-ameliyat sonrası bakım ünitesi COHb-deltaCOHb) dikkate alındı. COHb düzeylerine etki edebilecek yaş, cinsiyet, Amerikan Anesteziyoloji Derneği (ASA) fizik skoru, vücut kitle indeksi, sigara içme durumu, ameliyat türü, anestezi türü, hasta pozisyonu, taze gaz akışı (FGF), insuflasyon ve hava yolu basınçları karşılaştırıldı.

Bulgular: Ölçülen tüm zaman noktalarındaki COHb düzeyleri ile cinsiyet, ASA skorları, ameliyat tipi, anestezi tipi, pozisyon, FGF, insuflasyon basıncı ve hava yolu basıncı gibi faktörler arasında anlamlı bir ilişki bulunamadı. Sigara içenlerde başlangıçta (p=0,003), 1. saatte (p=0,006) ve pnömoperiton sonrası (p=0,009) COHb düzeyleri pasif içicilere, sigara içmeyenlere ve sigarayı bırakmış kişilere göre anlamlı derecede yüksekti.

Sonuç: Ameliyat süresinin uzun olması, farklı türde anesteziklerin kullanılması ve FGF'nin düşük olması COHb yüksekliği riskini artırmaz. Dolayısıyla robotik cerrahide hastanın pozisyonuna ve işlemin uzunluğuna bakılmaksızın farklı anestezik ilaçlar ve düşük FGF riski artırmadan kullanılabilir.

Anahtar Sözcükler: Robot destekli cerrahi, karbonmonoksit, karboksihemoglobin, robotik cerrahi

Address for Correspondence/Yazışma Adresi: Ülgen Öztürk Toyran, MD, Department of Anesthesiology and Reanimation, University of Health Sciences Türkiye, İstanbul Prof. Dr. Cemil Taşcıoğlu City Hospital, İstanbul, Türkiye
E-mail / E-posta: drulgenozturk@gmail.com
ORCID ID: orcid.org/0000-0003-1704-6390

Received/Geliş Tarihi: 23.05.2023
Accepted/Kabul Tarihi: 20.12.2023

INTRODUCTION

Carbon monoxide (CO) is an odorless gas that can significantly affect multiple organs and tissues in the body (1). CO exposure may occur under various circumstances, especially from sources such as car exhaust, smoking, and malfunctioning heating systems. Anesthesiologists should be especially cautious about the possibility of CO exposure during general endotracheal anesthesia. Because conventional carbon dioxide (CO_2) absorbents break down volatile anesthetic drugs, CO is typically produced within the anesthesia breathing circuit. The type of volatile agent used, anesthetic concentration, absorbent temperature, patient CO_2 production, chemical composition of the CO_2 absorbent used, and water content within the absorbent are significant factors that influence the level of CO production during anesthesia (2,3).

During various laparoscopic surgeries, the use of cautery can change abdominal CO_2 gas into CO gas. Peritoneal absorption can increase carboxyhemoglobin (COHb) levels. It has been noted that intra-abdominal CO levels can reach dangerous amounts although COHb levels can rise to considerable but non-risky values (4).

COHb evaluation is a test that determines the amount of CO bonded with hemoglobin in the blood. Therefore, the proportion of total blood hemoglobin was used to indicate COHb levels.

Robotic surgery is a minimally invasive procedure that allows surgeons to perform complex procedures with greater supervision, precision, and flexibility than regular surgery (5). Compared with conventional surgery, robotic surgery has many benefits, including smaller incisions, less blood loss, less discomfort, shorter hospital stays, and faster recovery times. In addition, it permits greater precision in actions and better vision of the surgical site, both of which can improve patient outcomes (6). Because robotic surgery is a modification of laparoscopic surgery, it necessitates the use of CO_2 pneumoperitoneum for intra-abdominal pathologies (6). Similar to laparoscopic surgery, CO_2 gas is inflated into the abdomen to create a closed chamber that allows intra-abdominal vision.

Although laparoscopic surgeries have been investigated for the conversion of abdominal CO_2 to CO and their impact on COHb levels, robotic surgeries have not been investigated. Our hypothesis is that prolonged pneumoperitoneum durations during robotic procedures, which are longer than those during laparoscopic procedures, would result in more CO_2 insufflation and, ultimately, higher COHb levels. Therefore, the goal of this study was to assess changes in COHb levels and investigate the factors affecting COHb levels in patients undergoing robotic surgery.

MATERIALS AND METHODS

The records of patients aged 18 years who underwent elective robotic surgery in the robotic operating room of the Gazi University Faculty of Medicine between December 2021 and December 2022 were retrospectively evaluated following the approval of the Gazi University Local Ethics Committee (approval number: 2023-259, date: 21.02.2023). Preoperative anesthesia evaluation, intraoperative anesthesia follow-up forms, and laboratory parameters of the patients included in the study were obtained from the hospital information system.

Patients with chronic obstructive pulmonary disease, restrictive pulmonary disease, a history of pulmonary infection in the last month, hematologic disease, allergy to the drugs used, pregnancy, or thoracic or ear-nose-throat (ENT) surgery were excluded. Patients for whom anesthesia forms and blood gas analyses were not accessible retrospectively were excluded from the study to ensure the consistency of the data.

Age, gender, weight, height, comorbid diseases, American Society of Anesthesiology (ASA) physical score, and smoking history were recorded from the preoperative anesthesia evaluation forms. Anesthetic agents used in the maintenance of anesthesia, type and duration of surgery, surgical position, insufflation and airway pressures, end-tidal carbon dioxide ($ETCO_2$) levels, and fresh gas flow (FGF) values were obtained from the anesthesia forms. The blood gas analyses used in the perioperative follow-up were systematically classified as post-induction, first hour, post-pneumoperitoneum, and postoperative care unit. pH, partial oxygen pressure, partial CO_2 pressure, hemoglobin, hematocrit, oxygen saturation, COHb, potassium, sodium, calcium, glucose, lactate, and bicarbonate levels were recorded.

In blood gas analysis, COHb values are obtained using spectrometric measurement method similar to those used in our hospital. We used COHb values measured during perioperative blood gas analysis follow-ups.

Alterations in COHb levels, the difference in postanesthesia care unit (PACU) COHb levels from baseline measurements (referred to as deltaCOHb), and the relationship between COHb levels at the time of measurement and age, gender, ASA scores, body mass index (BMI), smoking status, surgery type, anesthesia type, position, FGF, insufflation pressures, and airway pressures were assessed.

Statistical Analysis

Statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS), version 26.0 for Windows (SPSS Inc. Chicago, USA). Categorical variables were presented as counts and percentages, and continuous variables were reported as mean ± standard deviation. The normal distribution of variables was assessed using the Kolmogorov-Smirnov test. For normally distributed data, comparisons between two groups were performed using the independent t-test, and multiple groups were analyzed using One-Way ANOVA. Non-normally distributed data were compared using the Mann-Whitney U test for the two groups and Kruskal-Wallis test. Spearman's r data analysis was performed to evaluate the correlation. A statistical significance level of $p<0.05$ was set for all analyses.

RESULTS

The study analyzed data from 41 patients between the ages of 24 and 75 years and classified under ASA I-III. Twenty-eight patients were excluded for not meeting the inclusion criteria: 11 underwent thoracic surgery, 7 underwent ENT surgery, and 10 had respiratory disease infection in the past month (Figure 1).

The median duration of surgery was 240 min (interquartile range, 210-300). The baseline demographic and clinical characteristics of the patients are shown in Table 1.

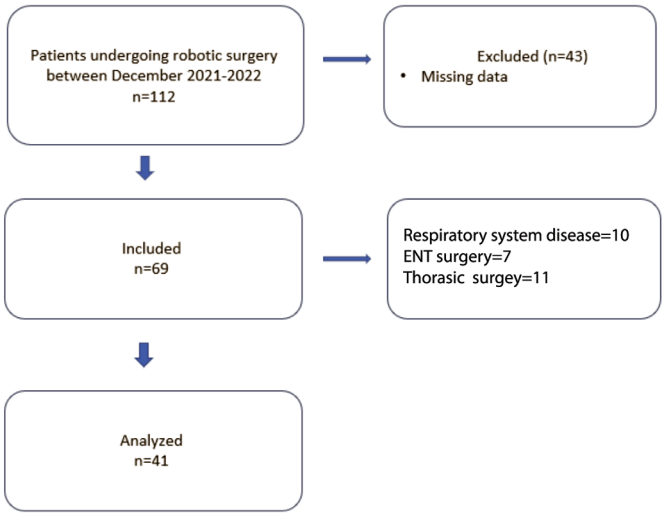


Figure 1. Flowchart of the study.
ENT: Ear-nose-throat.

Only five patients out of the entire patient population had increased COHb levels after surgery. Five patients, on the other hand, showed no change in deltaCOHb. In a large majority of cases (n=31), COHb levels decreased after surgery. The values of $ETCO_2$, partial pressure of CO_2, and COHb are provided in Table 2.

No significant relationship was found between COHb levels at all measured time points and factors such as gender, ASA scores, surgery type, anesthesia type, position, FGF, insufflation pressure, and airway pressure.

Age was found to be correlated with COHb levels, specifically baseline COHb, 1st hour COHb, post-pneumoperitoneum COHb, and deltaCOHb, but not with PACU COHb. Baseline COHb (r=-0.46, p=0.002), 1st hour COHb (r=-0.40, p=0.008), and post-pneumoperitoneum COHb (r=-0.39, p=0.01) were negatively correlated with age. However, there was a positive correlation between deltaCOHb and age (p=0.001, r=0.58).

Spearman's rank correlation analysis demonstrated a positive association between the change in COHb levels (deltaCOHb) and BMI (correlation coefficient, r=0.35, p=0.02) and age (p=0.001, r=0.58). Additionally, a moderate inverse correlation was observed between deltaCOHb and surgical duration (p=0.03, r=-0.34).

There was a significant difference between smoking status and COHb levels. Smokers had significantly higher COHb levels than passive smokers, non-smokers, and ex-smokers at baseline (p=0.003), 1st hour (p=0.006), and post-pneumoperitoneum COHb (p=0.009) levels. COHb change was also statistically significant in smokers compared with that in the other groups (p=0.001). COHb levels decreased more in smokers toward the end of surgery. No difference was observed in the PACU COHb levels. The COHb levels of the patients according to smoking status are shown in Table 3.

DISCUSSION

CO poisoning has historically been suggested to be caused by exposure of the patient to smoke from lasers and electrodesiccation during laparoscopic surgery (4). This retrospective study contributes to our understanding of COHb levels in robotic surgery by revealing several findings. First, our results provide additional evidence for earlier studies that showed how smoking affects COHb levels during laparoscopic procedures. Our research also shows that variables, including anesthetic type, patient position, and FGF, have no appreciable impact on COHb levels. These results provide a more thorough understanding of the complex dynamics of COHb levels during robotic surgery.

CO is produced endogenously but is also a common environmental pollutant; both sources contribute to the amount of COHb in the blood. COHb, which normally comprises less than 1-2% of the

Table 1. Demographic data

Age, years, mean ± SD	58.8±13.2
Gender, n (%)	
Male	26 (63.4)
Female	15 (36.6)
ASA, n (%)	
I	4 (9.7)
II	28 (68.2)
III	9 (21.9)
BMI, kg/m^2, mean ± SD	29.1±7.4
Surgery type, n (%)	
Prostate	21 (51.2)
Rectum	17 (41.5)
Sleeve gastrectomy	3 (7.3)
Anesthesia type, n (%)	
Desflurane	15 (36.6)
Sevoflurane	14 (34.1)
TIVA	12 (29.3)
Position, n (%)	
Trendelenburg	32 (78.0)
Lithotomy	5 (12.2)
Supine	4 (9.8)
Smoking status, n (%)	
No smoking	14 (34.9)
Active smoker	11 (25.6)
Ex-smoker	13 (32.6)
Passive smoker	3 (7.0)
Fresh gas flow, n (%)	
1 liter	16 (39.0)
2 liters	24 (58.5)
3 liters	1 (2.4)
Surgery time, minutes, mean ± SD	261.6±81.4
Insufliation pressures, mmHg, mean ± SD	14.3±1.5
Ppeak, mmHg, mean ± SD	28.4±4.6

ASA: American Society of Anesthesiology, BMI: Body mass index, Ppeak: Peak airway pressure, SD: Standard deviation.

Table 2. End-tidal carbon dioxide, partial pressure of carbon dioxide, and carboxyhemoglobin levels at time points

	Baseline	1st hour	Post-pneumoperitoneum	PACU
$ETCO_2$ (mmHg); median (IQR)	34 (31.5-36.5)	36 (33.0-38.0)	37.0 (34.25-40.75)	N/A
$PaCO_2$ (mmHg); median (IQR)	37.4 (34.0-40.9)	40.4 (37.7-43.9)	43.60 (39.2-48.4)	41.7 (38.4-44.6)
COHb (%); mean ± SD	2.5±0.5	2.5±0.4	2.4±0.4	2.3±0.4

PACU: Postanesthesia care unit, $ETCO_2$: End-tidal carbon dioxide, $PaCO_2$: Partial pressure of carbon dioxide, COHb: Carboxyhemoglobin, IQR: Interquartile range, SD: Standard deviation.

Table 3. Smoking status and carboxyhemoglobin levels at different time points

	Active smoker; median (IQR)	Passive smoker; median (IQR)	Ex-smoker; median (IQR)	No smoking; median (IQR)	p
Baseline COHb	2.9 (2.6-3.6)	2.2 (2.1-2.2)	2.3 (1.8-2.6)	2.5 (2.3-2.6)	0.003
1st hour COHb	2.8 (2.5-3.5)	2.2 (2.1-2.3)	2.3 (2.2-2.4)	2.4 (2.4-2.5)	0.006
COHb	2.6 (2.5-3.3)	2.1 (1.9-2.1)	2.2 (2.0-2.4)	2.3 (2.2-2.5)	0.009
PACU COHb	2.5 (2.2-2.9)	2.1 (2.0-2.1)	2.3 (2.2-2.6)	2.1 (2.1-2.4)	0.08
DeltaCOHb	-0.6 (-0.7-0.3)	-0.1 (-0.1-0.05)	-0.05(-0.2-0.7)	-0.2 (-0.4-0.2)	0.001

COHb: Carboxyhemoglobin, PACU: Postanesthesia care unit, DeltaCOHb: Difference in PACU COHb levels from baseline measurements, IQR: Interquartile range.

total hemoglobin, is the product of the reaction between CO and hemoglobin. COHb concentration can be used to estimate the amount of CO produced. Less than 2% of COHb is commonly considered normal in non-smokers, although values of 5-10% may suggest mild-moderate CO exposure, and levels of 25-35% are thought to be potentially life-threatening (7).

The peritoneal cavity is insufflated with gas, often CO_2, to provide exposure during laparoscopic surgery. Coagulation of tissue by electrocautery in the hypoxic environment of the abdominal cavity inflated with CO_2 during laparoscopic procedures has been hypothesized to produce CO (7).

In the peritoneal cavity during laparoscopic cholecystectomy, Beebe et al. (4) found CO at a median concentration of 345 ppm 5 min after the use of electrocautery and at a concentration of 475 ppm at the conclusion of the procedure, both of which were higher than the 35 ppm upper limit for an hour of exposure set by the Environmental Protection Agency (7). Despite the increase in CO, there was no evidence that these patients had significant CO absorption, as the COHg values were the same before, during, and on the day after surgery. While a comparable number of patients in our retrospective analysis experienced little to no change in COHb levels, in the majority of cases, COHb concentrations decreased compared with baseline. Soro et al. (8) assumed that laparoscopic surgery does not result in appreciable increases in COHb levels, even under closed-system anesthesia and without pulmonary CO elimination. This is most likely due to the low peritoneal absorption of CO. They concluded that adult patients who received pneumoperitoneum gas renewals regularly and electrocautery for normal durations did not experience CO intoxication. No patient displayed any evidence or clinical indications for CO poisoning according to Baum et al. (9). Furthermore, Strauss et al. (10) showed that during the first 6 h of closed-system anesthesia, COHb barely increased by 0.4% on average.

Exogenous CO is present in cigarette smoke and is absorbed by the lungs to produce COHb. The accuracy of both self-reporting and inhalation by passive smokers can be investigated using measurable COHb levels. COHb levels and self-reported smoking have a statistically significant relationship (11). Thus, the association between COHb changes and perioperative problems in smokers, non-smokers, and passive smokers was identified. We demonstrated a correlation between smoking status and COHb levels, which differed from that reported by Park et al. (12). Furthermore, compared with baseline levels, our study revealed an interesting finding of decreased PACU COHb levels among active smokers. We attributed this reduction to the use of high concentrations of inspired oxygen (FIO_2) from room air together with the provision of adequate and efficient mechanical ventilation during the prolonged duration of robotic surgery. These results highlight the critical need for adequate ventilation and the impact of increasing FIO_2 on reducing COHb levels in active smokers during robotic procedures.

Numerous studies and case reports have shown that CO production in the anesthetic circuit caused by dried absorbents leads to elevated COHb levels and clinical symptoms of CO toxicity (13-16). When volatile anesthetic drugs are degraded by conventional CO_2 absorbents without sufficient moisture, CO is produced within the anesthesia breathing circuit (13,17). Different inhalation anesthetics generate CO at varying rates, and CO exposure under anesthesia is indirectly correlated with FGF in the respiratory circuit (2,14,18). In contrast to the predicted associations, we found no association between COHb level, anesthetic type, and FGF. Park et al. (12) evaluated hemodynamic and respiratory parameters, COHb, and postoperative hepatic and renal function in patients who underwent prolonged laparoscopic procedures to compare the effects of minimal flow with those of high flow desflurane anesthesia. The COHb values in the minimal-flow group were significantly higher than those in the high-flow group. However, there was no difference in the COHb concentration from the baseline concentration in the minimal-flow group. They also found that none of the patients in either group had COHb concentrations greater than 1.5%. The researchers concluded that both minimal-flow and high-flow desflurane anesthesia are

equally safe and effective during prolonged laparoscopic surgery (12).

The findings of this study revealed that deltaCOHb had a similar propensity to increase with increasing BMI and age. This suggests that older and overweight individuals have an increased risk of developing elevated COHb levels following surgery. Nevertheless, it should be noted that this risk decreases when surgery lasts longer and patients receive more thorough ventilation. In other words, even in older and heavier patients, a lower risk of increased postoperative COHb levels is associated with longer surgical times and more successful ventilation methods.

Study Limitations

The limitations of our study include the inability to monitor changes in COHb levels with continuous measurements and the lack of follow-up on postoperative respiratory complications. Patient data were obtained through the hospital information system and anesthesia monitoring forms. These factors contribute to the constraints of our study.

CONCLUSION

In summary, our findings indicate that longer operation times, variations in anesthetic agents, and controlled FGF do not increase the likelihood of elevated COHb levels. This implies that laparoscopic surgery does not significantly increase the risk of heightened COHb levels, provided patients receive sufficient hyperventilation with oxygen concentrations between 40% and 80% and continuous efforts are made to effectively remove intra-abdominal smoke. Furthermore, the outcomes suggest that a range of anesthetic medications and reduced FGF can be safely used in robotic surgery, regardless of patient position or procedure duration. These findings underscore the importance of effective smoke extraction systems and appropriate ventilation strategies for reducing the occurrence of CO-related complications during laparoscopic surgery.

Ethics

Ethics Committee Approval: The records of patients aged 18 years who underwent elective robotic surgery in the robotic operating room of the Gazi University Faculty of Medicine between December 2021 and December 2022 were retrospectively evaluated following the approval of the Gazi University Local Ethics Committee (approval number: 2023-259, date: 21.02.2023).

Informed Consent: Retrospective study.

Author Contributions

Concept: S.E., Design: S.E., Supervision: G.İ., Resources: G.İ., Materials: Ö.E., Data Collection or Processing: Ö.E., Analysis or Interpretation: Ü.Ö.T., Literature Search: Ü.Ö.T., Writing: S.E., Critical Review: G.İ.

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

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

REFERENCES

1. Iqbal S, Clower JH, Hernandez SA, Damon SA, Yip FY. A review of disaster-related carbon monoxide poisoning: surveillance, epidemiology, and opportunities for prevention. Am J Public Health. 2012; 102: 1957-63.
2. Fang ZX, Eger EI 2nd, Laster MJ, Chortkoff BS, Kandel L, Ionescu P. Carbon monoxide production from degradation of desflurane, enflurane, isoflurane, halothane, and sevoflurane by soda lime and Baralyme. Anesth Analg. 1995; 80: 1187-93.
3. Fan SZ, Lin YW, Chang WS, Tang CS. An evaluation of the contributions by fresh gas flow rate, carbon dioxide concentration and desflurane partial pressure to carbon monoxide concentration during low fresh gas flows to a circle anaesthetic breathing system. Eur J Anaesthesiol. 2008; 25: 620-6.
4. Beebe DS, Swica H, Carlson N, Palahniuk RJ, Goodale RL. High levels of carbon monoxide are produced by electro-cautery of tissue during laparoscopic cholecystectomy. Anesth Analg. 1993; 77: 338-41.
5. Paul S, McCulloch P, Sedrakyan A. Robotic surgery: revisiting "no innovation without evaluation". BMJ. 2013; 346: f1573.
6. Marohn MR, Hanly EJ. Twenty-first century surgery using twenty-first century technology: surgical robotics. Curr Surg. 2004; 61: 466-73.
7. Wald NJ, Idle M, Boreham J, Bailey A. Carbon monoxide in breath in relation to smoking and carboxyhaemoglobin levels. Thorax. 1981; 36: 366-9.
8. Soro M, García-Pérez ML, Ferrandis R, Aguilar G, Belda EJ. Closed-system anaesthesia for laparoscopic surgery: is there a risk for carbon monoxide intoxication? Eur J Anaesthesiol. 2004; 21: 483-8.
9. Baum J, Sachs G, vd Driesch C, Stanke HG. Carbon monoxide generation in carbon dioxide absorbents. Anesth Analg. 1995; 81: 144-6.
10. Strauss JM, Bannasch W, Hausdörfer J, Bang S. Die Entwicklung von Carboxyhämoglobin während Langzeitnarkosen im geschlossenen Kreissystem [The evolution of carboxyhemoglobin during long-term closed-circuit anesthesia]. Anaesthesist. 1991; 40: 324-7.
11. Hart CL, Smith GD, Hole DJ, Hawthorne VM. Carboxyhaemoglobin concentration, smoking habit, and mortality in 25 years in the Renfrew/Paisley prospective cohort study. Heart. 2006; 92: 321-4.
12. Park SY, Chung CJ, Jang JH, Bae JY, Choi SR. The safety and efficacy of minimal-flow desflurane anesthesia during prolonged laparoscopic surgery. Korean J Anesthesiol. 2012; 63: 498-503.
13. Coppens MJ, Versichelen LF, Rolly G, Mortier EP, Struys MM. The mechanisms of carbon monoxide production by inhalational agents. Anaesthesia. 2006; 61: 462-8.
14. Tang CS, Fan SZ, Chan CC. Smoking status and body size increase carbon monoxide concentrations in the breathing circuit during low-flow anesthesia. Anesth Analg. 2001; 92: 542-7.
15. Frink EJ Jr, Nogami WM, Morgan SE, Salmon RC. High carboxyhemoglobin concentrations occur in swine during desflurane anesthesia in the presence of partially dried carbon dioxide absorbents. Anesthesiology. 1997; 87: 308-16.
16. Bonome C, Belda J, Alvarez-Refojo F, Soro M, Fernández-Goti C, Cortés A. Low-flow anesthesia and reduced animal size increase carboxyhemoglobin levels in swine during desflurane and isoflurane breakdown in dried soda lime. Anesth Analg. 1999; 89: 909-16.
17. Baxter PJ, Garton K, Kharasch ED. Mechanistic aspects of carbon monoxide formation from volatile anesthetics. Anesthesiology. 1998; 89: 929-41.
18. Wissing H, Kuhn I, Warnken U, Dudziak R. Carbon monoxide production from desflurane, enflurane, halothane, isoflurane, and sevoflurane with dry soda lime. Anesthesiology. 2001; 95: 1205-12.

Original Investigation | Özgün Araştırma

GMJ 2024;35:265-275

DOI: http://dx.doi.org/10.12996/gmj.2024.3923

Domestic Violence Against Pregnant Women: Prevalence and Related Factors

Gebe Kadınlara Yönelik Aile İçi Şiddet: Sıklık ve İlişkili Faktörler

Metin Pıçakçıefe, Şeyma Ata, Raziye Ülkü Kıcalı, M. Onur Vatandaş

Department of Public Health, Muğla Sıtkı Koçman University Faculty of Medicine, Muğla, Türkiye

ABSTRACT

Objective: Violence against women, especially during pregnancy, is a problem affecting the health of both the mother and fetus. The study evaluated the prevalence and associated factors of domestic violence in pregnant women who apply to a healthy life center (HLC).

Methods: The population of the cross-sectional and analytical study, in which the surveillance data were analyzed, consisted of 202 pregnant women who applied to the HLC of the district health directorate. The SPSS 22.0 package program was used to analyze the data. The data was examined using Fisher's exact test, Pearson's chi-square and $p<0.05$ was considered statistically significant.

Results: 2% of pregnant women were exposed to domestic violence before pregnancy and 5.4% of them were exposed to domestic violence during pregnancy. Of the participants, 0.5% were exposed to physical, 2.5% to verbal, 5% to psychological, 1.5% to economic, and 2.5% to social violence. Pregnant women living in rural areas (p=0.035), middle income (p=0.047), having a nuclear family structure (p=0.004), dissatisfaction with marriage life (p=0.001) and planned pregnancy (p=0.025) significantly increases the frequency of exposed to domestic violence.

Conclusion: The most important result of our study is the low frequency of exposure of pregnant women to domestic violence during pregnancy. The most common type of violence is psychological violence. Healthcare professionals should question domestic violence during pregnancy while conducting anamnesis and conduct a detailed examination about violence.

Keywords: Pregnant, women, domestic violence, health life center

ÖZ

Amaç: Kadına yönelik şiddet özellikle gebelik döneminde hem anne hem de fetüs sağlığını etkileyen bir sorundur. Bu çalışmanın amacı bir sağlıklı hayat merkezine başvuran gebelerin aile içi şiddetle karşılaşma sıklığının ve ilişkili faktörlerin değerlendirilmesidir.

Yöntemler: Sürveyans verisinin analiz edildiği, kesitsel ve analitik tipteki çalışmanın evrenini bir ilçe sağlık müdürlüğü sağlıklı hayat merkezine başvuran 202 gebe kadın oluşturmuştur. Verilerin analizinde SPSS 22.0 paket programı kullanılmıştır. Verilerin değerlendirilmesinde Fisher's exact test, Pearson's ki-kare kullanılmış ve $p<0,05$ anlamlılık sınırı kabul edilmiştir.

Bulgular: Gebelerin %2'si gebelik öncesi, %5,4'ü ise gebelik döneminde aile içi şiddete maruz kalmıştır. Katılanların %0,5'i fiziksel, %2,5'i sözel, %5'i psikolojik, %1,5'i ekonomik ve %2,5'i sosyal şiddete maruz kalmıştır. Gebelerin kırsal alanda yaşaması (p=0,035), gelir durumunun orta olması (p=0,047), çekirdek aile yapısına sahip olması (p=0,004), evlilik yaşamından memnun olmaması (p=0,001) ve gebeliğinin planlı olması (p=0,025) gebelikte aile içi şiddetle karşılaşma sıklığını anlamlı olarak artırmaktadır.

Sonuç: Çalışmamızın en önemli sonucu gebelerin gebelik döneminde aile içi şiddete maruz kalma sıklığının düşük olmasıdır. En çok karşılaşılan şiddet türü pisikolojik şiddettir. Sağlık çalışanları anamnez alırken gebelik döneminde aile içi şiddeti sorgulamalı ve şiddetle ilgili ayrıntılı muayene yapmalıdır.

Anahtar Sözcükler: Gebe, kadın, aile içi şiddet, sağlıklı hayat merkezi

Address for Correspondence/Yazışma Adresi: Metin Pıçakçıefe, MD, Department of Public Health, Muğla Sıtkı Koçman University Faculty of Medicine, Muğla, Türkiye
E-mail / E-posta: mpicakciefe@hotmail.com
ORCID ID: orcid.org/0000-0002-2877-7714

Received/Geliş Tarihi: 21.06.2023
Accepted/Kabul Tarihi: 13.05.2024

INTRODUCTION

Violence has existed since the beginning of mankind and can often occur as a result of unequal relations against nations, society, or physically, economically, culturally, or emotionally disadvantaged people. Violence affects the social order both at the institutional level (political, economic, cultural, educational, ethnic-racial) and in interpersonal relationships (familial, domestic, physical, sexual, psychological, moral) (1).

Violence against women is a violation of human rights, based on gender inequality, a public health problem, and an obstacle to sustainable development (2). Women around the world are at risk of gender-based violence, regardless of their country, ethnicity, class, religion, and economic and social status (3).

Acts of violence may be perceived differently in different societies or among social segments of a society because of the role of men and women in society and cultural diversity (1). Adolescent, young, ethnic and other minority, transgender, and disabled women are at higher risk for all forms of violence (2). Domestic violence against women continues to exist worldwide as a violation of human rights that has no cultural, geographical, religious, social, and economic boundaries. Domestic violence against women, which is an important social problem, not only affects women's physical and mental health but also prevents the development of their legal, social, political, and economic status (4).

Domestic violence against women is defined as a violation of health and human rights and continues to exist all over the world. Studies have shown that 95% of victims of domestic violence are women (5). Domestic violence, partner violence, and spousal violence are terms used to describe violence that occurs between people in a current or previous relationship. Intimate partner violence includes any behavior that can occur in many ways, such as physical, psychological, sexual, and economic violence, and that takes place with the aim of controlling the other person (6). This behavior model includes many types of abuse, such as physical, sexual, verbal, social, and economic abuse. Acts of domestic violence usually include verbally abusing the partner, psychological abuse, stalking, threatening with violence, throwing an object, pushing, slapping, kicking, hitting, beating, threatening with a weapon or using a weapon, depriving the partner of basic resources such as food, clothing, money, transportation, or health, and keeping the partner away from social activities (7). Globally, 30% of women have experienced physical and/or sexual violence by a partner during their lifetime (2).

It has been reported in the literature that the risk of domestic violence increases when a woman becomes pregnant. Although relationships before pregnancy are satisfactory, records show that violence may start in the early stages of pregnancy. Pregnancy is accepted as a period in which the risk of being exposed to violence increases because of factors such as ambivalent feelings during pregnancy, decreased sexual intercourse, and increased economic pressures (8). Because pregnancy is a period when women are vulnerable, the prevalence of violence in the population of pregnant women is important. Violence during pregnancy is of particular concern because of the potential negative consequences for both the mother and her unborn child (9). During pregnancy, women face physical and psychological changes that make them more sensitive or vulnerable, thus attracting more attention from their partners and family. However, violence is a reality in the lives of many of these women, often triggering losses that are irreparable for the mother and child (1). Worldwide, one in four people are physically or sexually abused during pregnancy, usually by their partner (10,11). Studies in the literature on this subject have reported that the prevalence of violence experienced by pregnant women during pregnancy in the general population is between 1% and 20% (12). Violence against women is a problem that affects both maternal and fetal health, especially during pregnancy. In addition, it may lead to many negative health problems, such as depression, posttraumatic stress disorder, preterm labor, miscarriage, fetal growth retardation, and low birth weight babies, and may even result in the death of the woman (13,14).

Violence against women, which maintains its importance at the global level, is one of the most important social problems in our country. In a study conducted in Türkiye, the rate of women who had been subjected to physical violence by their husbands or ex-husbands in any period of their lives was 36%, and the rate of women who had been subjected to sexual violence was 12% (15). One out of every ten women who have been pregnant at least once in Türkiye has experienced physical violence by her husband or intimate partner(s) during pregnancy (4).

Our research will enable the formulation of targeted policies and programs and the development of existing policies and programs to combat violence against women more effectively. In addition, the data obtained at the local level will contribute to national and international studies. However, the fact that the number of studies providing detailed information and data in this field in our country is quite low constitutes a major obstacle in combating violence against women. This study was planned to meet the need for data at the national level to combat violence against women more effectively and to formulate policies. This study aimed to evaluate the frequency of domestic violence and related factors in pregnant women who applied to a healthy life center (HLC).

MATERIALS AND METHODS

A cross-sectional, analytical-type research was planned. The study population consisted of 202 pregnant women who applied to the Muğla District Health Directorate HLC between June 15 and December 15, 2019. The researchers interviewed pregnant women at the Child, Adolescent, Women and Reproductive Health Services unit of the HLC. After explaining the purpose of the study, the questionnaire form was applied to those who agreed to participate in the study. The application of the questionnaire form, in which the face-to-face interview method was used, took approximately 15 min.

The dependent variable of our study was encounters with domestic violence during pregnancy. The situation of encountering violence was examined under the subheadings of physical, verbal, sexual, psychological (emotional), financial, and social violence.

The independent variables of our study were analyzed under the subheadings of sociodemographic, marital life, and pregnancy status variables: sociodemographic variables; age, age of, educational status, employment status, occupation, educational status of husband, employment status of husband, health insurance status, income status, place of residence, family type, presence of children, number of children, and number of people living at home. Variables

related to marital life; marriage type, marriage duration (years), marriage age, number of marriages, and satisfaction with marital life. Variables related to pregnancy status; pregnancy order, pregnancy week, and planned pregnancy status.

Ethical Approval

This study was conducted within the framework of ethical rules. Approval was obtained from the Human Research Ethics Committee of Muğla Sıtkı Koçman University (approval number: 122, date: 16.07.2019) and the Dean's Office of the Faculty of Medicine before the study. Students and resident physicians were informed that participation in the survey was voluntary.

Statistical Analysis

The data were first summarized using descriptive statistics. In addition to Kolmogorov-Smirnov and Shapiro-Wilk tests, conformity to normal distribution was checked according to histogram and other visual methods. Fisher's exact test and Pearson's chi-square test were used to determine whether the level of exposure to violence was different according to independent variables in the data obtained by counting. SPSS 22.0 package program was used for data analysis and $p<0.05$ significance limit was accepted.

RESULTS

When the distribution of pregnant women who applied to the HLC was evaluated according to their sociodemographic characteristics, the mean age of the applicants was 28.9±4.7 years, 89.1% were younger than 35 years, and the mean age of their husbands was 32.2±5.4 years, 70.8% were younger than 35 years. The education level of 60.4% of the participants was university level. 49.5% of the participants were employed. The occupation of 38.1% of the participants was housewife, 17.3% were self-employed, and 13.9% were teachers. The education level of 50.5% of the husbands of the participants was high school and below. 96.5% of the husbands of the respondents were employed. 95.5% of the respondents had health insurance for themselves or their husbands. 27.2% of the participants described their income status as good, 71.3% as fair, and 1.5% as poor. 74.3% of the respondents lived in urban areas. The family type of 93.6% of the participants was nuclear family. 64.9% of the participants had no children (Table 1).

When the distribution of pregnant women who applied to the HLC was evaluated according to their characteristics related to their marital status, 78.2% of the participants had civil and imam marriages. The mean duration of marriage was 4.2±3.8 years. While 28.7% had been married for 1 year, 54% had been married for more than 2 years. The mean age at marriage was 24.7±4.3 years. 57.4% of the participants were married at the age of 25 years or younger. It was the first marriage of 93.6% of the participants. 86.1% of the participants reported that they were satisfied with their marital life (Table 2).

When the distribution of pregnant women who applied to the HLC according to their pregnancy status was examined, 55% of the participants were in their first pregnancy. When the gestational week of the participants was analyzed, it was found that 42.6% were in the 2nd trimester and 40.1% were in the 3rd trimester. The pregnancies of 82.7% of the participants were planned pregnancies (Table 3).

Table 1. Distribution of pregnant women according to sociodemographic characteristics

Feature	Number (n)	Percent (%)
Age (year)		
<35	180	89.1
≥35	22	10.9
Age of husband (year)		
<35	143	70.8
≥35	59	29.2
Education status		
High school and below	80	39.6
University	122	60.4
Employment status		
Working	100	49.5
Not working	102	50.5
Profession		
Housewife	77	38.1
Health worker	22	10.9
Officer	22	10.9
Teacher	28	13.9
Self-employment	35	17.3
Engineer	7	3.5
Worker	7	3.5
Other	4	1.9
Education status of the husband		
High school and below	102	50.5
University	100	49.5
Employment status of the husband		
Working	195	96.5
Not working	7	3.5
Health insurance		
There is	193	95.5
No	9	4.5
Income status		
Good	55	27.2
Middle	144	71.3
Bad	3	1.5
Place of residence		
Urban	150	74.3
Break	52	25.7
Family type		
Nuclear family	189	93.6
Extended family	13	6.4
Child presence		
There is	71	35.1
No	131	64.9

Table 2. Distribution of pregnant women according to their characteristics related to marital status (n=202)

Status of marriage	Number (n)	Percent (%)
Form of marriage		
Civil marriage	44	21.8
Civil and religious marriage	158	78.2
Duration of marriage		
1 year	58	28.7
2 years	35	17.3
>2 years	109	54.0
Age at marriage		
≤25 years	116	57.4
>25 years	86	42.6
How many marriages		
First marriage	189	93.6
Two and above	13	6.4
Satisfaction with the married life		
Satisfied	174	86.1
Not satisfied	28	13.9

Table 3. Distribution of pregnant women according to pregnancy status (n=202)

Status of pregnancy	Number (n)	Percent (%)
Pregnancy sequence		
First pregnancy	111	55.0
Two and above	91	45.0
Pregnancy week		
1st trimester	35	17.3
2nd trimester	86	42.6
3rd trimester	81	40.1
Planned pregnancy status		
Yes	167	82.7
No	35	17.3

When the distribution of pregnant women who applied to the HLC according to the variables related to violence was evaluated, 2% of the participants reported that they were exposed to violence in the pre-pregnancy period. 5.4% of the participants reported being exposed to any type of domestic violence during pregnancy. Among the pregnant women who participated in our study, 0.5% were exposed to physical violence, 2.5% to verbal violence, 5% to psychological violence, 1.5% to economic violence, and 2.5% to social violence. None of the participants who were subjected to violence were sexually abused. Of those who were subjected to violence, 1.5% reported being subjected to violence by their husbands, 1.5% by their mothers-in-law, and 97% did not report by whom they were subjected to violence. None of the victims of violence suffered any injury because of this violence, nor did they receive any treatment. None of the victims of violence reported to the judicial authorities because of this violence (Table 4).

Table 4. Distribution of pregnant women according to variables related to violence (n=202)

Variables related to violence	Number (n)	Percent (%)
Exposure to violence before pregnancy		
Yes	4	2.0
No	198	98.0
Exposure to violence during current pregnancy		
Yes	11	5.4
No	191	94.6
Physical violence		
Yes	1	0.5
No	201	99.5
Verbal violence		
Yes	5	2.5
No	197	97.5
Psychological (emotional) violence		
Yes	10	5.0
No	192	95.0
Sexual violence		
Yes	0	0
No	202	100
Financial (economic) violence		
Yes	3	1.5
No	199	98.5
Social violence		
Yes	5	2.5
No	197	97.5
Number of encounters with violence		
1 time	5	2.5
2 and more	197	97.5
Person subjected to violence		
Wife	3	1.5
Mother-in-law	3	1.5
Unanswered	196	97.0
Injury because of violence		
Yes	0	0
No	202	100
Receiving treatment after violence		
Yes	0	0
No	202	100
Reporting to the judicial authorities after violence		
Yes	0	0
No	202	100

There was no significant difference between the age, educational status, employment status, and health insurance status of the pregnant women who participated in our study and the frequency of encountering domestic violence during pregnancy (p>0.05). There was no significant difference between the age, education level, employment status, and frequency of encountering violence during pregnancy of the husbands of the pregnant women who participated in our study (p>0.05).

Among the participants, the frequency of violence was found to be significantly higher among those with moderate income than among those with good and poor income (p=0.047).

Among the participants of our study, the frequency of exposure to violence among those living in rural areas was found to be significantly higher than that among those living in urban areas (p=0.035).

Among the participants, the frequency of violence among those with a nuclear family structure was found to be significantly higher than that among those with an extended family structure (p=0.004) (Table 5).

No significant correlation was found between the type of marriage, duration of marriage, age at marriage, and number of marriages of pregnant women who participated in our study and the frequency of domestic violence during pregnancy (p>0.05).

The frequency of violence among participants who were dissatisfied with their marital life was found to be significantly higher than that among those who were satisfied (p=0.001).

Among the pregnant women who participated in our study, the frequency of violence during pregnancy was found to be significantly higher in those who had not been exposed to violence before pregnancy compared to those who had been exposed to violence before pregnancy (p=0.004) (Table 6).

There was no significant relationship between the number of pregnancies of pregnant women who participated in our study and the frequency of domestic violence during pregnancy (p>0.05).

The frequency of domestic violence among the participants in the 2nd trimester was found to be significantly higher than that in the 1st and 3rd trimesters (p=0.025).

Among the participants of our study, the prevalence of domestic violence among those with planned pregnancies was found to be significantly higher than that among those without planned pregnancies (p=0.025) (Table 7).

DISCUSSION

While the mean age of the pregnant women who participated in our study was 28.9±4.7 years, it was found to be 28.4±4.4 years in a study on intimate partner violence against pregnant women in Manisa (16),

Table 5. Relationship between sociodemographic characteristics of pregnant women and their exposure to domestic violence

Features	Experience of domestic violence during pregnancy		
		(%)*	p-value
Age	<35 (8)	72.7	0.104**
	≥35 (3)	27.3	
Age of the husband	<35 (5)	45.5	0.084**
	≥35 (6)	54.5	
Education status	High school and below (3)	27.3	0.532**
	University (8)	72.7	
Employment status	Working (5)	45.5	0.769***
	Not working (6)	54.5	
Husband's education status	High school and below (4)	36.4	0.335***
	University (7)	63.6	
Husband's employment status	Working (11)	100	0.518***
	Not working (0)	0	
Presence of health insurance	There is (10)	90.9	0.443***
	None (1)	9.1	
Income status	Good (1)	9.1	**0.047***
	Medium (9)	81.8	
	Bad (1)	9.1	
Place of residence	Urban (5)	45.5	**0.035****
	Break (6)	54.5	
Family type	Nuclear family (8)	72.7	**0.004****
	Extended family (3)	27.3	

*: Percentage of exposure to domestic violence during pregnancy, **: Fisher's exact test, ***: Pearson's chi-square.

Table 6. Relationship between marital status characteristics of pregnant women and their exposure to domestic violence

Marriage status	Experience of domestic violence during pregnancy		
		(%)*	p-value
Form of marriage	Civil marriage (9)	81.8	1.000**
	Civil and religious marriage (2)	18.2	
Duration of marriage	1 year (2)	18.2	0.683***
	2 years (2)	18.2	
	>2 years (7)	63.6	
Age at marriage	≤25 years (5)	45.5	0.534**
	>25 years (6)	54.5	
How many marriages	First marriage (10)	90.9	0.712***
	Two or more (1)	9.1	
Satisfaction with the married life	Satisfied (5)	45.5	**0.001****
	Not satisfied (6)	54.5	
Exposure to violence before pregnancy	Yes (2)	18.2	**0.004****
	No (9)	81.8	

*: Percentage of exposure to domestic violence during pregnancy, **: Fisher's exact test, ***: Pearson's chi-square.

Table 7. Relationship between pregnancy characteristics of pregnant women and their exposure to domestic violence

Features	Experience of domestic violence during pregnancy		
		(%)*	p-value**
Pregnancy sequence	First pregnancy (6)	54.5	1.000**
	Two or more (5)	45.5	
Pregnancy week	1st trimester (1)	9.1	**0.025*****
	2nd trimester (9)	81.8	
	3rd trimester (1)	9.1	
Planned pregnancy state of being	Yes (6)	54.5	**0.025****
	No (5)	45.5	

*: Percentage of exposure to domestic violence during pregnancy, **: Fisher's exact test, ***: Pearson's chi-square.

26.5±0.2 years in another study conducted in Malatya (17) and 23.2±4.2 years in a study conducted in Tripura, India (18). It can be said that the results of our study are compatible with the literature.

In a study on intimate partner violence against pregnant women in Manisa, 90.5% of pregnant women were younger than 34 years of age (16), 66.8% of pregnant women were between 20 and 34 years of age in a study conducted in Colombia (19), and 85.6% were younger than 35 years of age in a study conducted in Tanzania (20). The results of our study are compatible with those of these studies.

In a study conducted in Manisa, it was found that 9.5% of pregnant women had university education (16), in a study conducted in Düzce, 5.7% had university education (21), in a study conducted in İzmir, 24.3% had high school and university education (22), in a study conducted in Colombia, 47.9% had university education (19), and in a study conducted in South Africa, 55.1% had university education (23). The result of our study is high compared with studies conducted in our country and abroad.

While the mean age of the husbands of the pregnant women who participated in our study was 32.2±5.4 years, the mean age of the husbands was found to be 31.1±5.6 years in a study conducted in Yozgat (24), 31.2±5.2 years in a study conducted in Çanakkale (25) and 29.6±4.4 years in a study conducted in Delhi, India (26). The results of the studies conducted in Yozgat, Çanakkale and India are similar to those of our study.

In a study conducted in Sivas, it was found that 45.7% of the husbands of the participants had a university education (27), in a study conducted in İstanbul, 33.9% of the husbands of pregnant women had a university education (28), and in a study conducted in İzmir, 30% had a high school education or higher (22). In a study conducted in India, it was found that 17.6% had a university education (18). The results of our study were higher than those of studies conducted in Türkiye and abroad.

In a study conducted in Yozgat, 51.8% of pregnant women lived in rural areas (24); in a study conducted in Malatya, 29.6% (17); in a study conducted in India, 72.2% (18); and in a study conducted in Colombia, 28.4% (19). The results of studies conducted in Türkiye and abroad differ.

In a study conducted in India, 88.9% (18) and in a study conducted in Malatya, 97.3% (17) of the pregnant women were housewives. These results were higher than those of our study.

In a study conducted in Tanzania, 25.3% of pregnant women were not working (20), in a study conducted in Manisa, 91.5% of pregnant women were not working (16), and in a study conducted in İzmir, 86.5% of pregnant women were not working (22). The results of the study conducted in Tanzania were lower than those of our study, while the results of the studies conducted in Manisa and İzmir were higher than those of our study.

In a study conducted in Manisa, most of the pregnant women had a moderate income level (16). In a study conducted in İzmir, 68.3% of pregnant women had a moderate income level (22), in a study conducted in Çanakkale, 76.1% had a good or moderate income level (25), and in a study conducted in Düzce, 72.3% of pregnant women had a moderate income level (21). These results are compatible with the results of our study.

The majority of the participants in our study had a nuclear family structure. In a study conducted in Çanakkale, 86.3% (25), in a study conducted in İzmir, 76.5% (22) and in a study conducted in Malatya, 58.6% (17) of the participants had a nuclear family structure. The results of our study are consistent with those of these studies.

In a study conducted in Manisa, 9.8% (16), in a study conducted in Düzce, 8.9% (21) and in a study conducted in İzmir, 28.3% (22) of pregnant women did not have health insurance.

In a study conducted in Manisa, 79.5% of the pregnant women had children (16), in a study conducted in Yozgat, 61.4% (24), in a study conducted in İzmir, 52.2% (22) and in another study conducted in Manisa, 61% of the pregnant women had children (29). These results are higher than those of our study.

In a study conducted in South Africa, the mean number of years of marriage was found to be 4.7±4.3 years (23), and in a study conducted in Çanakkale, it was found to be 4.9±7.1 years (25). These results are compatible with the results of our study.

In a study conducted in Manisa, 96.3% of pregnant women had both civil and religious marriages (29). The results of the study conducted in Manisa are compatible with the results of our study.

In a study conducted in Manisa, 85.5% of the pregnant women were in their first marriage (16). The results of the study conducted in Manisa are compatible with the results of our study.

In a study conducted in South Africa, 36.9% of pregnant women had their first pregnancy (23), in a study conducted in İstanbul 42.4% (28) and in a study conducted in İzmir 43% (22). These results are lower than those in our study.

In a study conducted in South Africa, 79.7% of pregnant women had unplanned pregnancies (23), in a study conducted in Manisa, 19% (16), in a study conducted in Manisa, 25.4% (28) in a study conducted in İstanbul, and 17% in a study conducted in İzmir (22). The result of the study conducted in South Africa is higher than that of our study. The results of the studies conducted in Manisa, İstanbul, and İzmir are compatible with our study.

In a study conducted in South Africa, the gestational week was found to be 23.8±5.6 weeks (23). In a study conducted in İzmir, 55.2% of the pregnant women were in the 3rd trimester (22). In a study conducted in Manisa, 38.3% of the pregnant women were in the 2nd trimester and 56.7% were in the 3rd trimester (29). The majority of the participants in our study were pregnant women in the 2nd and 3rd trimesters.

In a study conducted in India, 43.2% of pregnant women (18), in a study conducted in Manisa, 22.9% (16), and in another study conducted in Manisa, 32.7% (30) of pregnant women were reported to have been exposed to violence before pregnancy. In a study conducted in Çanakkale, 18.5% of pregnant women reported that they were exposed to physical violence before pregnancy (25). In our study, the frequency was found to be 2%. This may be due to the small number of participants in our study and differences in development, social, cultural, and even economic levels between countries and cities.

In our study, the prevalence of those who experienced domestic violence during pregnancy was 5.4%. In a study conducted in Malatya, 31.7% of pregnant women (17), in a study conducted in Düzce, 64.2% of pregnant women (21), in a study conducted in İstanbul, 50.8% of pregnant women (28), in a study conducted in Van, 64.6% of pregnant women (31), in a study conducted in Nigeria, 14.2% of pregnant women in a study conducted in Nigeria (32), 8.9% of pregnant women in a study conducted in Colombia (19), 21.3% of pregnant women in a study conducted in South Africa (23), 21.5% of pregnant women in a study conducted in Peru (33), and 4.3% of pregnant women in a study conducted in Sweden were exposed to domestic violence during pregnancy (34). When we look at the literature, although the result of the study conducted in Sweden is similar to the result of our study, the results of other studies are quite high. As seen in the literature, the frequency of violence varies between 4.3% and 64.6%, although it varies between countries. The results of our study are also within the range stated in the literature.

In our study, the prevalence of those who experienced domestic physical violence during pregnancy was 0.5%. In a study conducted in Manisa, 24.8% (30); in a study conducted in Çanakkale, 10.3% (25); in a study conducted in İzmir, 10.9% (22); in a study conducted in Mexico, 6.7% (35); in a study conducted in Pakistan, 12.6% (36); in a study conducted in Peru, 11.9% (33); and in a study conducted in China, 3.6% (37) were exposed to physical violence during pregnancy. As seen in the literature, the prevalence of physical violence varies between 3.6% and 24.8% although it varies between countries. The results of our study are below the range stated in the literature.

In our study, the prevalence of those who experienced domestic verbal violence during pregnancy was 2.5%. In a study conducted in Texas, 5.1% of pregnant women were exposed to verbal violence (38), in a study conducted in Yozgat, 1.6% (24), in a study conducted in India-Tripura, 40.6% (18) and in a study conducted in Nigeria, 66.2% (32). When the literature was examined, it was observed that the frequency of verbal violence among societies was in a wide range due to the existence of measurement and evaluation methods and cultural differences, and because it varied according to the perception of the individual.

In our study, the prevalence of those who experienced domestic psychological violence during pregnancy was 5%. In a study conducted in 20 large cities in the USA, 13.1% of pregnant women were exposed to psychological violence (39), in a study conducted

in Peru, 15.6% of pregnant women were exposed to psychological violence (33), in a study conducted in Düzce, 26.5% of pregnant women were exposed to psychological violence (21), and in a study conducted in Ethiopia, 14.6% of pregnant women were exposed to psychological violence (40). In our study, the frequency of those who were exposed to domestic psychological violence during pregnancy was found to be low compared with the literature. More studies on this subject are required.

The prevalence of sexual violence during pregnancy was found to be 4.3% in a study conducted in Düzce (21), 8.3% in a study conducted in İzmir (22), 32.5% in a study conducted in Sivas (27), 9.7% in a study conducted in Malatya (17), 3.9% in a study conducted in Peru (33) and 4.3% in a study conducted in China (41). When we looked at the literature, we observed that sexual violence during pregnancy was not questioned in some studies on violence in pregnant women. In the studies in which it was questioned, we mostly encountered information that its frequency was low. In our study, no one-encountered domestic sexual violence during pregnancy.

In our study, the most common type of violence was psychological violence. In studies conducted in Malatya, İstanbul, Düzce, İzmir, Sivas, USA, Pakistan, South Africa, India-Delhi, the most common type of violence encountered by pregnant women was psychological violence (17,21-23,26-28,36,39). In studies conducted in Nigeria, India-Tripura, and Texas, verbal violence was found to be the most common type of violence (18,32,38). In studies conducted in Van, Mexico, Peru, and Uganda, the most common type of violence was physical violence (31,33,35,42). When we look at the literature, pregnant women most frequently encounter psychological violence in studies on violence.

In a study conducted in Çanakkale, 33.3% of pregnant women were exposed to violence (25); in a study conducted in Malatya, 11.9% (17). In a study conducted in Sweden, 13% (34) and in a study conducted in Peru, 11.9% (33) were 35 years of age or older. These results are similar to those of our study.

In a study conducted in İzmir, 86.5% of pregnant women exposed to violence were non-working pregnant women (22). In a study conducted in Yozgat, 86.6% of the pregnant women exposed to violence were unemployed (24), in a study conducted in Tanzania 25.4% were unemployed (20) and in a study conducted in Peru 41.8% were unemployed (33). The results of our study were found to be higher than the results of studies conducted abroad and lower than the results of studies conducted in our country. According to studies conducted in our country, we found that most pregnant women exposed to violence were not working.

In our study, 54.5% of the pregnant women who encountered domestic violence had their first pregnancy, whereas in a study conducted in İzmir, the majority of the pregnant women who encountered violence had their first pregnancy (22). It was the first pregnancy of 39.3% in a study conducted in Peru (33), 36.9% in a study conducted in South Africa (23) and 38.8% in a study conducted in Delhi, India (26). The result of our study is higher than those of these studies.

In Uganda, İstanbul, and our study, no statistical significance was found between the frequency of violence during pregnancy and age (28,42). In studies conducted in South Africa, India-Delhi, Malatya, Çanakkale and İzmir, a significant relationship was found between violence during pregnancy and age (17,22,23,25,26). There is a need for further studies on this subject.

In our study, we did not find statistical significance between violence in pregnancy and educational level. The results of studies conducted in Sweden, South Africa, İstanbul, and Yozgat support our study (23,24,28,34). In studies conducted in Sivas, Malatya, İzmir, and Brazil, statistical significance was found between educational level and violence during pregnancy, contrary to the results of our study (17,22,27,43,44).

In a study conducted in Sivas, a significant correlation was found between the low education level of the husband and violence (27). In a study conducted in Malatya, it was observed that the frequency of violence was strongly associated with the educational level of the pregnant woman's husband (17). In a study conducted in Çanakkale, a significant relationship was found between the educational level of the husband and violence (25). In a study conducted in İzmir, a statistically significant difference was found between the low level of education of the pregnant woman's husband and the exposure of the pregnant woman to violence by her husband (22). In a study conducted in Mexico, a significant relationship was found between a low level of education and violence (35). In a study conducted in Pakistan, a significant relationship was found between the low educational level of the husband and violence (36). In a study conducted in Yozgat, no significant statistical difference was found between the educational level of the pregnant woman's husband and the encounter with violence (24). The results of our study are compatible with those of the study conducted in Yozgat. However, most literature contradicts the results of our study.

In a study conducted in Delhi, India, Malatya, and İstanbul, no significant difference was found between non-working and working pregnant women in terms of the frequency of violence (17,26,28). These results are compatible with the results of our study.

In a study conducted in Sivas, a significant relationship was found between the unemployment of the pregnant woman's husband and all types of violence against pregnant women (27). In studies conducted in Van and İzmir, no significant correlation was found between the employment status of the pregnant woman's husband and violence against pregnant women (22,31).

In a study conducted in Sivas, a significant relationship was found between lack of health insurance and exposure to violence (27). In a study conducted in İzmir, a significant relationship was found between lack of social security and physical and emotional violence (22). The results of the studies conducted in Sivas and İzmir are not compatible with the results of our study.

In studies conducted in Malatya, Van, İzmir, and Delhi, India, a relationship was found between the frequency of violence against pregnant women and family income (17,22,26,31). These results are compatible with the results of our study.

When the literature is examined, there are studies that found a significant relationship between family type and violence against pregnant women in line with our study (18,29,43-46). However, no relationship was found in some studies (17,24,26).

In studies conducted in India, Tripura, Ethiopia, and Çanakkale, a statistically significant relationship was found between place of residence and violence (18,25,40). The results of these studies

support the results of our study. However, unlike the results of our study, no significant difference was found between living in rural or urban areas and the frequency of violence in studies conducted in Malatya, Yozgat, and Delhi, India (17,24,26). There is a need for further studies on this subject.

In studies conducted in Manisa, Malatya, and Van, a statistically significant relationship was found between increasing the number of children of pregnant women and the frequency of violence (17,30,31). In a study conducted in Yozgat, no significant statistical difference was found between the number of children of pregnant women and exposure to violence, similar to the result of our study (24).

In a study conducted in Delhi, India, Malatya, Van and Manisa found a significant relationship between increased duration of marriage and exposure to violence (17,26,30,31). The results of these studies are contrary to the results of our study, but a study conducted in China supports our study (41).

In a study conducted in Çanakkale, a statistically significant relationship was found between marital status and violence during pregnancy (25). In studies conducted in İstanbul and Yozgat, similar to our study, no significant statistical relationship was found between the type of marriage and exposure to violence (24,28).

In a study conducted in İstanbul, 39.3% of pregnant women described their marriages as very good/good, but no significant relationship was found between exposure to violence during pregnancy and satisfaction with marital life (28). In our study, the frequency of those who were satisfied with their marital life was higher than that of the study conducted in İstanbul. In addition, unlike the study in İstanbul, we found that the frequency of exposure to violence was significantly higher in those who were dissatisfied with their marital life. There are not many studies on this subject in the literature.

While a statistically significant relationship was found between unplanned pregnancy and the frequency of violence in a study conducted in Malatya, Van and İzmir (17,22,31), in a study conducted in İstanbul, although it was observed that those with unplanned pregnancy were exposed to more violent behavior, no statistically significant relationship was found between exposure to violence during pregnancy and planned pregnancy (28). In a study conducted in Sivas, a statistically significant difference was found between unplanned pregnancy and exposure to physical and emotional violence, whereas no significant relationship was found with exposure to sexual and economic violence (27). In our study, the frequency of violence was significantly higher in women with planned pregnancy. It is seen that more studies are needed on this subject.

In studies conducted in Malatya and İstanbul, it was observed that pregnant women in the second trimester-encountered domestic violence significantly more frequently (17,28). These results are compatible with those of our study.

In studies conducted in Sweden and İzmir, no significant relationship was found between the order of pregnancy and violence (22,34). The results of our study are compatible with those of studies conducted in Sweden and İzmir. However, the result of a study conducted in Delhi, India is different from our study and the literature (26).

In studies conducted in Uganda, South Africa, İstanbul, and Van, it was determined that those who were exposed to violence before pregnancy were exposed to violence more frequently in their current pregnancies ($p<0.05$) (23,28,31,42,47).

Study Limitations

The collection of data from a single center is the most important limitation of our study. In addition, the fact that the population is not large may make it difficult to generalize the results obtained.

CONCLUSION

The most important result of our study is that the frequency of exposure to violence during pregnancy is low. Other important results of our study are that the type of violence experienced by pregnant women who were exposed to violence during pregnancy was mostly psychological violence, and no pregnant women were exposed to sexual violence. The fact that none of the pregnant women who were exposed to violence received any treatment after the violence and did not apply to judicial authorities can be said to be a remarkable finding. The frequency of exposure to domestic violence during pregnancy significantly increased if the pregnant women had moderate income, lived in rural areas, had a nuclear family structure, were dissatisfied with their marital life, and had a planned pregnancy.

Women apply to health institutions more frequently to receive health care during pregnancy. Therefore, healthcare professionals have important responsibilities in detecting domestic violence during pregnancy and in monitoring, treating, and rehabilitating those who experience violence. It may be recommended that healthcare professionals should question domestic violence during pregnancy and conduct a detailed examination related to violence while performing anamnesis. In addition, it would be useful to inform a wider audience to increase the application of disadvantaged groups to HLCs.

Ethics

Ethics Committee Approval: This study was conducted within the framework of ethical rules. Approval was obtained from the Human Research Ethics Committee of Muğla Sıtkı Koçman University (approval number: 122, date: 16.07.2019) and the Dean's Office of the Faculty of Medicine before the study.

Informed Consent: Students and resident physicians were informed that participation in the survey was voluntary.

Author Contributions

Surgical and Medical Practices: M.P., Ş.A., R.Ü.K., M.O.V., Concept: M.P., Design: M.P., Data Collection or Processing: M.P., Ş.A., Analysis or Interpretation: M.P., Ş.A., Literature Search: M.P., Ş.A., R.Ü.K., M.O.V., Writing: M.P., Ş.A.

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

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

REFERENCES

1. Santos SMAB, Oliveira ZM, Coqueiro RDS, Santos VC, Anjos K FD, Casotti CA. Prevalence and profile of pregnant women who suffered physical violence. Revista de Pesquisa: Cuidado é Fundamental Online. 2017; 9: 401-7.

2. WHO. Respect women: Preventing violence against women. Geneva: World Health Organization; 2019 (WHO/RHR/18.19). License: CC BY-MC-SA 3.0 IGO).
3. Pallitto CC, Garcia-Moreno C, Jansen HAFM, Ellsberg M, Heise L, Watts C, et al. Intimate partner violence, abortion, and unintended pregnancy: results from the WHO Multi-country Study on Women's Health and Domestic Violence. Int J Gynaecol Obstet. 2013; 120: 3-9.
4. Prime Ministry, General Directorate on the Status of Women. Domestic Violence against Women in Turkey. Elma Teknik Basım Matbaacılık. Ankara; 2009.
5. Ergönen AT, Ozdemir MH, Can IO, Sönmez E, Salaçin S, Berberoğlu E, Demir N. Domestic violence on pregnant women in Turkey. J Forensic Leg Med. 2009; 16: 125-9.
6. Espinosa L, Osborne K. Domestic violence during pregnancy: implications for practice. J Midwifery Womens Health. 2002; 47: 305-17.
7. Yanikkerem E, Karadaş G, Adigüzel B, Sevil U. Domestic violence during pregnancy in Turkey and responsibility of prenatal healthcare providers. Am J Perinatol. 2006; 23: 93-103.
8. Hilberman E, Munson K. Sixty battered women. Int J Victimol. 1978; 2: 460-70.
9. Taillieu TL, Brownridge DA. Violence against pregnant women: prevalence, patterns, risk factors, theories, and directions for future research. Aggression and Violent Behavior. 2010; 15: 14-35.
10. Gazmararian JA, Lazorick S, Spitz AM, Ballard TJ, Saltzman LE, Marks JS. Prevalence of violence against pregnant women. JAMA. 1996; 275: 1915-20.
11. Leung WC, Kung F, Lam J, Leung TW, Ho PC. Domestic violence and postnatal depression in a Chinese community. Int J Gynaecol Obstet. 2002; 79: 159-66.
12. Alhusen JL, Ray E, Sharps P, Bullock L. Intimate partner violence during pregnancy: maternal and neonatal outcomes. J Womens Health (Larchmt). 2015; 24: 100-6.
13. Stöckl H, Filippi V, Watts C, Mbwambo JK. Induced abortion, pregnancy loss and intimate partner violence in Tanzania: a population based study. BMC Pregnancy Childbirth. 2012; 12: 12.
14. Howard LM, Oram S, Galley H, Trevillion K, Feder G. Domestic violence and perinatal mental disorders: a systematic review and meta-analysis. PLoS Med. 2013; 10: e1001452.
15. Republic of Turkey Ministry of Family, Labor and Social Services. General Directorate on the Status of Women. Combating Violence against Women (2016-2020). National Action Plan. December; 2016.
16. Kapan M, Yanıkkerem E. Kırsal ve kentsel alanda yaşayan gebelerin depresyon, yalnızlık ve şiddete maruz kalma durumları. TAF Preventive Medicine Bulletin. 2015; 15: 431-9.
17. Karaoglu L, Celbis O, Ercan C, Ilgar M, Pehlivan E, Gunes G, et al. Physical, emotional and sexual violence during pregnancy in Malatya, Turkey. Eur J Public Health. 2006; 16: 149-56.
18. Bhattacharjya H, Deb D. Intimate partner violence against women during pregnancy in Tripura: a hospital based study. Int J Res Med Sci. 2014; 2: 84-90.
19. Jaraba SMR, Garcés-Palacio IC. Association between violence during pregnancy and preterm birth and low birth weight in Colombia: Analysis of the demographic and health survey. Health Care Women Int. 2019; 40: 1149-69.
20. Mahenge B, Likindikoki S, Stöckl H, Mbwambo J. Intimate partner violence during pregnancy and associated mental health symptoms among pregnant women in Tanzania: a cross-sectional study. BJOG. 2013; 120: 940-6.
21. Bolu F, Mayda AS, Yılmaz M. The frequency of violence experienced by pregnant women who applied to a university hospital obstetrics outpatient clinic and the factors affecting them. Nobel Medicus. 2014: 31; 64-70.
22. Sağkal T, Kalkım A, Uğurlu E, Kırmızılar N. The Situation of Pregnant Women Exposure to Violence by Husband and the Factors Associated With Violence. TAF Prev Med Bull. 2014; 13: 381-90.
23. Groves AK, Moodley D, McNaughton-Reyes L, Martin SL, Foshee V, Maman S. Prevalence, rates and correlates of intimate partner violence among South African women during pregnancy and the postpartum period. Matern Child Health J. 2015; 19: 487-95.
24. Gençer A, Onat T, Başer E, Kara M, Yalvaç ES. Prevalence of Violence Against Pregnant Women in Yozgat. Bozok Tıp Dergisi. 2018: 8; 59-64.
25. Taspinar A, Bolsoy N, Kaya F, Şirin A, Şirin G. Physical violence and affecting factors during pregnancy in Çanakkale. Family and Society. 2008; 4: 63-76.
26. Jain S, Varshney K, Vaid NB, Guleria K, Vaid K, Sharma N. A hospital-based study of intimate partner violence during pregnancy. Int J Gynaecol Obstet. 2017; 137: 8-13.
27. Güler N. Physical, emotional, sexual and economic violence inflicted on women by their husbands during pregnancy and related factors. Dokuz Eylül University School of Nursing Electronic Journal. 2010; 3: 72-7.
28. Göğüş T, Yıldız H. Status of violence in pregnancy according to the trimesters, effecting factors and perinatal results. TAF Prev Med Bull. 2013; 12: 657-64.
29. Muslu A. The relationship between domestic violence in pregnancy and postpartum depression; Manisa Sample. Celal Bayar University Institute of Health Sciences. Department of Midwifery. Master Thesis. Manisa: 2014.
30. Taşpınar A, Bolsoy N, Şirin A. Are pregnant women subjected to physical violence? Manisa Sample. Journal of Forensic Psychiatry. 2005; 2: 41-7.
31. Sahin HA, Sahin HG. An unaddressed issue: domestic violence and unplanned pregnancies among pregnant women in Turkey. Eur J Contracept Reprod Health Care. 2003; 8: 93-8.
32. Fawole AO, Hunyinbo KI, Fawole OI. Prevalence of violence against pregnant women in Abeokuta, Nigeria. Aust N Z J Obstet Gynaecol. 2008; 48: 405-14.
33. Perales MT, Cripe SM, Lam N, Sanchez SE, Sanchez E, Williams MA. Prevalence, types, and pattern of intimate partner violence among pregnant women in Lima, Peru. Violence Against Women. 2009; 15: 224-50.
34. Hedin LW, Janson PO. Domestic violence during pregnancy. Acta Obstetricia et Gynecologica Scandinavica. 2000: 79; 625-30.
35. Díaz-Olavarrieta C, Paz F, Abuabara K, Martínez Ayala HB, Kolstad K, Palermo T. Abuse during pregnancy in Mexico City. Int J Gynaecol Obstet. 2007; 97: 57-64.
36. Farid M, Saleem S, Karim MS, Hatcher J. Spousal abuse during pregnancy in Karachi, Pakistan. Int J Gynaecol Obstet. 2008; 101: 141-5.
37. Thananowan N, Heidrich SM. Intimate partner violence among pregnant Thai women. Violence Against Women. 2008; 14: 509-27.
38. Yost NP, Bloom SL, McIntire DD, Leveno KJ. A prospective observational study of domestic violence during pregnancy. Obstet Gynecol. 2005; 106: 61-5.
39. Charles P, Perreira K. Intimate partner violence during pregnancy and 1-year postpartum. Journal of Family Violence. 2007; 22: 609-19.

40. Belay S, Astatkie A, Emmelin M, Hinderaker SG. Intimate partner violence and maternal depression during pregnancy: A community-based cross-sectional study in Ethiopia. PLoS ONE. 2019; 14: 1-15.
41. Leung WC, Leung TW, Lam YYJ, Ho PC. The prevalence of domesticviolence against pregnant women in a Chinese community. International Journal of Gynecology and Obstetrics. 1999; 66: 23-30.
42. Clarke S, Richmond R, Black E, Fry H, Obol JH, Worth H. Intimate partner violence in pregnancy: a cross-sectional study from post-conflict northern Uganda. BMJ Open. 2019; 9: e027541.
43. Moraes CL, Reichenheim ME. Domestic violence during pregnancy in Rio de Janeiro, Brazil. Int J Gynaecol Obstet. 2002; 79: 269-77.
44. Bifftu BB, Guracho YD. Determinants of Intimate Partner Violence against Pregnant Women in Ethiopia: A Systematic Review and Meta-Analysis. Biomed Res Int. 2022; 2022: 4641343.
45. Dietz PM, Gazmararian JA, Goodwin MM, Bruce FC, Johnson CH, Rochat RW. Delayed entry into prenatal care: effect of physical violence. Obstet Gynecol. 1997; 90: 221-4.
46. Cokkinides VE, Coker AL, Sanderson M, Addy C, Bethea L. Physical violence during pregnancy: maternal complications and birth outcomes. Obstet Gynecol. 1999; 93 :661-6.
47. Ayeni OB, Tekbaş S. Prevalence, Frequency, and Affecting Factors of Intimate Partner Violence Against Pregnant Women in Osun State, Nigeria. Violence and Gender. 2022: 36-4.

GMJ 2024;35:276-280

DOI: http://dx.doi.org/10.12996/gmj.2023.3941

Retrospective Research of Clinical and Hematological Changes Occurred by del Nido Cardioplegia in the Perioperative Period of Patients who Underwent Open-Heart Surgery

Açık Kalp Cerrahisi Geçiren Hastalarda del Nido Kardiyoplejisinin Perioperatif Dönemde Neden Olduğu Klinik ve Hematolojik Değişikliklerin Retrospektif İncelenmesi

Abdullah Özer[1], Başak Koçak[2], Mustafa Arslan[3], Elif Şimşek[1], Aycan Özdemirkan[3], Yusuf Ünal[3], Erkan İriz[1], Hakan Zor[1], Levent Oktar[1]

[1]Department of Cardiovascular Surgery, Gazi University Faculty of Medicine, Ankara, Türkiye
[2]Clinic of Cardiovascular Surgery, Sivas Numune Hospital, Sivas, Türkiye
[3]Department of Anaesthesiology and Reanimation, Gazi University Faculty of Medicine, Ankara, Türkiye

ABSTRACT

Objective: Aortic cross-clamping and postischemic myocardial dysfunction are fundamentally related to myocardial protection during open-heart surgery. Various cardioplegia solutions have been developed because of this issue. del Nido cardioplegia (DNC) solution is one of these solutions and has a vital impact on metabolic markers and cardiac protection in individuals of all ages. This study aimed to examine the effects of DNC on the perioperative follow-up period after cardiac surgery.

Methods: Preoperative and postoperative variations in selected biochemical and hematological variables of 71 patients who underwent open-heart surgery in our medical faculty between 2018 and 2020 were retrospectively examined and compared with normal values. SPSS 20.0 statistical software was used, and a statistically significant difference was defined as $p<0.05$.

Results: Hemoglobin, platelet, albumin, and uric acid levels were significantly lower at the end of cardiopulmonary bypass and the postoperative 24th hour than in the preoperative period. At the end of the cardiopulmonary bypass and the postoperative 24th hour, aspartate aminotransferase and lactate dehydrogenase levels were significantly greater than those in the preoperative period. Remarkable increases in hemoglobin, albumin, urea, and platelets in the postoperative 24th hour compared with the end of cardiopulmonary bypass were noted. We also reported substantial differences in glucose, lactate, creatine kinase-MB, and troponin levels.

Conclusion: We found significant changes in different parameters critical for the perioperative period of open-heart surgery. Although

ÖZ

Amaç: Aortik kross klemp uygulaması ve postiskemik miyokardiyal disfonkiyon, açık kalp cerrahisinde miyokard korunması ile ilişkili temel konulardandır. Bunun bir sonucu olarak değişik kardiyolpleji solüsyonları geliştirilmiştir. Bu solüsyonlardan birisi olan del Nido kardiyoplejisi (DNC), her yaştan bireyde kardiyak koruma ve metabolik değerler üzerinde önemli bir etkiye sahiptir. Çalışmamız DNC'nin kardiyak cerrahi geçiren hastalarda perioperatif periyottaki etkilerini incelemeyi amaçlamaktadır.

Yöntemler: 2018 ile 2020 yılları arasında merkemizde açık kalp cerrahisi operasyonu geçiren 71 hastaya ait, seçili biyokimyasal ve hematolojik parametrelerin preoperatif ve postoperatif dönemdeki değişiklikleri retrospektif olarak incelendi, normal laboratuvar değerleri ile karşılaştırıldı. İstatistiksel inceleme için SPSS 20.0 yazılımı kullanıldı ve $p<0,05$ değeri anlamlı kabul edildi.

Bulgular: Hemoglobin, platelet, albümin ve ürik asit değerleri, pompa çıkışında ve postoperatif 24. saatte yapılan ölçümlerde preoperatif döneme göre anlamlı olarak düşük çıktı. Aspartat aminotransferaz ve laktat dehidrojenaz da ise pompa çıkışı ve postoperatif 24. saatte, preoparatif döneme oranla anlamlı yükseklik tespit edildi. Hemoglobin, platelet, albümin, üre değerlerinin postoperatif 24. saatte, pompa çıkışına göre önemli yüksekliği görüldü. Ayrıca glukoz, laktat, kreatin kinaz-MB ve troponin değerlerinde de anlamlı farklılıklar tespit edildi.

Sonuç: Açık kalp cerrahisinde perioperatif dönemde kritik olan farklı parametrelerde anlamlı farklılıklar tespit ettik. Çalışmamız neticesinde DNC'nin güvenilir bir seçenek olduğunu göstermemize rağmen yeni klinik çalışmalara ihtiyaç vardır.

Address for Correspondence/Yazışma Adresi: Başak Koçak, MD, Clinic of Cardiovascular Surgery, Sivas Numune Hospital, Sivas, Türkiye
E-mail / E-posta: drbasakkocak@gmail.com
ORCID ID: orcid.org/0000-0002-3767-0687

Received/Geliş Tarihi: 18.07.2023
Accepted/Kabul Tarihi: 08.08.2023

ABSTRACT

our study found DNC to be a safer option, additional research into clinical usage is required.

Keywords: del Nido cardioplegia solution, cardiopulmonary bypass, perioperative period

ÖZ

Anahtar Sözcükler: del Nido kardiyopleji solüsyonu, kardiyopulmoner bypass, perioperatif dönem

INTRODUCTION

All types of cardiac surgery keep their frequency related to cardiovascular disease as a fundamental cause of death worldwide. Although coronary bypass graft surgery (CABG) is one of the most common operations in the world (1), it still has high morbidity and mortality levels (2) correlated with myocardial protection and injury (3-5). Cardiopulmonary bypass (CPB) and aortic cross-clamping provide surgical teams with a bloodless and non-beating heart (6) but interrupt myocardial perfusion during CABG, valve, or congenital heart surgery. Inadequate or no blood supply to myocardial tissue means cessation of oxygen and loss of ATP production. At the end of this process, the Na^{+}/Ca^{++} exchanger's dysfunction causes Ca^{++} accumulation in the intracellular area (5). Different cardioplegia solutions, which have been used to prevent myocardial dysfunction as a result of myocardial ischemia and reperfusion, have been investigated by many researchers. One of these solutions is del Nido cardioplegia (DNC), named by Dr. Pedro del Nido (5). This solution has been developed for tolerating the vulnerability of immature myocyte after increasing intracellular calcium (7). It has long been used in the pediatric population. Today, there are many studies about DNC's reliability not only for pediatric patients but also; for adult patients. DNC, which mostly comprises potassium chloride, lidocaine, mannitol, magnesium sulfate, and sodium bicarbonate, is more dilute with a 4:1 ratio (crystalloid to blood) (2,8,9). Several studies have also shown that DNC outperforms other treatments in terms of cardiac protection lasting longer than 90 min with a single dosage application (5-7). This manuscript aimed to compare the perioperative changes in clinical and hematological parameters of patients who underwent open-heart surgery with DNC.

MATERIALS AND METHODS

Study Design and Patients

We retrospectively reviewed preoperative and postoperative changes in selected biochemical and hematological values of 71 patients who underwent open-heart surgery at our center between 2018 and 2020. Patients with active infection, older than 80 years, and those unable to cooperate were excluded from the study. The procedures in the experiment were carried out according to the permission of the Gazi University Institutional Local Animal Care and Use Ethics Committee (approval number: 585, date: 28.06.2021).

Demographic Data

The following variables were examined: age, gender, weight, height, ejection fraction (EF), presence of diabetes mellitus, ASA risk, EuroSCORE, and type of operation.

Perioperative Data

We looked to see if there were any significant differences in the time of CPB, aortic cross-clamp, intensive care unit (ICU) stay, extubation, and the amount of drainage and urine output within 24 h of admission to the ICU, all of which are critical clinical parameters for the postoperative period of open-heart surgery. We also discovered the need for defibrillation, a pacemaker, an intraortic balloon pump (IABP), inotropic agent support intraoperatively, and the presence of a cerebrovascular stroke, mortality, and atrial fibrillation within 24 h after surgery in the postoperative period.

Glucose and lactate levels were measured at four scheduled time points and compared with baseline laboratory values: before CPB, before and after the aortic cross-clamp was released, and after CPB was terminated.

Selected hematological and biochemical values, including hemoglobin, platelet count, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine, lactate dehydrogenase (LDH), and uric acid, were measured at baseline, after the termination of CPB, and 24 h after surgery, were noted additionally. These parameters were also compared with normal laboratory values.

The occurrence of atrial fibrillation, cerebrovascular stroke, mortality, drainage, and urine output within 24 h of ICU admission, duration to extubation, and length of ICU stay were all documented. Creatine kinase-MB (CK-MB) and troponin levels were also retrieved at three time points: preoperative, 1 h after surgery, and 24 h after surgery.

Statistical Analysis

Data were analyzed using SPSS. $P<0.05$ was the threshold for a statistically significant difference. The findings are presented as mean ± standard deviation and the median of the biochemical variables.

RESULTS

The mean age of the patients included was 60.12±9.10. There were 76.1% male patients and 17.23% female patients. When we checked the ASA Score of patients. ASA 3 was the most common, accounting for 80.3% of the patients. The mean EuroScore was 3.91±2.40. Table 1 displays the patients' demographic information.

There was a significant increase in CK-MB's postoperative first-hour level compared with the preoperative level ($p<0.0001$). On the other hand, we found a remarkable decrease in CK-MB's postoperative 24 h level compared with the postoperative first-hour level ($p<0.0001$). Increased troponin in the postoperative first hour and postoperative 24^{th} hour compared with the preoperative value was significant. In Table 2, Figure 1, troponin and CK-MB levels are shown.

As compared with before CPB, there was a significant increase in all planned time points of glucose and lactate levels (p<0.0001, p<0.0001, p<0.0001, respectively). Similarly, we found considerable

Table 1. Patients' demographic data [mean ± SD, n (%)]

Parameter	Mean ± SD
Age	60.12±9.10
Sex	
Male, n (%)	54 (76.1)
Female, n (%)	17 (23.9)
Weight (kg)	166.20±12.76
Diabetes mellitus	
Yes, n (%)	41 (57.7)
No, n (%)	30 (42.3)
Height (cm)	78.37±17.09
Ejection fraction (%)	54.56±8.94
Operation type	
CABG, n (%)	50 (70.4)
Other, n (%)	21 (29.6)
Euro score	3.91±2.40
ASA score	
ASA II, n (%)	5 (7)
ASA III, n (%)	57 (80.3)
ASA IV, n (%)	9 (12.7)

SD: Standard deviation, CABG: Coronary bypass graft surgery, ASA: American Society of Anesthesiologists.

Table 2. CK-MB and troponin data [median (minimum-maxium)]

Marker	Preoperative	Postoperative first hour	Postoperative first day
Troponin	20 (0.6-26,358)	2,807 (91-26,358)*	1,486 (50-26,358)*
CK-MB	16 (6.5-135)	48 (21-245)*	24.5 (7.7-122)&

*P<0.05: Compared to preoperative, &p<0.05: Compared to postoperative first hour, CK: Creatine kinase.

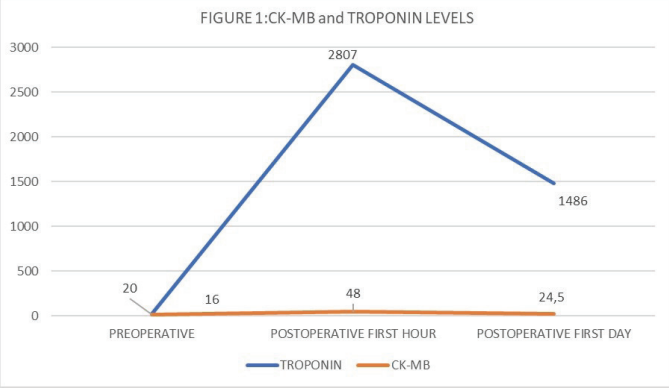


Figure 1. CK-MB and troponin levels.

CK: Creatine kinase.

Table 3. Glucose and lactate levels [median (minimum-maxium)]

	Glucose	Lactate
Before the CPB	135 (90-260)	1 (0.2-2.70)
Before releasing the aortic cross clamp	159 (103-258)*	1.6 (0.6-4)*
After releasing the aortic cross clamp	185 (109-260)*,&	2.1 (0.6-4.6)*,&
After the termination of CPB	186 (114-278)*,&	2.1 (0.5-4.6)*,&

CPB: Cardiopulmonary bypass, *p<0.05: Compared to preoperative, &p<0.05: Compared to before releasing the aortic cross clamp. CPB: Cardiopulmonary bypass.

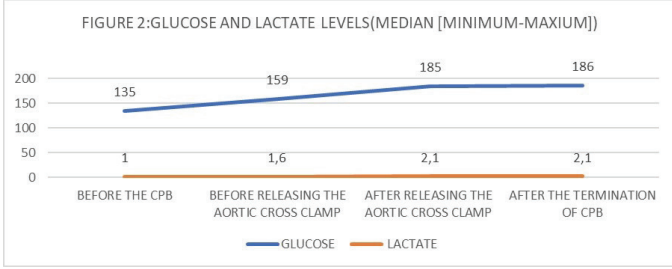


Figure 2. Glucose and lactate levels [median (minimum-maximum)].

CPB: Cardiopulmonary bypass.

Table 4. Cross-clamp time, CPB time, defibrillation, pacemaker, intra-aortic balloon pump, and inotropic agent usage [median (minimum-maximum), n (%)]

Variable	Mean ± SD
CPB time (minute)	115 (50-1451)
Cross-clamp (minute)	78 (37-821)
Defibrillation	
Yes, n (%)	13 (18.3)
No, n (%)	58 (81.7)
Pacemaker	
Yes, n (%)	17 (23.9)
No, n (%)	54 (76.1)
IABP	
Yes, n (%)	4 (5.6)
No, n (%)	67 (94.4)
Inotropic agent	
Yes, n (%)	54 (76.1)
No, n (%)	17 (23.9)

SD: Standard derivation, CPB: Cardiopulmonary bypass, IABP: Intraortic balloon pump.

Table 5. Extubation, ICU stay, amount of drainage, and urine [median (minimum-maximum)]

Data	
Extubation	*186 (114-278)*
ICU stay (hour)	48 (12-120)
Drainage (mililiter)	500 (50-2,900)
Urine (mililiter)	3,000 (640-6,000)

ICU: Intensive care unit.

Table 6. Biochemical and hematological data [mean ± SD, median (min.-max.)]

	Preoperative	End of CPB	Postoperative 24th h
Hemoglobin	12.81±1.99	8.37±1.03*	9.35±0.77*,&
Platelet	248507.04±75395.13	136126.76±50713.74*	168507.04±56629.84*,&
Albumin	3.96±0.49	2.20±0.36*	3.24±0.30*,&
AST	24 (13-878)	39 (11-128)*	45 (10-234)*
ALT	21 (9-1525)	25 (6-85)	29 (13-101)
BUN	17 (9-69)	16 (7-55)	20 (7.1-61)&
Creatinin	0.80 (0.4-7.7)	80 (0.3-6.1)	0.9 (0.3-4.9)
LDH	230 (113-1125)	323 (149-705)*	355 (204-908)*
Uric acid	5.80 (2.9-11)	5.25 (2.6-0.8)*	5.4 (2.1-10)*

*P<0.05: Compared to preoperative, &p< compared to end of CPB, SD: Standard deviation, min.: Minimum, max.: Maximum, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, BUN: Blood urea nitrogen, LDH: Lactate dehydrogenase, CPB: Cardiopulmonary bypass.

increases in glucose and lactate levels at two time points, which were after releasing the cross-clamp and after the termination of CPB, compared with before releasing the aortic cross-clamp (p<0.0001, p<0.0001, respectively) (Table 3, Figure 2).

During the intraoperative period, 18.3% of patients required defibrillation, and 76.1% of patients needed inotropic agent assistance. In 4 patients, an intra-aortic balloon pump was used, and 17 patients required a pacemaker. Table 4 shows that the mean aortic cross-clamp time was 78 (37-821) minutes and the mean CPB time were 115 (50-1451) minutes.

The mean extubation time was 186 min (114-278) and 48 h (12-120) were the average length of ICU stay. The total amount of tube drainage in the postoperative 24 h was 500 milliliters (50-2900) and urine was 3000 milliliters (640-6000) as shown in Table 5.

In the first 24 h following surgery, 7% of patients were noted with atrial fibrillation, whereas no cerebrovascular attack or death occurred. Table 6 summarizes the biochemical and hematological data of the patients, including hemoglobin, platelet count, albumin, AST, ALT, BUN, creatinine, LDH, and uric acid. There were significant decreases in hemoglobin, platelet, albumin, and uric acid levels at the end of CPB compared with preoperative values. Similarly, hemoglobin, platelet, albumin, and uric acid levels were remarkably lower at the postoperative 24 h compared to preoperative values. When compared with the end of CPB, BUN, albumin, platelet, and hemoglobin levels were higher in the postoperative 24 h. Another notable difference was in LDH levels greater in the postoperative 24 h and at the end of CPB compared with preoperative levels. At the end of CPB, AST was the only parameter to be significantly higher than its preoperative value. Likewise, AST was increased in postoperative 24 h compared to the preoperative value.

DISCUSSION

In this retrospective and single-center study, we presumed that DNC was a safe solution. The considerable decrease in troponin and CK-MB levels 24 h after surgery and the reduced use of defibrillation, IABP, and pacemakers corroborated our theory.

We did not collect the postoperative EF records. Therefore, the probable cause of increased inotropic drug administration could not be evaluated. On the other hand, we believe it can be associated with DNC's ingredients and their potential vasodilatation effects.

In several studies, the incidence of postoperative atrial fibrillation (POAF) varies. However, to the best of our knowledge, the incidence of POAF in the first 24 h following surgery has not been adequately documented. According to our data, atrial fibrillation was observed in 7% of patients in the 24 h following surgery; however, a lot of studies on POAF limit our comparison.

Cerebrovascular attack is one of the most serious consequences of open-heart surgery. Based on our findings, we believe that DNC has a protective effect against neurological problems; nevertheless, long-term results may be more definite to consider.

Only a few studies have shown a significant change in creatinine levels following open-heart surgery. We found no significant difference in creatinine levels in the current trial. In addition, we noted 3000 mL of total urine and a statistically meaningful increase in BUN at 24 h following surgery. Based on these findings, we conclude that DNC has no harmful effect on renal function. Another problem with our theory is the inability to precisely characterize postoperative renal insufficiency. Not only do patients need hemodialysis after surgery but also long-term outcomes for urine output might be parameters to consider.

Study Limitations

Our study has limitations on a few topics. Firstly, there were no patients under 18 or older than 80. On the other hand, all the patients had undergone elective surgeries, and we excluded patients with active infections.

CONCLUSION

DNC is a highly safe option for protecting the myocardium and organs. Future research is necessary.

Ethics

Ethics Committee Approval: The procedures in the experiment were carried out according to the permission of the Gazi University Institutional Local Animal Care and Use Ethics Committee (approval number: 585, date: 28.06.2021).

Informed Consent: Patient approval has not been obtained as it is performed on animals.

Author Contributions

Concept: A.Ö., B.K., M.A., E.Ş., A.Ö., Y.Ü., E.İ., H.Z., L.O., Design: A.Ö., B.K., M.A., E.Ş., A.Ö., Y.Ü., E.İ., H.Z., L.O., Data Collection or Processing: A.Ö., B.K., M.A., E.Ş., A.Ö., Y.Ü., E.İ., H.Z., L.O., Analysis or Interpretation: A.Ö., B.K., M.A., E.Ş., A.Ö., Y.Ü., E.İ., H.Z., L.O., Literature Search: A.Ö., B.K., M.A., E.Ş., A.Ö., Y.Ü., E.İ., H.Z., L.O., Writing: A.Ö., B.K., M.A., E.Ş., A.Ö., Y.Ü., E.İ., H.Z., L.O.

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

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

REFERENCES

1. Melly L, Torregrossa G, Lee T, Jansens JL, Puskas JD. Fifty years of coronary artery bypass grafting. J Thorac Dis. 2018; 10: 1960-7.
2. Yerebakan H, Sorabella RA, Najjar M, Castillero E, Mongero L, Beck J, et al. Del Nido Cardioplegia can be safely administered in high-risk coronary artery bypass grafting surgery after acute myocardial infarction: a propensity matched comparison. J Cardiothorac Surg. 2014; 9: 141.
3. Orak Y, Kocarslan A, Boran OF, Acipayam M, Eroglu E, Kirisci M, et al. Comparison of the operative and postoperative effects of del Nido and blood cardioplegia solutions in cardiopulmonary bypass surgery. Braz J Cardiovasc Surg. 2020; 35: 689-96.
4. Ginther RM Jr, Gorney R, Forbess JM. Use of del Nido cardioplegia solution and a low-prime recirculating cardioplegia circuit in pediatrics. J Extra Corpor Technol. 2013; 45: 46-50.
5. Sanetra K, Pawlak I, Cisowski M. Del Nido cardioplegia - what is the current evidence? Kardiochir Torakochirurgia Pol. 2018; 15: 114-8.
6. Si Guim G, Goh Wah Hoon C, Anne Lim C, Shoo Chay-Nancy H, Ai Li Ler A, Xuan Lim Q, et al. Use of del Nido Cardioplegia for Adult Heart Surgery: How Long Is Not Too Long? J Extra Corpor Technol. 2020; 52: 272-8.
7. Sorabella RA, Akashi H, Yerebakan H, Najjar M, Mannan A, Williams MR, et al. Myocardial protection using del nido cardioplegia solution in adult reoperative aortic valve surgery. J Card Surg. 2014; 29: 445-9.
8. Kim K, Ball C, Grady P, Mick S. Use of del Nido Cardioplegia for Adult Cardiac Surgery at the Cleveland Clinic: Perfusion Implications. J Extra Corpor Technol. 2014; 46: 317-23.
9. Haider A, Khwaja IA, Khan AH, Yousaf MS, Zaneb H, Qureshi AB, et al. Efficacy of Whole-Blood Del Nido Cardioplegia Compared with Diluted Del Nido Cardioplegia in Coronary Artery Bypass Grafting: A Retrospective Monocentric Analysis of Pakistan. Medicina (Kaunas). 2021; 57: 918.

GMJ 2024;35:281-285

DOI: http://dx.doi.org/10.12996/gmj.2024.3952

Where Do We Go Wrong in the Pharmacologic Treatment of Functional Constipation in Children?

Çocuklarda Fonksiyonel Kabızlığın Farmakolojik Tedavisinde Nerede Yanlış Yapıyoruz?

Neslihan Gürcan Kaya[1], Hakan Öztürk[2]

[1]Clinic of Pediatric Gastroenterology, University of Health Sciences Türkiye, Ankara Training and Research Hospital, Ankara, Türkiye
[2]Department of Pediatric Gastroenterology, Gazi University Faculty of Medicine, Ankara, Türkiye

ABSTRACT

Objective: Constipation is often inadequately treated in childhood, which can lead to psychological problems. This study aimed to evaluate the adequacy of prescribed drugs, dosing, usage, and responses to these drugs in children with functional constipation.

Methods: This research comprised children who had consulted a pediatric gastroenterologist for functional constipation and had previously undergone constipation therapy. The name of the drug used, duration of drug usage, method of measurement, and what they took the drug with, the dose of drug, and response to the drug were recorded.

Results: Eighty-seven percent of the patients had received lactulose treatment. Only 31% of the patients received a medication dose greater than 1 mL/kg. In 58.3% of cases, the duration of drug use was shorter than one month. There was not a response in 62.9% of cases, a partial response in 23.1%, and a full response in 13.8% of cases. When the patients were compared based on response, there was a significant difference in the duration of drug usage, what they drank the drug with, the daily dose of the drug, and the daily dosage per weight of the drug. Patients who measured the drug using a milliliter scale responded better. The response to the drug increased as the drug dosage per weight increased. Response was obtained in 73% of patients who had no response or partial response after drug or dose adjustments.

Conclusion: For a comprehensive response, parents must be carefully taught the dosage of the drug and how to measure it.

Keywords: Constipation, treatment, lactulose, treatment period, response

ÖZ

Amaç: Kabızlık çocukluk çağında sıklıkla yetersiz tedavi edilir ve bu durum psikolojik sorunlara yol açabilir. Bu çalışmada fonksiyonel kabızlığı olan çocuklarda reçete edilen ilaçların yeterliliği, dozajı, kullanımı ve bu ilaçlara verilen yanıtların değerlendirilmesi amaçlandı.

Yöntemler: Bu araştırma, fonksiyonel kabızlık nedeniyle pediatrik gastroenteroloji uzmanına başvuran ve daha önce kabızlık tedavisi gören çocukları kapsamaktadır. Kullanılan ilacın adı, kullanım süresi, ölçüm yöntemi, ilacı neyle aldığı, ilacın dozu ve ilaca verdiği yanıt kaydedildi.

Bulgular: Hastaların %87'si laktuloz tedavisi almıştı. Hastaların yalnızca %31'i 1 mL/kg'ın üzerinde ilaç dozu aldı. Olguların %58,3'ünde ilaç kullanım süresi bir aydan kısaydı. Olguların %62,9'unda yanıt alınamadı, %23,1'inde kısmi yanıt, %13,8'inde ise tam yanıt oluştu. Hastalar yanıta göre karşılaştırıldığında ilaç kullanım süreleri, ilacı neyle içtikleri, ilacın günlük dozu ve kilo başına günlük dozaj arasında anlamlı fark vardı. İlacı mililitre ölçeği kullanarak ölçen hastalar daha iyi yanıt verdi. Ağırlık başına ilaç dozajı arttıkça ilaca verilen yanıt da arttı. İlaç veya doz ayarlaması sonrasında yanıt alınamayan veya kısmi yanıt alınamayan hastaların %73'ünden yanıt alındı.

Sonuç: Kapsamlı bir müdahale için ebeveynlere ilacın dozajı ve bunun nasıl ölçüleceği dikkatle öğretilmelidir.

Anahtar Sözcükler: Kabızlık, tedavi, laktuloz, tedavi süresi, cevap

Address for Correspondence/Yazışma Adresi: Neslihan Gürcan Kaya, MD, Clinic of Pediatric Gastroenterology, University of Health Sciences Türkiye, Ankara Training and Research Hospital, Ankara, Türkiye
E-mail / E-posta: nesligurcan@hotmail.com
ORCID ID: orcid.org/0000-0002-1813-7780

Received/Geliş Tarihi: 26.07.2023
Accepted/Kabul Tarihi: 16.03.2024

INTRODUCTION

Constipation is a prevalent condition among children (1). Its reported prevalence ranges from 0.7-29.6%, accounting for 3% of all pediatric outpatient clinic visits and 25% of cases in pediatric gastroenterology (1,2). Although constipation can stem from many etiologies, 95% of children with constipation have no organic pathology and are defined as having functional constipation (3). The diagnosis of functional constipation can be made by a careful history and physical examination without the need for laboratory tests or other examinations (4,5). According to recommendations from the North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (NASPGHAN) and the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (ESPGHAN), polyethylene glycol (PEG) is the first-line treatment for pediatric constipation, and lactulose is recommended in cases in which PEG is unavailable (4). However, it has been shown that childhood constipation is usually not treated adequately (6). Inadequately managed constipation can result in significant abdominal discomfort, fecal incontinence, and subsequently psychological problems and social withdrawal among children (7). This study aimed to evaluate the adequacy of prescribed drugs, dosing, usage, and response to these drugs in children diagnosed with functional constipation.

MATERIALS AND METHODS

The study was conducted retrospectively, focusing on children who had visited the pediatric gastroenterology outpatient clinic of a training and research hospital between January 2020 and March 2021. The clinical data of patients were extracted from an electronic database. Patients between the ages of 1 and 18 years with functional constipation as defined by Rome IV diagnostic criteria, as well as those who had received any treatment for constipation prior to the visit to pediatric gastroenterology and after the visit to the pediatric gastroenterology clinic, the drug dosage had been corrected according to NASPGHAN and ESPGHAN recommendations, were included in this study (4). Patients with 2 or more of the following symptoms at least 4 days per month and criteria satisfied at least once per week for a minimum of 1 month with inadequate criteria for a diagnosis of irritable bowel syndrome are described as having functional constipation, according to Rome IV criteria: 2 or fewer defecations in the toilet per week in a child of a developmental age of at least 4 years, A minimum of one episode of fecal incontinence per week; a history of retentive posture or excessive volitional stool retention; painful and hard bowel movements; a big-diameter stool that can impede the toilet; and the appearance of a large fecal mass in the rectum. After a thorough examination, the symptoms cannot be explained entirely by another medical condition (8).

Patients under the age of one, with any organic condition for constipation or cerebral palsy, and those who had not received prior treatment were excluded from the study. In addition, patients who did not attend the follow-up visit were excluded.

Demographic information, including age and gender, of the patients was recorded. Anthropometric measurements such as height and weight standard deviation scores (SDS) were calculated using Turkish norms (9).

The duration of symptoms (1-3 months, 3-6 months, 6-12 months, and >12 months), the name of the drug administered, the duration of drug usage (1 month, 1-2 months, and >2 months), the method of measurement and what they took the drug with (tea spoon, dessert spoon, regular spoon or measuring with milliliters), the dosage of the drug, and the patient's response to the treatment were all documented.

Regarding treatment response, patients were categorized as follows:

- No response: If there was no improvement in stool shape, consistency, or painful defecation between or after treatments.

- Partial response: If there was some improvement in stool shape, consistency, and painful defecation between or after treatments, but not to the expected extent.

- Full response: If the patient experienced one or two soft bowel movements per day after treatment.

For measurement purposes, the teaspoon, dessert spoon, and regular spoon were considered equivalent to 2.5 milliliters (mL), 5 mL, and 10 mL, respectively.

The daily dosage of the drug was divided by the patient's body weight, and the dosage was calculated in milliliters per kilogram (mL/kg) accordingly.

The methodology for this study was approved by the Human Research Ethics Committee of the University of Malatya Turgut Özal (approval number: 2021/34).

Statistical Analysis

The variables were investigated using visual (histograms, probiblty plots) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk's test) to determine whether or not they are normally distributed. All variables were distributed normally. Normally distributed quantitative variables are presented as mean ± SD. Evaluation of differences between groups with respect to numerical variables was performed using t-test and one-way ANOVA if parametric test assumptions were checked. When the ANOVA test was significant, post-hoc tests were used to obtain the result between which pairs this difference was. Mann-Whitney U test was used to examine whether two samples given quantitative scale observations came from the same distribution.

Pearson's chi-square test was used to compare the ratios between the groups. Data were analyzed using IBM SPSS 16.0 statistical package program (IBM Corp., Armonk, NY, USA). A p-value of less than 0.05 were considered as statistically significant.

RESULTS

A total of 4,213 patients were initially assessed. Among them, 451 patients were consulted for constipation. Thirty-five of these patients were found to have constipation caused by an organic pathology, and those with cerebral palsy were excluded. Consequently, 416 patients were identified as having functional constipation according to the Rome IV criteria. From this group, 270 patients who had not previously used any medication for constipation were excluded. Additionally, 38 patients withdrew from the trial because of lack of follow-up or withdrawal of consent. A total of 108 patients were included in the study.

Among the 108 cases, 56 (51.9%) were girls. The mean age of patients was 4.4±3.7 years. The mean body weight SDS was -0.09±1 and mean height SDS was -0.09±0.9. Duration of symptom, used drug name, number of patients with disimpaction treatment given

or not given, numbers of what to give the drug with, duration of drug usage, and response to the drug given in Table 1.

When comparing patients based on their response categories (no response, partial response, and full response), no significant differences were observed in terms of gender, age, body weight SDS, height SDS, duration of symptoms, or name of the drug. However, significant differences were noted among the response groups when considering the duration of drug usage, method of drug measurement and what they drank the drug with, the daily dose of the drug, and the daily dose per weight of drug (Table 2).

When comparing the response groups based on the method of drug administration, a significant difference was found between those who utilized it with the milliliter sirup scale and those who used other spoons. The patients who had measured the drug in milliliters showed a better response rate. As the dosage of the drug and the dosage of the drug per weight increased, the response to the drug increased. After drug changes (lactulose, PEG) or dose adjustments, full response was obtained in 68 (73.1%) of 93 patients who did not respond or who had a partial response before.

DISCUSSION

Constipation is a common condition among children and is often challenging to manage (1,10). This study showed that choosing the first-line treatment prescribed to patients with functional constipation was appropriate according to the NASPGHAN and ESPGHAN recommendations. However, despite receiving the appropriate medication, approximately 80% of the patients did not respond to the appropriate drug. These findings suggest that both the dosage of the medication and its administration method play crucial roles for treating functional constipation. Specifically, increasing the drug dosage and dosage per weight, along with administering the drug using a milliliter scale, were associated with improved treatment responses. These observations highlight the significance of optimizing drug dosage and administration techniques for the management of functional constipation in children.

Table 1. Baseline characteristics of patients

Characteristics		Number of patients (%)
Gender	Girl	56 (51.9)
	Boy	52 (48.1)
Duration of symptoms	1-3 months	13 (12)
	3-6 months	31 (28.7)
	6-12 months	25 (23.1)
	>12 months	39 (36.1)
Used drug name	Lactulose	94 (87)
	Mineral oil	11 (10.2)
	Rectal laxatives	2 (1.9)
	Magnesium hydroxide	1 (0.9)
Disimpaction treatment	Not given	86 (79.6)
	Given	22 (20.3)
The method of measurement and administration of the drug	Tea spoon	22 (20.3)
	Dessert spoon	25 (23.1)
	Regular spoon	37 (34.3)
	Syrup scale milliliter	21 (19.4)
Duration of drug use	<1 month	63 (58.3)
	1 to 2 months	42 (38.9)
	>2 months	3 (2.8)
Respond to the drug	No response	68 (62.9)
	Partial response	25 (23.1)
	Full response	15 (13.8)

In this study, the vast majority of patients who visited pediatric gastroenterology were diagnosed with functional constipation according to the Rome IV criteria. Guidelines recommend PEG as the first-line maintenance treatment, with lactulose being an alternative if PEG is not available (4,11). In other studies, lactulose has been identified as one of the most powerful choices for the treatment of functional constipation (12,13). According to the Poddar et al. (14) study, lactulose and PEG are equally efficient in functional constipation. Similarly, Cao and Liu (15) showed that lactulose may successfully and safely treat chronic constipation in Chinese children. In this study, approximately 90% of the constipated patients received lactulose as the first-line treatment, indicating adherence to the recommended guidelines. However, despite the appropriate selection of first-line treatment, approximately 80% of the patients did not respond to the prescribed appropriate drug.

According to the guidelines of NASPGHAN and ESPGHAN, PEG with or without electrolytes is recommended as the first-line maintenance treatment for constipation, with a starting dose of 0.4 g/kg/day. The dose should be adjusted on the basis of the patient's clinical response. Alternatively, lactulose can be used as the first-line maintenance treatment, with a recommended dose of 1-2 g/kg, administered once or twice daily (4). The Canadian Pediatric Society, like NASPGHAN and ESPGHAN, advises lactulose at 1 mL/kg/day to 3 mL/kg/day in divided doses. Parents should be educated on adjusting the dosage based on the child's response to the stool softener. They are advised to gradually increase the dosage every 2 days until the child achieves one or two soft stools per day or to gradually decrease the dosage if the child experiences loose stools. It is important to inform parents that some leakage or soiling may occur at the beginning of therapy (5). A common reason for the lack of response to stool softening therapy is inadequate dosing (5). This study showed that choosing the first-line treatment prescribed to patients was appropriate. Even so, approximately 80% of the patients did not respond to the appropriate drug. This suggests that the underdose administered to patients is the first cause of the lack of response to correct stool softening therapy. Only 30% of the patients had used more than 1 mL/kg. The response to the drug increased as the dosage was increased in this trial. Response was obtained in 68 (73.1%) of 93 patients who did not respond or who had a partial response after drug (lactulose, PEG) or dose adjustments. This once again demonstrated the importance of dose regulation and appropriate drug changes.

Patients who received the medication measured using a milliliter scale had more accurate dosages than those who received it with a spoon. Approximately 75% of patients who received the medication in milliliters showed either partial or full response. These findings suggest that administering the medication using a milliliter dosage is more effective in eliciting a response than administering it with a spoon without a scale.

Table 2. Comparing baseline characteristics with response to drug

		No response (number or mean ± SD)	Partial response (number or mean ± SD)	Full response (number or mean ± SD)	p-value
Gender	Girl	35	9	8	0.40
	Boy	33	16	7	
Age		4.5±4.0	4.0±3.2	4.6±3.7	0.85
Body weight SDS		-0.10±1.07	0.04±0.99	-0.27±0.90	0.63
Height SDS		-0.14±0.94	-0.03±0.89	0.05±0.75	0.69
Duration of the symptoms	1-3 months	10	3	0	0.65
	3-6 months	17	8	6	
	6-12 months	15	7	3	
	>12 months	26	7	6	
Name of the drug	Lactulose	59	21	14	0.55
	Mineral oil	7	3	1	
	Magnesium hydroxide	0	1	0	
	Rectal laxatives	2	0	0	
Duration of drug use	<1 month	42	14	7	0.01
	1 to 2 months	26	11	5	
	>2 months	0	0	3	
The method of measurement and administration of the drug	Tea spoon	20	1	1	0.002
	Dessert spoon	18	6	1	
	Regular spoon	18	11	8	
	Syrup scale milliliter	10	6	5	
Daily dose of drug (mL)		11.2±7.7	18.1±10.6	24.6±11.8	0.001
Daily dose per weight (mL/kg)		0.66±0.43	1.07±0.58	1.4±0.61	0.001

SDS: Standard deviation scores.

According to the NASPGHAN and ESPGHAN guidelines, maintenance therapy for constipation should ideally last for a minimum of 2 months (4). However, in this study, approximately 90% of the patients had used the medication for less than 2 months. Insufficient duration of drug usage may be another reason for the lack of response observed in patients. Nonetheless, it is important to note that according to NASPGHAN and ESPGHAN, all constipation symptoms should be resolved for at least 1 month before discontinuing drugs (4). This underscores the significance of allowing an adequate period of time for drug usage before evaluating treatment response, highlighting insufficient duration of drug usage as the second most important factor contributing to patients' lack of response.

Study Limitations

The research's limitations were that it was a retrospective study with some patients.

CONCLUSION

In conclusion, constipation is a common and important problem in children. Constipation may be serious if not treated properly. No response is possible because the constipation drugs are not administered to the children in the proper manner and doses. To ensure a comprehensive response, the dosage of the drug and how they measure the drug must be disclosed to parents, and parents should be encouraged to alter the dose based on the response.

Ethics

Ethics Committee Approval: The methodology for this study was approved by the Human Research Ethics Committee of the University of Malatya Turgut Özal (approval number: 2021/34).

Informed Consent: Retrospective study.

Author Contributions

Concept: N.G.K., H.Ö., Design: N.G.K., Supervision: N.G.K., Resources: N.G.K., Materials: N.G.K., Data Collection or Processing: N.G.K., Analysis or Interpretation: H.Ö., Literature Search: N.G.K., Writing: N.G.K., Critical Review: N.G.K., H.Ö.

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

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

REFERENCES

1. van den Berg MM, Benninga MA, Di Lorenzo C. Epidemiology of childhood constipation: a systematic review. Am J Gastroenterol. 2006; 101: 2401-9.

2. Rasquin-Weber A, Hyman P, Cucchiara S, Fleisher D, Hyams J, Milla P, et al. Childhood functional gastrointestinal disorders. Gut. 1999; 45(Suppl 2): II60-8.
3. Loening-Baucke V. Chronic constipation in children. Gastroenterology. 1993; 105: 1557-64.
4. Tabbers M, DiLorenzo C, Berger M, Faure C, Langendam M, Nurko S, et al. Evaluation and treatment of functional constipation in infants and children: evidence-based recommendations from ESPGHAN and NASPGHAN. J Pediatr Gastroenterol Nutr. 2014; 58: 258-74.
5. Rowan-Legg A; Canadian Paediatric Society, Community Paediatrics Committee. Managing functional constipation in children. Paediatr Child Health. 2011; 16: 661-70.
6. Borowitz SM, Cox DJ, Kovatchev B, Ritterband LM, Sheen J, Sutphen J. Treatment of childhood constipation by primary care physicians: efficacy and predictors of outcome. Pediatrics. 2005; 115: 873-7.
7. Bernard-Bonnin AC, Haley N, Bélanger S, Nadeau D. Parental and patient perceptions about encopresis and its treatment. J Dev Behav Pediatr. 1993; 14: 397-400.
8. Hyams JS, Di Lorenzo C, Saps M, Shulman RJ, Staiano A, van Tilburg M. Functional Disorders: Children and Adolescents. Gastroenterology. 2016:S0016-5085(16)00181-5.
9. Neyzi O, Gunoz H, Furman A, Bundak R, Gokcay G, Darendeliler F, et al. Weight, height, head circumference and body mass index references for Turkish children. Cocuk Sagligi ve Hastaliklari Dergisi. 2008; 51: 1-14.
10. Youssef NN, Di Lorenzo C. Childhood constipation: evaluation and treatment. J Clin Gastroenterol. 2001; 33: 199-205.
11. Jarzebicka D, Sieczkowska-Golub J, Kierkus J, Czubkowski P, Kowalczuk-Kryston M, Pelc M, et al. PEG 3350 Versus Lactulose for Treatment of Functional Constipation in Children: Randomized Study. J Pediatr Gastroenterol Nutr. 2019; 68: 318-24.
12. Maydeo A. Lactitol or lactulose in the treatment of chronic constipation: result of a systematic. J Indian Med Assoc. 2010; 108: 789-92.
13. de Geus A, Koppen IJN, Flint RB, Benninga MA, Tabbers MM. An Update of Pharmacological Management in Children with Functional Constipation. Paediatr Drugs. 2023; 25: 343-58.
14. Poddar U, Singh S, Pawaria A, Srivastava A, Yachha SK. Aetiological spectrum, clinical differentiation and efficacy of polyethylene glycol over lactulose in children with constipation: Experience of 316 cases. J Paediatr Child Health. 2019; 55: 162-7.
15. Cao Y, Liu SM. Lactulose for the treatment of Chinese children with chronic constipation: A randomized controlled trial. Medicine (Baltimore). 2018; 97: e13794.

GMJ 2024;35:286-290

DOI: http://dx.doi.org/10.12996/gmj.2023.3953

A Retrospective Cohort Study with Blood Parameters for Early Estimation of Multiple Sclerosis: Ratio Suggestion

Multiple Sklerozun Erken Tahmini için Bazı Kan Parametreleriyle Bir Retrospektif Kohort Çalışması: Bir Oran Önerisi

Esra Tekin[1], Sibel Canbaz Kabay[2], Ayşegül Küçük[1]

[1]Department of Physiology, Kütahya Health Sciences University Faculty of Medicine, Kütahya, Türkiye
[2]Department of Neurology, Kütahya Health Sciences University Faculty of Medicine, Kütahya, Türkiye

ABSTRACT

Objective: Multiple sclerosis (MS) is a non-traumatic neurological disease that often affects young adults and causes disability. Because there is no curative treatment that can provide full recovery, it is important to make an early diagnosis and slow the course of disease with current drugs. This study aimed to evaluate and compare some blood parameters and their ratios to each other that can provide an advantage in early diagnosis.

Methods: We compared changes in blood parameters and their ratios to each other between healthy controls (95) and MS patients (95). The MS group was evaluated in three periods: the new diagnosis period, in which patients did not use any medication for MS, the relapse period, and the 6-month attack-free remission period.

Results: The results revealed that the neutrophil-to-lymphocyte ratio (NLR) and erythrocyte-to-lymphocyte ratio increased in MS patients, especially during the attack period. It was remarkable that the erythrocyte-lymphocyte ratio was significantly higher than that in the control group in all three MS periods.

Conclusion: NLR is considered a neuroinflammation marker. It is argued that NLR reflects systemic inflammation better than neutrophil and lymphocyte counts alone. In addition, in our data, the ratio of erythrocytes to lymphocytes was significantly higher in MS patients. Currently, there is no effective laboratory marker for the diagnosis of MS. We have concluded that complete blood count parameters and their ratios to each other can be biomarkers that can provide early diagnosis of MS.

Keywords: Multiple sclerosis, blood biomarkers, neutrophil-to-lymphocyte ratio, erythrocyte-to-lymphocyte ratio

ÖZ

Amaç: Multiple skleroz (MS), sıklıkla genç yetişkinleri etkileyen ve yeti kaybına neden olan travmatik olmayan bir nörolojik hastalıktır. Tam iyileşmeyi sağlayacak küratif bir tedavi olmadığı için erken tanı koymak ve mevcut ilaçlarla hastalığın seyrini yavaşlatmak önemlidir. Erken tanıda avantaj sağlayabilecek bazı kan parametrelerinin ve birbirlerine oranlarının değerlendirilmesi ve karşılaştırılması amaçlandı.

Yöntemler: Sağlıklı kontroller (95) ve MS hastaları (95) arasında kan parametrelerindeki değişiklikleri ve bunların birbirlerine oranlarını karşılaştırdık. MS grubu, MS için herhangi bir ilaç kullanılmayan yeni tanı dönemi, atak dönemi ve 6 aylık ataksız remisyon dönemi olmak üzere 3 dönemde değerlendirildi.

Bulgular: Sonuçlar, MS hastalarında özellikle atak döneminde nötrofil-lenfosit oranının (NLR) ve eritrosit-lenfosit oranının arttığını ortaya koydu. Üç MS döneminin tamamında eritrosit-lenfosit oranının kontrol grubuna göre anlamlı olarak yüksek olması dikkat çekiciydi.

Sonuç: NLR, bir nöroenflamasyon belirteci olarak kabul edilmektedir. NLR'nin sistemik enflamasyonu tek başına nötrofil ve lenfosit sayımlarından daha iyi yansıttığı düşünülmektedir. Ayrıca verilerimizde MS hastalarında eritrosit-lenfosit oranı anlamlı olarak daha yüksekti. MS tanısı için henüz etkili bir laboratuvar belirteci bulunmamaktadır. Bu çalışma sonucunda tam kan sayımı parametrelerinin ve birbirlerine olan oranlarının MS için erken tanı sağlayabilecek biyobelirteç olma potansiyeline sahip olduğu kanısına vardık.

Anahtar Sözcükler: Multiple skleroz, kan biyobelirteçleri, nötrofil-lenfosit oranı, eritrosit-lenfosit oranı

Address for Correspondence/Yazışma Adresi: Esra Tekin, MD, Department of Physiology, Kütahya Health Sciences University Faculty of Medicine, Kütahya, Türkiye
E-mail / E-posta: esratekin105@gmail.com
ORCID ID: orcid.org/0000-0002-9684-3277

Received/Geliş Tarihi: 27.07.2023
Accepted/Kabul Tarihi: 27.09.2023

INTRODUCTION

Multiple sclerosis (MS) is a central nervous system disease that does not have a blood-based biomarker that can provide a diagnosis and causes disability especially by affecting young adults (1). Because there is no curative treatment, it is important to make an early diagnosis and slow the course of the disease with current drugs. Early diagnosis and treatment of MS are important to avoid permanent neurological damage (2).

MS causes visual disturbances (such as optic neuritis), brain stem and spinal cord damage, and cortical damage in patients. Patients usually consult a doctor for weakness in the extremities, loss of sensation, and vision problems (3). The worldwide increase in the incidence of MS, which can cause these serious problems in patients, brings along socioeconomic problems (4). Therefore, it is important to conduct studies for developing curative treatment, early diagnosis, and rapid initiation of treatment.

It is stated that various genes, inflammatory processes, virus infections, and various exposures may be effective among the possible causes of MS, but its exact etiology is still unknown (5). Systemic inflammation is thought to be a factor in MS as in many other diseases. Systemic inflammation, which causes chronic neurodegeneration and activates proinflammatory cytokines and the immune system, also plays an important role in MS (6).

The ratios of complete blood count parameters to each other, especially the neutrophil-to-lymphocyte ratio (NLR), are being investigated for use in the early and easy diagnosis of various diseases (7,8). It has been argued that the NLR, which is considered a neuroinflammation marker, reflects systemic inflammation better than neutrophil and lymphocyte counts alone (9). In addition, different blood parameters such as platelet-to-lymphocyte ratio or monocyte-to-lymphocyte ratio have been tested as MS diagnostic markers (10,11). We suggest that the red blood cell (RBC) to lymphocyte ratio can be used to predict MS, and various other ratios.

For MS, which is difficult to diagnose, blood parameters that may be associated with the disease can be scanned during medical examination, and patients who are deemed necessary can be directed for further investigations. The use of these parameters may be beneficial in the referral of people with clinical symptoms of MS, who are at risk and suspected of the disease, for further examinations without wasting time. This study aimed to evaluate the suitability of blood parameters that can be used for the diagnosis of MS.

MATERIALS AND METHODS

Data Source

The blood test results of patients who were treated at Kütahya Health Sciences University, Evliya Çelebi Training and Research Hospital between 1 December 2017 and 1 December 2022 were retrospectively scanned for this study. Ethical approval for this study was obtained from the Ethics Committee of Non-Interventional Clinical Researches of Kütahya Health Sciences University Faculty of Medicine (approval number: 2022/07-05, date: 22.06.2022). In this study, which was conducted as a retrospective archive investigation, informed consent was not required. The individuals included in the study are described in Figure 1. This study was conducted in accordance with the Declaration of Helsinki.

Serum iron, hemoglobin, hematocrit, red cell distribution width (RDW), white blood cell (WBC), RBC, neutrophil, lymphocyte, NLR, RBC/lymphocyte, and RBC/neutrophil values of patients and healthy controls were investigated. Blood parameters were determined in the hospital laboratory using an AutoAnalyzer.

Study Cohort

Ninety-five healthy controls were included in this study. While selecting the control group, attention was paid to the fact that the individuals were between the ages of 20 and 70 years, had no MS diagnosis, and had no diagnosis of chronic heart, liver, and kidney disease or malignancy. The MS group included 95 patients aged between 20 and 70 years with a diagnosis of MS. The control group was formed to match the MS group in terms of age and gender (Table 1). MS group data were evaluated in three periods: the new diagnosis period, when the patients did not use any medication for MS (naive group), the relapse period, and the remission period without an attack for 6 months. The data of the same 95 MS patients included in the group during the drug-free, attack, and remission periods were compared with each other and with the healthy controls.

Statistical Analysis

Statistical analysis was performed using the Statistical Package of the Social Science (SPSS) program with version 20 (IBM SPSS Corp.; Armonk, NY, USA). Quantitative data are given as mean ± standard deviation. The conformity of the data to the normal distribution was determined by the Kolmogorov-Smirnov test. An unpaired t-test was used for normally distributed variables. The Mann-Whitney U test was used for variables that did not show normal distribution. The Wilcoxon test was used for pairwise comparisons of MS groups. P-value <0.05 was considered statistically significant.

RESULTS

Hemoglobin, hematocrit, and RBC values were found to be higher in the MS patient groups ($p<0.05$). Serum iron was significantly higher in the MS-remission group than in the control group ($p<0.05$). The RDW value was found to be higher in the control group than MS groups ($p<0.05$). Lymphocyte counts were low in the MS groups, whereas neutrophil counts were lower in the control group than in the MS groups ($p<0.05$) (Table 2, 3).

There was no difference in the RBC/neutrophil ratio between the groups, whereas the NLR was higher in the MS-relapse and MS-naive groups than in the control group ($p<0.05$). The RBC/lymphocyte ratio was found to be higher in all MS groups than in the control group ($p<0.05$) (Table 4).

In the comparison of MS groups within themselves, neutrophil and WBC counts were lower in the MS-remission group than in the relapse and naive groups. The lymphocyte count was higher in the MS-remission group than in the other groups and close to the control group ($p<0.05$). When the ratios were examined, while the NLR did not show a difference compared with the control in the MS-remission group, the RBC/Lymphocyte ratio had a significant difference ($p<0.05$) (Table 4).

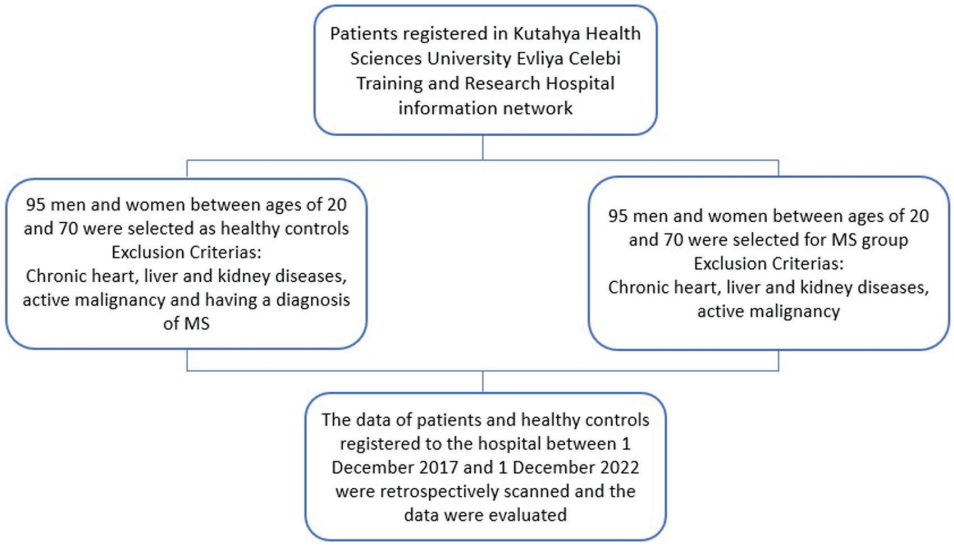


Figure 1. The workflow of this retrospective cohort study and the inclusion and exclusion of individuals.

Table 1. Age and sex characteristics

	MS group	Control group
Age*	37 (22-69) years	35 (21-65) years
Gender (male/female)	33/62	35/60

*Average value and bottom-top values, MS: Multiple sclerosis.

Table 2. Serum Fe, hemoglobin, hematocrit, and RDW levels

	Serum Fe	Hemoglobin	Hematocrit	RDW
Control	73.25±32.7	13.29±1.7	39.32±4.7	14.09±1.9
MS-naive	80.15±36.6	16.4±1.8*	41.61±4.6*	13.74±1.6*
MS-relapse	76.8±32.4*	14.01±1.7*,**	41.57±4.4*	13.68±1.6*
MS-remmission	83.16±36.5*	14.16±1.8*	41.92±4.8*	13.9±1.7

Data are given as mean ± standard deviation. *Compared to control group, p<0.05, **Compared to MS-naive group, p<0.05, MS: Multiple sclerosis, RDW: Red cell distribution width.

DISCUSSION

MS is a chronic autoimmune disease that may cause persistent neurological disabilities due to inflammation of the myelin sheath around the nerves (12). When MS is diagnosed, the disease may progress at different severities in patients. The prognosis varies depending on the type of MS and personal factors (13). Although there are drugs that show positive effects in the treatment of MS, there is no curative treatment yet (14). Therefore, it is important to diagnose and start treatment before permanent neurological damage occurs. Easily accessible and inexpensive blood parameters and their ratios can be used for the diagnosis of MS.

While a study found higher RBC levels in MS (15), a group of studies found unchanged RBC levels in MS (16,17). According to these studies, RBC and RBC-related blood parameters may increase in MS, but they do not tend to decrease (15-17). Similarly, in our study, hemoglobin, hematocrit, serum iron, and RBC levels were found to be higher in the MS groups. However, studies have indicated that the functions of erythrocytes may be insufficient and that erythrocyte deformability may decrease in MS patients (18-20).

The elevation in RBC and RBC-related parameters in MS seems to be because erythrocyte count is less affected by neuroinflammation and neuromodulatory drug use than leukocytes. However, despite high RBC, hemoglobin, and hematocrit levels, it is thought that erythrocytes may be dysfunctional in MS. Anemia of chronic disease, for example, causes suppression in the production of erythrocytes and shortening of life span, but leads to moderate anemia (21). As a result, we believe that the presence of inflammation impairs the functions of erythrocytes rather than reducing their number.

In addition, the fact that MS patients consult a doctor and perform tests more frequently compared with age- and gender-matched controls may have prevented possible anemia. Similarly, it is thought that before the diagnosis is made, there are frequent doctor visits in line with the complaints of MS patients.

Compared with leukocyte counts, erythrocyte counts are less affected by neuroinflammation caused by MS (22,23). In addition, neuromodulatory drugs used by MS patients may cause changes in the number of leukocyte types (23,24). Consistent with the higher RBC, hemoglobin, hematocrit, and serum Fe values in the MS groups, the RDW value was also higher in the control group.

In our data, the number of lymphocytes from leukocytes was low in the MS groups, whereas the number of neutrophils was high in the MS-naive and MS-relapse groups. However, the neutrophil count was similar to the control group in the MS-remission group. Therefore, NLR was found to be significantly higher in the MS-naive and MS-relapse groups than in the control group, while there was no

Table 3. WBC, RBC, neutrophils, and lymphocytes

	WBC	RBC	Neutrophil	Lymphocyte
Control	7.46±2.5	4.57±0.6	4.81±2.2	2.1±1
MS-naive	7.44±3.4	4.84±0.4*	4.94±2.9	1.79±0.8*
MS-relapse	7.65±3.4	4.81±0.4*	5.23±2.9	1.72±0.8*
MS-remmission	6.77±2.2*,**,***	4.86±0.3*	4.21±1.6*,**,***	1.94±0.7**

Data are presented as mean ± standard deviation. *Compared with control group, p<0.05, **Compared with MS-naive group, p<0.05. ***Compared with MS-relapse group, p<0.05. MS: Multiple sclerosis, WBC: White blood cell, RBC: Red blood cell.

Table 4. The ratios

	NLR	RBC/lymphocyte	RBC/neutrophil
Control	2.9±1.6	2.49±1	1.13±0.4
MS-naive	3.73±2.2*	3.8±1.6*	1.12±0.4
MS-relapse	4.22±2.1*	3.92±2.2*	1.1±0.3
MS-remmission	2.64±1.6	3.14±2*	1.29±0.4

Data are presented as mean ± standard deviation. *Compared with control group, p<0.05 MS: Multiple sclerosis, NLR: Neutrophil lymphocyte ratio, RBC: Red blood cell.

difference in the MS-remission group from the control. NLR is a marker recommended for use in various diseases to show inflammation (25-27). In our data, NLR was found to be higher in the MS-naive and MS-relapse groups. However, the lack of significant difference in the remission group suggests that it may not be sufficient for the diagnosis of MS patients who have not been diagnosed yet and who are not in the attack phase. In addition to neuroinflammation, the drugs used by MS patients change the number of neutrophils and lymphocyte leukocytes depending on the active substance of the drug. Therefore, it cannot be seen as a reliable biomarker that can make a definitive diagnosis (7,28). As a matter of fact, in various studies in the literature, no significant difference was found between the MS and control groups in terms of NLR (7). These differences may be because the patients are in an attack or remission period or the type of drug they use. However, the high NLR in MS patients seems to be due to a decrease in the lymphocyte count rather than an increase in the neutrophil count. Therefore, we suggest that the ratio of preserved erythrocytes to lymphocytes, which generally decreases in MS patients, although they are not functional, may be a more useful indicator.

Because the RBC/Lymphocyte ratio is both easily accessible and applicable and can be obtained in a minimally invasive way by venous blood collection, it may be beneficial for people with suspected MS. In people with symptoms suggestive of MS, the evaluation of this rate in primary health care institutions and referral for further evaluations if it is found to be high may facilitate the diagnosis. Studies should be carried out to be able to call the found value high and to determine the alarm value.

Conclusion

In this retrospective study, blood-based parameters and their ratios to each other were analyzed and evaluated to facilitate the diagnosis of MS. It was found that the MS groups at different periods differed from the control in various blood parameters. It was concluded that RBC/Lymphocyte, which can be used as a marker, may be more reliable for MS prediction than NLR. However, further studies should be conducted to determine at what value the RBC/Lymphocyte ratio should be referred to a neurologist for MS evaluations. In addition, the potential of this ratio to provide differential diagnosis of MS with other diseases and its specificity and sensitivity as a biomarker should be evaluated. The possible effects on post-diagnosis treatment strategies should also be determined by further studies.

Ethics

Ethics Committee Approval: Ethical approval for this study was obtained from the Ethics Committee of Non-Interventional Clinical Researches of Kütahya Health Sciences University Faculty of Medicine (approval number: 2022/07-05, date: 22.06.2022).

Informed Consent: Retrospective study.

Authorship Contributions

Concept: E.T., S.C.K., A.K., Design: E.T., S.C.K., A.K., Supervision: E.T., S.C.K., A.K., Resources: S.C.K., Materials: S.C.K., Data Collection or Processing: E.T., Analysis or Interpretation: E.T., A.K., Literature Search: E.T., A.K., Writing: E.T., A.K., Critical Review: E.T., S.C.K., A.K.

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

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

REFERENCES

1. Oh J, Vidal-Jordana A, Montalban X. Multiple sclerosis: clinical aspects. Curr Opin Neurol. 2018; 31: 752-9.
2. Lavorgna L, Borriello G, Esposito S, Abbadessa G, Capuano R, De Giglio L, et al. Impact of early diagnosis on clinical characteristics of an Italian sample of people with multiple sclerosis recruited online. Mult Scler Relat Disord. 2019; 27: 239-46.
3. Crayton HJ, Rossman HS. Managing the symptoms of multiple sclerosis: a multimodal approach. Clin Ther. 2006;28:445-60.
4. Dobson R, Giovannoni G. Multiple sclerosis - a review. Eur J Neurol. 2019; 26: 27-40.

5. Ward M, Goldman MD. Epidemiology and Pathophysiology of Multiple Sclerosis. Continuum (Minneap Minn). 2022; 28: 988-1005.
6. Voet S, Prinz M, van Loo G. Microglia in Central Nervous System Inflammation and Multiple Sclerosis Pathology. Trends in Molecular Medicine. 2019; 25; 112-23.
7. Gelibter S, Pisa M, Croese T, Dalla Costa G, Orrico M, Preziosa P, et al. Neutrophil-to-lymphocyte ratio: a marker of neuro-inflammation in multiple sclerosis? J Neurol. 2021; 268: 717-23.
8. Atılgan CÜ, Şendül SY, Kösekahya P, Çağlayan M, Alkan A, Güven D, et al. Evaluation of Neutrophil-to-Lymphocyte Ratio and Mean Platelet Volume in Patients with Active and Inactive Thyroid Orbitopathy. Sisli Etfal Hastan Tip Bul. 2018; 52: 26-30.
9. Hasselbalch IC, Søndergaard HB, Koch-Henriksen N, Olsson A, Ullum H, Sellebjerg F, et al. The neutrophil-to-lymphocyte ratio is associated with multiple sclerosis. Mult Scler J Exp Transl Clin. 2018; 4: 2055217318813183.
10. Carnero Contentti E, López PA, Criniti J, Pettinicchi JP, Cristiano E, Patrucco L, et al. Platelet-to-lymphocyte ratio differs between MS and NMOSD at disease onset and predict disability. Mult Scler Relat Disord. 2022; 58: 103507.
11. Huang WC, Lin HC, Yang YH, Hsu CW, Chen NC, Tsai WC, et al. Neutrophil-to-lymphocyte ratio and monocyte-to-lymphocyte ratio are associated with a 2-year relapse in patients with multiple sclerosis. Mult Scler Relat Disord. 2022; 58: 103514.
12. Marcus R. What Is Multiple Sclerosis? JAMA. 2022; 328: 2078.
13. Cortese R, Giorgio A, Severa G, De Stefano N. MRI Prognostic Factors in Multiple Sclerosis, Neuromyelitis Optica Spectrum Disorder, and Myelin Oligodendrocyte Antibody Disease. Front Neurol. 2021; 12: 679881.
14. Doshi A, Chataway J. Multiple sclerosis, a treatable disease. Clinical Medicine (London, England). 2016;16: s53-9.
15. Akaishi T, Misu T, Fujihara K, Nakaya N, Nakamura T, Kogure M, et al. White blood cell count profiles in multiple sclerosis during attacks before the initiation of acute and chronic treatments. Sci Rep. 2021; 11: 22357.
16. Kasprzycka W, Nieśpiałowska M, Jakubowska-Solarska. Blood count parameters in the course of multiple sclerosis. Journal of Transfusion Medicine. 2019; 12: 117-23.
17. Hon GM, Hassan MS, Rensburg RS, Erasmus RT, Matsha T. The hematological profile of patients with multiple sclerosis. Open Journal of Modern Neurosurgery. 2012; 2: 36-44.
18. Geiger M, Hayter E, Martin RS, Spence D. Red blood cells in type 1 diabetes and multiple sclerosis and technologies to measure their emerging roles. J Transl Autoimmun. 2022; 5: 100161.
19. Groen K, Maltby VE, Sanders KA, Scott RJ, Tajouri L, Lechner-Scott J. Erythrocytes in multiple sclerosis - forgotten contributors to the pathophysiology? Mult Scler J Exp Transl Clin. 2016; 2: 2055217316649981.
20. Simpson LO, Shand BI, Olds RJ, Larking PW, Arnott MJ. Red cell and hemorheological changes in multiple sclerosis. Pathology. 1987; 19: 51-5.
21. Ganz T. Molecular pathogenesis of anemia of chronic disease. Pediatr Blood Cancer. 2006; 46: 554-7.
22. Chmielewski PP, Strzelec B. Elevated leukocyte count as a harbinger of systemic inflammation, disease progression, and poor prognosis: a review. Folia Morphol (Warsz). 2018; 77: 171-8.
23. Schweitzer F, Laurent S, Fink GR, Barnett MH, Hartung HP, Warnke C. Effects of disease-modifying therapy on peripheral leukocytes in patients with multiple sclerosis. J Neurol. 2021; 268: 2379-89.
24. Manni A, Iaffaldano A, Lucisano G, D'Onghia M, Mezzapesa DM, Felica V, et al. Lymphocyte Count and Body Mass Index as Biomarkers of Early Treatment Response in a Multiple Sclerosis Dimethyl Fumarate-Treated Cohort. Front Immunol. 2019; 10: 1343.
25. Ustuntas G, Basat SU, Calik AN, Sivritepe R, Basat O. Relationship between Epicardial Fat Tissue Thickness and CRP and Neutrophil-Lymphocyte Ratio in Metabolic Syndrome Patients Over 65 Years. Sisli Etfal Hastan Tip Bul. 2021; 55: 405-11.
26. Qin B, Ma N, Tang Q, Wei T, Yang M, Fu H, et al. Neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR) were useful markers in assessment of inflammatory response and disease activity in SLE patients. Mod Rheumatol. 2016; 26: 372-6.
27. Bisgaard AK, Pihl-Jensen G, Frederiksen JL. The neutrophil-to-lymphocyte ratio as disease actvity marker in multiple sclerosis and optic neuritis. Mult Scler Relat Disord. 2017; 18: 213-7.
28. Dinoto A, Sartori A, Cheli M, Pasquin F, Baldini S, Bratina A, et al. Lymphopenia during treatment with dimethyl fumarate in patients with multiple sclerosis: Prevalence, predicting factors and clinical outcomes. Mult Scler Relat Disord. 2022; 57: 103357.

GMJ 2024;35:291-296

DOI: http://dx.doi.org/10.12996/gmj.2024.3985

Comparison of Preoperative Imaging and FNAB Results with Postoperative Pathology Results in Patients Undergoing AUS/FLUS

AUS/FLUS Nedeniyle Opere Edilen Hastalarda Preoperatif Görüntüleme ve İİAB Sonuçlarının Postoperatif Patoloji Sonuçlarıyla Kıyaslanması

Ferhat Demir, Oğuz Hançerlioğulları, Burak Uçaner, Mehmet Zeki Buldanlı, Murat Özkara

Department of General Surgery, University of Health Sciences Türkiye, Gülhane Training and Research Hospital, Ankara, Türkiye

ABSTRACT

Objective: Thyroid nodules are observed in 3-7% of the general population, of which 4-8% are detected by palpation and 10-41% by thyroid ultrasonography (USG). In this study, we aimed to make early surgical or follow-up decisions in patients with thyroid atypia of undetermined significance (AUS)/follicular lesion of undetermined significance based on demographic and clinical characteristics, sonographic findings, and laboratory tests.

Methods: Patients over the age of 18 years who were diagnosed with AUS and operated between August 2016 and August 2022 were included in the study. Patients under 18 years of age, those with missing data in the hospital automation system, and those with repeat fine-needle aspiration biopsy were excluded from the study.

Results: Sonographic features of malignant and benign cases were compared. In malignant cases, the diameter of the dominant nodule was smaller, which was significant in terms of malignancy. Multicentricity, edge irregularity, and presence of cervical lymph nodes on USG and American Thyroid Association high-risk cases were found to be significant regarding malignancy. "Taller than wide (TTW)" appearance on sonographic images of nodules was observed more frequently in malignant cases.

Conclusion: In regression analysis with age, gender, dominant nodule diameter, multicentricity, TTW shape, presence of calcification, presence of sonographic cervical lymph node, presence of lymphocytic thyroiditis in the parenchyma in the final pathology, and edge irregularity, the parameters TTW shape, presence of cervical lymph node, and presence of lymphocytic thyroiditis in the parenchyma were significant in favor of malignancy.

Keywords: Cancer of thyroid, AUS, thyroid nodule, thyroid neoplasms, ultrasound, biopsy

ÖZ

Amaç: Tiroid nodülleri genel popülasyonda %3-7 sıklıkla görülmekte olup; bunun %4-8'i palpasyon ile, %10-41'i ise tiroid ultrasonografi (USG) ile saptanmaktadır. Bu çalışmada tiroid önemi belirsiz atipi (AUS)/önemi belirsiz foliküler lezyon tanısı almış hastalarda demografik ve klinik özellikler, sonografik bulgular ve laboratuvar tetkikleri ile erken cerrahi kararı veya takip kararı vermeyi hedefledik.

Yöntemler: Çalışmaya; Ağustos 2016-2022 tarihleri arasında ince iğne aspirasyon biyopsisi (İİAB) sonucu AUS tanısı almış ve opere edilmiş 18 yaş üstü hastalar dahil edildi. On sekiz yaş altı hastalar, hastane otomasyon sisteminde verileri eksik olan hastalar ve İİAB tekrarı yapılmış hastalar çalışma dışı bırakıldı.

Bulgular: Malign ve benign olguların sonografik özellikleri karşılaştırıldı. Malign olgularda dominant nodül çapı daha küçük olduğu ve bunun malignite açısından anlamlı olduğu görüldü. Multisentrisite, kenar düzensizliği, USG'de servikal lenf nodu varlığı ve Amerikan Tiroid Derneği yüksek riskli olguların malignite açısından anlamlı olduğu görüldü. Nodüllerin sonografik görüntülerinde "Taller than wide (TTW)" görünümü malign olgularda daha sık izlendi.

Sonuç: Yaş, cinsiyet, dominant nodül çapı, multisentrisite, TTW şekil özelliği, kalsifikasyon varlığı sonografik servikal lenf nodu varlığı, nihai patolojide parankimde lenfositik tiroidit olması, kenar düzensizliği eklenerek regresyon analizi yapıldığında TTW şekil özelliği, servikal lenf nodu varlığı, lenfositik tiroidit parankim bulunması parametreleri malignite lehine anlamlı idi.

Anahtar Sözcükler: Tiroid kanseri, AUS, Tiroid nodülü, tiroid tümörleri, ultrason, biyopsi

Address for Correspondence/Yazışma Adresi: Burak Uçaner, MD, Department of General Surgery, University of Health Sciences Türkiye, Gülhane Training and Research Hospital, Ankara, Türkiye
E-mail / E-posta: burakucaner3838@gmail.com
ORCID ID: orcid.org/0000-0002-5420-3810

Received/Geliş Tarihi: 26.08.2023
Accepted/Kabul Tarihi: 13.05.2024

INTRODUCTION

Thyroid nodules are observed in 3-7% of the general population, of which 4-8% are detected by palpation and 10-41% by thyroid ultrasonography (USG) (1). 3-5% of these nodules are malignant (2).

According to the American Thyroid Association (ATA) guidelines, the clinical and diagnostic approach to nodules should include detailed anamnesis and physical examination, thyroid function tests should be performed, and thyroid fine-needle aspiration biopsy (FNAB) should be planned if necessary regarding the results. FNAB is an easy-to-access, fast, simple, cost-effective, and reliable method that is frequently used in the differentiation of benign and malignant nodules. FNAB and cytologic examination is the gold standard method for the differentiation of benign malignant nodules with 89-98% sensitivity and 92% specificity (3,4).

Although FNAB being the gold standard method, a comprehensive classification system has emerged as a necessity to avoid controversies on this issue. Therefore, the BETHESDA classification system was defined in 2007, and six categories were distinguished. Accordingly, it was categorized as 1) non-diagnostic, 2) benign, 3) atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS), 4) follicular neoplasia/suspected follicular neoplasia, 5) suspected malignancy, and 6) malignant (5).

BETHESDA 3 AUS/FLUS is reported in up to 7% of all thyroid FNABs, and studies have shown that 6-76% of malignancies can be found in these lesions following surgery, which is much higher than expected. This wide range in malignant lesion rates calls into question the accuracy of the National Cancer Institute's (NCI) BETHESDA classification. Thus, the approach to these lesions remains controversial (6,7).

In this study, we aimed to make early surgical or follow-up decisions in patients diagnosed with AUS/FLUS on the basis of demographic and clinical characteristics, sonographic findings, and laboratory tests. We also revealed the risks associated with histopathological aggressiveness findings in patients who underwent surgery.

MATERIALS AND METHODS

Ethics committee approval was obtained with the decision of the University of Health Sciences Türkiye, Gülhane Training and Research Hospital Clinical Research Ethics Committee (approval number: 2021/81, date: 15.12.2021). This study complies with the Declaration of Helsinki and the principles of Good Clinical Practice and does not contradict the ethical rules of subject research.

Patients over the age of 18 years who were diagnosed with AUS because of FNAB and operated between August 2016 and August 2022 were included in the study. Patients under 18 years of age, patients with AUS diagnosed by FNAB who were not operated on in our clinic, patients with missing data in the hospital automation system, patients who could not be reached by one-to-one interview technique, and patients with repeat FNAB were excluded from the study.

All patients admitted to University of Health Sciences Türkiye, Gülhane Training and Research Hospital, Department of General Surgery and who underwent thyroid FNAB were evaluated retrospectively. The information of 616 patients with a pathologic diagnosis of AUS was scanned through the hospital information system, and 321 patients were found to have undergone surgery in the general surgery clinic, and were included in the study.

Demographic characteristics (age, gender), body weight, height, and body mass index (BMI) of patients were recorded. We assessed comorbidity burden using the Charlson Comorbidity Index (CCI), which assigns a weighted score to each of the 17 comorbid conditions based on the relative risk of 1-year mortality. Preoperative TSH, fT3, fT4, thyroglobulin, and anti-TPO levels were recorded. The number of nodules, dominant nodule diameter, localization, multicentricity, multifocality, heterogeneity, presence of edge irregularity, echogenicity features, Taller than wide (TTW) shape, solid, cystic, mixed type, presence and nature of calcification (microcalcification, macrocalcification, presence of peripheral halo), and presence of cervical lymph nodes were obtained from hospital records as patients' preoperative ultrasonographic findings and classified as benign, low risk, intermediate risk, and high risk by ATA risk scoring based on these sonographic parameters. New TIRADS category information was collected from patients who underwent repeat FNAB. Information regarding the surgical procedure performed on the patients was obtained from the operating room records.

The final pathologic diagnosis of the postoperative thyroid material was recorded by dividing it into benign and malignant groups. The benign group included multinodular goiter, lymphocytic thyroiditis, NIFTP, and follicular adenoma, whereas papillary cancer, follicular cancer, and Hurthle cell cancer were included in the malignant group. Histopathologically, the presence of lymphovascular, capsular, and perineural invasion in the malignant group was examined, and reactive, malignant, and total lymph node counts were also analyzed. TNM staging of malignant cases was recorded.

Statistical Analysis

Statistical analyses were performed using SPSS version 22.0 package program. The conformity of the variables to the normal distribution was examined using visual (histograms and probability graphs) and analytical methods ("Kolmogorov-Smirnov test" and "Shapiro-Wilk tests"). Numerical variables determined according to normal distribution were analyzed by the "Independent groups t-test" between the two groups, and variables that were not normally distributed were analyzed by the "Mann-Whitney U test". Chi-square analysis and Fisher's exact test were used to compare categorical data. Multivariate analyses were performed using "Binary Logistic Regression analysis". Comparisons with p-values below 0.05 were considered statistically significant.

RESULTS

The mean age of the 321 patients included in the study was 46.7±12.3 years (18-88 years). Of these, 24.3% were over 55 years of age. Of the patients included in the study, 26.8% (n=86) were male and 73.2% (n=235) were female, with a female-to-male ratio of 2.7/1.

On ultrasonographic evaluation, multiple nodules were observed in 62.6% of the cases and single nodules in 37.4%. The median diameter of the dominant nodule was 19 mm. The most common nodule localization was in the right lobe (58.6%) and lower pole (51.7%). Heterogeneous appearance was observed in 49.8% of nodules. Moreover, multifocality was observed in 50.8%, multicentricity in 7.8%, margin irregularity in 23.4%, calcification in 29.3%, and cervical lymph node in 12.8%. In total, 74.8% of nodules had a solid appearance. Regarding the sonographic

features grouped in accordance with the criteria mentioned in the ATA guidelines, 17.4% of the cases had a high risk for malignancy (n=56), 9.3% (n=30) had an intermediate risk, 29.6% (n=95) had a low risk, 33.6% (n=108) had a very low risk, and 10% (n=32) had a benign risk category. All patients included in the study had AUS as the preoperative FNAB result. FNAB was repeated in 40.5% of these cases. According to the Bethesda classification, 6.2% were stage II, 65.4% were stage III, 27.7% were stage IV, and 0.8% were stage VI. Total bilateral thyroidectomy was performed in 77.6% of the patients, and lobectomy was performed in the remaining 22.4%. Postoperative histopathological results were evaluated. 37.1% were malignant (papillary carcinoma=112, Hürthle cell carcinoma=5, follicular carcinoma=2) and 62.9% were benign (multinodular goiter=93, follicular adenoma=82, NIFTP=14, thyroiditis=13). Lymphovascular invasion was observed in 13.4% of malignant cases, capsular invasion in 10.9%, and perineural invasion in 1.7%. Based on T staging, 78.2% of malignant cases were T1, 21% were T2, and 0.8% were T3.

The descriptive characteristics of the patients with malignant histopathology were compared with those with benign histopathology. Nonetheless, no difference was observed in terms of age, gender, BMI, or CCI (Table 1).

Sonographic features of malignant and benign patients were compared. In malignant cases, the diameter of the dominant nodule was smaller, which was significant in terms of malignancy (p<0.001). Multicentricity (p=0.014), edge irregularity (p<0.001), presence of cervical lymph nodes on USG (p=0.007), and ATA high-risk cases (p<0.001) were significant for malignancy. TTW appearance on sonographic images of nodules was observed more frequently in malignant cases (p<0.001). Table 2 shows the sonographic features of the malignant and benign cases.

There were 121 patients who had AUS as the first FNAB result, underwent repeat procedure, and underwent surgery. There was no difference between benign and malignant final pathology in these patients. Among these patients, the malignancy rate decreased in those whose BETHESDA stage remained the same, whereas malignancy was observed more frequently in those whose Bethesda stage changed (increased or decreased) (Table 3).

Sonographic features of patients with and without lymphovascular invasion were compared. Hypoechoic echogenicity (p<0.001), presence of calcifications (p<0.001), macrocalcifications (p=0.006),

Table 1. Comparison of the descriptive characteristics of benign and malignant cases

Characteristics	Benign, (n=202)	Malign, (n=119)	p-value
	n (%)	n (%)	
Age*	47.3±11.9	45.7±12.9	0.247†
<55 years old	150 (74.3)	93 (78.2)	0.432††
>55 years old	26 (21.8)	52 (25.7)	-
Gender			0.818††
Female	147 (72.8)	88 (73.9)	-
Male	55 (27.2)	31 (26.1)	-
BMI (kg/m²)*	27.1±4.2	27.2±5.3	0.871†
Charlson Comorbidity Index			0.154††
Mild (1-2)	175 (86.6)	110 (92.4)	-
Moderate (3-4)	23 (11.4)	9 (7.6)	-
Severe (5+)	4 (2.0)	0	-

†Student's t-test, ††Chi-square test, *Mean ± standard deviation, BMI: Body mass index.

Table 2. Comparison of the sonographic characteristics of benign and malignant cases

Characteristics	Benign, (n=202)	Malign, (n=119)	p-value
	n (%)	n (%)	
Number of nodules			0.401†
Single	72 (35.6)	48 (40.3)	-
Multiple	130 (64.4)	71 (59.7)	-
Dominant nodule diameter (mm)*	21 (4-90)	17 (2-70)	<0.001††
Localization			0.602†
Right lobe	116 (57.4)	72 (60.5)	-
Isthmus	7 (3.5)	6 (5.0)	-
Left lobe	79 (39.1)	41 (34.5)	-
Presence of multifocality	100 (49.5)	63 (52.9)	0.552†
Presence of multicentricity	10 (5.0)	15 (12.6)	0.014†
Nodule localization within the lobe			0.339†
Lower pole	110 (54.5)	56 (47.1)	-
Middle	69 (34.2)	44 (37.0)	-
Superior pole	23 (11.4)	19 (16.0)	-
Presence of heterogeneity	95 (47.0)	65 (54.6)	0.189†
Echogenicity			0.325†
Hyperechoic	49 (24.3)	21 (17.6)	-
Isoechoic	79 (39.1)	47 (39.5)	-
Hypoechoic	74 (36.6)	51 (42.9)	-
Presence of edge irregularity	30 (14.9)	45 (37.8)	<0.001†
Shape characteristics			<0.001†
Taller than wide (-)	180 (89.1)	13 (10.9)	-
Taller than wide (+)	22 (10.9)	106 (89.1)	-
Qualification			0.389†
Solid	146 (72.3)	94 (79.0)	-
Semisolid	20 (9.9)	8 (6.7)	-
Cystic	36 (17.8)	17 (14.3)	-
Presence of calcification	52 (25.7)	42 (35.3)	0.069†
Macrocalcification	10 (5.0)	7 (5.9)	0.719†
Peripheral halo	25 (12.4)	19 (16.0)	0.366†
Punctate microcalcification	17 (8.4)	16 (13.4)	0.152†
Presence of cervical lymph nodes	18 (8.9)	23 (19.3)	0.007†
Presence of ATA high risk	19 (9.4)	37 (31.1)	<0.001†

†Chi-square test, ††Mann-Whitney U test, *Median (minimum-maximum), ATA: American Thyroid Association.

and punctate microcalcifications (p=0.008) were significantly more frequent in cases with lymphovascular invasion. In total, 68.8% of patients with lymphovascular invasion and 25.2% of cases without lymphovascular invasion were in the ATA high-risk category. Lymphovascular invasion was observed more frequently in patients in the ATA high-risk category (p=0.001) (Table 4).

Predictors of malignancy were evaluated using multivariate analyses. The model was created with the variables that were found to be statistically significant in univariate analyses and considered clinically crucial based on the literature. The model included age, gender, dominant nodule diameter, presence of multicentricity, margin irregularity, shape (TTW), presence of calcification, presence of cervical lymph node, and lymphocytic surrounding tissue from thyroid parenchyma features other than the nodule examined in the specimen. The model was found to be significant (Nagelkerke R^2=0.696, X^2=228,475, p<0.001). Regression analysis showed that the TTW shape feature [p<0.001, odds ratio (OR): 70.52, 95% confidence interval (CI): 31.32-158.77] and presence of cervical lymph nodes (p=0.018, OR: 3.94, 95% CI: 1.26-12.31) were predictive of malignancy (Table 5).

DISCUSSION

To create a common language between cytopathologists and clinicians, the NCI defined the BETHESDA classification and categorized the FNAB result into four groups: non-diagnostic material, benign, AUS, FLUS, and malignant. The TIRADS 3 category of AUS/FLUS is a group that does not fit into any other category but contains nuclear abnormalities (8). This group accounts for approximately 4-15% of all FNABs. According to the literature, this group is operated with a rate of 6-48% and has a 5-15% risk of malignancy (9,10). It is assumed that radiological findings and cytopathological examination may be diagnostically helpful in making the malignant-benign distinction in thyroid nodules in intermediate cases (11).

In a 2015 meta-analysis by Straccia et al. (12), 145,920 FNAB cytology samples from 51 publications between 2009 and 2014 were examined, and the malignancy rate was found to be 23-31% in the postoperative pathology results of patients reported as having AUS. It was stated that this range showed a very heterogeneous distribution because the evaluation was performed in more than one center (12). According to the BETHESDA thyroid cytopathology reporting system, the expected malignancy rate for category 3 AUS/

Table 3. Analysis of cases with repeated FNAB

Characteristics	Benign, (n=202)	Malign, (n=119)	p-value
	n (%)	n (%)	
Repeat FNAB	74 (36.6)	47 (39.5)	0.609†
Bethesda (n=130)			0.039†
Stage II	3 (3.9)	5 (9.3)	
Stage III	57 (75.0)	28 (51.9)	
Stage IV	16 (21.1)	20 (37.0)	
Stage VI	0	1 (1.9)	

†Student t test, ††Chi-square test, FNAB: Fine-needle aspiration biopsy.

Table 4. Comparison of the sonographic characteristics of malignant cases with and without lymphovascular invasion

Characteristics	Lymphovascular invasion (-), (n=103)	Lymphovascular invasion (+), (n=16)	p-value
	n (%)	n (%)	
Number of nodules			0.765†
Single	41 (39.8)	7 (43.8)	
Multiple	62 (60.2)	9 (56.2)	
Dominant nodule diameter (mm)*	16 (2-65)	19 (5-70)	0.515††
Localization			0.652†
Right lobe	64 (62.1)	8 (50.0)	
Isthmus	5 (4.9)	1 (6.2)	
Left lobe	34 (33.0)	7 (43.8)	
Presence of multifocality	55 (53.4)	8 (50.0)	0.800†
Presence of multicentricity	15 (14.6)	0	0.217†††
Nodule localization within the lobe			0.191†
Lower pole	50 (48.5)	6 (37.5)	
Middle	35 (34.0)	9 (56.2)	
Superior pole	18 (17.5)	1 (6.2)	
Presence of heterogeneity	56 (54.4)	9 (56.2)	0.888†
Echogenicity			0.012†
Hyperechoic	16 (15.5)	5 (31.2)	
Isoechoic	46 (44.7)	1 (6.2)	
Hypoechoic	41 (39.8)	10 (62.5)	
Presence of edge irregularity	31 (30.1)	14 (87.5)	<0.001†
Shape characteristics			0.380†††
Taller than wide (-)	10 (9.7)	3 (18.8)	
Taller than wide (+)	93 (90.3)	13 (81.2)	
Qualification			0.469†
Solid	81 (78.6)	13 (81.2)	
Semisolid	8 (7.8)	0	
Cystic	14 (13.6)	3 (18.8)	
Presence of calcification	29 (28.2)	13 (81.2)	<0.001†
Macrocalcification	3 (2.9)	4 (25.0)	0.006†††
Peripheral halo	16 (15.5)	3 (18.8)	0.719†††
Punctate microcalcification	10 (9.7)	6 (37.5)	0.008†††
Cervical lymph node			0.176†
No	84 (81.6)	12 (75.0)	
Yes	19 (18.4)	4(25.0)	
ATA high-risk category	26 (25.2)	11 (68.8)	0.001†

†Chi-square test, ††Mann-Whitney U test, †††Fisher's exact test, *Median (minimum-maximum).

Table 5. Evaluation of the determinants of malignancy by regression analysis

Variable	B	p-value	OR	95% CI
Gender (female)	0.722	0.113	2.058	0.844-5.023
Age	-0.004	0.790	0.996	0.965-1.027
Dominant nodule diameter	-0.018	0.241	0.982	0.954-1.012
Multicentricity	1.254	0.086	3.503	0.839-14.621
Shape characteristics (taller than wide)	4.256	<0.001	70.523	31.324-158.77
Presence of calcification	0.045	0.915	1.046	0.458-2.389
Presence of cervical lymph nodes	1.373	0.018	3.949	1.266-12.317
Presence of lymphocytic thyroiditis	0.759	0.062	2.136	0.963-4.738
Edge irregularity	0.681	0.136	1.975	0.808-4.829

OR: Odds ratio, CI: Confidence interval.

FLUS is 5-15%. In other studies in the literature, however, Theoharis et al. (13) found the malignancy rate to be 48%, Layfield et al. (14) found 28%, and Broome and Solorzano (15) found 20%. In other studies, the malignancy rate for surgically confirmed cases was reported to range between 6% and 76% (16-18). It was reported as 37.1% in our study, which is compatible with the literature but higher than the updated BETHESDA thyroid cytopathology reporting system. The fact that there is a very wide range and high malignancy rate in the literature and in our study suggests that the malignancy rate predicted by BETHESDA should be re-evaluated.

In a study by Sahin et al. (19), the final pathology result was reported as papillary thyroid cancer most frequently in patients diagnosed with AUS (42%), and papillary thyroid cancer was emphasized as the most common cancer subtype for patients diagnosed with AUS. The rate of PTK in patients with AUS who underwent surgery and were diagnosed as malignant was reported to be 38%, whereas the same rate was 45.8% in the study by Luu et al. (20) and 48% in the study by Olson et al. (21). In our study, papillary thyroid cancer was found to be the most common thyroid cancer with a rate of 34.9% in patients with AUS, supporting the literature.

Jankovic et al. (22) investigated the relationship between lymphocytic thyroiditis and malignancy and reported significant results. A significant difference was found in our study between the presence of lymphocytic thyroiditis in the non-nodule thyroid parenchyma and malignancy on postoperative histopathologic examination (p=0.009) (22). In this study, the sonographic characteristics of the patients who were diagnosed with AUS and underwent surgery were analyzed by univariate analysis for each parameter, and a significant correlation was found between dominant nodule diameter <19 mm (p<0.001), multicentricity (p=0.014), edge irregularity (p<0.001), TTW shape feature (p<0.001) and presence of cervical lymph nodes on USG (p=0.007) and malignancy, on the other hand, single or multiple nodules, nodule localization and multifocality, presence of heterogeneity, hypoechoic, isoechoic, and hyperechoic, presence of solid, cystic components, macrocalcification and microcalcification, and presence of peripheral halo were not significantly associated with malignancy. In the multivariate analysis of malignancy-related factors, a significant difference was observed between the TTW shape (p<0.001) and the presence of cervical lymph nodes (p=0.018) and malignancy.

In a study in which 305 patients were evaluated, it was reported that age and gender did not make a statistically significant difference in terms of malignancy in patients who underwent surgery for AUS (23). In another study, 667 patients with AUS were evaluated, and it was observed that gender did not significantly affect malignancy (24). In a study conducted by Seo et al. (25) to determine the factors that increase the risk of malignancy in patients diagnosed with AUS, being older than 45 years of age, female gender, nodule localization, dominant nodule diameter less than 15 mm, and two or more FNABs with AUS were examined, and it was stated that two or more repeated FNABs reported as AUS increased the possibility of malignancy. Analysis of the effects of demographic data on malignancy in this study revealed that gender, age, and BMI did not affect malignancy.

Kaliszewski et al. (26) showed in a study of 342 patients that a TSH value below 2.5 mIU/L increased the risk of malignancy in patients with AUS, whereas other laboratory parameters had no effect on malignancy. In another study examining serum TSH, fT3, fT4, anti-TPO, and thyroglobulin levels, no difference was observed between laboratory parameters and malignancy (27). In this study, no difference was observed between the benign and malignant groups in TSH, fT3, fT4, anti-TPO, and thyroglobulin levels measured preoperatively.

Study Limitations

The limitations of our study include the retrospective design of the study, the lack of a single radiologist performing radiologic imaging examinations, the lack of a single pathologist evaluating FNAB and surgical specimens, and the lack of involvement of a single surgeon in the operative and clinical processes.

CONCLUSION

The nodules considered risky using the guidelines were significant for malignancy in our study in terms of classification based on the sonographic findings described by the ATA guidelines.

In multivariate analyses, univariate analyses, and in the literature, when regression analysis was performed by adding parameters that may be significant for malignancy (age, gender, dominant nodule diameter, multicentricity, TTW shape, presence of calcification, presence of sonographic cervical lymph nodes, presence of lymphocytic thyroiditis in the parenchyma on final pathology, edge irregularity), the parameters of TTW shape, presence of cervical lymph node, presence of lymphocytic thyroiditis parenchyma were significant indicators of malignancy. In conclusion, USG and the ATA risk score determined on the basis of ultrasonographic parameters were found to be highly effective guides in the determination of malignancy in patients diagnosed with AUS.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained with the decision of the University of Health Sciences Türkiye, Gülhane Training and Research Hospital Clinical Research Ethics Committee (approval number: 2021/81, date: 15.12.2021).

Informed Consent: Retrospective study.

Authorship Contributions

Concept: F.D., O.H., Design: M.Z.B., M.Ö., Supervision: O.H., M.Z.B., M.Ö., Resources: F.D., M.Ö., Materials: F.D., B.U., Data Collection or Processing: F.D., O.H., Analysis or Interpretation: F.D., B.U., Literature Search: O.H., M.Z.B., M.Ö., Writing: F.D., B.U., M.Z.B., Critical Review: F.D., O.H., B.U., M.Z.B., M.Ö.

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

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

REFERENCES

1. Cakir B, Aydin C, Korukluoğlu B, Ozdemir D, Sisman IC, Tüzün D, et al. Diagnostic value of elastosonographically determined strain index in the differential diagnosis of benign and malignant thyroid nodules. Endocrine. 2011; 39: 89-98.
2. Welker MJ, Orlov D. Thyroid nodules. Am Fam Physician. 2003; 67: 559-66.
3. Nikiforov YE, Ohori NP, Hodak SP, Carty SE, LeBeau SO, Ferris RL, et al. Impact of mutational testing on the diagnosis and management of patients with cytologically indeterminate thyroid nodules: a prospective analysis of 1056 FNA samples. J Clin Endocrinol Metab. 2011; 96: 3390-7.
4. Bonzanini M, Amadori P, Morelli L, Fasanella S, Pertile R, Mattiuzzi A, et al. Subclassification of the "grey zone" of thyroid cytology; a retrospective descriptive study with clinical, cytological, and histological correlation. J Thyroid Res. 2011; 2011: 251680.
5. Cibas ES, Ali SZ; NCI Thyroid FNA State of the Science Conference. The Bethesda System For Reporting Thyroid Cytopathology. Am J Clin Pathol. 2009; 132: 658-65.
6. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid. 2016; 26: 1-133.
7. Kholová I, Ludvíková M. Thyroid atypia of undetermined significance or follicular lesion of undetermined significance: an indispensable Bethesda 2010 diagnostic category or waste garbage? Acta Cytol. 2014; 58: 319-29.
8. Bongiovanni M, Spitale A, Faquin WC, Mazzucchelli L, Baloch ZW. The Bethesda System for Reporting Thyroid Cytopathology: a meta-analysis. Acta Cytol. 2012; 56: 333-9.
9. Rosario PW. Thyroid nodules with atypia or follicular lesions of undetermined significance (Bethesda Category III): importance of ultrasonography and cytological subcategory. Thyroid. 2014; 24: 1115-20.
10. Wong LQ, LiVolsi VA, Baloch ZW. Diagnosis of atypia/follicular lesion of undetermined significance: An institutional experience. Cytojournal. 2014; 11: 23.
11. Balentine CJ, Domingo RP, Patel R, Laucirica R, Suliburk JW. Thyroid lobectomy for indeterminate FNA: not without consequences. J Surg Res. 2013; 184: 189-92.
12. Straccia P, Rossi ED, Bizzarro T, Brunelli C, Cianfrini F, Damiani D, et al. A meta-analytic review of the Bethesda System for Reporting Thyroid Cytopathology: Has the rate of malignancy in indeterminate lesions been underestimated? Cancer Cytopathol. 2015; 123: 713-22.
13. Theoharis CG, Schofield KM, Hammers L, Udelsman R, Chhieng DC. The Bethesda thyroid fine-needle aspiration classification system: year 1 at an academic institution. Thyroid. 2009; 19: 1215-23.
14. Layfield LJ, Morton MJ, Cramer HM, Hirschowitz S. Implications of the proposed thyroid fine-needle aspiration category of "follicular lesion of undetermined significance": A five-year multi-institutional analysis. Diagn Cytopathol. 2009; 37: 710-4.
15. Broome JT, Solorzano CC. The impact of atypia/follicular lesion of undetermined significance on the rate of malignancy in thyroid fine-needle aspiration: evaluation of the Bethesda System for Reporting Thyroid Cytopathology. Surgery. 2011; 150: 1234-41.
16. Carr R, Ustun B, Chhieng D, Schofield K, Theoharis C, Hammers L, et al. Radiologic and clinical predictors of malignancy in the follicular lesion of undetermined significance of the thyroid. Endocr Pathol. 2013; 24: 62-8.
17. López Vázquez Y, Penín Álvarez M, San Miguel Fraile P, Barragáns Pérez M. Risk of malignancy in thyroid nodules with atipia of undetermined significance. Endocrinol Nutr. 2015; 62: 507-10.
18. Bongiovanni M, Piana S, Spitale A, Valli R, Carlinfante G, Gardini G. Comparison of the diagnostic accuracy of thyroid fine-needle aspiration in follicular-patterned lesions using a 5-tiered and a 6-tiered diagnostic system: a double-blind study of 140 cases with histological confirmation. Diagn Cytopathol. 2014; 42: 744-50.
19. Sahin M, Gursoy A, Tutuncu NB, Guvener DN. Prevalence and prediction of malignancy in cytologically indeterminate thyroid nodules. Clin Endocrinol (Oxf). 2006; 65: 514-8.
20. Luu MH, Fischer AH, Stockl TJ, Pisharodi L, Owens CL. Atypical follicular cells with equivocal features of papillary thyroid carcinoma is not a low-risk cytologic diagnosis. Acta Cytol. 2011; 55: 526-30.
21. Olson MT, Clark DP, Erozan YS, Ali SZ. Spectrum of risk of malignancy in subcategories of "atypia of undetermined significance." Acta Cytol. 2011; 55: 518-25.
22. Jankovic B, Le KT, Hershman JM. Clinical Review: Hashimoto's thyroiditis and papillary thyroid carcinoma: is there a correlation? J Clin Endocrinol Metab. 2013; 98: 474-82.
23. Turkyilmaz S, Ulusahin M, Celebi B, Cekic AB, Mungan S, Kucuktulu U, et al. Thyroid nodules classified as atypia or follicular lesions of undetermined significance deserve further research: Analysis of 305 surgically confirmed nodules. Cytopathology. 2017; 28: 391-9.
24. Suh YJ, Choi YJ. Strategy to reduce unnecessary surgeries in thyroid nodules with cytology of Bethesda category III (AUS/FLUS): a retrospective analysis of 667 patients diagnosed by surgery. Endocrine. 2020; 69: 578-86.
25. Seo JW, Jang AL, Suh SH, Park HS, Kang MK, Hong JC. Atypia of undetermined significance on thyroid fine needle aspiration - risk factors for malignancy. Clin Otolaryngol. 2017; 42: 234-8.
26. Kaliszewski K, Diakowska D, Rzeszutko M, Nowak Ł, Wojtczak B, Sutkowski K, et al. Assessment of Preoperative TSH Serum Level and Thyroid Cancer Occurrence in Patients with AUS/FLUS Thyroid Nodule Diagnosis. Biomedicines. 2022; 10: 1916.
27. Kayilioglu SI, Dinc T, Sozen I, Senol K, Katar K, Karabeyoglu M, et al. Thyroid nodules with atypia or follicular lesions of undetermined significance (AUS/FLUS): analysis of variables associated with outcome. Asian Pac J Cancer Prev. 2014; 15: 10307-11.

GMJ 2024;35:297-300

DOI: http://dx.doi.org/10.12996/gmj.2023.4000

Assessment of Blood Pressure Levels and Left Ventricular Functions of American Football Players in Türkiye

Türkiye'deki Amerikan Futbolu Oyuncularının Kan Basıncı Düzeyleri ve Sol Ventrikül Fonksiyonlarının Değerlendirilmesi

Özden Seçkin Göbüt, Serkan Ünlü, Burak Sezenöz

Clinic of Cardiology, Gazi University Hospital, Ankara, Türkiye

ABSTRACT

Objective: In this study, we aimed to evaluate the relationship between blood pressure, left ventricular (LV) mass, and systolic function in American football (AF) players in Türkiye using deformation imaging and to compare it with conventional echocardiographic methods.

Methods: AF players admitted to the Gazi University, Cardiology Unit between January 2021 and May 2023 were included in our study. The players were grouped as linemen or non-linemen according to their field positions. LV mass and ejection fraction (EF) were assessed using blood pressure measurements and conventional methods. Deformation analysis was performed by two-dimensional speckle tracking echocardiography, and LV torsion and global longitudinal strain values were calculated. Deformation analyses were compared with blood pressure and LV EF.

Results: Players in the lineman position had higher blood pressure measurements. Although both groups of players had similar and intact LV EF values, players in the lineman position had significant changes in LV mass, LV global longitudinal strain, and LV torsion in relation to blood pressure measurements.

Conclusion: AF players are at risk of hypertension. LV hypertrophy due to hypertension causes systolic dysfunction. Deformation analysis methods can detect subclinical myocardial damage even if the EF is intact.

Keywords: American football, hypertension, left ventricular hypertrophy, deformation analysis, longitudinal strain, torsion, seckel tracking echocardiography

ÖZ

Amaç: Bu çalışmamızda, Türkiye'deki Amerikan futbolu (AF) oyuncularında kan basıncı, sol ventrikül (LV) kütlesi ve sistolik fonksiyonu arasındaki ilişkiyi deformasyon görüntüleme yöntemi ile değerlendirmeyi ve konvansiyonel ekokardiyografik yöntemlerle karşılaştırmayı amaçladık.

Yöntemler: Çalışmamıza Ocak 2021-Mayıs 2023 tarihleri arasında Gazi Üniversitesi, Kardiyoloji Birimi'ne başvuran AF oyuncuları dahil edilmiştir. Oyuncular saha pozisyonlarına göre lineman veya non-lineman olarak gruplandırılmıştır. Kan basıncı ölçümleri ve konvansiyonel yöntemler ile LV kütlesi ile ejeksiyon fraksiyonları (EF) değerlendirilmiştir. İki boyutlu speckle tracking ekokardiyografi ile deformasyon analizleri yapılmış, LV torsiyon ve global longitidunal strain değerleri hesaplanmıştır. Deformasyon analizleri; kan basınçları ve LV EF ile karşılaştırılmıştır.

Bulgular: Lineman pozisyonundaki oyuncularda daha yüksek kan basıncı ölçümleri mevcuttu. Her iki grup oyuncuları benzer ve bozulmamış LV EF değerlerine sahip olmalarına rağmen, lineman pozisyonundaki oyuncularda LV kütle, LV gobal uzunlamasına strain ve LV torsiyonlarında, kan basıncı ölçümleri ile ilişkili olarak anlamlı değişiklikler mevcuttu.

Sonuç: AF oyuncuları hipertansiyon açısından risk altındadır. Hipertansiyona bağlı gelişen LV hipertrofisi sistolik disfonksiyona neden olur. Deformasyon analizi yöntemleri ile EF bozulmamıs dahi olsa subklinik miyokard hasarı tespit edilebilir.

Anahtar Sözcükler: Amerikan futbolu, hipertansiyon, sol ventrikül hipertrofisi, deformasyon analizi, uzunlamasına strain, torsiyon, benek takibi ekokardiyografi

Address for Correspondence/Yazışma Adresi: Özden Seçkin Göbüt, MD, Clinic of Cardiology, Gazi University Hospital, Ankara, Türkiye
E-mail / E-posta: ozden-seckin@hotmail.com
ORCID ID: orcid.org/0000-0001-7385-4419

Received/Geliş Tarihi: 07.09.2023
Accepted/Kabul Tarihi: 16.09.2023

INTRODUCTION

American football (AF) has gained significant popularity among university students in Türkiye. This physically demanding team sport induces specific physiological adaptations in the heart, a phenomenon commonly referred to as the "athlete's heart" (1). However, participation in AF also entails inherent health risks, most notably hypertension. Prior cross-sectional investigations have established a notable association between AF engagement and hypertension, with emerging indications that hypertension diagnosed in young athletes may persist throughout their lifetimes (2). Extended exposure to elevated blood pressure levels can result in organ damage, with hypertensive heart disease being a significant concern. Consequently, timely identification and effective management of hypertension in young athletes assume paramount importance. Hypertensive heart disease typically presents as concentric left ventricular (LV) hypertrophy, which is characterized by an increase in LV mass and wall thickness without a proportional enlargement of the LV cavity. This structural adaptation can exert significant implications for cardiac function (3). While conventional echocardiographic methods proficiently evaluate LV dimensions and geometry, their capacity to provide a comprehensive assessment of systolic function, which signifies the heart's ability to contract and effectively propel blood, may be somewhat limited. In response to this limitation, deformation imaging has emerged as a contemporary technique that offers a more comprehensive appraisal of systolic function by quantifying LV strain and strain rate. Strain denotes the percentage change in LV segment length during contraction, and strain rate quantifies the velocity of this alteration. Deformation imaging further enables the detection of subclinical systolic dysfunction and boasts superior reproducibility, irrespective of the ultrasound beam angle (4). During LV torsion, the base rotates in an overall clockwise direction and the apex rotates in a counterclockwise direction when viewed from the apex to the base. LV torsion is followed by rapid untwisting, which contributes to ventricular filling. Because LV torsion is directly related to fiber orientation, it may depict subclinical abnormalities in heart function. Recently, ultrasound speckle tracking was introduced for the quantification of LV torsion (5). This fast, widely available technique may contribute to a more rapid introduction of LV torsion as a clinical tool for the detection of myocardial dysfunction (4). Of course, two-dimensional speckle tracking echocardiography (2D-STE) has some limitations compared with to three-dimensional (3D). In three-dimensional speckle tracking echocardiography (3D-STE) analysis, it is possible to obtain images from a single apical window, free from geometric assumptions (6). However, accessibility to 3D-STE is unfortunately low.

The central hypothesis of this study posits that AF athletes exhibit elevated blood pressure levels, augmented LV mass, and diminished systolic function compared with their non-AF counterparts. Moreover, it is postulated that these parameters are interrelated. The primary objective of this research was to meticulously scrutinize the blood pressure profiles of AF athletes in Türkiye and unravel potential associations with cardiac function. To achieve this goal, we will harness deformation imaging in conjunction with conventional echocardiography techniques to provide a comprehensive assessment of cardiac function among AF athletes.

MATERIALS AND METHODS

Study Participants

The present study enrolled individuals aged 18 years and older with no significant medical history who presented at the Gazi University Cardiology Unit outpatient clinic for follow-up between January 2021 and May 2023. The ethical approval has been obtained from the Gazi University Clinical Research Ethics Committee (approval number: 273). A signed informed consent form has been obtained from each patient. The exclusion criteria comprised individuals with systolic heart failure, significant valvular pathology, pericardial disease, atrial fibrillation (AF), acute myocardial ischemia, pulmonary embolism, or any relevant medical history. A total of 46 patients met the inclusion criteria.

Player classification: Participants were categorized into two groups based on their field playing positions: lineman and non-linear.

Blood pressure measurement: Blood pressure readings were obtained in a clinical setting adhering to recommended guidelines. Measurements were taken using an appropriately sized cuff, following a 5-min rest period in a comfortable seated position. Each measurement was repeated three times, and the recorded blood pressure values were analyzed.

Echocardiographic evaluation: Echocardiographic assessments were conducted at rest by the same cardiologist using a General Electric Vivid E95 ultrasound system equipped with a 2D M5Sc-D probe (GE Vingmed Ultrasound). These evaluations adhered to the guidelines set forth by the American Society of Echocardiography. LV mass was calculated using an anatomically validated method, according to the formula LVM = 0.8 {1.04[(LVIDd + PWTd + SWTd)3 - (LVIDd)3]} + 0.6 g, where LVID is the LV internal dimension, PWT is the posterior wall thickness, SWT is the septal wall thickness, and d represents the end diastole. LV mass was normalized to both body surface areas. LV ejection fraction (EF) was calculated using the biplane Modified Simpson method. Multiple consecutive images were recorded over five cardiac cycles to obtain data sets of optimal image quality. These ECG-synchronized images were subsequently transferred to an offline EchoPac v201 (GE Vingmed ultrasound) workstation for in-depth analysis. The LV endocardial border, including the papillary muscles, was delineated using a dedicated software and further refined manually. The software facilitated dynamic analysis of the cardiac cycle. Torsion and global longitudinal strain (GLS) were determined by time curves using an 18-segment model. Images with inadequate tracking quality for more than two segments were excluded from the analysis.

Statistical Analysis

Continuous variables have been conveniently summarized as mean values accompanied by their respective standard deviations, whereas categorical data are presented as percentages or frequencies. To assess the normality of the distribution of continuous variables, the Kolmogorov-Smirnov test was employed. Parametric continuous variables were compared using the Student's t-test, whereas non-parametric continuous variables were analyzed using the Mann-Whitney U test. For the comparison of categorical variables, the chi-square (χ^2) test was used. A stringent criterion for statistical significance, denoted by a two-

tailed p-value of <0.05, was uniformly adopted throughout all analyses. Statistical analysis was conducted using SPSS version 23.0, developed by IBM Corp in Armonk, NY, USA.

RESULTS

The study encompassed a total of 46 players, with 16 classified as lineman players and 30 as non-lineman players. Table 1 provides an overview of the demographic, anthropometric, and clinical characteristics of the study cohorts. Notably, all participants were male, with a mean age of 24.8±2.2 years (ranging from; 23 to 27 years). Importantly, there were no statistically significant differences in age observed between the two groups. Notably, the comparison of systolic and diastolic blood pressure measurements, heart rates, and body weights revealed significant distinctions, with lineman players exhibiting higher values across these parameters (Table 1).

The study results, as outlined in Table 2, provide an overview of LV EF values and LV masses, both calculated using conventional echocardiographic methods and 2D-STE deformation analysis. Interestingly, no significant disparity was noted between the groups when assessing LV-EF using the modified Simpson method (p=0.96). However, when examining LV mass, LV GLS, and LV torsion, it was evident that lineman players exhibited significantly lower absolute measurements in these parameters (Table 2).

DISCUSSION

In this study, we investigated blood pressure and LV systolic function in AF players playing in different positions using 2D-STE. The findings of our study can be summarized as follows: (a) Although AF players had similar and intact LV-EF values, players in the lineman position had lower absolute LV GLS and higher LV torsion measurements. (b) Players in the lineman position had higher blood pressure measurements. There were significant changes in LV mass, LV GLS, and LV torsion in relation to blood pressure measurements.

Table 1. Demographic, anthropometric, and clinical features

Parameters	Lineman, (n=16)	Non-Lineman, (n=30)	p-value
Age (years)	24.8±1.9	24±2.1	0.68
SBP (mmHg)	135.7±10.7	117.1±7.8	<0.001
DBP (mmHg)	80.1±10.9	74.2±12	<0.001
Weight (kg)	115.7±14.5	85.5±13.1	<0.001

SBP: Systolic blood pressure, DBP: Diastolic blood pressure.

Table 2. Comparison of conventional echocardiographic parameters among the groups

Parameters	Lineman, (n=16)	Non-Lineman, (n=30)	p-value
LV mass (gram)	224±25	165±17	<0.001
LV-GLS (%)	19.7±4.7	-22.8±-4	<0.001
LV-torsion (°/cm)	1.7±0.6	2.6±0.8	<0.001
LV-EF (%)	64±7	63.9±6.8	0.960

LV: Left ventricle, GLS: Global longitudinal strain, EF: Ejection fraction.

Notably, while AF players exhibited relatively consistent and preserved LV-EF values, we observed intriguing disparities when examining specific player positions. In particular, players assigned to the lineman position exhibited lower absolute LV GLS values, indicative of potential subclinical myocardial dysfunction. Moreover, these lineman players demonstrated higher LV torsion measurements, further highlighting the unique physiological demands placed on athletes in this position.

To complement our findings on cardiac function, we also performed blood pressure measurements among AF players. Our results revealed that players in the lineman position had elevated blood pressure, which could be potentially attributed to the rigorous physical demands and strain experienced in this role. Interestingly, we uncovered significant correlations between blood pressure measurements and LV mass, LV GLS, and LV torsion, shedding light on the interplay between hemodynamics and myocardial function in these athletes.

Evaluation of Left Ventricular Contractility Function in AF Players by Conventional Echocardiography

Traditionally, the assessment of global LV systolic function has relied on the EF, measured by the Modified Simpson method. However, it is essential to emphasize that EF primarily serves as a marker of ventricular pump function rather than an indicator of myocardial contractility. This traditional metric often falls short in identifying subtle, subclinical myocardial dysfunction (7). Nonetheless, conventional echocardiography remains the initial imaging modality for evaluating ventricular function because of its non-invasive nature, ease of use, and cost-effectiveness.

Deformation Imaging and 2D Speckle Tracking Echocardiography for the Clinical Assessment of LV Function in Patients with AF

In our study, we recognized the limitations of conventional echocardiographic methods and their inability to provide comprehensive insights into subclinical LV function. Deformation imaging techniques, particularly 2D STE, have emerged as valuable tools for the early detection of myocardial dysfunction. Notably, tissue Doppler imaging, while valuable, assesses only longitudinal function and may be influenced by tethering effects. Conversely, STE offers multidimensional displacement analysis and excels in its ability to overcome passive contraction effects and angle independence, making it a superior choice (8). Moreover, STE operates at a frame rate conducive to routine echocardiography, enhancing its practicality in clinical settings. Our findings underscore the significance of AF players in the lineman position, revealing subtle myocardial damage as indicated by decreased absolute LV strain values. These values exhibited a negative correlation with blood pressure levels and LV masses, suggesting a complex interplay between physiological stressors and myocardial function. Notably, despite these findings of subclinical dysfunction, LV-EF measurements remained within the normal range, possibly because of compensatory mechanisms such as increased apical torsion.

Comparative Insights into the Role of Hypertension

The scarcity of studies focusing on hypertension and 2D-STE in AF players necessitates attention. Previous research by Crouse

et al. (2) observed significantly different blood pressure and LV mass ratios among AF players based on their training positions, highlighting the importance of considering position-specific physiological adaptations. Kansal et al. (9) also contributed to this body of knowledge by revealing statistically lower LV-GLS values in individuals with hypertension-induced LV hypertrophy in a study involving soccer players. Remarkably, these athletes exhibited similar and intact LV-EF values in the control group. Our study aligns with these findings, supporting the notion that in hypertensive heart disease, LV systolic dysfunction may manifest earlier than traditional echocardiographic parameters would suggest.

In this research endeavor, we investigated the relationship between systolic blood pressure and the occurrence of concentric hypertrophy among athletes with AF. Our study revealed compelling findings that shed light on the dynamic interplay between elevated blood pressure and cardiac structure and function: Our study revealed a noteworthy connection between increased systolic blood pressure and the development of concentric hypertrophy in AF players. This finding underscores the pivotal role of hemodynamics in shaping cardiac structure, particularly the heart's response to increased pressure loads. Concentric hypertrophy, characterized by thickening of the heart muscle, is a well-recognized adaptation to elevated blood pressure and represents an important aspect of hypertensive heart disease.

Structural changes and systolic function: Our investigation highlighted the ripple effect of structural changes associated with hypertensive heart disease on LV systolic function over time. This aspect is crucial because LV systolic function is a key determinant of the heart's ability to pump blood effectively. Hypertrophy and other structural alterations can lead to changes in systolic function, impacting an athlete's overall cardiac performance.

Early detection with LV-GLS: A groundbreaking aspect of our study was the use of 2D-STE to measure LV-GLS. This advanced imaging technique allows for the early detection of systolic dysfunction and provides a sensitive indicator of myocardial function. By measuring LV-GLS, we can identify subtle changes in systolic performance even before traditional parameters like EF show any abnormalities.

Prognostic implications: The implications of our findings extend beyond diagnosis and early detection. We propose that further investigations may establish the utility of LV-GLS values as a valuable prognostic tool in hypertensive heart disease. By tracking changes in LV-GLS over time, clinicians may gain valuable insights into an individual's prognosis, helping to guide treatment strategies and improve long-term outcomes for patients with hypertensive heart disease.

Study Limitations

It is essential to acknowledge the limitations of our study. Perhaps the most significant constraint was the relatively small sample size and the recruitment of participants from a single center. Although our findings provide valuable insights, future research with larger and more diverse cohorts would undoubtedly enhance our understanding of the complex relationships between position-specific demands, blood pressure, and LV function in AF players.

CONCLUSION

Our study contributes significantly to the understanding of the complex relationship between blood pressure, cardiac structure, and function in athletes with AF. This underscores the importance of monitoring blood pressure in these individuals to effectively detect and manage hypertensive heart disease. In addition, the potential of LV-GLS as an early detection and prognostic marker holds promise for improving patient care and outcomes in the realm of hypertensive heart disease. Further research in this direction may yield valuable clinical insights and inform evidence-based treatment approaches.

Ethics

Ethics Committee Approval: The ethical approval has been obtained from the Gazi University Clinical Research Ethics Committee (approval number: 273).

Informed Consent: A signed informed consent form has been obtained from each patient.

Author Contributions

Concept: Ö.S.G., S.Ü., B.S., Design: Ö.S.G., S.Ü., B.S., Data Collection or Processing: Ö.S.G., S.Ü., B.S., Analysis or Interpretation: Ö.S.G., S.Ü., B.S., Literature Search: Ö.S.G., S.Ü., B.S., Writing: Ö.S.G., S.Ü., B.S.

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

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

REFERENCES

1. Stout M. Athletes' heart and echocardiography: athletes' heart. Echocardiography. 2008; 25: 749-54.
2. Crouse SF, White S, Erwin JP, Meade TH, Martin SE, Oliver JM, et al. Echocardiographic and Blood Pressure Characteristics of First-Year Collegiate American-Style Football Players. Am J Cardiol. 2016; 117: 131-4.
3. Diamond JA, Phillips RA. Hypertensive heart disease. Hypertens Res. 2005; 28: 191-202.
4. Narayanan A, Aurigemma GP, Chinali M, Hill JC, Meyer TE, Tighe DA. Cardiac mechanics in mild hypertensive heart disease: a speckle-strain imaging study. Circ Cardiovasc Imaging. 2009; 2: 382-90.
5. Rüssel Iris K, Götte Marco JW, Bronzwaer Jean G, Knaapen P, Paulus Walter J, Rossum Albert C. Left ventricular torsion: an expanding role in the analysis of myocardial dysfunction. JACC: Cardiovascular Imaging. 2009; 2: 648-55.
6. Muraru D, Niero A, Rodriguez-Zanella H, Cherata D, Badano L. Three-dimensional speckle-tracking echocardiography: benefits and limitations of integrating myocardial mechanics with three-dimensional imaging. Cardiovasc Diagn Ther. 2018; 8: 101-17.
7. Mondillo S, Galderisi M, Mele D, Cameli M, Lomoriello VS, Zacà V, et al. Speckle-tracking echocardiography: a new technique for assessing myocardial function. J Ultrasound Med. 2011; 30: 71-83.
8. Collier P, Phelan D, Klein A. A Test in Context: Myocardial Strain Measured by Speckle-Tracking Echocardiography. J Am Coll Cardiol. 2017; 69: 1043-56.
9. Kansal MM, Lester SJ, Surapaneni P, Sengupta PP, Appleton CP, Ommen SR, et al. Usefulness of two-dimensional and speckle tracking echocardiography in "Gray Zone" left ventricular hypertrophy to differentiate professional football player's heart from hypertrophic cardiomyopathy. Am J Cardiol. 2011; 108: 1322-6.

GMJ 2024;35:301-309

DOI: http://dx.doi.org/10.12996/gmj.2024.4005

Elimination of Reactive Oxygen Species Formed by Chemotherapeutic Agent in Imatinib Resistant K562r Cell Line by Sweetgum Oil

İmatinib Dirençli K562r Hücre Hattında Kemoterapötik Ajanların Oluşturduğu Reaktif Oksijen Türlerinin Sığla Yağı ile Ortadan Kaldırılması

Melike Bügül Kılınçarslan[1], Onur Eroğlu[2]

[1]Department of Cancer Molecular Biology, Pamukkale University Faculty of Medicine, Denizli, Türkiye
[2]Department of Molecular Biology and Genetics, Bilecik Şeyh Edebali University Faculty of Science, Bilecik, Türkiye

ABSTRACT

Objective: The antibacterial, antioxidant, antiseptic, and anti-inflammatory properties of Sweetgum oil (SO), a resinous exudate obtained from the injured trunk of the Liquidambar orientalis tree and named locally as "SO", have been reported in many studies.

Methods: In this study, cytotoxic doses of imatinib and ponatinib combined with SO were applied to determine differences in reactive oxygen species (ROS) formation in resistant K562R and susceptible K562S cell lines and to observe the effects of ROS on autophagy. Cytotoxicity, ROS formation, DNA damage due to ROS, autophagy, and the expression of Atg4A, Atg5, LC3α/β proteins in cell lines were investigated. In the cytotoxicity studies, the IC_{50} values of SO in K562R and K562S cells were determined as 250 µg/mL and 150 µg/mL.

Results: 21.9% more ROS was observed in K562R cells. It was observed that the amount of ROS formed in the cells to which SO was applied was 28.8% less in K562R cells and 23.8% in K562S cells. In combined applications, ROS was decreased by 67.56% in K562R cells and by 60.9% in K562S cells. The effects of SO on autophagic activation were observed by fluorescence microscopy.

Conclusion: SO increased autophagic activation compared with ponatinib in K562R cells and decreased autophagic activation compared with imatinib in K562S cells. Expression levels of Atg4A, LC3α/β and Atg5 indicate that autophagy is induced and ROS formation is reduced in combined applications.

Keywords: K562R, K562S, Sweetgum oil, ROS, autophagy

ÖZ

Amaç: Liquidambar orientalis ağacının yaralı gövdesinden elde edilen ve yerel olarak "Sweetgum yağı (SO)" olarak adlandırılan reçineli bir eksüda olan SO'nun antibakteriyel, antioksidan, antiseptik ve anti-enflamatuvar özellikleri birçok çalışmada bildirilmiştir.

Yöntemler: Bu çalışmada, dirençli K562R ve duyarlı K562S hücre hatlarında ROS oluşumundaki farklılıkları belirlemek ve reaktif oksijen türlerinin (ROS) otofaji üzerindeki etkilerini gözlemlemek için SO ile kombine edilmiş sitotoksik imatinib ve ponatinib dozu uygulanmıştır. Hücre hatlarında sitotoksisite, ROS oluşumu, ROS'ye bağlı DNA hasarı, otofaji ve Atg4A, Atg5, LC3α/β proteinlerinin ekspresyon seviyeleri araştırıldı. Sitotoksisite çalışmalarında, SO'nun K562R ve K562S hücrelerindeki IC_{50} değerleri 250 µg/mL ve 150 µg/mL olarak belirlendi.

Bulgular: K562R hücrelerinde %21,9 daha fazla ROS gözlendi. SO uygulanan hücrelerde oluşan ROS'nin K562R hücrelerinde %28,8, K562S hücrelerinde ise %23,8 daha az olduğu gözlendi. Kombine uygulamalarda K562R hücrelerinde ROS'nin %67,56, K562S hücrelerinde ise %60,9 azaldığı görülmüştür. SO'nun otofajik aktivasyon üzerindeki etkileri floresan boyalarla boyanarak floresan mikroskopi ile gözlemlendi.

Sonuç: K562R hücrelerinde SO'nun ponatinibe göre otofajik aktivasyonu artırdığı, K562S hücrelerinde ise imatinibe göre otofajik aktivasyonu azalttığı gözlenmiştir. Atg4A, LC3α/β ve Atg5 proteinlerinin ekspresyon seviyeleri kombine uygulamada otofaji indüksiyonunun sağlandığını ve ROS oluşumunun azaldığını göstermektedir.

Anahtar Sözcükler: K562R, K562S, Sığla yağı, ROS, otofaji

Address for Correspondence/Yazışma Adresi: Melike Bügül Kılınçarslan, Department of Cancer Molecular Biology, Pamukkale University Faculty of Medicine, Denizli, Türkiye
E-mail / E-posta: melikebugul@gmail.com
ORCID ID: orcid.org/0000-0002-4530-1320

Received/Geliş Tarihi: 13.09.2023
Accepted/Kabul Tarihi: 19.01.2024

INTRODUCTION

Chronic myeloid leukemia (CML) is a myeloproliferative tumor in which a monoclonal, multipotent hematopoietic progenitor cell is caused by reciprocal translocation and subsequent proliferation. Because of the resulting Philadelphia chromosome, the oncogene BCR-ABL fusion protein exhibited increased tyrosine kinase activity (1). Additional chromosome numbers change from CML to conversion from chronic phase to acute phase. Extensive chromosome G-banding studies reveal host analytical coincidence and often an extra-health condition of trisomy 8, isochromosome 17q, trisomy 19, or Philadelphia chromosomes. The cell line K562, which was derived from CML, has been widely used since its first review in 1975 (2). The Philadelphia chromosome is present in 90% of CML cases. The remaining parts exhibit complex translocations (3).

Imatinib is a tyrosine kinase inhibitor that is used as first-line therapy for CML and is highly effective for treating CML patients (4,5). With the use of imatinib, it has been demonstrated that approximately 80% of CML patients in the chronic phase achieve complete cytogenetic remission during the 12-month treatment period (6). Expenditures of chronic phase CML are prolonged, and imatinib resistance develops, making the patient resistant to imatinib treatment (7,8). Ponatinib has been approved for the treatment of patients with CML resistant or intolerant to previous TKI therapy and patients with Ph+ (Philadelphia Chromosome) Acute Lymphoblastic Leukemia (9). Solutions reported by O'Hare et al. (10) in which parts of BCR-ABL T315I refills can be stored in ponatinib treatment-resistant CML patients.

Sweetgum oil (SO) is obtained from the liquidambar orientalis mill (Hamamelidaceae) and has been used as antiulcerogenic in Turkish folk medicine for centuries (11). The antibacterial activities of SO with *in vitro* techniques performed by Sağdiç et al. (12). Antioxidant activity was determined using the DPPH test by Topal et al. (13). The antioxidants of SO Suzek et al. (14) were studied *in vivo* in a summarized manner. Since SO has protective properties and acts as an antioxidant; thus, they are modified by the arrangement of their molecular tissues (14).

Liquidambar orientalis is widespread in the southwestern coastal regions of Türkiye in Köyceğiz, Fethiye, Marmaris, and Ula (14). SO contains 45% cinnamic acid. Cinnamic acid is a phenolic plant that has antioxidant, antibacterial, and anti-inflammatory properties produced with plant extracts containing cinnamic acid and propolis. However, some changes from these replaceable cinnamic acids are also observed to be protected from lipid peroxidation and damage by various oxidative components. Although there is no detailed information about its pharmacokinetics in the human body, SO has antioxidant, anti-inflammatory, and antimicrobial effects based on its cinnamic acid content (15).

Extraction of Sweetgum Oil

In order to extract oil from the sweetgum tree, the bark on the injured parts of the trees is first chipped and thinned. With a tool called a spoon, the wounds called veins are opened. The process of rejuvenating the wounds after a week is called sur. Two weeks after the procedure, the fat accumulated in the veins is removed with a spoon. This process lasts from mid-July to October's end. Chips containing bark and wood with oil are boiled in copper pots for 30-90 minutes with water. Then, SO is extracted by compressing it with a needle (15).

ROS are cellular molecules produced as a byproduct of mitochondrial oxidative metabolism or NADPH oxidase enzymes. ROS consists of radical and non-radical oxygen species including superoxide anion (O_2-), hydrogen peroxide (H_2O_2), and hydroxyl radical (-OH) (16). ROS at normal concentrations; they act as important second messengers that play a role in various signal transduction events that regulate the growth, proliferation, and differentiation of cells (17). ROS can cause DNA damage and thus induce transformation. Transformed cells are widely accepted to be associated with cancer because of their higher ROS production than normal cells. Increased ROS production; In addition to being associated with genomic instability and DNA damage, ROS performs a signaling function to promote cell proliferation and migration and contributes to leukemic cell transformation (16).

ROS and autophagy are key regulators of cellular homeostasis in the human body. Autophagy cooperates with ROS to maintain cellular homeostasis. Autophagy can be induced by ROS and inhibits ROS-induced damage to cells and tissues (17). Both oxidative stress and autophagy are described as protective and harmful pathways in response to cellular stressors. Direct redox-based regulation of autophagy occurs through H_2O_2-inhibited oxidation of Atg4 by H_2O_2, which suppresses delipidation of LC3-II. More slowly and indirectly, oxidative stress also regulates the transcription of Beclin 1 and LC3. The overproduction of ROS carries the risk of serious damage to the mitochondria and must be partially removed. This process occurs via selective autophagic degradation of damaged mitochondrial fragments, which is called "mitophagy" (18,19).

The aim of this study was to use SO, which has antioxidant activity reported in the literature, to eliminate ROS caused by the use of chemotherapeutic agents and thus prevent DNA damage in the K562R CML cell line, which has developed resistance to imatinib in the advanced stages, which is used as first- line treatment in the treatment of CML patients. to investigate its usage.

MATERIALS AND METHODS

SO, imatinib, and ponatinib were purchased commercially.

Cell Lines

The K562R cell line obtained from the pleural fluid of a 53-year-old female patient in the CML blast crisis period was grown in RPMI 1640 medium containing 10% FBS (Fetal Bovine Serum), 2 mM L glutamine, 1% penicillin/streptomycin and 1 µM imatinib at 37 °C in a 5% CO_2 incubator. The K562S cell line, an erythroid-myeloid precursor cell line, derived from a 53-year-old female patient in terminal blast crisis was grown in RPMI 1640 broth containing 10% FBS, 2 mM L glutamine, and 1% penicillin/streptomycin at 37 °C in a 5% CO_2 incubator.

Cytotoxicity Studies

Cytotoxic effects were determined using the methylthiazole diphenyl tetrazolium (MTT) method, which is a cell proliferation test based on the measurement of metabolic activity. An increase in mitochondrial succinate dehydrogenase activity is observed in cells proliferating according to their activities in mitochondria. MTT (Glentham Life

Sciences) dye is catalyzed by mitochondrial succinate dehydrogenase and reduced to dark blue formazan salts. Formazan formation occurs only in living cells with active mitochondria.

ROS Detection

An ABP ROS Assay Kit (catalog number: A057) was used for ROS detection. ROS generation was induced by incubation with 105 cells/mL chemicals. Negative controls were prepared without an inducing agent. For the positive control, a 50 mM tert-butyl hydroperoxide (TBHP) stock solution was prepared by adding 3.2 µL of 70% TBHP into 496.8 µL of phosphate buffered salt solution (PBS). The final concentration was then adjusted to 200 µM and incubated for 30-60 min under normal growth conditions. Dilute the H2DCFDA stock solution 1:1000 in pre-warmed buffer [Hanks' Balanced Salt solution (HBSS) or HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)] to detach cells from the growth medium to provide a final working concentration of 10 µM dye. Cells were incubated at 37 °C for 10-30 minutes. After removing the loading buffer, the cells were washed three times with prewarmed buffer (HBSS or HEPES). Cells were returned to pre-warmed growth medium and incubated at 37 °C for 10-20 minutes. It was immediately observed by fluorescence microscopy.

Determination of DNA Damage

DNA laddering tests were performed to determine DNA damage. Cells treated with imatinib, ponatinib, SO, or combined doses at IC_{50} values determined by the MTT assay and cells from the control group without the drug were incubated for 24 h. After incubation, the media from the flasks were collected into a 1.5 mL collection tube and centrifuged at 13,200 g and 40 °C for 5 min, and the supernatant was discarded. Flasks were washed with PBS, scraped, collected in the same collection tube, and centrifuged at 13,200 g for 2 min. After discarding the supernatant, 600 µL Lyzis Buffer (100 µL 10 mM tris-HCl + 200 µL 0.1 M EDTA + 500 µL 0.5% SDS + 7500 µL dH2O) was added to the pellet and left on ice for 1 h. After 1 h, the sample was centrifuged at 13,200 g for 10 min. The upper phase was taken into a new collection tube, 5 µL of RNase and 4 µL of Proteinase K were added and it was waited at 37 °C for 1 h. We centrifuged the mixture at 13,200 g for 15 min by adding phenolchloroform at a ratio of 1:1. The supernatant was taken into a new collection tube, and 1/10 of the volume of 3M Ammonium acetate pH: 5.2 and 2 times the volume of absolute ethanol were added. The samples were centrifuged at 13200 g for 2 min after incubating at 200 °C overnight. After the ethanol had dried, the pellet was dissolved in 25 µL of TE (Tris-EDTA). DNA sample was then mixed at a ratio of 1:5 in 150 mL of 3% agarose gel and loading dye were performed at 70 V for 120 min.

Hoecsht (33342) staining was performed to detect DNA damage. Cells were cultured not to exceed $1x10^6$ cells/mL. The test reagents were applied to the cells and incubated for 24 h. The cells were then separated from the medium and washed 2 times with 1X assay buffer. The supernatants were discarded and suspended with 100 µL of Hoecsht (33342) dye. After incubation at 37 °C for 30 min, the cells were washed with 1X assay buffer. It was suspended in 100 µL of 1X assay buffer. One drop of the cell suspension was applied on 1 slide and covered with a coverslip, and images were taken under a fluorescence microscope.

Determination of Autophagy

A CYTO-ID Autophagy Detection Kit (catalog no: ENZ-51031) was used to detect autophagy. Cells were cultured not to exceed $1x10^6$ cells/mL. Cells were collected via centrifugation, and test reagents and positive and negative controls were applied to the cells. After 24 h of incubation, cells were removed from the medium and washed 2 times with 1X assay buffer. The supernatant was discarded and suspended with 100 µL microscopy dual detection reagent. The cells were incubated at 37 °C for 30 min. Cells were washed with 1X assay buffer and suspended with 100 µL of 1X assay buffer. One drop of the cell suspension was applied on 1 slide and covered with a coverslip, and images were taken under a fluorescence microscope.

Immunoblotting Method

From the cell lysates, the protein concentrations determined by the Bradford test were taken into a new collection tube so that each protein sample contained an equal amount of protein. The proteins were denatured by adding 2X laemmli buffer to each collection tube at a ratio of 1:1 and incubating for 5 min at 95 °C. After denaturation, the samples were placed on ice. After the cell lysates, whose protein concentrations were determined, were loaded into the gel wells, the proteins were sorted by first passing through the loading gel and then through the dissociative gel via electric current. In order to detect the presence of the desired protein on the gel, the proteins listed according to their molecular weights were passed to the membrane with the help of an electric current. In order to show the protein sought on the membrane with the help of specific antibodies, primary and secondary antibody markings were made, and the desired proteins were shown.

Statistical Analysis

The data obtained are the average of at least 3 replicates. Data were analyzed using Graph Pad Prism 9.0 and Image J program. Independent t-tests were used for comparisons.

RESULTS

Cytotoxicity Studies

Because K562R cells are imatinib-resistant, cytotoxicity assays were performed on cells grown both with and without imatinib. To determine the effect of DMSO (dimethyl sulfoxide), in which the active ingredients of SO and imatinib are dissolved, on cell viability, cells were treated with DMSO, and it was observed that there was no significant effect on cell viability. The IC_{50} value of SO was determined as 250 µg/mL in both cases. The IC_{50} of ponatinib was determined as 0.015 µM in growth medium containing imatinib and 0.020 µM in growth medium without imatinib. In their combined application, cytotoxicity experiments were also performed in both imatinib-containing and imatinib-free growth media. In order to minimize the side effects of ponatinib, a lower dose of ponatinib was found to be

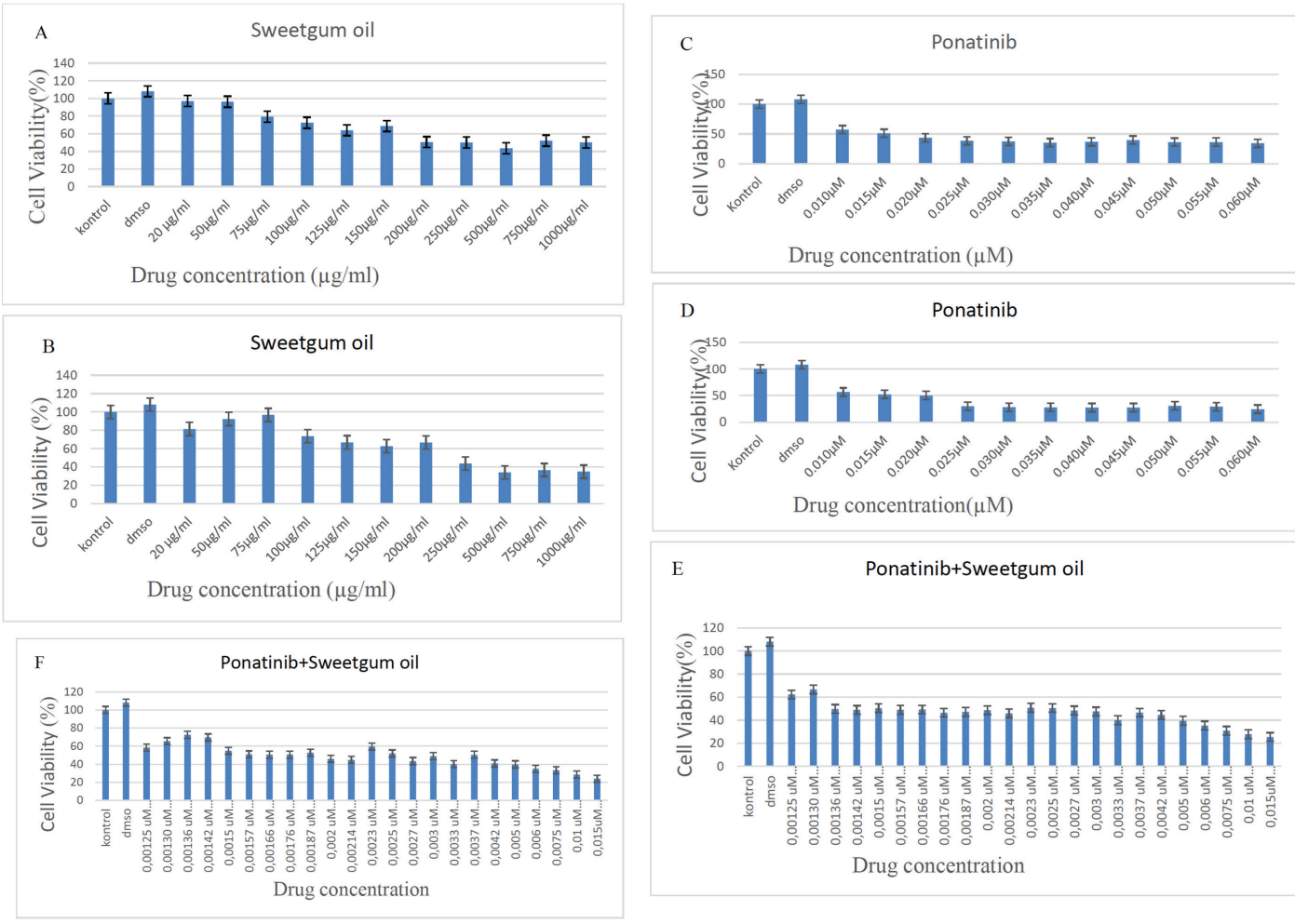


Figure 1. MTT results in K562R cells (A) Sweetgum oil MTT results in growth medium containing 1 µM imatinib in K562R cell line (IC_{50} value 250 µg/mL) (B) Sweetgum oil MTT results in growth medium containing imatinib in K562R cell line (IC_{50} value 250 µg/mL) (C) Containing 1 µM imatinib Ponatinib MTT results in growth medium (IC_{50} value 0.015 µM) (D) Ponatinib MTT results in growth medium without imatinib (IC_{50} value 0.020 µM) (E) Ponatinib and Sweetgum oil combined MTT results in growth medium containing 1 µM imatinib (IC_{50} value 0.0015 µM Ponatinib + 150 µg/mL Sweetgum oil) (F) Combined MTT results of ponatinib and Sweetgum oil in imatinib-free growth medium (IC_{50} value 0.0037 µM Ponatinib + 150 µg/mL Sweetgum oil).

effective (0.0015 µM instead of 0.0037 µM) when 150 µg/mL SO was used in the presence of imatinib, and the group containing low doses of ponatinib and imatinib was used for future experiments. The group was named combined 1. In order to assess the effect of imatinib in the experiments, another combined dose was also applied, and it was termed combined 2 (Figure 1-5).

In K562S cells, the IC_{50} value of SO was determined as 150 µg/mL, and the IC_{50} value of imatinib was determined as 10 µM. The combined IC_{50} value was 2 µM imatinib + 100 µg/mL SO. A smaller amount of SO was sufficient in K562S cells compared with K562R cells.

ROS Detection

It was determined that the amount of ROS formed in the group treated with SO in K562R cells was 28.8% less than the group treated with ponatinib. When combined applications are examined, ROS is 67.56% lower in combined applications than in single applications of SO (Figure 2).

In K562S cells, on the other hand, it was observed that the ROS formed in the group treated with SO was 23.8% lower than that in the group administered imatinib. In the combined application, the ROS was 60.9% less than that in the single application of SO (Figure 6).

Detection of DNA Damage

DNA breaks were visualized using agarose gel electrophoresis and Hoecsht (33342) dye using a fluorescence microscope 24 h after the application of determined doses of imatinib, ponatinib, and SO to K562R and K562S cells. It was observed that DNA damage increased with imatinib and ponatinib treatment compared with the control and SO groups (Figures 3, 7).

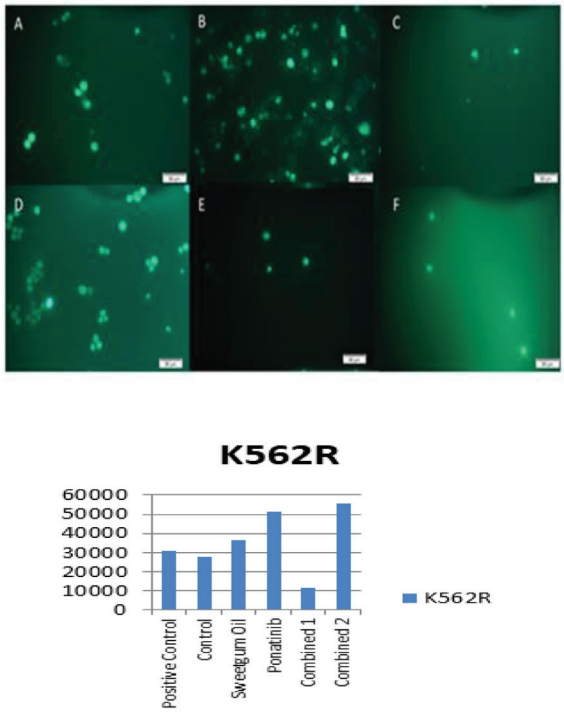


Figure 2. ROS graph of ROS in K562R cells with fluorescent microscope images (40x) (A) Positive control (B) Control (C) Sweetgum oil (D) Ponatinib (E) 0.0015 µM ponatinib + 150 µg/mIL Sweetgum oil (F) 0.0037 µM ponatinib + 150 µg/mL Sweetgum oil.

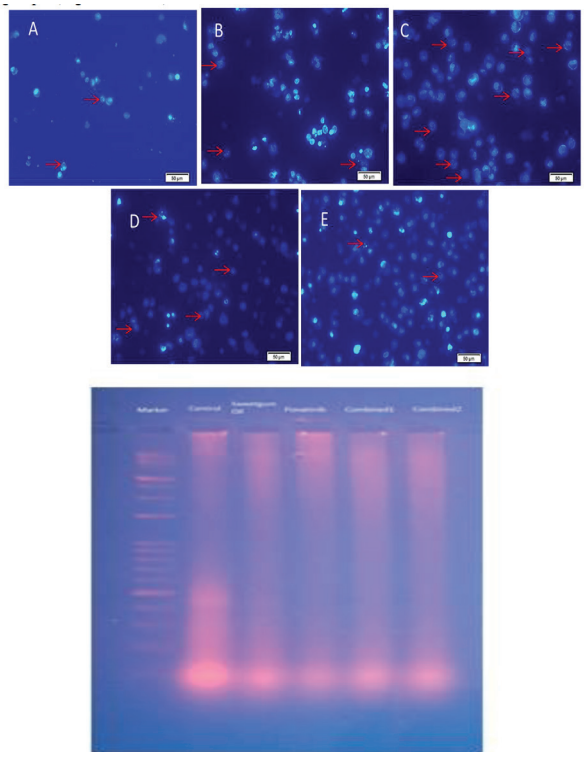


Figure 3. Demonstration of DNA breaks in K562R cells by fluorescent microscope (40x) and showing DNA breaks by agarose gel electrophoresis A) Control (group without any chemical application) B) Sweetgum oil C) Ponatinib D) 0.0015 µM Ponatinib + 150 µg/mL Sweetgum oil (E) 0.0037 µM ponatinib + 150 µg/mL Sweetgum oil (Arrows indicate DNA breaks).

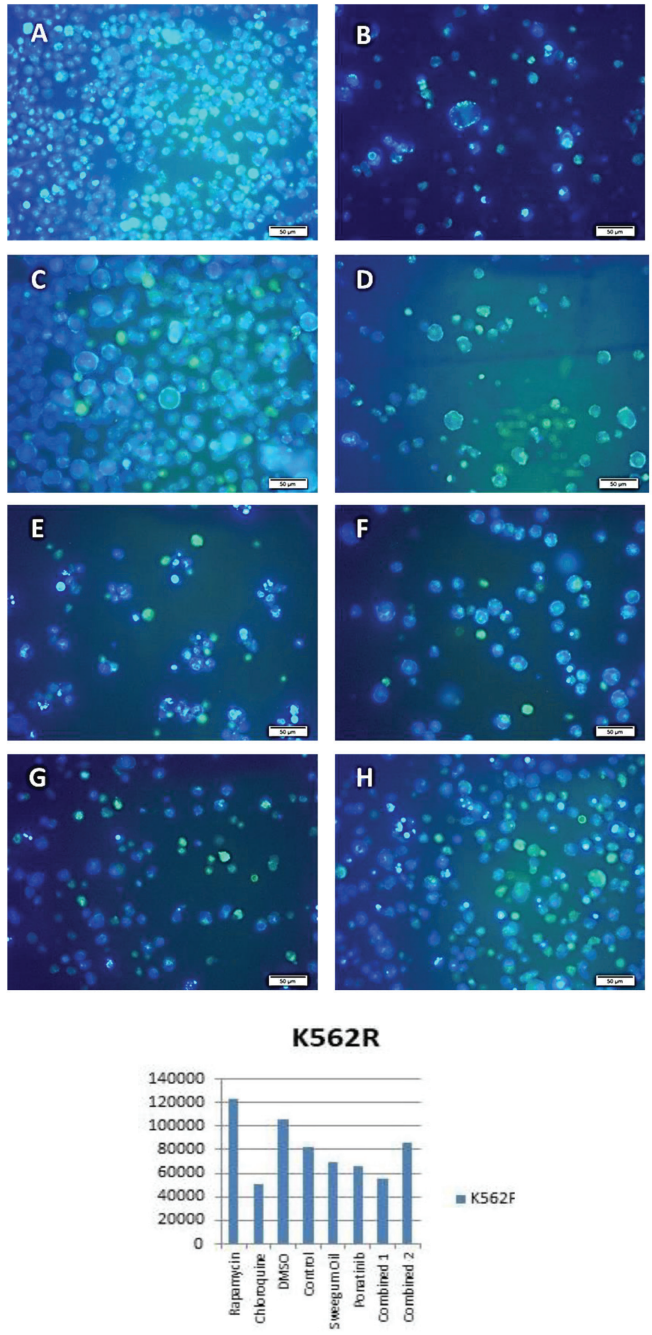


Figure 4. Graph of autophagic activation with fluorescent microscope images of autophagy formed in K562R cells at x40 magnification (A) Rapamycin (B) Chloroquine (C) DMSO (D) Control (E) Sweetgum oil (F) Ponatinib (G) 0.0015 µM Ponatinib + 150 µg/mL Sweetgum oil (H) 0.0037 µM ponatinib + 150 µg/mL Sweetgum oil.

Autophagy Determination

After K562R and K562S cells were incubated with the drugs at the indicated doses for 24 h, the kit procedure was applied, and images were obtained using a fluorescence microscope. It was observed that SO increased autophagic activation in K562R cells compared with ponatinib and decreased autophagic activation in K562S cells compared with imatinib (Figures 4, 8).

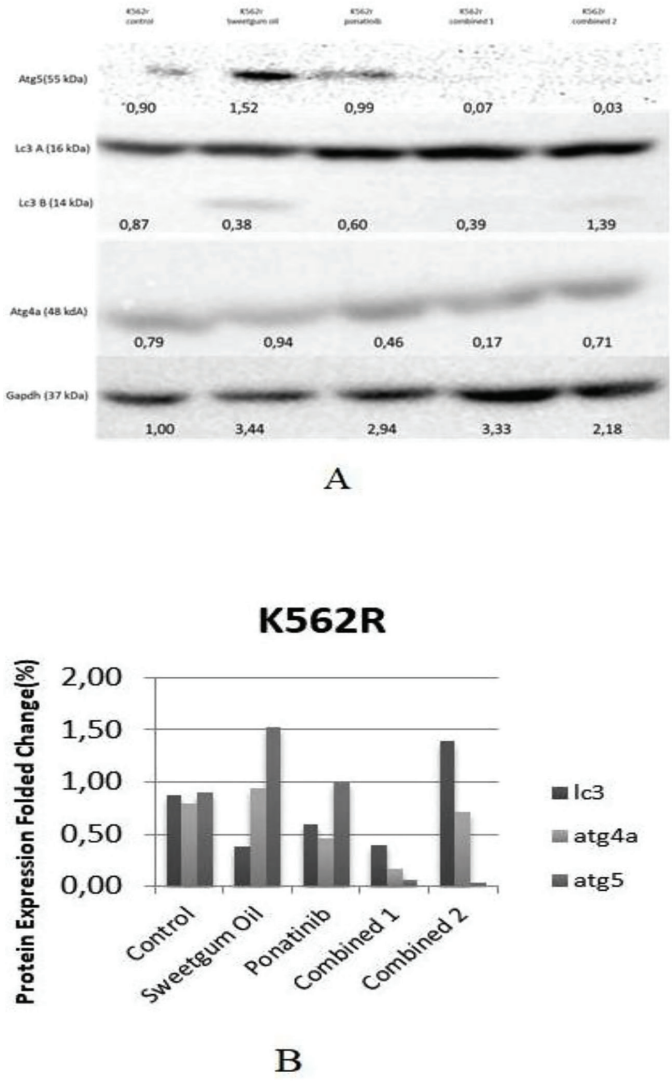


Figure 5. (A) Western blot images of Atg5, Atg4A, LC3α/β and GAPDH proteins in K562R cells (B) Folded change graph of Atg5, Atg4A, LC3α/β and GAPDH proteins in K562R cells.

Immunoblotting Method

In the western blotting experiment, the expression of LC3-I in K562R cells decreased in the SO-treated group compared with the control group. Cytosolic LC3-I is converted to LC3-II by adding phosphatidetetanolamine (PE), which means that autophagy is induced. An increase in Atg5 expression in SO means that autophagy is activated. As ROS levels decreased, Atg4A expression increased in the group treated with SO (Figure 5).

It was observed that LC3-I expression in K562S cells increased with the administration of SO and imatinib compared with that in the control group and decreased with the combined administration. As in K562R cells, SO appears to increase Atg5 expression in K562S cells. Autophagy induction appears to be achieved by combined treatment. Since ROS levels decreased in K562S cells, Atg4A expression also increased in the SO-treated group (Figure 9).

DISCUSSION

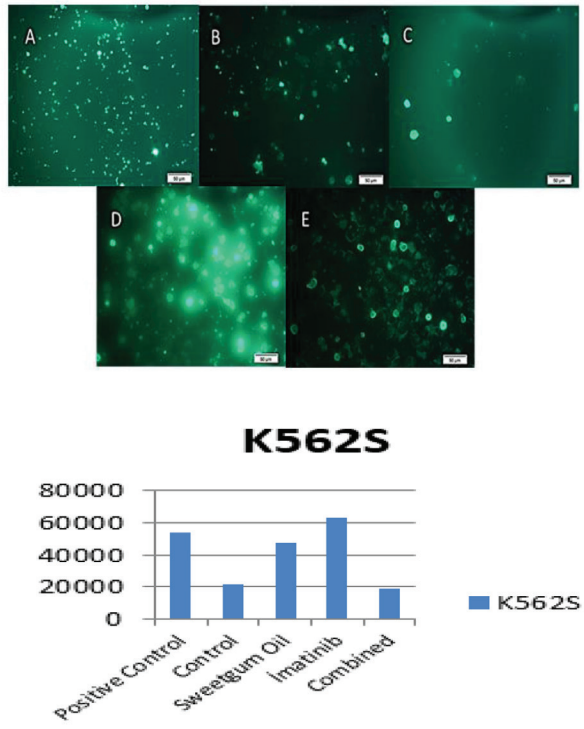


Figure 6. ROS plot of ROS in K562S cells with fluorescent microscope images (x40) (A) Positive control (B) Control (C) Sweetgum oil (D) Imatinib (E) 2 µM imatinib + 100 µg/mL Sweetgum oil.

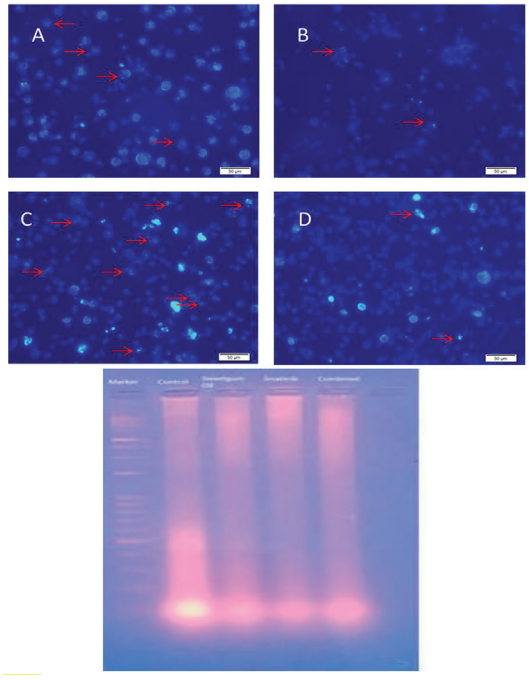


Figure 7. Demonstration of DNA breaks in K562S cells by fluorescent microscopy (x40) and DNA fragmentation by agarose gel electrophoresis (A) Control (the group that did not apply any chemicals) (B) Sweetgum oil (C) Imatinib (D) 2 µM imatinib + 100 µg/mL Sweetgum oil (arrows indicate DNA breaks).

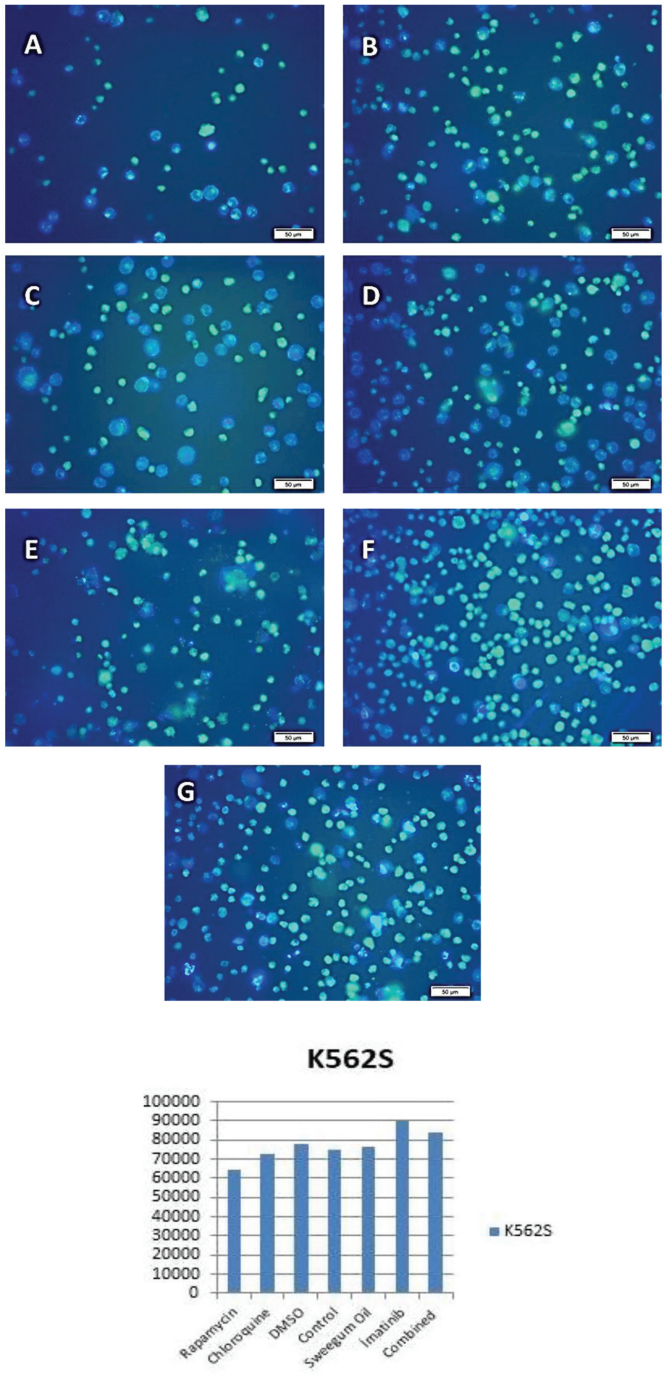


Figure 8. Graph of autophagic activation with fluorescent microscope images of autophagy formed in K562S cells at x40 magnification (A) Rapamycin (B) Chloroquine (C) DMSO (D) Control (E) Sweetgum oil (F) Imatinib (G) 2 µM imatinib + 100 µg/mL Sweetgum oil.

In this study, the effects of imatinib resistant K562R cell line and sensitive K562S cell lines, the effects of ROS on DNA resulting from the use of chemotherapeutic agents, and the effects of ROS on autophagy were investigated. Antioxidant activity of SO2 [Topal et al. (13) DPPH test]. Suzek et al. (14) extensively investigated the antioxidant activity of SO2 *in vivo*.

In this study, the MTT assay was performed to determine the

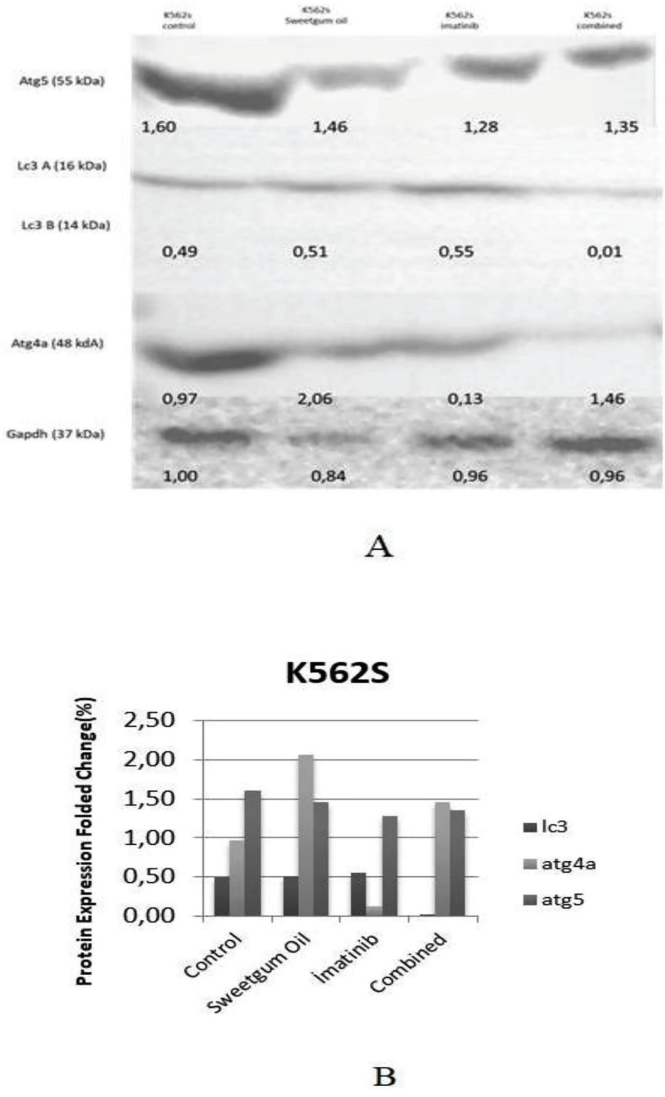


Figure 9. (A) Western blot images of Atg5, Atg4A, LC3α/β and GAPDH proteins in K562S cells (B) Folded change graph of Atg5, Atg4A, LC3α/β and GAPDH proteins in K562S cells.

cytotoxicity of the drug and SO in cell lines. Because of the MTT assay, a decrease in the viability of K562R and K562S cells was observed with increasing doses of SO (Figure 1, 10). In a previous study, it was shown that some substances in SO can have cytotoxic effects and can be a source of oxidative stress. In this study, it was emphasized that SO exerts its cytotoxic properties through DNA damage and is the source of the antimicrobial effect of SO (20). It was stated in a previous study that SO is a plant oil with antioxidant and oxidative effects (21). When SO was used, ROS formation in K562R and K562S cells was lower compared with ponatinib and imatinib. When combined applications were examined, ROS levels were decreased in K562R and K562S cells compared with the single application of SO. In this case, the application of SO together with tyrosine kinase inhibitors to the K562R and K562S cell lines has more positive effects on ROS than single application (Figure 2, 6). In addition, TKI- resistant cells may have higher ROS production than TKI-sensitive cells (22). When ROS production was compared between K562R cells showing

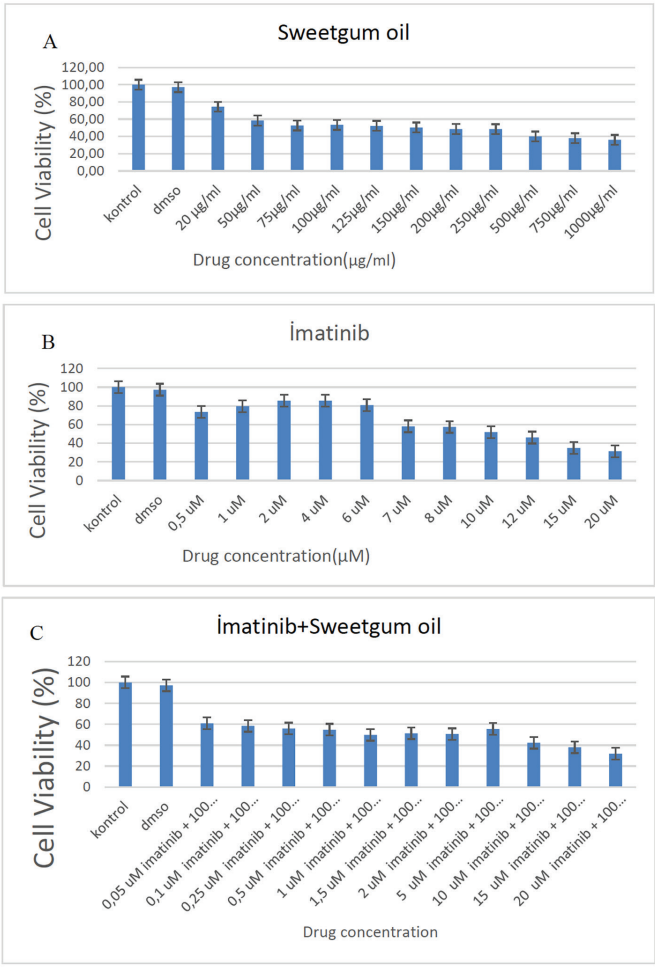


Figure 10. MTT results in K562S cells (A) MTT results of Sweetgum oil in K562S cell line (IC_{50} value 150 µg/mL) (B) MTT results of imatinib in K562S cell line (IC_{50} value 10 µM) (C) MTT results of combined imatinib and Sweetgum oil in K562S cell line (IC_{50} value 2 µM imatinib + 100 µg/mL Sweetgum oil).

TKI resistance and sensitive K562S cells not showing TKI resistance, more ROS was observed in K562R control cells compared with K562S control cells, which is consistent with the data in the literature. In this study, Hoecsht (33342) staining with the DNA laddering assay was performed to demonstrate ROS-induced DNA breakage and apoptosis (Figure 3, 7). More DNA breakage was observed in cells treated with imatinib and ponatinib in the K562R and K562S lines. These results demonstrate the relationship between ROS and DNA damage. Owing to its antioxidant properties, SO prevents the formation of ROS. ROS oxidizes cysteine amino acids in the catalytic region of Atg4A and inactivates Atg4A. It was observed that Atg4A expression was higher in cells treated with SO and decreased Atg4A expression in other groups with high ROS (Figure 5). An increase in Atg4A expression was observed in K562S cells treated with SO compared with the control group (Figure 9). These results support H2DCFDA and Hoecsht (33342) staining results. ROS and autophagy interact to maintain cellular homeostasis. Despite the increase in ROS in SO in K562R cells compared with control cells, it caused a decrease in autophagy activation, which was examined under fluorescence microscopy (Figure 4). The same is true for ponatinib. On the contrary, in K562S cells, autophagy increased with ROS increase (Figure 8). This suggests that the resistance formed in cells affects the working systems of ROS and autophagy.

When autophagy is induced in cells, it is converted to LC3-II by adding PE to cytosolic LC3-I and localizing to the LC3-II autophagosome membrane. Therefore, LC3-II expression is an indicator of autophagy (23). The expression levels of Atg5 and LC3 proteins were examined to assess autophagy. Under fluorescence microscopy, increased ROS levels suppressed autophagy in K562R cells. In autophagy, the elongation of the vesicle membrane and formation of a vesicle are catalyzed by the covalent attachment of the Atg12 protein to the Atg5 protein in the initial ubiquitin-like conjugation system. Atg5 bound to Atg12 associates with Atg16 and binds to the outer surface of the insulating membrane. Atg5 expression indicates membrane elongation. Atg5 expression in K562R and K562S cells was increased in the SO-treated group compared with the imatinib and ponatinib. autophagic activation is increased by SO in cells (20,23). In the western blot experiment, LC3-I expression was decreased in the SO group (Figure 5). It was observed that LC3-I expression in K562S cells increased with the administration of SO and imatinib compared with the control group, but decreased with the combined administration (Figure 9).

In studies using SO, it was shown that SO has cytotoxic and antitumor effects on K562R and K562S cells. SO reduced cell proliferation in a dose-dependent manner. Considering the results obtained in this study, which are in line with the literature, it can be seen that the chemotherapeutic agent causes DNA damage by creating oxidative stress while trying to kill cancer cells. At the same time, since antioxidants can exhibit oxidizing properties, attention should be paid to the dose of the antioxidant used. The use of antioxidants in cancer chemotherapy should not prevent the apoptosis or autophagic death of cancer cells. Elucidating signal transduction pathways and molecular mechanisms, including ROS and autophagy, that maintain homeostasis may provide new targets for cancer chemotherapy.

Study Limitations

Considering the results obtained in this study, which are in line with the literature, it can be seen that the chemotherapeutic agent causes DNA damage by creating oxidative stress while trying to kill cancer cells.

CONCLUSION

There is a need for studies examining the effects of SO on many biological processes, such as the cell cycle, homeostasis, migration, and angiogenesis, that support cancer chemotherapy.

Acknowledgments: We would like to thank Dr. Yüksel ÇETİN and Ayşen GÜNGÖR from Tubitak MAM Genetic Engineering and Biotechnology Institute for providing K562S cell lines. Also we would like to thank Assoc. Prof. Dr. Zeynep YÜCE and all the staff of the Dokuz Eylül University Medical Biology Department for providing the K562R cell lines for this study.

Ethics

Ethics Committee Approval: None.

Informed Consent: None.

Author Contributions

Concept: M.B.K., O.E., Design: M.B.K., O.E., Supervision: O.E., Resources: M.B.K., O.E., Data Collection or Processing: M.B.K., O.E., Analysis or Interpretation: M.B.K., O.E., Literature Search: M.B.K., O.E., Writing: M.B.K., O.E., Critical Review: M.B.K., O.E.

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

Financial Disclosure: This study was supported by Bilecik Şeyh Edebali University Scientific Research Coordination Unit (project numbered 2019-01.BŞEÜ.04-01).

REFERENCES

1. Schoepf AM, Salcher S, Obexer P, Gust R. Overcoming imatinib resistance in chronic myelogenous leukemia cells using non-cytotoxic cell death modulators. Eur J Med Chem. 2020; 185: 111748.
2. Gribble SM, Roberts I, Grace C, Andrews KM, Green AR, Nacheva EP. Cytogenetics of the chronic myeloid leukemia-derived cell line K562: karyotype clarification by multicolor fluorescence in situ hybridization, comparative genomic hybridization, and locus-specific fluorescence in situ hybridization. Cancer Genet Cytogenet. 2000; 118: 1-8.
3. Naumann S, Reutzel D, Speicher M, Decker HJ. Complete karyotype characterization of the K562 cell line by combined application of G-banding, multiplex-fluorescence in situ hybridization, fluorescence in situ hybridization, and comparative genomic hybridization. Leuk Res. 2001; 25: 313-22.
4. Clarkson B, Strife A, Wisniewski D, Lambek CL, Liu C. Chronic myelogenous leukemia as a paradigm of early cancer and possible curative strategies. Leukemia. 2003; 17: 1211-62.
5. Mauro MJ, Deininger MW. Chronic myeloid leukemia in 2006: a perspective. Haematologica. 2006; 91: 152.
6. Dong Y, Lin Y, Gao X, Zhao Y, Wan Z, Wang H, et al. Targeted blocking of miR328 lysosomal degradation with alkalized exosomes sensitizes the chronic leukemia cells to imatinib. Appl Microbiol Biotechnol. 2019; 103: 9569-82.
7. Druker BJ, O'Brien SG, Cortes J, Radich J. Chronic myelogenous leukemia. Hematology Am Soc Hematol Educ Program. 2002; 111-35.
8. Sawyers CL, Hochhaus A, Feldman E, Goldman JM, Miller CB, Ottmann OG, et al. Imatinib induces hematologic and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crisis: results of a phase II study. Blood. 2002; 99: 3530-9.
9. Huang WS, Metcalf CA, Sundaramoorthi R, Wang Y, Zou D, Thomas RM, et al. Discovery of 3-[2-(imidazo[1,2-b]pyridazin-3-yl)ethynyl]-4-methyl-N-{4-[(4-methylpiperazin-1-yl)methyl]-3-(trifluoromethyl) phenyl}benzamide (AP24534), a potent, orally active pan-inhibitor of breakpoint cluster region-abelson (BCR-ABL) kinase including the T315I gatekeeper mutant. J Med Chem. 2010; 53: 4701-19.
10. O'Hare T, Shakespeare WC, Zhu X, Eide CA, Rivera VM, Wang F, et al. AP24534, a Pan-BCR-ABL Inhibitor for Chronic Myeloid Leukemia, Potently Inhibits the T315I Mutant and Overcomes Mutation-Based Resistance. Cancer Cell. 2009; 16: 401-12.
11. Gurbuz I, Yesilada E, Demirci B, Sezik E, Demirci F, Baser KH. Characterization of volatiles and anti-ulcerogenic effect of Turkish sweetgum balsam (Styrax liquidus). J Ethnopharmacol. 2013; 148: 332-6.
12. Sağdiç O, Ozkan G, Ozcan M, Ozçelik S. A study on inhibitory effects of Siğla tree (Liquidambar orientalis Mill. var. orientalis) storax against several bacteria. Phytother Res. 2005; 19: 549-51.
13. Topal U, Sasaki M, Goto M, Otles S. Chemical compositions and antioxidant properties of essential oils from nine species of Turkish plants obtained by supercritical carbon dioxide extraction and steam distillation. Int J Food Sci Nutr. 2008; 59: 619-34.
14. Suzek H, Celik I, Dogan A, Yildirim S. Protective effect and antioxidant role of sweetgum (Liquidambar orientalis) oil against carbon tetrachloride-induced hepatotoxicity and oxidative stress in rats. Pharm Biol. 2015; 54: 451-7.
15. Aydıngöz M, Bulut S. Hidden Healing Potion of the Aegean: Sığla. AKU J Sci Eng. 2014; 14: 1-6.
16. Mi T, Wang Z, Mi T, Wang Z, Bunting KD. The Cooperative Relationship between STAT5 and Reactive Oxygen Species in Leukemia: Mechanism and Therapeutic Potential. Cancers (Basel). 2018; 10: 359.
17. Chen YF, Liu H, Luo XJ, Zhao Z, Zou ZY, Li J, et al. The roles of reactive oxygen species (ROS) and autophagy in the survival and death of leukemia cells. Crit Rev Oncol Hematol. 2017; 112: 21-30.
18. Fang Y, Wang J, Xu L, Cao Y, Xu F, Yan L, et al. Autophagy maintains ubiquitination-proteasomal degradation of Sirt3 to limit oxidative stress in K562 leukemia cells. Oncotarget. 2016; 7: 35692-702.
19. Van Erp AC, Hoeksma D, Rebolledo RA, Ottens PJ, Jochmans I, Monbaliu D, et al. The Crosstalk between ROS and Autophagy in the Field of Transplantation Medicine. Oxid Med Cell Longev. 2017; 2017: 7120962.
20. Kaya Çakir H, Eroglu O. In vitro anti-proliferative effect of capecitabine (Xeloda) combined with mocetinostat (MGCD0103) in 4T1 breast cancer cell line by immunoblotting. Iran J Basic Med Sci. 2021; 24: 1515-22.
21. Karadeniz B, Ulker Z, Alpsoy L. Genotoxic and cytotoxic effects of storax *in vitro*. Toxicol Ind Health. 2011; 29: 181-6.
22. Antoszewska-Smith J, Pawlowska E, Blasiak J. Reactive oxygen species in BCR-ABL1-expressing cells - relevance to chronic myeloid leukemia. Acta Biochim Pol. 2017; 64: 1-10.
23. Hamurcu Z, Delibaşı N, Geçene S, Şener EF, Dönmez-Altuntaş H, Özkul Y, et al. Targeting LC3 and Beclin-1 autophagy genes suppresses proliferation, survival, migration and invasion by inhibition of Cyclin-D1 and uPAR/Integrin β1/ Src signaling in triple negative breast cancer cells. J Cancer Res Clin Oncol. 2018; 144: 415-30.

GMJ 2024;35:310-314

DOI: http://dx.doi.org/10.12996/gmj.2024.4007

Does the Systemic Immune Inflammation Index and Plasma Atherogenic Index Predict Poor Prognosis in Patients Undergoing Mitral Balloon Valvuloplasty?

Mitral Balon Valvüloplasti Yapılan Hastalarda Sistemik İmmün İnflamasyon İndeksi ve Plazma Aterojenik İndeksi Kötü Prognozu Öngördürür mü?

Nergiz Aydın, Sefa Tatar, Ahmet Taha Şahin, Yakup Alsancak

Department of Cardiology, Necmettin Erbakan University, Meram Faculty of Medicine, Konya, Türkiye

ABSTRACT

Objective: In this study, we aimed to investigate the impact of the systemic immune-inflammation index (SII) and plasma atherogenic index (PAI) on adverse outcomes in patients undergoing percutaneous mitral balloon valvuloplasty (PMBV). A 5-year follow-up was conducted in patients with mitral stenosis who underwent PMBV, assessing all-cause mortality, major adverse cardiovascular events (MACE), surgical need for the mitral valve, and requirement for repeat PMBV.

Methods: This single-center retrospective study included 103 patients who underwent PMBV due to rheumatic mitral valve disease between January 2014 and January 2019. Demographic characteristics and pre- and postprocedural echocardiographic data of the patients were analyzed. The 5-year follow-up included assessments of all-cause mortality, MACE, surgical requirement for the mitral valve, and rates of repeat PMBV. Laboratory parameters at admission and at the third-month follow-up were recorded through a review of patient records.

Results: A total of 103 patients (75 females) with a mean age of 41.81±14.83 years were enrolled in the study. At admission, the SII was calculated as 8.49±6.27, PAI as 0.45±0.25, and the mitral valve area (MVA) as 1.16±0.19 cm2. Post-procedurally, MVA increased to 1.72±0.23 (p=0.001). At the third-month follow-up, SII was 7.77±7.16, and PAI was 0.47±0.25. While a statistically significant decrease was observed in SII post-procedure (p<0.001), no significant change was noted in PAI (p=0.843). When examining 5-year MACE rates in the deceased group, the SII at admission was 765.5±577.3, and at the third month, it was 794.2±907.6 (p>0.05). Regarding 5-year MACE rates in the deceased group, the PAI value at admission was 0.4±0.41, and at the third month, it was 0.4±0.42 (p>0.05).

Conclusion: In patients undergoing PMBV, SII is a significant parameter indicating a reduction in inflammation. However, both SII and PAI are

ÖZ

Amaç: Bu çalışmada sistemik immün-inflamasyon indeksi (SII) ve plazma aterojenik indeksinin (PAI) perkütan mitral balon valvüloplasti (PMBV) yapılan hastalarda kötü prognoza olan etkisini araştırmayı amaçladık. PMBV uygulanan mitral darlığı hastalarında 5 yıllık takipte tüm nedenlere bağlı mortalite, majör advers kardiyak olay (MACE), mitral kapağa cerrahi ihtiyacı, tekrar PMBV ihtiyacı değerlendirildi.

Yöntemler: Tek merkezli, retrospektif olan çalışmamız Ocak 2014 ve Ocak 2019 tarihleri arasında romatizmal mitral darlık nedeniyle PMBV uygulanan 103 hastayı içermektedir. Hastaların demografik özellikleri, işlem öncesi ve sonrası ekokardiyografik verileri incelendi. Hastaların 5 yıllık tüm nedenlere bağlı mortalite, MACE, mitral kapağa cerrahi ihtiyacı, tekrar PMBV oranlarına bakıldı. Hasta kayıtları üzerinden yapılan taramada başvuruda ve üçüncü ay kontrolde bakılan laboratuvar parametreleri kaydedildi.

Bulgular: Yaş ortalaması 41,81±14,83 yıl olan 103 hasta (75 kadın) çalışmaya dahil edildi. Başvuruda SII 8,49±6,27, PAI 0,45±0,25 ve mitral kapak alanı (MVA) 1,16±0,19 cm2 olarak hesaplandı. İşlem sonrası MVA 1,72±0,23 idi (p=0,001). İşlem sonrası üçüncü ayda SII 7,77±7,16 ve PAI 0,47±0,25 olarak hesaplandı. İşlem sonrası SII'de istatistiksel olarak anlamlı bir düşüş gözlenirken (p<0,001), PAI'de anlamlı bir değişiklik gözlenmedi (p=0,843). Beş yıllık MACE oranlarına bakıldığında mortal olan grupta başvuru anındaki SII 765,5±577,3, üçüncü aydaki SII 794,2±907,6 (p>0,05) olarak hesaplandı. Yine mortal olan grupta başvuru anındaki PAI değeri 0,4±0,41, üçüncü aydaki PAI 0,4±0,42 (p>0,05) olarak hesaplandı.

Sonuç: MBVP yapılan hastalarda SII inflamasyondaki azalmayı göstermesi açısından önemli bir parametredir. Ancak SII ve PAI, MBVP yapılan hastalarda 5 yıllık MACE sıklığını göstermesi açısından yetersizdir. Kötü prognozun gösterilmesi açısından ilave parametrelere ihtiyaç duyulmaktadır.

Address for Correspondence/Yazışma Adresi: Nergiz Aydın, MD, Department of Cardiology, Necmettin Erbakan University, Meram Faculty of Medicine, Konya, Türkiye
E-mail / E-posta: nrgz.ydn@hotmail.com
ORCID ID: orcid.org/0000-0003-3155-4076

Received/Geliş Tarihi: 19.09.2023
Accepted/Kabul Tarihi: 2.05.2024

ABSTRACT

insufficient for predicting the 5-year MACE frequency after PMBV. Additional parameters are required to indicate poor prognosis.

Keywords: Percutaneous mitral balloon valvuloplasty, mitral valve stenosis, systemic immune-inflammation index, plasma atherogenic index, MACE

ÖZ

Anahtar Sözcükler: Perkütan mitral balon valvüloplasti, mitral kapak darlığı, sistemik immün-inflamasyon indeks, plazma aterojenik indeks, MACE

INTRODUCTION

While the incidence of rheumatic heart disease has declined, rheumatic mitral valve stenosis (MS) remains a significant contributor to mortality and morbidity, particularly in developing countries. Percutaneous mitral balloon valvuloplasty (PMBV) is the preferred treatment for severely symptomatic patients. However, there is a notable lack of sufficient data regarding the long-term follow-up outcomes for individuals who have undergone PMBV. Procedural success is influenced by factors such as young age and favorable valve anatomy. Additionally, emerging evidence suggests that clinical outcomes post-PMBV depend not only on mitral valve anatomy but also on various clinical features (1). It has been established that chronic inflammation persists following rheumatic involvement. Recent studies investigating the presence of inflammation have identified new biomarkers such as the platelet-lymphocyte ratio, neutrophil-lymphocyte ratio, systemic immune-inflammatory index (SII), and plasma atherogenic index (PAI). SII has demonstrated predictive value for mortality in patients with conditions like hypertension, diabetes, and coronary artery disease (CAD). These biomarkers serve as indicators of inflammation and immune response, offering valuable insights into the ongoing inflammatory processes. Their relevance extends beyond rheumatic involvement, contributing to prognostic assessments in various medical conditions, including those mentioned earlier (2-4). Elevated SII levels before TAVI have demonstrated predictive value for major adverse cardiac events (MACE) and short-term mortality. This finding underscores the potential utility of SII as a prognostic marker in the context of TAVI, providing valuable insights into risk assessment for adverse cardiac events and mortality in the immediate post-procedural period (5). PAI is a index proposed by Dobiásová and Frohlich (6) in 2000. It is calculated as the logarithmic transformation of the triglyceride to high-density lipoprotein cholesterol ratio (TG/HDL-C) to base 10 (6). It influences the prognosis following percutaneous coronary intervention in patients with type 2 diabetes (7). In this study, our objective was to assess the prognostic impact of the SII and PAI on all-cause mortality, MACE, and the need for mitral valve surgery or repeat PMBV at the 5-year follow-up after PMBV.

MATERIALS AND METHODS

Our single-center, retrospective study included 103 patients who underwent mitral balloon valvuloplasty for rheumatic mitral stenosis between January 2014 and January 2019. Patients with known inflammatory disease, autoimmune disorders, chronic liver disease, malignancy, valve diseases requiring intervention other than mitral valve disease, presence of thrombus in the left atrium or atrial appendage, and active infections were excluded from the study. The demographic and medical characteristics of the patients were extracted from their medical records and the hospital's digital recording system. Routine laboratory parameters were assessed before the PMBV procedure and during the 3-month follow-up post-procedure, and the results were documented. In the echocardiography conducted prior to the procedure, measurements included the maximum and minimum gradients of the valve, valve area, Wilkins score, left atrium size, degrees of mitral and tricuspid regurgitation, and pulmonary artery pressure values. Post-procedure, the recorded parameters encompassed valve gradients, valve area, degrees of mitral and tricuspid regurgitation, and changes in pulmonary artery pressure. The SII was calculated using the neutrophil x platelet/lymphocyte formula (8); PAI was determined as the logarithm of the ratio of TG/HDL, calculated to the base 10 (6). Patients were assessed for all-cause mortality, MACE, necessity for mitral surgery, and recurrence of PMBV within a 5-year period following the procedure. Efforts were made to contact patients for whom information could not be accessed, using phone numbers registered in the hospital system. Patients with incomplete information during file review and those whose data could not be retrieved were excluded from the study.

This study was conducted in adherence to the Principles of the Declaration of Helsinki. Ethical approval was obtained from our Faculty's Clinical Research Ethics Committee (approval number: 3742, date: 15.04.2022).

Statistical Analysis

The research data were analyzed using SPSS 20.0. Descriptive statistics, such as average values for quantitative variables and the number of cases (percentage) for qualitative variables, were employed to present the results of the study based on the distribution of the data. Student's t-test for independent groups was used in normally distributed data for comparisons between two groups. In cases where the assumption of normality was not met among dependent groups, the Friedman test was employed for mean comparisons. For groups exhibiting non-normal distribution, the Kruskal-Wallis test was applied to assess the significance of differences among the averages of three or more groups. In all statistical tests, a p-value of 0.05 was considered statistically significant.

RESULTS

A total of 103 patients, including 75 women, with an average age of 41.81±14.83 years were enrolled in the study. Among the patients, 9.9% (n=10) had diabetes and 19.8% (n=20) had hypertension. Atrial fibrillation was identified in 44.5% (n=46) of the patients through electrocardiographic evaluation before the procedure (Table 1). The ejection fraction of the study participants was 59.08±3.19, and the Wilkins score was 8.42±0.93. The balloon size employed was

27.5±1.16 mm. In the echocardiographic assessment before the procedure, the maximum gradient was computed as 22.31±6.19, the mean gradient was 11.96±4.18, and the mitral valve area was determined as 1.12±0.26. Post-procedurally, a statistically significant decrease in the maximum and mean gradients in the valve area [(12.25±3.52; p<0.001), (5.76±1.88; p<0.001), (1.93±0.25; p<0.001)] was observed (Table 2). While the mean pulmonary artery pressure was 44.81±12.12 before the procedure, it decreased to 36.75±8.3 after the procedure (p<0.001). Although a statistically significant reduction in the SII was noted at the third-month follow-up [(852.176±631.896 vs. 772.9±714.68; p=0.009)], no significant change was observed in the PAI [(0.422±0.413 vs. 0.455±0.415; p=0.680)] (Table 3). When considering the 5-year MACE rates, in the mortality group, the SII at the time of admission was calculated as 765.5±577.3, and the SII at the third month was calculated as 794.2±907.6 (p>0.05). Similarly, in the mortality group, the PAI value at the time of admission was calculated as 0.4±0.41, and the PAI at the third month was calculated as 0.4±0.42 (p>0.05) (Table 4).

DISCUSSION

In the aftermath of acute rheumatic fever (ARF), autoantibodies activate complement proteins in susceptible individuals, initiating an inflammatory process that leads to cardiac damage. This inflammation affects all layers of the heart, with a particularly severe effect on valves originating from the endocardium. Although the inflammatory process affects all valves, it is notably more pronounced in the mitral valve. Chronic inflammation persists for years following an acute attack. In both ARF and chronic rheumatic valve patients, intralesional mononuclear cells predominantly secrete interferon, tumor necrosis factor (TNF), and Th1-type cytokines (9). This suggests that even during the chronic phase, these mononuclear cells continue to produce inflammatory cytokines. Previous studies have indicated that the levels of chronic inflammatory markers in patients with rheumatic valve disease are elevated compared with those in control groups (6). C-reactive protein (CRP) is a well-established marker of inflammation. The CRP levels were notably higher in the chronic rheumatic valve disease group than in the control group (10). Another study revealed a correlation between CRP levels, Wilkins score, and the severity of valve involvement (11). This finding represents additional evidence of ongoing inflammation. In patients with chronic rheumatic valve disease, inflammatory markers such as interleukin-6 (IL-6), IL-2, IL-8, TNF-alpha, fibrinogen, and CRP have been reported to exhibit a strong association with

Table 1. Demographic data of the patients

Variables	Before mitral balloon valvuloplasty
Female (n, %)	75 (75.2)
Age (mean ± SD)	41.81±14.83
DM (n, %)	10 (9.9)
HT (n, %)	20 (19.8)
COPD (n, %)	2 (1.9)
CKD (n, %)	4 (3.8)
AF (n, %)	46 (44.5)

DM: Diabetes mellitus, HT: Hypertension, COPD: Chronic obstructive pulmonary disease, CKD: Chronic kidney disease, AF: Atrial fibrillation, SD: Standard deviation.

Table 2. Comparison of echocardiographic data of the study group before and after PMBV

Variables	Before PMBV	After PMBV	P-value
Maximum gradient (mmHg)	22.31±6.19	12.25±3.52	<0.001
Mean gradient (mmHg)	11.96±4.18	5.76±1.88	<0.001
PAP (mmHg)	44.81±12.12	36.75±8.3	<0.001
MVA (cm^2)	1.12±0.26	1.93±0.25	<0.001

PAP: Pulmonary artery pressure MVA: Mitral valve area, PMBV: Percutaneous mitral balloon valvuloplasty.

Table 3. Laboratory values of the patients before the procedure and at the 3rd month of follow-up

Variables	Before PMBV	After PMBV	P-value
WBC (10^{-3}/u/L)	8,261±2,659	8,340±2,654	0.831
NEU (10^{-3}/u/L)	5,483±2,396	5,337±2,363	0.666
LYM (10^{-3}/u/L)	1,956±0.876	2,193±0.926	0.02
HGB (g/dL)	15,270±15,801	14,471±9,614	0.163
PLT (10^{-3}/u/L)	255.02±76,861	258.94±88,963	0.686
MO (10^{-3}/u/L)	0.471±0.487	0.516±0.231	0.516
SII	852,176±631,896	772.9±714.68	**0.009**
GFR (mL/dk)	89.51±16.72	88,505±17.18	0.793
BUN	33,643±14,352	35,676±26.29	0.149
CRE (mg/dL)	1.03±0.401	0.965±0.444	<0.001
NA (mmol/L)	138.52±2,336	139.01±2,252	0.055
K (mmol/L)	4,382±0.615	4,355±0.468	0.806
CA (mg/dL)	9.6±0.565	8.6±1,414	0.157
LDL (mg/dL)	112.43±35,774	105,637±35.00	0.230
TG (mg/dL)	143,315±74,662	147.99±86,669	0.920
HDL (mg/dL)	44,538±10,605	44,431±10,141	0.473
T. col (mg/dL)	183,295±44.00	175,171±43,456	0.423
PAI	0.422 ± 0.413	0.455 ± 0.415	0.680

WBC: White blood cell, NEU: Neutrophil, LYM: Lymphocyte, PLT: Platelet, GFR: Glomerular filtration rate, BUN: Blood urea nitrogen, CRE: Creatinin, NA: Sodium K: Potassium CA: Calcium, T. col: Total cholesterol, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, SII: Systemic immun inflammation index, PAI: Plasma atherogenic index.

Table 4. Relationship between pre-PMBV and 3rd month control SII and PAI values and 5-year MACE

Variables	MACE (+)	MACE (-)	P	T
SII (before the procedure)	765.7±577.3	897.7±655.4	0.304	1.034
SII (third month)	794.2±907.6	767.4±551.9	0.857	-0.180
PAI (before the procedure)	0.4±0.41	0.42±0.41	0.784	0.274
PAI (third month)	0.4±0.42	0.49±0.41	0.317	1.006

PMBV: Percutaneous mitral balloon valvuloplasty, SII: Systemic immun inflammation index, PAI: Plasma atherogenic index, MACE: Major adverse cardiovascular events.

the severity of valve involvement. Furthermore, these markers are linked to valve calcification and deterioration according to the New York Heart Association (NYHA) class (12).

Despite technological advancements, the Wilkins score remains a crucial tool for effectively selecting candidates for PMBV. In its early years, PMBV was primarily administered to patients with low Wilkins scores, aligning with favorable acute outcomes. However, as technical expertise developed and experience increased, indications expanded to encompass patients with intermediate scores (13). Clinical outcomes after PMBV have been documented to depend on various factors, in addition to mitral valve anatomy (14,15). The key determinants influencing long-term outcomes after successful PMBV include suboptimal valve patency, age, and prior unfavorable NYHA functional class (1). The SII is a marker of systemic inflammation calculated using the formula neutrophil platelet/lymphocyte. Elevated SII values correlate with adverse outcomes and increased MACE rates in patients with severe aortic stenosis undergoing TAVI (5). In another study, it was reported that the incidence of contrast-mediated nephropathy after TAVI was higher in the group with elevated SII (16). We observed a statistically significant decrease in SII values in the 3rd month following successful PMBV compared with pre-procedure levels. This reduction may be attributed to the decrease in inflammation resulting from the reduction in increased left atrial pressure after PMBV. Throughout the 5-year follow-up, the primary endpoints of the study, including all-cause mortality, MACE, the need for mitral valve surgery, and repeat PMBV, were not found to be correlated with the pre-procedure and 3-month control SII values.

Another aspect to consider is the role of inflammatory mechanisms in the progression of CAD and atheroma formation. Dyslipidemia stands out as one of the most critical factors contributing to CAD. Low-density lipoprotein cholesterol has been demonstrated to be highly susceptible to oxidative damage, consequently promoting the development of atherosclerotic lesions (17). Elevated PAI, calculated as the logarithmic transformation of the ratio of TG/HDL-C to the base 10, has been linked to an increased risk of atherosclerosis and coronary heart disease (18,19). PAI has been demonstrated to exhibit a correlation with CAD severity, and it also predicts MACE over a 3-year period, even in the absence of traditional risk factors (20). Although there is no established clear-cut threshold indicating high risk, PAI levels were observed to be <0.1 in umbilical cord samples, young children, and healthy women. Conversely, values up to 0.4 were identified in individuals with high atherosclerotic risk factors (19). In various studies, a PAI level <0.1 was categorized as low risk, 0.1-0.24 was deemed intermediate risk, and >0.24 was classified as high risk (17-19). In a separate study, a PAI level <0.11 was categorized as low risk, 0.11-0.21 was designated as medium risk, and >0.21 was identified as high risk (21). A robust association has been demonstrated between the development and severity of mitral annular calcification (MAC) and PAI (22). Considering the evident impact of inflammation on atheroma formation and the established role of PAI as a predictor of atherosclerosis, we investigated the variations in PAI values before and after PMBV in patients with mitral stenosis. The mean PAI value for patients with mitral stenosis before the procedure was determined as 0.422±0.413. This value exceeded the literature's threshold, indicating a high risk of CAD (17-19,21). The PAI value measured in the third month after the procedure did not exhibit a significant difference from the pre-procedure value. This may be attributed to the prolonged impact of lipid metabolism. Given the relatively short duration of our study, it is plausible that no disparity was observed in the results. It is evident that a longer follow-up period is essential for a comprehensive evaluation of effectiveness. PAI values measured before PMBV and at 3 months were not identified as effective markers in predicting MACE in the 5-year follow-up.

Study Limitations

The limitations of our study include its retrospective design with a small number of patients and incomplete documentation of the short- and long-term anti-inflammatory drugs used by the patients during the follow-up period.

CONCLUSION

The data obtained support the persistence of a chronic inflammatory response in patients with rheumatic mitral stenosis, consistent with existing studies in the literature. While the ability of the SII to indicate a reduction in inflammation in patients undergoing PMBV is noteworthy, both SII and PAI are insufficient in predicting the 5-year frequency of MACE. Additional parameters need to be evaluated for an accurate determination of poor prognosis.

Ethics

Ethics Committee Approval: Ethical approval was obtained from our Faculty's Clinical Research Ethics Committee (approval number: 3742, date: 15.04.2022).

Informed Consent: Retrospective study.

Author Contributions

Concept: S.T., Design: N.A., Supervision: A.T.Ş., Resources: Y.A., Materials: N.A., Data Collection or Processing: A.T.Ş., Analysis or Interpretation: S.T., Literature Search: Y.A., Writing: N.A., Critical Review: S.T.

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

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

REFERENCES

1. Meneguz-Moreno RA, Costa JR Jr, Gomes NL, Braga SLN, Ramos AIO, Meneghelo Z, et al. Very long term follow-up after percutaneous balloon mitral valvuloplasty. JACC Cardiovasc Interv. 2018; 11: 1945-52.
2. Cao Y, Li P, Zhang Y, Qiu M, Li J, Ma S, et al. Association of systemic immune inflammatory index with all-cause and cause-specific mortality in hypertensive individuals: Results from NHANES. Front Immunol. 2023; 14: 1087345.
3. Chen C, Chen Y, Gao Q, Wei Q. Association of systemic immune inflammatory index with all-cause and cause-specific mortality among individuals with type 2 diabetes. BMC Cardiovasc Disord. 2023; 23: 596.
4. Candemir M, Kiziltunç E, Nurkoç S, Şahinarslan A. Relationship Between Systemic Immune-Inflammation Index (SII) and the Severity of Stable Coronary Artery Disease. Angiology. 2021; 72: 575-81.

5. Tosu AR, Kalyoncuoglu M, Biter Hİ, Cakal S, Selcuk M, Çinar T, et al. Prognostic Value of Systemic Immune-Inflammation Index for Major Adverse Cardiac Events and Mortality in Severe Aortic Stenosis Patients after TAVI. Medicina (Kaunas). 2021; 57: 588.
6. Dobiásová M, Frohlich J. Nový aterogenní index plazmy (AIP) odpovídá poměru triglyceridů a HDL-cholesterolu, velikosti částic lipoproteinů a esterifikacní rychlosti cholesterolu: změny po lécbě lipanorem [The new atherogenic plasma index reflects the triglyceride and HDL-cholesterol ratio, the lipoprotein particle size and the cholesterol esterification rate: changes during lipanor therapy]. Vnitr Lek. 2000; 46: 152-6.
7. Qin Z, Zhou K, Li Y, Cheng W, Wang Z, Wang J, et al. The atherogenic index of plasma plays an important role in predicting the prognosis of type 2 diabetic subjects undergoing percutaneous coronary intervention: results from an observational cohort study in China. Cardiovasc Diabetol. 2020; 19: 23.
8. Chen JH, Zhai ET, Yuan YJ, Wu KM, Xu JB, Peng JJ, et al. Systemic immune-inflammation index for predicting prognosis of colorectal cancer. World J Gastroenterol. 2017; 23: 6261-72.
9. Guilherme L, Cury P, Demarchi LM, Coelho V, Abel L, Lopez AP, et al. Rheumatic heart disease: proinflammatory cytokines play a role in the progression and maintenance of valvular lesions. Am J Pathol. 2004; 165: 1583-91.
10. Gölbasi Z, Uçar Ö, Keles T, Sahin A, Çagli K, Çamsari A, et al. Increased levels of high sensitive C-reactive protein in patients with chronic rheumatic valve disease: evidence of ongoing inflammation. Eur J Heart Fail. 2002; 4: 593-5.
11. Alyan O, Metin F, Kacmaz F, Ozdemir O, Maden O, Topaloglu S, et al. High levels of high sensitivity C-reactive protein predict the progression of chronic rheumatic mitral stenosis. J Thromb Thrombolysis. 2009; 28: 63-9.
12. Davutoglu V, Celik A, Aksoy M. Contribution of selected serum inflammatory mediators to the progression of chronic rheumatic valve disease, subsequent valve calcification and NYHA functional class. J Heart Valve Dis. 2005; 14: 251-6.
13. Wilkins GT, Weyman AE, Abascal VM, Block PC, Palacios IF. Percutaneous balloon dilatation of the mitral valve: an analysis of echocardiographic variables related to outcome and the mechanism of dilatation. Heart. 1988;60:299–308.
14. Tomai F, Gaspardone A, Versaci F, Ghini AS, Altamura L, De Luca L, et al. Twenty year follow-up after successful percutaneous balloon mitral valvuloplasty in a large contemporary series of patients with mitral stenosis. Int J Cardiol. 2014; 177: 881-5.
15. Palacios IF, Sanchez PL, Harrell LC, Weyman AE, Block PC. Which patients benefit from percutaneous mitral balloon valvuloplasty? Prevalvuloplasty and postvalvuloplasty variables that predict long-term outcome. Circulation. 2002; 105: 1465-71.
16. Ertem AG, Ozen Y, Yuksekkaya B, Erdol AM, Erdoğan M, Demirtas K, et al. Association of the Novel Inflammatory Marker Systemic Immune-Inflammation index and Contrast-Induced Nephropathy in Patients Undergoing Transcatheter Aortic Valve Replacement for Severe Aortic Stenosis. Angiology. 2022; 73: 422-30.
17. Wu TT, Gao Y, Zheng YY, Ma YT, Xie X. Atherogenic index of plasma (AIP): a novel predictive indicator for the coronary artery disease in postmenopausal women. Lipids Health Dis. 2018; 17: 197.
18. Dobiásová M. AIP--aterogenní index plazmy jako významný prediktor kardiovaskulárního rizika: od výzkumu do praxe [AIP--atherogenic index of plasma as a significant predictor of cardiovascular risk: from research to practice]. Vnitr Lek. 2006; 52: 64-71.
19. Dobiásová M, Frohlich J. The plasma parameter log (TG/HDL-C) as an atherogenic index: correlation with lipoprotein particle size and esterification rate in apoB-lipoprotein-depleted plasma (FER(HDL)). Clin Biochem. 2001; 34: 583-8.
20. Mangalesh S, Yadav P, Dudani S, Mahesh NK. Atherogenic index of plasma predicts coronary artery disease severity and major adverse cardiac events in absence of conventional risk factors. Coron Artery Dis. 2022; 33: 523-30.
21. Fernández-Macías JC, Ochoa-Martínez AC, Varela-Silva JA, Pérez-Maldonado IN. Atherogenic Index of Plasma: Novel Predictive Biomarker for Cardiovascular Illnesses. Arch Med Res. 2019; 50: 285-94.
22. Uzunget SB, Sahin KE. Atherogenic index of plasma is an independent predictor of mitral annular calcification. BMC Cardiovasc Disord. 2022; 22: 511.

GMJ 2024;35:315-324

DOI: http://dx.doi.org/10.12996/gmj.2024.4030

Antiviral Drugs Used to Treat COVID-19 are not Liver Safe: A Comparative Experimental Study

COVID-19 Tedavisinde Kullanılan Antiviral Ajanlar Karaciğer İçin Güvenilir Değil: Karşılaştırmalı Deneysel Çalışma

Asiye Aslı Emniyet Sert, Cemile Merve Seymen, Gülnur Take Kaplanoğlu

Department of Histology and Embryology, Gazi University Faculty of Medicine, Ankara, Türkiye

ABSTRACT

Objective: This study aimed to determine whether the medication alone might be effective in the drug-induced liver damage that was reported during coronavirus disease-2019 (COVID-19) in healthy rats.

Methods: Thirty-three 8-10 week old Wistar albino male rats were separated into seven groups: the sham control groups for intravenous, subcutaneous, and gavage stress; the acyclovir, hydroxychloroquine, anakinra, and favipiravir groups. At the end of the experimental period, hematoxylin-eosin and silver impregnation histochemical, tumour necrosis factor-alpha (TNF-α)/interleukin-1 beta (IL-1β)/IL-6 immunohistochemical stainings were performed on liver tissue. Data were supported statistically.

Results: Morphological degeneration were observed in both the classic liver lobule and portal triad regions with drug administration. The intensity of the reticular fibers was found to be decreasing in the medicament groups, especially around the vena centralis. TNF-α, IL-1β, and IL-6 immunoreactivities were found to be significantly higher in the antiviral drug-administered groups than in the sham control groups.

Conclusion: It is concluded that liver damage was reported for treating COVID-19 triggered by the medicines applied.

Keywords: Antiviral agents, coronavirus, liver, TNF-α, IL-1β, IL-6

ÖZ

Amaç: Koronavirüs hastalığı-2019 (COVID-19) hastalarında ilaca-bağlı rapor edilen karaciğer hasarında, tedavi süresince kullanılan ajanların tek başlarına etken oluşturup oluşturmadıklarını sağlıklı sıçanlar üzerinde incelenmesi, çalışmamızda amaçlamıştır.

Yöntemler: Otuz üç adet 8-10 haftalık Wistar albino cinsi erkek sıçan intravenöz, subkutan ve gavaj stresi sham kontrol grupları, asiklovir grubu, hidroksiklorokin grubu, anakinra grubu ve favipiravir grubu olmak üzere yedi gruba ayrılmıştır. Hematoksilen-eozin ile gümüş impregnasyon yöntemleri ile histokimyasal; tümör nekroz faktörü-alfa (TNF-α)/interlökin-1 beta (IL-1β)/IL-6 ile de immünohistokimyasal boyamalar deney bitiminde alınan karaciğer dokuları üzerinde uygulanmıştır. Veriler istatistiksel olarak değerlendirilmiştir.

Bulgular: Çalışma sonucunda, klasik karaciğer lobülünde ve portal triad alanlarında ilaç uygulamalarına bağlı dejenerasyonlar tespit edilmiştir. Retiküler lif yoğunluğunun vena centralis çevresinde azaldığı; TNF-α, IL-1β ve IL-6 immün pozitivitelerinin ise sham kontrol gruplarına karşın, antiviral ajan uygulanan gruplarda arttığı görülmüştür.

Sonuç: COVID-19 tedavisi sırasında rapor edilen karaciğer hasarının, uygulanan ajanlar tarafından tetiklendiği kanısına varılmıştır.

Anahtar Sözcükler: Antiviral ajanlar, koronavirüs, karaciğer, TNF-α, IL-1β, IL-6

Address for Correspondence/Yazışma Adresi: Cemile Merve Seymen, MD, Department of Histology and Embryology, Gazi University Faculty of Medicine, Ankara, Türkiye
E-mail / E-posta: cmerveseymen@gmail.com
ORCID ID: orcid.org/0000-0002-8945-3801

Received/Geliş Tarihi: 25.10.2023
Accepted/Kabul Tarihi: 13.05.2024

INTRODUCTION

One of the most significant worldwide health crises at present is coronavirus disease-2019 (COVID-19), a respiratory viral infection brought on by a coronavirus (1,2). Globally, there have been more than 700 million COVID-19 cases, resulting in six million fatalities (3). Public health is being threatened by the COVID-19 pandemic, which has led to severe acute respiratory syndrome (SARS). Although this virus, according to existing clinical data, not only causes respiratory disorders and affects the lungs, but severe patients may exhibit a systemic and multi-organ disease. It also causes histopathological alterations in many non-respiratory organs, including the kidney, liver, brain, and heart (4,5).

Given these facts, antiviral drugs are in the spotlight because of their crucial role in treating SARS, but they are also required to stop the virus from spreading throughout the body, which will result in non-respiratory damage (6). Drug repurposing is another effective strategy for finding new clinical uses for already approved applications, in addition to drug development initiatives (7). In that case, antiviral medicaments that have been prescribed for other viral infections were acutely administrated to patients for a predefined period until COVID-19 vaccines were created (8).

One of the antiviral drugs administered to patients during that time was acyclovir, which is mostly used to treat herpes virus infections. This medicine inhibits viral DNA polymerase through the phosphorylation of the acyclovir compound (9,10). Another is the immunosuppressive drug anakinra, which is used to treat rheumatoid arthritis (RA). The cytokine storm cascade is significantly inhibited by this interleukin-1 (IL-1) receptor antagonist, which binds to both IL-1 alpha (α) and IL-1 beta (β) receptors (11-13). Owing to its immunosuppressive effects, hydroxychloroquine has been used to treat autoimmune diseases like lupus and RA and to prevent and treat malaria. It has also been suggested as a treatment for COVID-19 (14-16). It is well known that this medicine affects endosomal activity and raises intracellular pH. Wide-ranging secondary effects result from this action, which also alters membrane stability, interferes with lysosomal function and autophagy, disrupts signaling pathways, and inhibits transcriptional activity. By inhibiting toll-like receptor signaling and cytokine synthesis at the cellular level, this drug can decrease immunological activation (15,17). Finally, favipiravir, the first antiviral medicine utilized against COVID-19, was specifically used to treat influenza virus. It is a RNA-dependent RNA polymerase inhibitor that is activated in cells in its phosphoribosylated form and suppresses viral RNA polymerase activity (18-20).

Along with all this information, research has shown that the use of medicaments (particularly anakinra and favipiravir) (21,22) in the treatment of COVID-19 patients has been linked to drug-induced liver or hepatocellular damage (23,24). Direct viral damage, drug-induced hepatotoxicity, systemic inflammation, underlying liver disease, or hypoxia are some of the mechanisms of liver injury that have been proposed but not yet proven (25).

In this research, we sought to determine whether the medication alone might be effective in the drug-induced liver damage that was observed in COVID-19 patients through healthy rats. Using literature reviews as our guide, we chose the most widely used antiviral drugs for the treatment of COVID-19 patients. To achieve this, we compared the possible structural degenerations throughout the liver and examined the expression of tumor necrosis factor-alpha (TNF-α)/IL-1β/IL-6 in the tissue, which are known to be effective in many pathological pathways in the liver from fibrosis to necrosis.

MATERIALS AND METHODS

Animal studies were conducted in accordance with ethical standards and were approved by the Institutional Animal Ethics Committee Guidelines of Gazi University (approval number: G.Ü.ET-22.038).

Chemicals

All medications used in the experimental procedure were purchased from local pharmacies.

Animals

In this study, thirty-three 8-10 weeks old Wistar albino male rats were employed (Gazi University Faculty of Medicine Experimental Animal Breeding and Experimental Research Center, Ankara, Türkiye). They were housed in clean, sterile polypropylene cages with access to water and normal rat food under a 12-h light/dark cycle. They were housed six to a cage in an air-conditioned animal room with a temperature of 22±3 °C and 55±10% humidity. Before the start of the research, the animals were exposed to laboratory conditions for 4 weeks.

Experimental Design

The animals were separated independently into seven groups. A detailed summary of the administrations submitted to the experimental groups is shown in Table 1.

The administration doses were proportional to the masses of the experimental animals, even though the drug doses and termination of an experiment for the study were the same as those used in humans. On the basis of the COVID-19 Guidelines of the National Ministry of Health and the medicine prospectuses, dosages and durations were chosen. The sham control groups were initially planned to have six subjects for each; however, this was reduced to three at the ethics committee's request.

The animals were sedated with 45 mg/kg ketamine and 5 mg/kg xylazine, and liver tissue were harvested at the end of the experiment.

Histochemical Analysis

Liver tissue samples were preserved for 72 h in 10% neutral formaldehyde. The samples were washed in tap water, dehydrated with ascending alcohols, clarified in xylene, and embedded in paraffin. Hematoxylin-eosin and silver impregnation were performed on 4 µm thick liver sections. For the silver impregnation, a silver impregnation for reticulum kit (04-040801 Bio Optica, Lot: .0210, Milano, Italy) was used, which was obtained from standard commercial suppliers.

Immuno-Histochemical Analysis

4 µm cross sections of the liver tissue were taken on slides. The pieces were rehydrated after the paraffin was removed. A citrate buffer was used to achieve heat-induced antigen retrieval at pH 6.0. Endogenous peroxidase activity was blocked with 3% H2O2 (Lot: O2Q46013, Thermo Scientific, Waltham, MA) and a serum

blocking solution (Lot: PHL547, Thermo Scientific, Waltham, MA) were performed for 10 min. The sections were incubated with primary antibodies against TNF-α (SantaCruz: sc130349; 1:100); IL-1β (SantaCruz: sc7884; 1:100); and IL-6 (Santa Cruz: sc1265; 1:100) overnight at +4 °C. All primary antibodies were diluted with Large Volume UltrAb Diluent (Lot: UD51273, Thermo Scientific, Waltham, MA). Biotinylated secondary antibody and streptavidin peroxidase (Lot: PHL547, Thermo Scientific, Waltham, MA) were incubated with tissues for 15 min at room temperature. Phosphate buffer saline was used to wash the slides between each step. The binding sites of the antibody were visualized using DAB (Lot: HDX57664, Thermo Scientific, Waltham, MA). After washing in water and alcohol and clarifying in xylene, the slides were counterstained with Harris' hematoxylin and coated with balsam.

The density and intensity of the TNF-α, IL-1β, and IL-6 stainings were evaluated in the liver using a light microscope equipped with a digital camera (DM4000B Image Analyze System; Leica, Wetzlar, Germany), a Leica DFC280 plus camera, and a LAS software program (Leica). We used the following semi-quantitative IHC scoring system to assess the TNF-α, IL-1β, and IL-6 staining intensity; (0) no staining, (1) weak staining, (2) moderate to weak staining, (3) moderate staining, (4) moderate to strong staining, and (5) strong staining. Two independent observers, blinded to the treatment protocol, evaluated the immunostaining scores separately. The H-score was calculated as H-score = Σpi (i + 1), where is the intensity of the TNF-α, IL-1β, and IL-6 staining with values of 0-5, and Pi is the percentage of stained cells for each "i" intensity (26).

To create a single sham control group with six subjects for statistical analysis, two subjects were randomly chosen from each of the sham control groups (from the sham control groups 1-3), and their data were collected.

Statistical Analysis

H-score values derived from immunohistochemical scores were evaluated using the SPSS version 20.0 Software (SPSS Inc., Chicago, IL). All data were provided as mean ± standard deviation. ANOVA and Duncan's post-hoc tests were used to analyze the differences between the groups. A p-value of 0.05 was considered statistically significant.

RESULTS

Histochemical Results

The classic liver lobule (that includes vena centralis, endothelium, radially arranged hepatocytes, sinusoidal structures) and the portal triad (that includes portal vein, hepatic artery, bile excretory duct extension, and intermediate connective tissue) were both observed in their normal appearance through all three sham control groups, in accordance with microscopic examinations (Figures 1, 2, Table 2).

Dilatation was observed in the vena centralis of the acyclovir group. Degeneration was observed in some of the hepatocytes, and it was found that the nuclei of these types of hepatocytes were abolished, and the cell borders were also difficult to detect. The presence of condensed/pyknotic nuclei associated with the necrotic alteration identified in some hepatocytes was the most remarkable observation in this group. The vena centralis was also dilated in the hydroxychloroquine group, and it was remarkable that the normal radial organization of the hepatocytes, particularly in the regions around the vena centralis, was disrupted and a mass of cells was created. The cell morphologies of this type of hepatocyte were not readily evident. In these regions, the endothelium-lining the vena centralis was not easily visible and the sinusoids were dilated. This group also included hepatocytes with pyknotic nuclei. Around the

Table 1. Experimental design

Experimental groups	Number of subjects	Administrations	Termination of the experiment
Sham control-1 (gavage stress)	(n=3)	0.5 cc 0.09% sterile saline by gavage	5 days
Sham control-2 [intravenous (i.v.) injection stress]	(n=3)	0.5 cc 0.09% sterile saline by i.v. injection	7 days
Sham control-3 (subcutaneous [s.c.] injection stress)	(n=3)	0.5 cc 0.09% sterile saline by s. c. injection	7 days
Acyclovir	(n=6)	2x10 mg/kg acyclovir (dissolved in 0.09% sterile saline) (2 times a day, seperated by 12 hours) i.v. injection	7 days
Hydroxychloroquine	(n=6)	2x400 mg/kg hydroxychloroquine (dissolved in 0.09% sterile saline) (2 times a day, seperated by 12 h for the day 1) 2x200 mg/kg hydroxychloroquine (dissolved in 0.09% sterile saline) (2 times a day, seperated by 12 h for the day 2-5) gavage	5 days
Anakinra	(n=6)	4x100 mg/kg anakinra (dissolved in 0.09% sterile saline) (4 times a day, seperated by 6 hours) s.c. injection	7 days
Favipiravir	(n=6)	2x1600 mg/kg favipiravir (dissolved in 0.09% sterile saline) (2 times a day, seperated by 12 h for the day 1) 2x600 mg/kg favipiravir (dissolved in 0.09% sterile saline) (2 times a day, seperated by 12 h for the day 2-5) gavage	5 days

Table 2. Scores for each group based on degenerative criteria

	Sham control-1	Sham control-2	Sham control-3	Acyclovir	Hydroxychloroquine	Anakinra	Favipiravir
Dilatation in V. centralis				++	+	++	++
Dilatation in the sinusoids					+	+	+
Congestion				+	++	++	+
Radial disorganization					++	+	++
Degeneration in hepatocyte morphology				+	+	+	+
Pyknotic nuclei				++	+		
Karyolysis						+	++
Vacuolar degeneration						+	+++
Infiltration					+	+	
Fibrosis						++	++
Dilatation in the portal vein				+	++	++	+++
Hyalinization						+	+
Reticular fibers	+++	+++	+++	++	++	+	+

vena centralis, infiltration was visible. In the anakinra-administered group, vena centralis and sinusoids were dilated, and infiltration and an increase in the connective tissue that extended to the sinusoids were also distinguished. A few cells had pyknotic nuclei that could be observed. The karyolysis in this group that was compatible with the deletion of the intranuclear chromatin material seen in some hepatocytes was the most distinguishing characteristic of this group. Some hepatocytes were found to have vacuolar degeneration. The vena centralis and sinusoids exhibited significant dilatation in the favipiravir group, and a hyalinized structure that extended to the sinusoids. The typical radial arrangement of the hepatocytes was disrupted, and a mass of cells was formed in almost all of the hepatocytes. A loss of nuclei indicating karyolysis was observed in certain hepatocytes. Hepatocytes displayed a vacuous structure that may have been caused by organelle degeneration (Figure 1, Table 2).

When the portal triad region was examined for each group, the group that received acyclovir show minimal portal vein dilatation. The portal vein in the hydroxychloroquine group had severe dilatation and congestion. In addition to the severe dilatation and congestion through the portal vein after anakinra administration, hyalinization of the connective tissue in this region was observed. Administration of favipiravir led to intense dilatation of the portal vein and an increase in connective tissue in this region. However, it was noted that the fibers were not yet organized in this connective tissue area (Figure 2, Table 2).

The distribution of reticular fibers in the sinusoidal regions, particularly around the vena centralis, was found to be intense in all three sham control groups during the silver impregnation tests. The intensity of the reticular fibers was found to decrease in the experimental groups from acyclovir to hydroxychloroquine, anakinra, and favipiravir at a discontinuous level. The gold-yellow staining in the anakinra and favipiravir groups caused by increased collagen fibers and potential fibrosis despite decreased reticular fibers was another remarkable discovery of silver impregnation (Figure 3, Table 2).

Immuno-Histochemical Results

Immunoreactivities were distinguished at the cytoplasmic level through the classic liver lobule cells in TNF-α, IL-1β, and IL-6 immunohistochemical staining. Very weak TNF-α immuno-reactivity was observed in some hepatocytes in all three sham control groups. Weak immunoreactivity was observed in the acylovir and hydroxychloroquine groups, whereas the anakinra group showed weak to moderate immunoreactivity. In addition, more intense immunoreactivity was observed in the hepatocytes located around the vena centralis. Moderate immunoreactivity was observed in the hepatocytes around the vena centralis, whereas other hepatocytes showed weak immunoreactivity in certain places and moderate immunoreactivity in others (Figure 4). According to statistical findings, TNF-α immuno-reactivities were found to be significantly higher in the antiviral drug-administered groups than in the sham control group ($p<0.05$). However, no statistically significant difference was detected between the antiviral drug-administered groups ($p>0.05$) (Graphic 1).

Very weak IL-1β immuno-reactivity was observed in some hepatocytes in all three sham control groups. In the acyclovir group, hepatocytes had weak to moderate immunoreactivity, whereas strong immunoreactivity was observed in Kupffer cells. Moderate immunoreactivity in the hydroxychloroquine group and moderate to strong immunoreactivity were observed in the anakinra group. In addition, strong IL-1β immuno-reactivity was observed in relatively few Kupffer cells in the anakinra group compared with the acyclovir group. In the favipiravir group, strong immunoreactivity was observed in the hepatocytes around the vena centralis, whereas other hepatocytes showed moderate to strong immunoreactivity in certain places. Immunoreactive Kupffer cell distributions were the same as those in the acyclovir group (Figure 5). According to statistical findings, IL-1β immuno-reactivities were found to be significantly higher in the antiviral drug-administered groups than in the sham control group ($p<0.05$). Additionally, significantly higher IL-1β immuno-reactivities were observed in the anakinra

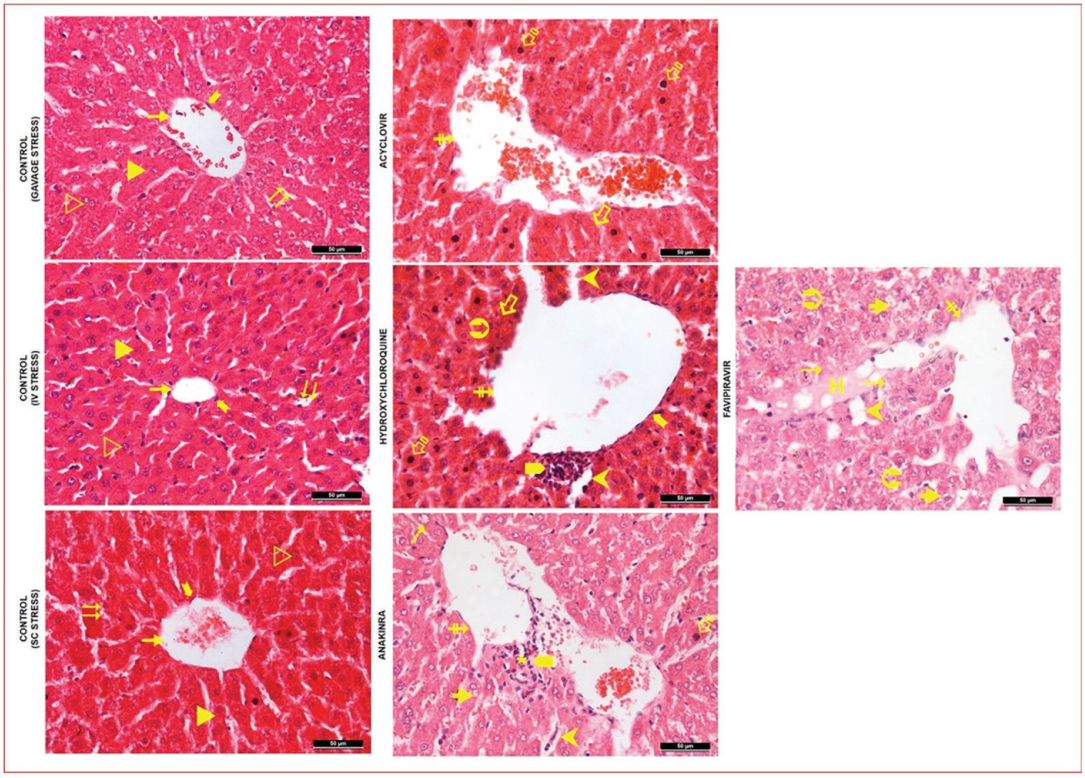


Figure 1. Liver sections (classical lobule) for each group showed ➙: Vena centralis, ▶: Hepatocyte, ⇉: Sinusoid, ➡: Endothelium, ▷: Nucleus, ‡: Dilatation in vena centralis, ⇨: Degeneration in hepatocyte morphology, ⇪: Pyknotic nucleus, ⇩: Radial disorganization, ➤: Dilatation in sinusoids, ➡: Infiltration, *: Fibrosis, ⇢: Karyolysis, ➧: Vacuolar degeneration, ⇔: Congestion, H: Hyalinization (hematoxylin-eosin x400).

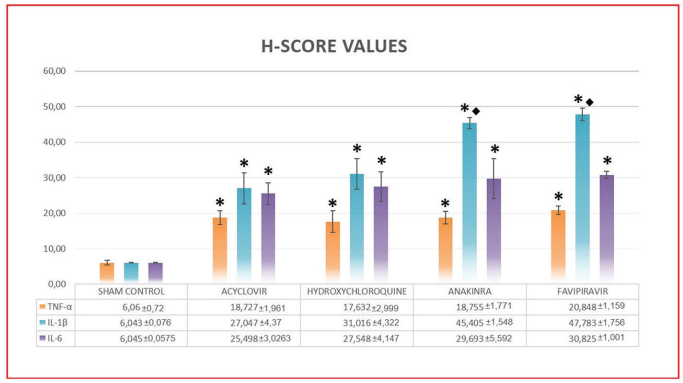
and favipiravir groups than in the acyclovir and hydroxychloroquine groups (p<0,05) (Graphic 1).

For all three sham control groups, very weak IL-6 immunoreactivity was observed in a few hepatocytes. Moderate immunoreactivity in hepatocytes and strong immunoreactivity in Kupffer cells were determined in the acyclovir group. The hepatocytes around the vena centralis showed moderate to strong immunoreactivities for the other three groups. The hydroxychloroquine group had weak to moderate immunoreactivity of hepatocytes in certain places, whereas the final two groups showed moderate immunoreactivities in these regions (Figure 6). According to statistical findings, IL-6 immunoreactivities were found to be significantly higher in the antiviral drug-administered groups than in the sham control group (p<0.05). However, no statistically significant difference was detected between the antiviral drug-administered groups (p>0.05) (Graphic 1).

DISCUSSION

COVID-19 mostly affects the lungs, but it can directly/indirectly cause virus-induced liver injury, whose mechanisms are currently under investigation (27). The first study that reported abnormal liver tests in patients with COVID-19 was reported by Chen et al. (28). Liver injury is indicated by high bilirubin levels and abnormal alanine transaminase/aspartate transaminase (ALT/AST) levels (1).

The mechanisms underlying the link between severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) and liver injury are complex and include direct cholangiocyte damage caused by SARS-CoV-2, immune overactivation and systemic inflammation, ischemia/reperfusion and hypoxia/reoxygenation injuries, and drug-induced liver injury (4,29). The majority of the data show that systemic inflammation is more likely to be the cause of hepatic injury during SARS-CoV-2 infection than to be triggered by a cytopathic effect that targets liver cells. In patients with COVID-19, viral RNA can be found in the liver tissue; however, infection of the liver cells has not yet been proven (2). Additionally, a number of drugs, biological agents,



Graphic 1. Comparative statistical graphs showing H-score analyses of immunostainings. (*) Statistically significant groups comparison to the sham control group, (♦) Statistically significant groups comparison to the acyclovir and hydroxychloroquine groups.

TNF-α: Tumour necrosis factor-alpha, IL-1β: Interleukin-1 beta.

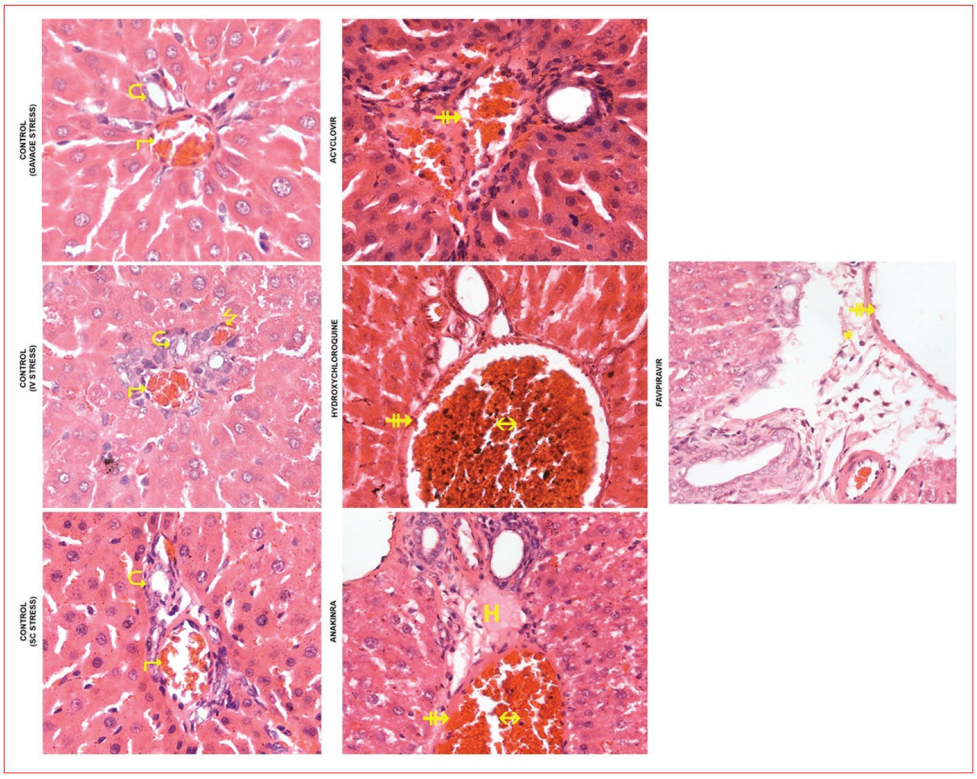


Figure 2. Liver sections (portal triad) for each group showed ⇝: Hepatic artery, ↱: Portal vein, ↳: Bile duct, ⇞: Dilatation in the portal vein, *: fibrosis (hematoxylin-eosin x400).

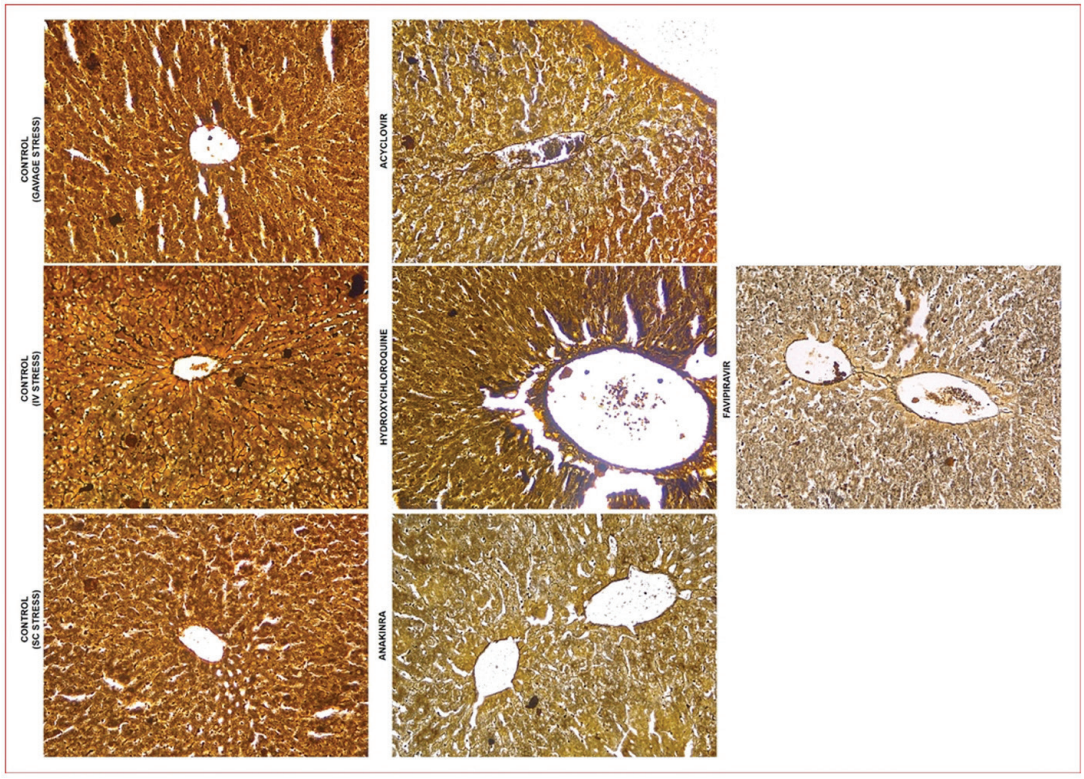


Figure 3. Liver sections for each group showed the reticular fibers, which were anastomosed in the tissue and stained black (silver Impregnation x200).

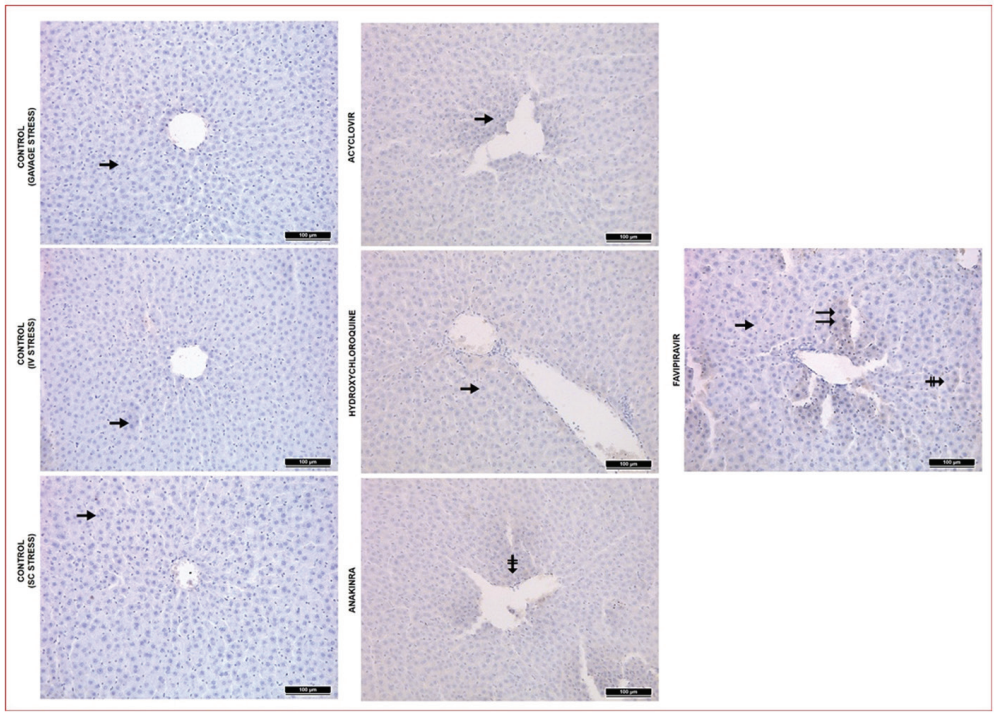


Figure 4. Liver sections of each group for TNF-α immuno-histochemistry staining showed ➙: Weak immuno-reactivity, ‡: Weak to moderate immuno-reactivity, ⇉: Moderate immuno-reactivity, ⇨: Moderate to strong immuno-reactivity, ➡: Strong immunoreactivity (immunperoxidase hematoxylin x200).
TNF-α: Tumour necrosis factor-alpha.

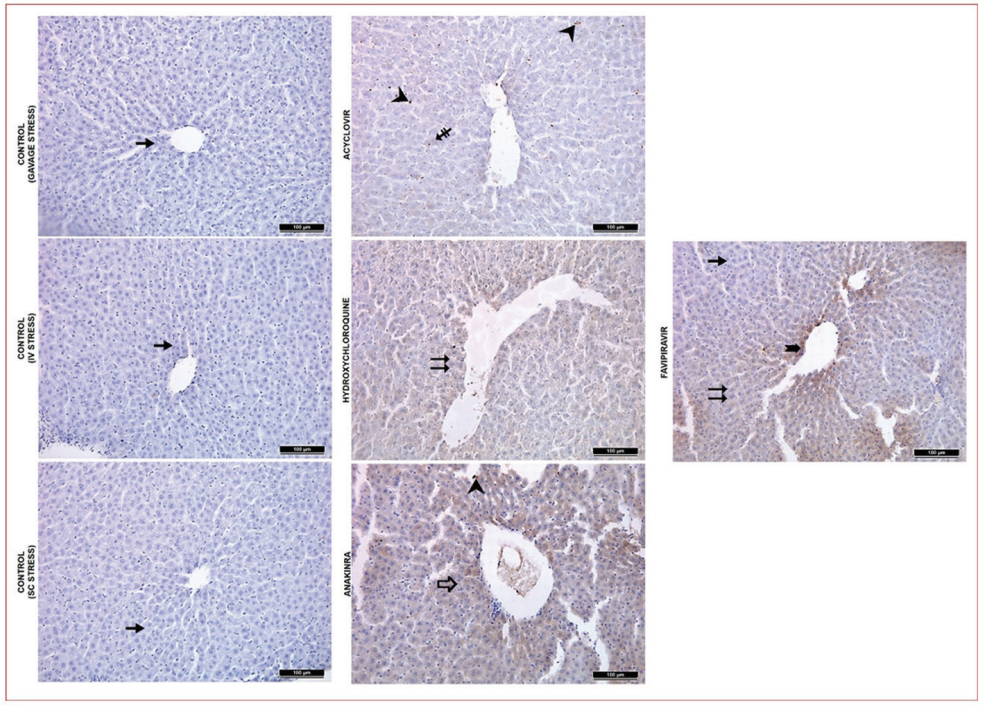


Figure 5. Liver sections of each group for IL-1β immuno-histochemistry staining showed ➙: Weak immuno-reactivity, ‡: Weak to moderate immuno-reactivity, ⇉: Moderate immuno-reactivity, ⇨: Moderate to strong immuno-reactivity, ➡: Strong immuno-reactivity, ➤: Strong immunoreactivity in Kupffer cells (immunperoxidase hematoxylin x200).
IL-1β: Interleukin-1 beta.

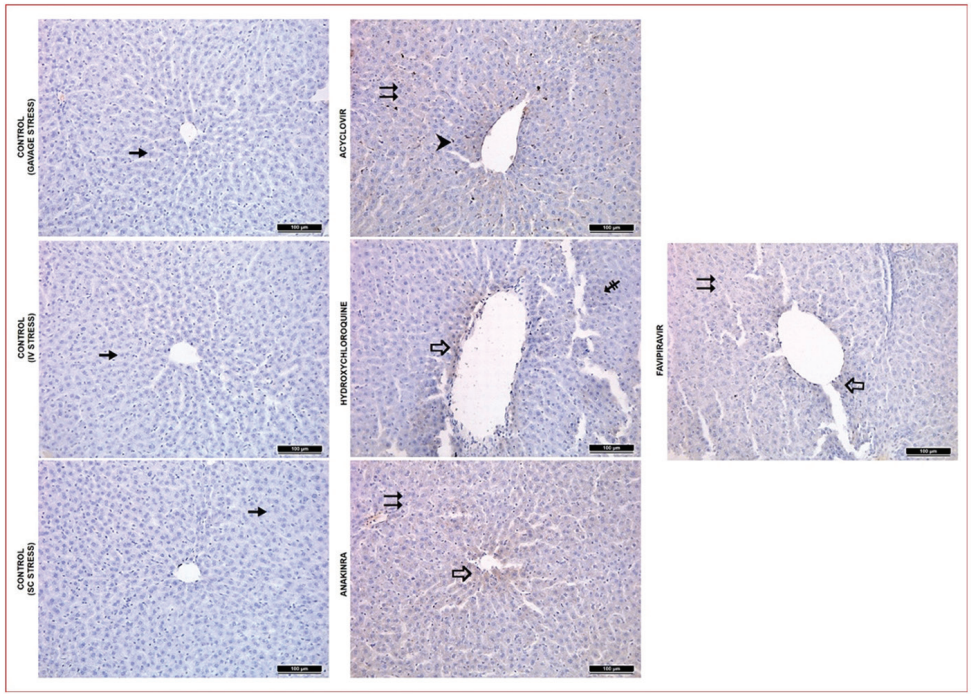


Figure 6. Liver sections of each group for IL-6 immunohistochemistry staining showed ➙: Weak immuno-reactivity, ⇞: Weak to moderate immuno-reactivity, ⇉: Moderate immuno-reactivity, ⇨: Moderate to strong immuno-reactivity, ➠: Strong immuno-reactivity, ➤: Strong immunoreactivity in Kupffer cells (immunperoxidase hematoxylin x200).

IL-6: Interleukin-6.

and new compounds, whose effectiveness in COVID-19 is under examination, have also been demonstrated to have the potential to lead to or worsen liver damage (27).

Drug-induced liver injury in hospitalized patients with SARS-CoV-2 infection was evaluated by Mihai et al. (25), and a significant increase in aminotransferase levels was observed during hospitalization, indicating drug-related hepatotoxicity. Indirect bilirubin increased and hepatocellular damage occurred, particularly ALT measurements were found to be higher than five times the upper limit of normal, and were both associated with the administration of potentially hepatotoxic medications (25). Other viewpoints in the study reviewed by Sodeifian et al. (23) claim that some research findings might indicate a direct role of drugs, whereas others could not. However, many studies found that medication administration may have caused liver damage, according to the same review (23).

Using literature reviews as our guide, we chose the most widely used antiviral drugs for treating COVID-19 patients as acyclovir, hydroxychloroquine, anakinra, and favipiravir in the present study.

If studies on liver damage associated with the administration of these drugs are to be investigated, Taylor et al. (21) indicated that the IL-1 receptor antagonist anakinra caused acute liver failure during the treatment of adult-onset Still's disease. The patient was administered 100 mg anakinra twice daily for 9 days by s.c. during the treatment. High bilirubin and ALT/AST levels were observed after administration (21). Khani et al. (12) used anakinra in COVID-19 treatment and finally reported that more patients had increased liver enzymes and thromboembolic events.

Similar to these studies describing anakinra-induced liver injury, in the current investigation, the anakinra-administered group was identified by vena centralis and sinusoids dilatation, infiltration, vacuolar degeneration, pyknotic nuclei, and karyolysis in the hepatocytes. In this group, the region of the portal triad also showed congestion and hyalinization of the connective tissue.

The first case of favipiravir-induced cholestatic liver injury was reported by Yamazaki et al. (30). The patient received 6000 mg/day of favipiravir on day 1 and 2400 mg/day on days 2-14. After the administrations, they found elevated alkaline phosphatase, ɣ-glutamyl transpeptidase, and total bilirubin levels, which suggested cholestatic liver injury. Finally, they concluded that this could happen when high doses of favipiravir were administered, especially in people with compromised liver function (30).

Kumar et al. (22) reported drug-induced liver injury in patients who used favipiravir for 2 weeks and 12 days during COVID-19 treatment. They evaluated a cholestatic liver biochemistry profile following laboratory results in a patient who used favipiravir for 2 weeks. They also noted moderate hepatocellular cholestasis and inflammation generated by lymphocytes and a few eosinophils in the portal area in the patient's percutaneous liver biopsies. In laboratory tests of another patient, they discovered markedly increased liver enzymes, and they reported favipiravir-induced acute hepatitis in this case (22).

Similar to the biopsy findings reported in this study, we also observed severe hepatocyte degeneration and a rise in connective tissue in the portal triad through our favipiravir-administered group.

Examining the expression of molecules linked to tissue degeneration is unquestionably one of the most important ways to determine the mechanism of liver damage.

The conventional view of hepatocyte cell death during liver injury is that it either occurred by programed cell death (apoptosis) or uncontrolled cell death (necrosis). Multiple cell death processes, such as necroptosis, proptosis, and autophagic cell death, can be induced after acute and chronic liver injury (31). Pyknotic nuclei associated with necrotic alterations were observed in drug-administered groups, and nuclei appearances compatible with karyolysis were differentiated in the anakinra and favipiravir groups according to the present investigation.

The inflammatory process starts with the production of TNF-α from macrophages, which is followed by an increase in IL-1, IL-6, and IL-8 expressions. IL-1β is also produced by macrophages, just like TNF-α. Therefore, all of these cytokines are referred to as proinflammatory cytokines because they are produced early on and initiate the inflammatory response (32-35). In keeping with this knowledge, we found that all three antibodies (TNF-α, IL-1β, and IL-6) increased in the drug-administered groups and that IL-1β was also significantly expressed in liver Kupffer cells in these groups.

TNF-α and IL-1β, the two main cytokines, contribute to the production of IL-6, and all three cytokines are linked to chronic inflammation. TNF-α increases the release of acute phase proteins from the liver together with IL-1 and IL-6 through hepatocytes, in addition to playing a role in the necrosis-induced death of tumors (36-38). The hallmarks of chronic liver disease are hepatocyte loss, inflammation, and liver fibrosis. TNF-α plays an essential role in acute and chronic liver inflammation that leads to liver fibrosis as well as apoptosis and proliferation (35). In the current investigation, drug-administered groups with elevated TNF-α expression, particularly in the anakinra and favipiravir groups, were observed to have an increase in connective tissue associated with fibrosis. We supported this finding using the silver impregnation method.

TNF-α and IL-1β both exert effects on the vascular endothelium, which results in thrombus/coagulation and edema by increasing cell permeability as a result of endothelial disruption. IL-1β alone can cause tissue damage, but the incidence of damage increases with TNF-α (36-38). In particular, in the hydroxychloroquine and anakinra groups, where significant expressions of IL-1β were found in the current investigation, endothelium degeneration surrounding the vena centralis, infiltration, and congestion due to potential endothelial injury in the portal vein were reported.

Yoshigai et al. (39) observed that IL-1β stimulation increased the TNF-α expression in rat hepatocyte cultures. In addition, organ failure and higher mortality rates are linked to TNF-α and IL-6 acting together (36-38). TNF-α, IL-1, and IL-6 suggest mediating hepatic damage in hepatitis through animal models (40,41). Rex et al. (34) reported that the action of hepatocytes during the liver inflammatory response is significantly affected by cytokines produced by macrophages. According to research, lipopolysaccharide stimulation causes the liver to release TNF-α and IL-1β, which then triggers the fas ligand-induced apoptotic pathway (34). The release of proinflammatory cytokines after myocardial infarction contributes to tissue repair and damage adaptation; however, over time, these cytokines cause permanent damage to the heart, according to a study by Turner et al. (42) This study also showed that 0.1-10 ng/mL TNF-α induction increased the mRNA levels of IL-1β and 6 in human cardiac fibroblasts.

Although numerous studies in the literature demonstrate drug-induced liver injury using laboratory tests under COVID-19 conditions, there is no research indicating the histological impact of antiviral drugs used in COVID-19 treatment on the liver. Taken together, liver damage was reported in the treatment of COVID-19 triggered by the drug applied, and among these medications, anakinra and favipiravir, and especially favipiravir, were more closely related to the degeneration according to the present study. It was concluded that TNF-α, IL-1β, and IL-6, but especially IL-1β, play an active role in these degeneration.

CONCLUSION

Consequently, this study represents the first histological study in this field. Our research was carried out on experimental animals because collecting postmortem tissues is problematic both in terms of ethics and time required. However, expanding the research on liver cell culture or liver organoids in vitro will make an essential contribution to the literature.

Ethics Committee Approval: Animal studies were conducted in accordance with ethical standards and were approved by the Institutional Animal Ethics Committee Guidelines of Gazi University (approval number: G.Ü.ET-22.038).

Informed Consent: Patient approval has not been obtained as it is performed on animals.

Author Contributions

Concept: C.M.S., G.T.K., Design: C.M.S., G.T.K., Data Collection or Processing: A.A.E.S., C.M.S., Analysis or Interpretation: A.A.E.S., C.M.S., G.T.K., Literature Search: A.A.E.S., C.M.S., Writing: C.M.S., Critical Review: C.M.S., G.T.K.

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

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

REFERENCES

1. Dawood DRM, Salum GM, El-Meguid MA. The Impact of COVID-19 on Liver Injury. Am J Med Sci. 2022; 363: 94-103.
2. D'Ardes D, Boccatonda A, Cocco G, Fabiani S, Rossi I, Bucci M, et al. Impaired coagulation, liver dysfunction and COVID-19: Discovering an intriguing relationship. World J Gastroenterol. 2022; 28: 1102-12.
3. World Health Organization. WHO Coronavirus (COVID-19) Dashboard-2023 [cited 11 April 2023]. Available from: https://www.covid19.who.int
4. Du M, Yang S, Liu M, Liu J. COVID-19 and liver dysfunction: Epidemiology, association and potential mechanisms. Clin Res Hepatol Gastroenterol. 2022; 46: 101793.
5. Seymen CM. The other side of COVID-19 pandemic: Effects on male fertility. J Med Virol. 2021; 93: 1396-1402.

6. Beheshtirouy S, Khani E, Khiali S, Entezari-Maleki T. Investigational antiviral drugs for the treatment of COVID-19 patients. Archives of Virology. 2022; 167: 751-805.
7. Rudrapal M, Khairnar SJ, Jadhav AG. Drug Repurposing (DR): An emerging approach in drug discovery in Badria AF (ed). Drug repurposing - hypothesis, molecular aspects and therapeutic applications. IntechOpen. 2020.
8. Heidary F, Madani S, Gharebahgi R, Asadi-Amoli F. Acyclovir as a potential add-on therapy in COVID-19 treatment regimens. Pharmaceutical Sciences. 2021; 27: 68-77.
9. Baker VS. Acyclovir for SARS-CoV-2: An old drug with a new purpose. Clinical Practice. 2021; 18: 1584-92.
10. Keating MR. Antiviral agents for non-human immunodeficiency virüs infections. Mayo Clin Proc 1999; 74: 1266-1283
11. King A, Vail A, O'Leary C, Hannan C, Brough D, Patel H, et al. Anakinra in COVID-19: important considerations for clinical trials. Lancet Rheumatol. 2020; 2: e379-81.
12. Khani E, Shahrabi M, Rezaei H, Pourkarim F, Afsharirad H, Solduzian M. Current evidence on the use of anakinra in COVID-19. Int Immunopharmacol. 2022; 111: 109075.
13. Kyriakoulis KG, Kollias A, Poulakou G, Kyriakoulis IG, Trontzas IP, Charpidou A, et al. The Effect of Anakinra in Hospitalized Patients with COVID-19: An Updated Systematic Review and Meta-Analysis. J Clin Med. 2021; 10: 4462.
14. RECOVERY Collaborative Group; Horby P, Mafham M, Linsell L, Bell JL, Staplin N, Emberson JR, et al. Effect of Hydroxychloroquine in Hospitalized Patients with Covid-19. N Engl J Med. 2020; 383: 2030-40.
15. Meyerowitz EA, Vannier AGL, Friesen MGN, Schoenfeld S, Gelfand JA, Callahan MV, et al. Rethinking the role of hydroxychloroquine in the treatment of COVID-19. FASEB J. 2020; 34: 6027-37.
16. Nina PB, Dash AP. Hydroxychloroquine as prophylaxis or treatment for COVID-19: What does the evidence say? Indian J Public Health. 2020; 64: S125-7.
17. Ibáñez S, Martínez O, Valenzuela F, Silva F, Valenzuela O. Hydroxychloroquine and chloroquine in COVID-19: should they be used as standard therapy? Clin Rheumatol. 2020; 39: 2461-5.
18. Agrawal U, Raju R, Udwadia ZF. Favipiravir: A new and emerging antiviral option in COVID-19. Med J Armed Forces India. 2020; 76: 370-6.
19. Bosaeed M, Alharbi A, Mahmoud E, Alrehily S, Bahlaq M, Gaifer Z, et al. Efficacy of favipiravir in adults with mild COVID-19: a randomized, double-blind, multicentre, placebo-controlled clinical trial. Clin Microbiol Infect. 2022; 28: 602-8.
20. Hassanipour S, Arab-Zozani M, Amani B, Heidarzad F, Fathalipour M, Martinez-de-Hoyo R. The efficacy and safety of Favipiravir in treatment of COVID-19: a systematic review and meta-analysis of clinical trials. Sci Rep. 2021; 11: 11022.
21. Taylor SA, Vittorio JM, Martinez M, Fester KA, Lagana SM, Lobritto SJ, et al. Anakinra-Induced Acute Liver Failure in an Adolescent Patient with Still's Disease. Pharmacotherapy. 2016; 36: e1-4.
22. Kumar P, Kulkarni A, Sharma M, Rao PN, Reddy DN. Favipiravir-induced Liver Injury in Patients with Coronavirus Disease 2019. J Clin Transl Hepatol. 2021; 9: 276-8.
23. Sodeifian F, Seyedalhosseini ZS, Kian N, Eftekhari M, Najari S, Mirsaeidi M, et al. Drug-Induced Liver Injury in COVID-19 Patients: A Systematic Review. Front Med (Lausanne). 2021; 8: 731436.
24. Li X, Wang W, Yan S, Zhao W, Xiong H, Bao C, et al. Drug-induced liver injury in COVID-19 treatment: Incidence, mechanisms and clinical management. Front Pharmacol. 2022; 13: 1019487.
25. Mihai N, Tiliscan C, Visan CA, Stratan L, Ganea O, Arama SS, et al. Evaluation of Drug-Induced Liver Injury in Hospitalized Patients with SARS-CoV-2 Infection. Microorganisms. 2022; 10: 2045.
26. Seymen CM, Yar Sağlam AS, Elmazoğlu Z, Arık GN, Take Kaplanoğlu G. Involvement of endometrial IGF-1R/IGF-1/Bcl-2 pathways in experimental polycystic ovary syndrome: Identification of the regulatory effect of melatonin. Tissue Cell. 2021; 73: 101585.
27. Vitrone M, Mele F, Durante-Mangoni E, Zampino R. Drugs and liver injury: a not to be overlooked binomial in COVID-19. J Chemother. 2022; 34: 207-20.
28. Chen N, Zhou M, Dong X, Qu J, Gong F, Han Y, et al. Epidemiological and clinical characteristics of 99 cases of 2019 novel coronavirus pneumonia in Wuhan, China: a descriptive study. Lancet. 2020; 395: 507-13.
29. Hu WS, Jiang FY, Shu W, Zhao R, Cao JM, Wang DP. Liver injury in COVID-19: A minireview. World J Gastroenterol. 2022; 28: 6716-31.
30. Yamazaki S, Suzuki T, Sayama M, Nakada TA, Igari H, Ishii I. Suspected cholestatic liver injury induced by favipiravir in a patient with COVID-19. J Infect Chemother. 2021; 27: 390-2.
31. Eguchi A, Wree A, Feldstein AE. Biomarkers of liver cell death. J Hepatol. 2014; 60: 1063-74.
32. Cao Q, Batey R, Pang G, Russell A, Clancy R. IL-6, IFN-gamma and TNF-alpha production by liver-associated T cells and acute liver injury in rats administered concanavalin A. Immunol Cell Biol. 1998; 76: 542-9.
33. De Cesaris P, Starace D, Riccioli A, Padula F, Filippini A, Ziparo E. Tumor necrosis factor-alpha induces interleukin-6 production and integrin ligand expression by distinct transduction pathways. J Biol Chem. 1998; 273: 7566-71.
34. Rex J, Lutz A, Faletti LE, Albrecht U, Thomas M, Bode JG, et al. IL-1β and TNFα Differentially Influence NF-κB Activity and FasL-Induced Apoptosis in Primary Murine Hepatocytes During LPS-Induced Inflammation. Front Physiol. 2019; 10: 117.
35. Yang YM, Seki E. TNFα in liver fibrosis. Curr Pathobiol Rep. 2015; 3: 253-261.
36. Galley HF, Webster NR. The immuno-inflammatory cascade. British Journal of Anaesthesia. 1996; 77: 11-16.
37. Kudo O, Fujikawa Y, Itonaga I, Sabokbar A, Torisu T, Athanasou NA. Proinflammatory cytokine (TNFalpha/IL-1alpha) induction of human osteoclast formation. J Pathol. 2002; 198: 220-7.
38. Leyva-López N, Gutierrez-Grijalva EP, Ambriz-Perez DL, Heredia JB. Flavonoids as Cytokine Modulators: A Possible Therapy for Inflammation-Related Diseases. Int J Mol Sci. 2016; 17: 921.
39. Yoshigai E, Hara T, Inaba H, Hashimoto I, Tanaka Y, Kaibori M, et al. Interleukin-1β induces tumor necrosis factor-α secretion from rat hepatocytes. Hepatol Res. 2014; 44: 571-83.
40. Pennington HL, Hall PM, Wilce PA, Worrall S. Ethanol feeding enhances inflammatory cytokine expression in lipopolysaccharide-induced hepatitis. J Gastroenterol Hepatol. 1997; 12: 305-13.
41. Batey R, Cao Q, Madsen G, Pang G, Russell A, Clancy R. Decreased tumor necrosis factor-alpha and interleukin-1alpha production from intrahepatic mononuclear cells in chronic ethanol consumption and upregulation by endotoxin. Alcohol Clin Exp Res. 1998; 22: 150-6.
42. Turner NA, Mughal RS, Warburton P, O'Regan DJ, Ball SG, Porter KE. Mechanism of TNFalpha-induced IL-1alpha, IL-1beta and IL-6 expression in human cardiac fibroblasts: effects of statins and thiazolidinediones. Cardiovasc Res. 2007; 76: 81-90.

Original Investigation | Özgün Araştırma

GMJ 2024;35:325-331

DOI: http://dx.doi.org/10.12996/gmj.2024.4075

Cytochrome P450 *2J2*7* Single-nucleotide Polymorphism and Nocturnal Hypertension in an Elderly Turkish Population

Yaşlı Türk Popülasyonunda Sitokrom P450 *2J2*7* Tek Nükleotid Polimorfizmi ve Gece Hipertansiyonu

Elif Hilal Vural[1], Arzu Güneş Granberg[2], Atiye Çengel[3] , Marja-Liisa Dahl[4], Hakan Zengil[5]

[1]Department of Medical Pharmacology, Lokman Hekim University Faculty of Medicine, Ankara, Türkiye
[2]Novartis Institute of Biomedical Research, Basel, Switzerland
[3]Department of Cardiology (Retired), Gazi University Faculty of Medicine, Ankara, Türkiye
[4]Department of Laboratory Medicine, Division of Clinical Pharmacology, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden
[5]Department of Medical Pharmacology (Retired), Gazi University Faculty of Medicine, Ankara, Türkiye

ABSTRACT

Objective: Blood pressure shows a physiological diurnal rhythm. Altered blood pressure circadian rhythm is more common in the elderly. Nocturnal hypertension (NH) and/or impaired nocturnal dipping profile are associated with increased mortality risk. The *CYP2J2*7* polymorphism is associated with decreased arachidonic acid metabolite levels and increased cardiovascular mortality risk in different populations. The aim of this study was to investigate whether NH was associated with *the CYP2J2*7* polymorphism in an elderly Turkish population.

Methods: Ambulatory blood pressure data were obtained from 120 elderly (78 women and 42 men, aged 60-105 years) volunteers during 24 h. The *CYP2J2*7 (G/T)* polymorphism was evaluated using a Taqman® Drug Metabolism Genotyping Assay kit and a TAQMAN ABI7900 device.

Results: The *CYP2J2*7 T*-allele frequency was 6.7% in our study population. The 24 h, day, and night systolic blood pressures were found to be 2-5 mmHg higher in *CYP2J2*7 T*-carriers (n=15) than in individuals with *the GG* genotype (n=105), however, the differences between the genotype groups were not statistically significant. The systolic blood pressure morning increase slope values of *the CYP2J2*7* carriers and *GG* genotype group were 8.6±2.0 (mean ± standard error of mean) and 5.7±0.5, respectively, i.e., 50.7% higher in *T*-carriers (p=0.039). There was no statistically significant difference in the frequency of NH with the *CYP2J2*7* polymorphism.

Conclusion: Considering the existing literature with our findings, the *CYP2J2*7* carrier status may indicate an increased risk of cardiovascular events. However, further studies are required to verify the significance of this finding.

Keywords: Nocturnal hypertension, *CYP2J2*7*, cytochrome

ÖZ

Amaç: Kan basıncı fizyolojik günlük bir ritim gösterir. Kan basıncının sirkadiyen ritminin değişmesi yaşlılarda daha sık görülür. Gece hipertansiyonu ve/veya bozulmuş gece düşüş profili, artan ölüm riskiyle ilişkilidir. *CYP2J2*7* polimorfizmi, farklı popülasyonlarda araşidonik asit metabolit seviyelerinin azalması ve kardiyovasküler mortalite riskinin artmasıyla ilişkilidir. Bu çalışmanın amacı yaşlı Türk toplumunda gece hipertansiyonunun *CYP2J2*7* polimorfizmi ile ilişkili olup olmadığını araştırmaktır.

Yöntemler: Ambulatuvar kan basıncı verileri 120 yaşlı (78 kadın ve 42 erkek, 60-105 yaş arası) gönüllüden 24 saat boyunca elde edilmiştir. *CYP2J2*7* (*G/T*) polimorfizmi, Taqman® İlaç Metabolizması Genotipleme Test kiti ve TAQMAN ABI7900 cihazı kullanılarak değerlendirilmiştir.

Bulgular: Çalışma popülasyonumuzda *CYP2J2*7 T*-allel frekansı %6,7 idi. *CYP2J2*7 T*-alleli taşıyıcılarında (n=15), *GG* genotipli bireylere (n=105) göre 24 saatlik, gündüz ve gece sistolik kan basınçları 2-5 mmHg daha yüksek bulunmuştur, ancak genotip grupları arasındaki fark istatistiksel olarak anlamlı değildi. *CYP2J2*7* taşıyıcıları ve *GG* genotip grubunun sistolik kan basıncı sabah artış eğimi değerleri sırasıyla 8,6±2,0 (ortalama ± ortalama standart hatası) ve 5,7±0,5 idi, yani *T*-alleli taşıyıcılarda %50,7 daha yüksekti (p=0,039). *CYP2J2*7* polimorfizmi ile gece hipertansiyon sıklığı arasında istatistiksel olarak anlamlı bir fark yoktu.

Sonuç: Bulgularımız ile mevcut literatür göz önüne alındığında, CYP2J2*7 taşıyıcılığı artmış kardiyovasküler olay riskine işaret edebilir. Ancak bu bulgunun önemini doğrulamak için daha ileri çalışmalara ihtiyaç vardır.

Anahtar Sözcükler: Gece hipertansiyonu, *CYP2J2*7*, sitokrom

Address for Correspondence/Yazışma Adresi: Elif Hilal Vural, MD, Department of Medical Pharmacology, Lokman Hekim University Faculty of Medicine, Ankara, Türkiye
E-mail / E-posta: elif.vural@lokmanhekim.edu.tr
ORCID ID: orcid.org/0000-0003-1309-6606

Received/Geliş Tarihi: 15.12.2023
Accepted/Kabul Tarihi: 14.03.2024

INTRODUCTION

Hypertension is a multifactorial and complex disease. Environmental factors and many candidate genes have been investigated to explore the etiology of hypertension, including angiotensinogen, angiotensin-converting enzyme, angiotensin II receptor types I and II, renin, renin-binding protein, and aldosterone synthase, in various ethnic populations (1). The physiological blood pressure pattern is time dependent, with an increase in the morning (activity) time and a decrease in the sleeping (rest) time (2). For both normotensive and hypertensive individuals, sleep time average blood pressure values are generally lower than their daytime blood pressure, by approximately 10-20% (3). Circadian rhythm abnormalities are associated with several cardiac and renovascular diseases (2). Various studies have reported that clock gene mutations, including *Bmal1, Clock, Per1/2/3,* and *Cry1/2,* are frequently expressed in individuals with altered circadian blood pressure profiles and impaired vascular hemodynamics, leading to myocardial infarction and stroke (4). Nighttime blood pressure change patterns vary among individuals who can be defined as dippers, non-dippers, super dippers, or rising (reverse dippers) (3,5,6). The results of the Ohasama study have shown that the circadian patterns of blood pressure without reduction during the nighttime, reversing dipping and non-dipping, are associated with increased mortality risk (7). Nocturnal hypertension (NH) is defined as high blood pressure at night (5). It has been shown that nighttime blood pressure reduction decreases with age (8). As reviewed by Tadic et al. (9), the prevalence of NH is influenced by demographic, clinical, and ethnic factors. The prevalence of NH has been reported as 10-60%, varying in different studies (10-12). Several studies have shown a relationship between cardiovascular mortality or morbidity and NH (9).

Among several other factors, arachidonic acid metabolites and monooxygenase play an important role in the etiopathogenesis of hypertension (13). Several studies have shown that 20-hydroxyeicosatetraenoic acid and epoxyeicosatrienoic acid (EETs) metabolites have an impact on the regulation of vascular tone and pathogenesis of hypertension (13). Because cytochrome P450 (CYP) enzymes are involved in the metabolism of arachidonic acids, variations in the CYP2C*, 2J, 4A,* and *4F* gene families have been studied for their relationship with blood pressure regulation (14). Wu et al. (15) showed that the CYP2J2 enzyme, which metabolizes arachidonic acid into EET, is highly expressed in the human heart. Furthermore, CYP2J2 mRNA levels have been found to be higher than CYP2C8 and CYP2C9 in human heart, whereas CYP2C9 mRNA levels were higher than CYP2J2 mRNA levels in human aorta, coronary artery, and especially in ischemic heart (16). These data was supported by the fact that CYP2J2 mRNA levels were the most expressed CYP450 isoform in human left heart ventricular samples (17). The CYP2C8, CYP2C9, and CYP2C19 enzymes have been shown to play substantial roles in the epoxidation of arachidonic acid and production of EETs (18,19). Node et al. (20) reported that CYP2J2 also catalyzes the formation of EETs, which have an anti-inflammatory effect on the bovine aortic vascular wall. CYP2J2 overexpression also increases tissue plasminogen activator (t-PA) expression in addition to having a vasodilator effect on vascular endothelial cells (21).

Of the several genetic variants of CYP2J2, the *CYP2J2*7* polymorphism is located 76 nucleotides upstream of the first nucleotide of the translation start codon and 50 nucleotides upstream of the transcription start site (22). This G-50T mutation leads to decreased arachidonic acid metabolism and production of 14,15-dihydroxyeicosatrienoic acid (23).

In addition to essential hypertension, the *CYP 2J2*7* polymorphism (24,25) has also been reported to be associated with an increased risk of myocardial infarction in Taiwanese (26).

The frequency of the *CYP2J2*7 T*-allele has been reported to vary between 2.1% and 17% in different ethnicities (22) 14.1% in African Americans, 7.7% in Caucasians (24) and 2.6% in Chinese (27).

As mentioned before, many studies have investigated the risk of organ damage in the presence of NH or have attempted to find the predictive role of gene mutations in this disease across different ethnic groups (4,9).

Because aging is a risk factor for NH and the role of cytochrome P450 enzyme genetic mutations in the regulation of arachidonic acid metabolism, regulation of blood pressure, and organ damage risk, the aim of our study was to investigate whether the *CYP2J2*7* polymorphism is associated with NH in an elderly Turkish population.

MATERIALS AND METHODS

The study was conducted with the permission of the Local Ethics Committee of Gazi University Faculty of Medicine (approval number: 193, date: 14.06.2006; no: 413/26.11.2007). A total of 120 elderly subjects (78 women and 42 men, aged 60-105 years) from two different nursing homes in Ankara. participated in the study on a voluntary basis. Subjects diagnosed with secondary hypertension or terminal cancer and those who failed to cooperate with study requirements were excluded. All volunteers signed an informed consent form before their participation.

Ambulatory blood pressure monitors (ABPM) were applied throughout the 24 h (Spacelabs 90207 model, Spacelabs Limited, Redmond, Washington, USA). Monitors were attached to the upper arm of the inactive volunteers. All monitors were set to measure blood pressure every 20 min between 06:00 and 24:00 and every 30 min between 24:00 and 6:00. During monitoring, the volunteers continued their daily activities and sleeping habits. The measurement limits of the monitors were arranged in such a way that in cases where systolic blood pressure was ≤70 mmHg or ≥220 mmHg, and diastolic blood pressure ≤40 mmHg or ≥150 mmHg, the measured value was considered as an incorrect reading and was automatically excluded from examination by the program. The records were accepted if at least 75% of the monitor measurements were successful during the 24 h examination period and if there was at least one successful reading per hour.

Monitoring records were analyzed using the ABPM-FIT (University of Heidelberg, Germany, version 2.2) and CV-SORT programs. Nocturnal and daytime blood pressures were calculated considering each volunteer's bedtime (resting period) and wake-up time.

Genetic Analysis

A 5 mL whole blood sample was collected and maintained at -20 °C until isolation of DNA. Genomic DNA was isolated from peripheric leucocytes using a QIAMP DNA Kit (Qiagen, Hilden, Germany) according to manufacturer's guidance and analyzed for

the *CYP2J2*7* (-76G>T) *(rs890293)* single nucleotide polymorphism (SNP) using a Taqman® Drug Metabolism Genotyping Assay kit (C_9581699_80) (Applied Biosystems, Foster City, CA, USA) and a TAQMAN ABI7900 (Applied Biosystems, Foster City, CA, USA) device. Taqman universal mixture, allelic discrimination mix, bovine serum albumin, and autoclaved distilled water were used to prepare a final reaction volume to 9 µl Master mix for each sample according to kit guidelines. Thermal Cycling conditions were applied as 50 °C, 2 min; 95 °C, 10 min and 45 cycles; 92 °C, 15 sec. and 60 °C, 1 min for the PCR reaction.

Data Analysis

Based on the analysis of the ABPM records, subjects were classified as NH or nocturnal normotensive (NN) based on the systolic blood pressure average ≥125 mmHg or below 125 mmHg, respectively, during resting period, according to the ABPM records. The subjects were also classified as dippers, non-dippers, or reverse dippers. Subjects with a nocturnal mean systolic blood pressure decrease of 10-20% compared with daytime levels were classified as Dippers, those with a decrease of less than 10% as non-dippers, and those with a mean systolic blood pressure increase during the night period compared with the daytime period were classified as reverse dippers.

Systolic and diastolic blood pressure morning increase slopes and night decrease slopes were also calculated on the basis of the ABPM data using mean blood pressure readings of each subject 3 h before and after wake-up time and 3 h before and after bedtime, respectively.

On the basis of the ABPM records, each subject's blood pressure load was also recorded with ABPM monitors. The load value is the percentage of the number of measurements over the limits in each period (28). The accepted limits were 140 mmHg (24 h period), 140 mmHg (daytime), and 125 mmHg (night time for systolic blood pressure).

Statistical Analysis

Statistical analysis was performed using Sigma Stat 3.1. Results are presented as mean ± standard error of mean. Student's t-test/Mann-Whitney U test were used for comparisons of the characteristics between the NH and normotensive subjects. The genotype frequencies of the groups were evaluated using Fisher's exact/chi-square test. Fisher's exact chi-square test was also used for categorical features. Statistical significance was set with p-value <0.05.

RESULTS

Of the 120 subjects included in the study, 47% (n=56) had NH (≥125/80 mmHg). The main characteristics of the volunteers are shown in Table 1. A total of 12% of the participants had diabetes mellitus. The majority of the participants, 37.5% (n=45) were on prescription for antihypertensive medication/medications. In addition, 19.6% (n=11) of NH and 14% (n=9) of NNs used more than one antihypertensive medication. Antihypertensive medications included beta blockers, calcium channel blockers, angiotensin II receptor antagonists, angiotensin receptor antagonist and diuretic combinations, angiotensin converting enzyme inhibitors (ACEI) and

Table 1. Main characteristics (n=120)

	Nocturnal hypertensive SBP ≥125 mmHg, (n=56)	Nocturnal normotensive SBP <125 mmHg, (n=64)	p
Age	82.3±1.0	79.7±1.3	**p>0.05**
Women/men	38/18	40/24	**p>0.05***
Body mass index	26.27	26.71	**p>0.05**
Systolic blood pressure (mmHg)			
24 h	137.1±1.4	118.3±1.3	**p<0.001**
Day	136.2±1.5	121.8±11.4	**p<0.001**
Night	137.8±1.5	111.1±1.9	**p<0.001**
Night load (%)	74.2±3.3	12.5±1.8	**p<0.001**
Morning blood pressure increases slope	4.9±0.6	7.1±0.7	**p<0.05**
Night blood pressure decrease slope	-5.7±0.8	-7.0±0.6	**p<0.05****
Diastolic blood pressure (mmHg)			
24 h	72.8±1.1	67.3±0.9	**p<0.001**
Day	73.5±1.2	69.8±1.0	**p<0.05**
Night	70.8±1.3	61.4±0.9	**p<0.001**
Night load (%)	22.7±3.6	4.6±1.0	**p<0.001**
Heart rate			
24 h	70.8±1.4	72.3±1.2	**p>0.05**
Day	72.9±1.5	75.0±1.3	**p>0.05**
Night	67.6±1.5	67.6±1.1	**p>0.05**

*Chi-square test, **Mann-Whitney U test, Student's t-test was used for other parameters, all results presented as mean ± standard error of mean. SNP: Single nucleotide polymorphism.

diuretics combinations, and ACEIs and alpha blockers in order of frequency.

*CYP2J2*7* SNP Allele and Genotype Frequency

Only one of the 120 participants was homozygous for the *CYP2J2*7 T*-allele, whereas 14 participants were heterozygous (G/T) and the rest (n=105) had the wild-type *GG* genotype. Homozygous and heterozygous T variant carriers were combined for statistical evaluation. The *T*-allele frequency was 6.7% [confidence interval (CI) 95%=0.4-0.8].

Allele frequencies and genotype distributions in the NH participants and those without NH groups are shown in Table 2.

24 h Blood Pressure Data in *CYP2J2*7* Genotype Groups

In this study, neither *CYP2J2*7* genotypes nor *T*-allele frequencies differed between the NH and NN groups as evaluated with Fisher's exact chi-square test [odds ratio (OR)=0.7; CI 95%=0.2-2.2, (p=0.8); (OR=0.7; CI %95=0.2-1.9), respectively].

The 24 h, day, and night systolic blood pressures were numerically 2-5 mmHg higher in *CYP2J2*7 T* carriers than in the *GG* genotype group (n=105) (Figure 1); however, the differences were not statistically significant (p>0.05).

Table 2. *CYP2J2*7* SNP allele and genotype frequency

	Nocturnal		**Nocturnal normotensive**		**p-value**
Genotype	**n**	**%**	**n**	**%**	
GG	50	89.3	55	85.9	**0.8**
GT	6	10.7	8	12.5	
TT	0	0	1	1.6	
	Nocturnal hypertensive group		**Without nocturnal hypertensive group**		
Allele	**n (%)**		**n (%)**		**Total n (%)**
G	106 (44)		118 (49)		224 (93.3)
T	6 (2.5)		10 (4.1)		16 (6.6)
			Total		240

*Percentages are presented as column percentages, SNP: Single nucleotide polymorphism.

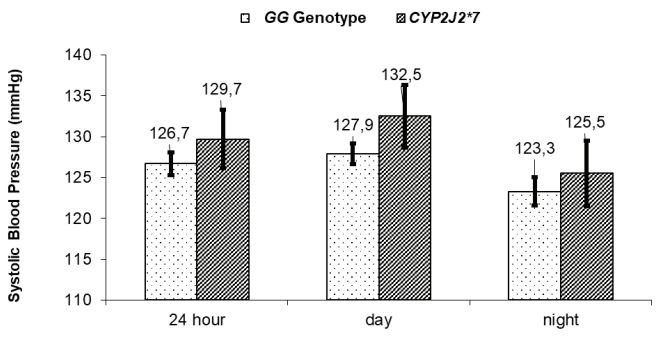


Figure 1. 24 h, day, and night systolic blood pressure of *CYP2J2*7* SNP carriers (n=15) and *GG* genotype (n=105) groups in the overall study population.

SNP: Single nucleotide polymorphism.

The systolic blood pressure morning increase slope values of *CYP2J2*7* carriers and the *GG* genotype were 8.6±2.0 and 5.7±0.5, respectively, i.e., 50.7% higher in *T*-carriers p=0.039 (Figure 2). However, the difference in systolic blood pressure night decrease slopes was not statistically significant between the groups (p>0.05).

Although the mean systolic blood pressure of NH *CYP2J2*7* carriers was 4-5 mmHg higher than that of NH *GG* genotype volunteers (Figure 3), this result was not statistically significant (p>0.05).

The systolic blood pressure load values of *CYP2J2*7* carriers tended to be higher than those in the *GG* group for 24 h, day and night, but again the difference did not reach statistical significance (p>0.05) (Figure 4).

No differences in diastolic blood pressure evaluations were found between *CYP2J2*7* carriers and the *GG* genotype group concerning morning increase, nighttime decrease, or heart rate (Table 3).

Dipping Patterns and *CYP2J2*7* Genotype

Of all participants, 27.5% were dippers, 52.5% were non-dippers, and 20 % were reverse dippers. No difference was observed between

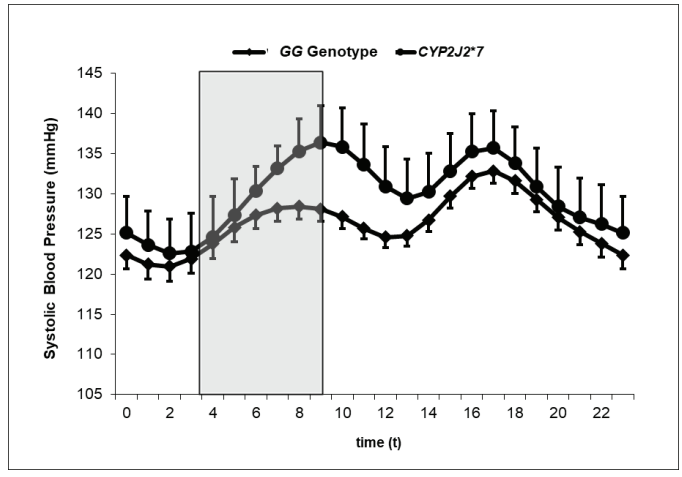


Figure 2. 24 h profile of systolic blood pressure of *CYP2J2*7* SNP carriers (n=15) and *GG* Genotype (n=105) subjects in the overall study population.

**Shaded area covers the 3 h before and after wake-up time of volunteers, SNP: Single nucleotide polymorphism.*

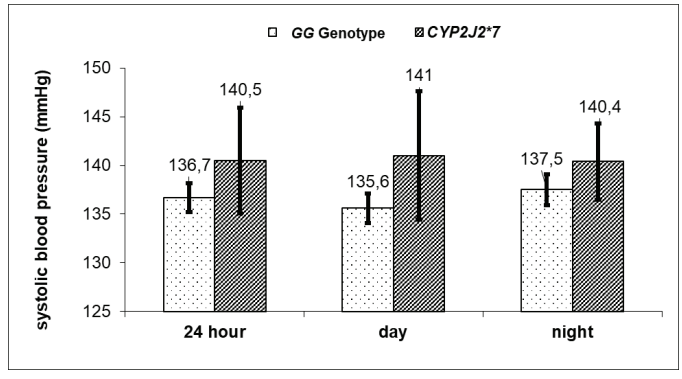


Figure 3. 24 h, day, and night systolic blood pressure values of "nocturnal hypertensive" *CYP2J2*7* SNP carriers (n=6) and *GG* genotype (n=50) groups.

SNP: Single nucleotide polymorphism.

the groups in terms of the frequency of those with and without polymorphism according to night-time blood pressure fall patterns (p>0.05). Although only the *CYP2J2*7* homozygote participant had a non-dipping profile, the distribution of the dipping patterns did not show a statistically significant difference between *CYP2J2*7* carriers and the *GG* genotype (Table 4).

DISCUSSION

In this study, there was a statistically significant association between morning slope and *CYP2J2*7* mutation carrier status. Several earlier studies have shown that sudden death and ventricular arrhythmias tend to occur in the morning time (29) and this has been explained by the impaired vascular endothelial function and increased prothrombotic activity observed in the morning hours, leading to an increased risk of cardiovascular events (30). In our study population, the morning systolic blood pressure increase slope of *CYP2J2*7 T*-carriers (n=15) was significantly higher than that in the group of *GG* subjects (p-value=0.039). Morning blood pressure surge is a substantial risk factor for target organ damage and a known trigger for cardiovascular events (31). Previously, the *CYP2J2*7* polymorphism was reported to be associated with premature myocardial infarction in Taiwanese patients in addition to smoking-related risk factors (26). It has been stated that more studies are needed regarding the clinical significance of morning blood pressure surge and even the clinical effectiveness of divided or timed doses of long-acting drugs in blood pressure control (32). Based on our findings, the carrier status for *CYP2J2*7* can be a predictor of increased morning systolic blood pressure slope and related risk of cardiovascular events. However, this aspect needs to be supported with long-term follow-up studies evaluating cardiovascular disease risk in association with the *CYP2J2* genotype.

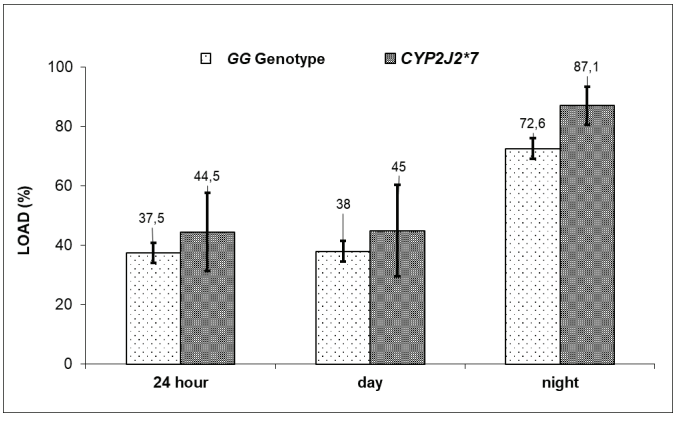


Figure 4. 24 h, day, and night systolic load values of *CYP2J2*7* SNP carriers (n=15) and *GG* genotype (n=105).

SNP: Single nucleotide polymorphism.

Table 3. Diastolic blood pressure values in relation to *CYP2J2*7* genotypes in the study population (n=120)

	***GG*, (n=105)**	***GT* or *TT*, (n=15)**	**p-value**
24 h	69.6±0.8	71.6±2.2	>0.05
Day	71.2±0.8	73.7±2.5	>0.05
Night	65.6±1.0	66.8±2.0	>0.05
Morning blood pressure increases slope	4.5±0.3	6.0±1.7	>0.05
Night blood pressure decrease slope	-4.9±0.4	-4.4±0.8	>0.05

The results of the Ohasama study in a population of Japanese subjects aged 40 years or older with approximately 10 years of follow-up of ambulatory blood pressure have shown that lower night-time mean blood pressure values predicted lower cerebrovascular and cardiovascular mortality rates (33). Moreover, the Dublin outcome study further supported that high values of nighttime blood pressure measurements affect mortality risk (34). Previously, a statistically significant association between *CYP2J2*7* polymorphism and hypertension has been reported in African-Americans, Caucasians, and Arabic people, respectively (24,35). Additionally, the association of *CYP2J2* polymorphism and essential hypertension has been reported in the Chinese Han population (36).

Based on the evidence in the literature concerning the association between hypertension and the *CYP2J2* genotype, we investigated ambulatory blood pressure data and the distribution of *CYP2J2*7* in a group of elderly subjects in two residential institutes in Ankara, Türkiye. We found no significant association between NH and either the *CYP2J2*7* genotype or *T*-allele frequency (Table 2). Although the 24 h, day, and night systolic blood pressure average were 2-5 mmHg higher in *CYP2J2*7* carriers (one homozygote and 14 heterozygote) than in *GG* genotype subjects (n=105) (Figure 1), the difference was not statistically significant (p>0.05). Similarly, no difference in diastolic blood pressure or heart rate was observed between *CYP2J7* genotype groups. This is most probably due to the major limitation of our study, namely, the size of the study population.

The CYP2J2 protein is highly expressed in vascular tissue and the heart and is involved in fatty acid metabolism, regulating vascular tone, inflammation, and cellular proliferation and angiogenesis (37). Spiecker et al. (23) reported that reduced basal CYP2J2 enzyme activity in turn might be related to coronary artery disease risk. In their study in healthy Caucasians, the allele frequency of *CYP2J2*7* was 10.6%. While King et al. (24) reported a higher frequency in African-American hypertensives (14.1%) than in Caucasians (7.7%), in a healthy Chinese volunteer population, an allele frequency

Table 4. Frequency of the *CYP 2J2*7* carrier status and dipping profile

	Frequency of dippers % (n)	**Frequency of non-dippers % (n)**	**Frequency of reverse dippers % (n)**
G/G	28 (29)	51 (54)	21 (22)
T carriers (*G/T* and *T/T*)	27 (4)	60 (9)	13 (2)
Total	28 (33)	53 (63)	20 (24)

Frequencies are defined as line percentages.

of 2.6% was reported (27). Li et al. (38) reported that the Chinese Uyghur healthy population *CYP2J2*7* allele frequencies are 3.45%. In our study population, the *CYP2J2*7* allele frequency was 6.7%, which was slightly higher than that reported in Chinese and similar to that reported in Western Caucasians.

White et al. (39) previously reported that over the 40% of load (percentage of high levels of blood pressure) were related to increased left ventricular mass and cardiac risk. On the other hand, it has been shown that systolic blood pressure load (>130 mmHg) is associated with a 14% increase in cardiac events (40). High blood pressure at night and impaired dipping profile, particularly riser pattern, were associated with overall cardiovascular diseases (41).

NH *CYP2J2*7* carriers' night systolic load (percentage of measurements >125 mmHg) was 87%, whereas the corresponding figure in *GG* carriers was 73%.

Study Limitations

Important limitations of our study are the small population size and the cross-sectional study design, leading to low power and lack of long-term observation data concerning cardiovascular disease risk. Moreover, there was only one subject homozygous for the *CYP2J2*7 T*-allele; therefore, we could not evaluate the impact of this genotype.

CONCLUSION

We have observed that *CYP2J2*7* polymorphism carrier status is related to an increased morning surge, which was reported to be one of the risk factors for cardiovascular diseases in earlier studies in the field. Considering the existing literature with our findings, it is further shown that the *CYP2J2*7* carrier status might indicate an increased risk of cardiovascular events. Evaluation of the *CYP2J2*7* genotype together with ambulatory blood pressure to identify the morning slope of individuals would be helpful to assess the risk of cardiovascular diseases and plan their blood pressure treatment accordingly.

Ethics

Ethics Committee Approval: The study was conducted with the permission of the Local Ethics Committee of Gazi University Faculty of Medicine (approval number: 193, date: 14.06.2006; no: 413/26.11.2007).

Informed Consent: All volunteers signed an informed consent form before their participation.

Author Contributions

Concept: E.H.V., A.Ç., H.Z., Design: E.H.V., A.Ç., H.Z., Data Collection or Processing: E.H.V., Analysis or Interpretation: E.H.V., Literature Search: A.G.G., M.L.D., Writing: E.H.V., A.G.

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

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

REFERENCES

1. Marteau JB, Zaiou M, Siest G, Visvikis-Siest S. Genetic determinants of blood pressure regulation. J Hypertens. 2005; 23: 2127-43.
2. Egstrand S, Mace ML, Olgaard K, Lewin E. The Vascular Circadian Clock in Chronic Kidney Disease. Cells. 2021; 10: 1769.
3. Smolensky MH, Hermida RC, Castriotta RJ, Portaluppi F. Role of sleep-wake cycle on blood pressure circadian rhythms and hypertension. Sleep Med. 2007; 8: 668-80.
4. Zhang J, Sun R, Jiang T, Yang G, Chen L. Circadian Blood Pressure Rhythm in Cardiovascular and Renal Health and Disease. Biomolecules. 2021; 11: 868.
5. Kario K. Nocturnal Hypertension: New Technology and Evidence. Hypertension. 2018; 71: 997-1009.
6. O'Brien E, Beevers G, Lip GY. ABC of hypertension. Blood pressure measurement. Part III-automated sphygmomanometry: ambulatory blood pressure measurement. BMJ. 2001; 322: 1110-4.
7. Ohkubo T, Imai Y, Tsuji I, Nagai K, Watanabe N, Minami N, et al. Relation between nocturnal decline in blood pressure and mortality. The Ohasama Study. Am J Hypertens. 1997; 10: 1201-7.
8. Staessen JA, Bieniaszewski L, O'Brien E, Gosse P, Hayashi H, Imai Y, et al. Nocturnal blood pressure fall on ambulatory monitoring in a large international database. The "Ad Hoc' Working Group. Hypertension. 1997; 29: 30-9.
9. Tadic M, Cuspidi C, Grassi G, Mancia G. Isolated Nocturnal Hypertension: What Do We Know and What Can We Do? Integr Blood Press Control. 2020; 13: 63-9.
10. Cuspidi C, Facchetti R, Bombelli M, Sala C, Tadic M, Grassi G, et al. Is night-time hypertension worse than daytime hypertension? A study on cardiac damage in a general population: the PAMELA study. J Hypertens. 2017; 35: 506-12.
11. Ogedegbe G, Spruill TM, Sarpong DF, Agyemang C, Chaplin W, Pastva A, et al. Correlates of isolated nocturnal hypertension and target organ damage in a population-based cohort of African Americans: the Jackson Heart Study. Am J Hypertens. 2013: 26; 1011-6.
12. Wang C, Deng WJ, Gong WY, Zhang J, Zhang QZ, Ye ZC, et al. Nocturnal Hypertension Correlates Better with Target Organ Damage in Patients with Chronic Kidney Disease than a Nondipping Pattern. J Clin Hypertens (Greenwich). 2015: 17; 792-801.
13. Sarkis A, Roman RJ. Role of cytochrome P450 metabolites of arachidonic acid in hypertension. Curr Drug Metab. 2004; 5: 245-56.
14. Capdevila JH, Wang W, Falck JR. Arachidonic acid monooxygenase: Genetic and biochemical approaches to physiological/pathophysiological relevance. Prostaglandins Other Lipid Mediat. 2015; 120: 40-9.
15. Wu S, Moomaw CR, Tomer KB, Falck JR, Zeldin DC. Molecular cloning and expression of CYP2J2, a human cytochrome P450 arachidonic acid epoxygenase highly expressed in heart. J Biol Chem. 1996; 271: 3460-8.
16. Delozier TC, Kissling GE, Coulter SJ, Dai D, Foley JF, Bradbury JA, et al. Detection of human CYP2C8, CYP2C9, and CYP2J2 in cardiovascular tissues. Drug Metab Dispos 2007; 35: 682-8.
17. Michaud V, Frappier M, Dumas MC, Turgeon J. Metabolic activity and mRNA levels of human cardiac CYP450s involved in drug metabolism. PLoS One. 2010; 5: e15666.
18. Rifkind AB, Lee C, Chang TK, Waxman DJ. Arachidonic acid metabolism by human cytochrome P450s 2C8, 2C9, 2E1, and 1A2: regioselective oxygenation and evidence for a role for CYP2C enzymes in arachidonic acid epoxygenation in human liver microsomes. Arch Biochem Biophys. 1995; 320: 380-9.
19. Bylund J, Ericsson J, Oliw EH. Analysis of cytochrome P450 metabolites of arachidonic and linoleic acids by liquid chromatography-mass spectrometry with ion trap MS. Anal Biochem. 1998; 265: 55-68.

20. Node K, Huo Y, Ruan X, Yang B, Spiecker M, Ley K, et al. Anti-inflammatory properties of cytochrome P450 epoxygenase-derived eicosanoids. Science. 1999; 285: 1276-9.
21. Node K, Ruan XL, Dai J, Yang SX, Graham L, Zeldin DC, et al. Activation of Galpha s mediates induction of tissue-type plasminogen activator gene transcription by epoxyeicosatrienoic acids. J Biol Chem. 2001; 276: 15983-9.
22. Berlin DS, Sangkuhl K, Klein TE, Altman RB. PharmGKB summary: cytochrome P450, family 2, subfamily J, polypeptide 2: CYP2J2. Pharmacogenet Genomics. 2011; 21: 308-11.
23. Spiecker M, Darius H, Hankeln T, Soufi M, Sattler AM, Schaefer JR, et al. Risk of coronary artery disease associated with polymorphism of the cytochrome P450 epoxygenase CYP2J2. Circulation. 2004; 110: 2132-6.
24. King LM, Gainer JV, David GL, Dai D, Goldstein JA, Brown NJ, et al. Single nucleotide polymorphisms in the CYP2J2 and CYP2C8 genes and the risk of hypertension. Pharmacogenet Genomics. 2005; 15: 7-13.
25. Polonikov AV, Ivanov VP, Solodilova MA, Khoroshaya IV, Kozhuhov MA, Ivakin VE, et al. A common polymorphism G-50T in cytochrome P450 2J2 gene is associated with increased risk of essential hypertension in a Russian population. Dis Markers. 2008; 24: 119-26.
26. Liu PY, Li YH, Chao TH, Wu HL, Lin LJ, Tsai LM, et al. Synergistic effect of cytochrome P450 epoxygenase CYP2J2*7 polymorphism with smoking on the onset of premature myocardial infarction. Atherosclerosis. 2007; 195: 199-206.
27. Wang H, Jiang Y, Liu Y, Lin C, Cheng G, Chen X, et al. CYP2J2*7 single nucleotide polymorphism in a Chinese population. Clin Chim Acta. 2006; 365: 125-8.
28. Zachariah PK, Sheps SG, Bailey KR, Wiltgen CM, Moore AG.Age-related characteristics of ambulatory blood pressure load and mean blood pressure in normotensive subjects. JAMA. 1991; 265: 1414-7.
29. Vicent L, Martínez-Sellés M. Circadian rhythms, cardiac arrhythmias and sudden death. Front Biosci (Landmark Ed). 2021; 26: 1305-11.
30. Thosar SS, Shea SA. Circadian control of human cardiovascular function. Curr Opin Pharmacol. 2021; 57: 89-97.
31. Kuwajima I, Mitani K, Miyao M, Suzuki Y, Kuramoto K, Ozawa T. Cardiac implications of the morning surge in blood pressure in elderly hypertensive patients: relation to arising time. Am J Hypertens. 1995; 8: 29-33.
32. Wang JG, Kario K, Park JB, Chen CH. Morning blood pressure monitoring in the management of hypertension. J Hypertens. 2017; 35: 1554-63.
33. Kikuya M, Ohkubo T, Asayama K, Metoki H, Obara T, Saito S, et al. Ambulatory blood pressure and 10-year risk of cardiovascular and noncardiovascular mortality: the Ohasama study. Hypertension. 2005; 45: 240-5.
34. Dolan E, Stanton A, Thijs L, Hinedi K, Atkins N, McClory S, et al. Superiority of ambulatory over clinic blood pressure measurement in predicting mortality: the Dublin outcome study. Hypertension. 2005; 46: 156-61.
35. Alghasham A, Ali A, Ismail H, Dowaidar M, Settin AA. CYP2J2 -50 G/T and ADRB2 G46A gene polymorphisms in Saudi subjects with hypertension. Genet Test Mol Biomarkers. 2012; 16: 1027-31.
36. Wu SN, Zhang Y, Gardner CO, Chen Q, Li Y, Wang GL, et al. Evidence for association of polymorphisms in CYP2J2 and susceptibility to essential hypertension. Ann Hum Genet. 2007; 71: 519-25.
37. Askari A, Thomson SJ, Edin ML, Zeldin DC, Bishop-Bailey D. Roles of the epoxygenase CYP2J2 in the endothelium. Prostaglandins Other Lipid Mediat. 2013; 107: 56-63.
38. Li H, He Y, Wang L, Xing S, Peng L, Yuan D, et al. Analysis of CYP2J2 mutations in the Chinese Uyghur population. Am J Transl Res. 2021; 13: 10871-81.
39. White WB, Dey HM, Schulman P. Assessment of the daily blood pressure load as a determinant of cardiac function in patients with mild-to-moderate hypertension. Am Heart J. 1989; 118: 782-95.
40. Wang N, Harris K, Hamet P, Harrap S, Mancia G, Poulter N, et al. Cumulative Systolic Blood Pressure Load and Cardiovascular Risk in Patients with Diabetes. J Am Coll Cardiol. 2022; 80: 1147-55.
41. Kario K, Hoshide S, Mizuno H, Kabutoya T, Nishizawa M, Yoshida T, et al. Nighttime Blood Pressure Phenotype and Cardiovascular Prognosis: Practitioner-Based Nationwide JAMP Study. Circulation. 2020; 142: 1810-20.

GMJ 2024;35:332-334

DOI: http://dx.doi.org/10.12996/gmj.2024.4143

Liver Transplantation for Liver-Originated Malignancy: A Single Center Experience

Karaciğer Kaynaklı Malignitelerde Karaciğer Nakli: Tek Merkez Deneyimi

Ramazan Kozan[1], M. Hakan Sözen[1,2], Aydın Dalgıç[1,2]

[1]Department of General Surgery, Gazi University Faculty of Medicine, Ankara, Türkiye
[2]Transplantation Center, Gazi University, Ankara, Türkiye

ABSTRACT

Objective: We aimed to evaluate liver transplantation (LT) effectiveness for liver-originated malignancies, focusing on hepatocellular carcinoma (HCC), at a single center.

Methods: Retrospective data review of LT cases between 2006 and 2023. Inclusion criteria: no extrahepatic involvement and liver-originated malignancy. Demographic characteristics, etiology, alpha-fetoprotein (AFP) levels, Milan Criteria compliance, pre-transplant treatments, complications, recurrence, and mortality were analyzed.

Results: Fourteen liver-originated tumors underwent LT, half of which were from deceased donors. Hepatitis B virus was the common etiology (71%). The median AFP level was 4 ng/mL. Fifty percent received pre-transplant therapy. Patient survival rates at 1, 3, and 5 years: 72%, 72%, 68% respectively. The recurrence-free survival rates for the same years were 93%.

Conclusion: LT, including living donor LT, is effective for liver-originated tumors, especially HCC. Encouraging survival rates align with the Milan and University of California, San Francisco Criteria. Despite limitations, ongoing research is vital for LT's role in liver cancer management, considering tumor size, positron emission tomography/computed tomography, grade, and AFP levels for candidate selection beyond the current criteria.

Keywords: Hepatocellular carcinoma, liver, liver transplantation, neuroendocrine tumor, primary liver tumor, transplant oncology

ÖZ

Amaç: Karaciğer kökenli tümörlerde tek bir merkezde yapılan, özellikle hepatosellüler karsinom (HCC) odaklı karaciğer nakli (LT) etkinliğini değerlendirmektir.

Yöntemler: 2006 ile 2023 yılları arasında gerçekleştirilen LT olgularına ait veriler retrospektif olarak incelendi. Dahil etme kriterleri; ekstrahepatik tutulumun olmaması ve karaciğer kökenli maligniteye bağlı nakillerdi. Demografik özellikler, etiyoloji, alfa-fetoprotein (AFP) seviyeleri, Milan Kriterleri uyum, pre-nakil tedavileri, komplikasyonlar, nüks ve mortalite analiz edildi.

Bulgular: On dört karaciğer kökenli tümör hastasına, yarısı kadaverik donörlerden olmak üzere LT yapıldı. Hepatit B virüs en yaygın etiyoloji (%71) olarak belirlendi. Medyan AFP düzeyi 4 ng/mL idi. Hastaların %50'si transplantasyon öncesi tedavi almıştı. Çalışmada 1, 3 ve 5 yıllık sağkalım oranları sırasıyla %72, %72 ve %68 olarak tespit edildi. Aynı yıllar için nükssüz sağkalım oranları %93'tü.

Sonuç: LT, özellikle HCC için etkili bir tedavi seçeneği olarak ortaya çıkmıştır. Umut verici sağkalım oranları, Milan ve University of California, San Francisco Kriterleri'yle uyumluluğu vurgular niteliktedir. Sınırlılıklara rağmen, karaciğer kanseri yönetiminde naklin rolünü doğrulamak ve mevcut kriterlerin ötesinde aday seçiminde tümör boyutu, pozitron emisyon tomografisi/bilgisayarlı tomografi sonuçları, tümör derecesi ve AFP düzeylerini dikkate almak için daha fazla araştırma gereklidir.

Anahtar Sözcükler: Hepatoselüler karsinom, karaciğer, karaciğer nakli, nöroendokrin tümör, primer karaciğer tümörü, transplant onkolojisi

Address for Correspondence/Yazışma Adresi: Ramazan Kozan, MD, Department of General Surgery, Gazi University Faculty of Medicine, Ankara, Türkiye
E-mail / E-posta: ramazankozan@gazi.edu.tr
ORCID ID: orcid.org/0000-0002-3835-8759

Received/Geliş Tarihi: 13.02.2024
Accepted/Kabul Tarihi: 21.02.2024

INTRODUCTION

Liver-originated malignancies represent a significant global burden and contribute substantially to cancer-related mortality. Liver transplantation (LT) has emerged as a pivotal therapeutic avenue for carefully selected patients with such tumors. Hepatocellular carcinoma (HCC), in particular, is a formidable health challenge, ranking as the sixth most prevalent cancer globally and the third leading cause of cancer-related deaths (1,2). Although liver resection serves as the primary curative modality for resectable HCC, its efficacy is hampered by a notable recurrence rate attributed to underlying hepatitis and cirrhosis. LT stands out as the gold standard radical intervention for HCC cases fulfilling established criteria, notably the Milan and University of California, San Francisco (UCSF) Criteria. Initially, many transplant centers favor liver resection for cases of resectable HCC with compensated liver function, reserving LT as a salvage option in scenarios involving disease recurrence or liver decompensation (3). However, owing to organ scarcity, the adoption of living donor liver transplantation (LDLT) for HCC has surged over the past decade, even extending to salvage settings with acceptable safety profiles. Against this backdrop, our study endeavors to scrutinize the efficacy of LT procedures for liver-originated malignancies and to appraise the insights gleaned from our institution's experience.

MATERIALS AND METHODS

Between 2006 and 2023, data of patients who underwent LT at the Gazi University Transplantation Center were retrospectively reviewed. The inclusion criteria comprised absence of extrahepatic involvement and transplantation due to liver-originated malignancy, while the exclusion criteria included inability to access patient archive and follow-up data. All data were retrospectively collected from patient charts and surgical files. Demographic characteristics (age and gender), donor type (living or deceased), tumor etiology and histopathology, alpha-fetoprotein (AFP) levels, compliance with the Milan Criteria, pre-transplant treatments [trans-arterial chemoembolization (TACE) or radiofrequency ablation (RF)], complications, recurrence, and mortality data were analyzed.

All procedures performed in this study were in compliance with the ethical standards of the institutional and/or national research committee, as well as with the principles outlined in the 1964 Helsinki and 2008 İstanbul Declarations; subsequent revisions or equivalent ethical standards. This study was approved by the Local Ethical Committee of Gazi University (approval number: 2024-194, date: 15.02.2024).

Statistical Analysis

All the statistical analysis was performed using SPSS software, version 20, (SPSS Inc., Chicago, IL, USA). Data are expressed as median and range. Relevant variables were analyzed using descriptive statistics. Survey analysis was conducted using the Kaplan-Meier estimator test.

RESULTS

LT has been performed on 14 liver-originated tumors at the Gazi University Transplantation Center in Ankara, Türkiye, since 2006. There were 9 (64%) male and 5 (36%) female recipients with a median age of 45 years (range, 19-61 years). Seven (50%) out of 14 LTs were performed from deceased donors (Table 1).

The etiology of the tumors was hepatitis B virus (n=10), cryptogenic (n=2), hepatitis C virus (n=1), and neuroendocrine tumor (n=1), respectively. The tumor origin included neuroendocrine (n=1) and HCC (n=13). Six (43%) out of 14 recipients were outside the Milan Criteria. One (7%) patient had been downgraded to the Milan Criteria. The median AFP level was 4 ng/mL (range, 1.4-399 ng/mL) (Table 1).

All patients in this group underwent positron emission tomography/computed tomography (PET/CT) to confirm the absence of extrahepatic involvement. The tumor was invisible on CT/magnetic resonance (MR) and was found in the liver explant in 1 (7%) patient. Seven (50%) patients received pre-transplant adjuvant therapy as TACE (n=6) and RF (n=1) (Table 1). None of the patients underwent liver surgery for tumors before LT. Only 1 patient was downgraded while outside the Milan Criteria and was added to the national waiting list.

The tumor sizes of patients outside the Milan Criteria were 25, 15, 8, and 8 cm (four lesions: total 11.5 cm), respectively. Two (14%) patients died after transplantation because of sepsis, hepatic artery

Table 1. Demographic and transplantation characteristics

Characteristics	Results
Age (year)	Median 45 (range; 19-61)
Gender	
Male	9 (64%)
Female	5 (36%)
Donor type	
Deceased	7 (50%)
Live	7 (50%)
Tumor histopathology	
Neuroendocrine	1 (7%)
Hepatocellular carcinoma	13 (93%)
Tumor etiology	
Hepatit B virus	10 (72%)
Cryptogenic	2 (14%)
Hepatit C virüs	1 (7%)
Neuroendocrine	1 (7%)
Pre-transplant therapy	7 (50%)
Tran-sarterial chemoembolization	6
Radiofrequency ablation	1
Milan criteria	
Inside	8 (57%)
Outside	6 (43%)
Alpha-fetoprotein level (ng/mL)	Median 4 (range; 1.4-399)
Follow-up (months)	Median 171 (range; 96-225)
Mortality	2 (14%)
Hepatic artery thrombosis	1
Tumor recurrence	1

thrombosis (n=1), and tumor recurrence (n=1). One recurrence was observed 6 months after transplantation in this patient group. All remaining patients are doing well with a median follow-up of 171 months (range, 96-225) (Table 1).

The patient survival rates for 1, 3, and 5 years are 72%, 72%, and 68%, respectively. The recurrence-free survival rates at 1, 3, and 5 years are 93%, 93%, and 93%, respectively.

DISCUSSION

The ethical dilemma of expanding transplantation criteria for cancer patients is complicated by the potential for cancer recurrence, a risk absent in non-malignant transplant candidates. While no models suggest a percentage of recurrence-free survival necessary to justify expansion criteria, this study demonstrated a 72% recurrence-free survival at 5 years, comparable to the 74% and 54% observed in the Milan Criteria group (3,4). Allograft survival is a potential concern for liver transplant recipients with a history of HCC requiring both chemotherapy and immunosuppression, with a lack of literature standardization on advantageous modalities (1-4).

The principal challenge is identifying preoperative criteria to select tumors with favorable biology and patients achieving a 5-year survival meeting or exceeding 75%, as advocated by the Barcelona group (3). Currently, preoperative tumor staging relies on an up-to-date CT or MR scan within 6 months of LT. However, the crucial role of tumor biology, especially histological grade and lymphovascular invasion, suggests biopsy and histological examination before LT in all cases. Despite concerns about patient acceptance, sampling error, and technical complications in cirrhotic and coagulopathic patients, the purported risk of tumor dissemination is minimal with proper patient selection and meticulous attention to the biopsy technique. The development of a reliable, noninvasive method to identify aggressive tumor biology without biopsy remains a fertile area for technological research (4-6).

The results of our study highlight the efficacy of LT as a viable treatment option for liver-originated tumors, particularly HCC. The favorable patient survival rates at 1, 3, and 5 years underscore the potential curative impact of transplantation in carefully selected cases, aligning with established criteria such as Milan and UCSF (4). The use of PET/CT for thorough pre-transplant assessment demonstrated its significance in confirming the absence of extrahepatic involvement, aiding in more precise patient selection.

Notably, the inclusion of LDLT in our study reflects the pragmatic response to organ shortages, extending the application of transplantation to salvage scenarios.

Study Limitations

It is crucial to acknowledge the limitations of our study. The relatively small sample size and single-center retrospective design may influence the generalizability of our findings. In addition, the presence of tumor recurrence, albeit infrequent, emphasizes the ongoing challenges in achieving long-term success.

CONCLUSION

Our study supports LT, including LDLT, as a valuable and life-extending treatment for liver-originated tumors. The encouraging survival rates warrant further exploration and consideration in the evolving landscape of liver cancer management. Despite the promising outcomes, ongoing research and multicenter studies are essential to validate our findings and address the inherent limitations, thereby ensuring a comprehensive understanding of the role of transplantation in this complex patient population.

We have a small patient group in this study, but we believe that, regardless of the tumor size, PET/CT scans, tumor grade, and AFP levels are possible parameters of the biological behavior of tumors, which will help in decision-making about the inclusion or exclusion of LT candidates with HCC beyond the current selection criteria.

Ethics

Ethics Committee Approval: This study was approved by the Local Ethical Committee of Gazi University (approval number: 2024-194, date: 15.02.2024).

Informed Consent: Retrospective study.

Author Contributions

Concept: R.K., M.H.S., A.D., Design: R.K., M.H.S., A.D., Supervision: R.K., M.H.S., A.D., Data Collection or Processing: R.K., M.H.S., Analysis or Interpretation: R.K., M.H.S., Literature Search: M.H.S., A.D., Writing: R.K., M.H.S., Critical Review: R.K., M.H.S.

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

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

REFERENCES

1. Rumgay H, Arnold M, Ferlay J, Lesi O, Cabasag CJ, Vignat J, et al. Global burden of primary liver cancer in 2020 and predictions to 2040. J Hepatol. 2022; 77: 1598-606.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021; 71: 209-49.
3. Reig M, Forner A, Rimola J, Ferrer-Fàbrega J, Burrel M, Garcia-Criado Á, et al. BCLC strategy for prognosis prediction and treatment recommendation: The 2022 update. J Hepatol. 2022; 76: 681-93.
4. Dageforde LA, Fowler KJ, Chapman WC. Liver transplantation for hepatocellular carcinoma: current update on treatment and allocation. Curr Opin Organ Transplant. 2017; 22: 128-34.
5. Karakayali H, Moray G, Sozen H, Dalgic A, Emiroglu R, Haberal M. Expanded criteria for liver transplantation in patients with hepatocellular carcinoma. Transplant Proc. 2006; 38: 575-8.
6. Tabrizian P, Zeitlhoefler M, Hassan AT, Marino R. Immunotherapy for transplantation of hepatocellular carcinoma: the next frontier in adjunctive therapy. Curr Opin Organ Transplant. 2024; 29: 144-54.

GMJ 2024;35:335-337

DOI: http://dx.doi.org/10.12996/gmj.2023.3702

Reporting the Presence of an Incision Similar to a Cardiac Notch in the Right Lung: A Case Report

Sağ Akciğerde Kardiyak Çentik Benzeri Kesiğin Varlığının Bildirilmesi: Olgu Sunumu

Mehran Kamani[1], Ali Razavinasab[1], Seyed Hassan Eftekhar Vaghefi[2], Abbas Mohammadi[3], Reza Sadeghi[1]

[1]Sirjan School of Medical Sciences, Sirjan, Iran
[2]Anatomy Research Center, Kerman University of Medical Sciences, Kerman, Iran
[3]Department of Clinical Biochemistry, Afazalipour School of Medicine; Kerman University of Medical Sciences, Physiology Research Center, Kerman University of Medical Sciences; Sirjan School of Medical Sciences, Sirjan, Iran

ABSTRACT

The lungs are the main organs of respiration, and their main function is ventilation. The lungs, through oxygenation, convert venous blood into arterial blood. On the outer surface of the right lung, there were two deep fissures, one called the oblique fissure and the other called the horizontal fissure, with respect to the horizontal plate, which partitioned it into three upper, middle, and lower lobes. The anterior side of the lungs is thin and sharp and is located inside the rib-interstitial pleural sinus. In the present case, during the dissection of the cadaver of a 57-year-old man, it was observed that the right lung, along with its posterior border, does not have the usual geometry in the lungs. The posterior border of the right lung had a heart incision similar to that of the anterior border of the left lung. The lingual process was also visible on the posterior border of the right lung. The left lung was normal. The presence of a heart incision is important in terms of anatomical variation along the posterior border of the right lung in terms of lung pathologies and the therapeutic pattern of lung segmental resection.

Keywords: Case report, presence of notch, lingula, variation of lungs, anterior and posterior borders

ÖZ

Akciğerler solunumun ana organlarıdır ve ana işlevleri havalandırmadır. Akciğerler oksijenlenme yoluyla venöz kanı arteriyel kana dönüştürür. Sağ akciğerin dış yüzeyinde yatay plakaya göre biri oblik fissür, diğeri yatay fissür adı verilen ve onu üst, orta ve alt olmak üzere üç loba ayıran iki derin çatlak vardı. Akciğerlerin ön tarafı ince ve keskindir ve kaburga interstisyel plevral sinüsün içinde yer alır. Sunulan olguda 57 yaşındaki erkek hastanın kadavrasının diseksiyonu sırasında sağ akciğerin arka kenarıyla birlikte akciğerlerdeki alışılagelmiş geometriye sahip olmadığı görüldü. Sağ akciğerin arka sınırında, sol akciğerin ön sınırına benzer bir kalp kesisi vardı. Lingual süreç sağ akciğerin arka sınırında da görülüyordu. Sol akciğer normaldi. Kalp kesisinin varlığı sağ akciğer arka sınırı boyunca anatomik varyasyon, akciğer patolojileri ve akciğer segmental rezeksiyonunun tedavi şekli açısından önemlidir.

Anahtar Sözcükler: Olgu sunumu, çentik varlığı, dil, akciğer değişimi, ön ve arka sınırlar

INTRODUCTION

The right lung has three lobes, which are separated by oblique and horizontal fissures. The oblique fissure extends from the surface of the rib to the medial surface of the lung, both above and below the hilum. The left lung has two upper and lower lobes separated by an oblique fissure. The anterior border of the left lung forms a cardiac notch. At the lower end of the cardiac notch is a small process called the lingula. The lingula has two upper lingular (IV) and lower lingular (V) bronchopulmonary segments (1). The normal anatomy of the lung is often altered by certain variations, such as the presence of

Address for Correspondence/Yazışma Adresi: Mehran Kamani, MD, Sirjan School of Medical Sciences, Sirjan, Iran
E-mail / E-posta: mehrankamany@yahoo.com
ORCID ID: orcid.org/0000-0002-6955-4103

Received/Geliş Tarihi: 04.10.2022
Accepted/Kabul Tarihi: 30.10.2023

an accessory fissure, absence of a fissure, or an unusual notch in the lungs.

CASE REPORT

During dissection of the cadaver of a 57-year-old man, the author noted an abnormal right lung morphology with a cardiac notch in the posterior border. A lingual process was also observed in the posterior border of the right lung. The structure of the horizontal and oblique fissures of the right lung was normal. The structure of the pleura was normal. The hilum arrangement of the right lung was also anatomically normal (Figure 1). Informed consent was obtained.

DISCUSSION

In the fourth week of the embryonic period, the respiratory diverticulum appears as a ventral growth from the anterior end of the foregut endoderm. The diverticulum develops caudally toward the surrounding mesenchyme, forming two right and left buds. Each lung then grows through repeated dual branching of the secondary bronchi. After several generations of branching, the bronchopulmonary segments are organized (2). Cadavers are the best tool for studying the anatomy of any organ. Numerous researchers have identified and presented anomalous anatomy of the lungs in human cadavers (3,4). In a study of 1,200 pairs of lungs in 2013, incomplete oblique fissures were found in 25.6% of the right lungs and incomplete horizontal fissures in 17.1% of the right lungs. The oblique fissure was absent in 7.3% and 4.8% of the left and right lungs, respectively. There were no horizontal fissures in 45.2% of the right lungs (5). In 1999, Lukose et al. (6) conducted a study on the morphology of the lungs, which showed that 21% of the left lungs had incomplete oblique fissures. In addition, horizontal fissures were absent and incomplete in 10.5% and 21% of the left and right lung, respectively. In 1999, Bergman et al. (7) studied changes in the peripheral division of the right lung and the base of the right and left lungs, in which they reported that in 21% of cases there was no horizontal fissure and in 67% the right lung was incomplete. In this study, we observed a defect on the posterior side of the right lung that impairs bronchopulmonary segregation. This defect is important in lung surgery and the removal of bronchopulmonary segments. Understanding embryology and the formation of small bronchopulmonary segments of the lungs and pulmonary veins is clinically useful (8). In this investigation, the upper lobe of the right lung has two lingual parts on its posterior side, whereas it normally has three segments, the apical, posterior, and anterior bronchopulmonary segments. However, in this case, in addition to the above segments, two upper and lower lingual bronchopulmonary segments were also observed. Because of these variations, the accessory lobes and bronchopulmonary segments of the lungs can be misinterpreted on radiographs and computed tomography scans. They can also be confounded with certain clinical conditions such as linear atelectasis and pleural scarring (9,10). Anomalies detected in the right lung in this study are important for diagnostic radiology and bronchopulmonary segment resection.

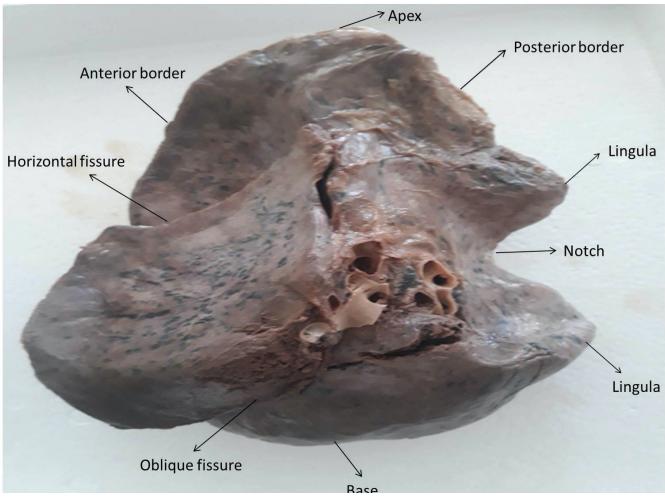


Figure 1. Sharp anterior border of the right lung. In this figure, the posterior border of the right lung has an unusual incision similar to the cardiac notch in the left lung.

Anomalies detected in the right lung in this study are important in diagnostic radiology and bronchopulmonary segment resection in pulmonary patients. It is important to identify the differences between lungs and atypical lungs. This knowledge helps physicians to accurately identify pathological conditions. This knowledge also helps surgeons remove pathology in the lungs. These variations should be considered before any surgery, such as segmental resection or lobectomy, to prevent further complications.

Ethics

Informed Consent: It was obtained.

Authorship Contributions

Concept: M.K., A.R., S.H.E.V., A.M., R.S., Design: M.K., A.R., S.H.E.V., A.M., R.S., Data Collection or Processing: M.K., A.R., S.H.E.V., A.M., R.S., Analysis or Interpretation: M.K., A.R., S.H.E.V., A.M., R.S., Literature Search: M.K., A.R., S.H.E.V., A.M., R.S., Writing: M.K., A.R., S.H.E.V., A.M., R.S.

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

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

REFERENCES

1. Dutta S, Mandal L, Mandal SK, Biswas J, Ray A, Bandopadhyay M. Natural fissures of lung: anatomical basis of surgical techniques and imaging. Natl J Med Res. 2013: 3; 117-21.
2. Anbusudar K, Dhivya S. Anatomical study on variations of fissures of lung. Indian Journal of Clinical Anatomy and Physiology. 2016: 3: 449-51.
3. Meenakshi S, Manjunath KY, Balasubramanyam V. Morphological variations of the lung fissures and lobes. Indian J Chest Dis Allied Sci. 2004; 46: 179-82.
4. Prakash, Bhardwaj AK, Shashirekha M, Suma HY, Krishna GG, Singh G. Lung morphology: a cadaver study in Indian population. Ital J Anat Embryol. 2010; 115: 235-40.
5. Mayuri J, Pradeep P, Vasudha N, Aparna T, Smita M. Anomalous lobar pattern of right lung: a case report. J Res Med Den Sci. 2013: 1: 80-1.
6. Lukose R, Paul S, Daniel M, Abraham SM, Alex ME, Thomas R, et al. Morphology of the lungs: variations in the lobes and fissures. Biomedicine- Trivandrum Then Taramani. 1999: 19: 227-32.

7. Bergman RA, Afifi AK, Miyauchi R. Variations in peripheral segmentation of right lung and the base of the right and left lungs. Illustrated Encyclopedia of Human Anatomic Variation. 2006.
8. Abe S, Yamamoto M, Noguchi T, Yoshimoto T, Kinoshita H, Matsunaga S, et al. Fetal development of the minor lung segment. Anat Cell Biol. 2014; 47: 12-7.
9. Nene AR, Gajendra KS, Sarma MVR. Lung lobes and fissures: a morphological study. Anatomy. 2011: 5; 30-8.
10. Khedekar D, S Hattangdi. Morphological variations of the lung: a cadaveric study in Mumbai population. Int J Anat Res. 2017: 5; 4313-6.

GMJ 2024;35:338-341

DOI: http://dx.doi.org/10.12996/gmj.2023.3791

X Chromosome Pericentric Inversion: Report of a Case with 46,X,inv(X)(p11.2q26) and a Mini-Review of the Literature

X Kromozomu Perisentrik Inversiyonları: 46,X,inv(X)(p11.2q26) Karyotipli Bir Olgunun Sunumu ve Literatürün Kısa Bir Derlemesi

Emine Göktaş, Betül Okur Altındaş, Ayşe Gül Zamani, Mahmut Selman Yıldırım

Department of Medical Genetics, Necmettin Erbakan University Faculty of Medicine, Konya, Türkiye

ABSTRACT

Pericentric inversions arise from double breaks on opposite arms of the chromosome, followed by 180° rotation and reintegration of the broken segment. Carriers of an inversion are mostly phenotypically healthy. However, they may have some clinical implications, including reproduction anomalies due to imbalanced gamete production. Here, we highlighted the phenotypical variability of X chromosome inversions by reporting a case. The female proband who had recurrent spontaneous abortions was admitted to the Medical Genetics polyclinic. After clinical evaluation, conventional Giemsa-banded karyotyping was performed. The result was 46,X,inv(X)(p11.2q26). Segregation analysis of the family members revealed that she inherited the pericentric inversion from her father and passed it on to her daughter. Detailed genetic counseling was provided to the family. The significance of X chromosome pericentric inversions in the literature was discussed with regard to their phenotypical relevance to enhance our understanding of clinical variability caused by chromosomal inversions.

Keywords: Pericentric inversions, X chromosome, habitual abortus, chromosome rearrangement

ÖZ

Perisentrik inversiyon; bir kromozomun zıt kolları üzerinde bulunan iki farklı bölgenin kırılması ve ardından bu kırık parçanın 180° dönerek yeniden aynı kromozoma birleşmesiyle oluşan kromozomal yeniden düzenlenmedir. İnversiyon taşıyıcıları sıklıkla fenotipik olarak sağlıklıdır. Ancak dengesiz gamet oluşumuna bağlı üreme bozukluklarının da dahil olduğu bazı klinik etkilenmeler görülebilir. Burada, oldukça nadir görülen kromozom X inversiyonuna sahip bir olgu sunulmuştur. Tekrarlayan spontan düşükleri olan kadın hasta Tıbbi Genetik polikliniğine başvurdu. Klinik değerlendirme sonrası, Giemsa-bantlama ile kromozom analizi gerçekleştirildi. Karyotip 46,X,inv(X)(p11.2q26) şeklinde raporlandı. Ailedeki segregasyon araştırıldığında, hastadaki perisentrik inversiyonun babasından kalıtıldığı ve hastanın yaşayan kız çocuğunda da mevcut olduğu görüldü. Aile bireylerine detaylı genetik danışma verildi. Olgumuzu, kromozomal inversiyonların yol açabileceği klinik çeşitliliği vurgulamak için literatürdeki X kromozomu perisentrik inversiyonlarının kısa bir derlemesiyle birlikte tartıştık.

Anahtar Sözcükler: Perisentrik inversiyon, X kromozomu, tekrarlayan düşükler, kromozom yeniden düzenlenmeleri

INTRODUCTION

Chromosome inversions are abnormal structural rearrangements arising from double breaks on the same chromosome followed by the segment between these breakpoints reversed and reattached. According to the localization of the breakpoints, there are two types of inversions: paracentric and pericentric. In paracentric inversions, both breaks occur on the same chromosome arm. In contrast, in pericentric inversions, which are more commonly observed, breaks are on the different sides of the centromere. Therefore, the inverted fragment includes the centromere. Inversions including the pericentric heterochromatin region on chromosomes 1, 9, 16, and Y are commonly regarded as polymorphisms. Breakpoints in these inversions are believed to be located in non-coding areas or repeat regions, causing them to be clinically benign. On the other hand, inversions that arise de novo and affect euchromatic regions may have direct health implications (1).

Address for Correspondence/Yazışma Adresi: Betül Okur Altındaş, MD, Department of Medical Genetics, Necmettin Erbakan University Faculty of Medicine, Konya, Türkiye
E-mail / E-posta: betul.okurr@gmail.com
ORCID ID: orcid.org/0000-0002-1754-4827

Received/Geliş Tarihi: 11.01.2023
Accepted/Kabul Tarihi: 18.09.2023

Inversion of an autosomal chromosome is observed in approximately 1%-2% of the general population. The significance of the clinical outcome is expected to be correlated with the size of the inverted segment. However, X chromosome inversions are extremely rare, with an estimated incidence of 1/28,000-30,000 and their phenotypic implications are much more complicated (2).

Because of the conservation of the total genetic material, inversions are usually not associated with severe morphological effects; however, carriers may have reproduction issues, including infertility and recurrent pregnancy losses, because of gametes with an unbalanced karyotype. If one of the parents is known to be an inversion carrier, the risk of imbalanced gamete formation has been estimated as 5-10% and the risk of having offspring with malformations is around 1%. These risk ratios depend on the size of the inverted fragment. If the region distally located to the inversion is smaller, the clinical findings are expected to be less severe because of lower gene content (3). Here we describe a case with an X-chromosome pericentric inversion presenting with habitual abortus.

Case Presentation

A 35-year-old female patient was admitted to the medical genetics polyclinic with a complaint of recurrent pregnancy losses (in November 2021). She had five spontaneous pregnancies. The first two resulted in two healthy children who were born during the term. However, the next three pregnancies resulted in an abortion at 5, 7, and 7 weeks, respectively. She had no other health complaints, and there was no consanguinity. Physical examination revealed a mild/moderate symmetrical short stature (measured height was 158 cm). Her husbands evaluation revealed no symptoms or findings. To understand the etiology of the habitual abortus, karyotype analysis from peripheral blood was performed on both spouses. Metaphase chromosomes obtained from peripheral blood lymphocyte cultures were evaluated using high-resolution G-banding techniques; using the Leica Biosystems imaging system (IL, USA). At least 10 karyotypes at the 450-600 band resolution level were analyzed, and at least 50 metaphase cells were counted in each specimen. Results were reported according to the International System for Human Cytogenetic Nomenclature 2020 (ISCN-2020). While her husband's karyotype was normal; the patient had a pericentric X chromosome inversion, described as 46,X,inv(X)(p11.2q26) (Figure 1, 2). To perform a segregation analysis, a conventional cytogenetic investigation was performed on the parents, two brothers, and both offsprings of the patient. It was shown that the proband's father and daughter were also carriers of the X chromosome inversion, and the other family members had normal karyotypes. No individual with a recombinant chromosome was observed in the family (Figure 3). Appropriate informed consent was obtained from the family members for sharing their and their children's data.

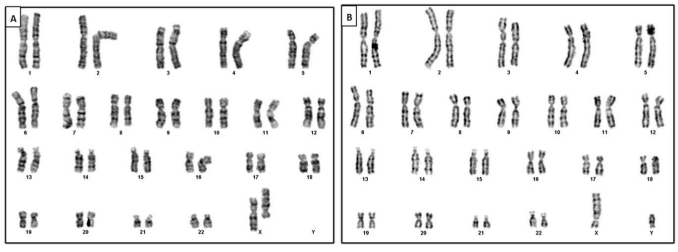


Figure 1. Karyotypes of two patients. Metaphase chromosomes from cultured lymphocytes were banded using conventional G-banding techniques and then arranged into homologous pairs. (A) The proband's karyotype is 46,X,inv(X)(p11.2q26)pat. (B) Karyotype of the proband's father is 46,Y,inv(X)(p11.2q26).

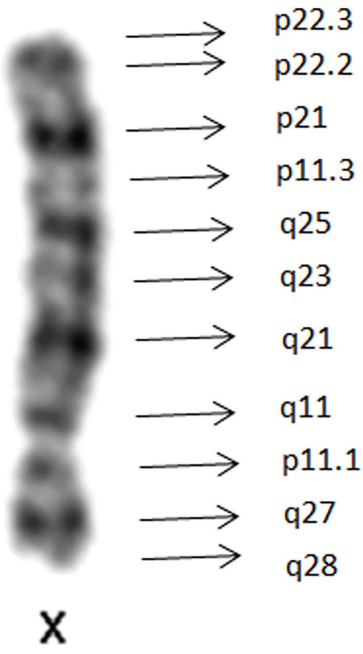


Figure 2. The figure depicting the X chromosome pericentric inversion. Breakpoints are located at p11.2 and q26.

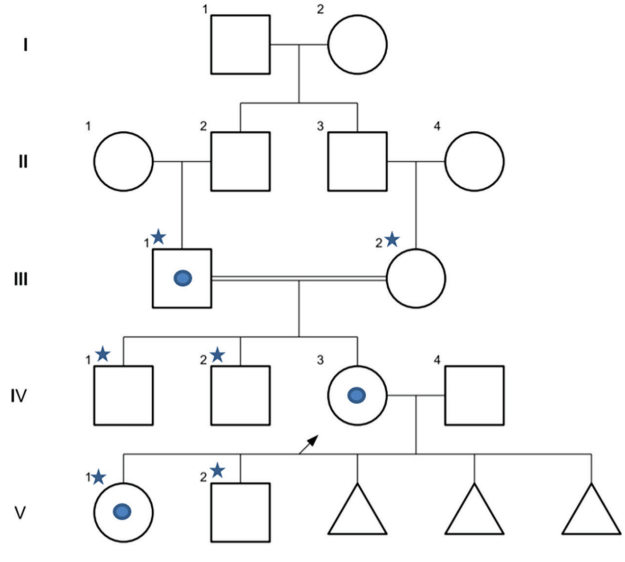


Figure 3. Pedigree of the proband's family. Individuals for whom cytogenetic evaluations are available are indicated using an Asterix. Males are represented by squares and females by circles. Triangles represent miscarriages. The blue dots depict individuals who harbored pericentric X chromosome inversion.

DISCUSSION

Pericentric inversions occur after two breaks are formed at each side of a centromere of a chromosome, which in turn causes that segment to be reversed 180° and reattached afterward. While autosomal chromosomal inversions are quite common, X chromosome inversions that may have a wide variety of clinical implications are rarely seen (4). In the literature, the most commonly identified breakpoints of the short arm of the X chromosome are the p11, p21, and p22 banding regions, whereas breakpoints located in the q arm are highly variable. Duckett and Young (5) reported a short-statured case with a recombinant X chromosome caused by maternal pericentric inversion in 1988. Cytogenetic analysis revealed the mother's karyotype as 46,X,inv(X)(p11.2q26); the same as our index patient. She was described as healthy; however, she had two first-trimester miscarriages and one daughter with a recombinant X chromosome (5). Because of the retained balance of the genetic material, carriers of an inversion are usually phenotypically healthy. However, it is difficult for the inverted chromosome to align during meiosis in germ cells. Because of an unequal crossing-over, the inversion carrier's reproductive potential can be affected. The possible gametes are the normal gamete, the one with the inverted X chromosome, and two different types of recombinant gametes; one with trisomy for the distal portion of the short arm and monosomy for the distal part of the long arm and one with monosomy of the distal portion of the short arm and trisomy of the distal part of the long arm. In the literature, females with an inverted X chromosome are associated with infertility, recurrent first-trimester pregnancy losses, and the birth of a child with genetic abnormalities (6). In this study, it is shown that the proband inherited the inverted X chromosome from her father and passed it on to her daughter. She had habitual abortus in the early weeks of pregnancy, which was consistent with the literature. According to the report of Mattei et al. (7), the Xq13-->26 critical region should be uninterrupted to preserve normal ovarian function. Our patient had this segment intact; therefore, her regular menstruation periods and spontaneous pregnancies can be regarded as supporting evidence for this hypothesis. Phenotypic implications of inversion carriers depend on the localization of breakpoints. In the literature, X-chromosome inversions have been associated with severe oligospermia. Ge et al. (6) reported two brothers with oligospermia whose karyotypes were 46,Y,inv(X)(p22.3;q22). This fracture site affects the Xp22::Xpter pseudoautosomal region 1 (PAR1), where homologous recombination of X and Y chromosomes occurs during meiosis in spermatogenic cells, causing severe oligospermia (6). In this study, breakpoints were not located in the PARs, and the proband's father had no difficulty having children. In the literature, a serious hemophilia A phenotype was also reported in a male proband whose karyotype was 46,Y,inv(X),(p11.21;q28)mat; this was caused by the interruption of the factor 8 gene localized in Xq28 region (1). In this case, the breakpoints excluded Xq28; therefore, inversion carriers had no bleeding disorder findings. Duchenne muscular dystrophy (DMD) is the largest known human gene encoding the dystrophin protein and is localized in the Xp21.2 region. Loss-of-function variations of this gene cause progressive DMD clinic. Approximately one-third of the patients additionally have an intellectual disability with an unclear mechanism. Tran et al. (8) reported a DMD case with an intellectual disability whose karyotype was 46,Y,inv(X)(p21.2;q28). The researchers found that the KUCG1 gene in the Xq28 region may be the cause of the disability through discontinuation. Family members in our study were evaluated for muscular disease or mental deficiency, and no symptoms or findings were revealed. In 2017, Wu et al. (9) defined a patient with inv(X)(p21q13) karyotype who had hypohidrotic ectodermal dysplasia. The EDA gene causing X-linked hypohidrotic ectodermal dysplasia, which is characterized by hypotrichosis, hypohidrosis, and hypodontia, is localized in the Xq13.1 region. Because of pericentric X chromosome inversion, interruption of this gene resulted in the phenotype (9).

The aforementioned studies clearly show that carriers of X chromosome inversions have phenotypic variability, depending on the breakpoints of the inversion. To estimate possible clinical features, it is crucial to define the inverted chromosomal regions and the genes localized at the breakpoints. Then, one must evaluate the correlation between these data and the clinical findings of the patient. A detailed evaluation is also important to predict any symptoms that might occur in the offspring. In the family we investigated, the carriers of the inversion were phenotypically healthy, and another case in the literature, which shares the same breakpoints. No family members were observed to harbor a recombinant X chromosome. However, the spontaneous abortions might have happened because of the recombinant X chromosome, through an intrauterine death. Because they have only one X chromosome, male embryos with nullisomic X chromosome fragments may not be able to develop or they might be expected to undergo abortion.

Detailed genetic counseling was given to the family, along with information about possible gamete production. It was emphasized that pregnancy losses could be due to this inversion. Clinical follow-up for inversion carriers is recommended for any future inversion-associated findings. Considering that future pregnancies can result in abortions or genetic abnormalities, the family was informed about preimplantation genetic testing and prenatal diagnosis techniques.

X chromosome inversions are very rarely observed in the general population, and inversion carriers are mostly phenotypically healthy. However, the interrupted genes localized at the breakpoints can cause a wide variety of clinical findings. One should keep in mind this variability when giving genetic counseling or when approaching a patient.

Ethics

Author Contributions

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

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

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

REFERENCES

1. Xin Y, Zhou J, Ding Q, Chen C, Wu X, Wang X, et al. A pericentric inversion of chromosome X disrupting F8 and resulting in haemophilia A. J Clin Pathol. 2017; 70: 656-61.

2. Kaiser P. Pericentric inversions. Problems and significance for clinical genetics. Hum Genet. 1984; 68: 1-47.
3. Honeywell C, Argiropoulos B, Douglas S, Blumenthal AL, Allanson J, McGowan-Jordan J, et al. Apparent transmission distortion of a pericentric chromosome one inversion in a large multi-generation pedigree. Am J Med Genet A. 2012; 158A: 1262-8.
4. Madan K. Balanced structural changes involving the human X: effect on sexual phenotype. Hum Genet. 1983; 63: 216-21.
5. Duckett DP, Young ID. A recombinant X chromosome in a short statured girl resulting from a maternal pericentric inversion. Hum Genet. 1988; 79: 251-4.
6. Ge Y, Sha Y, Cai M, Chen X, Sha Y, Xu X. Pedigree analysis of two brothers with severe oligozoospermia caused by maternal inv(X) (p22.3, q22) chromosome abnormality. Andrologia. 2020; 52: e13602.
7. Mattei MG, Mattei JF, Ayme S, Giraud F. X-autosome translocations: cytogenetic characteristics and their consequences. Hum Genet. 1982; 61: 295-309.
8. Tran TH, Zhang Z, Yagi M, Lee T, Awano H, Nishida A, et al. Molecular characterization of an X(p21.2;q28) chromosomal inversion in a Duchenne muscular dystrophy patient with mental retardation reveals a novel long non-coding gene on Xq28. J Hum Genet. 2013; 58: 33-9.
9. Wu T, Yin B, Zhu Y, Li G, Ye L, Liang D, et al. First report on an X-linked hypohidrotic ectodermal dysplasia family with X chromosome inversion: Breakpoint mapping reveals the pathogenic mechanism and preimplantation genetics diagnosis achieves an unaffected birth. Clin Chim Acta. 2017; 475: 78-84.