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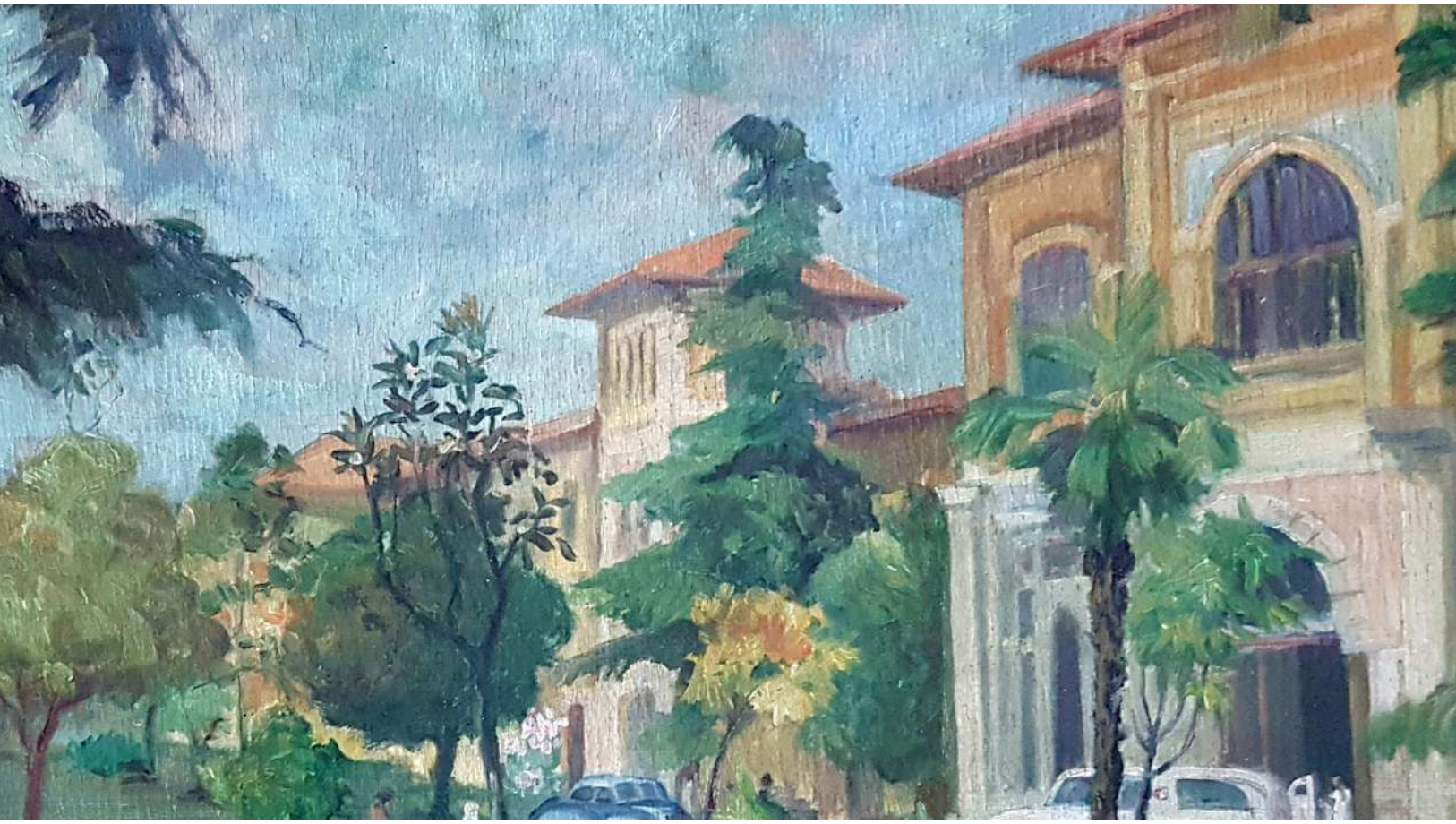

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Limitations, drawbacks, and the shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

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Table 1. Limitations for each manuscript type

Type of manuscript	Word limit	Abstract word limit	Reference limit	Table limit	Figure limit
Original Article	3500	350 (Structured)	40	6	6
Review Article	5000	350	50	6	10
Case Report	1500	200	15	No tables	5

Tables

Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above each table. Abbreviations used in the table should be defined below the table by footnotes (even if they are defined within the main text). Tables should be created using the word processing software "insert table" command and they should be arranged clearly to provide easy reading. Data presented in tables should not be a repetition of the data presented within the main text but should support the main text.

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Philips SJ, Whisnant JP. Hypertension and Stroke. In: Laragh JH, Brenner BM (editors). Hypertension pathophisiology, diagnosis, and management. 2nd ed. New York: Raven Press, 1995: 465–78.

Books with a Single Author:

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Sweetman SC. Martindale the Complete Drug Reference. 34th ed. London: Pharmaceutical Press; 2005.

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

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Author. Title. Type. Place Published: Institution; Year Date. Report No.: Report Number.

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

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Kaplan SI. Post-hospital home health care: elderly access and utilization (dissertation). St Louis (MO): Washington Univ; 1995.

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Author. Title. Alternate Title Year Date Accessed.doi: DOI. [Epub ahead of print].

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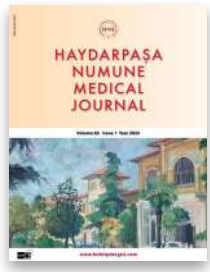

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ORIGINAL ARTICLE

The Significance of Sleep Electroencephalography in Demonstrating Epileptiform Pathology

Ezgi Nazlı, Yılmaz Çetinkaya, Buse Rahime Hasırcı Bayır

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Abstract

Introduction: We aimed to investigate the superiority of examining sleep electroencephalography (EEG) of patients with normal routine EEGs in detecting pathological activity.

Methods: In this study, sleep EEGs of 138 patients, who had previously undergone routine EEG examination and had normal results, conducted at Haydarpasa Numune Training and Research Hospital EEG Laboratory between January 1st, 2022 and January 1st, 2023, were analyzed.

Results: A total of 138 patients' sleep EEGs were evaluated in the study, with 70 (50.8%) of them being female. Among these, 26 (18.8%) showed pathological findings. Among the 26 patients with pathological sleep EEGs (13 females), 23 (88.5%) had a diagnosis of Epilepsy, and 21 (80.8%) were using antiseizure medication. Among the pathological EEGs, 5 (19.2%) showed organization disorder, 7 (26.9%) showed non-specific slow wave paroxysms, 6 (23.1%) showed focal epileptiform discharges, and 8 (30.8%) showed generalized epileptiform discharges.

Discussion and Conclusion: Time is crucial in the diagnosis and treatment of epilepsy. Conducting sleep EEG alongside routine EEG can contribute to the diagnosis, determination of seizure type, and early initiation of treatment.

Keywords: Electroencephalography; seizure; sleep.

Electroencephalography (EEG) is a crucial laboratory tool in diagnosing epilepsy, classifying patients with a confirmed epilepsy diagnosis, selecting appropriate treatment, determining prognosis, and monitoring disease progression [1]. The gold standard laboratory method for diagnosing epilepsy is long-term video EEG monitoring, which is particularly recommended when antiseizure medications fail, and seizures persist [2]. However, this method is neither practical nor cost-effective for every patient presenting with epileptic seizures in an outpatient setting. Therefore, routine (standard) EEG is preferred. Routine EEG is the simplest and most affordable EEG test, typically lasting 20–30 min. Its primary purpose in epilepsy diagnosis is to detect interictal epileptic discharges (IEDs). Standard activation methods which enhance the likelihood of capturing IEDs or even seizures are often employed. The most commonly used activation methods include photic stimulation, hyperventilation, and sleep deprivation (staying awake until late the previous night) [3].

Sleep and sleep deprivation are frequently utilized activation techniques in epilepsy assessments. Sleep EEG examinations in epilepsy patients aim to observe seizures,

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determine seizure type, identify the epileptic focus, and analyze when epileptic activity intensifies during sleep [4]. Studies have consistently demonstrated an increase in detecting epileptic discharges during sleep. It has been reported that both sleep deprivation and sleep itself can trigger seizures in epilepsy patients, with the majority occurring during sleep, particularly in the Non-Rapid Eye Movement phase [5-8]. This study aims to assess the increased detection rate of pathological activity in patients with normal routine EEGs by analyzing their sleep EEG recordings.

Materials and Methods

This study retrospectively initially analyzed sleep EEG recordings of 251 patients aged 18–70 years who underwent EEG at the EEG Laboratory of Haydarpaşa Numune Training and Research Hospital between January 01, 2022, and January 01, 2023. The inclusion criteria required both sleep EEG and prior normal routine EEG recordings in the database. The patients included either had a confirmed epilepsy diagnosis or were suspected of having epilepsy. Patients were excluded if their previous routine EEG recordings were unavailable in the hospital database, if the sleep EEG recording was technically inadequate or incomplete, or if essential clinical data were missing. In addition, patients who did not or could not provide informed consent were excluded.

EEG recordings were performed using silver cup electrodes affixed to the scalp with conductive paste, following the 10–20 electrode placement system. Data acquisition was conducted with Nicolet EEG, Nicolet Ver. 5.11 equipment (Natus Neurology Incorporated, Middleton, WI, USA) using a 0.5 Hz high-pass, 70 Hz low-pass, and 50 Hz notch filter. Routine EEG recordings lasted 30 min, while sleep EEG recordings lasted 60 min. Activation procedures included repeated eye opening and closing, hyperventilation, and intermittent photic stimulation at frequencies ranging from 5 Hz to 30 Hz, first with eyes open and then closed for 5 s. Patients were required to undergo 24 h of sleep deprivation before sleep EEG recordings. All EEG recordings were independently reviewed by two experienced neurologists specialized in clinical neurophysiology, and discrepant interpretations were resolved by consensus.

The patients' gender, epilepsy diagnosis status, and use of antiseizure medication were recorded. Pathological EEG findings were categorized into four groups: organization disorder, slow-wave paroxysm, focal epileptiform discharge, and generalized epileptiform discharge.

Ethical approval for the study was granted by the ethics committee on September 25, 2023, with the decision number HNEAH-KAEK 2023/175, in accordance with the ethical standards of the Declaration of Helsinki.

Statistical Analysis

For data analysis, the Statistical Package for the Social Sciences version 25 was used. Descriptive statistics were performed, and categorical variables were expressed as percentages.

Results

A total of 251 sleep EEG recordings were reviewed. Of these, 90 patients were excluded due to the unavailability of previous routine EEG recordings in the hospital database. An additional 23 patients were excluded because of incomplete clinical data, technically inadequate EEG recordings, or lack of informed consent.

The final study cohort consisted of 138 patients, including 70 female patients (50.8%). The mean age of the study population was 38.41±16.97 years. Among these, 26 (18.8%) exhibited pathological findings. Of the 26 patients with pathological sleep EEGs (13 females-50%, mean age: 34.42±16.05), 23 (88.5%) had a confirmed epilepsy diagnosis, and 21 (80.8%) were using antiseizure medication. The distribution of pathological EEG findings was as follows: organization disorder in 5 cases (19.2%), nonspecific slow-wave paroxysms in 7 cases (26.9%), focal epileptiform discharges in 6 cases (23.1%), and generalized epileptiform discharges in 8 cases (30.8%), as shown in Figure 1. Demographic and clinical characteristics of the study population are presented in Table 1.

Table 1. Demographic and clinical characteristics of the study population

	Mean±Standard deviation or number (%) or ratio
Total cohort (n=138)	
Age (years)	38.41±16.97
Sex=Female:Male ratio	70:68
Pathological sleep electroencephalography (n=26)	
Age (years)	34.42±16.05
Sex=Female:Male ratio	13:13
Confirmed epilepsy diagnosis	23 (88.5)
Antiseizure medication use	21 (80.8)

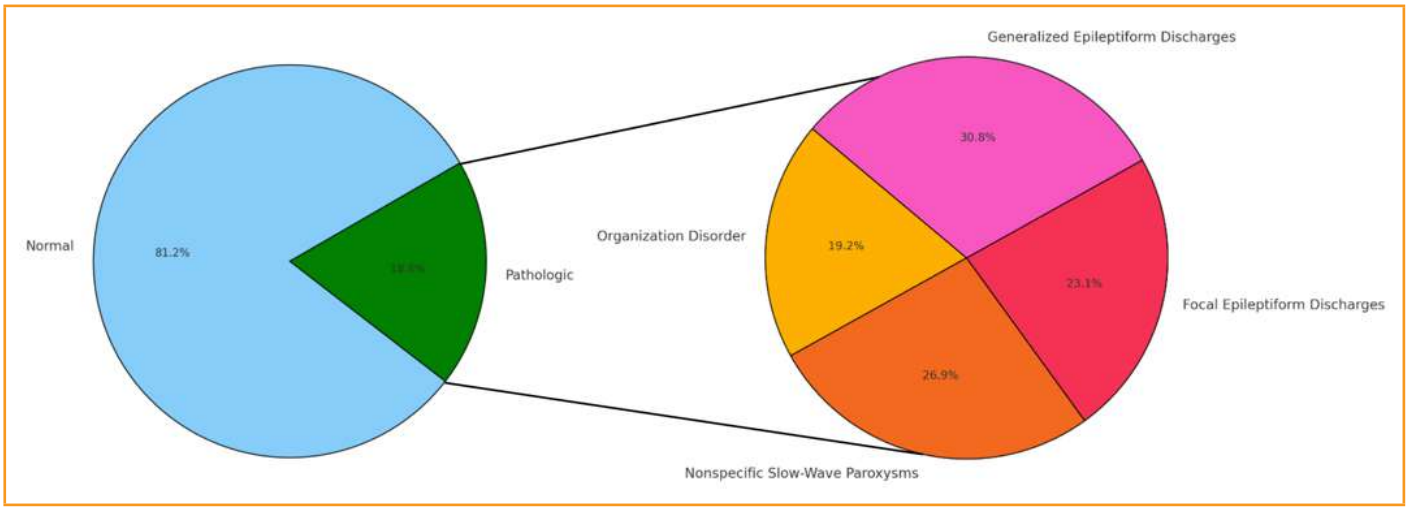

Figure 1. Pie of pie chart: Distribution of Sleep electroencephalography (EEG) recordings and distribution of pathological EEG results.

Discussion

EEG records cerebral bioelectrical activity through electrodes placed on the scalp and is the most significant laboratory tool for epilepsy diagnosis, seizure classification, and patient monitoring [1-4]. The detection rate of interictal pathology using EEG ranges between 19% and 84% [9]. A clinical study conducted in a neonatal population reported that only 48 (27%) of 177 clinically suspected seizure episodes had a corresponding electrographic seizure [10].

Routine EEG has an initial sensitivity of approximately 50%, which increases to 82–92% with repeated studies [11,12]. Extended EEG recordings can further enhance sensitivity. One study found that only 36% of patients exhibited IEDs within the first 20 min of long-term EEG monitoring, whereas 89% exhibited IEDs within the first 24 h [13]. Another study comparing repeated 30-min EEGs with extended 2-h EEGs found similar diagnostic accuracy in patients with previously normal routine EEGs [14]. In the present study, neither repeated routine EEG nor prolonged awake EEG was requested for patients with normal routine EEGs.

Sleep recordings play a crucial role in epilepsy diagnosis, particularly for patients with suspected epilepsy. EEG recordings after sleep deprivation or during sleep are frequently employed. Various activation methods are used to enhance the detection of epileptic pathology [15]. Numerous studies have demonstrated that sleep EEG recordings significantly improve diagnostic accuracy by triggering epileptiform discharges when routine awake EEG results are normal or inconclusive [4,5,6,7,16,17]. In this study, retrospective evaluation of sleep EEGs recorded within previously specified timeframe revealed that clinicians often opted for sleep EEG as a secondary diagnostic tool following normal routine EEG results.

A study by Meritam et al. [18] in 2018 reported abnormal findings in the sleep EEG recordings of 35 out of 156 patients (22%) with normal routine EEGs. Another study found IEDs in 41.1% of routine EEGs and 56.2% of all-night sleep EEGs [17]. Pratt et al. [19] examined 14 epilepsy patients with normal EEGs and identified pathology in 41% of patients after performing a second EEG recording following 24–26 h of sleep deprivation. Similarly, Bechinger et al. [20] found an activation rate of 37%, Scollo-Lavizzari et al. [21] reported 47%, Roby et al. [22] found 33%, Schwarz and Zangemeister [23] reported 38%, Arne-Bes et al. [24] found 41%, and Degen et al. [25] observed 57%. Rodin et al. [9] summarized a literature review indicating an activation incidence of approximately 45% following sleep deprivation. In this study, 18% of patients with normal routine EEGs exhibited pathological findings in their sleep EEGs. Among those with pathological findings, 88.5% had epilepsy, and 80.8% were on antiseizure medication.

The discrepancy between our findings and those reported in the literature regarding the detection of pathological sleep EEG findings may be attributed to the inclusion of not only epilepsy-diagnosed patients but also those suspected of having epilepsy, the relatively short duration of sleep EEG recordings compared to certain studies, and possibly insufficient selectivity during the sleep EEG request process. Previous work has also emphasized that the clinical utility of EEG depends strongly on appropriate patient selection and indication, which may partly explain differences in diagnostic yield across cohorts and settings [26].

In the literature, EEG recordings during sleep, wakefulness, and combined wakefulness and sleep have been comparatively evaluated. Some studies have classified patients undergoing sleep EEG based on epilepsy type and analyzed IEDs according to these classifications [5,6,7,18,27-30].

Limitation

Limitations of the study include its single-center design and retrospective nature. Increasing the sample size and conducting subgroup analyses based on factors such as seizure type and antiseizure medication use could yield more robust and high-quality results.

Conclusion

The findings of this study suggest that in patients suspected of epilepsy based on clinical history, performing an EEG after 24 h of sleep deprivation, incorporating both wakefulness and sleep recordings, may be a more practical and time-efficient approach for detecting pathology and guiding treatment compared to routine EEG. By incorporating sleep EEG into routine diagnostic algorithms, clinicians can increase diagnostic accuracy, enhance early intervention strategies, and improve individualized treatment approaches, especially in patients who experience nocturnal seizures, those with a high clinical suspicion of epilepsy, drug-resistant epilepsy patients, and those in whom routine EEG is inconclusive.

Disclosure

Ethics Committee Approval: This study was approved by the Haydarpaşa Numune Training and Research Hospital Ethics Committee (Date: 25.09.2023, Decision no: HNEAH-KAEK 2023/175).

Informed Consent: Informed consent has been obtained from the patients.

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References

1. Kane N, Acharya J, Benickzy S, Caboclo L, Finnigan S, Kaplan PW, et al. A revised glossary of terms most commonly used by clinical electroencephalographers and updated proposal for the report format of the EEG findings. Revision 2017. Clin Neurophysiol Pract 2017;2:170–85. Erratum in: Clin Neurophysiol Pract 2019;4:133. [CrossRef]
2. Benbadis SR, Tatum WO, Vale FL. When drugs don't work: an algorithmic approach to medically intractable epilepsy. Neurology 2000;55:1780–4. [CrossRef]
3. Benbadis SR. What type of EEG (or EEG-video) does your patient need? Expert Rev Neurother 2015;15:461–4. [CrossRef]
4. Adachi N, Alarcon G, Binnie CD, Elwes RD, Polkey CE, Reynolds EH. Predictive value of interictal epileptiform discharges during non-REM sleep on scalp EEG recordings for the lateralization of epileptogenesis. Epilepsia 1998;39:628–32. [CrossRef]
5. Niedermeyer E. Sleep and EEG. In: Niedermeyer E, da Silva FL, editors. Electroencephalography. Basic principles, clinical applications and related fields. 3rd ed. Williams & Wilkins; 1993. p.153–65.
6. Jovanovic UJ. General considerations of sleep and sleep deprivation. In: Degen R, Rodin EA, editors. Epilepsy, Sleep and Sleep Deprivation. 2nd ed. Amsterdam: Elsevier; 1991. p.205–15.
7. Gunderson CH, Dunne PB, Feyer TL. Sleep deprivation seizures. Neurology (Minneapolis) 1973;23:678–86. [CrossRef]
8. Bennett DR, Mattson RH, Ziter FA, Calverley JR, Liske EA, Pratt KL. Sleep deprivation: neurological and electroencephalographic effects. Aerosp Med 1964;35:888–90.
9. Rodin EA, Luby ED, Gottlieb JS. The electroencephalogram during prolonged experimental sleep deprivation. Electroencephalogr Clin Neurophysiol 1962;14:544–51. [CrossRef]
10. Acar DB, Bulbul A, Uslu S. Current overview of neonatal convulsions. Sisli Etfal Hastan Tip Bul 2019;53:1–6.
11. Marsan CA, Zivin LS. Factors related to the occurrence of typical paroxysmal abnormalities in the EEG records of epileptic patients. Epilepsia 1970;11:361–81. [CrossRef]
12. Salinsky M, Kanter R, Dasheiff RM. Effectiveness of multiple EEGs in supporting the diagnosis of epilepsy: an operational curve. Epilepsia 1987;28:331–4. [CrossRef]
13. Narayanan JT, Labar DR, Schaul N. Latency to first spike in the EEG of epilepsy patients. Seizure 2008;17:34–41. [CrossRef]
14. Foley CM, Legido A, Miles DK, Chandler DA, Grover WD. Long-term computer-assisted outpatient electroencephalogram monitoring in children and adolescents. J Child Neurol 2000;15:49–55. [CrossRef]
15. Mahuwala Z, Ahmadi S, Bozoky Z, Hays R, Agostini M, Ding K. Diagnostic yield of 2-hour EEG is similar with 30-minute EEG in patients with a normal 30-minute EEG. J Clin Neurophysiol 2019;36:204–8. [CrossRef]
16. Giorgi FS, Perini D, Maestri M, Guida M, Pizzanelli C, Caserta A, et al. Usefulness of a simple sleep-deprived EEG protocol for epilepsy diagnosis in de novo subjects. Clin Neurophysiol 2013;124:2101–7. [CrossRef]
17. Roupakiotis SC, Gatzonis SD, Triantafyllou N, Mantouvalos V, Chioni A, Zournas C, et al. The usefulness of sleep and sleep deprivation as activating methods in electroencephalographic recording: contribution to a long-standing discussion. Seizure 2000;9:580–4. [CrossRef]

18. Meritam P, Gardella E, Alving J, Terney D, Cacic Hribljan M, Beniczky S. Diagnostic yield of standard-wake and sleep EEG recordings. Clin Neurophysiol 2018;129:713–6. [CrossRef]
19. Pratt KL, Mattson RH, Weikers NJ, Williams R. EEG activation of epileptics following sleep deprivation: a prospective study of 114 cases. Electroencephalogr Clin Neurophysiol 1968;24:11–5. [CrossRef]
20. Bechinger D, Kriebel J, Schlager M. EEG following sleep deprivation--an important tool for diagnosis of epileptic seizures (author's transl). Z Neurol 1973;205:193–206. [Article in German] [CrossRef]
21. Scollo-Lavizzari G. Continuous EEG and EMG recordings during night sleep in a case of subacute sclerosing leucoencephalitis. Electroencephalogr Clin Neurophysiol 1968;25:170–4. [CrossRef]
22. Roby DS, Greenberg JO. Sleep deprivation and electroencephalographic abnormalities. J Clin Psychiatry 1978;39:542–3.
23. Schwarz JR, Zangemeister WH. The diagnostic value of the short sleep EEG and other provocative methods following sleep deprivation. J Neurol 1978;218:179–86. [CrossRef]
24. Arne-Bes MC, Calvet U, Thiberge M, Arbus L. Effects of sleep deprivation in an EEG study of epileptics. In: Sterman MB, Shouse MN, Passouant P, editors. Sleep and epilepsy. New York: Academic Press; 1982. p.441–52.
25. Degen R, Degen HE. The diagnostic value of the sleep EEG with and without sleep deprivation in patients with atypical absences. Epilepsia 1983;24:557–66. [CrossRef]
26. Agircan D, Bal M, Cekic M, Demir TG, Gocmen A, Ethemoglu O. From request to result: the clinical significance of EEG in a tertiary hospital in Turkey. Acta Medica Nicomedia 2024;7:288–92. [CrossRef]
27. Özer İŞ, Ağırcan C, Ertürk AY, Öztura İ, Baklan B. Epilepsi hastalarında rutin, bir saatlik ve tüm gece EEG'nin İED saptamaya katkısı. 38. Ulusal Klinik Nörofizyoloji EEG-EMG Kongresi; Muğla, Türkiye. 2022. p.1–2. [In Turkish]
28. Landau-Ferey J. A contribution to the study of nocturnal sleep in patients suspected of having epilepsy. Sleep Epilepsy 1982;32:421–9.
29. Chokroverty S. Sleep Disorders Medicine. An overview of sleep. Sleep EEG in adult. USA: Butterworth-Heinemann; 1995. p.429–53. [CrossRef]
30. Turanlı G, Allahverdiev A, Topçuoğlu MA, Yuzasif K, Sümer M, Renda Y. Uyku ve epilepsi. Türk Nörol Derg 1996;2:30–8. [Article in Turkish]

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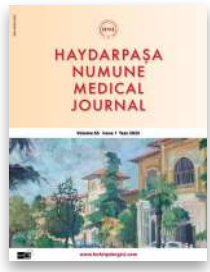

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ORIGINAL ARTICLE

The Effect of Demographic Characteristics, Psychological Traits, and Smoking Cessation Treatments on Smoking Cessation Success

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Abstract

Introduction: This study aimed to evaluate the effects of demographic factors, psychological profiles, and treatment modalities on smoking cessation outcomes.

Methods: A total of 181 patients who completed a 3-month follow-up at a smoking cessation clinic were included. Data collected comprised demographic characteristics, psychological assessments (General Self-Efficacy Scale [GSES], Hospital Anxiety and Depression Scale [HADS], and Fagerström Test for Nicotine Dependence [FTDN]), and treatment approaches, including nicotine replacement therapy (NRT), bupropion, varenicline, and cognitive behavioral therapy (CBT).

Results: Successful cessation was defined as continuous abstinence during follow-up. No significant association was found between cessation success and age, gender, smoking history, or motivation to quit. However, higher education levels and the absence of chronic illness were positively associated with success. Patients with chronic diseases exhibited lower GSES scores. Pharmacological treatments were more effective than CBT alone. Among pharmacological options, varenicline and NRT yielded higher success rates than bupropion, with no significant difference between varenicline and NRT. A negative correlation was observed between GSES and HADS scores.

Discussion and Conclusion: Educational attainment, absence of chronic illness, and pharmacological treatment were associated with higher smoking cessation success. Chronic diseases appear to reduce self-efficacy, and increased anxiety and depression may negatively impact cessation outcomes.

Keywords: Bupropion; cigarette smoking; self-efficacy; smoking cessation agents; smoking cessation; varenicline.

Tobacco use continues to be the primary cause of preventable morbidity and mortality globally [1]. This study aims to examine the factors affecting smoking cessation success and evaluate the effectiveness of different treatment protocols. The factors influencing smoking cessation success were evaluated under three main categories: descriptive characteristics, psychological characteristics, and pharmacological treatments applied. This study aims to explore the underlying causes of tobacco addiction, identify factors that facilitate or hinder smoking cessation, and evaluate the effectiveness of various treatment approaches.

Materials and Methods

This descriptive, cross-sectional study was conducted between March 15 and June 15, 2019, following ethics com-

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mittee approval. The study was approved by the Ethics Committee of Haydarpasa Numune Training and Research Hospital (approval date: March 18, 2019; decision number: HNEAH-KAEK 2019/29 [HNEAH KAEK 2019/KK/29]). The study was carried out in accordance with the ethical principles of the Declaration of Helsinki. A total of 210 patients who visited a smoking cessation outpatient clinic were enrolled, with 181 completing a 3-month follow-up. Exclusion criteria included psychiatric disorders, smoking duration under 2 years, and age under 18 or over 70.

Data were collected through face-to-face interviews using a structured demographic form, the Hospital Anxiety and Depression Scale (HADS), the Generalized Self-Efficacy Scale (GSES), and the Fagerström Test for Nicotine Dependence (FTND). Demographic variables included age, gender, education, marital and employment status, medical history, smoking habits, and cessation motivation. GSES assessed self-efficacy across three subdomains: initiation, persistence, and maintenance. FTND measured nicotine dependence, whereas HADS screened for anxiety and depression risk. Participants provided informed consent before assessment.

Smoking cessation success – defined as total abstinence during the 3-month period – was verified through clinic visits or follow-up phone interviews.

Statistical Analysis

All data were analyzed using IBM Statistical Package for the Social Sciences Statistics version 22. Descriptive statistics were presented as means and standard deviations for continuous variables and as frequencies and percentages for categorical variables. The Chi-square test was used to compare categorical variables between groups. The Kolmogorov–Smirnov test was applied to assess the normality of distribution for continuous variables. Independent samples t-test or Mann–Whitney U test was used for comparing continuous variables depending on the distribution. Binary logistic regression analysis was conducted to determine the predictors of smoking cessation success. P<0.05 was considered statistically significant.

Results

The findings were examined in three sections: Descriptive characteristics in the first section, psychological factors in the second section, and the smoking cessation treatment applied in the final section.

Evaluation of the Impact of Descriptive Characteristics on Smoking Cessation Success

When evaluating the smoking cessation status of all cases, among the 181 participants included in the study, 96 quit smoking, and 85 did not quit. The smoking cessation success rate in our study was 53%. The mean age of all cases was 40.21±12.55 years. The mean age of those who quit smoking was 40.81±12.34 years, whereas the mean age of those who did not quit is 39.53±12.81 years, with no statistically significant difference between the two groups (p=0.494).

The smoking cessation rate among individuals with a university or college education was significantly higher compared to those with only a primary school education (p=0.035). Smoking cessation success was found to be significantly higher in individuals without chronic diseases compared to those with chronic diseases, regardless of medication use (p=0.005).

The smoking cessation success rate among individuals without chronic diseases who use only dietary supplements was found to be significantly higher compared to those with chronic diseases who use medication (p_1=0.004) and those with chronic diseases who do not use any medication (p_2<0.001). The smoking cessation rate of individuals with both chronic diseases and regular medication use was found to be statistically significantly higher compared to those with chronic diseases but no medication use (p=0.022).

The detailed distribution and statistical analysis of demographic characteristics, presence of chronic diseases, regular medication or supplement use, and smoking cessation status are presented in Table 1.

The most common reason for visiting the smoking cessation clinic was personal choice (71.3%), followed by health-related concerns (15.5%) and doctor's recommendation (13.3%). Although no statistically significant difference was found, it was observed that the smoking cessation success rate was higher at 75% for those who visited the clinic upon a doctor's recommendation, compared to those who applied due to personal (50.4%) or health reasons (46.4%) (p=0.064).

Of the 18 individuals who visited the smoking cessation clinic upon a physician's recommendation, 7 (38.89%) had a chronic disease. In the subgroup analysis of participants with chronic conditions, no statistically significant difference was observed in smoking cessation success based on the reason for application (p=0.548). Nevertheless, among this group, those who were referred by a physician exhibited the highest quit rate (53.8%), followed by individuals who applied due to health concerns (41%) and those who applied by personal choice (35%).

Regarding the presence of an active smoker in the household, 43.6% of participants reported such exposure. The quit rate among those with an active smoker at home was 55.7%,

Table 1. Distribution and statistical analysis of demographic characteristics, presence of chronic diseases, regular medication or supplement use, and smoking cessation status

	Smoking cessation status		p
	Quit *n* (%)	Did not quit *n* (%)	
Gender			
Female	43 (50)	43 (50)	0.436
Male	53 (55.8)	42 (44.2)	
Marital status			
Married	62 (55.4)	50 (44.6)	0.426
Single	34 (49.3)	35 (50.7)	
Educational level			
Primary/High School	54 (47.4)	60 (52.6)	**0.035**
College/University	42 (63.6)	24 (36.4)	
Employment status			
Employed	62 (54.4)	52 (45.6)	0.636
Unemployed	34 (50.7)	33 (49.3)	
Presence of chronic disease			
Yes	31 (40.8)	45 (59.2)	**0.005**
No	65 (61.9)	40 (38.1)	
Regular medication use (excluding supplements)			
Yes	41 (53.9)	35 (46.1)	0.835
No	55 (52.4)	50 (47.6)	
Those with chronic diseases who do not use medication	1 (8.3)	11 (91.7)	**0.001**
Those with chronic diseases who use medication	30 (46.9)	34 (53.1)	
Those who do not have a disease but take supplements	11 (91.7)	1 (8.3)	

Chi-square test

Table 2. Evaluation of the reasons for visiting the smoking cessation clinic and the presence of active smokers in the family

	Smoking cessation status		p
	Quit *n* (%)	Did not quit *n* (%)	
Reason			
Doctor recommendation	18 (75)	6 (25)	0.064
Personal choice	65 (50.4)	64 (49.6)	
Health reasons	13 (46.4)	15 (53.6)	
Active smoker in the family			
Yes	44 (55.7)	35 (44.3)	0.528
No	52 (51)	50 (49)	

Chi-square test

Table 3. Comparison of smoking cessation status according to follow-up attendance

	Smoking cessation status		p
	Quit *n* (%)	Did not quit *n* (%)	
Follow-up attendees	79 (58.5)	56 (41.5)	**0.018**
Non-attendees	17 (37)	29 (63)	

Chi-square test

compared to 51% in those without. However, this difference was not statistically significant (p=0.528) (Table 2).

Smoking cessation rates differed significantly according to follow-up attendance; the cessation rate was 58.5% among follow-up attendees and 37.0% among non-attendees (p=0.018) (Table 3).

As shown in Table 4, the mean age at smoking initiation was 18.49±4.98 years (range: 6–37), with no significant difference between those who quit and those who did not. Similarly, the mean duration of smoking was 20.31±12.25 years (range: 2–55), and the mean number of cigarettes smoked per day was 22.18±9.34 (range: 2–60). None of these variables showed a significant association with smoking cessation outcomes.

As shown in Table 4, the most frequently cited motivations for smoking cessation were fear of future illness (55.2%) and existing disease (31.5%). The highest quit rates were observed among participants who cited physician recommendation (66.7%) and the desire to set an example (66.7%). However, these differences did not reach statistical significance (p=0.272 and p=0.331, respectively). Other reasons – including environmental concerns, economic factors, social pressure, and media influence – showed no significant association with cessation success (all p>0.05).

The information regarding the age of smoking initiation, smoking duration, daily cigarette consumption, and reasons for wanting to quit, along with their statistical analyses based on smoking cessation status, is displayed in Tables 4 and 5.

Evaluation of the Impact of Psychological Factors on Smoking Cessation Success

The mean anxiety and depression scores were 7.49±4.12 and 6.57±4.12, respectively. When HADS scores were compared according to smoking cessation status, no statistically significant differences were observed between individ-

Table 4. Evaluation of the age of smoking initiation, smoking duration, and the number of cigarettes smoked daily

	Smoking cessation status		p
	Quit	Did not quit	
	Q1-Q3 (median)	Q1-Q3 (median)	
Age at smoking initiation	16–20 (18)	15–20 (18)	0.911
Duration of smoking (years)	10–28 (20)	10–30 (17)	0.448
Daily cigarette consumption	20–25 (20)	20–30 (20)	0.167

Mann–Whitney U test

uals who quit and those who did not in terms of overall, anxiety, or depression scores. Using the clinical cutoff of ≥11 to define cases, the proportions of participants with anxiety or depression did not differ significantly between the groups (p>0.05). The evaluation of HADS scores and smoking cessation status is shown in Tables 6 and 7.

The mean score on the FTND among participants was 6.09±2.43 (range: 0–10). Analysis of smoking cessation outcomes based on dependence levels revealed the highest quit rate in the moderate dependence group (69.6%), followed by the very low (58.3%), low (55.1%), and high (51.0%) dependence groups. The lowest cessation rate was observed in the very high dependence group (46.7%). However, these differences were not statistically significant (p=0.444).

Table 5. Evaluation of the reasons for wanting to quit smoking

	Smoking cessation status			p
	Overall *n* (%)	Quit *n* (%)	Did not quit *n* (%)	
Due to a disease	57 (31.5)	29 (50.9)	28 (49.1)	0.693
Due to a thought of future illness	100 (55.2)	55 (55)	45 (45)	0.557
Because it harms the environment	59 (32.6)	35 (59.3)	24 (40.7)	0.239
Due to economic reasons	44 (24.3)	20 (45.5)	24 (54.5)	0.247
Media exposure	3 (1.7)	1 (33.3)	2 (66.7)	0.490
To set an example for others	18 (9.9)	12 (66.7)	6 (33.3)	+0.331
Because of the unpleasant smell	42 (23.2)	23 (54.8)	19 (45.2)	0.798
Due to the doctor's recommendation	21 (11.6)	14 (66.7)	7 (33.3)	+0.272
Due to social pressure	12 (6.6)	5 (41.7)	7 (58.3)	+0.605
Other reasons	11 (6.1)	5 (45.5)	6 (54.5)	+0.835

Chi-square test, +Continuity (Yates) correction

Table 6. Mean and standard deviation values of HADS scores by smoking cessation status

	Smoking cessation status	
	Quit Mean±SD	Did not quit Mean±SD
Overall	18.47±4.97	18.50±5.01
Anxiety	7.21±4.30	7.81±3.89
Depression	6.49±4.34	6.74±3.81

SD: Standard deviation, HADS: Hospital anxiety and depression scale

Table 7. Comparison of anxiety and depression levels (≥11) according to smoking cessation status

	Score	Smoking cessation status		p
		Quit (*n*=96) *n* (%)	Did not quit (*n*=85) *n* (%)	
Anxiety	≥11	21 (21.9)	23 (27.1)	0.524
Depression	≥11	17 (17.7)	12 (14.1)	0.650

Chi-square test

The mean FTND score of the cases was 6.09±2.43 (range: 0–10). As shown in Table 8, although quit rates varied across different levels of nicotine dependence, these differences were not statistically significant (p>0.05).

The total score of the GSES and its three factors – willingness to initiate behavior, willingness to expend effort in completing the behavior, and persistence in the face of adversity – were evaluated separately. The minimum, maximum, mean, and standard deviation values of the total scale and subdimension scores, along with the statistical evaluation, are presented in Table 9.

Table 8. Evaluation of smoking cessation status according to FTND scores

	Smoking cessation status		p
	Quit *n* (%)	Did not quit *n* (%)	
Very low	7 (58.3)	5 (41.7)	0.444
Low	20 (55.1)	17 (45.9)	
Moderate	16 (69.6)	7 (30.4)	
High	25 (51.0)	24 (49.0)	
Very high	28 (46.7)	32 (53.3)	

Chi-square test. FTND: Fagerström test for nicotine dependence

Table 9. Evaluation of the total GSES score and its three factor scores based on smoking cessation status

	Quit	Did not quit	p
	Mean±SD	Mean±SD	
Total GSES	53.49±9.37	52.98±7.96	0.694
Willingness to initiate behavior	28.56±5.82	27.81±5.19	0.363
Willingness to expend effort in completing the behavior	15.69±3.02	16±2.79	0.473
Persistence in the face of adversity	9.24±2.09	9.16±2.12	0.812

Student's t-test. GSES: General self-efficacy scale, SD: Standard deviation

When evaluating the two most common reasons for smoking cessation in terms of the total GSES score, the mean total GSES score of individuals who wanted to quit smoking due to an existing illness was significantly lower than that of those whose reason for quitting was not illness (p=0.049) (Table 10).

There was a weak negative correlation between the GSES total score and HADS-Anxiety (r=−0.195, p=0.009) and a weak-to-moderate negative correlation with HADS-Depression (r=−0.279, p<0.001). No correlation was observed between the GSES total score and FTND (r=0.006, p=0.934). According to conventional effect-size benchmarks (Cohen: r≈0.10=small, 0.30=medium, 0.50=large), the associations with anxiety and depression are of small magnitude (Table 11).

Evaluation of the Effect of Pharmacological and Non-Pharmacological Treatment Methods on Smoking Cessation Success

The smoking cessation rate among individuals receiving pharmacological treatment was found statistically significantly higher than that of those receiving non-pharmacological treatment (p<0.001) (Table 12). The smoking cessation rate among individuals receiving cognitive behavioral therapy (CBT) was statistically significantly lower than those receiving varenicline, bupropion, and nicotine replacement therapy (NRT) (p<0.001). The statistical significance was found as follows: p_1<0.001, p_2<0.001, p_3=0.026. The smoking cessation rate of individuals using bupropion was found statistically significantly lower than those using varenicline and NRT (p_1=0.017, p_2=0.020, p<0.05).

Logistic regression analysis was performed to identify independent predictors of smoking cessation success and to control for potential confounding factors among the studied variables. A series of univariate logistic regression analyses was conducted to identify individual factors associated with smoking cessation (Table 12). Pharmacological treatment was the strongest predictor of quitting; in addition, higher education level and attending follow-up visits were associated with smoking cessation. In contrast, having a chronic disease and ap-

Table 10. Evaluation of individuals who want to quit smoking due to illness and/or concern about future illness according to GSES

	Total GSES	p
	Mean±SD	
Desire to quit smoking due to illness		
No	54.11±8.48	**0.049**
Yes	51.37±9.01	
Desire to quit smoking due to the thought of future illness		
No	54.34±7.97	0.128
Yes	52.36±9.23	

Student's t-test. GSES: General self-efficacy scale, SD: Standard deviation

Table 11. Evaluation of the relationship between GSES, HADS, and FTND scores

	Total GSES	
	r	p
HADS anxiety	−0.195	**0.009**
HADS depression	−0.279	**<0.001**
FTND	0.006	0.934

Pearson correlation analysis. HADS: Hospital anxiety and depression scale, FTND: Fagerström test for nicotine dependence, GSES: General self-efficacy scale

Table 12. Evaluation of smoking cessation status based on the treatment method used

	Smoking cessation status		p
	Quit n (%)	Not quit n (%)	
Treatment used			
Varenicline	41 (78.8)	11 (21.2)	[1]<0.001
NRT	41 (77.4)	12 (22.6)	
Bupropion	6 (42.9)	8 (57.1)	
Cognitive behavioral therapy	8 (12.9)	54 (87.1)	
Treatment type			
Pharmacological	88 (73.9)	31 (26.1)	[2]<0.001
Non-pharmacological	8 (12.9)	54 (87.1)	

[1]Chi-square test, [2]Continuity (Yates) correction. NRT: Nicotine replacement therapy

Table 13. Univariate logistic regression results

Variable	OR	95% CI	p
Follow-up attendees	2.407	1.207–4.796	**0.0126**
Age	1.008	0.985–1.032	0.4915
Gender	0.831	0.463–1.491	0.5344
Marital status	1.276	0.700–2.329	0.4262
Educational level	1.977	1.062–3.678	**0.0315**
Employment status	1.157	0.632–2.118	0.6358
Presence of chronic disease	0.424	0.232–0.775	**0.0053**
Active smoker in the family	1.209	0.670–2.180	0.5286
Reason (personal choice)*	0.339	0.126–0.908	**0.0314**
Reason (health reasons)*	0.289	0.088–0.945	**0.0401**
Age at smoking ınitiation	1.001	0.944–1.062	0.9615
Duration of smoking (years)	1.007	0.983–1.031	0.5886
Daily cigarette consumption	0.973	0.942–1.005	0.0968
HADS-anxiety score	0.755	0.382–1.491	0.4178
HADS-depression score	1.309	0.586–2.927	0.5118
Fagerström score	0.916	0.810–1.035	0.1587
Total GSES score	1.007	0.974–1.041	0.6921
Pharmacological treatment	19.161	8.207–44.735	**<0.001**

OR: Odds ratio, CI: Confidence interval, (*) Reference category: Doctor's recommendation, GSES: General self-efficacy scale, HADS: Hospital anxiety and depression scale

Table 14. Multivariate logistic regression results

Variable	OR	95% CI	p
Follow-up attendees	1.295	0.514–3.260	0.5832
Age	1.004	0.908–1.112	0.9311
Gender	0.801	0.304–2.110	0.6531
Marital status	1.556	0.603–4.018	0.3608
Educational level	2.868	1.106–7.439	**0.0303**
Employment status	0.742	0.294–1.872	0.5275
Presence of chronic disease	0.452	0.186–1.102	0.0807
Active smoker in the family	1.838	0.772–4.377	0.1690
Reason (personal choice)*	0.368	0.098–1.382	0.1387
Reason (health reasons)*	0.174	0.037–0.814	**0.0263**
Age at smoking ınitiation	0.965	0.848–1.097	0.5820
Duration of smoking (years)	1.006	0.911–1.111	0.9063
Daily cigarette consumption	0.976	0.918–1.036	0.4204
HADS-anxiety score	0.555	0.188–1.641	0.2873
HADS-depression score	2.614	0.706–9.677	0.1503
Fagerström score	0.960	0.755–1.220	0.7371
Total GSES score	1.026	0.976–1.078	0.3136
Pharmacological treatment	23.783	8.873–63.744	**<0.001**

OR: Odds ratio, CI: Confidence interval, (*) Reference category: Doctor's recommendation. GSES: General self-efficacy scale, HADS: Hospital anxiety and depression scale

plying to the program voluntarily or for health-related reasons (compared to a doctor's recommendation) were associated with lower odds of quitting. A marginal association was observed for daily cigarette consumption. No statistically significant relationships were found for age, age at smoking initiation, duration of smoking, gender, marital status, employment status, presence of smokers in the family, anxiety, depression, Fagerström score, or Total GSES score (Table 13).

In the multivariate logistic regression analysis (Table 14), after controlling for potential confounding variables, pharmacological treatment (odds ratio [OR]=23.783, 95% confidence interval [CI]: 8.873–63.744, p<0.001) and higher educational level (OR=2.868, 95% CI: 1.106–7.439, p=0.0303) remained independently associated with smoking cessation success. Furthermore, quitting for health reasons was also found to be a significant predictor of success (OR=0.174, 95% CI: 0.037–0.814, p=0.0263). Other variables, including age, gender, marital status, presence of chronic disease, and psychological scale scores, were not significantly associated with smoking cessation in the adjusted model.

Discussion

Various background factors may influence the success of smoking cessation. For instance, younger individuals often perceive smoking as a means of socialization, whereas older individuals may believe that quitting will not lead to significant health improvements. While men are more likely to have social relationships with other smokers [2], women are affected by hormonal cycles such as menstruation and menopause [3]. Therefore, when evaluating demographic characteristics, we believe that a more individualized and detailed analysis is warranted.

Studies on the impact of education level on smoking cessation success yield conflicting results. While some suggest no significant effect [4,5], others show a positive association between higher education and increased cessation rates [6]. This may be due to greater awareness of smoking's harms and treatment effectiveness among more educated individuals. In our logistic regression analysis, higher educational level remained an independent predictor of smoking cessation success after adjusting for potential confounders. This finding supports the notion that educational attainment enhances individuals' health literacy and

engagement with cessation programs, ultimately facilitating better outcomes.

Although it is thought that individuals with chronic diseases are more successful in the smoking cessation process due to their motivation to regain lost health, many studies, including ours, demonstrate the opposite. Studies have shown that smoking cessation rates are lower in patients diagnosed with chronic obstructive pulmonary disease compared to those without the diagnosis [7] and that individuals with lung cancer make fewer attempts to quit smoking [8].

In our study, although the referral rate by physicians among individuals with chronic diseases was low (16.7%), smoking cessation success was higher in those referred by physicians (53.8%) compared to those who applied on their own (35%) or due to health concerns (41%). Among all participants, those referred by a physician achieved the highest quit rate of 75% (p=0.064). This finding aligns with existing literature suggesting that brief but effective counseling by healthcare professionals can significantly improve cessation outcomes.[9] These results underscore the importance of systematic and structured physician referrals as an effective and sustainable strategy in tobacco control, especially in motivating behavior change among patients with chronic diseases.

In addition, participants who attended at least one follow-up control visit demonstrated significantly higher cessation success compared to those who did not. Regular follow-up visits likely enhance motivation, reinforce behavioral change, and provide opportunities for physicians to adjust treatment and address withdrawal symptoms promptly. These findings emphasize the critical role of continued follow-up and active monitoring within primary care settings to sustain smoking abstinence.

In our study, individuals with chronic diseases demonstrated lower self-efficacy perceptions. Smoking is one of the major factors contributing to the development of chronic diseases and may also lead to a decrease in self-efficacy perception. A low self-efficacy perception leads individuals to become more easily overwhelmed by hopelessness, decreasing their motivation and resilience to overcome challenges [10]. In summary, chronic diseases, smoking, and low self-efficacy perception reinforce each other, creating a vicious cycle of cause and effect. Approaches aimed at increasing individuals' self-efficacy perception can break this cycle and strengthen smoking cessation success.

On the other hand, another important reason for the low smoking cessation success in individuals with chronic diseases may be their lack of adequate attention to their health condition. Indeed, in our study, the highest smoking cessation success was observed in individuals who, even without a chronic disease diagnosis, aimed to improve their health using supplements such as vitamins, ginseng, and zinc, and gave utmost importance to their health. Conversely, the lowest smoking cessation success was found in individuals with chronic diseases who did not even comply with the medical treatments they needed, supporting this idea.

The presence of smokers within a family can negatively affect not only the behavioral patterns but also the physical health of other members. For example, a study conducted on participants with smoking spouses demonstrated that an increase in the spouse's daily cigarette consumption was significantly associated with a decline in the participants' pulmonary function test results [11]. This situation, which serves as a negative role model especially for children and adolescents, may also increase access to cigarettes, thereby making smoking more appealing [12]. Conversely, individuals who directly observe the harmful consequences of smoking, such as coughing or cancer, are more likely to avoid smoking. However, existing studies predominantly focus on the adverse health effects of smoking.

Self-efficacy belief represents an individual's belief in their ability to intervene in their personal life, environmental events, and emotional state [13]. People with high self-efficacy believe they can control potential threats, whereas those with low self-efficacy believe they are unable to manage such threats and experience high levels of anxiety [13]. According to the social cognitive relapse model, a person's ability to resist smoking while coping with withdrawal symptoms is directly related to their level of self-confidence [14].

In line with this view, some studies have shown that individuals diagnosed with depression use the positive reinforcing effects of smoking to manage their negative moods, resulting in significantly higher levels of smoking behavior compared to others, making them more likely to be unsuccessful in quitting smoking [15-17].

In accordance with these views, our study found that individuals with low self-efficacy beliefs exhibited higher levels of depression and anxiety, and the amount of cigarettes smoked per day was significantly higher compared to others. These findings suggest that self-efficacy, depression, and smoking behavior are intertwined and influence each other. This supports the Bayesian network model, which suggests that psychological factors are interrelated and that a generalized study may not provide accurate results regarding smoking cessation success [18].

In the "maintenance" stage of the transtheoretical model of behavior change, individuals demonstrate high self-confidence in sustaining smoking abstinence, having successfully completed the action stage [19]. Increased self-confi-

dence can reduce the anxiety and hopelessness individuals may have regarding their inability to quit smoking, boosting their motivation and self-efficacy, which positively influences their ability to sustain smoking cessation. However, excessively high self-confidence and self-efficacy may lead individuals to view nicotine dependence as a problem that can be easily overcome, which could reduce the seriousness with which they treat the issue, delay their smoking cessation efforts, or cause them to transition between stages of change. The maintenance stage is one where transitions between lapses, relapses, and other stages of change can occur, and relapse is one of the major challenges in tobacco control [20].

Contrary to most previous studies, our findings did not reveal a significant association between FTND scores and smoking cessation success. This may be attributed to the limited sample size, potential limitations of the FTND scale in capturing the multifaceted aspects of nicotine dependence, and the homogeneity of the study population, leading to reduced variability in FTND scores. Given the positive correlations reported in numerous studies, further research involving more diverse populations is warranted.

Smoking cessation treatment is a specialized intervention that includes pharmacological therapy as well as CBT [21]. Consistent with our findings, numerous studies have demonstrated that pharmacological interventions significantly enhance smoking cessation success and are more effective than CBT alone [22]. Among these pharmacological treatments, varenicline has been shown to yield higher cessation success rates compared to bupropion or NRT [23,24]. According to the Turkish Medicines and Medical Devices Agency (TMMDA), varenicline is currently authorized for use in Türkiye under the trade name Dupons, manufactured by Abdi İbrahim Pharmaceuticals Inc. It is listed among the actively marketed medicinal products (TMMDA, 2025) [25].

No statistically significant difference in smoking cessation success was observed between varenicline and NRT in our study, contrary to existing literature. This may be due to a limited sample size and unmeasured treatment adherence. Further research with larger samples and adherence assessment is needed.

Cytisine, similar to varenicline, acts as a competitive antagonist of the α4β2 nicotinic acetylcholine receptor, thereby reducing nicotine binding to the receptor [26]. It is the most recently introduced smoking cessation treatment in our country. Access to novel therapies, such as cytisine, for smoking cessation will be facilitated through further research.

Limitation

The limitations of this study include a small sample size, a lack of treatment adherence assessment, and limited generalizability due to the homogeneous nature of the participants. In addition, psychological assessments may have been affected by individual variability.

Conclusion

Education positively influences the decision to quit smoking; therefore, all individuals, regardless of varying smoking patterns or challenges, should be encouraged to quit.

The presence of chronic illness reduces self-efficacy beliefs, which in turn decreases the motivation to quit smoking and negatively impacts the process. Individuals with high HADS scores often have low self-efficacy beliefs, so when approaching them, doctors should focus on a motivating approach rather than discussing the negative effects of smoking. FTND and HADS can aid treatment selection, but further research is needed to better predict cessation success.

Pharmacological therapies are critical to effective cessation and should be confidently used when not contraindicated. Enhancing healthcare providers' knowledge of these treatments and expanding access through more cessation clinics is essential. Ongoing scientific research is vital to better understand smoking addiction and improve intervention strategies.

Disclosure

Ethics Committee Approval: This study was approved by the Haydarpaşa Numune Training and Research Hospital Ethics Committee (Date: 18.03.2019, Decision no: HNEAH-KAEK 2019/29 [HNEAH KAEK 2019/KK/29]).

Informed Consent: Informed consent has been obtained from the participants.

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References

1. World Health Organization. WHO report on the global tobacco epidemic, 2023: Protect people from tobacco smoke.

WHO; 2023. Available at: https://www.who.int/publications/i/item/9789240077164. Accessed Apr 22, 2026.
2. Hanley-Jones S, Greenhalgh EM, Scollo M. Interventions tailored for age and/or gender. In: Greenhalgh EM, Scollo MM, Winstanley MH, editors. Tobacco in Australia: Facts and issues. Melbourne: Cancer Council Victoria; 2022. Available at: https://www.tobaccoinaustralia.org.au/chapter-7-cessation/7-19-interventions-tailored-for-age-and-or-gender. Accessed Apr 22, 2026.
3. Weinberger AH, Smith PH, Allen SS, Cosgrove KP, Saladin ME, Gray KM, et al. Systematic and meta-analytic review of research examining the impact of menstrual cycle phase and ovarian hormones on smoking and cessation. Nicotine Tob Res 2015;17:407–21. [CrossRef]
4. Arguder E, Karalezli A, Hezer H, Kılıç H, Er M, Hasanoglu HC, et al. Sigara bırakma başarısını etkileyen faktörler. Turk Toraks Derg 2013;14:81–7. [Article in Turkish]
5. Ministry of Health, Republic of Turkey. Global adult tobacco survey Turkey 2012. Ministry of Health, Republic of Turkey; 2014. Available at: https://hsgm.saglik.gov.tr/depo/birimler/tutun-ve-madde-bagimliligi-ile-mucadele-db/dokumanlar/Raporlar/GATS-2012-ENG-25-07-2014.pdf. Accessed Apr 22, 2026.
6. Tillgren P, Haglund BJ, Lundberg M, Romelsjö A. The sociodemographic pattern of tobacco cessation in the 1980s: results from a panel study of living condition surveys in Sweden. J Epidemiol Community Health 1996;50:625–30. [CrossRef]
7. Solak ZA, Başoğlu ÖK, Erdinç E. Kronik obstrüktif akciğer hastalığı olgularında sigarayı bırakma başarısı. Tuberk Toraks Derg 2006;54:43–50. [Article in Turkish]
8. Sanderson Cox L, Patten CA, Ebbert JO, Drews AA, Croghan GA, Clark MM, et al. Tobacco use outcomes among patients with lung cancer treated for nicotine dependence. J Clin Oncol 2002;20:3461–9. [CrossRef]
9. Chirila SI, Grasa CN, Gurgas L, Cristurean CV, Hanzu-Pazara L, Hangan T. Evaluating the effectiveness of brief interventions for smoking cessation performed by family doctors. Medicina (Kaunas) 2024;60:1985. [CrossRef]
10. Bandura A. Self-efficacy. In: Friedman H, editor. Encyclopedia of Human Behavior. Vol. 4. New York: Academic Press; 1998. p.71–81.
11. Sirin O, Egici MT, Ozturk GZ. Evaluation of pulmonary function tests results of non-smoker women whose husbands smoke. Eur J Hum Health 2022;2:42–50. [CrossRef]
12. Yüksel H, Tuzcu Ö. Lise öğrencileri arasında sigara, alkol ve uyuşturucu madde kullanımını etkileyen sosyo-demografik faktörler. Dumlupınar Univ Sos Bilim Derg 2024;26:117–31. [Article in Turkish] [CrossRef]
13. Bandura A. Self-efficacy. In: Weiner IB, Craighead WE, editors. The Corsini Encyclopedia of Psychology. 4th ed. Hoboken (NJ): Wiley; 2010. [CrossRef]
14. Gwaltney CJ, Metrik J, Kahler CW, Shiffman S. Self-efficacy and smoking cessation: a meta-analysis. Psychol Addict Behav 2009;23:56–66. [CrossRef]
15. Smith CJ, Fischer TH. Particulate and vapor phase constituents of cigarette mainstream smoke and risk of myocardial infarction. Atherosclerosis 2001;158:257–67. [CrossRef]
16. Giovannucci E, Colditz GA, Stampfer MJ, Hunter D, Rosner BA, Willett WC, et al. A prospective study of cigarette smoking and risk of colorectal adenoma and colorectal cancer in U.S. women. J Natl Cancer Inst 1994;86:192–9. [CrossRef]
17. Anda RF, Williamson DF, Escobedo LG, Mast EE, Giovino GA, Remington PL. Depression and the dynamics of smoking. A national perspective. JAMA 1990;264:1541–5. [CrossRef]
18. Yang X, Li S, Pan L, Wang Q, Li H, Han M, et al. Assessment of successful smoking cessation by psychological factors using the Bayesian network approach. Psychol Health Med 2016;21:652–61. [CrossRef]
19. Prochaska JO, DiClemente CC. Stages and processes of self-change of smoking: toward an integrative model of change. J Consult Clin Psychol 1983;51:390–5. [CrossRef]
20. Redding CA, Rossi JS, Rossi SR, Velicer WF, Prochaska JO. Health behavior models. Int Electron J Health Educ 2000;3:180–93.
21. Egici MT, Ozturk GZ, Bukhari MH, Toprak D. Smoking cessation with medication and behaviour therapy. Pak J Med Sci 2017;33:1459–62. [CrossRef]
22. Hartmann-Boyce J, Chepkin SC, Ye W, Bullen C, Lancaster T. Nicotine replacement therapy versus control for smoking cessation. Cochrane Database Syst Rev 2018;5:CD000146. [CrossRef]
23. Gonzales D, Rennard SI, Nides M, Oncken C, Azoulay S, Billing CB, et al. Varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, vs sustained-release bupropion and placebo for smoking cessation: a randomized controlled trial. JAMA 2006;296:47–55. [CrossRef]
24. Tanrıverdi H, Altuntaş M, Demir Ö, Afşar BB, Çelikiz M. Success rates of pharmacological therapies used for smoking cessation and factors that affect smoking cessation rates. Eur J Gen Med 2015;12:125–30. [CrossRef]
25. Türkiye İlaç ve Tıbbi Cihaz Kurumu. SKRS E-reçete ilaç ve diğer farmasötik ürünler listesi. Ankara: Türkiye İlaç ve Tıbbi Cihaz Kurumu. Erişim adresi: https://www.titck.gov.tr/dinamikmodul/43. Erişim tarihi Apr 22, 2026.
26. Tutka P, Vinnikov D, Courtney RJ, Benowitz NL. Cytisine for nicotine addiction treatment: a review of pharmacology, therapeutics and an update of clinical trial evidence for smoking cessation. Addiction 2019;114:1951–69. [CrossRef]

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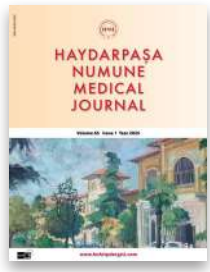

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ORIGINAL ARTICLE

The Effect of Non-Hydrochlorothiazide Diuretic Therapy on Symptoms and Diastolic Parameters in Obese Patients with Bendopnea

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Abstract

Introduction: Bendopnea is defined as the onset of dyspnea within 30 s of bending forward and is linked to increased right atrial and post-capillary wedge pressures. Diuretics reduce intravascular volume by inhibiting sodium reabsorption, thereby lowering cardiac filling pressures. This study aimed to evaluate the effects of non-hydrochlorothiazide (HCTZ) diuretics on symptoms and diastolic parameters in obese patients with bendopnea.

Methods: This retrospective, single-center study included 120 obese patients presenting with bendopnea to the cardiology outpatient clinic. Group 1 comprised patients who either received no medication or were prescribed only HCTZ. Group 2 included those treated with non-HCTZ diuretics (spironolactone, spironolactone + HCTZ, or indapamide). After 1 month, symptom status and clinical parameters were reassessed.

Results: The mean age was 57±9.1 years, with 90% female participants. At 1-month follow-up, bendopnea frequency was significantly lower in Group 2 compared to Group 1 (16.1% vs. 96.6%; p<0.001). Improvements were also observed in systolic mean blood pressure, NT-proBNP levels, and E/e' values in patients in Group 2.

Discussion and Conclusion: Non-HCTZ diuretics significantly alleviated bendopnea symptoms and improved diastolic parameters in obese patients. These findings suggest that non-HCTZ diuretics may offer symptomatic and hemodynamic benefits in this population.

Keywords: Bendopnea; diastolic function; diuretic; hydrochlorothiazide; obesity.

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Bendopnea was first identified in patients with heart failure with reduced ejection fraction (HFrEF) and has been recognized as a symptom associated with the hemodynamic impairment of heart failure (HF). It is defined as the onset of dyspnea within 30 s of bending forward [1]. Its pathophysiology has been attributed to increased right atrial pressure and post-capillary wedge pressure (PCWP) during bending. As patients bend forward, the elevated PCWP leads to pulmonary congestion, resulting in dyspnea [1]. A published study has demonstrated that HF patients with bendopnea have higher mortality rates compared to those without bendopnea [2]. Subsequent studies have shown that bendopnea can occur not only in HFrEF but also in patients with normal ejection fraction (EF) [3,4]. In a study investigating the prevalence of bendopnea in individuals without HF, female sex, obesity, and hypertension (HT) were found to be associated with bendopnea [4]. In a study including patients with an preserved EF, adverse outcomes were more frequently observed in those with bendopnea [3]. There are no studies in the literature regarding treating patients with bendopnea. Diuretics exert their effect by inhibiting sodium reabsorption at various sites within the nephron, thereby reducing intravascular volume. It has been demonstrated that the intravascular volume loss induced by diuretics leads to a reduction in cardiac filling pressures [5]. A study has shown that left ventricular hypertrophy (LVH) resulting from increased intracardiac pressure in hypertensive patients is reduced with diuretic therapy. However, similar effects have also been observed with other antihypertensive agents [6]. A recent study found that hydrochlorothiazide (HCTZ) reduced LVH significantly more in patients receiving HCTZ-like diuretics and HCTZ-potassium-sparing diuretic combinations compared to those receiving HCTZ alone [7]. For these reasons, we aimed to investigate the effects of non-HCTZ diuretics on symptomatic, diastolic parameters in obese patients with Bendopnea.

Materials and Methods

This retrospective, single-center study included patients over the age of 18 who presented with bendopnea complaints to the cardiology outpatient clinic of our hospital between March 01, 2020, and September 01, 2022. Among these patients, the following were excluded: Those with exertional dyspnea, left ventricular EF <50%, current use of diuretics other than HCTZ, moderate to severe valvular heart disease, a history of coronary artery disease, primary pulmonary parenchymal or obstructive disease, pregnancy, a history of malignancy, end-stage renal or liver disease, atrial fibrillation, a known diagnosis of HF, those whose data could not be accessed from the hospital records, and those who did not attend the follow-up visit 1 month later. The remaining 120 patients were designated as the final study group. The research protocols, aligned with the Declaration of Helsinki, received approval from the local ethics board, and all participating patients gave their consent. Our study was approved by the Ethics Committee of Tokat Gaziosmanpaşa University on January 19, 2023, with approval number 23-KAEK-011.

For the bendopnea test, the patient was instructed to sit and rest in a chair for 5 min. Then, they bent forward as if tying their shoelaces or putting on socks. The test was initiated, and if dyspnea occurred within 30 s, the patient was asked to report the symptom onset time in seconds to the physician. The test was stopped at 30 s. If dyspnea developed within this period, the test was considered positive. Conversely, if no dyspnea occurred within 30 s, the test was considered negative. Demographic data, blood parameters, baseline echocardiographic (transthoracic echocardiography [TTE]) findings, systolic ambulatory blood pressure (ABP) averages, body mass index (BMI), and waist circumference measurements were recorded for patients with a positive bendopnea test. Blood sampling was performed following an overnight fast. Laboratory assessments included measurements of hemoglobin, hematocrit, creatinine, albumin, total protein, glomerular filtration rate, potassium, ferritin, and N-terminus pro brain natriuretic peptide (NT-proBNP). Complete blood counts were analyzed using a Coulter Counter LH Series device (Beckman Coulter Inc., Hialeah, Florida, USA). Biochemical parameters were determined using a molecular analyzer (Roche Diagnostics, Mannheim, Germany).

TTE examinations were conducted using a Vivid S70N ultrasound platform (GE HealthCare Technologies, Chicago, Illinois, USA). All studies were digitally stored and later evaluated with dedicated offline analysis software. The investigator who performed the imaging was blinded to the treatment assignment.

The TTE protocol consisted of standard two-dimensional imaging in addition to spectral, color, and tissue Doppler modalities. Left ventricular systolic performance was assessed by calculating the EF through the modified biplane Simpson method. To assess diastolic function, transmitral inflow parameters – including the E and A waves and the E/A ratio – were obtained.

Pulsed-wave tissue Doppler velocities were additionally measured at end-expiration from the apical four-chamber view at both the septal and lateral portions of the mitral annulus. Early (e′) and late (a′) diastolic velocities were determined, and the E/e′ ratio was derived as a non-invasive estimate of left ventricular filling pressure.

HT was considered present when systolic blood pressure was ≥140 mmHg and/or diastolic blood pressure was ≥90 mmHg on a minimum of two separate measurements, or when the patient was receiving antihypertensive therapy. Diabetes mellitus (DM) was identified either by a fasting plasma glucose concentration exceeding 126 mg/dL, by a random glucose measurement above 200 mg/dL, or by the current use of any glucose-lowering medication.

Patients with bendopnea who were either not prescribed any medication or were initiated on HCTZ diuretics were classified as Group 1, while those prescribed non-HCTZ diuretics (spironolactone, spironolactone + HCTZ, or indapamide) were classified as Group 2. These groups were compared based on baseline demographic data, BMI, systolic mean values from ABP monitoring, waist circumference, E/e' ratio, NT-proBNP levels, and various blood parameters. After 1 month, the same patient groups were reassessed for bendopnea symptoms, NT-proBNP levels, E/e' ratio, and systolic mean values from ABP monitoring, and the results were compared again.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences software (version 21.0, SPSS Inc., Chicago, Illinois, USA) on the Windows platform. Continuous variables were reported as mean±standard deviation or as median with interquartile range (25^{th}–75^{th} percentiles), whereas categorical parameters were summarized as counts and percentages. The assumption of normal distribution was evaluated by the Kolmogorov-Smirnov test. Comparisons between categorical variables were carried out using either the Chi-square test or Fisher's exact test, while the Student's *t*-test or Mann–Whitney U test was applied for continuous data, depending on distribution characteristics. A two-tailed p<0.05 was considered statistically significant.

Results

The mean age of the 120 patients included in our study was 57±9.1 years, and 90% were female. Demographic and clinical data of the patients are listed in Table 1, and Group 1 and Group 2 patients were compared. Patients in Group 2 were significantly older than patients in Group 1 (*p*:0.016). Group 2 patients had significantly more women (p<0.001) and more patients with diabetes (p<0.001) than Group 1 patients. Patients in Group 2 had significantly higher waist circumference and BMI compared to Group 1 (*p*<0.001).

The evaluation of the two groups in terms of laboratory and other test parameters is given in Table 2. Creatinine, hemoglobin, hematocrit, and total protein levels were significantly higher in Group 1 than in Group 2 (p<0.05). NT-Pro BNP levels, E/e', and systolic ABP were significantly higher in Group 2 compared to Group 1 (p<0.05).

Table 1. Initial admission demographic and clinical data

Variables*	Group 1 *n*=58	Group 2 *n*=62	p
Age, years	55.28±10.06	59.30±7.66	0.016
Females	46 (79.3)	62 (100.0)	<0.001
DM	12 (20.7)	34 (54.8)	<0.001
Hypertension	28 (48.3)	34 (54.8)	0.472
LVEF, %	60.0 (60.0–62.0)	60.0 (60.0–62.0)	0.283
Tobacco use	8 (13.8)	2 (3.2)	0.037
Waist circumference, cm	102.0 (98.5–108.5)	111.5 (106.0–117.0)	<0.001
BMI, kg/m^2	32.4 (29.4–37.8)	43.2 (38.4–48.6)	<0.001
Bendopnea	58 (100.0)	62 (100.0)	
HT medication use	26 (44.8)	30 (50.0)	0.353

DM: Diabetes mellitus, LVEF: Left ventricular ejection fraction, BMI: Body mass index, HT: Hypertension. *P*<0.05 shows statistical significance. *Data are shown as number (percentage), mean±standard deviation or median (25^{th}–75^{th} percentiles)

Table 2. Initial admission blood and other test data

Variables*	Group 1 *n*=58	Group 2 *n*=62	p
Creatinin, mg/dL	0.7 (0.6–0.9)	0.6 (0.5–0.8)	0.015
Ferritin, ng/mL	48.0 (17.0–89.0)	38.5 (17.0–59.0)	0.304
Hemoglobin, g/dL	14.0 (13.2–14.5)	12.7 (11.4–13.7)	<0.001
Hematocrit, %	41.50 (39.60–44.00)	39.00 (37.00–41.00)	0.001
Total protein, g/L	75.0 (72.0–77.0)	71.0 (66.0–76.0)	0.008
Albumin, g/L	43.0 (41.0–46.0)	42.0 (40.0–45.0)	0.216
GFR, mL/min/1.73 m^2	102.0 (96.0–108.0)	102.0 (75.0–110.0)	0.462
Potassium, mEq/L	4.5 (4.2–4.7)	4.7 (4.5–4.8)	0.051
NT- proBNP levels, pg/mL	12.1 (10.0–20.0)	17.2 (11.8–30.5)	0.003
E/e'	8.0 (6.0–9.1)	10.0 (8.0–12.0)	0.001
Systolic ABP, mmHg	132.0 (124.0–141.0)	140.0 (134.0–155.0)	0.001

ABP: Ambulatory blood pressure, GFR: Glomerular filtration rate, NT-proBNP: N-terminus pro brain natriuretic peptide. *P*<0.05 shows statistical significance. *Data are shown as number (percentage), mean±standard deviation or median (25^{th}–75^{th} percentiles)

Table 3. Second admission

Variables*	Group 1 *n*=58	Group 2 *n*=62	p
Bendopnia	56 (96.6)	10 (16.1)	<0.001
NT-proBNP levels	13.0 (10.6–21.0)	16.0 (12.0–38.0)	0.055
E/e'	8.0 (6.0–9.0)	8.0 (7.0–10.0)	0.115
Systolic ambulatory BP	131.5 (124.0–141.0)	134.0 (123.0–163.0)	0.148

BP: Blood pressure, NT-proBNP: N-terminus pro brain natriuretic peptide. *P*<0.05 shows statistical significance. *Data are shown as number (percentage), mean±standard deviation or median (25^{th}–75^{th} percentiles)

The control parameters for both groups at the 1-month follow-up are presented in Table 3. At the 1-month follow-up, bendopnea was significantly less frequent in Group 2 compared to Group 1 (10 [16.1%] vs. 56 [96.6%]; p<0.001). No significant differences were found between the two groups for other parameters assessed after 1 month.

Discussion

In our study, we aimed to understand whether the use of non-HCTZ diuretics in patients with bendopnea provides benefit in terms of symptomatic and other clinical parameters in this patient group. Our study is the first study to investigate this issue in the literature.

In our study, significantly less bendopnea was found in the group using non-HCTZ diuretics (Group 2) compared to the other group (Group 2) at the outpatient clinic control 1 month later (10 [16.1%]–56 [96.6%]; p<0.001). All patients were obese in our study. When the patient groups with bendopnea in the literature were analyzed, it was found that obesity and overweight rates were high in patients with bendopnea, in parallel with our study [2,3]. Waist circumference and BMI of the patients in Group 2 were significantly higher than the other group. This showed that non-HCTZ diuretics are preferred more in patients with high BMI in clinical practice. Again in our study, it was observed that non-HCTZ diuretics were given to older patients, more women, and more patients with DM. In addition, it was observed that non-HCTZ diuretics were preferred in patients with higher NT-proBNP values, patients with higher E/e' values, and patients with higher mean systolic ABP. Considering these results, it was found that significantly more non-HCTZ diuretics were used in clinically higher-risk patient groups.

In a meta-analysis investigating which diuretic group was more effective in reducing LVH, 38 randomized trials including HCTZ and non-HCTZ diuretics were evaluated. At the end of this study, it was found that non-HCTZ diuretics reduced LVH significantly more. As a result, this result was thought to be due to the fact that HCTZ causes a lower systolic blood pressure drop than non-HCTZ diuretics and has a shorter half-life [8,9]. In addition, according to some data in the literature, non-HCTZ diuretics have been suggested to reduce cardiovascular mortality more than thiazide. The reasons for this are thought to be that indapamide reduces free oxygen radicals and has an inhibitory effect on platelet aggregation [9], chlorthalidone reduces platelet aggregation and vascular permeability [10]; and potassium-sparing diuretics/HCTZ combination reduces sodium and retains potassium more effectively [11,12]. In the study by Thibodeau et al. [1], it was found that patients with bendopnea had higher intracardiac pressures. There is data in the literature indicating that diuretics reduce intracardiac pressures by decreasing afterload [5]. In our study, we found that the group of patients with bendopnea who received non-HCTZ diuretics showed a significant symptomatic improvement compared to the group that received HCTZ or no diuretics. This result is observed as consistent with the findings of the studies mentioned above. In addition, although the non-thiazide diuretic group initially had significantly higher parameters such as E/e' and NT-proBNP compared to the other group, the lack of any difference between the two groups at the 1-month follow-up suggests that non-thiazide diuretics may provide both echocardiographic, biochemical, and symptomatic benefits in patients with bendopnea.

Limitation

The main limitation of our study is its retrospective design and the small sample size. Due to the unavailability of single-agent HCTZ in our country, patients receiving HCTZ also used this molecule in combination with ACE inhibitors and ARBs, which may have influenced the results. Furthermore, due to the unavailability of certain diuretics such as chlorthalidone, amiloride, and triamterene, these were not used in any of the patients. Therefore, the results of this study may not be applicable to all drug groups. Additionally, since the patients in our study were primarily obese and female, the applicability of these results to non-obese patients and males is uncertain, and further studies are needed to confirm these findings. In our study, diastolic function was assessed using only the E/e' ratio in TTE and blood NT-proBNP levels. The absence of other diastolic TTE parameters, in particular, constitutes a significant limitation. Similarly, in our study, the choice of diuretics prescribed to patients was left to the discretion of the physicians, which may have introduced bias.

Conclusion

Non-HCTZ diuretics significantly reduced bendopnea in obese patients presenting with bendopnea complaints. In

addition, positive effects were observed on systolic mean blood pressure, NT-proBNP levels, and diastolic parameters such as E/e'. We believe that starting non-HCTZ diuretics in obese patients with bendopnea symptoms would be beneficial in clinical practice.

Disclosure

Ethics Committee Approval: This study was approved by the Tokat Gaziosmanpaşa University Ethics Committee (Date: 19.01.2023, Decision no: 23-KAEK-011).

Informed Consent: Informed consent was obtained from all subjects involved in the study.

Conflict of Interest: The authors declare that there is no conflict of interest.

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References

1. Thibodeau JT, Turer AT, Gualano SK, Ayers CR, Velez-Martinez M, Mishkin JD, et al. Characterization of a novel symptom of advanced heart failure: bendopnea. JACC Heart Fail 2014;2:24–31. [CrossRef]
2. Thibodeau JT, Jenny BE, Maduka JO, Divanji PH, Ayers CR, Araj F, et al. Bendopnea and risk of adverse clinical outcomes in ambulatory patients with systolic heart failure. Am Heart J 2017;183:102–7. [CrossRef]
3. Gasanova AD, Dragunov DO, Sokolova AV, Arutyunov GP. Risk assessment of development of the major adverse cardiac events in patients with chronic heart failure with a preserved and ıntermediate ejection fraction in the presence of a Bendopnea symptom. Kardiologiia 2020;60:48–53. [Article in Russian] [CrossRef]
4. Baeza-Trinidad R, Isaula-Jimenez O, Peinado-Adiego C, Mosquera-Lozano JD. Prevalence of bendopnea in general population without heart failure. Eur J Intern Med 2018;50:e21–2. [CrossRef]
5. Stevenson LW, Bellil D, Grover-McKay M, Brunken RC, Schwaiger M, Tillisch JH, et al. Effects of afterload reduction (diuretics and vasodilators) on left ventricular volume and mitral regurgitation in severe congestive heart failure secondary to ischemic or idiopathic dilated cardiomyopathy. Am J Cardiol 1987;60:654–8. [CrossRef]
6. Sheridan DJ, Kingsbury MP, Flores NA. Regression of left ventricular hypertrophy; what are appropriate therapeutic objectives? Br J Clin Pharmacol 1999;47:125–30. [CrossRef]
7. Roush GC, Abdelfattah R, Song S, Ernst ME, Sica DA, Kostis JB. Hydrochlorothiazide vs chlorthalidone, indapamide, and potassium-sparing/hydrochlorothiazide diuretics for reducing left ventricular hypertrophy: A systematic review and meta-analysis. J Clin Hypertens (Greenwich) 2018;20:1507–15. [CrossRef]
8. Ernst ME, Carter BL, Goerdt CJ, Steffensmeier JJ, Phillips BB, Zimmerman MB, et al. Comparative antihypertensive effects of hydrochlorothiazide and chlorthalidone on ambulatory and office blood pressure. Hypertension 2006;47:352–8. [CrossRef]
9. Roush GC, Ernst ME, Kostis JB, Tandon S, Sica DA. Head-to-head comparisons of hydrochlorothiazide with indapamide and chlorthalidone: antihypertensive and metabolic effects. Hypertension 2015;65:1041–6. [CrossRef]
10. Woodman R, Brown C, Lockette W. Chlorthalidone decreases platelet aggregation and vascular permeability and promotes angiogenesis. Hypertension 2010;56:463–70. [CrossRef]
11. Spiers DR, Wade RC. Double-blind parallel study of a combination of chlorthalidone 50 mg and triamterene 50 mg in patients with mild and moderate hypertension. Curr Med Res Opin 1996;13:409–15. [CrossRef]
12. Lammintausta R, Kanto J, Mäntylä R. Urinary electrolyte profiles after amiloride, hydrochlorthiazide and the combination. J Int Med Res 1978;6:401–5. [CrossRef]

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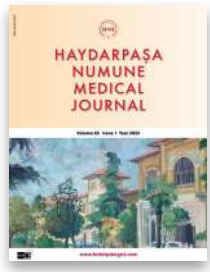

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ORIGINAL ARTICLE

Comparative Cardiovascular Safety Profile of Semaglutide, Liraglutide, and Tirzepatide: A Pharmacovigilance Analysis Based on The FDA Adverse Event Reporting System Reports

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Abstract

Introduction: Glucagon-like peptide-1 receptor (GLP-1) agonists and the dual glucose-dependent insulinotropic polypeptide/GLP-1 agonist tirzepatide are increasingly prescribed for type 2 diabetes and obesity, with clinical trials suggesting favorable cardiovascular (CV) profiles. However, post-marketing comparative data on CV adverse events (CVAEs) across these agents remain limited.

Methods: We extracted FDA adverse event reporting system (FAERS) reports between 2020 and 2025 where semaglutide, liraglutide, or tirzepatide appeared as the sole suspect drug. Reports with at least one CVAE – defined across four domains (arrhythmia, ischemic, hemodynamic, thromboembolic) were identified. We calculated CVAE proportions, summarized CVAE spectra, and compared serious outcomes (hospitalization, life-threatening events, death) between agents using $\chi 2$ tests and risk ratios.

Results: Among 26,930 monotherapy reports, 1,765 (6.6%) included ≥1 CVAE. CVAE proportions were highest for liraglutide (12.8%), followed by semaglutide (9.5%) and tirzepatide (5.3%) ($p<0.0001$). Common CVAEs included palpitations, heart rate increases, blood pressure fluctuations, and syncope. Major ischemic or thrombotic events were rare across all drugs. Serious outcomes occurred in 36.0% of CVAE reports for liraglutide, 29.7% for semaglutide, and 31.3% for tirzepatide ($p=0.38$). Tirzepatide reports had a higher proportion of life-threatening events (9.0%) than liraglutide (4.8%) or semaglutide (4.4%) ($p=0.0013$).

Discussion and Conclusion: Semaglutide, liraglutide, and tirzepatide show comparable CV safety profiles in FAERS data, with most CVAEs reflecting known pharmacologic effects rather than severe toxicity. To our knowledge, this is the first FAERS-based study to directly compare these agents' CVAE patterns, offering complementary insight to existing clinical trial evidence.

Keywords: FDA adverse event reporting system; glucagon-like peptide-1 receptor agonists; liraglutide; pharmacovigilance; semaglutide; tirzepatide.

Glucagon-like peptide-1 receptor agonists (GLP-1 RAs) and the dual glucose-dependent insulinotropic polypeptide (GIP)/GLP-1 agonist tirzepatide are structurally modified analogues of endogenous GLP-1 that provide sustained activation of the GLP-1 receptor and, in the case of tirzepatide, combined agonism at GLP-1 and GIP receptors [1]. Their broad tissue distribution in the pancreas, gastrointestinal tract, cardiovascular (CV) system, and central nervous system underpins pleiotropic effects ranging from glucose-lowering and weight reduction to potential organ protection [2,3].

CV outcome trials (CVOTs) have shown that several GLP-1 RAs reduce major adverse cardiovascular events (MACEs) in high-risk populations, while tirzepatide has demonstrated CV safety and may confer benefit in selected cohorts [4,5]. At the same

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time, GLP-1–based therapies are known to increase resting heart rate modestly and influence blood pressure through effects on weight, natriuresis, and autonomic balance [6,7].

Spontaneous reporting systems such as the FDA adverse event reporting system (FAERS) are a key component of post-marketing pharmacovigilance. FAERS aggregates adverse event (AE) reports submitted by healthcare professionals, manufacturers, and patients, and has been widely used to characterize safety signals [8]. Using CV outcomes from randomized trials as a benchmark, post-marketing data can help to identify rare or unexpected CV harms and to compare reporting patterns between agents in routine care. Building on recent FAERS-based work that evaluated neurological AEs with GLP-1 RAs using a standardized pharmacovigilance framework, we aimed to characterize and compare the CV AE (CVAE) profiles of semaglutide, liraglutide, and tirzepatide. Specifically, this study (i) quantified the proportion of monotherapy reports for each agent that contained at least one CVAE, (ii) described the distribution of key CV AEs across drugs, and (iii) compared the frequency of serious outcomes among CVAE reports.

Materials and Methods

Ethics Statement

This study used publicly available, de-identified data from the FAERS database; therefore, institutional ethics approval and informed consent were not required. The analysis was conducted in accordance with present recommendations for the reporting of pharmacovigilance disproportionality analyses based on individual case safety reports.

Data Source

FAERS is a spontaneous reporting system maintained by the U.S. Food and Drug Administration to monitor the safety of marketed drugs and biologics. Its standard structure includes demographic information, drug exposures, adverse reactions, patient outcomes, reporter type, therapy timelines, and indications. CVAEs are coded using the Medical Dictionary for Regulatory Activities, in which CVAEs are organized hierarchically into higher-level terms and system organ classes [9]. For the present analysis, we used a curated line listing derived from FAERS, containing individual reports in which semaglutide, liraglutide, or tirzepatide appeared as a suspect agent. Each row represented one unique report and included information on suspect product active ingredients for CVAEs.

Data Extraction and Case Selection

Data extraction followed a pre-defined protocol analogous in structure to that used in recent GLP-1 RA pharmacovigilance studies, with adaptations for the CV focus of this work [10]. We first identified all reports in which semaglutide, liraglutide, or tirzepatide was listed in the file as a suspect product. To reduce confounding from multiple suspect drugs, we then restricted the dataset to monotherapy reports by requiring that the "Suspect Product Active Ingredients" field contain exactly one active ingredient: Semaglutide, liraglutide, or tirzepatide. Reports with more than one suspect active ingredient (e.g., combinations with another GLP-1 RA, insulin, or a sodium-glucose transport 2 inhibitor) were excluded.

Definition of CV AEs

A report was classified as CVAE-positive if at least one AE corresponded to a CV concept within the following domains: (1) Arrhythmia and rate-related events: palpitations, heart rate increased, tachycardia, bradycardia, atrial fibrillation, non-specific arrhythmia, ventricular tachycardia, cardiac arrest, cardiac or heart failure. (2) Ischemic and vascular events: myocardial infarction, acute myocardial infarction, angina, ischemic heart disease, stroke, cerebrovascular accident, transient ischemic attack. (3) Hemodynamic disturbances: Hypertension, blood pressure increased, hypotension, blood pressure decreased, orthostatic hypotension, syncope, circulatory collapse, shock. (4) Thromboembolic events: Pulmonary embolism, deep vein thrombosis, arterial thrombosis, and embolism. This four-domain framework was chosen to capture both frequent functional CV disturbances and clinically serious ischemic or thromboembolic outcomes in a reproducible pharmacovigilance structure.

For descriptive analyses, we later tabulated selected AEs of particular clinical interest (e.g., palpitations, heart rate increased, hypertension, hypotension, syncope, chest pain, atrial fibrillation, myocardial infarction, pulmonary embolism, and deep vein thrombosis) among CVAE-positive reports for each drug.

Seriousness and Clinical Outcomes

Seriousness outcomes were identified as Hospitalization, Life-threatening, Death, Disability, Congenital anomaly, and other serious outcomes. Within the subset of CVAE-positive reports, we created indicator variables denoting hospitalization (presence of "Hospitalized"), life-threatening events (presence of "Life Threatening"), and death (presence of "Died"). These outcomes were evaluated only among CVAE-positive reports and compared between drugs.

Statistical Analysis

We performed a descriptive pharmacovigilance analysis focused on within-cohort comparisons of CVAE reporting between semaglutide, liraglutide, and tirzepatide. For each

drug, the primary metric was the proportion of monotherapy reports with ≥1 CVAE, calculated as the number of CVAE-positive reports divided by the total number of monotherapy reports for that agent. Approximate 95% confidence intervals (CIs) for proportions were derived using the normal approximation to the binomial distribution. To assess whether CVAE proportions differed across the three drugs, we constructed a 3×2 contingency table (drug×CVAE status) and applied a χ^2 test. Pairwise comparisons were then conducted using 2×2 χ^2 tests, and risk ratios (RRs) with 95% CIs were calculated to quantify relative differences in CVAE reporting between liraglutide versus tirzepatide, semaglutide versus tirzepatide, and liraglutide versus semaglutide. Within the CVAE-positive subset, we compared the proportions of reports with hospitalization, life-threatening events, and death between drugs using χ^2 tests.

Results

Overall CVAE Reporting in Monotherapy Reports

A flowchart of the study is given in Figure 1. Among 26,930 monotherapy FAERS reports that named semaglutide, liraglutide, or tirzepatide as the sole suspect active ingredient, 1,765 reports (6.6%) contained at least one CVAE. The distribution of total monotherapy reports, CVAE-positive reports, and resulting proportions is summarised in Table 1. Liraglutide accounted for 978 monotherapy reports, of which 125 were CVAE-positive, yielding a CVAE proportion of 12.8% (95% CI 10.7–14.9). Semaglutide contributed 6,232 monotherapy reports and 595 CVAE-positive reports, for a proportion of 9.5% (95% CI 8.8–10.3). Tirzepatide accounted for the largest number of monotherapy reports (19,720), with 1,045 CVAE-positive reports and a CVAE proportion of 5.3% (95% CI 5.0–5.6). The global χ^2 test comparing CVAE proportions across the three drugs was significant (χ^2=203.8, p<0.0001), indicating that the likelihood of a report containing a CVAE differed by agent. Pairwise comparisons showed that CVAEs were reported approximately 2.4-fold more often for liraglutide than for tirzepatide (RR 2.41, 95% CI 2.03–2.87) and approximately 1.8-fold more often for semaglutide than for tirzepatide (RR 1.80, 95% CI 1.64–1.98). Liraglutide also showed a higher CVAE proportion than semaglutide (RR 1.34, 95% CI 1.12–1.60).

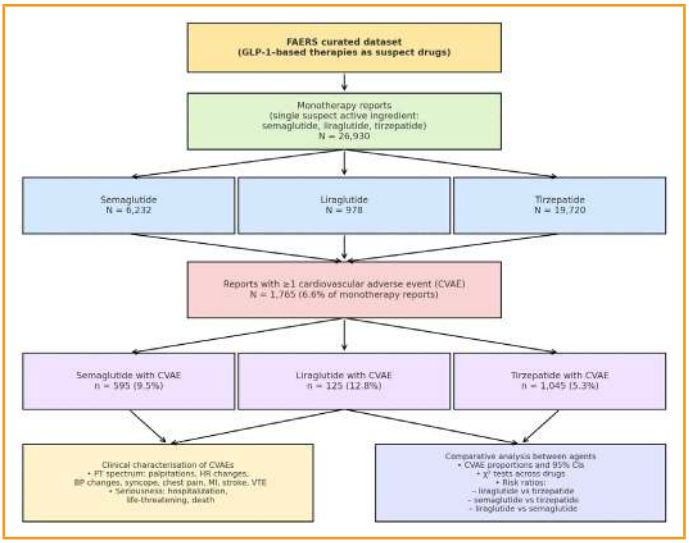

Figure 1. Flowchart of case selection and analytic strategy for cardiovascular adverse events (CVAEs) associated with GLP-1–based therapies in FAERS.

Table 1. Distribution of monotherapy reports and cardiovascular adverse events (CVAEs) by drug

Drug	Total monotherapy reports	Reports with ≥1 CVAE (n)	CVAE proportion (%)	95% CI (%)
Liraglutide	978	125	12.8	10.7–14.9
Semaglutide	6,232	595	9.5	8.8–10.3
Tirzepatide	19,720	1,045	5.3	5.0–5.6
Total	26,930	1,765	6.6	–

Global χ^2 for difference in CVAE proportions across drugs=203.8, p<0.0001. Relative to tirzepatide, RR (95% CI) for CVAE reporting: liraglutide 2.41 (2.03–2.87); semaglutide 1.80 (1.64–1.98). Liraglutide vs semaglutide: RR 1.34 (1.12–1.60).
CVAE: Cardiovascular adverse event; CI: Confidence interval; RR, relative risk.

Spectrum of CVAE

Across all three agents, the qualitative CVAE pattern was similar and dominated by rhythm- and hemodynamic-related AEs. As summarized in Table 2, palpitations, heart rate increased, blood pressure changes, syncope, and chest pain were among the most frequently reported CV AEs in the CVAE-positive subset. Palpitations were reported in 21 liraglutide, 53 semaglutide, and 124 tirzepatide CVAE reports. Heart rate increased appeared in 4, 43, and 90 reports for liraglutide, semaglutide, and tirzepatide, respectively. Hypertension AEs were more frequent in semaglutide CVAE reports (*n*=64) than in liraglutide (*n*=10) or tirzepatide (*n*=44), whereas hypotension and syncope occurred more often with tirzepatide (hypotension 77, syncope 95) than with semaglutide (20 and 25) or liraglutide (8 and 9). Chest pain was present in 10, 43, and 72 CVAE reports for liraglutide, semaglutide, and tirzepatide, respectively. Events reflecting major ischemic disease and venous thromboembolism were comparatively infrequent. Myocardial infarction or acute myocardial infarction was reported in 6 liraglutide,

Table 2. Selected cardiovascular adverse events (CVAEs) among Liraglutide, Semaglutide and Tirzepatide

CVAE	Liraglutide (n)	Semaglutide (n)	Tirzepatide (n)
Palpitations	21	53	124
Heart rate increased	4	43	90
Hypertension	10	64	44
Hypotension	8	20	77
Blood pressure increased	11	39	63
Blood pressure decreased	3	30	54
Syncope	9	25	95
Chest pain	10	43	72
Atrial fibrillation	8	32	36
Myocardial infarction	6	22	37
Cerebrovascular accident / ischaemic stroke	7	18	29
Pulmonary embolism	2	16	49
Deep vein thrombosis	2	9	29

CVAE: Cardiovascular adverse event

22 semaglutide, and 37 tirzepatide CVAE reports, while cerebrovascular accident or ischemic stroke AEs occurred in 7, 18, and 29 reports, respectively. Pulmonary embolism and deep vein thrombosis were also uncommon in absolute numbers.

Serious Outcomes among CVAE-Positive Reports

Within CVAE-positive reports, we examined the frequency of hospitalization, life-threatening outcomes, and death, stratified by agent (Table 3). Hospitalization was recorded in 36.0% of liraglutide CVAE reports (45/125), 29.7% of semaglutide CVAE reports (177/595), and 31.3% of tirzepatide CVAE reports (327/1,045). The χ^2 test for hospitalization proportions did not identify a statistically significant difference between drugs (approximately χ^2=1.93, p=0.38). Life-threatening outcomes were noted in 6 liraglutide (4.8%), 26 semaglutide (4.4%), and 94 tirzepatide (9.0%) CVAE reports (χ^2≈13.35, p=0.0013). Deaths occurred in 3 liraglutide (2.4%), 17 semaglutide (2.9%), and 29 tirzepatide (2.8%) CVAE reports (χ^2=0.08, p=0.96).

Table 3. Serious outcomes among CVAE-positive reports

Outcome	Liraglutide (n=125)	Semaglutide (n=595)	Tirzepatide (n=1.045)
Hospitalized (n, %)	45 (36.0%)	177 (29.7%)	327 (31.3%)
Life-threatening (n, %)	6 (4.8%)	26 (4.4%)	94 (9.0%)
Died (n, %)	3 (2.4%)	17 (2.9%)	29 (2.8%)

Global χ^2 for hospitalization across drugs≈1.93, p≈0.38 (no significant difference). Global χ^2 for life-threatening events≈13.35, p≈0.0013 (higher proportion for tirzepatide). Global χ^2 for death≈0.08, p≈0.96 (no significant difference).

Discussion

The predominance of palpitations, tachycardia, blood pressure changes, and syncope in the FAERS reports is consistent with known pharmacologic effects of GLP-1 receptor agonism. GLP-1-based therapies can modestly increase resting heart rate and alter autonomic balance, a chronotropic effect that provides a plausible mechanism for palpitations or a sensation of rapid heartbeat reported by patients [11]. Indeed, clinical studies have documented that drugs, such as liraglutide can raise heart rate by a few beats per minute relative to placebo [12]. In addition, GLP-1 receptor agonists promote weight loss and have natriuretic and vasodilatory effects, which generally lead to modest reductions in blood pressure on average [13,14]. Evidence from large-scale CV outcome trials provides reassuring context: GLP-1 receptor agonists have demonstrated CV benefits or neutrality in high-risk patients. Trials such as LEADER (liraglutide) and SUSTAIN-6 or SELECT (semaglutide) showed significant reductions in MACE and CV mortality compared to placebo [15].

In our study, the observed differences in CVAE reporting rates between agents should be interpreted with caution. Liraglutide and semaglutide have been used for many years, often in older patients with long-standing diabetes and co-morbid CV disease, who might be more prone to (or more closely monitored for) CV symptoms. Tirzepatide, being newer, has rapidly gained use, particularly in patients with obesity (including those without severe CV history); in such individuals, the baseline risk of CV events may be lower, and the *sheer volume of reports* for this popular drug (dominated by other adverse effects, such as gastrointestinal events) may dilute the proportion that is CV-related. Therefore, clinicians should not interpret the lower FAERS CVAE proportion for tirzepatide as definitive evidence of superior cardiac safety. All three agents appear comparably safe from a CV standpoint, so therapeutic decisions should prioritize factors such as glycemic control, weight loss efficacy, patient comorbidities, accessibility, and tolerance of other side effects.

Limitation

This study has some important limitations to keep in mind. FAERS is a voluntary reporting system, so not every AE gets reported – especially the milder ones. What's more, the number of reports does not tell us how many people actually took each drug, so we cannot calculate the true risk of side effects. The patients using these medications may also differ: Those on liraglutide or semaglutide might have had more severe disease or heart conditions, while tirzepatide users are often newer and healthier. Although we focused on monotherapy cases to reduce this variation, some differences likely remain. We only compared the drugs to each other, not to all other medications, so our results show how they relate within the GLP-1 class – not their absolute risk. Also, no formal adjustment for multiple comparisons was applied, and therefore, *p*-values should be interpreted as exploratory.

Conclusion

In this pharmacovigilance study utilizing the FAERS database, we found that semaglutide, liraglutide, and tirzepatide exhibit largely comparable CV safety profiles, with most reported AEs involving mild hemodynamic or rhythm-related symptoms such as palpitations, transient changes in heart rate or blood pressure, and occasional syncope. Notably, serious ischemic or arrhythmogenic events were uncommon across all agents. To our knowledge, this is the first study to conduct a direct, head-to-head comparative analysis of CV AEs among these three incretin-based therapies using spontaneous report data. While randomized trials have established the CV efficacy of these agents, our findings contribute real-world post-marketing safety data that enhance present understanding of their tolerability in clinical practice.

Disclosure

Ethics Committee Approval: In this study, publicly available, de-identified data obtained from the FAERS database were used; therefore, approval from an institutional ethics committee was not required.

Informed Consent: Since this study used publicly available, de-identified data obtained from the FAERS database, informed consent was not required.

Conflict of Interest: The author declares that there is no conflict of interest.

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References

1. Baker DE, Walley K, Levien TL. Tirzepatide. Hosp Pharm 2023;58:227–43. [CrossRef]
2. Ma J, Hu X, Zhang W, Tao M, Wang M, Lu W. Comparison of the effects of Liraglutide, Tirzepatide, and Retatrutide on diabetic kidney disease in db/db mice. Endocrine 2025;87:159–69. [CrossRef]
3. Jastreboff AM, Aronne LJ, Ahmad NN, Wharton S, Connery L, Alves B, et al. Tirzepatide once weekly for the treatment of obesity. N Engl J Med 2022;387:205–16. [CrossRef]
4. Gera A, Latif F, Borra V, Naz S, Mittal V, Ayoobkhan FS, et al. Efficacy of glucagon-like peptide-1 receptor agonists for prevention of stroke among patients with and without diabetes: A meta-analysis with the SELECT and FLOW trails. Int J Cardiol Heart Vasc 2025;57:101638. [CrossRef]
5. Bonfioli GB, Pagnesi M, Tomasoni D, Rakisheva A, Metra M. Understanding the role of glucagon-like peptide-1 receptor agonists in the treatment of heart failure. Card Fail Rev 2025;11:e19. [CrossRef]
6. Olgar Y, Durak A, Turan B. Acute GLP-1 agonism induces arrhythmogenic electrical activity in aged mice heart through impaired cellular Na+ and Ca2+ handlings: the role of CK2 hyperphosphorylation. Anatol J Cardiol 2025;29:83–94. [CrossRef]
7. Greco C, Santi D, Brigante G, Pacchioni C, Simoni M. Effect of the glucagon-like peptide-1 receptor agonists on autonomic function in subjects with diabetes: a systematic review and meta-analysis. Diabetes Metab J 2022;46:901–11. [CrossRef]
8. U.S. Food & Drug. FDA Adverse event monitoring system (AEMS) public dashboard. Available at: https://www.fda.gov/drugs/fdas-adverse-event-reporting-system-faers/fda-adverse-event-reporting-system-faers-public-dashboard. Accessed Nov 21, 2025.
9. Mozzicato P. MedDRA: An overview of the medical dictionary for regulatory activities. Pharmaceut Med 2009;23:65–75. [CrossRef]
10. Chen H, Liu S, Gao S, Shi H, Yan Y, Xu Y, et al. Pharmacovigilance analysis of neurological adverse events associated with GLP-1 receptor agonists based on the FDA Adverse Event Reporting System. Sci Rep 2025;15:18063. [CrossRef]
11. Sun F, Wu S, Guo S, Yu K, Yang Z, Li L, et al. Impact of GLP-1 receptor agonists on blood pressure, heart rate and hypertension among patients with type 2 diabetes: A systematic review and network meta-analysis. Diabetes Res Clin Pract 2015;110:26–37. [CrossRef]
12. Tougaard RS, Jorsal A, Tarnow L, Hansson NH, Kistorp C, Schou M, et al. Heart rate increases in liraglutide treated chronic heart failure patients: association with clinical parameters and adverse events. Scand Cardiovasc J 2020;54:294–9. [CrossRef]

13. Marx N, Husain M, Lehrke M, Verma S, Sattar N. GLP-1 receptor agonists for the reduction of atherosclerotic cardiovascular risk in patients with type 2 diabetes. Circulation 2022;146:1882–94. [CrossRef]
14. Joseph J. Sustained effects of glucagon-like peptide-1 (GLP-1) agonists on blood pressure in obesity and type 2 diabetes: a longitudinal case study. Cureus 2025;17:e88893. [CrossRef]
15. Kalayci A, Januzzi JL, Mitsunami M, Tanboga IH, Karabay CY, Gibson CM. Clinical features modifying the cardiovascular benefits of GLP-1 receptor agonists: a systematic review and meta-analysis. Eur Heart J Cardiovasc Pharmacother 2025;11:552–61. [CrossRef]

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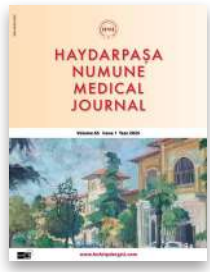

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ORIGINAL ARTICLE

Zinc, Copper, and Oxidative Stress in Diabetes: Insights From an Oral Glucose Tolerance Test Study

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Abstract

Introduction: Zinc (Zn) and copper (Cu) play important roles in glucose metabolism and redox balance. This study evaluated serum Zn Cu and oxidative stress markers during an oral glucose tolerance test and examined their relationships with glucose dynamics.

Methods: We studied 195 adults including 138 healthy controls and 57 individuals with newly diagnosed diabetes. Blood samples were obtained at baseline T0 at 60 min T1 and at 120 min T2. Glucose insulin Zn Cu total oxidant status (TOS) and related biochemical parameters were measured. Between group comparisons were performed and correlation heatmaps were presented descriptively.

Results: Glucose levels were higher in the diabetes group than in controls at T1 and T2 with $p<0.001$. At T1 zinc was higher in patients than in controls 84.14 versus 77.95 mcg/dL with ($p=0.010$) while groups were similar at baseline and at T2. Cu declined after the glucose load in both groups. TOS was lower during the test in both groups in a descriptive manner and between group differences were not significant at any time point. Lipid profile insulin cortisol and Homeostatic Model Assessment of Insulin Resistance did not differ significantly between groups. Correlation patterns suggested more stable relationships in controls and weaker more variable links among trace elements and oxidative markers in diabetes.

Discussion and Conclusion: Glucose dysregulation was evident in diabetes whereas trace element and oxidative stress responses to the glucose challenge were modest. The higher zinc level at T1 in patients may reflect short term mobilization or redistribution. These findings support the need for longitudinal and interventional studies to clarify the roles of Zn and Cu in dysglycemia and to assess the translational value of dynamic trace element measurements.

Keywords: Copper; diabetes mellitus; oral glucose tolerance test; oxidative stress; zinc.

Diabetes mellitus is a chronic metabolic disease with substantial morbidity that requires lifelong management. It encompasses Type 1 diabetes (autoimmune β-cell destruction), Type 2 diabetes (insulin resistance with relative insulin deficiency), and gestational diabetes (hyperglycemia first recognized in pregnancy, which confers later Type 2 diabetes [T2D] risk).

Beyond glycemia, alterations in trace elements and redox balance are increasingly implicated in diabetes pathophysiology. Zinc (Zn) contributes to insulin processing, storage, and signaling [1-4], whereas copper (Cu), a redox-active metal, supports key enzymatic reactions and, when dysregulated, may amplify reactive oxygen [5-10].

Recent syntheses indicate that Zn exerts clinically relevant effects on glycemic control in T2D. Meta-analyses and systematic reviews consistently report reductions in fasting blood sugar, hemoglobin A1C (HbA1c), and insulin resistance with Zn supplementation [11-14]. In individuals with

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prediabetes, Zn appears to improve fasting glucose and glucose tolerance and may delay progression to overt T2D [15].

In contrast, the role of Cu in dysglycemia remains complex and inconsistent. A recent systematic review reported heterogeneous associations between Cu intake or status and T2D outcomes, which suggests that both deficiency and excess may be harmful and reflects the dual antioxidant and pro-oxidant actions of Cu [16]. Broader trace-element reviews indicate that higher Cu exposure can intensify oxidative stress, a recognized driver of diabetic complications [17]. Circulating Cu concentrations also appear higher in gestational diabetes, particularly in late pregnancy and in Asian populations [18].

Oxidative stress remains central to diabetes pathophysiology. Pooled analyses show increased lipid and DNA oxidation markers and reduced antioxidant defenses in T2D [19,20]. Comparable redox disturbances are reported in gestational diabetes and are linked to adverse perinatal outcomes, with partial mitigation through glycemic control and antioxidant support [21,22].

Dynamic changes in trace elements and redox markers during a standardized glycemic challenge remain incompletely defined. Using the oral glucose tolerance test (OGTT) as a controlled physiological stressor, we obtained serial measurements of serum Zn, Cu, and oxidative stress biomarkers in newly diagnosed diabetes and normoglycemic controls, quantified their relations with glucose excursions, and evaluated shifts in correlation networks. This time-resolved design focuses on early disease, extends prior cross-sectional work, and aims to refine mechanistic understanding while informing the potential clinical utility of trace-element assessment in dysglycemia.

Materials and Methods

This study was conducted at the Bezmialem Vakif University Health Application and Research Center and was approved by the Bezmialem Vakif University Research Ethics Committee (Approval No: 11195). All procedures involving human participants were conducted in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Participants

Adults aged 18–65 years who were referred for routine metabolic evaluation were screened. Exclusion criteria were liver kidney or endocrine disorders, prior gastrointestinal surgery or malabsorption, chronic infection, autoimmune disease, malignancy, psychiatric disease, pregnancy, lactation, alcohol abuse, active smoking, anemia, thrombocytosis, leukocytosis and inflammatory disease. Use of drugs that affect glucose metabolism trace element balance or oxidative stress including antioxidant supplements, antihypertensives, lipid lowering agents and immunomodulators led to exclusion. Body mass index and body composition were not collected, and this is acknowledged in the limitations.

OGTT Protocol

Participants fasted for at least 12 h. Fasting plasma glucose was measured at baseline. Individuals with fasting plasma glucose above 126 mg/dL did not undergo the glucose challenge in line with the World Health Organization guidance. Eligible participants received a standard OGTT solution that contained 75 g of anhydrous glucose dissolved in 300 mL of water. Blood samples were obtained at baseline (before OGTT), T_0 at 1 h (60 min), T_1, and at 2 h (120 min), T_2 after intake. During the procedure, participants were instructed to refrain from smoking, food intake other than water, and strenuous physical activity. A total of 195 participants were enrolled with 57 newly diagnosed diabetes cases defined as 2 h glucose of at least 200 mg/dL and 138 normoglycemic controls.

Sample Collection

Blood was collected into trace element tubes to minimize exogenous Zn and Cu. Samples were processed on a clean bench with powder-free gloves and without metallic instruments in the workspace. Serum was separated within 1 h of collection by centrifugation. Aliquots were transferred with metal-free pipette tips into metal-free polypropylene vials. All aliquots were stored at −86°C. Only one freeze-thaw cycle was permitted.

Biochemical Assays

Total oxidant status (TOS), paraoxonase activity, and arylesterase activity were measured using commercial kits from Rel Assay Diagnostics Gaziantep Turkey. Serum Zn and Cu were quantified using colorimetric methods from the same manufacturer. Additional measurements included glucose insulin cortisol 25 hydroxy vitamin D iron total cholesterol high density lipoprotein cholesterol and low density lipoprotein (LDL) cholesterol on an Architect c16000 analyzer Abbott Diagnostics Abbott Park Illinois United States. Homeostatic model assessment of insulin resistance values were calculated as (Fasting insulin [μU/mL] × Fasting glucose [mg/dL])/405.

Statistical Analysis

Analyses were performed with IBM Statistical Package for the Social Sciences Statistics for Windows version 22. Dis-

tributions were inspected with histograms and Q-Q plots. Normality was assessed with the Shapiro-Wilk test. Skewed variables were analyzed after log transformation and are reported as transformed or back-transformed values as appropriate. Homogeneity of variance was checked with the Levene test, and non-parametric alternatives were used when assumptions were not met.

Between-group contrasts at each time point used Welch *t*-tests. Within-group changes across time points used paired *t*-tests. Primary comparisons were organized into predefined families for each biomarker and family wise error rate was controlled with Bonferroni adjustment within each endpoint family. Two-sided alpha was set at 0.05.

Outliers were screened with the interquartile range rule. Sensitivity analyses with winsorization were used to evaluate robustness in the presence of large standard deviations. Missing data were not imputed, and analyses used available cases.

A priori calculations targeted a medium effect size with Cohen d of 0.5, with alpha of 0.05 and a power of 0.80, which yielded a minimum of 64 participants per group. The diabetes group included 57 participants, and the control group included 138 participants. The shortfall in the diabetes group and its potential impact on borderline effects are acknowledged in the limitations.

Results

The study included a total of 195 participants (138 controls and 57 patients with diabetes), with a mean age of 32.87±10.07 years. Biochemical parameters were assessed at three time points: before OGTT (T_0), 60 min (T_1), and 120 min (T_2) following the OGTT. Baseline characteristics and T_0 laboratory measurements are presented in Table 1.

At T_0, fasting glucose levels were similar between the groups (p=0.729). At T_1, Zn was 84.14 µg/dL in patients and 77.95 µg/dL in controls with (p=0.010). At T_0, the groups were similar with p=0.720, and at T_2 groups were similar with *P* = 0.602. Cu declined after the glucose load in both groups, and between-group differences were not significant at T_1 or T_2. TOS values were lower at T_1 and T_2 than at T_0 in both groups in a descriptive manner, and between-group differences were not significant at T_0, T_1, or T_2. Group comparisons at T_1 and T_2 for all analytes are summarized in Table 2.

Catalase paraoxonase and arylesterase showed no significant between-group differences at T_0, T_1, or T_2. Cortisol did not differ between groups at any time point, and at T_0, lipid parameters, iron, and vitamin D were comparable between groups.

Temporal changes in serum Zn, Cu, and TOS levels are illustrated in Figure 1, showing modest dynamic responses over the OGTT period.

Table 1. Biochemical parameters at T_0 (before OGTT) in control and patient groups

Parameter	Control mean (SD) (*n*=138)	Patient mean (SD) (*n*=57)	p
Age	32.23 (10.27)	33.44 (9.74)	0.445
HOMA-IR	1.86 (0.92)	2.18 (2.31)	0.155
Cholesterol (mg/dL)	201.12 (47.23)	194.99 (42.69)	0.375
HDL (mg/dL)	56.05 (14.65)	55.20 (16.64)	0.736
LDL (mg/dL)	122.55 (33.51)	118.66 (36.61)	0.489
Fe (µg/dL)	64.49 (55.74)	58.59 (28.39)	0.324
Vitamin D (ng/mL)	20.65 (16.48)	19.97 (14.66)	0.777
Glucose (mg/dL)	88.54 (11.06)	89.14 (10.93)	0.729
Cortisol (µg/dL)	19.91 (6.94)	19.89 (7.11)	0.981
Insulin (µU/mL)	9.79 (9.93)	8.39 (3.89)	0.154
Cu (µg/dL)	171.16 (58.94)	168.91 (63.02)	0.816
Zn (µg/dL)	83.15 (16.33)	82.29 (14.67)	0.720
TOS	34.72 (92.29)	42.65 (95.87)	0.594
ARES (U/L)	150.12 (36.85)	146.95 (36.00)	0.578
Catalase (U/L)	16.58 (14.97)	18.46 (17.66)	0.479
PON (U/L)	0.70 (0.05)	1.10 (13.66)	0.323

HOMA-IR: Homeostatic model assessment of insulin resistance; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; Fe: Iron; TOS: Total oxidative status; ARES: Arylesterase, PON: Paraoxonase; Cu: Copper; Zn: Zinc; OGTT: Oral glucose tolerance test.

Table 2. Biochemical parameters at T_1 (60 min) and T_2 (120 min) after OGTT

Parameter	T_1			T_2		
	Control mean (SD)	Patient mean (SD)	p	Control mean (SD)	Patient mean (SD)	p
Glucose (mg/dL)	139.96 (40.95)	259.98 (18.01)	<0.001	105.37 (25.27)	220.91 (14.54)	<0.001
Cortisol (µg/dL)	16.32 (6.46)	16.52 (6.94)	0.850	14.48 (6.19)	14.31 (6.15)	0.862
Insulin (µU/mL)	60.27 (41.25)	65.13 (45.09)	0.497	44.19 (38.27)	53.41 (35.20)	0.105
Cu (µg/dL)	148.61 (53.13)	149.86 (58.47)	0.889	148.02 (53.25)	152.00 (58.09)	0.655
Zn (µg/dL)	77.95 (14.10)	84.14 (15.42)	0.010	84.54 (14.94)	83.34 (14.51)	0.602
TOS*	24.40 (10.51)	23.86 (7.90)	0.693	22.51 (11.83)	23.79 (12.27)	0.502
ARES (U/L)	148.71 (39.15)	149.26 (42.40)	0.932	150.40 (40.39)	145.77 (43.85)	0.492
Catalase (U/L)	20.60 (16.21)	18.76 (9.00)	0.308	19.93 (17.35)	17.53 (10.11)	0.224
PON (U/L)	0.72 (0.08)	0.69 (0.18)	0.192	0.72 (0.08)	0.71 (0.09)	0.259

*TOS is expressed as µmoL H_2O_2 equivalent per liter. TOS: Total oxidative status; ARES: Arylesterase; PON: Paraoxonase; Cu: Copper; Zn: Zinc; OGTT: Oral glucose tolerance test.

Figure 2 presents Pearson correlation heatmaps of biochemical parameters in the Control and Patient groups at each time point (T_0, T_1, T_2). At T_0, stronger positive correlations among lipid parameters and oxidative stress markers were observed in the Control group. In contrast, the Patient group displayed weaker and more variable correlations, particularly involving catalase and antioxidant response elements. In controls, Zn and Cu showed a consistent inverse pattern within and across time points. Zn was temporally stable with r values around 0.65–0.76 across T_0 T_1 and T_2. Cu showed even higher temporal stability with r values around 0.81–0.89. The Zn to Cu coupling was negative with coefficients typically between −0.38 and −0.71. TOS showed only weak links with the other variables. In patients, Zn displayed lower temporal stability with r values around 0.10–0.38 while Cu stability remained high with r values around 0.85–0.89. The inverse Zn to Cu pattern persisted and was modest within the same time window and stronger across different time points, generally between −0.33 and −0.57. Associations of Zn with glycemic markers after the glucose load were small and inconsistent.

Notably, Zn showed a transient positive correlation with insulin and glucose in the Patient group at T_1, suggesting a possible acute metabolic interaction in response to glucose intake. These patterns diminished at T_2.

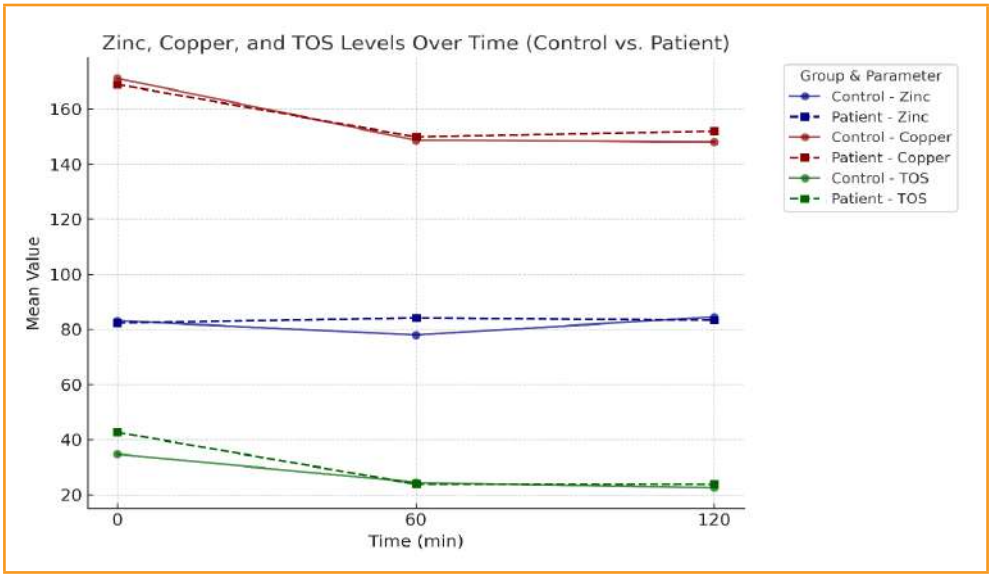

Figure 1. Temporal trends of zinc, copper, and total oxidative status levels in patient and control groups.

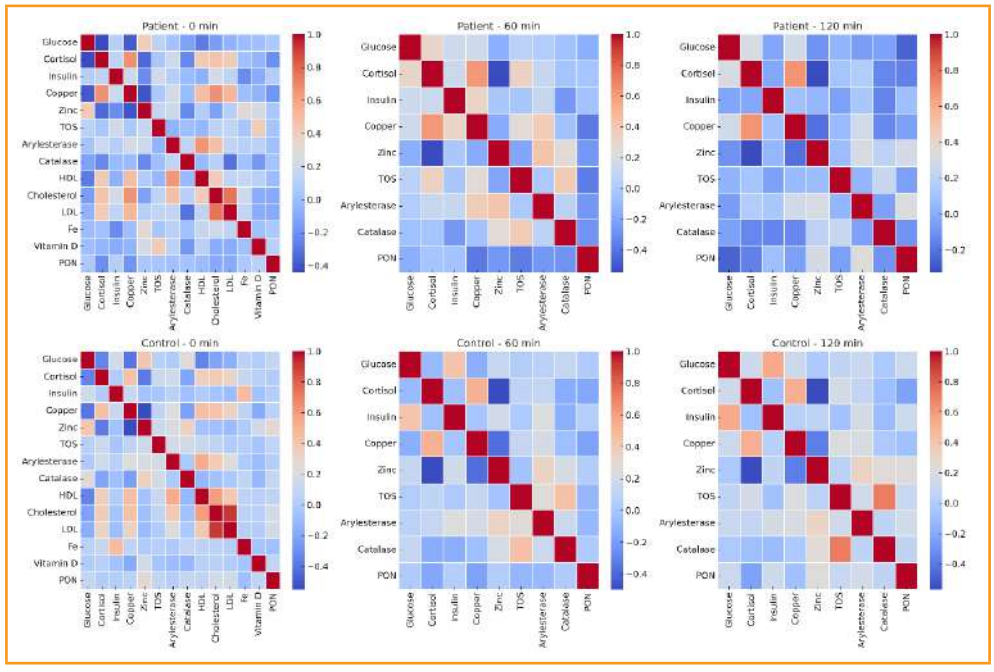

Figure 2. Correlation heatmaps of biochemical parameters in patient and control groups at T0 (before oral glucose tolerance test [OGTT]), T1 (60 min after OGTT), and T2 (120 min after OGTT) (The color scale encodes r from −1 to +1).

Overall, the correlation maps suggest more stable and coordinated metabolic regulation in healthy individuals, whereas diabetic patients exhibit disrupted trace element and oxidative stress interactions during OGTT. Sensitivity analyses using winsorization did not materially change the direction or significance of the primary results.

Discussion

This study explored the interplay between glucose metabolism, oxidative stress, and trace elements (Zn and Cu) in individuals undergoing an OGTT. The findings reveal significant alterations in glucose levels post-OGTT among patients with diabetes, yet only modest and transient changes in trace element concentrations and oxidative stress markers, indicating a potentially attenuated oxidative response to glucose intake in this population.

Previous research has highlighted the contribution of oxidative stress to metabolic dysfunction. Bielawska et al.[23] reported a decrease in oxidized LDL levels post-OGTT in individuals with increased body mass, possibly due to enhanced lipoprotein clearance or redistribution. In this cohort, TOS values were lower during the test in a descriptive manner, and between-group differences were not significant at any time point.

Age and physiological context may influence oxidative responses. Katsa et al. [24] demonstrated that glucose excursions in children with excess body weight elevated antioxidant enzyme activities, such as superoxide dismutase and glutathione peroxidase 3, potentially reflecting a compensatory antioxidant response. In contrast, the lack of significant TOS change observed in our adult cohort may indicate reduced antioxidant adaptability or a delayed oxidative response. Meta analytic syntheses indicate that lipid and DNA oxidation markers are elevated and antioxidant defenses are reduced in T2D and similar redox disturbances are seen in gestational diabetes with partial mitigation by glycemic control and antioxidant support [19-22]. Against that background, our descriptive decrease in TOS during the test without between-group differences suggests that TOS alone may be insufficient to capture short-term redox dynamics in adults. The omission of superoxide dismutase and glutathione peroxidase limits comparability with the broader literature and may understate acute antioxidant responses. Future work that combines standardized enzymatic panels with serial sampling could reconcile acute challenge data with chronic redox alterations reported in pooled analyses.

Zn plays a multifaceted role in glucose metabolism through its effects on insulin signaling, β-cell function, and redox regulation [25-27]. Consistent with previous reports linking Zn deficiency to impaired glucose tolerance in gestational diabetes mellitus and polycystic ovary syndrome [28-30], at T_1 Zn was higher in patients than in controls with (p=0.010) while groups were similar at T_0 and at T_2. This transient rise may reflect Zn mobilization or utilization during acute glucose handling, aligning with its insulin-mimetic prop-

erties. However, T_0 Zn levels were similar between groups, and overall fluctuations were modest. Our OGTT finding of higher Zn in patients at 60 min should be interpreted alongside recent evidence that chronic Zn supplementation improves fasting glucose HbA1c and insulin resistance in T2D and may benefit individuals with prediabetes [11-15]. Those syntheses address sustained intake and longer time scales whereas our design probes acute physiology. The transient difference we observe is consistent with short term mobilization or redistribution and does not imply baseline deficiency or treatment benefit. Whether dynamic Zn responses during a glucose challenge predict longitudinal glycemic trajectories remains an open question that requires prospective validation.

Cu has also been implicated in diabetes pathophysiology, though findings remain inconsistent. While several studies reported elevated Cu levels in individuals with T2DM [31-33]. Our results showed no significant differences in serum Cu between groups and a comparable decline after glucose intake. These discrepancies may stem from differences in population characteristics, disease duration, or unmeasured confounders such as medication use or inflammatory status. Our null between group contrasts and the uniform post-load decline in Cu align with the heterogeneity summarized in a recent systematic review which indicates that both low and high Cu exposure may be detrimental and reflects the dual antioxidant and pro oxidant actions of Cu [16]. Broader trace element reviews link higher Cu exposure to heightened oxidative stress, which is implicated in complications of diabetes [17]. Elevated circulating Cu reported in gestational diabetes likely reflects a different physiological context including pregnancy related changes and population specific factors [18]. Differences in diet inflammation and the partitioning of Cu between ceruloplasmin-bound and non-ceruloplasmin pools were not measured in our study and may contribute to variation across cohorts.

Given Cu's role in redox cycling and its association with oxidative damage, its dysregulation has been proposed as a therapeutic target. Studies have linked elevated Cu levels with impaired fasting glucose, gestational diabetes, and increased diabetes-related complications [34-36]. Accordingly, Cu chelation therapy has been hypothesized as a strategy to mitigate disease progression [37-39].

While our findings do not demonstrate a disturbance in basal Cu homeostasis, the dynamic response to the glucose load warrants further investigation. This study is observational and cannot establish causality. The diabetes subgroup was slightly smaller than the a priori target, therefore borderline effects should be interpreted with caution. Despite prespecified families and Bonferroni adjustment, residual type I error remains possible given the number of tests. We did not measure superoxide dismutase or glutathione peroxidase, which limits interpretability of the redox profile. Body mass index and body composition were not collected, which constrains adjustment for adiposity-related confounding.

The study has notable strengths. We evaluated glucose, trace elements, and oxidative stress markers at multiple OGTT time points in newly diagnosed diabetes and normoglycemic controls. The design adds by tracking serial Zn, Cu and oxidative stress markers under a standardized glycemic challenge and by comparing correlation network structure between groups.

Limitation

The slightly smaller than planned diabetes subgroup may have limited statistical power for detecting borderline associations, although the analyses were conducted using prespecified statistical procedures and multiplicity control. The absence of BMI data, together with the lack of additional antioxidant enzyme measurements such as superoxide dismutase and glutathione peroxidase, constrained adjustment for adiposity-related confounding and limited a more comprehensive characterization of the acute redox response during OGTT.

Conclusion

In this cohort, glucose dysregulation was clear in diabetes, whereas trace element and oxidative stress responses to the glucose challenge were relatively stable. Zn was higher in patients than in controls at T_1 and otherwise similar across groups and time points, which may reflect short-term mobilization or redistribution. Cu declined after the glucose load in both groups without between-group differences. Measurement of Zn or Cu during the OGTT is not ready for clinical use. Prospective studies with standardized assays and clinical end points are needed to define the translational value of dynamic trace element responses and to test whether modulation of Zn or Cu homeostasis can improve glycemic control.

Disclosure

Ethics Committee Approval: This study was approved by the Bezmialem Vakif University Research Ethics Committee (Date: 12.07.2018, Decision no: 11195).

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References

1. Chausmer AB. Zinc, insulin and diabetes. J Am Coll Nutr 1998;17:109–15. [CrossRef]
2. Ely MR, Kenefick RW, Cheuvront SN, Chinevere T, Lacher CP, Lukaski HC, et al. The effect of heat acclimation on sweat microminerals: artifact of surface contamination. Int J Sport Nutr Exerc Metab 2013;23:470–9. [CrossRef]
3. Powell SR. The antioxidant properties of zinc. J Nutr 2000;130(5S Suppl):1447S–54S.
4. Tapiero H, Tew KD. Trace elements in human physiology and pathology: zinc and metallothioneins. Biomed Pharmacother 2003;57:399–411. [CrossRef]
5. Uriu-Adams JY, Keen CL. Copper, oxidative stress, and human health. Mol Aspects Med 2005;26:268–98. [CrossRef]
6. Cakina S, Aydın B, Beyazit F. Evaluation of thiol/disulfide homeostasis in patients with gestational diabetes mellitus. Gynecol Endocrinol 2020;36:1006–09. [CrossRef]
7. Jha JC, Banal C, Chow BS, Cooper ME, Jandeleit-Dahm K. Diabetes and kidney disease: role of oxidative stress. Antioxid Redox Signal 2016;25:657–84. [CrossRef]
8. Kaneto H, Xu G, Fujii N, Kim S, Bonner-Weir S, Weir GC. Involvement of c-Jun N-terminal kinase in oxidative stress-mediated suppression of insulin gene expression. J Biol Chem 2002;277:30010–8. [CrossRef]
9. Volpe CMO, Villar-Delfino PH, Dos Anjos PMF, Nogueira-Machado JA. Cellular death, reactive oxygen species (ROS) and diabetic complications. Cell Death Dis 2018;9:119. [CrossRef]
10. Yamamoto M, Yamato E, Toyoda S, Tashiro F, Ikegami H, Yodoi J, et al. Transgenic expression of antioxidant protein thioredoxin in pancreatic beta cells prevents progression of type 2 diabetes mellitus. Antioxid Redox Signal 2008;10:43–9. [CrossRef]
11. Wang Z, Ronsmans C, Woolf B. Triangulating evidence for the causal impact of single-dose zinc supplement on glycemic control for type-2 diabetes2021. MedRxiv 2021. [CrossRef]
12. Nazari M, Nikbaf-Shandiz M, Pashayee-Khamene F, Bagheri R, Goudarzi K, Hosseinnia NV, et al. Zinc supplementation in individuals with prediabetes and type 2 diabetes: a GRADE-assessed systematic review and dose-response meta-analysis. Biol Trace Elem Res 2024;202:2966–90. [CrossRef]
13. Ghaedi K, Ghasempour D, Jowshan M, Zheng M, Ghobadi S, Jafari A. Effect of zinc supplementation in the management of type 2 diabetes: A grading of recommendations assessment, development, and evaluation-assessed, dose-response meta-analysis of randomized controlled trials. Crit Rev Food Sci Nutr 2024;64:9228–39. [CrossRef]
14. Daneshvar M, Ghaheri M, Safarzadeh D, Karimi F, Adib-Hajbagheri P, Ahmadzade M, et al. Effect of zinc supplementation on glycemic biomarkers: an umbrella of interventional meta-analyses. Diabetol Metab Syndr 2024;16:124. [CrossRef]
15. Jayawardena R, Ranasinghe P, Kodithuwakku W, Dalpatadu C, Attia J. Zinc supplementation in prediabetes mellitus. Minerva Endocrinol (Torino) 2022;47:334–43. [CrossRef]
16. Eljazzar S, Abu-Hijleh H, Alkhatib D, Sokary S, Ismail S, Al-Jayyousi GF, et al. The role of copper intake in the development and management of type 2 diabetes: a systematic Review. Nutrients 2023;15:1655. [CrossRef]
17. Bjørklund G, Dadar M, Pivina L, Doşa MD, Semenova Y, Aaseth J. The role of zinc and copper in insulin resistance and diabetes mellitus. Curr Med Chem 2020;27:6643–57. [CrossRef]
18. Lian S, Zhang T, Yu Y, Zhang B. Relationship of circulating copper level with gestational diabetes mellitus: a meta-analysis and systemic review. Biol Trace Elem Res 2021;199:4396–409. [CrossRef]
19. Banik S, Ghosh A. The association of oxidative stress biomarkers with type 2 diabetes mellitus: A systematic review and meta-analysis. Health Sci Rep 2021;4:e389. [CrossRef]
20. S S, Hegde SV, Agarwal SV, Ns D, Pillai A, Shah SN, et al. Biomarkers of oxidative stress and their clinical relevance in type 2 diabetes mellitus patients: a systematic review. Cureus 2024;16:e66570.
21. Zheng ZX, Feng X, Zhuang L. The effect of oxidative stress and antioxidants treatment on gestational diabetes mellitus outcome: a scoping review. Cell Biochem Biophys 2024;82:3003–13. [CrossRef]
22. Chatzakis C, Sotiriadis A, Tsakmaki E, Papagianni M, Paltoglou G, Dinas K, et al. The effect of dietary supplements on oxidative stress in pregnant women with gestational diabetes mellitus: a network meta-analysis. Nutrients 2021;13:2284. [CrossRef]
23. Bielawska L, Wysocka E, Baszczuk A, Dżumak A, Ludziejewska A, Cymerys M, et al. The effect of 75 grams of glucose during OGTT on plasma markers of lipid and lipoprotein peroxidation, oxidized LDL and thiobarbituric acid reactive substances, in people with increased body mass. Metabolites 2023;13:483. [CrossRef]
24. Katsa ME, Kostopoulou E, Nomikos T, Ioannidis A, Sarris V, Papadogiannis S, et al. The response of antioxidant enzymes and antiapoptotic markers to an oral glucose tolerance test (OGTT) in children and adolescents with excess body weight. Int J Mol Sci 2023;24:16517. [CrossRef]
25. Tang X, Shay NF. Zinc has an insulin-like effect on glucose transport mediated by phosphoinositol-3-kinase and Akt in 3T3-L1 fibroblasts and adipocytes. J Nutr 2001;131:1414–20. [CrossRef]

26. Mahboob M, Rahman MF, Grover P. Serum lipid peroxidation and antioxidant enzyme levels in male and female diabetic patients. Singapore Med J 2005;46:322–4.
27. Prasad AS, Bao B, Beck FW, Kucuk O, Sarkar FH. Antioxidant effect of zinc in humans. Free Radic Biol Med 2004;37:1182–90. [CrossRef]
28. Bo S, Lezo A, Menato G, Gallo ML, Bardelli C, Signorile A, et al. Gestational hyperglycemia, zinc, selenium, and antioxidant vitamins. Nutrition 2005;21:186–91. [CrossRef]
29. Wang Y, Tan M, Huang Z, Sheng L, Ge Y, Zhang H, et al. Elemental contents in serum of pregnant women with gestational diabetes mellitus. Biol Trace Elem Res 2002;88:113–8. [CrossRef]
30. Guler I, Himmetoglu O, Turp A, Erdem A, Erdem M, Onan MA, et al. Zinc and homocysteine levels in polycystic ovarian syndrome patients with insulin resistance. Biol Trace Elem Res 2014;158:297–304. [CrossRef]
31. Zargar AH, Shah NA, Masoodi SR, Laway BA, Dar FA, Khan AR, et al. Copper, zinc, and magnesium levels in non-insulin dependent diabetes mellitus. Postgrad Med J 1998;74:665–8. [CrossRef]
32. Kazi TG, Afridi HI, Kazi N, Jamali MK, Arain MB, Jalbani N, et al. Copper, chromium, manganese, iron, nickel, and zinc levels in biological samples of diabetes mellitus patients. Biol Trace Elem Res 2008;122:1–18. [CrossRef]
33. Walter RM Jr, Uriu-Hare JY, Olin KL, Oster MH, Anawalt BD, Critchfield JW, et al. Copper, zinc, manganese, and magnesium status and complications of diabetes mellitus. Diabetes Care 1991;14:1050–6. [CrossRef]
34. Yin J, Wang X, Li S, Zhu Y, Chen S, Li P, et al. Interactions between plasma copper concentrations and SOD1 gene polymorphism for impaired glucose regulation and type 2 diabetes. Redox Biol 2019;24:101172. [CrossRef]
35. Li P, Yin J, Zhu Y, Li S, Chen S, Sun T, et al. Association between plasma concentration of copper and gestational diabetes mellitus. Clin Nutr 2019;38:2922–7. [CrossRef]
36. Qiu Q, Zhang F, Zhu W, Wu J, Liang M. Copper in diabetes mellitus: a meta-analysis and systematic review of plasma and serum studies. Biol Trace Elem Res 2017;177:53–63. [CrossRef]
37. Cooper GJ, Phillips AR, Choong SY, Leonard BL, Crossman DJ, Brunton DH, et al. Regeneration of the heart in diabetes by selective copper chelation. Diabetes 2004;53:2501–8. [CrossRef]
38. Hamada Y, Nakashima E, Naruse K, Nakae M, Naiki M, Fujisawa H, et al. A copper chelating agent suppresses carbonyl stress in diabetic rat lenses. J Diabetes Complications 2005;19:328–34. [CrossRef]
39. Keegan A, Cotter MA, Cameron NE. Effects of chelator treatment on aorta and corpus cavernosum from diabetic rats. Free Radic Biol Med 1999;27:536–43. [CrossRef]

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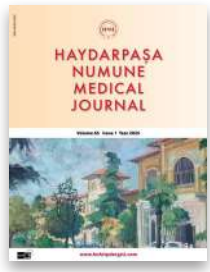

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ORIGINAL ARTICLE

Assessing the Effectiveness and Safety of Surgical and Medical Management in First-Trimester Pregnancy Loss

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Abstract

Introduction: To compare the short- and long-term outcomes of medical (misoprostol) and surgical (vacuum aspiration) management in patients with first-trimester pregnancy loss.

Methods: This retrospective cohort study included patients diagnosed and treated for spontaneous abortion under 14 gestational weeks between January 2019 and January 2025 at a tertiary center. Patients were divided into two groups according to treatment modality: Medical (misoprostol) or surgical (vacuum aspiration). Demographic characteristics, treatment success rates, hospitalization duration, hemoglobin/hematocrit changes, complication rates, and subsequent fertility outcomes were compared.

Results: A total of 368 patients were included, with 114 receiving medical treatment and 254 undergoing surgical management. The overall success rate was significantly higher in the surgical group (99.2%) compared with the medical group (50%) ($p<0.001$). The surgical group had a shorter hospital stay ($p=0.014$), lower hemoglobin drop ($p<0.001$), and fewer complications ($p=0.01$). The most common complications in the medical group were retained products of conception and pelvic infection. Treatment success was consistently higher with surgical management across both missed and incomplete abortion subtypes ($p<0.001$).

Discussion and Conclusion: Surgical management with vacuum aspiration appears more effective and safer than medical management with misoprostol for first-trimester pregnancy losses. These findings may support individualized decision-making based on gestational age, clinical stability, and resource availability. Limitations include the retrospective design and lack of standardized misoprostol protocols.

Keywords: Fertility outcome; first-trimester pregnancy loss; misoprostol; pregnancy loss; treatment outcome; vacuum aspiration.

Pregnancy loss, also known as spontaneous abortion or miscarriage, is defined as the termination of an intrauterine pregnancy before the 20th week of gestation and occurs in approximately 15–20% of clinically recognized pregnancies [1,2]. The majority of these losses – nearly 80% – take place during the first trimester of pregnancy [3,4]. Accepted treatment options for early pregnancy loss include expectant management, medical therapy, and surgical evacuation. Although these approaches differ significantly in terms of the process, each has been shown to be effective and generally well tolerated by patients. Present evidence does not support the superiority of any one method in terms of long-term reproductive outcomes. Therefore, patients should be thoroughly counseled on the potential risks and benefits of each option to support individualized, preference-based decision-making.

Expectant management is a conservative approach involving the spontaneous expulsion of pregnancy tissue without medical or surgical intervention. Medical therapy involves pharmacological uterine evacuation using prostaglandin analogs, most commonly misoprostol. Typically, a

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single dose of 800 µg misoprostol is administered vaginally, with additional doses considered in cases of incomplete expulsion. Surgical management entails active evacuation of the uterus, usually through vacuum aspiration or dilatation and curettage (D&C), both of which can be performed with or without anesthesia. While surgical evacuation has long been considered the gold standard for first-trimester miscarriage management due to its high success rate, it is also associated with increased risks of complications, such as hemorrhage, uterine perforation, and pelvic infection [5,6]. In contrast, medical management is less invasive, more cost-effective, does not require anesthesia, and may reduce endometrial trauma; however, it carries a higher likelihood of treatment failure and longer hospital stay in some cases [7,8].

Therefore, this study aims to compare the clinical outcomes of medical management using misoprostol and surgical management through vacuum aspiration in patients with first-trimester spontaneous abortions up to 14 weeks of gestation. Particular attention is given to patient-centered and clinically relevant parameters, including treatment success rates, complication incidence, duration of hospitalization, need for additional interventions, patient satisfaction, and subsequent fertility outcomes. By comprehensively evaluating these factors, the study seeks to inform clinical decision-making and identify the most appropriate and effective treatment approach for optimizing the management of early pregnancy loss.

Materials and Methods

The present retrospective observational cohort study was conducted at the Gülhane Training and Research Hospital. A retrospective review and analysis of the medical records and clinical findings of patients diagnosed with first-trimester pregnancy loss (gestational age ≤14 weeks) between 1 January 2019 and 31 January 2025 was conducted. Ethical approval for the study was obtained from the tertiary center Gülhane Training and Research Hospital (approval date April 10, 2025, number: 2025/75) and the study adhered to the principles outlined in the Declaration of Helsinki.

Patients were included in the study if they had a diagnosis of first-trimester spontaneous abortion, received treatment with either medical (misoprostol) or surgical (vacuum aspiration) methods, and had complete and accessible medical records detailing demographic characteristics, treatment processes, and follow-up information. Patients diagnosed with ectopic pregnancy, molar pregnancy, or septic abortion, those managed expectantly, those requiring additional interventions following initial treatment, and those with incomplete or missing medical data were excluded.

Pregnancy loss terminology was based on standard definitions: Anembryonic pregnancy was defined as a nonviable gestation characterized by an empty gestational sac without a yolk sac or embryo, possibly following embryonic resorption. Fetal demise was used to describe pregnancies of 10 weeks or greater in which a fetus was visualized but lacked cardiac activity. The term "missed abortion" was replaced by "asymptomatic pregnancy loss," describing nonviable pregnancies in the absence of symptoms. "Incomplete miscarriage" indicated the presence of retained products of conception in the uterus after pregnancy loss.

Medical management was carried out in accordance with institutional protocols using misoprostol, either alone or as part of a combination regimen when available.

According to the 2017 International Federation of Gynecology and Obstetrics (FIGO) guidelines on misoprostol-only regimens for reproductive health, the recommended dosage and route vary depending on indication and gestational age.

For incomplete pregnancy loss, 600 µg orally, 400 µg sublingually, or 400–800 µg vaginally may be administered as a single dose. For missed pregnancy loss, 800 µg intravaginally or 600 µg sublingually may be administered every 3 h until expulsion occurs [9].

In our study, since only first-trimester pregnancy losses (<14 weeks) were included, the treatment protocol was planned in accordance with these FIGO 2017 recommendations, considering both the indication and gestational age.

All administrations were performed under clinical supervision in the hospital setting. Surgical treatment consisted of vacuum aspiration. Treatment success was defined as complete evacuation of pregnancy tissue without the need for further intervention. Complications evaluated included infection, hemorrhage, uterine perforation, and retained products requiring additional procedures. Gestational age was determined by ultrasound measurement of crown–rump length at initial presentation.

Binary logistic regression analysis was performed to identify independent predictors of treatment success. Variables with $p<0.10$ in univariate analysis (age, gestational age, type of pregnancy loss, hemoglobin change, and treatment modality) were entered into the model. Adjusted odds ratios (OR) with 95% confidence intervals (CI) were reported.

Statistical Analysis

Data, including maternal demographics, obstetric history, clinical findings, treatment modalities, and outcomes, were extracted from patient records and analyzed. Statistical analysis was conducted using the Statistical Package

for the Social Sciences version 25.0 (IBM Corp., Chicago, IL, USA). Normality of continuous variables was assessed with the Shapiro–Wilk test and histograms. Normally distributed variables were presented as mean±standard deviation, and non-normally distributed data as median (minimum–maximum). Categorical data were summarized as frequencies and percentages. Comparisons between groups were performed using the chi-square or Fisher's exact test for categorical variables and Student's t-test or Mann-Whitney *U*-test for continuous variables, depending on the distribution. Binary logistic regression was used to assess causality. A p<0.05 was considered statistically significant.

This study was conducted and reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.

Results

A total of 368 patients diagnosed with first-trimester pregnancy loss were included in the study. As illustrated in the flowchart (Fig. 1), patients were divided into two groups according to the treatment modality: Medical management with misoprostol (*n*=114) and surgical management with vacuum aspiration or D&C (*n*=254). Baseline demographic characteristics, clinical findings, treatment outcomes, and follow-up data were systematically analyzed. Primary outcomes included treatment success, hospitalization duration, and complication rates, while secondary outcomes involved time to subsequent conception and need for additional intervention. The comparative analysis of these parameters is presented below.

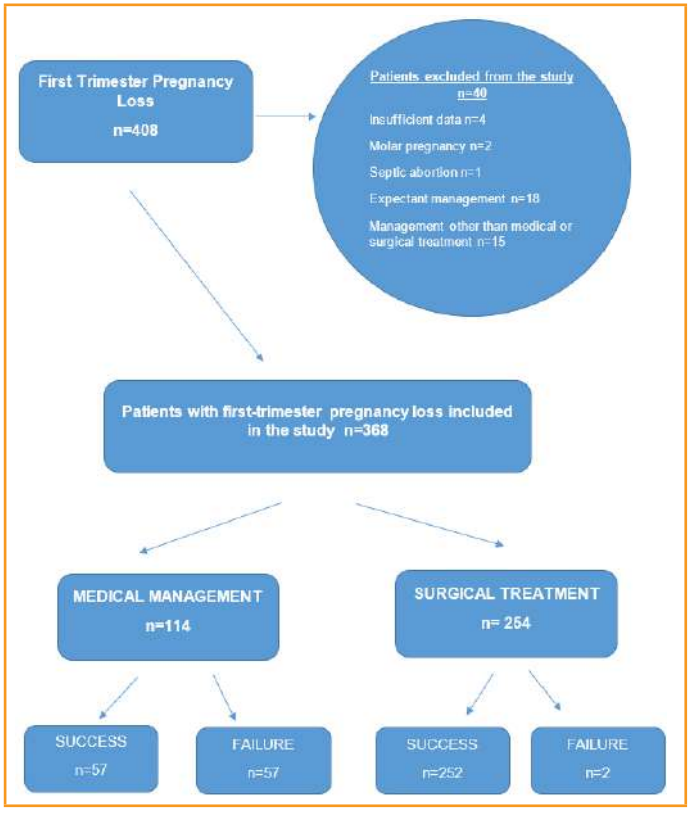

Figure 1. Patient flow diagram for study cohort.

Table 1 summarizes the baseline demographic and clinical characteristics of patients included in the study. A total of 368 patients with first-trimester pregnancy loss were included, of whom 114 received medical management, and 254 underwent surgical treatment. The median age of patients was comparable between the groups (30 [18–43] years in the medical group vs. 31 [18–47] years in the surgical group, p=0.12). There were no significant differences between the two groups in terms of gravida, parity, number of abortions, or number of living children (p>0.05 for all).

The median gestational age was significantly higher in the medical management group compared to the surgical group (11 weeks vs. 9 weeks, p<0.001). Anemia was observed in 9.6% of patients in the medical group and 6.3% in the surgical group (p=0.28). Prior uterine surgical interventions were reported in 19.3% of the medical group and 27.6% of the surgical group, without a statistically significant difference (p=0.11).

Missed miscarriage was more frequently observed in the medical management group (83.3%) compared to the surgical group (74.8%), while incomplete miscarriage was more common in the surgical group (25.2%) than in the medical group (16.7%), though this difference did not reach statistical significance (p=0.072). Comorbidities were present in 26.3% and 25.3% of patients in the medical and surgical groups, respectively (p=0.75).

The outcomes of medical and surgical management in first-trimester pregnancy loss are summarized in Table 2. The pre-procedure hematocrit (Hct) level was significantly lower in the medical group (median 37 [24.9–44]) compared to the surgical group (median 37.7 [28.6–49.4], *p*=0.014). Post-procedure Hct levels were also significantly lower in the medical group (34.3 [16.3–42.2] vs. 36.2 [25.4–44.1], *p*<0.0001). The reduction in Hct was greater in the medical group (2.5 [0–13] vs. 1.1 [0–9.7], *p*<0.001), and the rate of ≥10% decrease in Hct was significantly higher (36.8% vs. 18.9%, *p*<0.001) (Table 2).

The median duration of inpatient stay was longer in the medical group (2 days [2–17]) compared to the surgical group (1 day [1–8], *p*=0.014). The success rate was markedly lower in the medical group (50%) than in the surgical group (99.2%, *p*<0.001). Complication rates were also higher in the medical group (8.8%) than in the surgical group (2.4%, *p*=0.01) (Table 2). In this study, conception delay was defined as the absence of pregnancy within 12 months of

Table 1. Demographic characteristics of patients with first-trimester pregnancy loss

	Medical management (n=114)	Surgical management (n=254)	p
Age (years)	30 (18-43)	31 (18-47)	0.12
Gravida	2 (1-11)	2 (1-11)	0.45
Parity	1 (0-5)	1 (0-6)	0.26
Abortion	0 (0-10)	0 (0-10)	0.98
Living	1 (0-5)	1 (0-6)	0.27
Gestational age (weeks)	11 (5-14)	9 (5-14)	**<0.001**
Anemia n (%)	11 (9.6%)	16 (6.3%)	0.28
Prior Uterine Surgical Interventions n (%)	22 (19.3%)	70 (27.6%)	0.11
Type of miscarriage n (%)			
Missed	95 (83.3%)	190 (74.8%)	0.072
Incomplete	19 (16.7%)	64 (25.2%)	
Comorbidity n (%)	30 (26.3%)	93 (25.3%)	0.75

*Data are presented as median (range) for continuous variables and number (percentage) for categorical variables. P-values were calculated using the Mann–Whitney U test (continuous variables) and Chi-square test (categorical variables).

Table 2. Combined clinical and reproductive outcomes and treatment success rates

Outcome	Medical management (n=114)	Surgical management (n=254)	p
Pre-op Hct level (median, range)	37 (24.9–44)	37.7 (28.6–49.4)	0.014
Post-op Hct level (median, range)	34.3 (16.3–42.2)	36.2 (25.4–44.1)	<0.001
Reduction in Hct (median, range)	2.5 (0–13)	1.1 (0–9.7)	<0.001
≥10% Reduction in Hct, n (%)	42 (36.8)	48 (18.9)	<0.001
Duration of inpatient stay (median days, range)	2 (2–17)	1 (1–8)	0.014
Treatment success rate, n (%)	57 (50)	252 (99.2)	<0.001
Overall complications, n (%)	10 (8.8)	6 (2.4)	0.01
Conception delay >12 months, n (%)	4 (8.9)	9 (10.6)	0.76

Data are presented as median (range) for continuous variables and as number (percentage) for categorical variables. p-values were calculated using the Mann–Whitney U test for continuous variables and the Chi-square test for categorical variables. A p-value <0.05 was considered statistically significant. Hct: hematocrit

unprotected intercourse in women who wished to conceive. It was recorded in 8.9% (*n*=4) of the patients managed medically and 10.6% (*n*=9) of those who underwent surgical treatment, with no statistically significant difference between groups (p=0.76) (Table 2).

The success rates of the treatment methods varied significantly depending on the type of miscarriage. Among patients with missed miscarriage, surgical management achieved a significantly higher success rate (98.9%) compared to medical treatment (49.5%) (p<0.001). Similarly, in cases of incomplete miscarriage, all patients undergoing surgical evacuation experienced successful outcomes (100%), whereas the success rate for medical management was markedly lower at 47.4% (p<0.001). These findings suggest that surgical intervention is substantially more effective than medical treatment in ensuring complete uterine evacuation across different miscarriage types.

Multivariate logistic regression identified treatment modality (surgical vs. medical) as the only significant predictor of treatment success (adjusted OR=12.5, 95% CI: 5.3–29.4, *p*<0.001). None of the other variables – including age, gravidity, parity, gestational age, or baseline hemoglobin – showed a significant association with treatment outcome.

Although major complications were rare in both groups, the medical management group exhibited a relatively higher risk profile. Pelvic infection was observed in 2.6% of patients in the medical group versus 0.4% in the surgical group. Uterine bleeding occurred in 1.8% and 0.4%, respectively. One patient in the medical group (0.9%) required a blood transfusion, whereas none in the surgical group did.

A single case of chorioamnionitis (0.9%) was also reported in the medical group. Notably, retained products of conception were significantly more frequent in the medical group (5.3%) compared to the surgical group (0.8%).

Discussion

Miscarriage is a frequent complication of early pregnancy, affecting many women of reproductive age [9,10]. In low- and middle-income countries, where maternal and neonatal mortality rates remain high, it can cause severe morbidities, such as hemorrhage, infection, and even death [11]. Thus, evaluating treatment methods that are both effective and accessible is essential. Among surgical approaches, vacuum aspiration is the most widely used and is recommended in the World Health Organization's safe abortion guidelines [12]. Misoprostol, a synthetic prostaglandin E1 analogue, is also widely used for cervical ripening and labor induction, acting on uterine smooth muscle to promote contractions and cervical remodeling [13]. Studies comparing surgical and medical management of early pregnancy loss indicate that both methods are generally effective and safe, but their advantages and limitations depend on patient characteristics and healthcare resources. In this study, we compared medical and surgical management of first-trimester pregnancy loss with respect to success rates, complication profiles, and follow-up requirements.

In a large retrospective cohort of 30,146 women in Los Angeles, the success rate of medical abortion at or before 9 weeks was 99.6%, compared to 99.8% with surgical abortion [14]. Other studies report similar results. In Nepal, among 160 women, complete abortion occurred in 93.15% of those managed with manual vacuum aspiration versus 85.14% with medical treatment [15]. A 2022 study of 342 women found medical success rates of 94.3% for pregnancies ≤6 weeks and 97% for 6–8 weeks [16]. A Cochrane network meta-analysis of 39 randomized controlled trials confirmed vacuum aspiration was more effective than medical management for missed abortion (relative risk [RR]: 1.51; 95% CI: 1.14–2.01) [17]. Similarly, Ireland et al.[18] showed that combining mifepristone with misoprostol was highly effective in first-trimester termination. In our cohort, surgical management achieved a 99.2% success rate, while the medical group had a markedly lower rate of 50% (p<0.001). The lack of mifepristone use may explain the reduced effectiveness, as misoprostol alone is associated with lower success and greater need for additional interventions.

Although both medical and surgical methods are considered safe and effective, unplanned surgical aspiration due to persistent pregnancy, pain, or prolonged bleeding was more frequent in the medical group (2.1% vs. 0.6%). The medical group also showed lower success and higher complication rates (8.8% vs. 2.4%, p=0.01), and longer hospitalization (2 vs. 1 day, p=0.014). A review by El-Refaey and Templeton confirmed that mifepristone–misoprostol regimens are effective but often cause prolonged bleeding, pain, nausea, and gastrointestinal side effects, whereas surgical methods carry risks, such as uterine perforation or cervical laceration [19]. Our findings support these observations, showing incomplete abortion and retained products were significantly more frequent with medical treatment (5.26% vs. 0.8%). Similar reports note longer bleeding, more follow-up, and higher revision rates in medical cases [15,20]. A prospective study of 838 women also found higher surgical revision in the medical group (7.4% vs. 3.0%, p<0.001) [20]. Meta-analysis evidence supports that surgery reduces the need for additional intervention (RR: 0.19; 95% CI: 0.10–0.37) and shortens hospital stay [17]. In our cohort, pelvic infection was also more common in the medical group (2.63% vs. 0.4%). These complications, together with longer monitoring needs, highlight that although non-invasive, medical management may require more frequent follow-up and additional interventions, affecting patient satisfaction and healthcare resources.

Our findings, although differing from the high success rates reported in large cohorts, highlight that medical treatment may not always match the efficacy of surgical methods. Outcomes appear influenced by patient selection and the availability of pharmacological agents. While the overall safety profile aligns with previous literature, the lower success and higher complication rates with misoprostol alone suggest it may not be appropriate for all populations.

In our study, the median gestational age was significantly higher in the medical group (11 weeks vs. 9 weeks). This factor may have contributed to the greater blood loss and Hct reduction observed in that group, as advanced gestational age is associated with larger uterine volume and placental surface area. Similar observations have been reported in previous studies linking gestational age to increased bleeding during medical management.

The present research provides real-world evidence from a relatively large, single-center cohort, systematically evaluating treatment success, complication rates, and hospital stay durations. Such evidence is particularly valuable in low- and middle-income settings, where treatment access and follow-up may vary. By directly comparing outcomes in both missed and incomplete miscarriages, these findings clarify the practical advantages and limitations of each approach, offering guidance for individualized treatment and healthcare resource allocation.

Regarding fertility outcomes, conception delay was similar between groups (8.9% medical vs. 10.6% surgical, p=0.76).

These results are consistent with previous research showing no significant differences in fertility after early pregnancy loss. For instance, a cohort of 203 women found comparable pregnancy rates between groups at 6 months (68.0% vs. 65.1%) [21], while a trial following 604 women for up to 9 years showed no differences in natural conception rates (97.7%), time to pregnancy, or live birth rates [22].

Nevertheless, the potential risk of intrauterine adhesions (IUAs) after surgical procedures should not be overlooked. Blind techniques, such as dilation and curettage or vacuum aspiration, may increase the risk of endometrial trauma, whereas misoprostol could offer a fertility-preserving alternative [23]. In one study, IUAs were absent in the misoprostol group but occurred in 7.7% of women who underwent D&C.[24]. Although our study did not detect significant differences in fertility outcomes, the theoretical advantage of preserving endometrial integrity supports considering long-term reproductive goals when selecting treatment.

Limitation

Several limitations should be acknowledged. Its retrospective design may have introduced selection bias, and reliance on medical records could have resulted in incomplete data. Being conducted in a single tertiary center also limits generalizability. Treatment allocation was not randomized but based on clinical judgment or patient preference, which may have influenced outcomes. In the medical group, the exclusive use of misoprostol might have contributed to lower success rates. Furthermore, fertility outcomes were partly based on patient self-reports, potentially underestimating subtle reproductive issues. Despite these limitations, the study's relatively large sample size over an extended period, along with subgroup analyses, strengthens its clinical relevance. Evaluating both short- and long-term outcomes provides a more comprehensive understanding of treatment effectiveness.

Conclusion

Overall, the findings indicate that vacuum aspiration as a surgical approach for managing first-trimester pregnancy loss is associated with significantly higher success rates, lower complication rates, and a shorter duration of follow-up compared to medical management. These findings underscore the clinical advantages of surgical treatment, particularly in terms of patient safety and healthcare efficiency. In resource-constrained settings, where medication availability or compliance may be limited, surgical management should be considered the preferred method due to its reliability, cost-effectiveness, and rapid resolution of symptoms.

Disclosure

Ethics Committee Approval: This study was approved by the Gülhane Training and Research Hospital Ethics Committee (Date: 10.04.2025, Decision no: 2025/75).

Informed Consent: Patient consent was waived due to the retrospective nature of the study.

Conflict of Interest: The authors declare that there is no conflict of interest.

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References

1. Al-Alami Z, Abu-Huwaij R, Hamadneh S, Taybeh E. Understanding miscarriage prevalence and risk factors: insights from women in Jordan. Medicina 2024;60:1044. [CrossRef]
2. Cunningham FG, Leveno KJ, Dashe JS, Hoffman BL, Spong CY, Casey BM, editors. Williams Obstetrics. 26th ed. New York (NY): McGraw Hill; 2022.
3. National Institute for Health and Care Excellence (NICE). Ectopic pregnancy and miscarriage: diagnosis and initial management. NICE clinical guideline no. 154. Manchester (UK): NICE; 2012.
4. Magnus MC, Wilcox AJ, Morken NH, Weinberg CR, Håberg SE. Role of maternal age and pregnancy history in risk of miscarriage: prospective register based study. BMJ 2019;364:l869. [CrossRef]
5. Cubo AM, Soto ZM, Haro-Pérez A, Hernández Hernández ME, Doyague MJ, Sayagués JM. Medical versus surgical treatment of first trimester spontaneous abortion: a cost-minimization analysis. PLoS One 2019;14:e0210449. [CrossRef]
6. Nwafor JI, Agwu UM, Egbuji CC, Ekwedigwe KC. Misoprostol versus manual vacuum aspiration for treatment of first-trimester incomplete miscarriage in a low-resource setting: A randomized controlled trial. Niger J Clin Pract 2020;23:638–46. [CrossRef]
7. Abbas D, Chong E, Raymond EG. Outpatient medical abortion is safe and effective through 70 days gestation. Contraception 2015;92:197–9. [CrossRef]
8. Trinder J, Brocklehurst P, Porter R, Read M, Vyas S, Smith L. Management of miscarriage: expectant, medical, or surgical?

Results of randomised controlled trial (miscarriage treatment (MIST) trial). BMJ 2006;332:1235–40. [CrossRef]
9. FIGO Working Group on Misoprostol for the Prevention of Postpartum Hemorrhage. *Misoprostol-only recommended dosages for reproductive health indications*. International Federation of Gynecology and Obstetrics (FIGO); 2017.
10. Shahraki-Sanavi F, Sahab-Negah S, Nili S, Mangolian Shahrbabaki P, Ansari-Moghaddam A, Fereidouni M, et al. Evaluating miscarriage incidence after COVID-19 vaccination. Sci Rep 2025;15:20387. [CrossRef]
11. Izulla P, Muriuki A, Kiragu M, Yahner M, Fonner V, Nitu SNA, et al. Proximate and distant determinants of maternal and neonatal mortality in the postnatal period: A scoping review of data from low- and middle-income countries. PLoS One 2023;18:e0293479. Erratum in: PLoS One 2024;19:e0304260. [CrossRef]
12. Safe Abortion: Technical and Policy Guidance for Health Systems. 2nd ed. Geneva: World Health Organization; 2012.
13. Ramadan M, Bashour G, Eldokmery E, Alkhawajah A, Alsalhi K, Badr Y, et al. The efficacy and safety of oral and vaginal misoprostol versus dinoprostone on women experiencing labor: A systematic review and updated meta-analysis of 53 randomized controlled trials. Medicine (Baltimore) 2024;103:e39861. [CrossRef]
14. Upadhyay UD, Desai S, Zlidar V, Weitz TA, Grossman D, Anderson P, et al. Incidence of emergency department visits and complications after abortion. Obstet Gynecol 2015;125:175–83. [CrossRef]
15. Giri KP, Dangal G. Comparison of medical abortion with manual vacuum aspiration in termination of pregnancy up to nine weeks. J Nepal Health Res Counc 2020;18:116–9. [CrossRef]
16. Dethier D, Soon R, Ronquillo T, Wong Z, Tschann M. Comparison of medication abortion outcomes at less than and greater than 6 weeks gestation. Contraception 2024;140:110691. [CrossRef]
17. Ghosh J, Papadopoulou A, Devall AJ, Jeffery HC, Beeson LE, Do V, et al. Methods for managing miscarriage: a network meta-analysis. Cochrane Database Syst Rev 2021;6:CD012602. [CrossRef]
18. Ireland LD, Gatter M, Chen AY. Medical compared with surgical abortion for effective pregnancy termination in the first trimester. Obstet Gynecol 2015;126:22–8. [CrossRef]
19. Ashok PW, Kidd A, Flett GM, Fitzmaurice A, Graham W, Templeton A. A randomized comparison of medical abortion and surgical vacuum aspiration at 10-13 weeks gestation. Hum Reprod 2002;17:92–8. [CrossRef]
20. Bačíková A, Sehnal B, Pojarová M, Kolářová Z, Zikán M. Comparison of the quality of life between women undergoing medical and surgical terminations of pregnancy. Ceska Gynekol 2021;86:304–10. [CrossRef]
21. Tzur Y, Samueloff O, Raz Y, Bar-On S, Laskov I, Tzur T. Conception rates after medical versus surgical evacuation of early miscarriage. Fertil Steril 2021;115:118–24. [CrossRef]
22. Tam WH, Tsui MH, Lok IH, Yip SK, Yuen PM, Chung TK. Long-term reproductive outcome subsequent to medical versus surgical treatment for miscarriage. Hum Reprod 2005;20:3355–9. [CrossRef]
23. Weeks A, Alia G, Blum J, Winikoff B, Ekwaru P, Durocher J, et al. A randomized trial of misoprostol compared with manual vacuum aspiration for incomplete abortion. Obstet Gynecol 2005;106:540–7. [CrossRef]
24. Tam WH, Lau WC, Cheung LP, Yuen PM, Chung TK. Intrauterine adhesions after conservative and surgical management of spontaneous abortion. J Am Assoc Gynecol Laparosc 2002;9:182–5. [CrossRef]

HAYDARPAŞA NUMUNE MEDICAL JOURNAL

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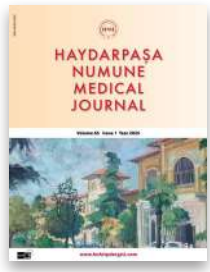

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ORIGINAL ARTICLE

Clinical Significance of Tumor Location in Urothelial Carcinoma of The Bladder

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Abstract

Introduction: Bladder cancer is the most common malignancy of the urinary tract, occurring more frequently in men than in women. While pathological features are major prognostic determinants, the effect of tumor localization remains unclear. This study aimed to investigate the relationship between bladder tumor location, stage, recurrence, and progression.

Methods: A retrospective review was conducted of 452 patients diagnosed with urothelial carcinoma who underwent transurethral resection of the bladder tumor between 2016 and 2021. Demographics, tumor location, pathological stage, recurrence, and progression were analyzed. The bladder was divided into eight regions for mapping. Chi-square and Fisher's exact tests were used; $p<0.05$ was considered significant.

Results: The mean age was 64.5 years, with a mean follow-up of 34.5 months. High-grade tumors were found in 65% of cases. The lateral walls were the most common sites, whereas the trigone and base were the least common. Some tumors were significantly associated with high-grade and muscle invasion. Overall recurrence was 41%, and progression was 10.6%. Recurrence was significantly higher in tumors of the posterior wall.

Discussion and Conclusion: Findings indicate that dome tumors are linked to higher stage and grade, whereas posterior wall tumors carry increased recurrence risk. Tumor location may have prognostic value in bladder cancer, warranting further studies.

Keywords: Bladder cancer; location; progression; recurrence; transurethral resection of the bladder tumor; tumor grade.

Bladder cancer ranks as the seventh most frequently diagnosed cancer in men worldwide and tenth when both sexes are considered together [1]. It stands out as the most common cancer of the urinary tract. Several risk factors have been identified for bladder cancer, including age, gender, race, and exposure to certain carcinogens. Among these carcinogens, the most common exposure is cigarette smoke [2,3]. The incidence of bladder cancer rises particularly after the age of 65. Moreover, it occurs about 4 times more frequently in men than in women. In the white race, the incidence is approximately twice as high as in the black race [4]. About 90% of bladder cancers arise from the urothelial epithelium [5]. Approximately 75% of patients present with disease limited to the mucosa (stage pTa, carcinoma *in situ* [CIS]) or submucosa (stage pT1); this rate is even higher in younger patients (under the age of 40). Compared to muscle-invasive tumors (pT2-4), patients with pTa, pT1, and CIS tumors have a higher long-term survival

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rate and a lower risk of cancer-specific mortality [6]. Bladder tumors show wide variation in their course, and appropriate diagnosis, staging, and grading require pathological evaluation. Pathological grading forms the basis of staging. The mainstay of pathological diagnosis is the transurethral resection of the bladder tumor (TUR-BT). Although tumor stage, grade, size, and multifocality are well-established determinants of disease behavior in bladder cancer, the potential clinical significance of intravesical tumor location remains controversial. It has not been clearly defined in patients undergoing primary TUR-BT.

Previous studies have suggested that tumors arising from specific regions of the bladder may be associated with distinct pathological characteristics; however, the available evidence is limited and inconsistent [7,8].

In this study, we aimed to retrospectively investigate whether the location of the tumor within the bladder in patients who underwent primary TUR-BT and were diagnosed with urothelial carcinoma is associated with certain parameters, such as grade, prognosis, recurrence, and multifocality.

Accordingly, this study was designed as a hypothesis-generating analysis to explore the association between intravesical tumor location and clinicopathological characteristics in a real-world TUR-BT population.

Materials and Methods

Ethics committee approval for our study was obtained on January 18, 2022, with file number 2022/5, from the Bezmialem Vakif University Non-Interventional Research Ethics Committee. The study was conducted in accordance with the principles of the Declaration of Helsinki. Data from a total of 452 patients who underwent TUR-BT and were diagnosed with "urothelial carcinoma" between January 2016 and August 2021 in the Urology Department of Bezmialem Vakif University Faculty of Medicine Hospital were retrospectively analyzed. Patients with histopathologically confirmed urothelial carcinoma and available follow-up data after primary TUR-BT were included in the study. The study included 61 female and 391 male patients. Post-operative follow-up was performed according to the contemporary European Association of Urology guideline recommendations valid at the time of patient management. Patients with non-muscle-invasive bladder cancer underwent cystoscopic surveillance at regular intervals, including an initial cystoscopy at 3 months after TUR-BT, followed by periodic cystoscopies based on individual risk stratification. Patients diagnosed with muscle-invasive bladder cancer were referred for further oncological evaluation and multidisciplinary management according to institutional practice. Due to the retrospective design of the study, detailed data regarding intravesical therapy regimens and adjuvant treatments were not consistently available and were therefore not included in the analysis. Patients with incomplete pathological records, missing tumor localization data, non-urothelial histology, or inadequate follow-up were excluded. The parameters recorded included patient age, gender, tumor location, pathological stage of the primary tumor, development of progression or recurrence, and total tumor size. Recurrence was defined as the appearance of a new tumor on control cystoscopies after complete resection. Progression was defined as an increase in pathological stage compared with the primary tumor. For patients with an indication for re-TUR in the 1st month, pathology results showing a tumor were not considered recurrence. Given that the study population included both non-muscle-invasive and muscle-invasive tumors, recurrence and progression were evaluated based on pathological findings rather than treatment outcomes.

For bladder mapping, eight different groups were created based on the intravesical location of the tumor: Right lateral wall (including right ureteric orifice), left lateral wall (including left ureteric orifice), posterior wall, dome, bladder neck, trigone, bladder base, and cases in which the bladder was completely filled with tumor. Possible differences in pathological stage, progression, and recurrence according to tumor location were evaluated using Pearson's Chi-square and Fisher's exact tests. In addition, whether tumor size and gender were significantly associated with pathological grade, progression, recurrence, and tumor location was investigated.

Statistical Analysis

All statistical analyses were performed using IBM Statistical Package for the Social Sciences Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA). $P<0.05$ was considered statistically significant.

Due to limitations in available clinicopathological variables, multivariate regression analyses could not be reliably performed.

Results

The mean age at primary diagnosis of the patients included in the study was 64.48±12.42 years. The mean follow-up period was 34.49±29.49 months. Low-grade tumors were detected in 148 cases and high-grade tumors in 304 cases. Among the high-grade tumors, 142 were pTa, 134 were pT1, and 28 were pT2. While 284 patients had a single tumor, 168 had multiple tumors within the bladder. During follow-up, recurrence was observed in 187 patients, and progression was observed in 48 of these. In the primary

pathology of the patients who experienced progression, 12 had low-grade tumors, and 36 had high-grade tumors. Right and left lateral wall involvement rates were 52.8% and 41.8%, respectively, making these the most common locations. The percentages of cases involving the trigone and bladder base were 5.3% and 4.6%, respectively, which were the least common locations. Tumors were observed in the dome in 41 patients and in the bladder neck in 26 patients. Tumors of the posterior wall were found in 64 patients, and in 11 patients, the bladder was filled with tumors. Tumors involving more than one bladder region were recorded separately for each involved anatomical location. Therefore, individual patients with multifocal disease could be represented in more than one localization category. Accordingly, the total number of tumors analyzed according to intravesical location exceeded the total number of patients included in the study, reflecting a tumor-based rather than patient-based localization analysis. Figure 1 shows the distribution of tumor locations within the bladder.

Table 1 presents the relationship between the location of the tumor within the bladder and the stage of the primary tumor.

In our study, of the 41 tumors involving the dome, 34 were high-grade tumors. The frequency of high-grade tumors in the dome was significantly increased (p=0.025). Furthermore, of the 41 dome tumors, 8 were high-grade pT2. The frequency of high-grade pT2 tumors was significantly higher in tumors located in the dome (p<0.001). Among the 11 cases where the bladder was filled with tumor, 3 had high-grade pT2 tumors, which were also significantly higher (p=0.025). No statistically significant association between tumor grade and intravesical location was observed in other bladder regions.

Table 1. Relationship between tumor location and grade

	Low-grade pTa	High-grade pTa	High-grade pT1	High-grade pT2	Total
Right lateral wall	83	78	66	12	239
Left lateral wall	56	62	62	9	189
Dome	7	11	15	8	41
Posterior wall	21	17	21	5	64
Bladder neck	8	8	9	1	26
Base	6	8	9	1	24
Trigone	7	5	9	0	21
Bladder filled with a tumor	3	3	2	3	11

When evaluating the relationship between tumor location and recurrence or progression, it was observed that recurrence in tumors of the posterior wall was significantly increased (p=0.02), while no other statistically significant associations were detected. Table 2 examines the relationship between tumor location, recurrence, and progression.

Regarding gender, although the number of female patients was lower than that of male patients, a significant association was observed between female gender and tumors filling the bladder (p=0.009). Of the 452 patients, 11 had bladders filled with tumors. Of these, 5 were female.

The mean tumor size was calculated as 29.08±21.22 mm. In tumors larger than 3 cm, the frequency of recurrence was significantly higher (p<0.001); similarly, the frequency of progression was also significantly increased (p=0.019). Multivariate regression analyses were planned; however,

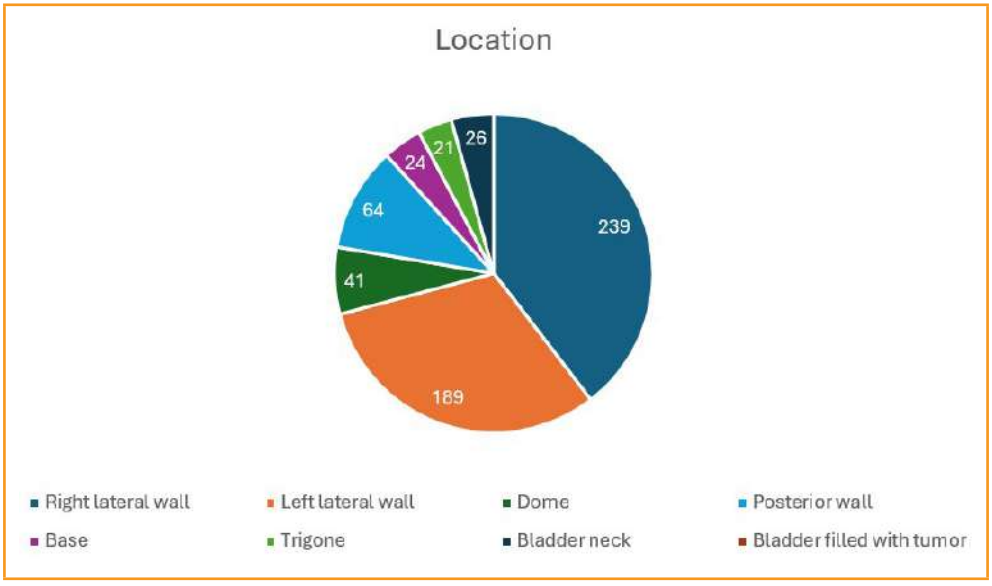

Figure 1. Distribution of tumor locations within the bladder.

Table 2. Relationship between tumor location, recurrence, and progression

	Recurrence	p	Progression	p
Right lateral wall	98	0.867	21	0.180
Left lateral wall	81	0.587	26	0.066
Dome	20	0.312	7	0.180
Posterior wall	35	0.020	9	0.335
Bladder neck	13	0.357	4	0.506
Base	13	0.191	4	0.306
Trigone	9	0.887	1	0.714
Bladder filled with a tumor	6	0.374	3	0.101

due to missing data on several established prognostic variables, including intravesical therapy status, CIS, lymphovascular invasion, and variant histology, a reliable multivariate model could not be constructed.

Discussion

Although limited data are available in the literature on whether the location of urothelial carcinoma of the bladder affects the prognosis and other parameters of the disease, it has been investigated by some researchers. Studies have aimed to predict prognosis according to the location of the tumor within the bladder in patients who underwent radical cystectomy. In the study by Weiner et al., [7] tumors of the trigone and bladder neck were associated with increased lymph node involvement, whereas tumors in the dome were detected at a higher stage. Another study reported that tumors involving the bladder neck may have a higher risk of recurrence after intravesical immunotherapy [8].

Stephenson et al. [9] evaluated the initial tumor locations of newly diagnosed bladder cancer patients, similar to our study. They found that tumors located in the bladder neck were associated with a worse prognosis. In addition, tumors located in the dome were observed to be of higher grade, whereas those located in the lateral wall and ureteric orifices were of lower grade. In a study by Takashi et al., [10] high-grade non-muscle-invasive bladder cancers were evaluated, and location was shown to have no effect on survival.

In the studies by Weiner et al. [7] and Stephenson et al., [9] tumors located in the bladder neck were found to have a poor prognosis, whereas in our study, no statistically significant difference was observed in terms of recurrence and prognosis in bladder neck tumors. In contrast, the finding of higher-grade tumors and a significantly higher frequency of muscle-invasive tumors in the dome suggested the hypothesis that tumors in the dome might be more difficult to visualize during cystoscopy and, therefore, may be overlooked, being diagnosed at more advanced stages after repeated cystoscopies. Similarly, in the study by Stephenson et al., [9] dome tumors were detected at higher T stages compared to lateral wall tumors, supporting the possibility that tumors in this location may produce later symptoms than those in the lateral wall.

In our study, the risk of recurrence in the posterior wall was observed to be significantly higher, raising the possibility that residual tumor tissue may remain due to the greater difficulty of posterior wall resections during TUR-BT. Since there is limited literature available on this topic, further studies are needed for a more accurate interpretation.

In addition, we found that bladders filled with tumors were significantly more common in female patients. Although bladder cancer is more common in men, the probability of detecting higher T-stage bladder cancer at initial diagnosis is higher in women [11]. In a study, the time (in days) from first hematuria to the diagnosis of bladder cancer was calculated in male and female patients with hematuria caused by bladder cancer and was found to be significantly longer in women (85.4 vs. 73.6 days; $p<0.001$). This is thought to be because hematuria in women is often initially evaluated as a urinary tract infection, and imaging of the bladder is not performed [12]. The high rate of bladders filled with tumors observed in female patients in our study is similar to these findings in the literature.

In another large-volume study, the relationship between the original intravesical location of bladder cancer and the presence of metastasis was investigated. Parallel to our study, the most common involvement was of the lateral wall, followed by the posterior wall. The highest metastasis rate was observed in patients with bladder neck involvement [13]. In the study by Jancke et al., [14] a significant association was found between tumor recurrence and pTa/pT1 bladder cancers involving the ureteric orifices. In our bladder mapping, the ureteric orifices were included in the right and left lateral wall regions, and the trigone was mapped separately. In our study, no statistically significant increase in recurrence was observed in lateral wall involvement, including the orifices.

In the study by Dutta et al., [15] the effect of tumor location on survival in bladder adenocarcinoma cases was investigated, and poorer outcomes were found in cases involving the trigone, ureteric orifices, and bladder neck. We investigated the effect of tumor location only in patients with transitional cell carcinoma of the bladder.

There are also studies in the literature on urinary tract tumors other than bladder cancer that highlight the rela-

tionship between tumor location and prognosis [16,17]. In a study evaluating 149 patients, it was reported that the distal ureteral location of upper urinary tract transitional cell carcinoma was associated with a better prognosis compared to proximal location, regardless of stage [16]. The fact that tumor location may be important not only for bladder cancer but also for ureter and other urinary system tumors makes our study even more meaningful and highlights the need for more comprehensive studies on the subject.

In the study by Wedel et al., [18] tumors involving the lateral walls were found to be significantly associated with lymph node positivity, whereas posterior wall involvement had the least association with lymph node positivity. In our study, the effects of tumor location on T stage, progression, and recurrence were evaluated; however, its effect on metastasis, lymph node involvement, and survival was not evaluated. We accept this as a limitation of our study.

Limitation

Due to its retrospective design, detailed information regarding intravesical and adjuvant treatments was not consistently available, preventing inclusion of these variables in multivariate analyses. Furthermore, lymph node involvement, metastatic disease, and survival outcomes were not assessed. Despite these limitations, our cohort represents one of the larger single-center series evaluating bladder tumor location, and the findings may contribute to a better understanding of the relationship between intravesical tumor location and tumor characteristics.

Conclusion

In our study, it was found that transitional cell carcinoma of the bladder frequently involved the lateral walls; tumors involving the dome were of higher grade and more frequently muscle-invasive. In addition, tumors involving the posterior wall were associated with a higher risk of recurrence. In female patients, muscle invasion and bladders completely filled with tumors were found to be significantly more common. Although tumor location alone should not be considered a definitive prognostic factor, awareness of location-specific characteristics may contribute to improved clinical assessment and follow-up strategies.

Disclosure

Ethics Committee Approval: This study was approved by the Bezmialem Vakif University Ethics Committee (Date: 18.01.2022, Decision no: 2022/5).

Informed Consent: As the study was retrospective and all patient identifiers were anonymized, informed consent was not obtained.

Conflict of Interest: The authors declare that there is no conflict of interest.

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Authorship Contributions: Concept – F.G., E.C.C., B.M.K., Y.Ö., C.A., K.K., R.G., Y.Ö.İ.; Design – F.G., E.C.C., B.M.K., Y.Ö., C.A., K.K., R.G., Y.Ö.İ.; Supervision – F.G., E.C.C., B.M.K., Y.Ö., C.A., K.K., R.G., Y.Ö.İ.; Fundings – Y.Ö.İ., R.G.; Materials – F.G., R.G., Y.Ö.İ.; Data collection and/or processing – B.M.K., C.A., K.K.; Analysis and/or interpretation – F.G., R.G., Y.Ö.İ.; Literature review – F.G.; Writing: E.C.C., Y.Ö.; Critical review – F.G.

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References

1. International Agency for Research on Cancer, World Health Organization. Estimated number of new cases in 2020, worldwide, both sexes, all ages. Available at: https://amlo-biosciences.com/today/data/factsheets/populations/900-world-fact-sheets.pdf. Accessed Apr 30, 2026.
2. Dobruch J, Oszczudłowski M. Bladder cancer: current challenges and future directions. Medicina (Kaunas) 2021;57:749. [CrossRef]
3. 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. [CrossRef]
4. Charlton ME, Adamo MP, Sun L, Deorah S. Bladder cancer collaborative stage variables and their data quality, usage, and clinical implications: a review of SEER data, 2004-2010. Cancer 2014;120(Suppl 23):3815–25. [CrossRef]
5. World Health Organization. Urinary and male genital tumours. IARC, Lyon, France, 2022.
6. Burger M, Catto JW, Dalbagni G, Grossman HB, Herr H, Karakiewicz P, et al. Epidemiology and risk factors of urothelial bladder cancer. Eur Urol 2013;63:234–41. [CrossRef]
7. Weiner AB, Desai AS, Meeks JJ. Tumor location may predict adverse pathology and survival following definitive treatment for bladder cancer: a national cohort study. Eur Urol Oncol 2019;2:304–10. [CrossRef]
8. Vukomanovic I, Colovic V, Soldatovic I, Hadzi-Djokic J. Prognostic significance of tumor location in high-grade non-muscle-invasive bladder cancer. Med Oncol 2012;29:1916–20. [CrossRef]
9. Stephenson WT, Holmes FF, Noble MJ, Gerald KB. Analysis of bladder carcinoma by subsite. Cystoscopic location may have prognostic value. Cancer 1990;66:1630–5. [CrossRef]
10. Takashi M, Murase T, Mizuno S, Hamajima N, Ohno Y. Multivariate evaluation of prognostic determinants in bladder cancer patients. Urol Int 1987;42:368–74. [CrossRef]
11. Liu S, Yang T, Na R, Hu M, Zhang L, Fu Y, et al. The impact of female gender on bladder cancer-specific death risk after radical cystectomy: a meta-analysis of 27,912 patients. Int Urol Nephrol 2015;47:951–8. [CrossRef]

12. Dobruch J, Daneshmand S, Fisch M, Lotan Y, Noon AP, Resnick MJ, et al. Gender and bladder cancer: a collaborative review of etiology, biology, and outcomes. Eur Urol 2016;69:300–10. [CrossRef]
13. Park HK. Do histology and primary tumor location influence metastatic patterns in bladder cancer? Curr Oncol 2023;30:9078–89. [CrossRef]
14. Jancke G, Rosell J, Jahnson S. Tumour location adjacent to the ureteric orifice in primary Ta/T1 bladder cancer is predictive of recurrence. Scand J Urol 2016;50:33–8. [CrossRef]
15. Dutta R, Abdelhalim A, Martin JW, Vernez SL, Faltas B, Lotan Y, et al. Effect of tumor location on survival in urinary bladder adenocarcinoma: A population-based analysis. Urol Oncol 2016;34:531.e1–6. [CrossRef]
16. van der Poel HG, Antonini N, van Tinteren H, Horenblas S. Upper urinary tract cancer: location is correlated with prognosis. Eur Urol 2005;48:438–44. [CrossRef]
17. Favaretto RL, Shariat SF, Chade DC, Godoy G, Adamy A, Kaag M, et al. The effect of tumor location on prognosis in patients treated with radical nephroureterectomy at Memorial Sloan-Kettering Cancer Center. Eur Urol 2010;58:574–80. [CrossRef]
18. Wedel SA, Jones J, Tsaur I, Ackermann H, Beecken WD, Jonas D, et al. Association of intravesical tumor location with metastases to the pelvic lymph nodes in transitional cell cancer of the bladder. Am J Med Sci 2010;339:341–4. [CrossRef]

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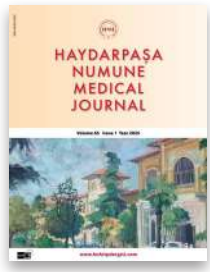

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ORIGINAL ARTICLE

Evaluation of Kawasaki Disease Cases in A Tertiary Care Center in Terms of Diagnosis, Treatment, and Complications

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Abstract

Introduction: Kawasaki disease (KD) is the leading cause of acquired heart disease in children, and timely diagnosis is crucial to prevent cardiac complications. We aimed to evaluate the clinical and demographic characteristics of patients diagnosed with KD, reveal differences between complete and incomplete cases, and determine risk factors associated with cardiac involvement.

Methods: This retrospective study included 30 patients who were diagnosed with KD. Patients were classified as having complete KD (cKD) or incomplete KD (iKD) based on standard diagnostic criteria. Statistical analyses, including logistic regression and receiver operating characteristic (ROC) analysis, were performed to explore the predictors of cardiac involvement.

Results: The study included 30 patients (73.3% male, n=22). 70% of the patients had cKD. Cardiac involvement was found in 40% of patients, with mitral regurgitation and coronary artery dilatation being the most common. Patients with cKD had a longer duration of fever (p=0.047), higher C-reactive protein (p=0.018) and alanine aminotransferase levels (p=0.044), lower hemoglobin levels (p=0.029), and more frequent cardiac involvement (p=0.049) than those with iKD. The duration of fever was significantly higher in those with cardiac involvement (p=0.035). ROC analysis identified fever duration as a significant predictor of cardiac involvement (area under the curve=0.727, p=0.038), with an optimal cutoff of 3.5 days yielding 91.7% sensitivity and 72.2% specificity.

Discussion and Conclusion: Fever duration, particularly over 3.5 days, is strongly associated with cardiac complications in KD. These findings highlight the need for early recognition and timely treatment to improve cardiovascular outcomes.

Keywords: Coronary aneurysm; diagnosis; fever; mitral valve insufficiency; Mucocutaneous lymph node syndrome.

Kawasaki disease (KD) is an acute, systemic vasculitis, especially in children under 5 years of age, whose etiology has not yet been fully elucidated [1]. KD, considered the most common cause of acquired heart disease in childhood, is a significant source of morbidity due to the risk of serious cardiac complications [2,3]. The fact that the disease often presents with nonspecific symptoms in the early period of the disease causes the diagnosis to not be made on time; this increases the risk of permanent cardiac sequelae, especially coronary artery aneurysms [4-6].

The diagnosis of KD is largely based on clinical findings, owing to the lack of a specific biomarker. However, the presence of incomplete cases that do not fully meet the diagnostic criteria and the similarity of the clinical picture of KD with common respiratory tract infections make differential diagnosis very difficult [7-9]. Therefore, delays in diagnosis and treatment may adversely affect both the acute course of the disease and long-term cardiac prognosis.

In this environment of clinical uncertainty, multisystem inflammatory syndrome (MIS-C), which was identified during

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the COVID-19 pandemic and overlaps with certain clinical features with KD, has also come to the fore as a condition to be considered in the differential diagnosis [10-12]. Although MIS-C shares some similar symptoms with KD, it is differentiated by different immunopathogenesis and age distribution; however, clinical similarities may lead to diagnostic confusion, especially in the early stages of the disease. Therefore, increasing clinical awareness for the timely recognition of KD and early initiation of treatment has become one of the main factors determining the prognosis of the disease [13].

In this study, we aimed to evaluate the clinical and demographic characteristics of our patients diagnosed with KD, to reveal the differences between complete KD (cKD) and incomplete cases (iKD), and to determine the possible risk factors associated with the development of cardiac involvement. We assume that cKD cases exhibit a more pronounced inflammatory response and a higher likelihood of cardiac involvement compared to iKD.

Materials and Methods

Patients and Data Collection

This retrospective study was conducted by reviewing the medical records of 30 patients with KD at a tertiary care center between 2010 and 2020. The diagnosis of KD was based on clinical criteria that included fever lasting more than 5 days, skin rash, oral mucosal changes, bilateral non-purulent conjunctivitis, extremity signs, and acute non-suppurative cervical lymphadenopathy [14]. Patients who met at least five of the six criteria were classified as having cKD, and those who met four or fewer criteria were classified as having iKD [15]. All pediatric patients diagnosed with KD were screened consecutively. Patients with complete clinical, laboratory, and echocardiographic data were included in this study. Patients with missing data, patients transferred to another center before follow-up was completed, and patients who refused treatment were excluded from the study.

Clinical data, including gender, age at diagnosis, duration of diagnosis, season of diagnosis, fever, conjunctivitis, oral mucosal changes, cervical lymphadenopathy, rash, sterile pyuria, aseptic meningitis, and gallbladder hydrops, were obtained retrospectively from hospital records. In addition, KD treatment, echocardiography findings, abdominal ultrasonography (USG) results (normal/hepatomegaly/splenomegaly/hepatosplenomegaly), and laboratory values at admission (white blood cell [WBC] count [4–10×10^9/L], hemoglobin level [11.5–15.5 g/dL], platelet [PLT] count [150–450×10^9/L], C-reactive protein [CRP] [<5 mg/L], erythrocyte sedimentation rate [ESR] [<20 mm/h], alanine aminotransferase [ALT] [<40 U/L], aspartate aminotransferase [AST] [<40 U/L], and sodium [Na] levels [135–145 mmol/L]) were recorded.

Echocardiographic Assessment

Transthoracic echocardiography was performed in all patients at the time of diagnosis, before the initiation of IVIG therapy, using a pediatric echocardiography system equipped with an appropriate phased-array transducer. All examinations were performed and interpreted by the same experienced pediatric cardiologist. Echocardiographic abnormalities were evaluated according to the American Heart Association guidelines [16]. Dilatation was defined as an internal diameter ≥3 mm in children younger than 5 years or ≥4 mm in children aged 5 years and older, or a segmental diameter ≥1.5 times that of an adjacent segment; aneurysm was defined as an internal diameter ≥4 mm or ≥1.5 times that of an adjacent segment. Diffuse enlargement of the left main coronary artery without a discrete aneurysmal segment was classified as ectasia. Mitral regurgitation was defined as the detection of a systolic regurgitant jet from the left ventricle into the left atrium on color Doppler imaging, primarily in the apical four-chamber view, and pericardial effusion was defined as the visualization of an echo-free space between the visceral and parietal pericardial layers during diastole on transthoracic echocardiography.

Ethics Committee Approval

This study was conducted in compliance with the Helsinki Declaration as well as local laws and regulations. Due to the retrospective nature of the study, obtaining written informed consent from the patients was not required. This study was approved by the ethics committee (date/no.: March 15, 2021–HNHEAH-KAEK 2021/93) and obtained from the Institutional Review Board of the Haydarpasa Numune Training and Research Hospital.

Statistical Analysis

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) for Windows, version 26.0 (SPSS Inc., Chicago, IL). Categorical variables are presented as numbers with their corresponding percentages and were evaluated using the chi-square test. The normality of data was assessed using the Kolmogorov–Smirnov test. Accordingly, Student's *t*-test was used for normally distributed variables, and the Mann–Whitney U test was used for non-normally distributed variables. Continuous variables were expressed as mean±standard deviation for normally

distributed data and as median (minimum–maximum) for non-normally distributed data.

Logistic regression analyses were performed to identify independent risk factors predicting cardiac and coro-

Table 1. Comparison of patients with complete and incomplete Kawasaki Disease

	Patients with cKD (*n*=21)	Patients with iKD (*n*=9)	p
Age (years) (median [min-max])	5 (1–11)	5 (1–8)	0.819
Time to diagnosis (days) (median [min-max])	5 (3–12)	5 (3–12)	0.746
Male gender (*n*, %)	16 (76.2)	6 (66.7)	0.666
Season of diagnosis (*n*, %)			
Spring	7 (33.3)	3 (33.3)	1.0
Summer	6 (28.6)	2 (22.2)	1.0
Autumn	4 (19)	2 (22.2)	1.0
Winter	4 (19)	2 (22.2)	1.0
Clinical findings (*n*, %)			
Duration of fever (days) (median [min-max])	5 (3–10)	3 (2–10)	**0.047**
Fever (*n*, %)	21 (100)	9 (100)	*
Mucosal changes (*n*, %)	21 (100)	9 (100)	*
Rash (*n*, %)	20 (95.2)	8 (88.9)	0.517
Cervical lymphadenopathy (*n*, %)	18 (85.7)	7 (77.8)	0.622
Bilateral non-purulent conjunctivitis (*n*, %)	19 (90.5)	3 (33.3)	**0.003**
Sterile pyuria (*n*, %)	4 (19)	1 (11.1)	1.0
Aseptic meningitis (*n*, %)	4 (19)	1 (11.1)	1.0
Gallbladder hydrops (*n*, %)	3 (14.3)	1 (11.1)	1.0
Echocardiography (*n*, %)	11 (52.4)	1 (11.1)	**0.049**
Mitral regurgitation (*n*, %)	5 (23.8)	0 (0)	0.286
Left coronary artery dilatation (*n*, %)	3 (14.3)	0 (0)	0.534
Coronary artery aneurysm (*n*, %)	2 (9.5)	0 (0)	1.0
Pericardial effusion (*n*, %)	1 (4.8)	1 (11.1)	0.517
Left coronary artery ectasia (*n*, %)	1 (4.8)	0 (0)	1.0
Laboratory findings at admission			
White blood cell count ($\times 10^9$/L) (mean±SD)	13.1±4.3	12.8±5.5	0.834
Hemoglobin (g/dL) (median [min-max])	11 (9–13)	12 (11–13)	**0.029**
Platelet count ($\times 10^9$/L) (mean±SD)	315.5±144.1	429.4±216.9	0.100
CRP (mg/L) (median [min-max])	130 (11–600)	40 (10–130)	**0.018**
ESR (mm/h) (mean±SD)	75.9±18.9	58.4±36.3	0.093
ALT (IU/L) (median [min-max])	21 (7–360)	15 (9–194)	**0.044**
AST (IU/L) (median [min-max])	32 (12–182)	26 (17–113)	0.147
Sodium (mmol/L) (median [min-max])	136 (130–140)	136 (130–138)	0.783
Abdominal ultrasonography (*n*, %)			
Hepatomegaly (*n*, %)	2 (9.5)	0 (0)	1.0
Splenomegaly (*n*, %)	1 (4.8)	0 (0)	1.0
Hepatosplenomegaly (*n*, %)	1 (4.8)	0 (0)	1.0

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; cKD: complete Kawasaki disease; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; iKD: Incomplete Kawasaki disease; SD: Standard deviation. * Statistical comparison was not applicable because all patients in both groups presented with this finding.

Table 2. Comparison of patients with and without cardiac involvement, and with and without coronary artery involvement

	Cardiac involvement			Coronary artery involvement		
	Yes (*n*=12)	**No (*n*=18)**	**p**	**Yes (*n*=6)**	**No (*n*=24)**	**p**
Age (years) (median [min-max])	4 (1–10)	5.5 (1–12)	0.881	3 (1–8)	5.5 (1–11)	0.333
Time to diagnosis (days) (median [min-max])	6.5 (3–12)	5 (3–12)	0.100	5.5 (3–7)	5 (3–12)	0.915
Male gender (*n*, %)	10 (83.3)	12 (66.7)	0.419	5 (83.3)	17 (70.8)	1.0
Type (*n*, %)						
cKD	11 (91.7)	10 (55.6)	**0.049**	6 (100)	15 (62.5)	0.141
iKD	1 (8.3)	8 (44.4)	**0.049**	0 (0)	9 (37.5)	0.141
Clinical findings (*n*, %)						
Duration of fever (days) (median [min-max])	6 (3–10)	4 (2–10)	**0.035**	5 (3–7)	5 (2–10)	0.916
Fever (*n*, %)	12 (100)	18 (100)	*	6 (100)	24 (100)	*
Mucosal changes (*n*, %)	12 (100)	18 (100)	*	6 (100)	24 (100)	*
Rash (*n*, %)	11 (91.7)	17 (94.4)	1.0	6 (100)	22 (91.7)	1.0
Cervical lymphadenopathy (*n*, %)	9 (75)	16 (88.9)	0.364	5 (83.3)	20 (83.3)	1.0
Bilateral non-purulent conjunctivitis (*n*, %)	10 (83.3)	12 (66.7)	0.419	5 (83.3)	17 (70.8)	1.0
Sterile pyuria (*n*, %)	1 (8.3)	4 (22.2)	0.622	0 (0)	5 (20.8)	0.553
Aseptic meningitis (*n*, %)	4 (33.3)	1 (5.6)	0.128	2 (33.3)	3 (12.5)	0.254
Gallbladder hydrops (*n*, %)	1 (8.3)	3 (16.7)	0.632	0 (0)	4 (16.7)	0.557
Laboratory findings						
White blood cell count ($\times10^9$/L) (mean±SD)	14.3±4.2	12.2±4.8	0.226	13.2±3.8	13±4.9	0.939
Hemoglobin (g/dL) (median [min-max])	11 (9–12)	11 (9–13)	0.226	11 (10–12)	11 (9–13)	0.247
Platelet count ($\times10^9$/L) (mean±SD)	405±235	312±108	0.155	381±188	341±172	0.626
CRP (mg/L) (median [min-max])	135 (11–410)	91 (10–600)	0.298	115 (11–190)	100 (10–600)	0.876
ESR (mm/h) (mean±SD)	74.3±21.4	68.2±29	0.539	80.1±22.3	68.2±26.7	0.327
ALT (IU/L) (median [min-max])	26.5 (11–360)	16 (7–180)	0.068	27 (12–360)	18 (7–194)	0.275
AST (IU/L) (median [min-max])	38.5 (18–165)	27.5 (12–182)	0.408	46.5 (24–165)	27.5 (12–182)	0.275
Sodium (mmol/L) (median [min-max])	137 (130–140)	136 (130–139)	0.505	138 (133–140)	136 (130–139)	0.074
Abdominal ultrasonography (*n*, %)						
Hepatomegaly (*n*, %)	1 (8.3)	2 (11.1)	1.0	1 (16.7)	2 (8.3)	0.501
Splenomegaly (*n*, %)	1 (8.3)	1 (5.6)	1.0	0 (0)	2 (8.3)	1.0
Hepatosplenomegaly (*n*, %)	1 (8.3)	0 (0)	0.400	0 (0)	1 (4.2)	1.0

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; cKD: Complete Kawasaki disease; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; iKD: Incomplete Kawasaki disease. * Statistical comparison was not applicable because all patients in both groups presented with this finding.

nary artery involvement. First, a univariate binary logistic regression analysis was performed for all possible variables. Although none of the variables reached statistical significance in the univariate logistic regression analysis, clinically relevant variables that have been consistently associated with cardiac involvement in the literature were included in the multivariate model. This approach was adopted to avoid excluding potentially important predictors due to limited statistical power and to adjust for possible confounding effects. Odds ratios and 95% confidence intervals (CI) were calculated for each variable.

Receiver operating characteristic (ROC) analysis was performed to determine the diagnostic value of laboratory parameters in predicting cardiac involvement. The ROC curve was constructed for each parameter, and the area under the curve (AUC) was calculated. The level used to determine statistical significance was set at $p<0.05$.

Table 3. Risk factors for cardiac involvement

	Cardiac involvement					
	Univariate analysis			Multivariate analysis		
	OR	95% CI	p	OR	95% CI	p
Age	0.979	0.769–1.247	0.863	1.068	0.797–1.432	0.660
Time to diagnosis	1.218	0.850–1.745	0.282	0.407	0.106–1.567	0.191
Male gender	2.500	0.410–15.230	0.320	2.912	0.382–22.180	0.302
Duration of fever	1.535	0.970–2.431	0.067	3.890	0.788–19.196	0.095
White blood cell count	1.000	1.000–1.000	0.224			
Hemoglobin	0.627	0.305–1.287	0.203			
Platelet count	1.000	1.000–1.000	0.175			
CRP	1.002	0.996–1.008	0.471	0.999	0.992–1.007	0.886
ESR	1.009	0.981–1.039	0.525			
ALT	1.006	0.995–1.017	0.271			
AST	1.008	0.990–1.026	0.372			
Sodium	1.044	0.802–1.358	0.751			

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CI: Confidence interval; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; OR: Odds ratio.

Results

Baseline Characteristics, Clinical and Laboratory Findings of the Cohort

The median age at disease onset among the 30 patients diagnosed with KD was 5 (1–11) years, the median time to diagnosis was 5 (3–12) days, and 73.3% (*n*=22) were male. The most commonly diagnosed season was spring (*n*=10, 33.3%). Of the patients, 70% (*n*=21) had cKD and 30% (*n*=9) had iKD. The median duration of fever was 5 (2–10) days. Clinical findings included fever (*n*=30, 100%), mucosal changes (*n*=30, 100%), rash (*n*=28, 93.3%), cervical lymphadenopathy (*n*=25, 83.3%), bilateral non-purulent

Table 4. Risk factors for coronary artery involvement

	Coronary artery involvement					
	Univariate analysis			Multivariate analysis		
	OR	95% CI	p	OR	95% CI	p
Age	0.837	0.602–1.163	0.288	0.852	0.584–1.241	0.404
Time to diagnosis	0.860	0.523–1.417	0.555	0.811	0.483–1.361	0.428
Male gender	2.059	0.202–20.959	0.542	4.034	0.283–57.441	0.303
Duration of fever	0.977	0.609–1.569	0.924			
White blood cell count	1.000	1.000–1.000	0.936			
Hemoglobin	0.650	0.278–1.524	0.322			
Platelet count	1.000	1.000–1.000	0.616			
CRP	0.998	0.990–1.006	0.679	1.002	0.992–1.012	0.697
ESR	1.018	0.983–1.053	0.321			
ALT	1.006	0.996–1.017	0.236			
AST	1.007	0.989–1.027	0.443			
Sodium	1.459	0.900–2.367	0.126	1.667	0.917–3.032	0.094

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CI: Confidence interval; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; OR: Odds ratio.

Table 5. Receiver operating characteristic (ROC) analysis for cardiac involvement

Variables	AUC	S.E.	p	OR (95% CI)	Cut-off	Sensitivity	Specificity
Age	0.484	0.115	0.882	0.258–0.709			
Time to diagnosis	0.676	0.106	0.108	0.468–0.884			
Duration of fever	0.727	0.100	**0.038**	0.532–0.922	3.5	91.7%	72.2%
WBC	0.595	0.106	0.385	0.386–0.803			
Hemoglobin	0.373	0.103	0.244	0.171–0.574			
PLT	0.597	0.117	0.374	0.368–0.827			
CRP	0.613	0.114	0.300	0.389–0.838			
ESR	0.604	0.106	0.341	0.397–0.811			
ALT	0.699	0.099	0.069	0.505–0.893			
AST	0.590	0.111	0.409	0.373–0.807			
Sodium	0.572	0.115	0.512	0.347–0.797			

AUC: Area under the curve; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CI: Confidence interval; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate; OR: Odds ratio; PLT: Platelet count; SE: Standard error; WBC: White blood cell count.

conjunctivitis (*n*=22, 73.3%), sterile pyuria (*n*=5, 16.7%), aseptic meningitis (*n*=5, 16.7%), and gallbladder hydrops (*n*=4, 13.3%). Echocardiography findings included mitral regurgitation (*n*=5, 16.7%), left coronary artery dilatation (*n*=3, 10%), coronary artery aneurysm (*n*=2, 6.7%), pericardial effusion (*n*=2, 6.7%), and left coronary artery ectasia (*n*=1, 3.3%). The mean WBC was 13±4.6×10^9/L, median hemoglobin was 11 (9–13) g/dL, mean PLT count was 349.7±173.5×10^9/L, median CRP was 100 (10–600) mg/L, mean ESR was 70.6±26 mm/h, median ALT was 19 (7–360) IU/L, median AST was 28.5 (12–182) IU/L, and median sodium was 136 (130–140) mmol/L at the time of diagnosis. Abdominal USG revealed hepatomegaly (*n*=3, 10%), splenomegaly (*n*=2, 6.7%), and hepatosplenomegaly (*n*=1, 3.3%). Second-dose IVIG treatment was administered to 6.6% of the patients (*n*=2).

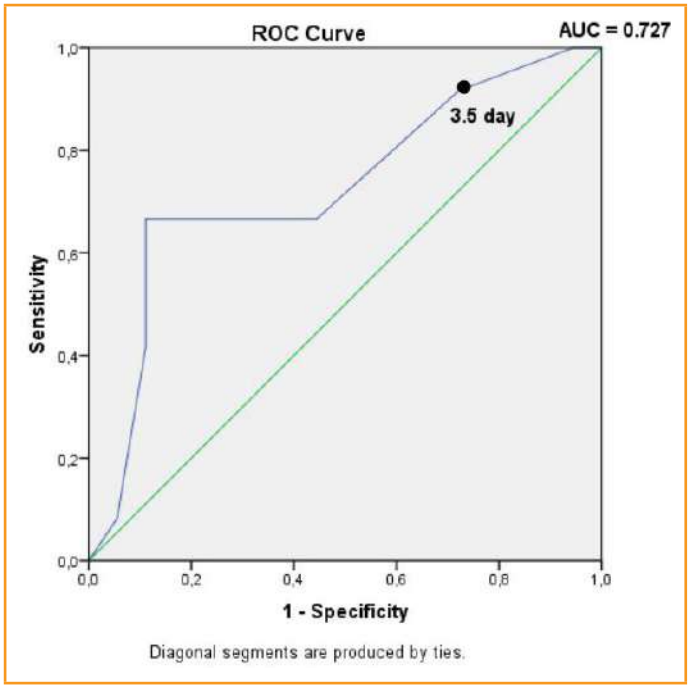

Figure 1. Receiver operating characteristic curve of fever duration for predicting cardiac involvement in patients with Kawasaki disease (area under the curve=0.727; optimal cut-off=3.5 days).

Comparison of Patients with Complete and iKD

The duration of fever (5 [3–10] days vs. 3 [2–10 days], p=0.047), bilateral non-purulent conjunctivitis (*n*=19, 90.5% vs. *n*=3, 33.3%; p=0.003), and cardiac involvement (*n*=11, 52.4% vs. *n*=1, 11.1%; p=0.049) were significantly higher in cKD patients compared to iKD patients. CRP levels (130 [11–600] mg/L vs. 40 [10–130] mg/L; p=0.018) and ALT levels (21 [7–360] IU/L vs. 15 [9–194] IU/L; p=0.044) were significantly higher in cKD patients compared to iKD patients, whereas hemoglobin levels (11 [9–13] g/dL vs. 12 [11–13] g/dL; p=0.029) were significantly lower in cKD patients. No statistically significant differences were observed in other demographic, clinical, or laboratory characteristics between the two groups (Table 1).

Risk Factors for Cardiac and Coronary Artery Involvement

The duration of fever (6 [3–10] days vs. 4 [2–10 days], p=0.035) was significantly higher in those with cardiac involvement compared to those without cardiac involvement (Table 2). No statistically significant risk factors were found among all variables evaluated for cardiac and coronary artery involvement (p>0.05) (Tables 3 and 4).

ROC Analysis for Cardiac Involvement

In the ROC analysis, only the duration of fever reached statistical significance as a predictor of cardiac involvement (AUC=0.727; 95% CI: 0.532–0.922; p=0.038). The optimal cutoff value was 3.5 days, which yielded a sensitivity of 91.7% and a specificity of 72.2% (Fig. 1). None of the other clinical or laboratory variables demonstrated a significant discriminatory ability (p>0.05) (Table 5).

Discussion

In this study, the demographic, clinical, and laboratory characteristics of 30 pediatric patients diagnosed with KD were evaluated, and factors associated with cardiac involvement were analyzed. cKD patients compared to iKD patients had a longer duration of fever, higher CRP and ALT levels, and lower hemoglobin levels. Cardiac involvement, particularly mitral regurgitation and coronary artery abnormalities, was more frequently observed in cKD cases. The duration of fever was significantly longer in patients with cardiac involvement. The duration of fever longer than 3.5 days was found to be a determining cut-off value for cardiac involvement. These findings indicate that fever duration represents not only a disease severity marker but also a practical diagnostic and prognostic parameter for the early identification of patients at an increased risk of cardiac complications.

KD occurs in all age groups, but most cases are detected in children under 5 years of age [17,18]. While rare in infants under 6 months due to maternal antibodies [19], cases have been reported in this group [20,21]. The disease is more prevalent in winter and spring due to infectious agents and environmental factors [22]. Males are more affected due to genetic or immunologic predisposition, with a male-to-female ratio of 1.5:1–1.7:1 [18], though this difference is not significant in adult cases [23]. In our cohort, over half of the patients were under five, about three-quarters were male, and most diagnoses occurred in spring, aligning with the literature on age, gender, and seasonal patterns.

In the acute phase, mucocutaneous findings with prolonged fever were predominant. Oral mucosal changes and conjunctivitis are seen in most cases, while cervical lymphadenopathy occurs less frequently [24,25]. Although fever and mucocutaneous findings were predominant in our cohort, conjunctivitis was the least frequent. This may be related to the presentation stage, subtle ocular findings in young age groups, and different interpretations, but may also reflect the limited sample size. Some of the patients may develop gastrointestinal symptoms, hepatobiliary involvement, aseptic meningitis, irritability, and pulmonary complications [26-28]. Our study also found that rare findings, such as aseptic meningitis and gallbladder hydrops, were consistent with the literature.

No diagnostic laboratory tests are specific for KD. In the acute phase, elevated CRP and ESR, thrombocytosis, leukocytosis, normochromic normocytic anemia, and hyponatremia are the most common [29]. Hypoalbuminemia, elevated transaminases, sterile pyuria, and eosinophilia are supporting findings [30,31]. Hepatomegaly and splenomegaly are rare but are reported more frequently in incomplete cases or cases with macrophage activation syndrome [32]. In our study, rates of elevated CRP (100%) and ESR (100%), anemia (60%), leukocytosis (53.3%), hyponatremia (26.7%), elevated transaminases (ALT: 26.7%; AST: 23.3%), sterile pyuria (16.7%), and organomegaly (hepatomegaly: 10%; splenomegaly: 6.7%; hepatosplenomegaly: 3.3%) matched the literature. Thrombocytosis (26.7%) was lower than the reported rates, possibly due to evaluation in the acute stage.

cKD is observed more frequently and is relatively easy to diagnose. In contrast, iKD reaches 48% in some series, due to diagnostic difficulties and increased clinical awareness [17,18]. More than half of our study patients had cKD, consistent with the literature. Fever duration, conjunctivitis, cardiac involvement rates, and CRP and ALT levels were significantly higher in cKD patients compared to iKD patients, aligning with previous studies [33,34]. However, hemoglobin levels were significantly lower in the cKD group compared to the iKD group, contradicting the literature [35]. This finding in our cohort may be related to a more abrupt and intense systemic inflammatory response in patients with cKD, potentially contributing to the earlier development of inflammation-related anemia. The detection of higher CRP and ALT levels in the cKD group also supported the presence of an increased inflammatory burden.

Children without early IVIG treatment face a high risk for serious cardiac complications. Coronary artery aneurysms, myocarditis, pericarditis, and endocarditis are the main cardiac complications; mitral and aortic regurgitation cause significant morbidity [36]. Risk factors for cardiac involvement include male gender, age below 1 year, prolonged fever duration, high CRP and ESR levels, hyponatremia, and IVIG refractoriness [37]. In our study, cardiac involvement occurred in approximately half of the cases, with mitral regurgitation being the most common. This differs from the literature and may be due to echocardiography in the acute period revealing valvular lesions earlier, whereas coronary artery pathologies become evident in the subacute or late periods. The significant relationship between fever duration and cardiac involvement is consistent with the literature, and it has been observed that the risk of complications increases markedly

in children with a fever duration of ≥3.5 days. Therefore, fever duration can be considered a clinical parameter that may contribute to early risk classification in children with KD and may guide the timing of cardiac evaluation.

In the initial treatment, an anti-inflammatory dose of acetylsalicylic acid (ASA) is administered with IVIG unless there are contraindications. After a fever-free period of 48–72 h, ASA was reduced to an anti-aggregate dose [38]. In refractory cases with refractory fever, second-dose IVIG treatment and systemic glucocorticoids, cyclosporine, tumor necrosis factor alpha inhibitors, or ulinastatin may be added as additional options [39,40]. In the literature, the need for a second dose of IVIG was reported in approximately 15% of cases [38]. In our study, this rate was 6.66%, which is lower than the rates reported in the literature. This difference may be explained by the relatively early diagnosis, rapid initiation of treatment, and milder clinical features of our patient group.

Limitation

The main limitation of our study was its retrospective and single-center design, which limited the generalizability of the results. In addition, the relatively small sample size limits the statistical power of the study and increases the risk of type II error, which should be taken into account when interpreting the results. However, the comparative analysis of complete and incomplete cases stands out as a strength contributing to the literature. Despite the limitations of this study, the findings contribute to the literature from a clinical perspective.

Conclusion

Our study provides clinically relevant diagnostic and prognostic insights by revealing the clinical features of KD and the risk factors associated with cardiac involvement. In particular, the relationship between fever duration and cardiac complications highlights the critical role of early recognition and timely treatment initiation in preventing adverse cardiac outcomes. The low requirement for a second dose of IVIG with early diagnosis and rapid initiation of treatment further supports the beneficial effects of early diagnosis and prompt therapeutic intervention, likely reflecting increased clinical awareness. Nevertheless, confirmation of these findings in larger, multicenter prospective studies is warranted to strengthen the evidence base for the optimal management of KD.

Disclosure

Ethics Committee Approval: This study was approved by the Haydarpaşa Numune Training and Research Hospital Ethics Committee (Date: 15.03.2021, Decision no: HNHEAH-KAEK 2021/93).

Informed Consent: Due to the retrospective nature of the study, obtaining written informed consent from the patients was not required.

Conflict of Interest: The authors declare that there is no conflict of interest.

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References

1. Wang CL, Wu YT, Liu CA, Kuo HC, Yang KD. Kawasaki disease: infection, immunity and genetics. Pediatr Infect Dis J 2005;24:998–1004. [CrossRef]
2. Conti G, Giannitto N, De Luca FL, Salpietro A, Oreto L, Viola I, et al. Kawasaki disease and cardiac involvement: an update on the state of the art. J Biol Regul Homeost Agents 2020;34(4 Suppl. 2):47–53.
3. Wood L, Tulloh R. Kawasaki disease: diagnosis, management and cardiac sequelae. Expert Rev Cardiovasc Ther 2007;5:553–61. [CrossRef]
4. Ermolaeva YA, Grishkevich IR, Doroshenko IV, Zgerskaya TV, Samoilova IG, Gay SA, et al. Challenges of timely Kawasaki disease diagnosis in 2-month-old child: clinical case. Current Pediatrics 2025;24:26–36. [CrossRef]
5. Geraldi RS, Carvalho RA. Kawasaki disease, a clinical challenge: literature review. Int J Health Sci 2023;3:1–6. Available from: https://atenaeditora.com.br/catalogo/artigo-revista/doenca-de-kawasaki-um-desafio-clinico-revisao-de-literatura-2. Accessed May 5, 2026. [CrossRef]
6. Osiejewska A, Wojtachnio D, Bartoszewicz J, Grądzik A, Nowakowska I, Kudan M, et al. Kawasaki disease: a comprehensive review. J Educ Health Sport 2022;12:317–27. [CrossRef]
7. Datta S, Maiti S, Das G, Chatterjee A, Ghosh P. Incomplete Kawasaki disease-a diagnostic and therapeutic challenge. J College Med Sci Nepal 2013;9:30–5. [CrossRef]
8. Idris I, Awadelkarim AM, Saad E, Dayco J, Beker S. Incomplete Kawasaki disease in an infant: a case report and literature review. Cureus 2022;14:e22122. [CrossRef]
9. Aljishi FM, Alsinan A, Almadan B, Almokhtar R, Alsinan T. Diagnosing incomplete Kawasaki disease. Saudi J Med Med Sci 2018;6:172–3. [CrossRef]
10. Lee S, Kim D, Kim BJ, Rhim JW, Lee SY, Jeong DC. Comparison of COVID-19-associated multisystem inflammatory syndrome in children (MIS-C) and Kawasaki disease shock syndrome: case reports and literature review. J Rheum Dis 2023;30:272–7. [CrossRef]

11. Marzano AV, Cassano N, Moltrasio C, Verdoni L, Genovese G, Vena GA. Multisystem inflammatory syndrome in children associated with COVID-19: a review with an emphasis on mucocutaneous and kawasaki disease-like findings. Dermatology 2022;238:35–43. [CrossRef]
12. Prtorić L, Didović D, Valenčak-Ignjatić I, Šokota A, Stemberger-Marić L. 16 Comparison of children with kawasaki disease and MIS-C. Arch Dis Childhood 2021;106:3–7. [CrossRef]
13. Morishita KA, Goldman RD. Current approach to the evaluation and management of incomplete Kawasaki disease in the emergency department. Pediatr Emerg Care 2020;36:537–41. [CrossRef]
14. Ayusawa M, Sonobe T, Uemura S, Ogawa S, Nakamura Y, Kiyosawa N, et al. Revision of diagnostic guidelines for Kawasaki disease (the 5th revised edition). Pediatr Int 2005;47:232–4. [CrossRef]
15. Sonobe T, Kiyosawa N, Tsuchiya K, Aso S, Imada Y, Imai Y, et al. Prevalence of coronary artery abnormality in incomplete Kawasaki disease. Pediatr Int 2007;49:421–6. [CrossRef]
16. McCrindle BW, Rowley AH, Newburger JW, Burns JC, Bolger AF, Gewitz M, et al. Diagnosis, treatment, and long-term management of Kawasaki disease: a scientific statement for health professionals from the american heart association. Circulation 2017;135:e927–99. Erratum in: Circulation 2019;140:e181–4.
17. Isaacs D. Attending and speaking at scientific meetings. J Paediatr Child Health 2017;53:325–6. [CrossRef]
18. Shivalingam G, Prashanth GP, Hebbal K, Aguiar R. Clinical presentation and cardiovascular outcome in complete versus incomplete Kawasaki disease. Indian Pediatr 2017;54:844–7. [CrossRef]
19. Du ZD, Zhao D, Du J, Zhang YL, Lin Y, Liu C, et al. Epidemiologic study on Kawasaki disease in Beijing from 2000 through 2004. Pediatr Infect Dis J 2007;26:449–51. [CrossRef]
20. Kossiva L, Papadopoulos M, Lagona E, Papadopoulos G, Athanassaki C. Myocardial infarction in a 35-day-old infant with incomplete Kawasaki disease and chicken pox. Cardiol Young 2010;20:567–70. [CrossRef]
21. Stepanovskyy YS, Klymyshyn YI, Moshkina TV, Saftuk SM, Tykhonenko NO, Bondarenko AV. An incomplete form of Kawasaki disease in a 2-month-old girl. How the diagnostic algorithm works in practice. Infus Chemother 2024;7:45–51. [CrossRef]
22. Burns JC, Cayan DR, Tong G, Bainto EV, Turner CL, Shike H, et al. Seasonality and temporal clustering of Kawasaki syndrome. Epidemiology 2005;16:220–5. [CrossRef]
23. Boodoosingh N, Seecheran R, Varachhia S, Mack N, Minocha V, Giddings S, et al. Incomplete Kawasaki disease in an adult South Asian patient. J Investig Med High Impact Case Rep 2018;6:2324709618792028. [CrossRef]
24. Newburger JW. Therapy of acute Kawasaki disease. Cardiol Young 1991;1:254–5. [CrossRef]
25. Kawasaki T. Clinical features of Kawasaki syndrome. Pediatr Int 1983;25:79–87. [CrossRef]
26. Egala A, Hariharan R, Sarangarajan S, Puntambekar A, Lama MV, Ramamoorthy JG, et al. An uncommon cause of spontaneous pneumothorax in a febrile infant: Atypical Kawasaki disease. Pediatr Pulmonol 2022;57:2571–3. [CrossRef]
27. Sengler C, Gaedicke G, Wahn U, Keitzer R. Pulmonary symptoms in Kawasaki disease. Pediatr Infect Dis J 2004;23:782–4. [CrossRef]
28. Egritas O, Nacar N, Hanioglu S, Soyer T, Tezic T. Early but prolonged gallbladder hydrops in a 7-month-old girl with Kawasaki syndrome: Report of a case. Surg Today 2007;37:162–4. [CrossRef]
29. Zabeen F, Hassan MQ, Nazrin T, Farheen C, Mohammad Iqbal AS, Hossain MI, et al. Presentation and outcome of children with Kawasaki disease: experience in a tertiary care hospital in Bangladesh. Dhaka Shishu (Children) Hospital J 2023;38:70–8. [CrossRef]
30. Mărginean CO, Meliț LE, Gozar L, Mărginean CD, Mărginean MO. Incomplete refractory Kawasaki disease in an infant-a case report and a review of the literature. Front Pediatr 2018;6:210. [CrossRef]
31. Huang YH, Kuo HC, Tsai CM. High eosinophil counts aid in diagnosing Kawasaki disease in febrile children. Eur Heart J 2024;45(Suppl 1):ehae666.3103. [CrossRef]
32. Miselli F, Mastrolia MV, Simonini G, Trapani S, Calabri GB. Splenomegaly in Kawasaki disease: a pitfall in diagnosis. J Pediatr 2023;257:113359. [CrossRef]
33. Malimban J, Garcia RD, Francisco-Mallari MR. A comparative study of pediatric patients with complete vs. incomplete Kawasaki disease in a tertiary hospital: an eleven year review. PIDSP J 2022;23:55–63. [CrossRef]
34. Perrin L, Letierce A, Guitton C, Tran TA, Lambert V, Koné-Paut I. Comparative study of complete versus incomplete Kawasaki disease in 59 pediatric patients. Joint Bone Spine 2009;76:481–5. [CrossRef]
35. Ha KS, Jang GY, Lee J, Lee KC, Son CS. Laboratory markers in incomplete Kawasaki disease according to coronary artery outcome. Korean Circ J 2018;48:287–95. [CrossRef]
36. Dominguez SR, Anderson MS, El-Adawy M, Glodé MP. Preventing coronary artery abnormalities: a need for earlier diagnosis and treatment of Kawasaki disease. Pediatr Infect Dis J 2012;31:1217–20. [CrossRef]
37. Charoonrochana S, Klaiklueng K, Somanundana R. Prediction of resistant to intravenous immunoglobulin (IVIG) treatment in patients with Kawasaki disease in the tertiary care hospital. 2021. [CrossRef]
38. Newburger JW, Fulton DR. Kawasaki disease. Curr Treat Options Cardiovasc Med 2007;9:148–58. [CrossRef]
39. Kanai T, Ishiwata T, Kobayashi T, Sato H, Takizawa M, Kawamura Y, et al. Ulinastatin, a urinary trypsin inhibitor, for the initial treatment of patients with Kawasaki disease: a retrospective study. Circulation 2011;124:2822–8. [CrossRef]
40. Furuno K. The addition and subtraction of treatment for Kawasaki disease. Pediatr Int 2021;63:751. [CrossRef]

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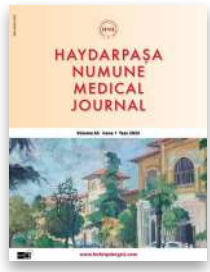

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ORIGINAL ARTICLE

Low Seroprevalence of *Toxoplasma Gondi*i in People Living With Human Immunodeficiency Virus: Is it Possible to Ignore?

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Abstract

Introduction: Immunocompromised individuals, especially people living with human immunodeficiency virus (HIV), are vulnerable to severe complications from latent *Toxoplasma gondii* reactivation. This study investigated *T. gondii* seropositivity in people living with HIV and its association with demographic and clinical factors to identify high-risk groups.

Methods: Between January 1, 2020, and June 1, 2025, 1189 people living with HIV from the infectious diseases outpatient clinic were included. Demographic data, CD4+ T lymphocyte counts, HIV ribonucleic acid levels, and *T. gondii* immunoglobulin G (IgG)/immunoglobulin M results were retrospectively collected, and seropositivity rates and their associated factors were statistically analyzed.

Results: Of the 1189 people living with HIV included in the study, 88.7% were male (n=1055) and 11.3% were female (n=134), with a mean age of 38.2±9.6 years. Serological evaluation revealed that *T. gondii* IgG antibodies were positive in 31.4% of the patients (n=374). Among the study population, 118 patients (9.9%) had a $CD4^+$ T lymphocyte count below 100 cells/mm^3 at the time of admission. Of these patients, 48 (40.7%) were seropositive for *T. gondii* IgG. In contrast, among the 1071 patients with a CD4+ T cell count ≥100 cells/mm^3, 326 (30.4%) were IgG positive. *T. gondii* seropositivity was significantly higher in patients with CD4+ T lymphocyte counts <100 cells/mm^3, and this difference was statistically significant (p=0.030). Seropositivity rates increased significantly with age (p<0.001), lowest in 18–25 (16.5%) and highest in ≥50 years (64.88%).

Discussion and Conclusion: Our study highlights the combined impact of age-related cumulative exposure and environmental factors on the epidemiology of *T. gondii* infection among people living with HIV. Higher seronegativity in younger patients indicates reduced exposure, potentially increasing the risk of primary infection in the immunosuppressed. Thus, *T. gondii* serological screening remains essential, especially in those with low $CD4^+$ counts.

Keywords: $CD4^+$ T lymphocyte; Human immunodeficiency virus; Seroprevalence; *Toxoplasma gondii*.

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Toxoplasmosis is a zoonotic disease caused by *Toxoplasma gondii*, an intracellular protozoan parasite. It is estimated that approximately 30% of the global human population is infected with *T. gondii*, with seroprevalence rates reaching up to 54.7% in low-income countries, whereas dropping to around 26% in high-income countries. The infection is particularly more prevalent in developing countries, where it poses significant public health concerns [1]. In immunocompetent people, toxoplasmosis is usually self-limiting and asymptomatic. However, in immunocompromised patients, it can become a life-threatening condition with potentially fatal outcomes. People living with human immunodeficiency virus (HIV) are at especially high risk for *T. gondii* infection due to their suppressed immune systems.

In the literature, the seroprevalence of *T. gondii* among people living with HIV has been reported to range between 35% and 60%, with a significantly increased risk of infection associated with low $CD4^+$ T cell counts (<200 cells/mm^3) [2-4]. One study found that people with $CD4^+$ T cell counts below 100 cells/mm^3 had a 28-fold increased risk of developing cerebral toxoplasmosis [5]. The immune response to *T. gondii* is highly complex and variable due to the genetic heterogeneity of the host. Despite a robust cellular immune response, the parasite forms tissue cysts containing the bradyzoite form, which establishes latent infection. This latent form can reactivate and cause clinical disease when the immune system is suppressed [6]. During the chronic phase, ongoing immune activation may lead to activation-induced cell death of $CD4^+$ T lymphocytes, which play a key role in the defense against toxoplasmosis. Similarly, $CD8^+$ T lymphocytes, which exhibit cytotoxic activity against *T. gondii*, are also affected by HIV infection, resulting in functional impairment of two critical components of cellular immunity [7]. In this context, although HIV infection does not directly trigger the development of toxoplasmic encephalitis (TE), it increases susceptibility by facilitating the reactivation of latent *T. gondii* infection. Therefore, according to HIV management guidelines from Europe (EACS) and the United States (DHHS/NIH-CDC-IDSA), serological screening for *T. gondii* immunoglobulin G (IgG) antibodies is recommended for all people diagnosed with HIV, to predict and prevent opportunistic infections. For those with $CD4^+$ T cell counts below 200/µL, prophylaxis with trimethoprim-sulfamethoxazole is advised to prevent cerebral toxoplasmosis [8].

T. gondii infection is a widespread public health concern on a global scale. Seroprevalence rates vary significantly across populations, depending on factors such as age, immune status, hygiene conditions, dietary habits, and environmental exposure. Among people living with HIV, these rates are reported to be even more variable. For instance, while the seropositivity rate is approximately 8.2% in Japan, it can reach up to 31.6% in Greece. This variation highlights the influence of both geographical and environmental factors on exposure to *T. gondii* infection [9,10].

In studies conducted in Turkey, the seroprevalence of *T. gondii* among people living with HIV has been reported at varying rates. For example, in a retrospective analysis of 114 people living with HIV followed between 2020 and 2022 in Şanliurfa, *T. gondii* IgG antibody positivity was 57.9% [11]. In another study conducted in Istanbul, the reported rate was 41.6% [12]. This variability may be attributed to differences in patient populations, lifestyle factors, and regional hygiene conditions across the study centers. Determining the seroprevalence of *T. gondii* among people living with HIV is important for understanding the epidemiological aspects of the infection as well as for guiding clinical management. Moreover, such data can guide clinicians in identifying patients who require close monitoring for prophylaxis, thereby helping to prevent unnecessary medication use. In this context, people living with HIV who are seronegative for *T. gondii* IgG and immunoglobulin M (IgM) represent a population at risk, and early identification and education of these individuals is crucial for implementing preventive measures and reducing the likelihood of primary infection.

The primary objective of this study is to determine the seropositivity rate of *T. gondii* among people living with HIV and thereby elucidate the seroepidemiological status at our center. Identifying the prevalence of latent *T. gondii* infection through serological markers will contribute to timely and accurate clinical decision-making aimed at preventing severe complications, such as TE, which may arise with progressive immunosuppression in advanced stages of HIV infection.

Materials and Methods

This study was conducted in accordance with the principles of the Declaration of Helsinki and with the approval of the Non-Interventional Clinical Research Ethics Committee of Haydarpaşa Numune Training and Research Hospital, dated July 1, 2025 (Decision number: 2025/86).

In this study, the medical records of 1540 people living with HIV who presented to the Infectious Diseases and Clinical Microbiology Outpatient Clinic of the University of Health Sciences Haydarpaşa Numune Training and Research Hospital between January 1, 2020, and June 1, 2025, and whose HIV diagnosis was confirmed by the Western Blot test, were reviewed. Patients under 18 years of age and 351 patients whose medical records were unavailable were excluded. A total of 1189 people living with HIV with accessible *T. gondii* serological data were included in the study. Demographic data (age, sex, marital status, and nationality), $CD4^+$ T lymphocyte counts, HIV ribonucleic acid (RNA) levels, and *T. gondii*

IgG and IgM serology results were retrospectively obtained from patient files and the hospital information management system. *T. gondii* IgG and IgM levels were measured using the enzyme-linked immunosorbent assay method on Roche Cobas e411 automated analyzers. According to the manufacturer, the IgG assay has a reported sensitivity and specificity of 99% each, whereas the IgM assay has a reported sensitivity of 95% and specificity of 96%. $CD4^+$ T lymphocyte counts were determined through flow cytometry, and HIV RNA levels were assessed by polymerase chain reaction. Based on the data obtained, *T. gondii* seropositivity rates were calculated, and their associations with clinical and demographic variables were evaluated through statistical analyses.

Statistical Analysis

The data of 1189 people living with HIV were analyzed using IBM Statistical Package for the Social Sciences Statistics for Windows, Version 26.0. Descriptive statistics were reported as number of observations (*n*), percentage (%), mean±standard deviation (SD) ($\bar{x}$±SD), and median (Q1–Q3). Comparisons between categorical variables were performed using Pearson's Chi-square (χ^2) test. *Post hoc* analyses for Pearson's χ^2 tests were performed by examining adjusted standardized residuals for each cell. When the overall χ^2 test was significant, pairwise comparisons of column proportions were conducted, and a Bonferroni correction was applied to control for multiple testing. The distribution of continuous variables was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests, and Q-Q plots. For comparisons of continuous variables between two groups, the independent-samples *t*-test was used for variables showing a normal distribution, and the Mann–Whitney U test was used for variables not normally distributed. P<0.05 was considered statistically significant.

Results

The demographic and clinical data of 1189 people living with HIV included in the study were evaluated. Of the patients, 1055 (88.7%) were male, and 134 (11.3%) were female. The mean age of the patients was 38.2±9.6 years, ranging from 18 to 80 years. As a result of serological analyses, *T. gondii* IgG antibody was found to be positive in 374 patients (31.4%) and negative in 815 patients (68.6%). No patient was found to be positive for *T. gondii* IgM antibody. The mean age of seropositive patients was 40.5±9.2 years, whereas the mean age of seronegative patients was 31.0±8.7 years. The difference between the groups was statistically significant (p<0.001) (Table 1).

Table 1. *T. gondii* seroprevalence by demographic and immunological characteristics in people living with HIV

Variable	Total (%)	*T. gondii* IgG negative (%)	*T. gondii* IgG positive (%)	p
Gender‡				
Male	1055 (88.7)	733 (89.9)	322 (86.1)	0.065
Female	134 (11.3)	82 (10.1)	52 (13.9)	
Age (years)*	38.2±9.6	31.0±8.7	40.5±9.2	<0.001
$CD4^+$ T cell count‡ (cells/mm^3)				
<100	118 (9.9)	70 (8.6)	48 (12.8)	0.015
100–200	124 (10.4)	78 (9.6)	46 (12.3)	
201–350	261 (22.0)	171 (21.0)	90 (24.1)	
351–500	288 (24.2)	205 (25.2)	83 (22.2)	
>500	398 (33.5)	291 (35.7)	107 (28.6)	
Marital status‡				
Single	875 (73.6)	633 (77.7)	242 (64.7)	<0.001
Married	314 (26.4)	182 (22.3)	132 (35.3)	
Nationality‡				
Foreign	44 (3.7)	30 (3.7)	14 (3.7)	> 0.999
Turkish	1145 (96.3)	785 (96.3)	360 (96.3)	
HIV RNA† (IU/mL)	201.964 (43.562–895.444)	196.124 (41.643–901.771)	219.290 (47.329–877.180)	0.591

T. gondii: *Toxoplasma gondii*, IgG: immunoglobulin G, HIV RNA: Human immunodeficiency virus ribonucleic acid, IU/mL: International units per milliliter. *Student's *t*-test was used for normally distributed continuous variables (age). †Mann–Whitney U test was used for non-normally distributed continuous variables (HIV RNA). ‡Chi-square (χ^2) test was used for categorical variables

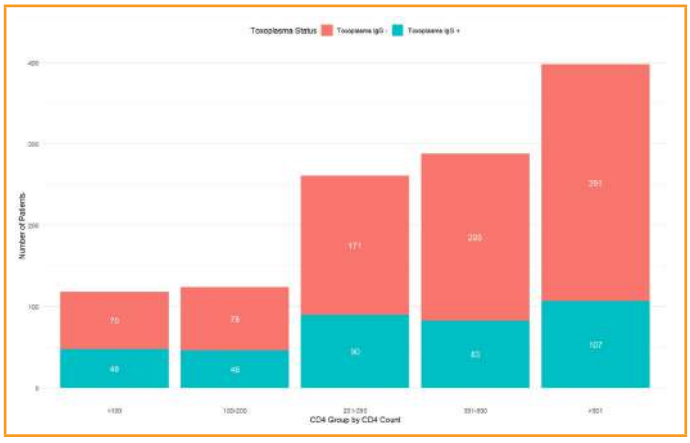

Figure 1. *Toxoplasma gondii* immunoglobulin G seropositivity according to CD4$^+$ T lymphocyte countst.

When *T. gondii* IgG seropositivity rates were evaluated according to age groups, it was observed that seropositivity significantly increased with age. The lowest seropositivity rate was observed in the 18–25 age group (16.52%), whereas the highest rate was found in individuals aged over 50 years (64.88%). The detailed age-related distribution is presented in Table 2. Notably, a marked increase in seropositivity was observed among patients aged 50 years and older. Considering all age groups, the overall seropositivity rate was calculated as 31.45% (*n*=372). The association between age group and *T. gondii* IgG seropositivity was statistically significant (p<0.001) (Table 2). *Post hoc* evaluation using adjusted standardized residuals indicated fewer seropositive cases than expected in the 18–25 and 26–35 groups and more seropositive cases than expected in the 36–50 and >50 groups; Bonferroni-corrected comparisons supported that the observed difference was driven primarily by the elevated seropositivity in the 36–50 and >50 groups compared with younger age categories.

The mean CD4$^+$ T lymphocyte count was determined to be 412 cells/mm^3 (IQR: 275–588), and the median HIV RNA level was 13,500 IU/mL (IQR: 2000–88,000). Among the patients included in the study, 118 (9.9%) had a CD4$^+$ T lymphocyte count below 100 cells/mm^3 at the time of presentation. *T. gondii* IgG seropositivity was detected in 48 (40.7%) of these patients. Among the 1071 patients with a CD4$^+$ T lymphocyte count ≥100 cells/mm^3, 326 (30.4%) were IgG positive. *T. gondii* seropositivity was higher in patients with a CD4$^+$ T lymphocyte count <100 cells/mm^3, and this difference was found to be statistically significant (p=0.030). The distribution of *T. gondii* IgG seropositivity according to CD4$^+$ T lymphocyte counts is shown in Figure 1.

When the relationship between marital status and *T. gondii* IgG seropositivity was evaluated, it was found that 875 patients (73.6%) were single and 314 patients (26.4%) were married. Among single patients, 242 (27.7%) were seropositive for *T. gondii* IgG, whereas 132 married patients (42.0%) were found to be seropositive. Marital status was observed to have an impact on seropositivity; the seropositivity rate was 42.0% among married patients and 27.7% among single patients (p<0.001) (Table 1).

Of the 1189 people living with HIV included in the study, 96.3% (*n*=1.145) were citizens of the Republic of Türkiye, whereas 3.7% (*n*=44) were foreign nationals. The *T. gondii* IgG seropositivity rate was found to be similar in both groups. IgG seropositivity was detected in 14 (31.8%) of the foreign nationals and in 360 (31.4%) of the Turkish citizens (Table 1).

Discussion

In this study, the *T. gondii* IgG seroprevalence among people living with HIV was found to be 31.4%. This rate is notably lower compared to previous studies conducted in Türkiye in different years. For example, in a study conducted by Şenoğlu et al. [13] in Istanbul in 2018, the reported seroprevalence was 43.5%. In a systematic review by Yenilmez and Çetinkaya in 2019, the seroprevalence was reported as 43.5% among people with a CD4$^+$ T lymphocyte count <200 cells/mm^3 and 40.03% among those with ≥200 cells/mm^3 [14]. In a more recent study conducted in 2024 in Istanbul, which evaluated 916 people living with HIV, the seroprevalence was recorded as 36.5% [15]. In light of these

Table 2. Comparison of Toxoplasma IgG positivity by age group (*n*=1189)

Age group (years)	Positive	Negative	Total	Percentage positive	p
18–25^a	40	202	242	16.52	<0.001
26–35^a	96	336	431	22.27	
36–50^b	153	231	385	39.74	
>50^c	85	46	131	64.88	
Total	374	815	1189	31.45	

Pairwise comparisons were performed using the Chi-square test with Bonferroni correction. Superscripts denote statistically significant differences between groups in pairwise comparisons. IgG: immunoglobulin G

data, the 31.4% seroprevalence observed in our study remains lower compared to the rates reported in previous studies conducted in Türkiye.

In addition, a large-scale meta-analysis examining *T. gondii* seroprevalence among people living with HIV across 37 countries reported a global average seropositivity rate of 44.2%. When the distribution by country was examined, the lowest seropositivity rate was reported in the Netherlands at 3.7%. In comparison, the rates were recorded as 10% in both Canada and the United States, 35% in Germany, 36% in Italy, 23% in France, and 23% in Brazil. Among North African and Middle Eastern countries, seropositivity rates were reported as 88% in Libya, 62% in Morocco, and 40% in Iran. The highest seroprevalence was reported in Ethiopia at 92% [16]. Considering the findings of this comprehensive study, the 31.4% seropositivity rate identified in our center falls below the global average and appears to be comparable to the rates reported in several European countries.

There may be several important reasons for this discrepancy. The relatively low seroprevalence observed in our study may be partly explained by the predominance of male participants (88.7%). A similar male predominance has been reported in other studies from Türkiye, with 86% in Şenoğlu et al., [13] 88.5% in Ergen et al., [12] and 81.6% in Damar Çakirca et al.[11] It is generally recognized that women have a higher likelihood of contact with domestic animals, particularly cats, which may increase exposure to *T. gondii*. Therefore, the low proportion of female participants might have contributed to the overall lower seroprevalence observed in our cohort. In addition, the mean age of participants was 38.2 years, and 16.5% were between 18 and 25 years old. Seropositivity rates were lower among younger patients, suggesting that *T. gondii* exposure increases with age and that younger generations may have a lower cumulative risk of infection. In parallel, the earlier diagnosis of HIV infection in recent years and the fact that people living with HIV tend to present to healthcare services with higher CD4 counts may provide a protective advantage against opportunistic infections.

In our study, the seropositivity rate in the 18–25 age group was only 16.5%, whereas it was 22.3% in the 26–35 age group, 39.7% in the 36–50 age group, and 64.9% in those older than 50 years. Notably, the predominance of *T. gondii* IgG seronegativity in younger age groups is striking. This finding raises the question highlighted in the title of our study: "Are we eliminating Toxoplasma?" It supports the hypothesis that *T. gondii* exposure has declined over time due to improvements in hygiene conditions, food safety, reduced animal contact, and the adoption of more conscious behavioral practices. However, this trend may increase the risk of primary infection in seronegative people who later become immunosuppressed. Therefore, monitoring seronegative patients and reconsidering prophylactic strategies in high-risk groups are of clinical importance. Similarly, international studies have reported that *T. gondii* seropositivity in people living with HIV increases with age, with lower seroprevalence rates observed in younger patients [17,18].

In particular, the seropositivity rate of 62.8% observed in patients over 50 years of age demonstrates that exposure to the infection increases cumulatively over the course of life and that seroprevalence rises markedly with age. Similarly, a recent study conducted in people living with HIV (*n*=247, November 2022–November 2024) identified age as an independent risk factor for seroprevalence, reporting the highest IgG seropositivity rate of 66.7% in the 61–80 age group and showing that the risk of infection increases significantly with advancing age. This study also confirmed that age substantially increases Toxoplasma seroprevalence in people living with HIV [19]. Moreover, the higher seropositivity rates observed in elderly patients can be explained by the largely subclinical course of infection and the persistence of detectable IgG antibodies for many years [20].

In our study, *T. gondii* seropositivity was detected in 40.7% of people living with HIV with $CD4^+$ T lymphocyte counts below 100 cells/mm^3. This finding highlights the risk of reactivation of latent *T. gondii* infections in patients with advanced immunodeficiency. Consistent with the literature, low $CD4^+$ cell counts (<200 cells/mm^3), particularly those falling below 100 cells/mm^3, have been emphasized as a critical threshold for the development of TE [2]. Accordingly, current HIV management guidelines published in Europe and the United States recommend routine screening for *T. gondii* IgG antibodies in patients at the time of HIV diagnosis to anticipate opportunistic infections, and initiation of primary prophylaxis in patients with $CD4^+$ counts below 200 cells/mm^3 [8]. Our findings reinforce the clinical importance of these recommendations, demonstrating that the high seroprevalence observed in people living with HIV who have $CD4^+$ counts below 100 cells/mm^3 underscores the need for proactive monitoring and the implementation of appropriate prophylactic strategies in this population.

The high seropositivity observed among patients with $CD4^+$ T lymphocyte counts below 100 cells/mm^3 underscores the ongoing clinical relevance of latent toxoplasmosis as a potential cause of reactivation. In this patient group, continued serological monitoring and the timely initiation of prophylactic therapy are critical for improving clinical outcomes. Moreover, meta-analyses in the literature have demonstrated that seroprevalence is significantly higher in patients with profound immunosuppression. For example, in a global meta-analysis conducted by Wang et al., [17] *T. gondii* seroprev-

alence was 42.1% in people living with HIV compared with 32.0% in the control group, and this difference was statistically significant (odds ratio=1.92; 95% confidence interval 1.44–2.55) [17]. These findings suggest that reduced CD4+ T-cell counts play a crucial role in the reactivation and clinical manifestation of latent *T. gondii* infection. Rather than affecting baseline seroprevalence, immune status appears to determine the likelihood and severity of disease. In addition, individuals with advanced immunosuppression may be more prone to acquiring new infections due to increased susceptibility to environmental exposure.

The findings of this study demonstrate a significant association between *T. gondii* seroprevalence and marital status. The seropositivity rate was 42.0% in married patients compared with 27.7% in single patients, and this difference was statistically significant (p<0.001). Several seroprevalence studies in people living with HIV have similarly reported higher seropositivity rates among married patients, with age and certain sociodemographic factors identified as potential contributors. For instance, a study conducted among women living with HIV in Ethiopia found *T. gondii* IgG seroprevalence rates of 93.8% in married patients and 86.2% in single patients [21]. One of the primary explanations for this difference relates to age: The mean age of married patients is typically higher than that of single patients, and seropositivity naturally increases with cumulative exposure over time. In addition, environmental factors such as engagement in gardening activities and ownership of domestic animals, particularly cats, may increase the risk of infection in married people. Indirect contact with cat feces or soil can contribute to higher seropositivity rates. Furthermore, families with children may spend more time in parks, gardens, or sandboxes, thereby increasing environmental exposure. Collectively, these findings suggest that married patients may be at greater risk of *T. gondii* infection due to shared living conditions and heightened environmental exposure.

Among the 1189 people living with HIV included in the study, 96.3% (*n*=1.145) were Turkish citizens, whereas 3.7% (*n*=44) were foreign nationals. The *T. gondii* IgG seropositivity rate was similar in both groups. IgG seropositivity was detected in 14 foreign nationals (31.8%) and in 360 Turkish citizens (31.4%). No significant association was observed between nationality and *T. gondii* seropositivity (p=1.000). This finding may be explained by the small number of foreign nationals in our cohort and the fact that they largely reside under the same geographic and socioeconomic conditions as the Turkish patients.

In conclusion, the markedly lower seropositivity rates observed in younger age groups in our study indicate a progressive decline in *T. gondii* exposure in the general population. This trend suggests that, in the future, people living with HIV may face an increased susceptibility to primary infection and its potential severe complications. Therefore, preventive education is of paramount importance, particularly for seronegative patients. People living with HIV who test negative for *T. gondii* IgG should be counseled on effective protective measures against the parasite. They should avoid direct contact with soil or materials potentially contaminated with cat feces, thoroughly wash fruits and vegetables, ensure that drinking water is obtained from safe sources, and strictly avoid consuming raw or undercooked meat. In addition, patients should be advised to wash kitchen utensils and surfaces that come into contact with raw meat with soapy water and to adhere to general hygiene practices [8,22,23].

In this context, the early identification and education of *T. gondii*–seronegative people living with HIV should be considered a key preventive health strategy to reduce the risk of infection and potential complications.

Limitation

This study has some limitations. First, its retrospective single-center design may limit the generalizability of the findings. Second, information on potential risk factors for Toxoplasma gondii exposure, such as dietary habits, cat contact, and environmental factors, was unavailable. Finally, the absence of follow-up clinical data prevented evaluation of the relationship between serological status and subsequent toxoplasmosis. Despite these limitations, the large sample size provides valuable epidemiological data on T. gondii seroprevalence among people living with HIV in Türkiye.

Conclusion

Our study highlights the combined impact of age-related cumulative exposure and environmental factors on the epidemiology of *T. gondii* infection among people living with HIV. The predominance of seronegativity in younger age groups suggests a declining exposure to the parasite in the general population. It indicates that this infection may become increasingly rare in the future. However, this decline should not be overlooked, as it simultaneously increases the risk of primary infection in immunocompromised patients. Therefore, performing serological screening for *T. gondii* in people living with HIV with low CD4+ T lymphocyte counts is a critical preventive measure to avoid life-threatening complications, such as encephalitis.

Disclosure

Ethics Committee Approval: This study was approved by the Haydarpaşa Numune Training and Research Hospital Ethics Committee (Date: 01.07.2025, Decision no: 2025/86).

Informed Consent: We confirm that each participant provided informed consent before participating in the study.

Conflict of Interest: The authors declare that there is no conflict of interest.

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References

1. Foroutan M, Dalvand S, Daryani A, Ahmadpour E, Majidiani H, Khademvatan S, et al. Rolling up the pieces of a puzzle: A systematic review and meta-analysis of the prevalence of toxoplasmosis in Iran. *Alex J Med* 2018;54:189–96. [CrossRef]
2. Luft BJ, Remington JS. Toxoplasmic encephalitis in AIDS. Clin Infect Dis 1992;15:211–22. [CrossRef]
3. Montoya JG, Boothroyd J, Kovacs J. Toxoplasma gondii. In: Mandell G, Dolin A, Bennett J, editors. Principles and practice of infectious diseases. Philadelphia, PA: Elsevier; 2017. p.424–5.
4. Lampejo T. Toxoplasma gondii infection in HIV-infected pregnant women: epidemiology and risks of mother-to-child transmission. Pan Afr Med J 2022;42:275.
5. Bazmjoo A, Bagherzadeh MA, Raoofi R, Taghipour A, Mazaherifar S, Sotoodeh H, et al. Toxoplasma gondii, HBV, and HCV co-infection and their correlation with CD4 cells among Iranian HIV-positive patients. Immun Inflamm Dis 2023;11:e794. [CrossRef]
6. Filisetti D, Candolfi E. Immune response to Toxoplasma gondii. Ann Ist Super Sanita 2004;40:71–80.
7. Fauci AS, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL, Loscalzo J, editors. Toxoplasma infections: Harrison's principles of internal medicine. 19th ed. New York: McGraw- Hill; 2015.
8. Clinicalinfo.HIV.gov. Panel on Opportunistic Infections in Adults and Adolescents with HIV. Guidelines for the prevention and treatment of opportunistic infections in adults and adolescents with HIV: Toxoplasma gondii encephalitis. 2024. Available from: https://clinicalinfo.hiv.gov/sites/default/files/guidelines/archive/adult-adolescent-oi-2024-10-08.pdf. Accessed May 5, 2026.
9. Hoshina T, Horino T, Saiki E, Aonuma H, Sawaki K, Miyajima M, et al. Seroprevalence and associated factors of Toxoplasma gondii among HIV-infected patients in Tokyo: A cross sectional study. J Infect Chemother 2020;26:33–7. [CrossRef]
10. Voyiatzaki C, Zare Chormizi AD, Tsoumani ME, Efstathiou A, Konstantinidis K, Chaniotis D, et al. Seroprevalence of *Toxoplasma gondii* among HIV positive patients under surveillance in Greek infectious disease units: a screening study with comparative evaluation of serological methods. Pathogens 2024;13:375. [CrossRef]
11. Damar Çakırca T. Şanlıurfa ilinde HIV/AIDS: 114 olgunun retrospektif analizi, tek merkez deneyimi. J Harran Univ Med Fac. 2022;19:594–9. [Article in Turkish] [CrossRef]
12. Ergen P, Yılmaz-karadağ F, Aydın Ö. Seroprevalence of toxoplasma gondii in HIV-infected patients admitted to a university hospital. J Health Sci Med 2020;3:372–6. [CrossRef]
13. Şenoğlu S, Yeşilbağ Z, Altuntaş Aydın Ö, Kumbasar Karaosmanoğlu H, Kart Yaşar K. Toxoplasma gondii IgG seroprevalence in patients with HIV/AIDS. Turkiye Parazitol Derg 2018;42:175–9. [CrossRef]
14. Yenilmez E, Çetinkaya RA. Difference in *Toxoplasma gondii* seroprevalence rates due to low and high CD4 counts in patients with HIV/AIDS. Turkiye Parazitol Derg 2019;43(Suppl 1):1–7. [CrossRef]
15. Yağcı Çağlayık D, Sili U, Tükenmez Tigen E, Ertürk Şengel B, Can B, Tunçel T, et al Investigation of toxoplasma seropositivity in adult individuals infected with HIV followed at Marmara University Pendik Training and Research Hospital, 2024. Turk Hij Den Biyol Derg 2024;81:409–18. [Article in Turkish] [CrossRef]
16. Safarpour H, Cevik M, Zarean M, Barac A, Hatam-Nahavandi K, Rahimi MT, et al. Global status of Toxoplasma gondii infection and associated risk factors in people living with HIV. AIDS 2020;34:469–74. [CrossRef]
17. Wang ZD, Liu HH, Ma ZX, Ma HY, Li ZY, Yang ZB, et al. *Toxoplasma gondii* infection in immunocompromised patients: a systematic review and meta-analysis. Front Microbiol 2017;8:389. [CrossRef]
18. Manuel L, Comia IR, Miambo RD, Sousa IM, Cuboia N, Carimo A, et al. Seroprevalence and risk factors for *Toxoplasma gondii* infection in people living with HIV: a cross-sectional study from maputo central hospital, mozambique. Res Rep Trop Med 2025;16:43–53. [CrossRef]
19. Kürkçü MF, Bakır A, Kaba SBT, Kuşabbi İA, Usluca S. Toxoplasma gondii coinfection in HIV-positive patients at a tertiary care hospital. Curr HIV Res 2025. [CrossRef]
20. Montoya JG, Liesenfeld O. Toxoplasmosis. Lancet 2004;363:1965–76. [CrossRef]
21. Shimelis T, Tebeje M, Tadesse E, Tegbaru B, Terefe A. Sero-prevalence of latent Toxoplasma gondii infection among HIV-infected and HIV-uninfected people in Addis Ababa, Ethiopia: A comparative cross-sectional study. BMC Res Notes 2009;2:213. [CrossRef]
22. Marín-García PJ, Planas N, Llobat L. *Toxoplasma gondii* in foods: prevalence, control, and safety. Foods 2022;11:2542. [CrossRef]
23. Rani S, Pradhan AK. Evaluating uncertainty and variability associated with Toxoplasma gondii survival during cooking and low temperature storage of fresh cut meats. Int J Food Microbiol 2021;341:109031. [CrossRef]

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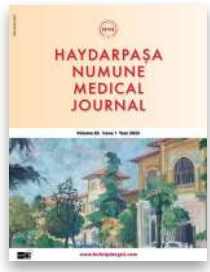

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ORIGINAL ARTICLE

Congenital Heart Defects in Twin Pregnancies: A Retrospective Analysis of Clinical Characteristics and Risk Factors

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Abstract

Introduction: Congenital heart defects (CHDs) are among the most common congenital anomalies. An increased frequency of CHDs has been reported in twin pregnancies. This study aims to determine the frequency, types, and contributing factors of CHDs in twin births.

Methods: This retrospective study included twin pregnancies in which both fetuses underwent at least one echocardiographic examination at the pediatric cardiology outpatient clinic. Data were collected from hospital records and included maternal age, use of assisted reproductive technology (ART), mode of delivery, pregnancy-related risk factors (gestational diabetes mellitus, preeclampsia, hypertension, and hypothyroidism), gestational age at birth, birth weight, and presence and type of CHD. Data were analyzed using Microsoft Excel.

Results: The study included 498 twin infants (252 female, 50.6%) and 249 mothers. Among the cases, 116 (23.3%) were monochorionic, and 26.1% were conceived through in vitro fertilization, with the remainder being spontaneous pregnancies. A total of 34 infants (6.83%) were diagnosed with CHDs. In four pregnancies, both twins were affected, whereas in the remaining cases, only one twin was affected. Most CHDs consisted of septal defects and right ventricular outflow tract obstructions, whereas 12 cases had more complex anomalies.

Discussion and Conclusion: In line with previous literature, this study found an increased frequency of CHDs in twin pregnancies. No significant difference in CHD frequency was observed between ART and spontaneous twin pregnancies. No specific risk factor was identified as a distinguishing feature between cases with and without CHDs.

Keywords: Congenital heart defect; in vitro fertilization; twin baby; twin pregnancies.

Multiple pregnancies have been reported to occur in approximately 2–3% of all births [1,2].

With the increasing maternal age and the widespread use of assisted reproductive technologies (ART), the incidence of multiple pregnancies has reportedly doubled in recent decades [1,3,4].

Congenital heart defects (CHDs) are the most commonly detected congenital anomalies, with an estimated preva-

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lence of 7–9/1000 live births [5]. It has been reported that the incidence of CHDs is higher in multiple pregnancies compared to singleton pregnancies. Among multiple pregnancies, the incidence of CHDs is higher in dizygotic twins than in singletons, and even higher in monozygotic twins than in dizygotic twins. The highest rates of CHDs have been reported in monozygotic twin pregnancies complicated by twin-to-twin transfusion syndrome (TTTS) [6].

Several factors have been associated with the increased incidence of CHDs in multiple pregnancies, including genetic predisposition, prematurity, advanced maternal age, and the increased use of ART [7]. For this reason, it is recommended that twin pregnancies – especially monozygotic ones – undergo both prenatal and postnatal evaluation with echocardiography [7-9].

This study aims to determine the frequency and types of CHDs, maternal characteristics, and associated risk factors in twin pregnancies referred to the pediatric cardiology outpatient clinic for evaluation and assessed through echocardiography.

Materials and Methods

This retrospective, single-center study included twin infants and their mothers who presented to the pediatric cardiology outpatient clinic between 2018 and 2024 for various reasons and underwent at least one echocardiographic evaluation. Cases in which one twin was lost *in utero* or where both twins did not undergo echocardiographic evaluation were excluded. Only cases in which both twins had echocardiographic examinations and were born after the 24th week of gestation were included in the study.

Maternal characteristics, including maternal age at delivery, gravidity, parity, and pregnancy-related risk factors (such as hypertension, preeclampsia, gestational diabetes mellitus, and hypothyroidism) were recorded. It was also noted whether the pregnancy was spontaneous or achieved through *in vitro* fertilization (IVF), the chorionicity of the pregnancy, the mode of delivery (vaginal or cesarean), and whether the newborns were admitted to the neonatal intensive care unit. The gestational age and birth weights of the infants were documented.

The gestational ages at birth were classified as <28 weeks, 28–32 weeks, 32–37 weeks, and >37 weeks. Birth weights were categorized as 1000–1500 g, 1500–2500 g, and >2500 g. If there was a weight difference >20% between the twin pair, the twins were classified as discordant (large and small); otherwise, they were considered concordant. According to their growth status, the infants were categorized as small for gestational age (SGA), appropriate for gestational age (AGA), or large for gestational age (LGA).

Statistical Analysis

All echocardiographic evaluations were retrospectively reviewed from hospital records. Minor findings potentially related to prematurity, such as small atrial septal defects (ASDs) that closed spontaneously within a short period, small muscular ventricular septal defects (VSDs), or a patent ductus arteriosus (PDA), were not included in the CHD group. Cases that required follow-up, intervention, or surgery were classified as having a CHD. In cases with multiple CHDs, classification was based on the major defect.

A dataset of 498 twin patients was analyzed to determine associations between CHDs and other patient characteristics. Maternal age was treated as a continuous variable, whereas other variables – including sex, birth week, birth weight, gravidity, parity, twin type, consanguinity, and echocardiographic findings – were categorized as categorical variables.

All statistical analyses and tests were conducted using the Statistical Package for the Social Sciences (SPSS) version 30 (IBM SPSS Statistics, IBM Corporation, Armonk, NY, USA) on a personal computer. Data storage, preprocessing, and charts were created using MS Excel 2016. The normality of variable distributions was assessed through the Kolmogorov–Smirnov test, histograms, coefficient of variation, Detrended Q-Q Plots, and analyses of skewness and kurtosis.

$P<0.05$ was considered statistically significant. Group comparisons were made using the independent sample T-test and Mann–Whitney U-test (due to non-Gaussian data distribution). A Chi-squared test was applied for frequency data analysis, and the Fisher–Freeman–Halton Exact Test was also used for additional comparisons.

Multivariate logistic regression analysis was performed; however, due to the relatively small number of CHD cases (n=34) in relation to the number of independent variables included in the model, excessively wide confidence intervals were observed for several factors, which indicates low statistical precision and a high risk of model overfitting. To avoid presenting potentially unstable estimates, multivariate analysis results were not included in the manuscript.

Ethical Approval

Ethical approval for this study was obtained from the Zeynep Kamil Maternity and Children's Diseases Training and Research Hospital Clinical Research Ethics Committee (Approval number: 110; Date: August 23, 2023). This study was performed in accordance with the standards of ethics outlined in the Declaration of Helsinki, 2013. All participants provided informed written consent (or written assent with parental consent, for minors) before participation in this study.

Results

This study included 498 twin infants, comprising 252 females (50.6%) and 246 males (49.4%). The mean gestational age at birth was 33.3±3.1 weeks, and the mean birth weight was 2021.15±628.71 g. Among these infants, 399 (80.1%) had a history of admission to the neonatal intensive care unit.

A total of 249 mothers participated in the study. The mean maternal age was 30.73±5.46 years (range: 18–49 years). Of these, 233 mothers (93.57%) delivered through cesarean section, whereas 16 (6.43%) had vaginal deliveries. The distribution of gravidity and parity among the mothers is presented in Figure 1.

Of the cases, 130 (26.1%) were born from pregnancies conceived through IVF, whereas 368 (73.9%) were from spontaneous twin pregnancies. In terms of chorionicity, 116 infants (23.3%) were monochorionic, and 382 (76.7%) were dichorionic twins. The birth characteristics of the infants by chorionicity are presented in Table 1. The characteristics of IVF-conceived and spontaneous twin pregnancies are shown in Table 2.

Pearson's Chi-square test was applied to assess the relationships between chorionicity and sex (p=0.882), gestational age at birth (p=0.069), birth weight (p=0.122), and growth classification (SGA/AGA/LGA) (p=0.923). No statistically significant associations were found between chorionicity and any of these variables.

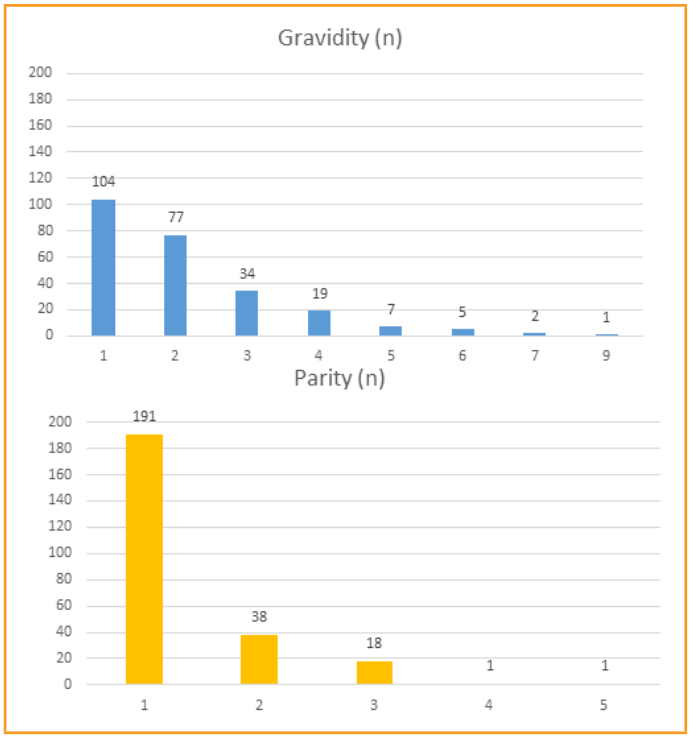

Figure 1. Patient flow diagram for study cohort.

The relationship between maternal age and chorionicity was assessed by the independent samples Kruskal–Wallis and Mann–Whitney U tests, revealing a statistically significant difference (p=0.006). This result indicates that maternal age is a significant factor influencing chorionicity type (monochorionic vs. dichorionic), suggesting that maternal age should be considered when evaluating chorionicity type.

Pearson's Chi-square test revealed no statistically significant associations between conception type (IVF vs. spontaneous) and the variables of sex (p=0.73), birth weight (p=0.625), or weight discordance (p=0.109).

However, the same test indicated a statistically significant difference in gestational age categories between IVF and spontaneously conceived infants (p=0.006), suggesting a meaningful difference in gestational age at birth between the two groups.

In addition, a significant difference was observed between conception type and chorionicity (p<0.001), indicating that the distribution of chorionicity differs significantly between IVF and spontaneous pregnancies.

Table 1. Birth characteristics by chorionicity

	Monochorionic n	%	Dichorionic n	%	p
Sex (Male/female)					
Female	58	50.0	194	50.79	p>0.05
Male	58	50.0	188	49.21	
Gestational age					
<28 weeks	4	3.45	26	6.81	p>0.05
28–32 weeks	26	22.41	66	17.28	
32–37 weeks	78	67.24	236	61.78	
>37 weeks	8	6.90	54	14.14	
Birth weight					
<1 kg	7	6.03	19	4.97	p>0.05
1.0–1.5 kg	21	18.10	46	12.04	
>1.5–2.5 kg	72	62.07	232	60.73	
>2.5 kg	16	13.79	85	22.25	
SGA/AGA/LGA					
SGA	18	15.52	55	14.4	p>0.05
AGA	93	80.17	308	80.63	
LGA	5	4.31	19	4.97	
Maternal age	29.41	(18–39)	31.14	(19–49)	p<0.05

SGA: Small for gestational; AGA: Appropriate for gestational age; LGA: Large for gestational age.

Table 2. Characteristics of *in vitro* fertilization conceived and spontaneous twin pregnancies

	***In vitro* fertilization**		**Spontaneous**		**p**
Sex	***n***	**%**	***n***	**%**	
Female	57	43.85	195	52.99	p>0.05
Male	73	56.15	173	47.01	
Gestational age					
<28 weeks	10	7.69	20	5.43	p<0.05
28–32 weeks	34	26.15	58	15.76	
32–37 weeks	78	60.00	236	64.13	
>37 weeks	8	6.15	54	14.67	
Birth weight					
<1 kg	9	6.92	17	4.62	p>0.05
1.0–1.5 kg	20	15.38	47	12.77	
>1.5–2.5 kg	76	58.46	228	61.96	
>2.5 kg	25	19.23	76	20.65	
Birth weight discordance					
Larger discordant twin	8	6.15	38	10.33	p>0.05
Smaller discordant twin	8	6.15	38	10.33	
Concordant twins	114	87.69	292	79.35	
Chorionicity					
Dichorionic	114	87,69	268	72,83	p<0.05
Monochorionic	16	12,31	100	27,17	
Maternal age	Mean	Min-Max	Mean	Min-Max	
	32.43	22–49	30.14	18–43	*p*<0.05

Independent samples Kruskal–Wallis and Mann–Whitney U tests applied to examine the relationship between maternal age and conception type also revealed a statistically significant difference (p<0.001). These findings indicate a significant association between maternal age and the method of conception (IVF or spontaneous), suggesting that maternal age is distributed differently across the two groups or that certain maternal age ranges are more associated with either IVF or spontaneous conception.

A total of 34 infants (6.83%) were found to have CHDs. In 4 twin pairs, both infants had a CHD, whereas in 26 cases, only one twin was affected. The mean maternal age in this group was 30.52±6.05 years (range: 20–41 years). Among the mothers who gave birth to at least one infant with a CHD, 8 (26.67%) had conceived through IVF, and 22 (73.33%) had spontaneous twin pregnancies.

The mean gestational age of twins with CHDs was 34.47±3.16 weeks (range: 26–40 weeks), and the mean birth weight was 2166.82±545.78 g (range: 950–3870 g).

The specific CHDs identified by echocardiography are presented in Table 3.

In addition, 86 infants (17.27%) had findings such as small ASDs that closed spontaneously within a few months, small muscular VSDs, or a PDA – defects considered to be related to prematurity and developmental stage. These cases were not included in the CHD group.

Among the twin pairs in which both infants had CHDs, two were monochorionic, and the other two were dichorionic. Their clinical characteristics are detailed in Table 4.

The characteristics of infants with and without CHDs are summarized in Table 5.

Pearson's Chi-square test showed no statistically significant differences between infants with and without CHDs regarding sex (p=0.177), chorionicity (p=0.650), or conception type (IVF vs. spontaneous; p=0.723). The Fisher–Freeman–Halton Exact Test revealed no significant differences in birth weight (p=0.396) or weight discordance (p=0.146) between the two groups.

However, the Fisher–Freeman–Halton Exact Test showed a statistically significant difference in gestational age groups

Table 3. Distribution of congenital heart defects identified by echocardiography

Congenital heart defects	***n***	**%**
Atrial septal defect±Pulmonary stenosis	10	29.41
Atrial septal defect+Ventricular septal defect	3	8.82
Ventricular septal defect±Pulmonary stenosis	3	8.82
Pulmonary stenosis	2	5.88
Ventricular septal defect – Pulmonary atresia	3	8.82
Tetralogy of Fallot/Double outlet right ventricle	2	5.88
Ventricular septal defect+Aortic hypoplasia/ Coarctation of the aorta	3	8.82
Patent ductus arterosus	1	2.94
Double inlet left ventricle+Transposition of great arteries+Coarctation of aorta	1	2.94
Aortic valve pathology+Aortic stenosis±Atrial septal defect	2	5.88
Truncus arteriosus	1	2.94
Intact ventricular septum – Pulmonary atresia	1	2.94
Double aortic arch	1	2.94
Hypoplastic left heart syndrome	1	2.94
Total	34	100

Table 4. Characteristics of twin pairs in which both infants had congenital heart defects

Case	Sex	Gestational age	Birth weight (gr)	Birth weight discordance (<%20)	Chorionicity	Congenital heart defect	Maternal age	Gravidity	Parity	Risk factor
1a	F	35+0	2560	No	DC	Moderate secundum ASD	28	2	1	0
1b	M		2560			Moderate secundum ASD				
2a	F	37+3	2230	No	DC	Aortic arch hypoplasia+CoA+LV hypoplasia	34	5	4	Preeclampsia
2b	M		2240			DORV+PS+ToF				
3a	F	35+0	2500	No	MC	VSD-PA	34	4	3	0
3b	F		2300			ASD-PDA				
4a	F	32+3	1680	No	MC	Large secundum ASD+PS+PLSVC	24	2	2	0
4b	F		1950			Moderate secundum ASD				

MC: Monochorionic; DC: Dichorionic; CHD: Congenital heart defect; ASD: Atrial septal defect; CoA: Coarctation of aorta; LV: Left ventricle; DORV: Double outlet right ventricle; PS: Pulmonary stenosis; ToF: Tetralogy of Fallot; VSD: Ventricular septal defect; PA: Pulmonary atresia; PDA: Patent ductus arteriosus; PLSVC: Persistent left superior vena cava.

Table 5. Comparison of characteristics between twins with and without congenital heart defects

	Congenital heart disease (*n*=34)		No congenital heart defect		p
Sex	*n*	%	*n*	%	
Female	21	61.76	231	49.78	p>0.05
Male	13	38.24	233	50.22	
Gestational age					
<28 weeks	2	5.88	28	6.03	p<0.05
28–32 weeks	3	8.82	89	19.18	
32–37 weeks	19	55.88	295	63.58	
>37 weeks	10	29.41	52	11.21	
Birth weight					
<1 kg	3	8.82	23	4.96	p>0.05
1.0–1.5 kg	2	5.88	65	14.01	
>1.5–2.5 kg	23	67.65	281	60.56	
>2.5 kg	6	17.65	95	20.47	

	Congenital heart disease (*n*=34)		No congenital heart defect		p
	n	%	*n*	%	
Birth weight discordance					
Larger discordant twin	1	2.94	45	9.70	p>0.05
Smaller discordant twin	6	17.65	40	8.62	
Concordant twins	27	79.41	379	81.68	
Chorionicity					
Dichorionic	25	73.53	357	76.94	p>0.05
Monochorionic	9	26.47	107	23.06	
Mode of conception					
In vitro fertilization	8	23.53	122	26.29	p>0.05
Spontaneous	26	76.47	342	73.71	

between infants with and without CHDs (p=0.024). This finding suggests a potential association between gestational age and the presence of CHDs, which may be of clinical importance.

Of the mothers, 121 (48.59%) had no identifiable risk factors (including gestational diabetes mellitus, gestational hypertension, preeclampsia, hypothyroidism, or IVF conception). 86 mothers (34.54%) had at least one risk factor, 32 (12.85%) had two risk factors, and 10 (4.02%) had three

Table 6. Maternal risk factors during pregnancy

Maternal risk factors	*n*	%
Gestational diabetes mellitus	50	20.08
Gestational hypertension/Preeclempsia	41	16.47
Hypothyroidism	25	10.04
In vitro fertilization	65	26.10

different risk factors concurrently. The distribution of maternal risk factors is presented in Table 6.

Discussion

Multiple pregnancies occur in approximately 2–3% of all births. In the United Kingdom, it has been reported that 20–30% of these are monochorionic. [1,4]. Overall, congenital anomalies are reported to be 1.3 times more common in twin infants compared to singletons [1,4].

CHDs are the most common type of congenital anomaly. While the incidence is reported as 7–9/1000 live births in the general population, this rate increases to approximately 20/1000 live births in twin pregnancies [5]. The prevalence is higher in monochorionic compared to dichorionic twins, particularly in monochorionic diamniotic pregnancies. In cases complicated by TTTS, which occurs in about 10–15% of monochorionic diamniotic twins, the incidence of CHDs increases by 12–13 times compared to the general population [5].

In a meta-analysis of 12 studies, the rate of CHDs in monochorionic twins was reported as 59/1000 live births – about 6 times higher than the general rate [3]. In TTTS cases, this rate rises to as much as twelvefold [3]. A study conducted in Taipei found that the incidence of CHDs in dichorionic twins was similar to that in singletons, whereas it was 5 times higher in monochorionic twins [10]. Chorionicity has been reported to have a stronger influence on the development of CHDs than zygosity [11]. The Danish registry, covering the years 1977–2001 and including a random sample without considering zygosity, reported a CHD rate of 5% in twin pregnancies compared to singletons [11]. The CHD rate in our study population, at 6.83%, is consistent with these findings.

Factors such as genetic predisposition, prematurity, advanced maternal age, and the use of ART have been discussed as potential contributors to the increased prevalence of CHDs. An increased incidence of CHDs has been reported in IVF pregnancies [12]. It has been suggested that periconceptional and mechanical manipulations, embryo freezing, the use of various culture media, and differing durations in culture environments may affect the gastrulation phase and the development of the heart, which is the first organ to form. In addition, in individuals requiring fertility treatment, both underlying subclinical genetic abnormalities and higher maternal age – as observed in our study and known to be associated with an increased risk of chromosomal anomalies – may contribute to the pathogenesis of CHDs [12]. However, contrary to the literature, there was no significant difference in the incidence of CHDs between IVF and spontaneous twin pregnancies in our study.

In this study, certain cardiac findings common in preterm infants, such as a PDA and small ASDs that closed during early follow-up, were excluded from the CHD category to focus specifically on primary structural heart defects. While this approach ensures a more precise evaluation of significant congenital anomalies, it may lead to lower reported prevalence rates compared to studies that utilize broader diagnostic criteria, including all minor prematurity-related shunts. Therefore, when comparing our findings with the existing literature, it is essential to consider these definition-based differences; the exclusion of these transient physiological shunts explains our lower CHD prevalence but provides a more accurate representation of true structural defects in twin cohorts.

In multiple pregnancies, CHDs are more frequently septal defects such as ASD and VSD, as well as congenital anomalies such as right ventricular outflow tract obstruction (RVOTO). The pathogenesis of RVOTO, particularly in monochorionic twins with TTTS, has been explained by hemodynamic changes. However, the relatively low frequency of pathologies such as aortic stenosis and aortic coarctation, which would also be expected to be influenced by hemodynamic changes, necessitates a cautious interpretation of these associations [13]. More complex pathologies such as tetralogy of Fallot and transposition of the great arteries have been reported to occur at similar rates as in singleton pregnancies [3]. In a study by the Danish group, while an overall increase in CHDs was noted in twins, no complicated CHDs were identified. Consistent with previous studies, the most common types of CHDs in our study group were septal defects and pulmonary stenosis. However, in our group, 12 out of 34 cases presented with more complex CHDs compared to the literature. This may be attributed to our center being a tertiary perinatology center, where more complicated cases are referred.

Although monochorionic twins are genetically identical, the concordance of congenital anomalies – defined as the presence of CHD in both twins of a pair – has been reported at a rate of <20% [14]. In dichorionic twins, the majority of anomalies are discordant, and the risk of CHD in each twin is similar to that of singletons. In our study, a CHD was iden-

tified in only one twin in 26 pregnancies. Among these discordant cases, only 7 had a birth weight difference >20%, and in 6 of those, the affected twin was the smaller one. In 4 of the 30 pregnancies with CHDs, both twins were affected. There were no significant differences in chorionicity, maternal age, or birth weights between the twins in these cases. Among these four concordant cases, in two pregnancies (one monochorionic, one dichorionic), both twins had the same type of CHD, whereas in the other two, each twin had a different type of CHD.

When examined in terms of IVF status, maternal age, birth weight, and gestational age, there were no statistically significant differences between cases with and without CHDs. When maternal risk factors were analyzed in detail (Table 5), no significant distinguishing criterion was identified between mothers of twins with and without CHDs. In only one of the concordant CHD cases, the mother had preeclampsia. These findings suggest that the etiology of CHDs cannot be explained solely by hemodynamic mechanisms and the commonly discussed risk factors as aforementioned, and multiple additional factors, such as epigenetic mechanisms, uterine and environmental factors, may be involved.

Limitation

Although our study is comprehensive due to its inclusion of a large cohort of twins, it has certain limitations. These include its retrospective design, being conducted in a single center, and the inclusion of cases that are presented only to the pediatric cardiology outpatient clinic.

Another limitation is that some of the twin cases evaluated in our clinic were born in other centers, leading to missing obstetric data such as zygosity and the presence or absence of TTTS. Moreover, the absence of a comparison group with singleton births is also considered a limitation.

However, this study is valuable as it highlights an increased incidence of CHDs in twin pregnancies, the presence of different and more complex CHD types compared to other study populations, the predominance of discordant CHDs, and the absence of an increased CHD rate in IVF pregnancies.

Conclusion

In conclusion, in line with the literature, CHDs were increased in both monochorionic and dichorionic twin pregnancies in our study. The pathophysiological mechanisms behind the increase in CHDs in twin pregnancies are not yet fully understood, though genetic factors, abnormal placentation, maternal factors, environmental influences, and epigenetic factors are all thought to contribute.

In monochorionic pregnancies, hematological disturbances such as TTTS, delayed division of the fertilized ovum, and unequal distribution of cells to the two fetuses may lead to differing cardiac development. In dichorionic pregnancies, when associated with ART, factors related to the procedure and unequal placental development may play a role.

This topic continues to present many unresolved questions that warrant further research. We hope that future studies will provide deeper insights into the underlying pathophysiological mechanisms contributing to the development of CHDs in twin pregnancies.

Our study has several limitations. First, the retrospective design and the fact that the study population consisted of patients referred to a tertiary pediatric cardiology clinic may introduce selection bias. Second, the exclusion of certain prematurity-related lesions (e.g., PDA and small ASDs) might result in a lower prevalence rate compared to some studies in the literature.

Disclosure

Ethics Committee Approval: This study was approved by the Zeynep Kamil Maternity and Children's Diseases Training and Research Hospital Ethics Committee (Date: 23.08.2023, Decision no: 101).

Informed Consent: All participants provided informed written consent (or written assent with parentalconsent, for minors) prior to participation in this study.

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References

1. Weber MA, Sebire NJ. Genetics and developmental pathology of twinning. Semin Fetal Neonatal Med 2010;15:313–8. [CrossRef]
2. Aviram A, Lipworth H, Asztalos EV, Mei-Dan E, Melamed N, Cao X, et al. Delivery of monochorionic twins: lessons learned from the Twin Birth Study. Am J Obstet Gynecol 2020;223:916.e1–e9. [CrossRef]
3. Gijtenbeek M, Shirzada MR, Ten Harkel ADJ, Oepkes D, C Haak M. Congenital heart defects in monochorionic twins: a

systematic review and meta-analysis. J Clin Med 2019;8:902. [CrossRef]
4. Glinianaia SV, Rankin J, Wright C. Congenital anomalies in twins: a register-based study. Hum Reprod 2008;23:1306–11. [CrossRef]
5. Balasubramanian R, Vuppalapati S, Avanthika C, Jhaveri S, Peddi NC, Ahmed S, et al. Epidemiology, genetics and epigenetics of congenital heart diseases in twins. Cureus 2021;13:e17253. [CrossRef]
6. McPherson E, Korlesky C, Hebbring S. Cardiac anomalies in liveborn and stillborn monochorionic twins. Clin Med Res 2020;18:58–67. [CrossRef]
7. Molin J, Paerregaard MM, Pihl C, Thygesen CB, Pietersen A, Dannesbo S, et al. Cardiac findings in newborn twins. Acta Paediatr 2023;112:2050–9. [CrossRef]
8. Donofrio MT, Moon-Grady AJ, Hornberger LK, Copel JA, Sklansky MS, Abuhamad A, et al. Diagnosis and treatment of fetal cardiac disease: a scientific statement from the American Heart Association. Circulation 2014;129:2183–242. Erratum in: Circulation 2014;129:e512. [CrossRef]
9. Springer S, Mlczoch E, Krampl-Bettelheim E, Mailáth-Pokorny M, Ulm B, Worda C, et al. Congenital heart disease in monochorionic twins with and without twin-to-twin transfusion syndrome. Prenat Diagn 2014;34:994–9. [CrossRef]
10. Chen CJ, Wang CJ, Yu MW, Lee TK. Perinatal mortality and prevalence of major congenital malformations of twins in Taipei city. Acta Genet Med Gemellol (Roma) 1992;41:197–203. [CrossRef]
11. Panagiotopoulou O, Fouzas S, Sinopidis X, Mantagos SP, Dimitriou G, Karatza AA. Congenital heart disease in twins: The contribution of type of conception and chorionicity. Int J Cardiol 2016;218:144–9. [CrossRef]
12. Tararbit K, Houyel L, Bonnet D, De Vigan C, Lelong N, Goffinet F, et al. Risk of congenital heart defects associated with assisted reproductive technologies: a population-based evaluation. Eur Heart J 2011;32:500–8. [CrossRef]
13. Groene SG, Tollenaar LSA, Middeldorp JM, Lopriore E. Neonatal management and outcome in complicated monochorionic twins: What have we learned in the past decade and what should you know? Best Pract Res Clin Obstet Gynaecol 2022;84:218–28. [CrossRef]
14. Imany-Shakibai H, Yin O, Russell MR, Sklansky M, Satou G, Afshar Y. Discordant congenital heart defects in monochorionic twins: Risk factors and proposed pathophysiology. PLoS One 2021;16:e0251160. [CrossRef]

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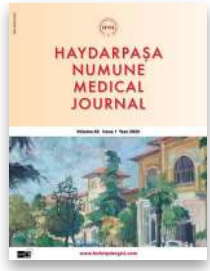

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ORIGINAL ARTICLE

Prognostic Factors and Biomarker Performance in Predicting Mortality and Complications After Surgical Repair of Perforated Peptic Ulcer: A Retrospective Cohort Study

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Abstract

Introduction: Perforated peptic ulcer (PPU) remains a life-threatening surgical emergency with substantial morbidity and mortality. Early identification of high-risk patients is essential for guiding operative strategy and optimizing perioperative care. This study evaluated clinical, biochemical, and operative predictors of post-operative complications and 30-day mortality and assessed the prognostic performance of routine biomarkers.

Methods: A retrospective cohort study was conducted including 114 adults who underwent surgical repair for intraoperatively confirmed PPU between May 2022 and September 2025 at a tertiary center in Istanbul, Türkiye. Demographics, American Society of Anesthesiologists (ASA) class, symptom-to-surgery interval, operative details, and baseline laboratory values (lactate, C-reactive protein [CRP], albumin, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio) were analyzed. Primary outcomes were 30-day post-operative complications and all-cause 30-day mortality. Multivariable logistic regression identified independent predictors, and receiver operating characteristic (ROC) analysis evaluated biomarker performance.

Results: Post-operative complications occurred in 28.1% of patients, with a 30-day mortality of 7.9%. Complications were significantly lower after laparoscopic repair (10.7%) than open repair (32.9%), with the open group showing more adverse baseline biomarker profiles. On multivariable analysis, elevated lactate (≥2.5 mmol/L; odds ratio [OR] 8.33) and higher ASA class (II–IV; OR 2.51) independently predicted complications, while advanced age (≥53 years; OR 1.085/year) and hypoalbuminemia (<31 g/L; OR 0.805) predicted 30-day mortality. ROC analysis showed excellent discrimination for age (area under the curve [AUC] 0.883) and albumin (AUC 0.895) in predicting mortality, and both lactate and CRP strongly predicted selection of an open surgical approach.

Discussion and Conclusion: Advanced age, elevated ASA class, and biochemical abnormalities – particularly elevated lactate and low serum albumin – are independent predictors of post-operative complications and mortality following PPU repair. Incorporation of these readily available markers into pre-operative assessment may improve risk stratification, guide operative approach selection, and optimize perioperative management. Prospective studies are warranted to validate thresholds and develop standardized protocols.

Keywords: Albumin; biomarkers; complications; lactate; laparoscopic repair; mortality; perforated peptic ulcer; surgical outcomes

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Peptic ulcer disease (PUD) continues to represent an important global health concern, with a reported lifetime prevalence of 5–10% and an annual incidence of 1.5–3%, affecting nearly 4 million individuals worldwide [1-3]. Although its incidence has declined with the widespread use of proton pump inhibitors and eradication of Helicobacter pylori, complications still occur in 10–20% of patients. Among these, perforated peptic ulcer (PPU) is one of the most severe, occurring in 2–14% of cases and carrying a lifetime risk of approximately 5% [2,3]. PPU is associated with high mortality, with short-term rates of 11–30% and long-term mortality up to 25%. Delays in surgical intervention beyond 24 h may increase mortality to nearly 50% [2,4].

Diagnosis of PPU relies on clinical assessment supported by imaging. While erect chest radiography can detect pneumoperitoneum, computed tomography offers greater sensitivity and overall diagnostic accuracy [5]. Early surgical intervention is critical to limit progression to generalized peritonitis, sepsis, and potential mortality [3,4,6]. While open repair has traditionally been the standard approach, laparoscopic repair is increasingly preferred due to lower morbidity and shorter hospital stay [2,5-7].

Given the variability in clinical presentation, accurate pre-operative risk stratification is crucial. Several clinical and biochemical parameters – including age, symptom-to-surgery interval, albumin, lactate, C-reactive protein (CRP), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and white blood cell (WBC) count – have been associated with post-operative complications, need for open surgery, and mortality in PPU [8-10]. Lactate, together with WBC and CRP, plays a key role in early diagnosis, risk stratification, and monitoring of sepsis, with elevated levels predicting adverse outcomes [11]. Post-operative trends in CRP and NLR may facilitate early detection of complications, while albumin reflects nutritional and physiological reserve, informing perioperative management [12].

This study aimed to identify clinical, biochemical, and operative factors associated with post-operative complications and 30-day mortality following PPU repair and to evaluate the predictive performance of key biomarkers – lactate, CRP, albumin, NLR, and PLR – for risk stratification and surgical decision-making.

Materials and Methods

Study Design and Population

This retrospective, single-center cohort study was carried out at Başakşehir Çam and Sakura City Hospital, a high-volume tertiary referral center in Istanbul, Türkiye. The study included all patients who underwent surgical treatment for PPU between May 2022 and September 2025.

Adult patients (≥18 years) with an intraoperatively confirmed diagnosis of acute PPU were eligible for inclusion, regardless of whether they were treated with an open or laparoscopic approach. Patients were excluded if the perforation was due to non-peptic causes, such as trauma or malignancy, or if key clinical, laboratory, or operative data were missing, preventing reliable statistical analysis.

Data Collection

Patient data were obtained from the hospital's electronic medical records, including clinical documentation, laboratory findings, and operative reports. The following variables were collected:

- Demographic data: Age and sex.
- Clinical parameters: American Society of Anesthesiologists (ASA) score and time from symptom onset to surgery (days).
- Surgical details: Operative approach (laparoscopic or open) and duration of surgery (minutes).
- Hospital course: Length of hospital stay (days).
- Baseline laboratory values: Lactate, CRP, serum albumin, NLR, and PLR.

Surgical Technique and Perioperative Care

Laparoscopic repair was performed using a standard three-port technique, with primary closure of the perforation reinforced by an omental patch. Open repair was carried out through midline laparotomy, also using primary closure and an omental patch, either with the Graham or modified Graham technique. All patients underwent standardized pre-operative resuscitation and received intravenous antibiotics and proton pump inhibitor therapy in accordance with institutional protocols.

Outcomes

The primary outcome was the occurrence and type of post-operative complications within 30 days. These included surgical site infection, incisional hernia, bile leak, hematoma, evisceration, pleural effusion, deep vein thrombosis, intra-abdominal abscess, fever, atelectasis, and disseminated intravascular coagulation.

Secondary outcomes included all-cause perioperative and 30-day mortality, which were used as measures of short-term survival and overall surgical effectiveness. Length of hospital stay and type of surgical approach (laparoscopic, open, or converted) were also evaluated to explore their impact on recovery and morbidity.

In 10 cases, procedures initially started laparoscopically were converted to open surgery due to the presence of

generalized peritonitis, where continuation of laparoscopy was considered neither safe nor effective. These patients were analyzed according to the intention-to-treat principle and were therefore included in the open surgery group.

Comparative analyses between surgical approaches were performed to identify factors associated with increased risk of complications and mortality. Receiver operating characteristic (ROC) curve analysis and multivariable logistic regression were used to assess the diagnostic performance and prognostic value of key biomarkers – lactate, CRP, albumin, NLR, and PLR – in predicting the need for open surgery, post-operative complications, and mortality. Optimal cut-off values for lactate (>2.5 mmol/L) and albumin (<31 g/L) were determined using the Youden Index derived from ROC analysis.

Ethical Approval and Consent to Participate

This study was conducted in accordance with the ethical standards of both institutional and national research committees, as well as the principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Ethics Committee of Başakşehir Çam and Sakura City Hospital (Approval No: E-96317027-514.10-282238896; Date: July 21, 2025).

Given the retrospective nature of the study, the requirement for informed consent was waived. All patient data were anonymized before analysis to ensure confidentiality.

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences version 27.0 (IBM Corp., Armonk, NY, USA). The distribution of continuous variables was assessed using the Shapiro–Wilk test. Variables that did not follow a normal distribution were reported as median and interquartile range (IQR), while categorical variables were expressed as frequencies and percentages (n [%]).

For group comparisons, non-normally distributed continuous variables were analyzed using the Mann–Whitney U test or Kruskal–Wallis H test, as appropriate. Categorical variables were compared using the Pearson Chi-square test or Fisher's exact test. When multiple pairwise comparisons were performed, the Bonferroni correction was applied.

ROC curve analysis was used to evaluate diagnostic performance. Forward stepwise logistic regression analysis was conducted to identify independent predictors of outcomes. A $p<0.05$ was considered statistically significant.

Results

A total of 114 patients with PUD were included in the study. The median age was 45 years (IQR: 30–55), and the majority were male (78.9%, *n*=90), while 21.1% (n=24) were female. The median time from symptom onset to surgery was 1 day (IQR: 1–3). Median operative time was 90 min (IQR: 70–110), and the median length of hospital stay was 5 days (IQR: 5–7). Post-operative complications occurred in 28.1% of patients (n=32), and the overall mortality rate was 7.9% (n=9) (Table 1).

Comparison of Patients According to Surgical Method

When patients were compared based on surgical approach (open, laparoscopic, and converted), no significant differences were observed in age, sex, duration of symptoms, ASA classification, operative time, or length of hospital stay (all $p>0.05$). However, complication rates differed significantly between groups ($p=0.035$), occurring in 32.9% of patients undergoing open repair compared with 10.7% in the laparoscopic group.

Significant differences were also found in lactate ($p<0.001$), CRP ($p=0.013$), and PLR ($p=0.049$) levels across groups. Bonferroni-adjusted pairwise comparisons showed that both lactate and CRP levels were significantly higher in the open surgery group than in the laparoscopic group. Although ASA scores did not differ significantly ($p=0.133$), the proportion of patients with ASA III–IV status was nearly twice as high in the open group (34.2%) compared with the laparoscopic group (17.9%). While not statistically significant, this difference is clinically relevant and suggests a potential selection bias, with laparoscopic repair more frequently performed in physiologically stable patients (Table 2).

Diagnostic Markers Associated with Open Surgery

ROC curve analysis identified lactate, CRP, and PLR as predictors of the need for open surgery. Among these, lactate demonstrated the highest diagnostic performance, followed by CRP and PLR. The area under the curve (AUC) values were 0.760 (95% confidence interval [CI]: 0.664–0.855) for lactate, 0.680 (95% CI: 0.569–0.790) for CRP, and 0.652 (95% CI: 0.533–0.772) for PLR. The corresponding ROC curves are presented in Figure 1.

Multivariable logistic regression analysis further showed that elevated lactate (≥2.5 mmol/L) (odds ratio [OR]: 8.332, 95% CI: 2.276–30.497, $p=0.001$) and elevated CRP (≥11.4 mg/L) (OR: 3.606, 95% CI: 1.367–9.517, $p=0.010$) were independent predictors of open surgery (Table 3).

Factors Associated with Complications and Mortality

Patients who developed complications had significantly higher ASA scores ($p=0.020$) and were more likely to under-

Table 1. Characteristics of patients diagnosed with peptic ulcer perforation

Variables	Statistics
Number of patients	114
Age (year)	
M (IQR)/(Minimum-maximum)	45 (30–55)/(19–90)
Sex, *n* (%)	
Male	90 (78.9)
Female	24 (21.1)
Duration of symptoms (days)	
M (IQR)/(Minimum-maximum)	1 (1–3)/(1–30)
American Society of Anesthesiologists score	
I-II	82 (71.9)
III-IV	32 (28.1)
Surgical technique, *n* (%)	
Open surgery	76 (66.7)
Laparoscopic surgery	28 (24.6)
Conversion to open surgery	10 (8.8)
Surgery duration (minutes)	
M (IQR)/(Minimum-maximum)	90 (70–110)/(10–200)
Length of hospital stay (days)	
M (IQR)/(Minimum-maximum)	5 (5–7)/(1–33)
Complication, *n* (%)	32 (28.1)
Type of complication *n* (%)	
Surgical site infection	10 (8.8)
Incisional hernia	4 (3.5)
Bile leak	4 (3.5)
Hematoma	2 (1.8)
Evisceration	2 (1.8)
Pleural effusion	4 (3.5)
Deep vein thrombosis	1 (0.9)
Abscess	1 (0.9)
Fever	2 (1.8)
Atelectasis	1 (0.9)
Disseminated intravascular coagulation	1 (0.9)
Mortality, *n* (%)	9 (7.9)
Lactate	
M (IQR)/(Minimum-maximum)	2.2 (1.5–3.2)/(0.6–16)
Albumin	
M (IQR)/(Minimum-maximum)	41 (36–45)/(21–54)
C-reactive protein	
M (IQR)/(Minimum-maximum)	29.5 (4.6–114.9)/ (0.1–814.8)
Neutrophil-to-lymphocyte ratio	
M (IQR)/(Minimum-maximum)	9.5 (5.7–17.7)/ (1.3–58.1)
Platelet-to-lymphocyte ratio	
M (IQR)/(Minimum-maximum)	305.7 (178–462)/ (33.9–2080)

M: Median; IQR: Interquartile range.

go open surgery (p=0.019). In multivariable analysis, both higher ASA class (II–IV) (OR: 2.510, 95% CI: 1.028–6.128, p=0.043) and open surgical approach (OR: 3.846, 95% CI: 1.055–14.018, p=0.041) were identified as independent risk factors for post-operative complications (Table 4).

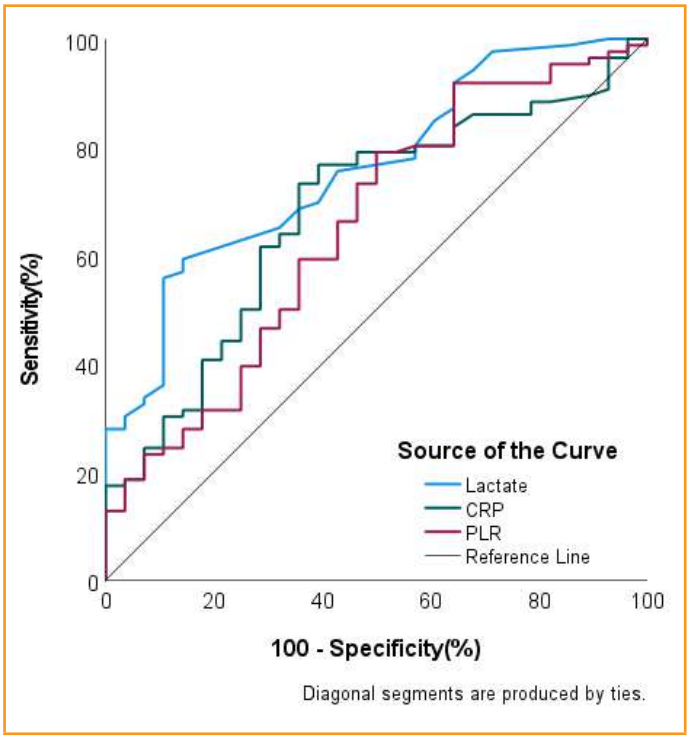

Figure 1. Receiver operating characteristic curve graph for the international normalized ratio/Albumin ratio according to deaths in patients diagnosed with hepatocellular carcinoma.

Table 2. Distribution of patients' characteristics according to surgical type

Variables	Open surgery[a]	Laparoscopic surgery[b]	Conversion to open surgery[c]	p	Bonferroni test (adjusted *p*)		
	n (%)/M (IQR)	*n* (%)/M (IQR)	*n* (%)/M (IQR)		a versus b	a versus c	b versus c
Number of patients	76	28	10				
Age (year)	47 (31–59)	40 (29–49)	44 (26–55)	0.292[a]			
Sex				0.163[b]			
Male	56 (73.7)	25 (89.3)	9 (90)				
Female	20 (26.3)	3 (10.7)	1 (10)				
Duration of symptoms (days)	1 (1–3)	1 (1–2)	1 (1-5)	0.142[a]			
ASA score				0.133[b]			
I-II	50 (65.8)	23 (82.1)	9 (90)				
III-IV	26 (34.2)	5 (17.9)	1 (10)				
Surgery duration (minutes)	85 (65–109)	90 (71–111)	94 (88–96)	0.567[a]			
Hospital stay (days)	6 (5–8)	5 (5-6)	5 (5–6)	0.414[a]			
Complication	25 (32.9)	3 (10.7)	4 (40)	**0.035**[b]			
Mortality	9 (11.8)	0 (0)	0 (0)	0.117[b]			
Lactate	2.6 (1.8–4.1)	1.7 (0.9–2.2)	2.1 (1.5–2.8)	**<0.001**[a]	**<0.001**	0.716	0.416
Albumin	40 (33.3–45)	42.5 (37.3–44)	44 (36.8–47)	0.221[a]			
CRP	34.4 (11.8–143)	5.5 (2.2–41.1)	19.1 (3.2–107)	**0.013**[a]	**0.010**	>0.999	0.852
NLR	9.6 (5.7–17.9)	9.8 (6.2–22.8)	8.5 (5.3–15.3)	0.839[a]			
PLR	321.9 (206–516)	194.1 (115.4–408.6)	278.9 (146.4–563.6)	**0.049**[a]	**0.043**	>0.999	0.836

[a]Kruskal–Wallis test, [b]Fisher's exact test, M: Median; IQR: Interquartile range; p: Probability value; ASA: American Society of Anesthesiologists; CRP: C-Reactive protein; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio.

Table 3. Multivariable logistic regression analysis of independent variables associated with open surgery

Variables (Step2)	B	SE	OR (95% CI)	p	Model summary
Lactate (≥2.5)	2.120	0.662	8.332 (2.276–30.497)	**0.001**	χ^2=26.593
CRP (≥11.4)	1.283	0.495	3.606 (1.367–9.517)	**0.010**	R^2=0.310
Constant	–0.211	0.353	0.809	0.549	

B: Unstandardized coefficient; SE: Standard error; OR: Odds ratio; CI: Confidence interval; CRP: C-Reactive protein; p: Probability value; Method: Forward Stepwise (Likelihood Ratio); Excluded variables: PLR (p=0.528), χ^2: Chi-square, R^2: R-squared

Mortality analysis revealed that patients who died were significantly older (p<0.001), had longer symptom duration before surgery (p=0.017), higher lactate levels (p=0.002), lower albumin levels (p<0.001), and higher CRP levels (p=0.004) compared to survivors (Table 5). Multivariable logistic regression identified advanced age (OR: 1.085, 95% CI: 1.016–1.159, p=0.015) and low albumin levels (OR: 0.805, 95% CI: 0.660–0.983, p=0.033) as independent predictors of mortality (Table 4).

Performance of Biomarkers in Predicting Mortality

ROC curve analysis demonstrated strong diagnostic performance for age (AUC: 0.883; 95% CI: 0.813–0.952), albumin (AUC: 0.895; 95% CI: 0.796–0.993), and lactate

Table 4. Multivariable logistic regression analysis of independent variables associated with complications and mortality

Dependent variable: Presence of complication (1=Yes; 0=No)					
Variables (Step2)	**B**	**SE**	**OR (95% CI)**	**p**	**Model summary**
ASA score (II-IV)	0.920	0.455	2.510 (1.028–6.128)	**0.043**	χ^2=10.392
Type of open surgery	1.347	0.660	3.846 (1.055–14.018)	**0.041**	R^2=0.125
Constant	–3.258	0.854	0.038	<0.001	
Age	0.082	0.034	1.085 (1.016–1.159)	**0.015**	χ^2=36.964
Lactate	0.382	0.203	1.466 (0.985–2.181)	0.059	R^2=0.652
Albumin	–0.217	0.102	0.805 (0.660–0.983)	**0.033**	
Constant	–0.608	4.115	0.545	0.883	

B: Unstandardized coefficient; SE: Standard error; OR: Odds ratio; CI: Confidence interval; p: Probability value; Method: Forward stepwise (Likelihood Ratio); Excluded variables in the model predicting mortality: Symptom duration (p=0.071), ASA score (p=0.225), Sex (0.272), and CRP (p=0.208), Excluded variables in the model predicting complications: Age (p=0.876) and Sex (p=0.308); χ^2: Chi-square, R^2: R-squared

(AUC: 0.808; 95% CI: 0.627–0.989) in predicting mortality. An age cutoff of ≥53 years yielded 100% sensitivity and 74.3% specificity, while an albumin cutoff of <31 g/L provided 66.7% sensitivity and 96.2% specificity (Table 6). The ROC curves for mortality prediction are shown in Figure 2.

Table 5. Comparison of complication and mortality rates according to patient characteristics

Variables	***n***	**Complication**		**p**	**Mortality**		**p**
		No (*n*=82)	**Yes (*n*=32)**		**No (*n*=105)**	**Yes (*n*=9)**	
		***n* (%)/M (IQR)**	***n* (%)/M (IQR)**		***n* (%)/M (IQR)**	***n* (%)/M (IQR)**	
Age (year)	114	43 (29–51)	52 (34–62)	0.066[c]	43 (29–53)	61 (57–75)	**<0.001**[c]
Sex				0.095[a]			0.091[b]
Male	90	68 (75.6)	22 (24.4)		85 (94.4)	5 (5.6)	
Female	24	14 (58.3)	10 (41.7)		20 (83.3)	4 (16.7)	
Duration of symptoms (day)	114	1 (1–2)	2 (1-3)	0.185	1 (1–2)	3 (2–5)	**0.017**[c]
ASA score				**0.020**[a]			**<0.001**[b]
I-II	82	64 (78)	18 (22)		81 (98.8)	1 (1.2)	
III-IV	32	18 (56.3)	14 (43.8)		24 (75)	8 (25)	
Surgical method				**0.019**[a]			0.110[b]
Laparoscopic	28	25 (89.3)	3 (10.7)		28 (100)	0(0)	
Open surgery	86	57 (66.3)	29 (33.7)		77 (89.5)	9 (10.5)	
Surgery duration (minutes)	114	90 (70–100)	95 (71–126)	0.153[c]	90 (70–105)	105 (55–145)	0.412[c]
Lactate	114	2.2 (1.5–2.8)	2.4 (1.5–3.8)	0.391[c]	2.2 (1.5–2.8)	7.8 (2.3–9.7)	**0.002**[c]
Albumin	114	41 (36–45)	40 (33–45)	0.477[c]	41 (36–45)	29 (25–36)	**<0.001**[c]
CRP	114	22.9 (3.4–73.7)	55.6 (7.6–146.8)	0.064[c]	25 (3.5–90)	147.7 (43.7–409.8)	**0.004**[c]
NLR	114	9 (5.6–19.5)	10 (7.3–15.2)	0.975[c]	10.2 (5.8–17.9)	6.7 (5.2–9.1)	0.148[c]
PLR	114	267.5 (169.7–430.8)	344.5 (207.8–543.6)	0.081[c]	305.3 (170.8–449)	325.7 (223.3–1099.1)	0.209[c]
Complication							0.709[b]
No	82				76 (92.7)	6 (7.3)	
Yes	32				29 (90.6)	3 (9.4)	

[a]Pearson Chi-Square, [b]Fisher's exact test; [c]Mann–Whitney U test, M: Median, IQR: Interquartile range, p: Probability value; ASA: American Society of Anesthesiologists; CRP: C-reactive protein; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio.

Table 6. Receiver operating characteristic analysis of age and albumin for evaluating diagnostic performance in patients with mortality

Variables	AUC (95% CI)	Cut-off	Sensitivity (%)	Specificity (%)	Accuracy (%)
Age	0.883 (0.813–0.952)	≥53	100	74.3	77.2
Albumin	0.895 (0.796–0.993)	<31	66.7	96.2	93.9
Lactate	0.808 (0.627–0.989)	≥4.4	66.7	91.4	89.5

AUC: Area under the curve; CI: Confidence interval.

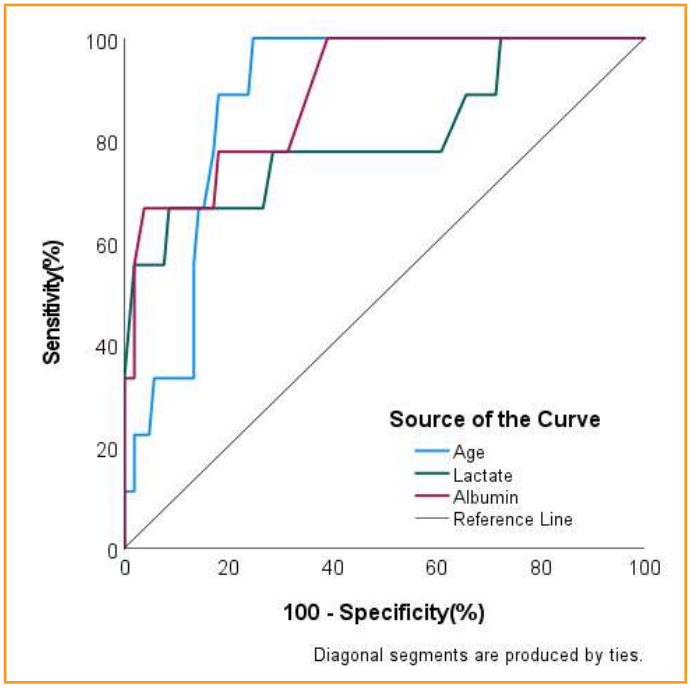

Figure 2. Receiver operating characteristic curves evaluating the diagnostic performance of age, lactate, and albumin for mortality.

Discussion

In this study, we examined surgical outcomes and identified pre-operative factors associated with morbidity and mortality in patients undergoing emergency repair for acute PPU. Although laparoscopic repair was associated with lower rates of post-operative complications, this finding should be interpreted with caution. Patients selected for laparoscopy had significantly lower lactate and CRP levels, suggesting less severe systemic inflammation and better physiological status at presentation. Therefore, the apparent advantage of laparoscopy likely reflects appropriate patient selection rather than a clear superiority of the technique itself. While laparoscopic repair is safe and feasible in hemodynamically stable patients, open surgery remains indispensable in those with advanced sepsis or physiological instability [2,7,13].

The fundamental principles of surgical management for PPU have remained consistent over time, with primary closure reinforced by an omental patch continuing to be the standard approach in both open and laparoscopic procedures [3,14,15]. As a result, improvements in patient outcomes depend less on technical innovation and more on early recognition of physiological deterioration. In this context, reliable risk assessment is essential for guiding resuscitation strategies, surgical timing, and post-operative care [16].

Various clinical scoring systems have been developed to support prognostic assessment in PPU. The Boey score, although simple and widely used, has shown limited predictive accuracy in more recent patient populations [17]. The PULP score incorporates a broader range of clinical and biochemical variables and offers improved mortality prediction; however, both systems fall short in capturing the dynamic physiological changes that often characterize emergency presentations [18,19].

In our cohort, advanced age, higher ASA class, elevated pre-operative lactate, and low serum albumin levels emerged as independent predictors of poor outcomes. These routinely available parameters provide a practical way to assess systemic inflammation, tissue hypoperfusion, and overall physiological reserve. A lactate level ≥2.5 mmol/L was strongly associated with both post-operative complications and the need for open surgery, highlighting its value as an early marker of sepsis and impaired perfusion [20,21]. Similarly, hypoalbuminemia (<31 g/L) was closely linked to mortality, reflecting its role as an indicator of both nutritional status and systemic inflammatory burden [22]. The contributions of age and ASA class further emphasize the increased vulnerability of elderly and comorbid patients to septic stress.

Biochemical markers also influenced the choice of surgical approach. Patients selected for laparoscopic repair generally had lower lactate, CRP, and PLR values, whereas those with more pronounced inflammatory responses were more likely to undergo open surgery. Multivariable analysis confirmed that lactate ≥2.5 mmol/L and CRP ≥11.4 mg/L independently predicted the need for open surgery or conversion. These findings likely reflect the severity of peritonitis and tissue edema, which can limit the safety and feasibility of minimally invasive techniques. Incorporating these thresholds into pre-operative assessment may sup-

port more informed surgical decision-making and improve perioperative safety.

Our results are consistent with previous studies highlighting the prognostic value of biochemical markers in gastrointestinal emergencies [15]. In addition, inflammatory indices such as PLR and NLR may further refine risk stratification and contribute to more individualized and timely management strategies [23].

Limitation

This study has inherent limitations, including its retrospective, single-center design, which may introduce selection bias. In addition, the relatively small number of mortality events limits the statistical power of subgroup analyses. Moreover, factors such as surgeon experience and variations in post-operative management may have influenced the outcomes. Nonetheless, the use of a well-defined patient cohort, comprehensive biochemical assessment, and multivariable analysis enhances the reliability of our findings.

Despite these limitations, age, serum lactate, and serum albumin proved to be consistent and objective predictors of adverse outcomes. ROC analysis demonstrated strong discriminative ability for albumin and age in predicting mortality, supporting their potential use in simplified clinical decision-making tools. Future prospective, multicenter studies are needed to validate these findings and to explore their integration into standardized perioperative care pathways.

Conclusion

Pre-operative age, serum lactate, and albumin are strong, objective predictors of complications and 30-day mortality in PPU surgery. Laparoscopic repair is safe in hemodynamically stable patients, while open surgery remains necessary for those with severe physiological compromise. Incorporating these biomarkers into pre-operative assessment can optimize surgical decision-making and improve perioperative outcomes.

Disclosure

Ethics Committee Approval: This study was approved by the Başakşehir Çam and Sakura City Hospital Ethics Committee (Date: 21.07.2025, Decision no: E-96317027-514.10-282238896).

Informed Consent: Given the retrospective nature of the study, the requirement for informed consent was waived. All patient data were anonymized before analysis to ensure confidentiality.

Conflict of Interest: The authors declare that there is no conflict of interest.

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Authorship Contributions: Concept – A.B.; Design – A.B., M.Y.; Supervision – A.B., A.L.I.; Data collection and/or processing – M.Y.; Analysis and/or interpretation – A.B., A.L.I.; Literature review – A.B., M.Y., A.L.I.; Writing – A.B.; Critical review – A.L.I.

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References

1. Wadewitz E, Friedrichs J, Grilli M, Vey J, Zimmermann S, Kleeff J, et al. Approaches for the treatment of perforated peptic ulcers: a network meta-analysis of randomised controlled trials - study protocol. BMJ Open 2024;14:e082732. [CrossRef]
2. Gavriilidis P, Schena CA, Di Saverio S, Hromalik L Jr, Eryilmaz M, Catena F, et al. Alternative treatments to treat perforated peptic ulcer: a systematic review and network meta-analysis of randomized controlled trials. World J Emerg Surg 2025;20:31. [CrossRef]
3. Stettler GR, Follette CJ, Avery MD. Surgical management of perforated peptic ulcers. Curr Surg Rep 2025;13:9. [CrossRef]
4. Hoshi H, Endo A, Ito K, Akutsu T, Odera H, Shiraki H, et al. Analysis of the characteristics and management of perforated peptic ulcer from 2011 to 2022: A multicenter and retrospective descriptive study. Ann Gastroenterol Surg 2025;9:464–75. [CrossRef]
5. Chung KT, Shelat VG. Perforated peptic ulcer - an update. World J Gastrointest Surg 2017;9:1–12. [CrossRef]
6. Boyd-Carson H, Doleman B, Cromwell D, Lockwood S, Williams JP, Tierney GM, et al. Delay in source control in perforated peptic ulcer leads to 6% increased risk of death per hour: a nationwide cohort study. World J Surg 2020;44:869–75. [CrossRef]
7. Patel PY, Akhani MK, Rathod R, Baria B. Post-operative outcomes of laparoscopic versus open repair of perforated duodenal ulcer: a systematic review. Cureus 2025;17:e92021. [CrossRef]
8. Møller MH, Adamsen S, Thomsen RW, Møller AM. Preoperative prognostic factors for mortality in peptic ulcer perforation: a systematic review. Scand J Gastroenterol 2010;45:785–805. [CrossRef]
9. Basile-Filho A, Lago AF, Menegueti MG, Nicolini EA, Rodrigues LAB, Nunes RS, et al. The use of APACHE II, SOFA, SAPS 3, C-reactive protein/albumin ratio, and lactate to predict mortality of surgical critically ill patients: A retrospective cohort study. Medicine (Baltimore) 2019;98:e16204. Erratum in: Medicine (Baltimore) 2019;98:e16675. [CrossRef]

10. Møller MH, Shah K, Bendix J, Jensen AG, Zimmermann-Nielsen E, Adamsen S, et al. Risk factors in patients surgically treated for peptic ulcer perforation. Scand J Gastroenterol 2009;44:145–52. [CrossRef]
11. Mikkelsen ME, Miltiades AN, Gaieski DF, Goyal M, Fuchs BD, Shah CV, et al. Serum lactate is associated with mortality in severe sepsis independent of organ failure and shock. Crit Care Med 2009;37:1670–7. [CrossRef]
12. Mayank S, Ekka NM, Sinha DK, Kumar B, Kumar N, Ranjan A, et al. Predictive accuracy of c-reactive protein as an early indicator of leakage after anastomosis and primary repair in gastrointestinal surgery. Cureus 2024;16:e71107. [CrossRef]
13. Sokhal BS, Mohamedahmed A, Zaman S, Wuheb AA, Abdalla HE, Husain N, et al. Laparoscopic versus open repair for peptic ulcer perforation: a systematic review, meta-analysis and trial sequential analysis of randomised controlled trials. Time to conclude! Ann R Coll Surg Engl 2025;107:331–45. [CrossRef]
14. Chan KS, Ng STC, Tan CHB, Gerard G, Oo AM. A systematic review and meta-analysis comparing postoperative outcomes of laparoscopic versus open omental patch repair of perforated peptic ulcer. J Trauma Acute Care Surg 2023;94:e1–13. [CrossRef]
15. Sartelli M, Catena F, Abu-Zidan FM, Ansaloni L, Biffl WL, Boermeester MA, et al. Management of intra-abdominal infections: recommendations by the WSES 2016 consensus conference. World J Emerg Surg 2017;12:22. [CrossRef]
16. Desoky AA, Ayoub MT, Mostafa NM, Hashem EM, Mohammed MA. Short-term outcome following postoperative enhanced recovery implementation in patients with perforated peptic ulcer. Eur J Med Res 2025;30:234. [CrossRef]
17. Gulzar JS, Paruthy SB, Arya SV. Improving outcome in perforated peptic ulcer emergency surgery by Boey scoring. Int Surg J 2016;3:2120–8. [CrossRef]
18. Meidiansyah NS, Manawan EEU, Legiran. Comparison of the accuracy of boey score and PULP score in prediction of gastric perforation mortality at Dr. Mohammad Hoesin general hospital palembang. Sriwijaya J Surg 2022;5:448–96.
19. Patel S, Kalra D, Kacheriwala S, Shah M, Duttaroy D. Validation of prognostic scoring systems for predicting 30-day mortality in perforated peptic ulcer disease. Turk J Surg 2019;35:252–8. [CrossRef]
20. Toppo S, Gaurav K, Mondal R, Kumar K, Kumar K. Study on the preoperative and postoperative levels of serum lactate to estimate morbidity and mortality in cases of bowel perforation at a tertiary care hospital in Jharkhand, India. Cureus 2025;17:e84748. [CrossRef]
21. Falter F, Tisherman SA, Perrino AC, Kumar AB, Bush S, Nordström L, et al. Serial lactate in clinical medicine - a narrative review. J Intensive Care Med 2026;41:175–85. [CrossRef]
22. Thorsen K, Søreide JA, Søreide K. What is the best predictor of mortality in perforated peptic ulcer disease? A population-based, multivariable regression analysis including three clinical scoring systems. J Gastrointest Surg 2014;18:1261–8. [CrossRef]
23. Bezzerri V, Putignani L, Mantuano E, Polini A, Navarini L, Vomero M, et al. A focus on inflammatory and bacterial biomarkers in secondary peritonitis. Cells 2025;14:1653. [CrossRef]

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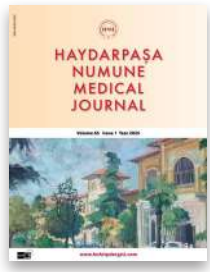

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ORIGINAL ARTICLE

The Relationship and Prognostic Significance of INR/Albumin Ratio with Radiological Data and AFP in Patients with Hepatocellular Carcinoma

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Abstract

Introduction: This study aimed to evaluate the relationship between the international normalized ratio-to-albumin ratio (IAR) and alpha-fetoprotein (AFP) levels, radiological findings, and prognosis in patients with hepatocellular carcinoma (HCC).

Methods: A retrospective analysis was conducted on 115 HCC patients admitted between 2010 and 2023. IAR was calculated as INR divided by albumin. Patients were categorized into high (≥0.288) and low (<0.288) IAR groups. Radiological findings, AFP levels, Child-Pugh (CP) classification, model for end-stage liver disease score, tumor characteristics, and survival were compared between groups. The optimal IAR cutoff for predicting mortality was determined using receiver operating characteristic curve analysis.

Results: A statistically significant positive correlation was observed between IAR and MELD score ($p<0.001$) and AFP levels ($p=0.021$). The optimal IAR cutoff was 0.267, with 80.2% sensitivity and 52.9% specificity. Patients with higher IAR had higher MELD scores, more tumors, worse CP classification, and higher mortality ($p<0.05$ for all).

Discussion and Conclusion: IAR is significantly associated with disease severity, tumor burden, and prognosis in HCC patients. It may serve as a simple, inexpensive, and non-invasive biomarker to guide early diagnosis, monitoring, and prognostic assessment in clinical practice.

Keywords: Albumin; hepatocellular carcinoma; international normalized ratio.

Hepatocellular carcinoma (HCC) is the most common primary malignant tumor of the liver, originating from hepatocytes. Worldwide, it ranks sixth in incidence among all cancers and third in mortality [1]. The main etiological factors include chronic infections with Hepatitis B and C viruses, long-term alcohol consumption, and non-alcoholic steatohepatitis. Cirrhosis develops in most cases and significantly contributes to the risk of HCC [2]. Screening is particularly cost-effective in high-risk populations, with ultrasonography (USG) recommended every 6–12 months [3]. Alpha-fetoprotein (AFP) is commonly used alongside USG for HCC surveillance, but AFP alone is insufficient for diagnosis due to the influence of other diseases and conditions [4]. Liver biopsy is definitive but often limited due to underlying cirrhosis. Therefore, diagnosis is frequently based on characteristic findings on dynamic contrast-enhanced computed tomography or magnetic resonance imaging [4]. Despite advances in imaging, surgery, and post-operative treatments, HCC remains highly lethal.

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Various tumor markers, including AFP, as well as molecular and genetic biomarkers, have been investigated for diagnosis and follow-up. AFP, widely used for over 60 years, has limitations such as normal levels in early HCC, prompting the search for more reliable markers. Among these, the international normalized ratio-to-albumin ratio (IAR), albumin/globulin ratio, C-reactive protein/albumin ratio, albumin/bilirubin ratio, des-gamma-carboxy prothrombin, AFP-L3, alpha-fucosidase, and glypican have shown potential.

Survival is closely related to tumor stage and liver disease severity. The Child-Pugh (CP) staging system and model for end-stage liver disease (MELD) score are commonly used to assess liver function and prognosis [5,6]. Recent research focuses on simple, inexpensive, non-invasive markers such as IAR. This study aimed to evaluate the relationship between IAR and radiological findings, AFP levels, and prognosis in HCC patients.

Materials and Methods

This study was designed as a retrospective study. Ethical approval was obtained from the Medical Specialization Education Board of the Haydarpaşa Numune Health Application and Research Center, University of Health Sciences (decision number E-62977267-771-223954832, dated September 7, 2023) and from the Haydarpaşa Numune Hospital Clinical Research Ethics Committee (decision number HNEAH-KAEK 2023/171/4296, dated September 25, 2023). All procedures were conducted in accordance with the ethical standards of the institutional research committee and the principles of the 1964 Declaration of Helsinki and its later amendments.

Patients diagnosed with HCC who were admitted to the hospital between January 1, 2010, and August 1, 2023, were retrospectively evaluated. A total of 115 patients who met the inclusion and exclusion criteria during the study period were included in the analysis. The inclusion criteria were defined as being 18 years of age or older and having a confirmed diagnosis of HCC. Patients with conditions that could lead to hypoalbuminemia were excluded from the study. These conditions included protein–energy malabsorption, inflammatory bowel disease, chronic enteritis, acute severe systemic inflammation, chronic inflammatory diseases, any additional malignant neoplasm, and patients receiving treatment for burns. In addition, patients with renal dysfunction defined as a glomerular filtration rate below 60 mL/min according to the MDRD calculation, patients diagnosed with nephrotic syndrome, those receiving diuretic therapy due to volume overload associated with heart failure, and patients with uncontrolled diabetes mellitus were excluded. Uncontrolled diabetes mellitus was defined as recurrent fasting hyperglycemia >300 mg/dL within 3 months before or after laboratory measurements or glycated hemoglobin levels >11%. Patients receiving anticoagulant therapy or those with known bleeding disorders were also excluded from the study.

The demographic characteristics of the included patients were recorded. Laboratory and clinical data obtained within 20 days before and after the first multiphase contrast-enhanced cross-sectional imaging that radiologically detected HCC were retrospectively evaluated. The collected data included age, sex, presence of cirrhosis, platelet count, international normalized ratio (INR), albumin, aspartate aminotransferase, alanine aminotransferase, creatinine, bilirubin, tumor size, number of tumors, AFP level, and mortality status.

The CP stage and MELD score were calculated for all patients. Based on the obtained data, the IAR was calculated, and patients were divided into two groups according to the IAR value: High IAR (≥0.288) and low IAR (<0.288). Radiological findings, AFP levels, and prognostic parameters were compared between these two groups.

Outcome Variables

The primary outcome variables were the comparison of radiological findings and AFP levels between patients with high IAR (≥0.288) and those with low IAR (<0.288). The secondary outcome variable was the evaluation of the relationship between IAR and the prognosis of patients with HCC developing on the basis of chronic liver disease.

Statistical Analysis

The sample size was calculated using G*Power version 3.1.9.4 software. Based on an effect size of 0.6, a confidence level of 95%, and a statistical power of 80%, it was determined that at least 45 patients should be included in each group.

Statistical analyses were performed using IBM Statistical Package for the Social Sciences Statistics software (IBM Corp., Armonk, NY, USA). Descriptive statistics were expressed as mean, standard deviation, median, frequency, percentage, minimum, and maximum values. Pearson correlation analysis was used to evaluate the relationships between quantitative variables with normal distribution, while Spearman correlation analysis was used for variables without normal distribution. The independent samples *t*-test was used to compare normally distributed quantitative variables between two groups, whereas the Mann–Whitney U test was applied for non-normally distributed variables. The Chi-square test was used for comparisons of categorical variables. A $p<0.05$ was considered statistically significant.

Table 1. ROC curve result for the INR/albumin ratio according to deaths in patients diagnosed with HCC

Area under the curve (95% confidence interval)	Standard error	Threshold value	Sensitivity (%)	Specificity (%)	p
0.683 (0.576–0.791)	0.055	0.267	80.2	52.9	**0.002***

*Statistically significant (p<0.05). ROC: Receiver operating characteristic; INR: International normalized ratio; HCC: Hepatocellular carcinoma.

Results

The study included 115 patients diagnosed with HCC. Their ages ranged from 39 to 82 years, with a mean age of 64.03±9.66 years. Of these, 26 patients (22.6%) were female and 89 (77.4%) were male. All patients (100%) had underlying liver cirrhosis. The etiology of cirrhosis was hepatitis B virus in 52 patients (45.2%), hepatitis C virus in 21 patients (18.3%), and other causes in 42 patients (36.5%).

According to CP classification, 71 patients (61.7%) were Child A, 38 patients (33.0%) were Child B, and 6 patients (5.2%) were Child C. The median MELD score was 8 (7–11). 70 patients (60.9%) had a single tumor, while 45 patients (39.1%) had two or more tumors. The median tumor size was 55 mm (26–93). Tumor size was <5 cm in 51 patients (44.3%) and 5 cm or greater in 64 patients (55.7%). The number of affected liver segments ranged from 1 to 4.

During the follow-up, 34 patients (29.6%) survived, while 81 patients (70.4%) died. AFP levels were significantly higher in male patients (151.47 µg/L [5.85–2252]) compared to female patients (9.80 µg/L [3.49–198.25], p=0.034).

The INR/albumin ratio (IAR) was below 0.288 in 49 patients (42.6%) and 0.288 or above in 66 patients (57.4%). Among patients with an IAR below 0.288, 35 patients (71.4%) were

Table 2. Comparison of age, gender, prognostic parameters, and radiological findings according to HCC patients' INR/Albumin ratio being below and above 0.267

	Those with an INR/albumin ratio of 0.267 or above (*n*=83, %72.2)	Those with an INR/albumin ratio below 0.267 (*n*=32, %27.8)	p
Age (year)	64.51±9.27[a]	62.78±10.65[a]	0.394[d]
Gender $_{n,\%}$			
Female	20 (24.1)	6 (18.8)	0.539[e]
Male	63 (75.9)	26 (81.3)	
Child-Turcotte-Pugh (CTP) classification			
Child A	40 (48.2)	31 (96.9)	**<0.001**[e,*]
Child B	37 (44.6)	1 (3.1)	
Child C	6 (7.2)	0 (0)	
MELD score	10 (8–12)[b]	7 (6.25–8)[b]	**<0.001**[e,*]
Tumor number			
1	45 (54.2)	25 (78.1)	**0.019**[e,*]
2 and above	38 (45.8)	7 (21.9)	
Tumor size (mm)	57 (28–92)[b]	47.50 (23.50–96.75)[b]	0.433[f]
Tumor size			
<5 cm	35 (42.2)	16 (50)	0.449[e]
5 cm and above	48 (57.8)	16 (50)	
Number of segments affected by the tumor	1 (1–4)[c]	1 (1–4)[c]	0.884[f]
State of survival			
Alive	17 (20.5)	17 (46.9)	**0.001**[e,*]
Death	66 (79.5)	17 (53.1)	

[a]Mean±standard deviation, [b]Median (interquartile range), [c]Median (minimum-maximum), [d]Independent *t*-test, [e]Chi-square test, [f]Mann–Whitney U test. *Statistically significant (p<0.05). INR: International normalized ratio; HCC: Hepatocellular carcinoma.

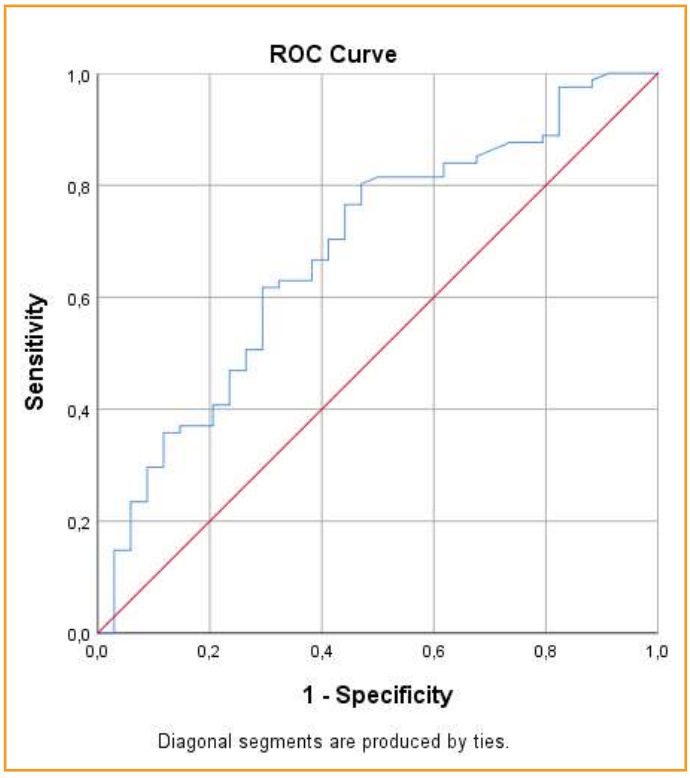

Figure 1. Receiver operating characteristic curve graph for the international normalized ratio/Albumin ratio according to deaths in patients diagnosed with hepatocellular carcinoma.

Child A, compared to 36 patients (54.5%) in the high IAR group; the difference was statistically significant (p<0.001). Furthermore, 37 patients (75.5%) in the low IAR group had a single tumor, whereas 29 patients (43.9%) in the high IAR group had a single tumor (p=0.017).

Patients with an IAR ≥0.288 had a higher median MELD score of 10 (8–13) compared to 7 (6–9) in the low IAR group (p<0.001). Mortality was also significantly higher in the high IAR group: 53 patients (80.3%) died in the high IAR group versus 28 patients (57.1%) in the low IAR group (p=0.007) (Fig. 1).

Receiver operating characteristic (ROC) curve analysis for predicting mortality showed an area under the curve of 0.683 (standard error=0.055, p=0.002). The optimal IAR cutoff was determined to be 0.267, with a sensitivity of 80.2% and specificity of 52.9% (Table 1).

Using the IAR cutoff of 0.267, 40 patients (74.1%) in the low IAR group were Child A, compared to 32 patients (48.5%) in the high IAR group (p<0.001). Tumor count also differed significantly, with 42 patients (77.8%) in the low IAR group having a single tumor versus 28 patients (42.4%) in the high IAR group (p=0.019). The median MELD score in patients with IAR ≥0.267 was significantly higher at 10 (8–13), compared to 7 (6–9) in those with IAR <0.267 (p<0.001) (Table 2).

Discussion

This study examined the relationship between the IAR measured in 115 HCC patients and AFP levels, prognostic scores, and radiological data. We found a statistically significant positive correlation between the IAR and AFP values, as well as the MELD score. Significant differences were also observed between the IAR and the CP classification, mortality status, and tumor count. Although no statistically significant relationship was found between the IAR and tumor size or the number of affected segments, a significant association was observed with tumor count.

Hepatitis B and hepatitis C infections remain the primary etiological factors of HCC, while alcohol consumption, non-alcoholic fatty liver disease, autoimmune hepatitis, and hemochromatosis also contribute. In our study, hepatitis B was responsible for 45.2% of cases and hepatitis C for 18.3%, consistent with regional epidemiological patterns and similar to the findings of Ülger et al [7].

AFP, although widely used for diagnosis and follow-up of HCC, can be elevated in various other conditions, including other cancers (for example, teratoblastoma, embryonal carcinoma, hepatoblastoma) and chronic liver diseases, and is influenced by factors such as gender, race, and geography [8,9]. In our study, AFP levels were higher in male patients than in females, consistent with previous reports.

The use of IAR in HCC is relatively novel, with limited studies available. Haruki et al [10]. first evaluated IAR in HCC patients in 2018 and found it could predict short- and long-term outcomes following hepatic resection, using a cutoff of 0.288. Our study determined a slightly lower cutoff of 0.267. Using both thresholds, we observed significant correlations between IAR, AFP levels, and CP class, supporting previous findings. Similar correlations between IAR and MELD score have been reported in patients with hepatitis B-related decompensated cirrhosis [11,12]. Our findings reinforce that IAR is associated with both liver function and tumor burden in HCC.

Radiological assessment remains central to HCC diagnosis, particularly in patients with underlying cirrhosis where biopsy may be limited [13]. In our study, the IAR correlated significantly with tumor count but not with tumor size or the number of affected segments, a finding that differs from some previous reports [9,10,14]. This discrepancy may be due to the smaller sample size in our study compared to multicenter investigations. When examining tumor counts, our study included 70 patients (60.9%) with a single tumor and 45 patients (39.1%) with multiple tumors, whereas other studies reported higher proportions of patients with a single tumor. This difference in tumor distribution may have influenced the variations in results.

Limitation

The main limitations of our study include its retrospective and single-center design, relatively small patient cohort, and inability to calculate precise mortality rates due to the lack of exact death dates. Despite these limitations, the study provides valuable insights into the prognostic potential of IAR in HCC.

Conclusion

Our findings indicate that the IAR is significantly associated with AFP levels, CP classification, MELD score, tumor count, and patient survival status in HCC. Given these results, IAR may serve as a useful, inexpensive, and non-invasive prognostic marker in clinical practice. The observed relationship between IAR and tumor count differs from previous studies, highlighting the need for further research. Multicenter prospective studies with larger patient populations are warranted to validate the diagnostic, follow-up, and prognostic utility of the IAR in HCC patients.

Disclosure

Ethics Committee Approval: This study was approved by the Haydarpaşa Numune Training and Research Hospital Ethics Committee (Date: 25.09.2023, Decision no: HNEAH-KAEK 2023/171/4296).

Informed Consent: The study was conducted retrospectively using patient medical records and does not require voluntary consent.

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References

1. 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. [CrossRef]
2. Global Burden of Disease Liver Cancer Collaboration; Akinyemiju T, Abera S, Ahmed M, Alam N, Alemayohu MA, Allen C, et al. The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level: results from the global burden of disease study 2015. JAMA Oncol 2017;3:1683–91.
3. Heimbach JK, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, Roberts LR, et al. AASLD guidelines for the treatment of hepatocellular carcinoma. Hepatology 2018;67:358–80. [CrossRef]
4. Forner A, Reig M, Bruix J. Hepatocellular carcinoma. Lancet 2018;391:1301–14. [CrossRef]
5. Child CG III, Turcotte JG. Surgery and portal hypertension. In: Child CG III, ed. The liver and portal hypertension. Philadelphia: WB Saunders; 1964. p.50–64.
6. Freeman RB Jr, Wiesner RH, Harper A, McDiarmid SV, Lake J, Edwards E, et al. The new liver allocation system: moving toward evidence-based transplantation policy. Liver Transpl 2002;8:851–8. [CrossRef]
7. Ülger Y, Delik A. Prognostic value of international normalized ratio-to-albumin ratio and ferritin level in chronic liver patients with hepatocellular carcinoma. J Gastrointest Cancer 2022;53:1028–33. [CrossRef]
8. Lau HL, Linkins SE. Alpha-fetoprotein. Am J Obstet Gynecol 1976;124:533–54. [CrossRef]
9. Baig JA, Alam JM, Mahmood SR, Baig M, Shaheen R, Sultana I, et al. Hepatocellular carcinoma (HCC) and diagnostic significance of A-fetoprotein (AFP). J Ayub Med Coll Abbottabad 2009;21:72–5.
10. Haruki K, Shiba H, Saito N, Horiuchi T, Shirai Y, Fujiwara Y, et al. Risk stratification using a novel liver functional reserve score of combination prothrombin time-international normalized ratio to albumin ratio and albumin in patients with hepatocellular carcinoma. Surgery 2018;164:404–10. [CrossRef]
11. Zhang T, Ye B, Shen J. Prognostic value of albumin-related ratios in HBV-associated decompensated cirrhosis. J Clin Lab Anal 2022;36:e24338. [CrossRef]
12. Cai M, Han Z, He X, Zhang J. Usefulness of international normalized ratio to albumin ratio for evaluation of mortality in hepatitis b virus-associated decompensated cirrhosis. Biomed Res Int 2021;2021:6664574. [CrossRef]
13. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. J Hepatol 2018;69:182–236. Erratum in: J Hepatol 2019;70:817. [CrossRef]
14. Deng Y, Pang Q, Miao RC, Chen W, Zhou YY, Bi JB, et al. Prognostic significance of pretreatment albumin/globulin ratio in patients with hepatocellular carcinoma. Onco Targets Ther 2016;9:5317–28. [CrossRef]

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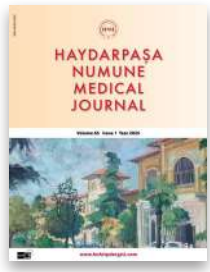

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ORIGINAL ARTICLE

The Effect of Omalizumab Therapy on Systemic Inflammatory Parameters in Chronic Spontaneous Urticaria

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Abstract

Introduction: Chronic spontaneous urticaria (CSU) is a disorder characterized by the triggering of mast cells and basophils and is thought to involve systemic inflammatory responses. Hematological parameters obtained from the complete blood count, particularly the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), have been considered potential biomarkers associated with systemic inflammatory processes. The purpose of this study was to examine the impact of omalizumab therapy on inflammatory parameters, including C-reactive protein (CRP), NLR, and PLR in patients diagnosed with CSU.

Methods: Data from 44 individuals with CSU who were followed at the Dermatology Clinic of Haydarpaşa Numune Training and Research Hospital between June 2022 and June 2024 and received omalizumab therapy for at least 1 year were retrospectively analyzed in this observational study. CRP, NLR, and PLR levels were compared before treatment, at the 6th month of therapy, and at the end of the 1st year. In addition, the relationship between baseline urticaria activity score (UAS7) and these inflammatory parameters was assessed.

Results: The study population had an average age of 48.57±13.43 years. Among the participants, 65.9% were women and 34.1% were men. Throughout the omalizumab treatment period, CRP and NLR levels demonstrated a statistically significant decrease ($p<0.001$). In addition, PLR values also decreased, with a notable reduction observed at the 6th month of therapy ($p=0.029$). Analysis of relationships between variables revealed a moderate positive association between baseline UAS7 values and both CRP and PLR levels, while a robust positive association was demonstrated between baseline UAS7 and NLR ($r=0.864$; $p<0.001$).

Discussion and Conclusion: These findings may be associated not only with clinical improvement but also with reductions in certain systemic inflammatory markers. The strong association between baseline NLR and disease activity indicates that NLR may serve as a practical, accessible biomarker in CSU.

Keywords: Chronic spontaneous urticaria; C-reactive protein; lymphocytes; neutrophils; omalizumab.

Urticaria is a condition characterized by pruritic, erythematous, transient wheals (urtica) lasting <24 h and/or angioedema involving the skin. Depending on the disease course, urticaria can be classified as acute or chronic, and according to triggering factors as spontaneous or inducible. Acute urticaria is typically characterized by the presence of urticarial plaques and/or angioedema for <6 weeks, whereas in chronic urticaria these symptoms persist for longer than 6 weeks [1]. The exact pathogenesis of the disease has yet to be fully clarified; however, pathophysiological processes such as autoimmunity, autoallergy, inflammation, and coagulation are thought to play important roles. According to the current EAACI/GA^{2}LEN/EuroGuiDerm/APAAACI guideline, non-sedating H1 antihistamines are recommended as initial therapy for chronic spontaneous urticaria (CSU), and the dose may be increased up to

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fourfold if adequate disease control cannot be achieved [1].

Omalizumab, a monoclonal antibody targeting immunoglobulin E (IgE), is licensed for the treatment of individuals with urticaria who do not achieve adequate benefit from second-generation H1 antihistamine therapy. It has been demonstrated to be both effective and safe in the management of CSU. The recommended initial dose of omalizumab is 300 mg administered every 4 weeks [2,3].

Over the past few years, various inflammatory markers determined from complete blood count parameters have been increasingly used in clinical studies to evaluate systemic inflammation. Higher C-reactive protein (CRP) concentrations and greater neutrophil-to-lymphocyte ratios (NLR) have been linked to several inflammatory conditions [4,5].

This study aimed to evaluate CRP, NLR, and platelet-to-lymphocyte ratio (PLR) levels before treatment, at 6 months, and at 1 year in patients with CSU treated with omalizumab at our institution, and to investigate the effect of omalizumab on systemic inflammatory parameters.

Materials and Methods

This retrospective, observational study aimed to analyze the data of patients who were diagnosed with CSU in the Dermatology Clinic of Haydarpaşa Numune Training and Research Hospital between June 2022 and June 2024 and who received omalizumab therapy due to inadequate response to maximum-dose oral antihistamine treatment.

The study was approved by the Ethics Committee of Haydarpaşa Numune Training and Research Hospital (Approval No: HNEAH-GAEK 2024/142, HNEAH-GAEK/KK/2024/142) and was conducted in accordance with the principles of the Declaration of Helsinki.

The study population included a total of 44 individuals aged 18–80 years with CSU, who received omalizumab treatment for at least 1 year. Patients with comorbid conditions that could affect blood cell counts, such as infection, malignancy, hematological disorders, or recent surgery, as well as those who used systemic corticosteroids or immunosuppressive therapy during the study period, were excluded. NLR and PLR were derived from the ratios of neutrophils and platelets to lymphocytes, respectively. CRP, NLR, and PLR values were obtained from laboratory results recorded before the initiation of omalizumab therapy. Omalizumab was administered at a dose of 300 mg every 4 weeks, and the same parameters were reassessed at the 6th month and at the end of the 1st year of treatment. Thus, pre-treatment, 6-month, and 1-year values were compared to evaluate the effect of omalizumab therapy on systemic inflammatory parameters.

Concomitant antihistamine use during omalizumab treatment was recorded for all patients.

Statistical Analysis

Statistical analyses were performed using IBM Statistical Package for the Social Sciences Statistics version 26.0 (IBM Corp., Armonk, NY, USA) and Microsoft Excel 2024 (Microsoft Corporation, Redmond, WA, USA).

Categorical variables were presented as frequencies (*n*) and corresponding percentages (%). The distribution of continuous variables was visually explored through histogram and Q–Q plot inspections, and the assumption of normality was additionally investigated using the Shapiro–Wilk test. Continuous data demonstrating a normal distribution were reported as mean±standard deviation, whereas variables that did not conform to normality were summarized as median with the observed minimum and maximum values.

Sex-based differences in CRP, NLR, and PLR were analyzed with distribution-appropriate statistical tests, namely the independent samples t-test for data that met the assumption of normality and the Mann–Whitney U-test for data that did not meet the assumption of normality. Temporal changes in CRP, NLR, and PLR (baseline, 6 months, and 1 year) were evaluated using repeated-measures analysis of variance for normally distributed data, whereas the Friedman test was applied to variables that did not meet the normality assumption. When a significant time effect was detected, two-by-two comparisons were performed with Bonferroni-adjusted *post hoc* tests.

Relationships between data were examined using parametric or non-parametric correlation methods depending on data distribution. Pearson correlation analysis was applied to normally distributed variables, whereas Spearman rank correlation analysis was preferred for variables that did not meet the assumption of normality.

A probability value below 0.05 was accepted as evidence of statistical significance.

Results

A cohort of 44 patients confirmed to have CSU and followed at our institution between June 2022 and June 2024 who received omalizumab treatment for a minimum duration of 1 year were recruited for the study. The study included participants with a mean age of 48.57±13.43 years, of whom 29 (65.9%) were female, and 15 (34.1%) were male.

Antihistamine use during omalizumab treatment was absent in 56.8% of patients (*n*=25), whereas 43.2% (*n*=19) continued antihistamine therapy concurrently.

Statistically significant differences were observed in CRP values measured before treatment, at the 6-month and 12-month follow-ups (p<0.001). Bonferroni-adjusted pairwise comparisons showed significant reductions both at the 6-month and 12-month follow-ups compared with pre-treatment values. The present findings indicate that omalizumab therapy may be correlated with a decrease in CRP levels, a marker of systemic inflammation (Table 1).

When NLR values were evaluated, the mean value decreased from 2.92±1.50 before treatment to 2.07±0.62 at the 6th month and 2.08±1.37 at the 1st year. The change over time was observed to be statistically significant (p<0.001), and pairwise comparisons demonstrated significant reductions both at the 6th month and at the 1st year compared with pre-treatment values (Table 1).

PLR values were 133.56±43.87 before treatment, 112.98±31.66 at the 6th month, and 120.45±41.45 at the 1st year. A significant time effect was observed (p=0.029), and *post hoc* analysis indicated that this significance was primarily driven by the comparison between pre-treatment and the 6th month (*P*=0.005). No meaningful differences were detected between pre-treatment and the 1st year or between the 6th month and the 1st year (Table 1). These findings suggest that the decrease in PLR may be more pronounced during the early phase of treatment.

A moderately strong positive association that reached statistical significance was observed between the urticaria activity score (UAS7) score assessed at treatment initiation and both CRP and PLR levels (r=0.507; p<0.001 and r=0.473; p<0.001, respectively; [Table 2]). In contrast, a robust positive association was identified between baseline UAS7 and NLR values (r=0.864; p<0.001; [Table 2]). These findings suggest that NLR may have a greater potential to reflect clinical disease activity compared with CRP and PLR.

No sex-related differences were detected in CRP, NLR, or PLR levels at baseline, the 6-month evaluation, or the 1-year follow-up (all p>0.05).

Table 1. Pairwise comparison results of the time effect for CRP, NLR, and PLR values

CRP	p*
Pre-treatment – 6th month	**<0.001**
Pre-treatment – 1st year	**<0.001**
6th month – 1st year	0.099
NLR	**p***
Pre-treatment – 6th month	**<0.001**
Pre-treatment – 1st year	**<0.001**
6th month – 1st year	1.000
PLR	**p***
Pre-treatment – 6th month	**0.005**
Pre-treatment – 1st year	0.454
6th month – 1st year	0.750

*Bonferroni-adjusted *p*-values, CRP: C-reactive protein; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio.

Table 2. Relationship between UAS7 score and CRP, NLR, and PLR

	UAS7	
	r	p
CRP	0.507	<0.001
NLR	0.864	<0.001
PLR	0.473	0.001

r: Spearman correlation coefficient, UAS7 score: Urticaria activity score, CRP: C-reactive protein; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio.

Discussion

CSU is a complex inflammatory disease in which histamine and various proinflammatory mediators play a central role. [1] Currently, CSU is considered not to be limited solely to local mast cell activation; systemic inflammatory responses are also believed to contribute to the pathogenesis of the disease.[1] Omalizumab neutralizes free IgE and downregulates FcεRI receptor expression on mast cells and basophils, thereby suppressing inflammatory activation. The clinical efficacy of omalizumab in individuals with chronic urticaria who are resistant to antihistamine therapy has been demonstrated in randomized controlled trials, which have reported significant reductions in symptoms [2,3].

Several studies have supported the presence of systemic inflammation in patients with chronic urticaria. In particular, acute phase reactants, including CRP and certain cytokines, have been shown to be elevated in individuals with chronic urticaria and may be associated with disease activity. In the study by Ucmak et al.,[4] CRP and interleukin-6 levels were observed to be significantly higher in cases with chronic urticaria in comparison with healthy individuals, and these parameters were reported to correlate with the UAS7. Similarly, in our study, the moderate positive correlation observed between baseline CRP levels and the UAS7 score supports these findings.

More recently, inflammatory markers obtained from complete blood count parameters have also been investigated for the evaluation of systemic inflammatory response. According to a study by Ozer et al., [5] in individuals with bullous pemphigoid, the NLR was observed to be significantly higher compared with healthy individuals, and this param-

eter was suggested to be a potential indicator of systemic inflammatory activity in inflammatory skin diseases. In the study by Weissmann et al., [6] NLR and PLR values were reported to be correlated with the disease severity in patients with chronic urticaria, suggesting that these parameters may be useful in evaluating disease activity. Similarly, in our study, the correlation observed between baseline NLR and PLR values and baseline UAS7 scores is consistent with these findings and suggests that these parameters may reflect disease activity and systemic inflammation in chronic urticaria.

Studies investigating biomarkers that may predict treatment response in CSU have also increased in recent years. Calzari et al. [7] emphasized the importance of exploring clinical and laboratory markers that could predict early response to antihistamine and omalizumab therapy among individuals with CSU. In the study by Tarkowski et al., [8] hematological markers such as NLR, PLR, and the systemic immune-inflammation index were evaluated, and although these parameters could not be considered definitive biomarkers for predicting treatment response in chronic urticaria, they were suggested to be useful in assessing their relationship with disease activity. Similarly, in the study conducted by Aktaş et al., several biomarkers that might predict the response of patients with CSU to omalizumab treatment were investigated; however, no definitive conclusions regarding their clinical applicability were reached.

Ertaş et al. demonstrated that certain hematological parameters and inflammatory indices changed during the course of treatment in individuals with CSU receiving omalizumab.

Hematological inflammatory indices have been shown to reflect systemic inflammation not only in chronic urticaria but also in other dermatological inflammatory diseases. In a systematic review and meta-analysis including individuals with psoriasis, Ye et al. reported that NLR and PLR were significantly greater in cases compared with healthy individuals, suggesting that these parameters may serve as low-cost biomarkers reflecting the presence of disease.

Similarly, according to a study by Kearney et al., [12] in patients with hidradenitis suppurativa, NLR and PLR values were observed to be lower in patients receiving biologic therapy compared with those receiving other treatments, and a significant reduction in inflammatory indices was observed with adalimumab treatment. These findings suggest that hematological inflammatory indices may reflect treatment-related changes in systemic inflammation.

Similar findings have also been reported in other chronic inflammatory dermatological diseases, such as atopic dermatitis. In a study by Jiang et al., NLR and PLR levels were found to be significantly greater in individuals with atopic dermatitis compared with healthy individuals, and these parameters were reported to show a positive correlation with the SCORing Atopic Dermatitis index, which reflects disease severity.

In our study, the changes observed in NLR and PLR values following omalizumab therapy suggest that these parameters may reflect treatment-related inflammatory changes in patients with CSU.

Limitation

Some limitations of this study should be acknowledged. First, the retrospective nature of the study and its single-center setting may restrict the generalizability of the results. Furthermore, a limited number of participants and the lack of a control group represent additional important limitations of this study. Nevertheless, the evaluation of inflammatory parameters at pre-treatment, the 6th month, and the 1st year represents one of the strengths of the study, as it allows the assessment of changes in these parameters over time.

Conclusion

This study demonstrated that omalizumab therapy in individuals with CSU was associated with a significant reduction in CRP and NLR levels. Furthermore, the strong correlation observed between baseline NLR values and baseline UAS7 scores suggests that NLR may serve as a practical biomarker reflecting disease activity. These findings support the notion that omalizumab may not only reduce clinical symptoms but also exert regulatory effects on systemic inflammatory processes. Nevertheless, large-scale prospective multicenter studies are required to validate the clinical usefulness of inflammatory indices as reliable biomarkers in routine clinical practice.

Disclosure

Ethics Committee Approval: This study was approved by the Haydarpaşa Numune Training and Research Hospital Ethics Committee (Date: 18.11.2024, Decision no: HNEAH-GAEK 2024/142, HNEAH-GAEK/KK/2024/142).

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References

1. Zuberbier T, Abdul Latiff AH, Abuzakouk M, Aquilina S, Asero R, Baker D, et al. The international EAACI/GA²LEN/EuroGuiDerm/APAAACI guideline for the definition, classification, diagnosis, and management of urticaria. Allergy 2022;77:734–66. [CrossRef]
2. Maurer M, Rosén K, Hsieh HJ, Saini S, Grattan C, Gimenéz-Arnau A, et al. Omalizumab for the treatment of chronic idiopathic or spontaneous urticaria. N Engl J Med 2013;368:924–35. [CrossRef]
3. Kaplan A, Ledford D, Ashby M, Canvin J, Zazzali JL, Conner E, et al. Omalizumab in patients with symptomatic chronic idiopathic/spontaneous urticaria despite standard combination therapy. J Allergy Clin Immunol 2013;132:101–9. [CrossRef]
4. Ucmak D, Akkurt M, Toprak G, Yesilova Y, Turan E, Yıldız I. Determination of dermatology life quality index, and serum C-reactive protein and plasma interleukin-6 levels in patients with chronic urticaria. Postepy Dermatol Alergol 2013;30:146–51. [CrossRef]
5. Ozer I, Balevi S, Ataseven A. Can neutrophil/lymphocyte ratio be used as a marker in the diagnosis of bullous pemphigoid? Selcuk Med J 2018;34:65–9. [CrossRef]
6. Weissmann S, Burrack N, Golan-Tripto I, Horev A. Increased neutrophil-lymphocyte ratio and platelet-lymphocyte ratio in chronic and severe urticaria. Acta Derm Venereol 2024;104:adv23932. [CrossRef]
7. Calzari P, Favale EM, Cugno M, Asero R, Marzano AV, Ferrucci SM. Predictors of early treatment response to antihistamines and omalizumab in chronic spontaneous urticaria. Front Allergy 2026;6:1728559. [CrossRef]
8. Tarkowski B, Ławniczak J, Tomaszewska K, Kurowski M, Zalewska-Janowska A. Chronic urticaria treatment with omalizumab-verification of NLR, PLR, SIRI and SII as biomarkers and predictors of treatment efficacy. J Clin Med 2023;12:2639. [CrossRef]
9. Aktaş ÖÖ, Çimşir DÖ, Bülbül EN, Şerbetçi N, Fendoğlu TZ, İnan E, et al. Biomarkers of response to omalizumab in patients with chronic spontaneous urticaria. Allergol Immunopathol (Madr) 2025;53:7–13. [CrossRef]
10. Ertaş R, Özyurt K, Karakükçü Ç, Akkuş MR, Özlü E, Avcı A, et al. Evaluation of platelet parameters and neutrophil/lymphocyte ratio during omalizumab treatment in patients with severe chronic spontaneous urticaria. Turk J Med Sci 2018;48:1255–62. [CrossRef]
11. Ye JH, Zhang Y, Naidoo K, Ye S. Neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in psoriasis: a systematic review and meta-analysis. Arch Dermatol Res 2024;316:85. [CrossRef]
12. Kearney N, McCourt C, Hambly R, Hughes R, O'Kane D, Kirby B. Association of biologic treatment in hidradenitis suppurativa with reduced neutrophil-lymphocyte ratio and platelet-lymphocyte ratio. JAMA Dermatol 2023;159:222–4. [CrossRef]
13. Jiang Y, Ma W. Assessment of neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio in atopic dermatitis patients. Med Sci Monit 2017;23:1340–6. [CrossRef]

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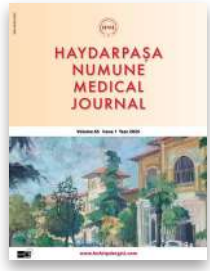

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ORIGINAL ARTICLE

The Effects of Citric Acid on Neural Tube Development in Chick Embryos

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Abstract

Introduction: Neural tube defects (NTDs) are common congenital malformations that cause lifelong disabilities, high medical costs, and significant mortality. This study aimed to evaluate the effects of citric acid, a widely used food additive, on neural tube development in chick embryos.

Methods: Fertilized, specific pathogen-free chicken eggs were randomly assigned to two groups (n=15 each). Group A (control) received no treatment, whereas Group B received 0.5 mM citric acid injected beneath the embryonic disc at Hamburger–Hamilton stage 9. After 72 h of incubation, embryo survival was recorded, and surviving embryos were examined macroscopically and histopathologically for NTDs. Statistical analysis was performed using Fisher's exact test.

Results: All embryos in the control group survived (15/15, 100%) and showed normal neural tube development. In the citric acid group, only 10/15 embryos survived (66.7%), and 9/10 (90%) of these exhibited NTDs, mainly cranial and caudal closure failures. Citric acid significantly reduced survival ($p<0.05$) and increased NTD incidence ($p<0.001$).

Discussion and Conclusion: Citric acid significantly reduced embryo survival and increased the incidence of NTDs in chick embryos. These findings suggest that citric acid may have embryotoxic and teratogenic effects, highlighting the need for further studies on the safety of widely used food additives during early pregnancy.

Keywords: Chick embryo, citric acid, food additives, neural tube defect, teratogenicity

Neural tube defects (NTDs) are among the most common congenital anomalies. They lead to lifelong disabilities and substantial medical care costs. Moreover, they represent a major public health problem that can cause perinatal and childhood mortality [1].

NTDs are clinical conditions that occur as a result of improper development of the neural tube. They rank second among all congenital anomalies in terms of frequency and may present with different clinical entities such as spina bifida and anencephaly. Anencephaly is characterized by the complete or partial absence of the cerebral hemispheres and cranium, whereas spina bifida results from the failure of the neural tube to close properly in the vertebral region [2,3].

Congenital anomalies, particularly during the 1st year of life, are among the leading causes of infant mortality.

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Among these anomalies, NTDs constitute the second most common group after congenital heart diseases [4].

A review of the literature reveals that the global incidence of NTDs is approximately 1–7/1000 live births, and they occur more frequently in females than in males. In Türkiye, the incidence of NTDs has been reported as 2.75/1000 live births [5].

The etiology of NTDs involves complex, multifactorial interactions between genetic and environmental factors. Despite their high incidence, the underlying causes of these defects are not fully understood [6,7].

Experimental studies have shown that exposure to alcohol, carbamazepine, salicylates, insulin, clomiphene, valproic acid, and chemotherapeutic agents may induce NTDs. Similarly, interaction with various teratogenic agents has also been found to increase the incidence of these disorders [8].

Citric acid ($C_6H_8O_7$) is a tricarboxylic acid found in the metabolism of aerobic organisms. It is widely used in many industrial fields, with approximately 70% of global production utilized in the food industry. Besides food manufacturing, citric acid is also used in the pharmaceutical, agricultural, cosmetic, chemical, and other industrial sectors. In the food industry, it primarily serves as a preservative, an acidity regulator, a flavor enhancer, and a synergist of antioxidant activity [9-11].

In this study, we aimed to evaluate the potential effects of citric acid on the development of NTDs in chicken embryos.

Materials and Methods

Study Design

This study was conducted in collaboration with the Department of Histology and Embryology, Hamidiye Faculty of Medicine, University of Health Sciences. In this experimental study, fertilized, pathogen-free chicken eggs were used for incubation and injection (Bornova Veterinary Control and Research Institute, İzmir, Türkiye). The eggs were randomly divided into two groups:

- Group A (Control): No treatment (n=15)
- Group B (Experimental): Injected with 0.5 mM (0.096 mg/L) citric acid (n=15).

Physiological saline was used as the vehicle for citric acid administration in the experimental group. The control group received no injection or treatment. Therefore, the study design did not include a sham-injected vehicle control group.

Ethical Approval

This study was conducted in accordance with the "Regulation of the Local Ethics Committee for Animal Experiments of the Health Sciences University of the Republic of Türkiye." According to Official Gazette No. 28914, dated February 15, 2014, chick-embryo experiments conducted before the last one-third of incubation are exempt from animal ethics approval.

Citric Acid Dose

The dose was determined based on the safe range (0.1–1.0 mM) reported in the literature for embryo culture. During the preliminary phase of the study, the drug was tested at low (0.1 mM), medium (0.5 mM), and high (1.0 mM) concentrations. The findings indicated that the minimally effective dose corresponded to the medium dose (0.5 mM); therefore, this dose was selected for use in the main experiment. Citric acid ($C_6H_8O_7 \cdot H_2O$, ZAG Kimya, CAS No: 5949-29-1, purity 99.5%, molecular weight 210.14 g/mol) was dissolved in sterile saline to prepare the desired concentration.

Incubation and Injection

The average egg weight was 65±2 g. Eggs were incubated for 24 h at a temperature of 37.2±0.1°C and 60–70% humidity. The study consisted of two groups, each containing 15 embryos. After 24 h of incubation, the embryos had reached stage 9 according to the Hamburger–Hamilton classification.[12] The eggshells were carefully opened under ×4 magnification, and the embryonic discs were identified. A volume of 0.1 mL of the prepared solution was injected beneath the embryonic disc using a 24-gauge syringe. After injection, the opened eggshells were sealed with sterile adhesive tape and incubated for an additional 72 h. At the end of this period, the eggs were reopened, and the embryos were carefully dissected from the extra-embryonic membranes. Embryo survival was recorded as intact vasculature and a visible heartbeat. In the control group, 15 embryos survived (100%), whereas in the citric acid group, 10 embryos survived (66.7%). All surviving embryos were examined macroscopically and histopathologically for neural tube development and closure.

Histopathological Evaluation

At the end of the incubation period, the eggs were opened, and the embryos were carefully transferred into 4% neutral buffered formalin for fixation. Following 48 h of fixation, the embryos were dehydrated through a graded ethanol series (70%, 96%, and 100%), each step lasting 15 min. The samples were then cleared in xylene and embedded in paraffin. From each paraffin block, serial sections of 5 µm thickness were obtained and mounted on glass slides. The sections were deparaffinized in xylene, rehydrated through a descending ethanol gradient, and subsequently stained

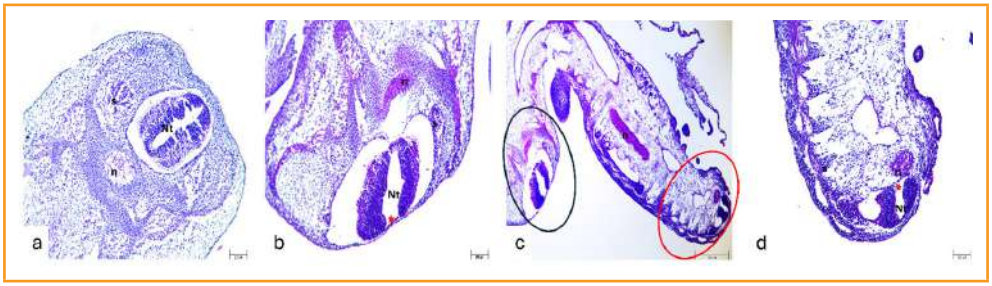

Figure 1. Macroscopic and histopathological evaluation of neural tube development in chick embryos. (a) Control embryo showing intact neural tube (Nt) with notochord (n) and somites (s). Scale bar=100 µm. (b) Cranial neural tube of a citric acid-treated embryo; the red star indicates closure failure. Scale bar=100 µm. (c) Citric acid group embryo; black circle corresponds to the magnified region in (b), red circle corresponds to the magnified region in (d). Scale bar=500 µm. (d) Caudal neural tube of a citric acid-treated embryo; the red star indicates closure failure. Scale bar=100 µm.

with hematoxylin and eosin. After staining, the slides were covered with coverslips using Entellan® (Merck) for permanent mounting. Microscopic evaluation was performed using a Zeiss Axiocam light microscope. Particular attention was given to the morphological organization of the neural tube and surrounding structures. Any disruption, discontinuity, or malformation observed in the neural tube organization was defined as an NTD.

Statistical Analysis

Analyses were performed using the Statistical Package for the Social Sciences v28.0 (IBM Corp., Armonk, NY). Fisher's exact test was applied. Survival rates and NTD incidence were analyzed independently. $P<0.05$ was considered statistically significant. A *post hoc* power analysis was performed using the observed difference in proportions for NTD incidence between the control and citric acid groups, with $\alpha=0.05$. The analysis indicated sufficient statistical power to detect the observed difference in NTD incidence. However, because no a priori power analysis was performed, this issue was acknowledged as a limitation. All evaluations were double-blinded.

Results

Chicken embryos treated with 0.5 mM citric acid were compared with controls for survival and neural tube development.

Macroscopic Findings

- Control group: All 15 embryos survived and showed normal neural tube development. No anomalies were detected (Fig. 1a)
- Citric acid group: Of 15 embryos, only 10 survived (66.7%). Among survivors, 9 (90%) showed NTDs, mostly as cranial and caudal closure failures (Fig. 1b-d).

Histopathological Findings

Control embryos exhibited intact neural tube morphology. In contrast, citric acid-treated embryos showed closure defects, notochord irregularities, incomplete ectodermal invagination, and impaired somite formation (Fig. 1a-d).

Statistical Findings

Table 1 summarizes survival and NTD incidence. Citric acid significantly reduced survival ($p<0.05$) and increased NTD incidence ($p<0.001$). Data are presented as the number of embryos and percentages. Survival was recorded at 72 h, and NTD incidence was calculated among surviving embryos. Fisher's exact test showed significantly lower survival ($p<0.05$) and higher NTD incidence ($p<0.001$) in the citric acid group compared with controls (Fig. 2 and Table 1).

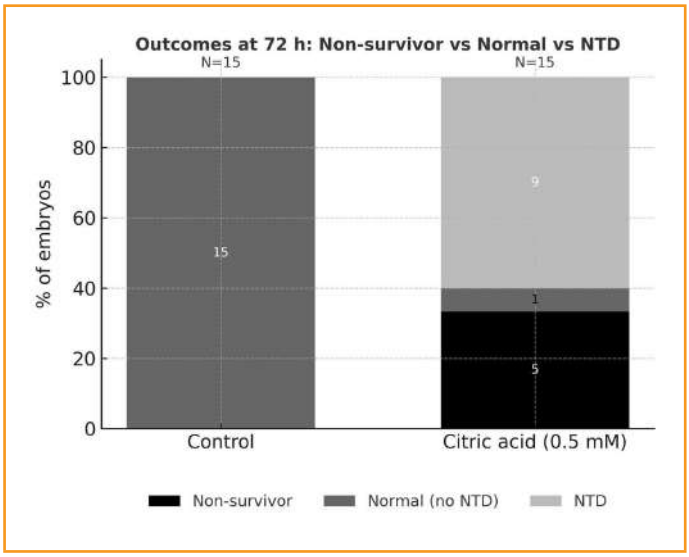

Figure 2. Outcome at 72 h: Non-survivor versus normal versus neural tube defect.

Table 1. Survival and incidence of NTDs in control and citric acid groups

Group	Eggs incubated	Survived at 72 h	Survival %	NTD incidence (survivors)	NTD % (survivors)
Control	15	15	100	0	0
Citric acid	15	10	66.7	9	90

NTDs: Neural tube defects

Discussion

NTDs, which arise from abnormal neurulation, are congenital malformations that can cause severe medical problems both prenatally and postnatally, often resulting in lifelong disability or even death. They also have significant financial and social implications [13].

All types of NTDs are characterized by defective midline closure of the neural tube. In these cases, the organization of neural tissue, surrounding bone, mesenchymal tissue, and skin is disrupted. However, embryogenesis involves highly complex and diverse processes [14].

Globally, the incidence of NTDs varies between 1 and 10/1,000 live births. Despite their relatively high frequency, their etiology is not fully elucidated [3].

Nutritional, genetic, and environmental factors play important roles in the development of NTDs [13,14]. Environmental contributors include socioeconomic status, maternal folate deficiency, maternal hyperthermia, and the use of certain drugs such as carbamazepine, valproic acid, diuretics, antihistamines, and sulfonamides [14].

Among these factors, the most significant cause of NTDs is folic acid deficiency before conception. Early folic acid supplementation can reduce the recurrence of NTDs by up to 71%, with a recommended daily intake of 0.4 mg for women without a history of NTDs and 4 mg for those who are carriers or who have had an affected child. Conversely, folate antagonists such as carbamazepine can double the incidence of myelomeningocele [15].

Since the introduction of periconceptional folic acid supplementation, the global prevalence of NTDs has markedly decreased. The American Academy of Pediatrics recommends that women of reproductive age consume 400 µg of folic acid daily to minimize NTD risk. Research indicates that preconceptional folic acid supplementation can reduce the incidence of NTDs by approximately 50% [4,16,17].

Genetic factors have also been implicated in NTD etiology, particularly polymorphisms of the 5,10-methylenetetrahydrofolate reductase gene. The common C677T polymorphism causes substitution of valine for alanine at position 222 of the enzyme, reducing enzyme activity and tissue folate levels while increasing plasma homocysteine concentrations. This polymorphism occurs in homozygous (TT) or heterozygous (CT) forms in approximately 10% and 38% of the population, respectively. The homozygous TT genotype shows a more pronounced effect, associated with an increased risk of NTDs and a modestly elevated risk of cardiovascular disease [15,18].

Several experimental studies have investigated the impact of environmental factors, food additives, and pharmaceuticals on neural tube development in chicken embryos [19-23]. However, despite the widespread use of food additives, there is limited literature exploring their potential effects on NTD formation. For instance, Emon et al [19]. investigated the effect of sodium benzoate on chicken embryos and reported that it did not induce NTD formation. In contrast, Ovalioglu et al [23]. found that tartrazine, a synthetic dye, caused neural tube closure defects in chick embryos. These findings underscore the importance of further studies investigating commonly used additives.

In the food industry, citric acid is a widely used additive in beverages, baked goods, jams, marmalades, cheeses, and canned foods, where it functions as an acidity regulator, flavor enhancer, preservative, and synergist for antioxidant activity [24-27].

In our study, we investigated the effects of citric acid – a commonly used food additive – on neural tube development in chick embryos. The findings suggest that citric acid exposure at the administered concentration may be associated with impaired neural tube closure in this experimental model. We used a single-dose protocol, which represents a limitation of this study. However, we believe the findings provide valuable insight for the literature. To the best of our knowledge, this is the first experimental study to suggest a possible association between citric acid exposure and neural tube closure defects in chick embryos. Further studies are warranted to elucidate the mechanism underlying neural tube closure failure associated with citric acid exposure.

Similarly, Çelik et al [24]. reported that citric acid induced DNA damage at high doses, suggesting potential genotoxicity.

Because pregnancy tests typically yield positive results only after the 2nd week of gestation, many women are unaware that they are pregnant during the critical period of neural tube closure. This lack of awareness makes it difficult

to avoid environmental teratogens, which may lead to NTD development. These observations should not be directly extrapolated to human pregnancy; however, they support the need for further experimental studies evaluating the developmental safety of commonly used food additives.

Food additives are extensively used for purposes such as flavor enhancement, preservation, coloring, acidity regulation, and nutritional supplementation. Given their widespread and continuous exposure in daily diets, large-scale research is necessary to assess their potential health risks.

Limitation

This study has several limitations. First, only a single dose of citric acid was tested; therefore, dose–response relationships could not be established. Second, the follow-up was limited to 72 h, preventing evaluation of later developmental stages. Third, the control group did not receive a sham injection, whereas the experimental group underwent subblastodermic injection of citric acid solution. Therefore, the possible influence of the injection procedure itself on embryonic survival or neural tube development cannot be completely excluded. Future studies should include a sham-injected vehicle control group to distinguish the specific effects of citric acid from those potentially related to the injection procedure. Fourth, the sample size was relatively small, with 15 embryos in each group, and no a priori power analysis was performed. Although the *post hoc* power analysis based on the observed NTD incidence indicated sufficient statistical power, the absence of an a priori sample size calculation limited the statistical planning and generalizability of the study. Therefore, the present results should be interpreted as preliminary and should be validated in larger experimental studies designed according to appropriate power analysis. Finally, while the chick embryo is a widely used and informative teratology model, it does not fully replicate mammalian or human embryogenesis.

Future Directions

Future studies should investigate the effects of different doses of citric acid, as well as longer incubation periods, to determine whether the observed defects persist or progress over time. In addition, molecular and cellular analyses will be necessary to elucidate the mechanisms by which citric acid disrupts neural tube closure. Comparative studies in mammalian models may further clarify the translational relevance of these findings to human pregnancy.

Conclusion

Our findings suggest that citric acid exposure at the administered concentration may be associated with impaired neural tube development and increased NTD formation in chick embryos. Although this study used only a single dose, which limits the scope of inference, it provides valuable preliminary evidence regarding the potential teratogenic effects of citric acid. To our knowledge, this is the first experimental study to suggest such an association, highlighting the need for further research to clarify the mechanisms underlying neural tube closure disruption.

These findings should not be directly extrapolated to human pregnancy; however, they highlight the need for further experimental research on the developmental effects of commonly used food additives.

Disclosure

Ethics Committee Approval: Ethical Approval: This study was conducted in accordance with the "Regulation of the Local Ethics Committee for Animal Experiments of the Health Sciences University of the Republic of Türkiye". Per Official Gazette No. 28914 (15 Feb 2014), chick-embryo experiments conducted before the last one-third of incubation are exempt from animal ethics approval.

Informed Consent: This type of study does not require informed consent.

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References

1. Kancherla V. Neural tube defects: a review of global prevalence, causes, and primary prevention. Childs Nerv Syst 2023;39:1703–10. [CrossRef]
2. Shookhoff JM, Gallicano GI. A new perspective on neural tube defects: folic acid and microRNA misexpression. Genesis 2010;48:282–94. [CrossRef]
3. Güran Ş, Çoban ZD. Nöral tüp defektinde etkilenen mekanizmalar, hastalığın moleküler biyolojisi ve genetiği. *Turkiye Klin J Pediatr* 2012;21:224–9. [Article in Turkish]
4. Özcan İH, Yücel M, Kahveci H. Retrospective examination of infants with congenital neural tube defect. Cureus 2023;15:e48886. [CrossRef]
5. Büyükbayrak EE, Akalın M, Dağçınar A, Sakar M. Açık spina bifidalı fetüslerde intrauterin cerrahi tedavi. *Maternal-Fetal Tıp*

ve Rehabilitasyon Derneği 2025:1–14. Available from: https://tmftp.org/uploads/acik-spina-bifidali-fetuslerde-intrauter-in-cerrahi-tedavi-uzman-gorusu.pdf. Accessed Jun 3, 2026.

6. Detrait ER, George TM, Etchevers HC, Gilbert JR, Vekemans M, Speer MC. Human neural tube defects: developmental biology, epidemiology, and genetics. Neurotoxicol Teratol 2005;27:515–24. [CrossRef]
7. Au KS, Ashley-Koch A, Northrup H. Epidemiologic and genetic aspects of spina bifida and other neural tube defects. Dev Disabil Res Rev 2010;16:6–15. [CrossRef]
8. Canbek İ. Spinal disrafizmler: meningosel ve meningomyelosel. In: Karabekir HS, ed. *Pediatrik Nöroşirürji – Konjenital Anomaliler.* 1st ed. Türkiye Klinikleri; 2025. p.45–51. [Article in Turkish]
9. Öztürk M, Erbaş M. Sitrik asit üretimi ve saflaştırılması. *Gıda* 2021;46:296–310. [CrossRef]
10. Amato A, Becci A, Beolchini F. Citric acid bioproduction: the technological innovation change. *Crit Rev Biotechnol* 2020;40:199–212. [CrossRef]
11. Francisco JCE, Rivera WL, Vital PG. Influences of carbohydrate, nitrogen, and phosphorus sources on citric acid production by the fungal endophyte *Aspergillus fumigatus* P3I6. *Prep Biochem Biotechnol* 2019;50:292–301. [CrossRef]
12. Hamburger V, Hamilton HL. A series of normal stages in the development of the chick embryo. 1951. Dev Dyn 1992;195:231–72. [CrossRef]
13. Çolak A. Spinal anomalilerde tanım, etyoloji ve sınıflandırma. In: Baykaner, Erşahin, Mutluer, Özek, editors. *Pediatrik Nöroşirürji.* 1st ed. Ankara: TND Yayınları; 2014. p.239–51. [In Turkish]
14. Duru S, Türkoğlu E. Miyelomeningosel. *Turk Neurosurg* 2013;23:226–32. [Article in Turkish]
15. Greenberg MS. Primer craniospinal anomalies. In: Greenberg MS, ed. *Greenberg's Handbook of Neurosurgery.*10th ed. Thieme; 2023. p.295–316. [CrossRef]
16. Klusmann A, Heinrich B, Stöpler H, Gärtner J, Mayatepek E, Von Kries R. A decreasing rate of neural tube defects following the recommendations for periconceptional folic acid supplementation. Acta Paediatr 2005;94:1538–42. [CrossRef]
17. Folic acid for the prevention of neural tube defects. American Academy of Pediatrics. Committee on Genetics. Pediatrics 1999;104:325–7. [CrossRef]
18. Kirke PN, Mills JL, Molloy AM, Brody LC, O'Leary VB, Daly L, et al. Impact of the MTHFR C677T polymorphism on risk of neural tube defects: case-control study. BMJ 2004;328:1535–6. [CrossRef]
19. Emon ST, Orakdogen M, Uslu S, Somay H. Effects of the popular food additive sodium benzoate on neural tube development in the chicken embryo. Turk Neurosurg 2015;25:294–7.
20. Simsek H, Colak A, Kaya S, Kutlay M, Cetinkal A, Haholu A, et al. The effects of Diesel exhaust particles on neural tube development in the early stage chicken embryo. Turk Neurosurg 2012;22:77–82. [CrossRef]
21. Cetinkal A, Cakir A. Does gabapentin affect neural tube development? experimental study using an early stage chick embryo model. Turk Neurosurg 2021;31:967–72. [CrossRef]
22. Temiz C, Temiz P, Demirel A, Sayin M, Umur AS, Ozer FD. Effect of sodium phenytoin concentration on neural tube development in the early stages of chicken embryo development. J Clin Neurosci 2009;16:307–11. [CrossRef]
23. Ovalioglu AO, Ovalioglu TC, Canaz G, Aydin AE, Arslan S, Sar M, et al. Effects of tartrazine on neural tube development in the early stage of chicken embryos. Turk Neurosurg 2020;30:583–7. [CrossRef]
24. Çelik DA, Toğay VA, Türel GY, Özçelik N. Evaluation of genotoxicity of citric acid, ascorbic acid, and sodium citrate used as food additives in human lymphocyte cells. *Med J SDU* 2022;29:486–92. [CrossRef]
25. Yılmaz S, Unal F, Yüzbaşıoğlu D, Aksoy H. Clastogenic effects of food additive citric acid in human peripheral lymphocytes. Cytotechnology 2008;56:137–44. [CrossRef]
26. Türkoğlu S. Genotoxicity of five food preservatives tested on root tips of Allium cepa L. Mutat Res 2007;626:4–14. [CrossRef]
27. Chen X, Lv Q, Liu Y, Deng W. Effects of the food additive, citric acid, on kidney cells of mice. Biotech Histochem 2015;90:38–44. [CrossRef]

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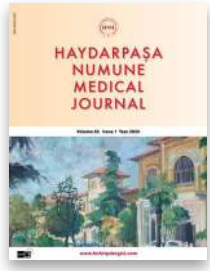

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ORIGINAL ARTICLE

Associations with Pathological Complete Response in Hormone Receptor-Negative and Human Epidermal Growth Factor Receptor 2-Positive Breast Cancer: A Real-World Study

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Abstract

Introduction: Hormone receptor-negative, human epidermal growth factor receptor 2-positive (HR–/HER2+) breast cancer is an aggressive subtype with high sensitivity to human epidermal growth factor receptor 2 (HER2)-targeted neoadjuvant therapy. Although pathologic complete response (pCR) is associated with improved outcomes, real-world predictors of pCR remain unclear. This study aimed to evaluate clinical and pathological factors associated with pCR in patients with HR–/HER2+ breast cancer.

Methods: This retrospective real-world study included 73 patients with HR–/HER2+ breast cancer treated with neoadjuvant systemic therapy. Clinicopathological features, laboratory parameters, and treatment regimens were collected. HER2 and hormone receptor status were assessed by immunohistochemistry and fluorescence in situ hybridization when indicated. pCR was defined as the absence of invasive disease in the breast and axillary lymph nodes. We examined potential clinical and pathological factors associated with pCR in HR–/HER2+ breast cancer.

Results: The median age was 50.9 years, and all patients were female. Dual HER2 blockade was administered in 63% of patients. Overall, pCR was achieved in 67.1% of cases. Comparison of baseline clinicopathological characteristics according to pCR showed that pre/peri menopausal patients achieved significantly higher pCR rates than postmenopausal patients (80.6% vs. 57.1%, p=0.030). In multivariate analysis, the presence of comorbidity (odds ratio [OR]: 7.44, p=0.012), age (OR: 0.88, p=0.003), menopausal status (OR: 5.31, p=0.011), and the number of neoadjuvant cycles (OR: 0.45, p=0.028) were identified as statistically significant predictors.

Discussion and Conclusion: Neoadjuvant therapy achieved high pCR rates in this real-world HR–/HER2+ breast cancer cohort. Menopausal status remained a significant clinical factor associated with pCR. Moreover, age, the number of neoadjuvant cycles, and comorbidity were also identified as significant factors. These real-world findings require validation in larger cohorts, and further prospective studies incorporating molecular biomarkers are warranted.

Keywords: Breast cancer, hormone receptor-negative, human epidermal growth factor receptor 2-positive, pathological complete response, predictors

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Breast cancer is the most common malignancy among women worldwide, and hormone receptor-negative, human epidermal growth factor receptor 2-positive (HR–/HER2+) tumors account for approximately 10–15% of all cases. Before the advent of human epidermal growth factor receptor 2 (HER2)-targeted therapies, this subtype was associated with aggressive biological behavior and poor clinical outcomes [1-3]. Neoadjuvant systemic therapy (NST) is now a cornerstone of treatment for early-stage HR–/HER2+ breast cancer, enabling tumor downstaging, increased rates of breast-conserving surgery, early assessment of treatment response, and treatment of micrometastasis. Pathologic complete response (pCR) has emerged as a strong surrogate marker for improved event-free and overall survival in this setting [4].

Among all breast cancer subtypes, HR–/HER2+ tumors demonstrate the highest sensitivity to anti-HER2–based neoadjuvant regimens. Large pooled analyses report pCR rates of up to 50–70% with contemporary chemotherapy combined with dual HER2 blockade using trastuzumab and pertuzumab [4,5]. Despite these favorable outcomes, substantial interpatient variability persists, suggesting that tumor- and patient-related factors modulate response to therapy.

Several clinical and pathological predictors of pCR – such as tumor size, nodal status, histologic grade, Ki-67 index, age, and type of neoadjuvant regimen – have been investigated, with inconsistent results across studies [6-8]. In particular, the predictive value of Ki-67 and baseline nodal involvement remains controversial. Given evolving treatment strategies and the widespread use of dual HER2 blockade, contemporary real-world data are needed to better define reliable predictors of pCR. In addition, HER2+ disease is heterogeneous; approximately 15% of patients are hormone receptor–negative (HR–) and exhibit distinct biological behavior.

Reliable assessment of pCR predictors is clinically critical, guiding treatment de-escalation in highly responsive patients and escalation strategies, such as adjuvant T-DM1, for those with residual disease after NST [9]. Consequently, this study evaluates clinical and pathological predictors of pCR in HR–/HER2+ breast cancer treated with contemporary neoadjuvant regimens, yielding real-world evidence to support personalized treatment.

Materials and Methods

Study Design and Patients

This real-world retrospective study included 73 patients aged 18 years and older who were diagnosed with HR–/HER2+ breast cancer and received neoadjuvant treatment at two specialized oncology centers between 2014 and 2024. Demographic and clinical characteristics were extracted from patients' medical records.

HER2 and hormone receptor status were assessed on pretreatment core-needle biopsy specimens using immunohistochemical (IHC) analysis. Patients with HER2 IHC scores of 0 or 1+ were classified as HER2-negative. Tumors with an equivocal HER2 score (2+) underwent fluorescence *in situ* hybridization to evaluate *HER2* gene amplification, and cases with confirmed amplification were considered HER2+. Tumors with a HER2 IHC score of 3+ were also regarded as HER2+. Estrogen receptor and progesterone receptor negativity were defined as nuclear staining in <1% of tumor cells.

Clinicopathologic variables collected included demographic data, Eastern Cooperative Oncology Group performance status, comorbidities, menopausal status, tumor characteristics (size, grade, histological subtype, lymph node involvement, and Ki-67 index), smoking status, laboratory parameters, treatment-related cardiotoxicity, number of neoadjuvant treatment cycles, and pCR. Neoadjuvant treatment regimens were recorded for all patients, all of whom received trastuzumab.

This study was approved by the Bezmialem Vakif University Hospital ethics committee (Approval No: 2025/454; December 27, 2025) and conducted in compliance with the Declaration of Helsinki.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, version 24.0 (SPSS Inc., Chicago, IL, USA). Categorical variables were summarized as frequencies and percentages, whereas continuous and ordinal variables were presented as mean±standard deviation, median, and range. The Kolmogorov–Smirnov test was applied to assess the normality of data distribution. Continuous variables were compared using Student's t-test or Mann–Whitney U test, depending on normality. Comparisons of categorical variables were conducted using Pearson's χ^2 test and Fisher's exact test. Analysis of variance was used to compare continuous variables across more than two independent groups to assess whether statistically significant differences existed between group means. Patient characteristics were evaluated using descriptive analyses. Logistic regression analysis was used for univariate analyses to identify factors associated with the outcomes of interest. $P<0.05$ was considered statistically significant. Variables with $p<0.2$ in univariate analysis were included in multivariate analysis.

Table 1. Baseline clinicopathological characteristics

Variable	*n* (%) or mean±SD	Variable	*n* (%) or mean±SD
Total patients	73	Clinical nodal status (%)	
Age (years, mean±SD)	50.92±11.78	cN0	13 (17.8)
Sex	Female:73 (100)	cN1	54 (74.0)
Body mass index (kg/m^2, mean±SD)	28.30±5.96	cN2	4 (5.5)
ECOG performance status (%)		cN3	3 (2.7)
0–1	64 (87.7)	Tumor grade (%)	
≥2	9 (12.3)	Grade 2	12 (16.4)
At least ≥1 comorbidity (%)		Grade 3	41 (56.2)
Yes	27 (37)	Not available	20 (27.4)
No	46 (63)	HER2 status (%)	
Smoking (%)		IHC 2+/FISH+	7 (9.6)
Yes	19 (26)	IHC 3+	66 (90.4)
No	54 (74)	Neoadjuvant treatment regimen (%)	
Menopausal status		AC → taxane+trastuzumab	27 (37)
Pre/perimenopausal	31 (42.4)	AC → taxane+trastuzumab+pertuzumab	38 (52)
Postmenopausal	42 (57.6)	Carboplatin+docetaxel+trastuzumab+pertuzumab	8 (11)
Time from diagnosis to treatment initiation (days, mean±SD)	28.93±29.07	Dual HER2 blockade	46 (63)
Clinical tumor size (mm, mean±SD)	36.62±20.15	Surgical procedure (%)	
Clinical T stage (%)		Mastectomy	32 (43.8)
cT1	10 (13.7)	Breast-conserving surgery	41 (56.1)
cT2	47 (64.4)	Treatment-related cardiotoxicity	3 (4.1)
cT3	11 (15.1)	Pathologic complete response (%)	
cT4	5 (6.8)	Yes	49 (67.1)
		No	24 (32.9)

ECOG: Eastern Cooperative Oncology Group; HER2: Human epidermal growth factor receptor 2; IHC: İmmunohistochemistry; FISH: Fluorescent *in situ* hybridization; pCR: Pathological complete response; AC: Adriamycin and doxorubicin plus cyclophosphamide.

Results

A total of 73 patients with HR–/HER2+ breast cancer who received neoadjuvant therapy were included in our study. The mean age was 50.92±11.78 and all the patients were female. The baseline characteristics of the 73 patients are shown in Table 1. Regarding neoadjuvant treatment regimens, 27 patients (37.0%) received anthracycline-based chemotherapy with doxorubicin plus cyclophosphamide (AC) followed by a taxane plus trastuzumab, 38 patients (52.1%) received AC followed by a taxane combined with trastuzumab and pertuzumab, and 8 patients (11.0%) were treated with carboplatin, docetaxel, trastuzumab, and pertuzumab. The proportion of patients who received dual HER2 blockade was 63% (*n*=46). All patients enrolled in the study received between 4 and 8 cycles of neoadjuvant therapy. The majority of patients, 62 (84.9%), completed 8 cycles of treatment. Mastectomy was performed in 32 patients (43.8%), while 41 patients (56.2%) underwent breast-conserving surgery. Treatment-related cardiotoxicity was observed in only 3 patients. pCR was achieved in 49 patients (67.1%), whereas 24 patients (32.9%) did not achieve pCR.

Univariate analysis of baseline clinicopathological characteristics was compared between patients who achieved a pCR and those who did not (non-pCR), as summarized in Table 2. Moreover, univariate analysis of pathological and laboratory parameters according to pCR status is presented in Table 3. In univariate analysis, pCR rates were numerically higher in patients receiving dual HER2 blockade, but no statistically significant association was observed (p=0.574).

Table 2. Comparison of baseline clinicopathological characteristics according to pathological complete response

Characteristics	pCR (+) (*n*, %) (*n*=49) (67.1%)	pCR (-) (*n*, %) (*n*=24) (32.9%)	p	Characteristics	pCR (+) (*n*, %) (*n*=49) (67.1%)	pCR (-) (*n*, %) (*n*=24) (32.9%)	p
At least ≥1 comorbidity				cT2	29 (59.2)	18 (75)	
Yes	15 (30.6)	12 (50)	p=0.089	cT3	8 (16.3)	3 (12.5)	
No	34 (69.4)	12 (50)		cT4	3 (6.2)	2 (8.3)	
ECOG performance status				HER2 status			
0–1	44 (89.8)	20 (83.3)	p=0.332	IHC 2+/FISH+	5 (10.2)	2 (8.3)	p=0.582
≥2	5 (10.2)	4 (16.7)		IHC 3+	44 (89.8)	22 (91.7)	
Smoking				Neoadjuvant treatment regimen			
Yes	14 (28.6)	5 (20.8)	p=0.341	AC→taxane+trastuzumab	18 (36.7)	9 (37.5)	p=0.514
No	35 (71.4)	19 (79.2)		AC→taxane+trastuzumab+pertuzumab	27 (55.1)	11 (45.8)	
Menopausal status				Carboplatin+docetaxel+trastuzumab+pertuzumab	4 (8.2)	4 (16.7)	
Pre/Perimenopausal	25 (51)	6 (25)	p=0.030	Dual HER2 blockade			
Postmenopausal	24 (49)	18 (75)		Yes	31 (63.3)	15 (62.5)	p=0.574
cNod status				No	18 (36.7)	9 (37.5)	
Positive	10 (20.4)	3 (12.5)	p=0.315	Surgical procedure			
Negative	39 (79.6)	21 (87.5)		Mastectomy	19 (38.8)	13 (54.2)	p=0.160
Tumor grade				Breast-conserving surgery	30 (61.2)	11 (45.8)	
Grade 2	5 (10.2)	7 (29.2)	p=0.211	Body mass index (kg/m^2)			
Grade 3	24 (48.9)	17 (70.8)		<30 kg/m^2	29 (59.2)	16 (66.7)	p=0.361
Clinical T stage				≥30 kg/m^2	20 (40.8)	8 (33.3)	
cT1	9 (18.3)	1 (4.2)	p=0.352				

HER2: Human epidermal growth factor receptor 2; IHC: Immunohistochemistry; FISH: Fluorescent *in situ* hybridization; pCR: Pathological complete response; AC: Adriamycin and doxorubicin plus cyclophosphamide.

Table 3. Association of continuous variables with pCR

Characteristics (mean±SD or median (IQR))	pCR (+)	pCR (–)	p
Age (years)	51.94±12.19	48.83±10.84	0.792
BMI (kg/m^2)	29.06±5.84	28.67±6.32	0.292
Clinical tumor size (mm)	34.82±18.54	40.29±23.09	0.279
Ki-67	44.26±21.6	42.13±16.02	0.671
Hemoglobin (g/dL)	11.5±1.22	11.4±1.24	0.749
Platelet (/mm^3)	265000 (68000)	264000 (92000)	0.326
Neutrophil (/mm^3)	4100 (2350)	3750 (2170)	0.332
Lymphocyte (/mm^3)	1950 (1100)	1610 (612)	0.080
NLR	2.07 (1.39)	2.44 (2.14)	0.619
Albumin (g/L)	4.22±0.32	4.42±0.76	0.128
Number of neoadjuvant cycles	7.78±0.74	7.38±1.24	0.093

BMI: Body mass index; NLR: Neutrophil lymphocyte ratio; PCR: Pathologic complete response.

Table 4. Multivariate analysis for pCR response

Characteristics	OR (95% CI)	p
Age (years)	0.87 (0.80–0.95)	0.003
Pre/perimenopause status	5.31 (1.47–19.15)	0.011
Number of neoadjuvant cycles	0.45 (0.22–0.91)	0.028
At least ≥1 comorbidity	7.44 (1.54–35.81)	0.012

PCR: Pathologic complete response; OR: Odds ratio, CI: Confidence interval.

Regarding surgical approach, breast-conserving surgery was more frequently performed in the pCR-positive group; however, the difference was not statistically significant (p=0.160). However, menopausal status was significantly associated with pCR achievement in the univariate analysis. Pre/perimenopausal patients (*n*=25/31) demonstrated a higher pCR rate compared with postmenopausal patients (*n*=24/42) (80.6% vs. 57.1%, p=0.030).

Variables with potential significance in the univariate analysis (p<0.20), including menopausal status, were included in the multivariate model. In multivariate logistic regression analysis, the presence of comorbidity (odds ratio [OR]: 7.44, 95% confidence interval [CI]: 1.55–35.82, p=0.012), age (OR: 0.88, 95% CI: 0.81–0.96, p=0.003), menopausal status (OR: 5.31, 95% CI: 1.47–19.15, p=0.011), and the number of neoadjuvant cycles (OR: 0.45, 95% CI: 0.22–0.92, p=0.028) were identified as statistically significant predictors. Multivariate analysis is shown in Table 4.

Discussion

HR–/HER2+ breast cancer represents a distinct biological subgroup within HER2+ disease and is frequently treated with neoadjuvant therapy. Response to neoadjuvant treatment, particularly the achievement of pCR, is an important prognostic indicator in this population. Understanding the magnitude of benefit from neoadjuvant therapy – especially in real-world settings – and identifying potential baseline predictors of treatment response are therefore clinically relevant. Hormone receptor negativity is a well-established predictive factor for pCR, with several studies consistently showing higher pCR rates in HR– tumors compared with hormone receptor-positive disease, particularly with HER2-targeted regimens incorporating trastuzumab with or without pertuzumab [5]. Given the aggressive biology of this subgroup, identifying additional predictors of response remains important. Although recent real-world analyses have begun to explore this, evidence is still limited and largely retrospective [10,11]. In addition, identifying reliable predictors of pCR has critical clinical implications. Achievement of pCR is strongly linked to improved event-free and overall survival in HER2+ breast cancer and serves as a key endpoint for guiding post-operative treatment decisions [4]. Patients with residual disease after neoadjuvant therapy may benefit from treatment escalation strategies, such as adjuvant T-DM1 [9], whereas those achieving pCR could be candidates for future treatment de-escalation approaches.

In this real-world analysis of patients with HR–/HER2+ breast cancer, we observed a high pCR rate of 67.1%, which is in line with results from large clinical trials and pooled analyses of contemporary anti-HER2–based neoadjuvant therapies [4,5]. When baseline clinical and pathological characteristics were evaluated, menopausal status emerged as the statistically significant factor associated with pCR, whereas other established clinicopathological parameters did not show a statistically significant association. However, when parameters that were potentially significant in the univariate analysis were evaluated together in the multivariate model, menopausal status retained its statistical significance, and three additional factors also reached statistical significance. Among these, each 1-year increase in age was associated with a 12% reduction in the likelihood of achieving pCR. Pre/perimenopausal status was associated with a 5.3-fold higher likelihood of pCR compared with postmenopausal status. Increasing age was associated with a lower likelihood of PCR, while pre/perimenopausal status was linked to higher response rates. However, the role of age remains controversial, as tumor biology is generally considered the primary determinant of treatment response. Therefore, the observed association may partly reflect underlying biological differences rather than a direct causal effect. In addition, comorbidity burden and the number of neoadjuvant cycles were also associated with pCR, underscoring the multifactorial nature of treatment response. These findings should be interpreted with caution and validated in larger prospective cohorts.

Despite the recognized predictive role of hormone receptor status itself, data specifically addressing predictive factors *within the* HR–/HER2+ *subgroup* remain limited. Most available studies have evaluated heterogeneous HER2+ populations and have treated hormone receptor status primarily as a binary variable, rather than performing subgroup-specific analyses focused exclusively on HR– patients [12,13]. Consequently, the determinants of neoadjuvant response in this biologically distinct subgroup are not yet fully characterized. This further highlights the importance of our study, which is based on real-world data and focuses on baseline characteristics.

The observed pCR rate in our cohort aligns with data from pivotal studies such as NeoSphere and TRYPHAENA, as well as the Food and Drug Administration pooled analysis,

which reported pCR rates ranging from 50% to 70% in HR–/HER2+ disease treated with chemotherapy plus dual HER2 blockade [4,5]. These findings confirm the high chemosensitivity and HER2-dependence of this subtype and support the effectiveness of current real-world treatment strategies outside of controlled clinical trial settings.

Our study highlights the association between menopausal status and pCR, showing higher rates in premenopausal and perimenopausal patients than in postmenopausal ones (80.6% vs. 57.1%, p=0.030). We also found that the menopausal status (OR: 5.31, p=0.011) was identified as a statistically significant predictor of pCR in the multivariate analysis. However, the role of menopausal status has been inconsistently observed; some studies suggest that younger age and premenopausal status may increase chemosensitivity, possibly due to higher tumor proliferation or differences in immune function [6,7], while others find no independent predictive value. These real-world data suggest that menopausal status may influence neoadjuvant response in HR–/HER2+ breast cancer, though underlying mechanisms remain unclear.

In contrast to several previous reports, we did not find a significant relationship between baseline Ki-67 index and pCR in our cohort. Although Ki-67 is widely studied as a marker of tumor proliferation and chemosensitivity, its predictive value in HER2+ breast cancer appears to be controversial,[6,8] possibly due to methodological variability in scoring, cutoff thresholds, and interobserver reproducibility. Our findings indicate that baseline Ki-67 alone has limited value as a reliable predictor of pCR in routine practice, especially with contemporary HER2-targeted neoadjuvant regimens. Similarly, traditional clinicopathological parameters such as tumor size, nodal status, and histological grade were not significantly associated with pCR, contrasting with Luz et al [14]. Although grade 3 tumors were more frequent among patients achieving pCR, this difference was not statistically significant. These results align with accumulating evidence that conventional anatomical staging parameters may have diminished predictive value in the era of effective HER2-directed therapies [7,8]. Importantly, baseline nodal involvement did not impair pCR rates, supporting that patients with node-positive disease can still benefit considerably from modern neoadjuvant treatment. Therefore, although pCR rates are relatively high in this HR–HER2+ subgroup, our findings provide an insight that the variability in response – why some patients achieve higher pCR while others do not – may be investigated beyond traditional clinicopathological features using various novel biomarkers [15,16].

In our study, regarding treatment regimens, no statistically significant association was detected between the type of neoadjuvant chemotherapy and pCR. Although pCR rates were numerically higher in patients receiving dual HER2 blockade, this difference did not reach statistical significance, possibly due to the limited sample size. However, the favorable pCR rates noted with trastuzumab and pertuzumab align with randomized trial data supporting dual HER2 blockade as the standard of care in high-risk early-stage HER2+ breast cancer [4,5]. In addition, routinely available laboratory and inflammatory markers – including neutrophil-to-lymphocyte ratio, lymphocyte count, and serum albumin – were not significantly associated with pCR in our data. Although immune-related biomarkers are increasingly considered as potential predictors of response in HER2+ disease, particularly through antibody-mediated mechanisms, our findings indicate that these routinely analyzed laboratory parameters have limited predictive value when assessed in a real-world setting.

Limitation

Several limitations of this study should be noted. The retrospective design and relatively small sample size may limit the ability to detect modest associations. In addition, missing pathological data for certain variables and heterogeneity in neoadjuvant treatment regimens could have affected the results. Finally, molecular biomarkers beyond commonly used clinicopathological factors – such as tumor-infiltrating lymphocytes or genomic alterations – were not evaluated and may offer additional predictive value.

Conclusion

This real-life study demonstrates high pCR rates in patients with HR–/HER2+ breast cancer treated with recent neoadjuvant therapy. Menopausal status remained a significant clinical factor associated with pCR. In addition, given the observed associations with age, number of neoadjuvant cycles, and comorbidity, we believe that these findings should be supported with larger-scale studies. Moreover, these findings emphasize the variability of treatment response in HR–/HER2+ breast cancer and highlight the need for prospective studies incorporating molecular and immune biomarkers to improve pCR prediction and guide personalized treatment strategies.

Disclosure

Ethics Committee Approval: This study was approved by the Bezmialem Vakif University Hospital Ethics Committee (Date: 27.12.2025, Decision no: 2025/454).

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References

1. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. CA Cancer J Clin 2024;74:12–49. Erratum in: CA Cancer J Clin 2024;74:203. [CrossRef]
2. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987;235:177–82. [CrossRef]
3. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci U S A 2001;98:10869–74. [CrossRef]
4. Cortazar P, Zhang L, Untch M, Mehta K, Costantino JP, Wolmark N, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet 2014;384:164–72. Erratum in: Lancet 2019;393:986. [CrossRef]
5. Gianni L, Pienkowski T, Im YH, Roman L, Tseng LM, Liu MC, et al. Efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with locally advanced, inflammatory, or early HER2-positive breast cancer (NeoSphere): a randomised multicentre, open-label, phase 2 trial. Lancet Oncol 2012;13:25–32. [CrossRef]
6. Ding J, Yang Y, Jiang L, Wu W, Shao Z. Predictive factors of pathologic complete response in HER2-positive and axillary lymph node positive breast cancer after neoadjuvant paclitaxel, carboplatin plus with trastuzumab. Oncotarget 2017;8:56626–34. [CrossRef]
7. Denkert C, von Minckwitz G, Darb-Esfahani S, Lederer B, Heppner BI, Weber KE, et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. Lancet Oncol 2018;19:40–50. [CrossRef]
8. Mukai H, Yamaguchi T, Takahashi M, Hozumi Y, Fujisawa T, Ohsumi S, et al. Ki-67 response-guided preoperative chemotherapy for HER2-positive breast cancer: results of a randomised Phase 2 study. Br J Cancer 2020;122:1747–53. [CrossRef]
9. von Minckwitz G, Huang CS, Mano MS, Loibl S, Mamounas EP, Untch M, et al. Trastuzumab emtansine for residual ınvasive HER2-positive breast cancer. N Engl J Med 2019;380:617–28. [CrossRef]
10. Freitas AJA, Causin RL, Varuzza MB, Hidalgo Filho CMT, Silva VDD, Souza CP, et al. Molecular biomarkers predict pathological complete response of neoadjuvant chemotherapy in breast cancer patients: review. Cancers (Basel) 2021;13:5477. [CrossRef]
11. Van Bockstal MR, Dano H, Benhaddi N, Dubois D, Vandervekken J, Van Marcke C, et al. Predictive markers for pathological complete response (pCR) after neo-adjuvant chemotherapy in HER2-positive breast carcinoma. Histol Histopathol 2024;39:153–64.
12. Chen H, Gui X, Zhou Z, Su F, Gong C, Li S, et al. Distinct ER and PR expression patterns significantly affect the clinical outcomes of early HER2-positive breast cancer: A real-world analysis of 871 patients treated with neoadjuvant therapy. Breast 2024;75:103733. [CrossRef]
13. Yang ZJ, Xin F, Chen ZJ, Yu Y, Wang X, Cao XC. Real-world data on neoadjuvant chemotherapy with dual-anti HER2 therapy in HER2 positive breast cancer. BMC Cancer 2024;24:134. [CrossRef]
14. Luz P, Lopes-Brás R, de Pinho IS, Patel V, Esperança-Martins M, Gonçalves L, et al. Predictive factors for pCR and relapse following neoadjuvant dual HER2-blockade in HER2+ breast cancer: an international cohort study. Clin Transl Oncol 2025;27:4160–9. [CrossRef]
15. Pop B, Popa C, Antone NZ, Achimas-Cadariu PA, Vlad IC, Morar-Bolba G, et al. Evaluating the effectiveness of neoadjuvant therapy in Her2-positive invasive breast cancer: a comprehensive analysis of 167 cases in Romania. Cancers (Basel) 2025;17:2312. [CrossRef]
16. Sun SX, Piotrowski MJ, Adesoye T, Mitchell MP, Garber HR, Teshome M, et al. Long-term outcomes and predictors of response in breast cancer patients with advanced nodal ınvolvement. J Am Coll Surg 2024;238:1–9. [CrossRef]

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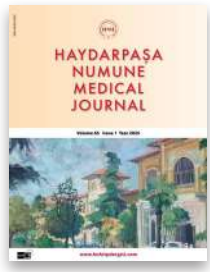

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CASE REPORT

Penile Hematoma After The Use of a Penile Chastity Cage for Sexual Gratification in An Individual Interested in Sadomasochism

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Abstract

A 32-year-old male presented with a penile hematoma after using a penile chastity cage for sexual gratification. Physical examination revealed distal penile edema and diffuse hematoma without urethral hemorrhage or incontinence. Doppler ultrasonography showed no cavernosal or fascial defect but demonstrated a subcutaneous hematoma. Management included ice application, Coban compression bandage, analgesic–anti-inflammatory treatment, and oral antibiotic prophylaxis. The bandage was changed daily; symptoms improved by 48 h, it was removed at 1 week, and complete resolution occurred by week 3. At 6 months, sexual function was normal. This case highlights potential risks of such devices and the successful conservative management of a penile hematoma.

Keywords: Bondage; discipline; sadism and masochism; chastity cage; paraphilia; penile hematoma; sadomasochism.

According to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, paraphilias include unusual sexual desires/behaviors that cause significant distress or functional impairment [1-4].Sadomasochism is defined as the pursuit of sexual gratification based on physical/psychological pain. Devices such as chastity cages may be used in these practices (Fig. 1). Prolonged or improper use can lead to anatomical trauma and functional impairment. This case presents the clinical management of a penile hematoma developing after the use of a chastity cage.

Case Report

A 32-year-old male presented with a penile hematoma and pain. His history revealed that he had used a chastity cage during bondage, discipline, sadism, and masochism practices and kept the device on for 48 h. After removal, he noticed bruising and presented within 6 h due to severe pain. Physical examination revealed edema and diffuse hematoma, especially in the distal penis (Fig. 2). There was no urethral hemorrhage or incontinence. Doppler ultrasonography showed no cavernosal or fascial defect but revealed a diffuse subcutaneous hematoma. Coagulation parameters were normal. Management included ice

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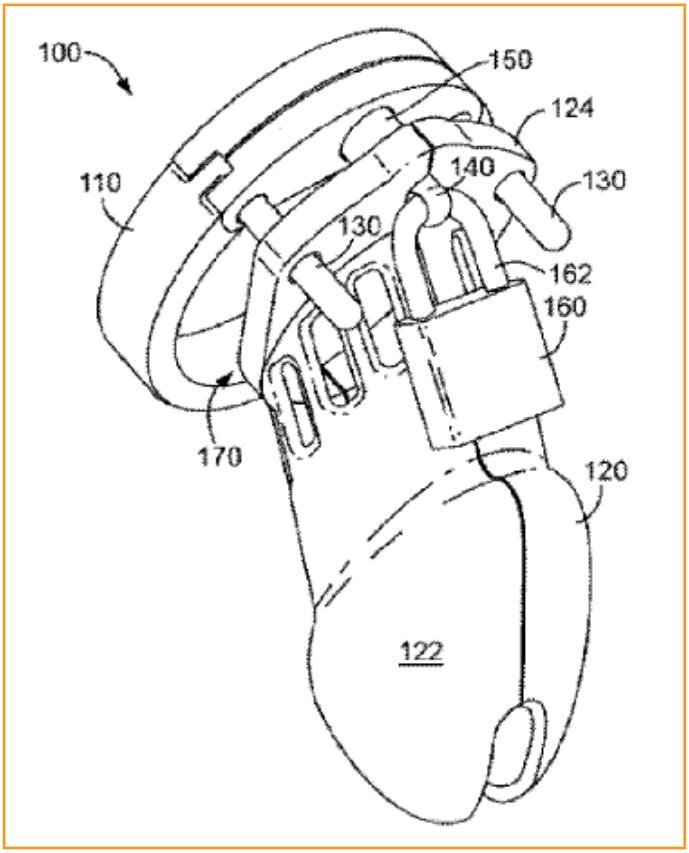

Figure 1. Chastity cage.

application, Coban compression bandage (self-adhesive elastic bandage), analgesic–anti-inflammatory treatment, and oral antibiotics. The bandage was changed daily; symptoms regressed at 48 h, the bandage was removed at 1 week, and complete resolution occurred by the 3rd week. At 6-month follow-up, no sexual dysfunction was detected. Written informed consent was obtained from the patient for publication of this case report and accompanying images.

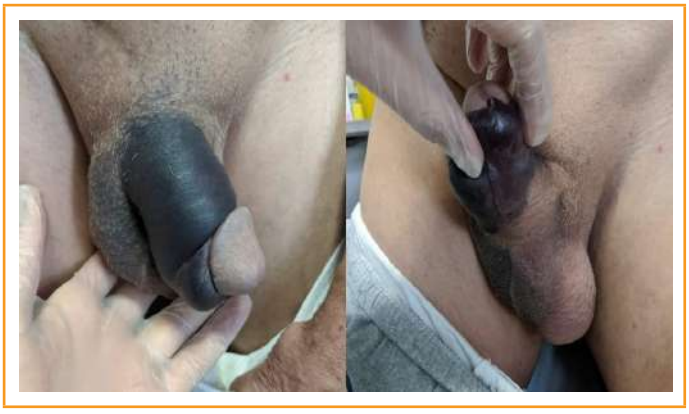

Figure 2. Penile hematoma.

Discussion

Penile hematoma due to chastity cage use can often be managed successfully with conservative treatment [2]. However, prolonged or careless use may result in complications such as infection, abscess, tissue necrosis, and later cavernous fibrosis with erectile dysfunction [3]. Early diagnosis and appropriate conservative management are critical. Psychosocial evaluation and counseling of individuals may help prevent recurrence of similar complications [1,4].

Conclusion

The use of chastity cages as part of sadomasochistic practices carries a significant risk of penile trauma, ranging from minor hematomas to potentially severe complications such as infection, necrosis, and erectile dysfunction. This case demonstrates that with timely diagnosis and appropriate conservative management, including compression bandage, anti-inflammatory medication, and prophylactic antibiotics, complete recovery can be achieved without long-term sequelae. Clinicians should remain vigilant regarding such injuries, conduct a thorough psychosocial assessment, and provide patient education to minimize the risk of recurrence and to ensure both physical and sexual health in the long term.

Disclosure

Ethics Committee Approval: This is a single case report, and therefore ethics committee approval was not required in accordance with institutional policies.

Informed Consent: Written informed consent was obtained from the patient for the publication of this case report and the accompanying images.

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References

1. Berner W, Briken P. Paraphilia, sexual preference disorders. Diagnosis, etiology, epidemiology, treatment and prevention. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz 2007;50:33–43. [Article in German] [CrossRef]
2. Bertini JE Jr, Corriere JN Jr. The etiology and management of genital injuries. J Trauma 1988;28:1278–81. [CrossRef]
3. Maigaard T, Trip MB. Acute urological trauma. Ugeskr Laeger 2023;185:V09220574.
4. First MB. DSM-5 and paraphilic disorders. J Am Acad Psychiatry Law 2014;42:191–201.

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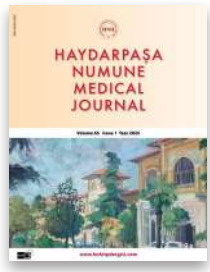

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CASE REPORT

Incidental Detection of a Tracheal Bronchus in a Patient with *Pneumocystis jirovecii* Pneumonia

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Abstract

A tracheal bronchus (TB) is a bronchial anomaly originating from the tracheal wall just above the main carina, before the bifurcation into the right and left main bronchi. Advancements in computed tomography and bronchoscopy technologies have led to a significant increase in the number of diagnosed cases. TB can be asymptomatic and detected incidentally but it may also be associated with certain cardiovascular and bronchopulmonary anomalies. *Pneumocystis jirovecii*, previously classified as a protozoan, is now classified as an opportunistic fungus. Like other opportunistic fungi, it typically causes infection in immunocompromised patients, and it is generally not expected to be pathogenic in immunocompetent individuals. In this case report, we describe the patient who was evaluated due to bilateral infiltrates in the lungs, where TB was detected during further evaluation. Following advanced diagnostic tests, the patient was diagnosed with *P. jirovecii* pneumonia, and the case is discussed in the context of the current literature.

Keywords: Broncopulmonary anomalies; *Pneumocystis jirovecii*; Pneumonia, Tracheal bronchus.

Tracheal bronchus (TB), first described by Sandifort in 1785, is a rare anomaly occurring in approximately 1% of the global population. It may coexist with other cardiovascular anomalies, or it may occur independently without any associated anomalies. The clinical features of TB usually depend on its size and any associated pathologies. It may be detected incidentally without any symptoms or non-specific symptoms such as cough, dyspnea, and chest pain [1,2] may be present.

The diagnosis of TB is often made in childhood; however, patients can rarely be diagnosed during adulthood. In adults, TB is generally asymptomatic, especially in the absence of coexisting anomalies; however, it may lead to bronchiectasis, chronic obstructive pulmonary disease, and recurrent pneumonias in some cases. Two primary diagnostic tools for TB are multi-detector chest computed tomography (CT) and fiberoptic bronchoscopy (FOB). While asymptomatic cases typically do not require treatment, special attention is needed in cases undergoing surgery, as endotracheal tubes could potentially obstruct the airway [3,4].

Pneumocystis jirovecii, previously classified as a protozoan, is now recognized as an opportunistic fungus based on ribosomal RNA analysis. It typically causes pneumonia in immunocompromised adults. *P. jirovecii* pneumonia (PJP) is usually characterized by bilateral multifocal alveolar infiltrates [5].

This case report presents a rare incidentally detected TB in a patient diagnosed with PJP, diagnosed during further investigation for bilateral lung infiltrations, and reviewed in the context of the current literature.

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Table 1. Laboratory values of the case

Parameter	Result	NV	Parameter	Result	NV
WBC (10^3 µL)	46.20	4.00–10.00	Creatinine (mg/dL)	1.03	0.7–1.2
Neutrophil count (10^3 µL)	20.45	2.00–7.00	ALT (U/L)	17	0–40
Neutrophil (%)	44.30	40.00–80.00	AST (U/L)	38	0–41
Lymphocyte count (10^3 µL)	24.3	0.80–4.00	LDH (U/L)	205	135–225
Lymphocyte (%)	52.6	10.00–50.00	Sedimentation mm/saat	22	0–15
Hemoglobin (g/dL)	13.9	13–17	CRP (µg/L)	250.57	0–5
Hemotocrit (%)	44	40.00–54.00	Procalcitonin (mg/dL)	1.06	<0.5
Galactomannan Antigen	1.348 (+)	0.5–15	RT-PCR for *Pneumocystis jirovecii*	Positive	No data
Anti-HIV	0.229	<1.0	CMV PCR (Iu/mL)	85	20–190000

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, CMV: Cytomegalovirus, CRP: C-Reaktif Protein, dL; Deciliter, EBV: Epstein–Bar virus, g: Gram, mL: Milliliter, mm: Milimeter, NV: Normal value, Ig: Immunoglobulin, WBC: Leukocyte, L: Liter LDH: Lactatedehydragenase, PCR: Polymerase chain reaction, RT: Reverse transcriptase, U: Unit

Case Report

A 75-year-old male patient, planned for oncological treatment due to a newly diagnosed diffuse infiltrative high-grade glial tumor in the brain, was evaluated for fever. Chest CT showed widespread infiltrations in both lungs, leading to further investigation for infectious pathologies and malignancies. He was referred to the chest diseases outpatient clinic for FOB and sample collection. The patient had a smoking history of 20 pack-years and medical history of benign prostatic hyperplasia, newly diagnosed glial tumor, and goiter. There were no significant findings in the family history, along with no history of tuberculosis exposure. Physical examination revealed bilateral crackles in all lung zones. The patient's temperature was 37.3°C, pulse rate was 70 beats/min, blood pressure was 118/65 mmHg, and SpO_2 was 97% on room air. Laboratory results are presented in Table 1.

A posterior-anterior chest X-ray showed multifocal non-homogeneous density increases in all lung zones (Fig. 1), while the chest CT revealed widespread ground-glass opacities (GGOs) in both lung parenchyma (Fig. 2). In addition, the trachea was observed to bifurcate into three branches at the level of the main carina (Fig. 3). The echocardiogram showed no findings suggestive of vascular anomalies. An abdominal ultrasound, performed for

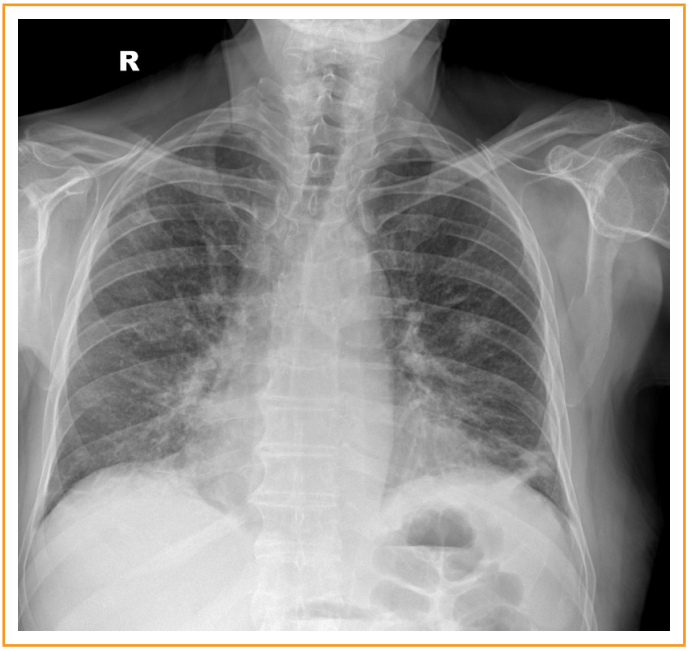

Figure 1. Bilateral pulmonary reticular infiltrates, most prominent in the mid-left zone and in the hilar area.

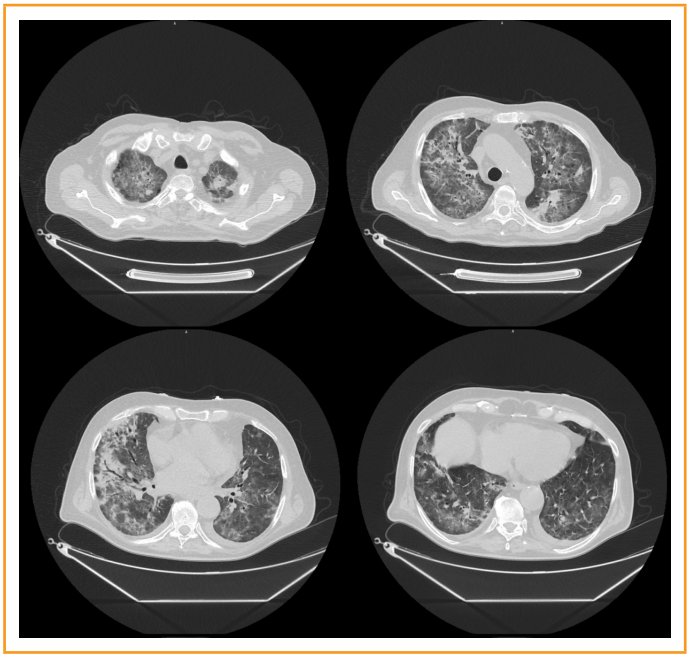

Figure 2. Widespread ground-glass densities and consolidated areas in all zones of both lungs.

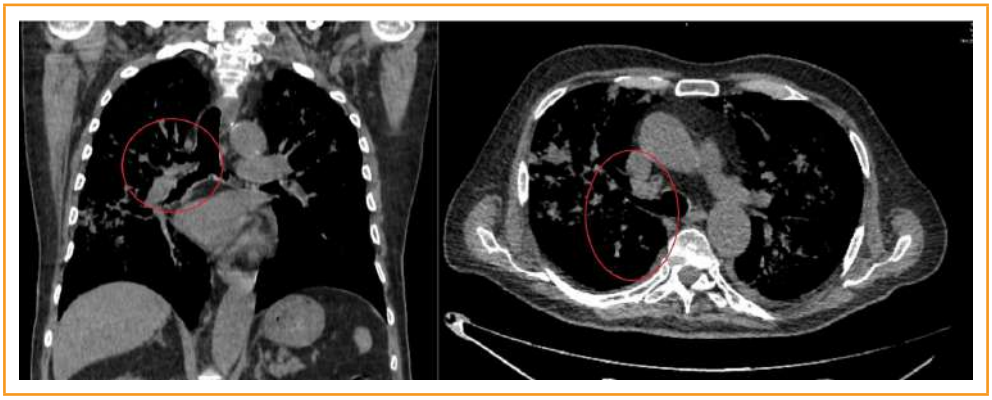

Figure 3. A tracheal bronchus originating from the right side of the trachea, just above the main carina, without separation of the right and left main bronchi.

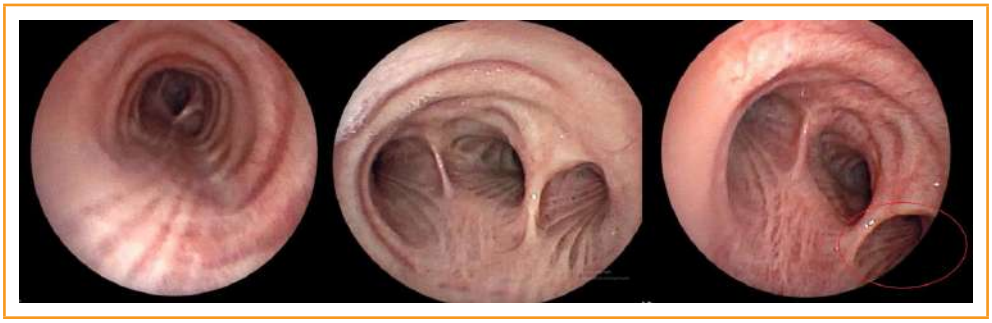

Figure 4. Bronchoscopic view of the tracheal bronchus.

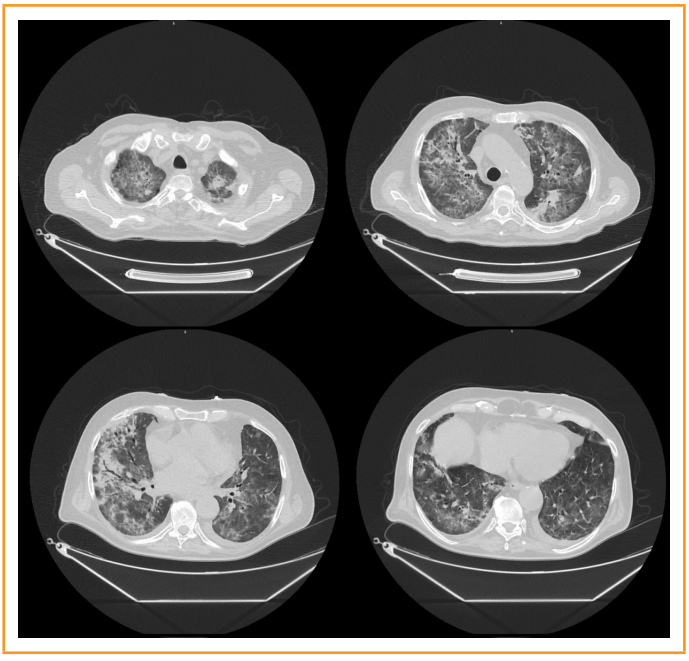

Figure 5. Classification of tracheal bronchi.

unrelated reasons, revealed that the right kidney had a lobulated contour and was larger than the left, and also demonstrated findings consistent with a duplex collecting system. FOB revealed a trachea bifurcating into three branches at the level of the main carina, with an accessory bronchus arising from the right main bronchus. No anomalies or endobronchial lesions were detected in the other segments and bronchi (Fig. 4).

The bronchial wash fluid tested positive for galactomannan antigen. In addition, polymerase chain reaction (PCR) analysis confirmed the presence of *P. jirovecii* (Table 1). Informed consent was obtained from the patient.

Discussion

TB is a rare anomaly, mostly diagnosed incidentally during chest CT scans performed for unrelated reasons. This report presents a rare case of TB detected incidentally in a patient diagnosed with PJP, with both radiological and bronchoscopic evidence of TB.

TB is classified into three types based on its size and location:

- Type I: Larger in diameter, nearly equal to the tracheal diameter above the main carina
- Type II: Arises proximally from the main carina and has a narrower diameter
- Type III: Originates from the region of the main carina itself (Fig. 5).

A review of the literature indicates that most cases are located on the right side, with an average distance from the main

carina ranging from 0 to 2 cm, and an average diameter of 1.7–12 mm. Type II is the most frequently observed anatomical variant [6,7]. Consistent with the literature, our case presented a Type II TB located on the right side, with a distance of 4 mm from the main carina and a diameter of 9 mm. TB can be detected incidentally, but it may also be associated with various cardiovascular and bronchopulmonary anomalies. Reported associations include rib abnormalities, pectus excavatum, congenital esophageal obstruction, tracheal stenosis, congenital cystic malformations, vertebral anomalies, pulmonary valve abnormalities, and pulmonary artery stenosis or dilation [8]. Down syndrome and Klippel–Feil syndrome have also been reported to have a higher prevalence of TB. In our case, apart from the duplex kidney appearance and dual collecting system detected on abdominal ultrasound, no other anomalies were found.

Although it is often diagnosed incidentally and remains asymptomatic in most cases, the presence of TB becomes significant in patients undergoing thoracic surgery. During the placement of an endotracheal tube or a double-lumen tube, obstruction of the TB may lead to intraoperative complications. Especially when one-lung ventilation is needed, the location and angle of the TB to the main carina should be carefully considered during double-lumen tube placement. Therefore, it is important to inform the necessary departments preoperatively. In selected cases, FOB can be used to guide tube placement [9]. Patients at risk for PJP include those who are HIV positive, immunocompromised (due to cancer, organ transplants, or immunomodulatory treatments), or have autoimmune and inflammatory diseases. PJP in immunocompetent individuals is extremely rare. The exact source of PJP in immunocompetent individuals remains unclear, with theories suggesting either a new infection or the reactivation of a latent childhood infection. Bilateral, diffuse GGOs are the predominant radiological finding in PJP. Therefore, the differential diagnosis should include atypical bacterial, viral, and certain fungal pneumonias, interstitial lung diseases, drug-induced pneumonitis, radiation injury, pulmonary edema, and other conditions causing diffuse alveolar damage. PCR analysis of respiratory samples is a useful diagnostic tool for *P. jirovecii*, as cultures are not typically available [10-13]. In our patient, who had a new diagnosis of a glial tumor, was HIV negative, and had not received chemotherapy or immunosuppressive treatment, PCR testing of bronchial wash fluid was positive, confirming the diagnosis of PJP. Although galactomannan antigen detection in bronchial wash fluid is not a primary diagnostic test for PJP, some studies have reported that its positivity in PJP has a sensitivity of 86–94% for confirming the diagnosis.

Conclusion

In conclusion, PJP should be considered in cases of widespread infiltrates in the lungs in immunocompetent individuals. Even though TB is incidentally detected in this case, clinicians should inform anesthesiologists during preoperative preparation to avoid complications and difficulties during intubation, ensuring special attention is paid to airway management.

Disclosure

Ethics Committee Approval: This is a single case report, and therefore ethics committee approval was not required in accordance with institutional policies.

Informed Consent: Informed consent was obtained from the patient.

Conflict of Interest: The authors declare that there is no conflict of interest.

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

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Authorship Contributions: Concept – Ç.D., İ.B.S.; Design – Ç.D., İ.B.S.; Supervision – Y.Ç.; Fundings – E.G.; Materials – C.D., İ.B.S.; Data collection and/or processing – E.G.; Analysis and/or interpretation – Y.Ç.; Literature review – E.G.; Writing – C.D., İ.B.S.; Critical review – C.D.

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References

1. Lam K, Hoang LV, Anh LV. Tracheal bronchus and associated pathologies detected by multidetector-row computed tomography in the Vietnamese population. J Clin Imaging Sci 2022;12:56. [CrossRef]
2. Shih FC, Lee WJ, Lin HJ. Tracheal bronchus. CMAJ 2009;180:783. [CrossRef]
3. Tsukamoto M, Hirokawa J, Hitosugi T, Yokoyama T. Airway management for a pediatric patient with a tracheal bronchus. Anesth Prog 2018;65:50–1. [CrossRef]
4. Laroia AT, Thompson BH, Laroia ST, van Beek E Jr. Modern imaging of the tracheo-bronchial tree. World J Radiol 2010;2:237–48. [CrossRef]
5. Apostolopoulou A, Fishman JA. The pathogenesis and diagnosis of *Pneumocystis jiroveci* pneumonia. J Fungi (Basel) 2022;8:1167. [CrossRef]
6. Conacher ID. Implications of a tracheal bronchus for adult anaesthetic practice. Br J Anaesth 2000;85:317–20. [CrossRef]
7. Kim H, Kim YT, Jou SS, Lee WH. True tracheal bronchus: classification and anatomical relationship on multi-detector computed tomography. J Korean Soc Radiol 2017;76:264–72. [CrossRef]

8. Pérez Ruiz E, Caro Aguilera P, Valdivielso AI, Sanchís Cárdenas S, Martínez García Y, Pérez Frías J. Tracheal bronchus diagnosed in children undergoing flexible bronchoscopy. Paediatr Respir Rev 2018;28:26–30. [CrossRef]
9. Friedlander DA, Panigrahi R, Palaniappan D. Tracheal bronchus with difficult lung ısolation: a case report. Semin Cardiothorac Vasc Anesth 2022;26:237–40. [CrossRef]
10. Rouis H, Moussa C, Houcine Y, Zendah I, Kwas H, Ghedira H, et al. *Pneumocystis jirovecii* pneumonia in a healthy immunocompetent patient: A case report and literature review. SAGE Open Med Case Rep 2023;12:2050313X231220802. [CrossRef]
11. Guarner J, Brandt ME. Histopathologic diagnosis of fungal infections in the 21st century. Clin Microbiol Rev 2011;24:247–80. [CrossRef]
12. Bateman M, Oladele R, Kolls JK. Diagnosing Pneumocystis jirovecii pneumonia: A review of current methods and novel approaches. Med Mycol 2020;58:1015–28. [CrossRef]
13. Zhou S, Linder KA, Kauffman CA, Richards BJ, Kleiboeker S, Miceli MH. Diagnostic Performance of Bronchoalveolar Lavage (1,3)-β-d-Glucan Assay for *Pneumocystis jirovecii* Pneumonia. J Fungi (Basel) 2020;6:200. [CrossRef]

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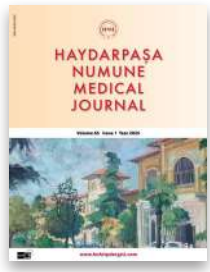

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CASE REPORT

CASPR-2 Antibody-Associated Cramp-Fasciculation Syndrome: A Case Report

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Abstract

Cramp-fasciculation syndrome is a rare form of peripheral nerve hyperexcitability (PNH). We report the case of a 79-year-old man who presented with widespread, visible, and persistent muscle twitching involving both upper and lower limbs, more prominently affecting the entire body, accompanied by a burning sensation in the feet for approximately 1 month. He also reported difficulty initiating sleep and frequent constipation. Clinical and electrophysiological evaluations demonstrated fasciculations and cramps. Further investigations identified positive contactin-associated protein-like 2 antibodies, a subtype of voltage-gated potassium channel antibodies, as the underlying etiology. Although fasciculations are commonly seen in motor neuron diseases and radiculopathies, they may also appear, though less frequently, as part of a PNH syndrome. Clinically, this condition represents a spectrum disorder, and autoimmune mechanisms are often implicated in its pathogenesis.

Keywords: Autoimmune; cramp; fasciculation.

Peripheral nerve hyperexcitability (PNH) syndromes constitute a group of rare disorders characterized by abnormal spontaneous electrical activity in muscles detected through electrophysiological studies, accompanied by muscle twitching of variable frequency and severity, as well as muscle stiffness. These syndromes may present as primary entities such as Isaac's syndrome, Morvan syndrome, or Cramp-fasciculation syndrome (CFS) but can also occur secondarily in association with central nervous system disorders or myopathies. CFS is among the rarest forms of PNH, clinically characterized by widespread fasciculations and involuntary movements resembling myokymia. Diagnosis is typically established by needle electromyography, an electrophysiological technique used to assess neuromuscular function. In this report, we describe a rare case of contactin-associated protein-like 2 (CASPR2) antibody-associated CFS, emphasizing its clinical and electrophysiological features.

Case Report

A 79-year-old man presented to the emergency department with a 10-day history of widespread muscle twitching, most prominent in the face and limbs, accompanied by burning pain in the feet, insomnia, and constipation. The

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twitching was continuous, clearly visible, and intense in the facial muscles and extremities. Although there were no features of restless legs syndrome, the symptoms caused nocturnal restlessness and significant sleep disturbance. The patient also experienced intermittent daytime muscle cramps.

He had a 15-year history of diabetes mellitus and had unintentionally lost approximately 15 kg over the previous 6 months. Additional complaints included neuropathic pain, insomnia, and recurrent constipation. Neurological examination revealed glove-and-stocking sensory loss and decreased vibration sense in both lower limbs. Deep tendon reflexes were normal in the upper limbs but reduced in the lower limbs. Fasciculations were observed in large muscle groups – most prominently in the bilateral vastus medialis and right deltoid muscles – which were irregular, arrhythmic, and persisted even during sleep.

Features of autonomic dysfunction included orthostatic hypotension, urinary frequency, incontinence, dribbling, and incomplete bladder emptying, despite normal prostate function. Abdominal causes were excluded in the emergency setting. Routine laboratory studies, including serum creatine kinase, were within normal limits. Craniospinal magnetic resonance imaging and cerebrospinal fluid analyses were unremarkable, with a negative immunoglobulin G index and thyroid antibodies.

Nerve conduction studies demonstrated reduced sensory nerve action potentials in the ulnar and median nerves, with prolonged distal latencies and slowed conduction velocities. Tibial and peroneal motor amplitudes were normal, while sural amplitudes were borderline low (Fig. 1 and Table 1). Needle electromyography (Table 2) of limb, paraspinal, and facial muscles revealed frequent fasciculations without sharp waves or fibrillation potentials, excluding acute or chronic denervation. No myokymic or neuro-

Table 1. Motor and sensory nerve conduction studies of the patient

Motor NCS		**Sensory NCS**	
Median nerve-R		Median nerve-R	
Distal latency (ms)	4.52	Distal latency (ms)	4.32
Amplitude (mV)		Amplitude (uV)	7.10
-Wrist	5.22	NCV (m/s)	30.1
-Elbow	5.73		
NCV (m/s)	49.5		
Ulnar nerve-R		Ulnar nerve-R	
Distal latency (ms)	3.68	Distal latency (ms)	2.40
Amplitude (mV)		Amplitude (uV)	9.80
-Wrist	9.43	NCV (m/s)	50.0
-Elbow	7.88		
NCV (m/s)	57.8		
Tibial nerve-R		Sural nerve-R	
Distal latency (ms)	6.65	Distal latency (ms)	2.90
Amplitude (mV)		Amplitude (uV)	5.80
-Ankle	5.04	NCV (m/s)	38.6
-Popliteal	5.46		
NCV (m/s)	42.5		
Peroneal nerve-R			
Distal latency(ms)	3.75		
Amplitude(mV)			
-Ankle	6.64		
-Head of fibula	6.09		
NCV (m/s)	48.5		

NCS: Nerve conduction study, NCV: Nerve conduction velocity, ms: Millisecond, mv: Millivolt, m/s: Meter/second.

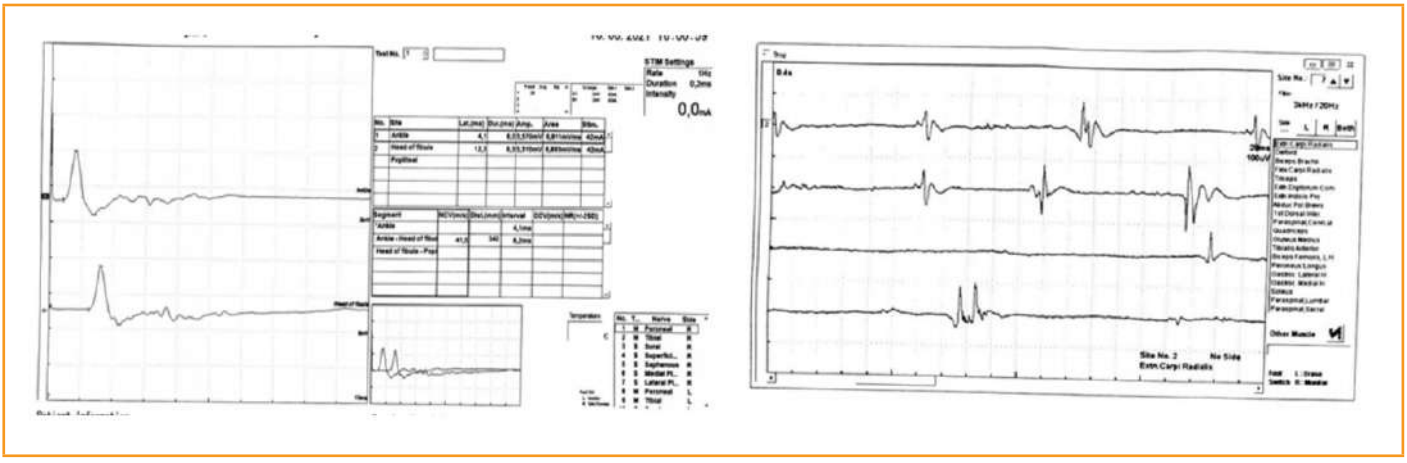

Figure 1. Nerve conduction and needle EMG examination of the patient.

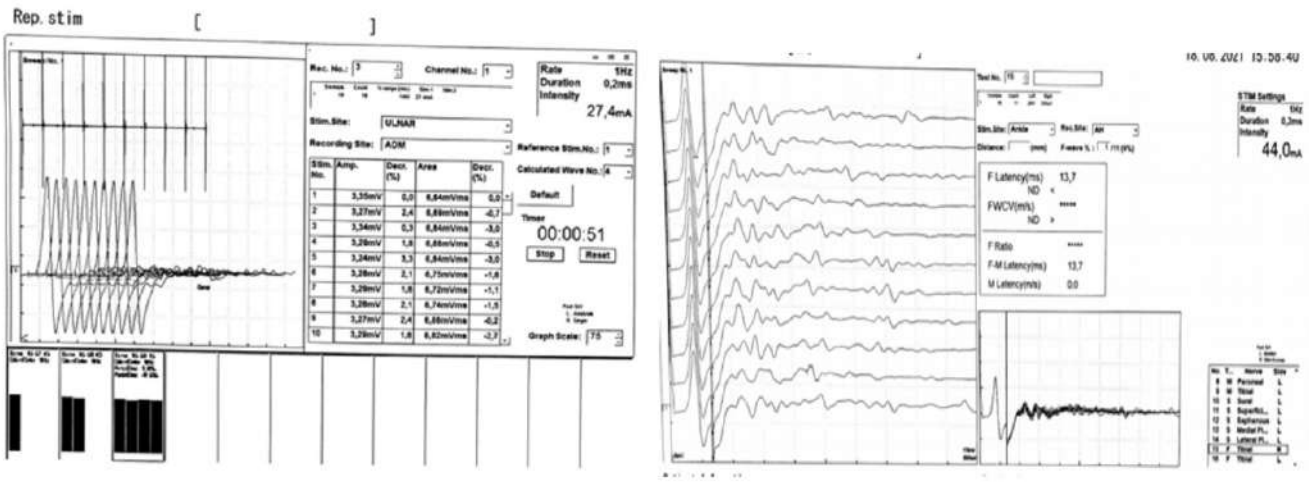

Figure 2. Repetitive nerve stimulation test and after-discharges following M waves in the patient.

myotonic discharges were observed. Some distal muscles showed mildly increased motor unit potential amplitudes with reduced recruitment. Based on these findings, the patient was followed for sensory axonal polyneuropathy, while motor neuron disease and radiculopathy were excluded.

During repetitive nerve stimulation (1–5 Hz) of the tibial nerve, after-discharges followed the initial compound muscle action potential (Fig. 2). RR-interval variability and sympathetic skin response studies also confirmed autonomic involvement.

Paraneoplastic evaluation revealed negative tumor markers and unremarkable systemic imaging. Upper and lower gastrointestinal endoscopies showed only atrophic gastritis. Whole-body positron emission tomography imaging demonstrated hypermetabolic mediastinal lymph nodes; however, the findings were inconclusive for differentiating between granulomatous and lymphoproliferative processes, prompting a pathology recommendation. PPD testing, sputum acid–fast bacilli, and serum angiotensin-converting enzyme levels were negative. Thoracic surgery consultation recommended mediastinoscopy, which the patient declined.

Table 2. Needle electromyography (EMG) study of the patient

Muscle/side		Fibs	Pos. Wave	Fasc.	Normal MUP	Poly	Low Amp.	High Amp.	Dur.	Recruit	Int. Patt
Deltoid	R	0	0	+2	0	N	0	0	N	Full	Full
Extn.Dig.Com	R	0	0	+2	0	N	0	+1	N	Full	Full
1st Dorsal Int	R	0	0	+2	0	N	0	+1	N	Full	Full
Triceps	R	0	0	+2	0	N	0	0	N	Full	Full
Hypoglossus	R	0	0	+3	0	N	0	0	N	Full	Full
Masseter	R	0	0	+3	0	N	0	0	N	Full	Full
Tib. Ant	B	0	0	+3	0	+1	0	+1	N	Reduced	Reduced
Gastroc. Med	B	0	0	+2	0	N	0	+1	N	Reduced	Reduced
Vastus Med	B	0	0	+2	0	N	0	+1	N	Full	Full
Rectus Fem.	B	0	0	+3	0	N	0	+1	N	Full	Full
Paraspinal, lumbar	B	0	0	+3	0	N	0	+1	N	Full	Full
Rectus abdominis	B	0	0	+3	0	N	0	+1	N	Full	Full

Fibs: Fibrillation potential; Pos.Wave: Positive wave; Fasc: Fasciculation potential; Poly: Polyphasic motor unit potentials; Amp.: Amplitude; Dur: Duration; Int. Patt.: Interference pattern; Extn.Dig.Com: Extensor digitorum communis; 1st Dorsal Int: First dorsal interosseous; Tib.Ant.: Tibialis anterior; Gastroc. Med: Gastrocnemius medialis; Vastus Med: Vastus medialis; Rectus Fem: Rectus femoris.

Although the paraneoplastic antibody panel was negative, autoimmune screening revealed positivity for CASPR2 antibodies. A diagnosis of CASPR2 antibody-associated CFS was made. Treatment with pregabalin resulted in a marked improvement in fasciculations and cramps. Given the potential risk of malignancy and the need for long-term disease monitoring, the patient was scheduled for regular follow-up.

Discussion

CFS is a diagnosis of exclusion, primarily established on clinical grounds. Its hallmark feature – fasciculation – necessitates a broad differential diagnosis. Previous studies have demonstrated that fasciculations may also occur in healthy individuals, often precipitated by stress, trauma, dehydration, medications, fatigue, alcohol consumption, or tobacco use [1-3]. However, they are more frequently observed in disorders of the anterior horn cells such as amyotrophic lateral sclerosis, muscular dystrophy, and spinal muscular atrophy, as well as in polyneuropathies, multifocal motor neuropathy, radiculopathies, plexopathies, and hereditary conditions including Huntington's disease, Fabry disease, and GM2 gangliosidosis. Fasciculations may also accompany paraneoplastic, infectious, and metabolic conditions and less commonly appear within the spectrum of PNH syndromes such as Isaacs, Morvan, and CFSs [1,4,5].

Electroclinical evaluation is essential, particularly to exclude acute or chronic denervation. In our patient, alternative etiologies were systematically ruled out. Imaging findings were normal, and needle electromyography demonstrated no denervation potentials, thereby excluding muscle, motor neuron, and central nervous system pathologies [6,7].

A study from 1991 highlighted the diagnostic utility of repetitive nerve stimulation, which demonstrated after-discharges following the initial motor response – indicating neuronal hyperexcitability – during tibial nerve stimulation at 0.5–5 Hz, recorded from the abductor hallucis muscle [8,9]. Similarly, in our case, supramaximal repetitive stimulations elicited after-discharges consistent with previously reported findings. Several studies have suggested that neuropathic pain, peripheral neuropathy, and autonomic dysfunction may form part of the PNH spectrum, most commonly attributed to autoimmune mechanisms involving antibodies to voltage-gated potassium channels (VGKCs), particularly CASPR2 [10].

VGKC-complex antibodies are also implicated in Isaacs and Morvan syndromes, which are characterized by neuromyotonia and limbic encephalitis, respectively. In our patient, however, electromyography revealed neither myokymic nor neuromyotonic discharges, and there were no clinical manifestations suggestive of Morvan syndrome, such as hallucinations, confusion, encephalopathy, or seizures.

Among the VGKC-complex antibodies, CASPR2 – a paranodal potassium channel-associated protein – has been identified as the principal antigenic target in CFS. CASPR2 antibodies are believed to disrupt peripheral nerve electrical stability, resulting in hyperexcitability [11]. Although antineuronal antibodies are detected in only about one-third of patients with CFS, testing for VGKC antibodies remains clinically recommended [12].

CFS is generally considered a benign condition; however, it has been associated with malignancies such as lung carcinoma and thymoma [13]. Earlier reports noted that carbamazepine effectively relieved fasciculations [14], and subsequent studies have demonstrated symptomatic improvement with gabapentin at doses of 300–600 mg daily, divided into two or three doses [15].

Conclusion

CFS is an exceedingly rare disorder that may present with non-specific and variable clinical manifestations. Central nervous system disorders, myopathies, and motor neuron diseases should be carefully considered in the differential diagnosis. Once the diagnosis is established through detailed neurological examination and electrophysiological evaluation, further investigations are warranted to identify potential underlying autoimmune, infectious, or neoplastic etiologies. Long-term follow-up is crucial for monitoring disease progression and detecting possible systemic associations.

Disclosure

Ethics Committee Approval: This is a single case report, and therefore ethics committee approval was not required in accordance with institutional policies.

Informed Consent: Written informed consent was obtained from the patient for the publication of this case report and any accompanying images.

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References

1. Walter TR. Benign fasciculation syndrome. J Pain Palliat Care Pharmacother 2015;29:54–5. [CrossRef]
2. Fermont J, Arts IM, Overeem S, Kleine BU, Schelhaas HJ, Zwarts MJ. Prevalence and distribution of fasciculations in healthy adults: Effect of age, caffeine consumption and exercise. Amyotroph Lateral Scler 2010;11:181–6. [CrossRef]
3. Mitsikostas DD, Karandreas N, Coutsopetras P, Piperos P, Lygidakis C, Papageorgiou C. Fasciculation potentials in healthy people. Muscle Nerve 1998;21:533–5. [CrossRef]
4. Mills KR. Characteristics of fasciculations in amyotrophic lateral sclerosis and the benign fasciculation syndrome. Brain 2010;133:3458–69. [CrossRef]
5. Finsterer J, Aliyev R. Fasciculations in human hereditary disease. Acta Neurol Belg 2015;115:91–5. [CrossRef]
6. De Wel B, Claeys KG. Neuromuscular hyperexcitability syndromes. Curr Opin Neurol 2021;34:714–20. [CrossRef]
7. Ahmed A, Simmons Z. Isaacs syndrome: A review. Muscle Nerve 2015;52:5–12. [CrossRef]
8. Tahmoush AJ, Alonso RJ, Tahmoush GP, Heiman-Patterson TD. Cramp-fasciculation syndrome: a treatable hyperexcitable peripheral nerve disorder. Neurology 1991;41:1021–4. [CrossRef]
9. Harrison TB, Benatar M. Accuracy of repetitive nerve stimulation for diagnosis of the cramp-fasciculation syndrome. Muscle Nerve 2007;35:776–80. [CrossRef]
10. Boyko M, Au KLK, Casault C, de Robles P, Pfeffer G. Systematic review of the clinical spectrum of CASPR2 antibody syndrome. J Neurol 2020;267:1137–46. [CrossRef]
11. Lancaster E, Huijbers MG, Bar V, Boronat A, Wong A, Martinez-Hernandez E, et al. Investigations of caspr2, an autoantigen of encephalitis and neuromyotonia. Ann Neurol 2011;69:303–11. [CrossRef]
12. Liewluck T, Klein CJ, Jones LK Jr. Cramp-fasciculation syndrome in patients with and without neural autoantibodies. Muscle Nerve 2014;49:351–6. [CrossRef]
13. Spagni G, Modoni A, Primiano G, Luigetti M, Sun B, Falso S, et al. Clinical, neurophysiological and serological clues for the diagnosis of neuromyotonia and distinction from cramp-fasciculation syndrome. Neuromuscul Disord 2023;33:636–42. [CrossRef]
14. Nirenberg MJ, Chaouni R, Biller TM, Gilbert RM, Paisán-Ruiz C. A novel TRPA1 variant is associated with carbamazepine-responsive cramp-fasciculation syndrome. Clin Genet 2018;93:164–8. [CrossRef]
15. Forcelini CM, Rotta FT, Posenato N, Rovani JS, Crusius PS, Mallmann AB, et al. Benign fasciculations responsive to gabapentin. Arq Neuropsiquiatr 2007;65:1015–7. [CrossRef]