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İSTANBUL KUZEY KLİNİKLERİ

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REVIEW

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Original Article ALLERGY & IMMUNOLOGY

Severe cutaneous adverse drug reactions due to antituberculosis drugs and their management

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ABSTRACT

OBJECTIVE: Tuberculosis and drug reactions due to antituberculosis drugs are important public health problems. Severe cutaneous adverse drug reactions (SCARs) due to antituberculosis drugs are factor that makes tuberculosis treatment difficultch-allenging. In this real-life study, we present our experience with SCARs due to antituberculosis drugs and their management.

METHODS: Patients hospitalized in the tuberculosis ward of tertiary care reference hospital between January 1, 2015, and September 1, 2023, were retrospectively reviewed. Patients who consulted the immunology and allergy clinic were included in the study.

RESULTS: A total of 4,039 patients were hospitalized with tuberculosis during the study period. A total of 316 (7.8%) patients were consulted, and out of these, eight (2.5%) patients were evaluated as SCARs. Of the eight patients, seven (87.5%) were diagnosed with drug reaction with eosinophilia and systemic symptoms (DRESS), and one was diagnosed with Stevens-Johnson syndrome (SJS) (12.5%). The peripheral blood eosinophil count of patients with DRESS ranged between 740 and 8,690 cells/μL. One patient tested positive for human immunodeficiency virus and developed SJS. Methylprednisolone intravenous 1 mg/kg and Fexofenadine 180 mg/day per oral were used in the treatment of SCARs in all cases. Ad-on treatment for three cases used mepolizumab (anti-IL5 monoclonal antibody) for DRESS. All patients were switched to an alternative treatment protocol and tolerated the new regimen well.

CONCLUSION: In cases with developed SCARs, a new treatment protocol consisting of different medications can be applied after the symptoms improve. DRESS was the most common SCAR of antituberculosis drugs. Monitoring the eosinophil count can help in early diagnosis. Systemic steroids and antihistamines may be effective in the treatment. Mepolizumab can be used with off-label approval in the treatment of DRESS cases to accelerate the treatment process.

Keywords: Antituberculosis drug hypersensitivity; drug reaction; drug reaction with eosinophilia and systemic symptoms (DRESS); mepolizumab; severe cutaneous drug reactions; stevens-johnson syndrome (SJS).

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Tuberculosis is a contagious and potentially lethal disease that causes significant health issues. Based on current treatment recommendations, 85% of patients can be cured when treated for four to six months [1]. Adverse drug reactions (ADRs) that occur during treatment are a significant public health concern for both the patient and the community. ADRs to antituberculosis drugs include hepatitis, cutaneous ADRs, nausea/vomiting, influen-

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za-like illness, and arthralgia [2]. In a previous study, the rate of immediate-type hypersensitivity reactions to first-line antituberculosis drugs was found to be 3.4% [3]. The prevalence of drug reactions in hospitalized tuberculosis patients was reported to be 7.8%. [4].

Severe cutaneous adverse reactions (SCARs) may involve only the skin or may also be life-threatening reactions. The definition of SCARs includes various entities such as generalized bullous fixed drug eruptions, acute generalized exanthematous pustulosis, drug reaction with eosinophilia and systemic symptoms (DRESS), and Stevens-Johnson Syndrome/toxic epidermal necrolysis (SJS/TEN) [5]. These non-immunoglobulin E-mediated hypersensitivity reaction patterns can range from simple cutaneous drug eruptions caused by immunological mechanisms to severe clinical presentations involving organs such as the liver or hematological abnormalities [6]. In the database of the Korean Registry of SCAR, it was reported that antituberculosis drugs were involved in the etiology of 53 (6.7%) out of 783 SCAR patients [7]. The frequency of DRESS caused by antimycobacterial drugs ranked 8th (6.2%) among drug classes [8].

There are various diagnostic and therapeutic approaches for different conditions in SCARs. Managing SCARs associated with antituberculosis drugs presents challenges such as time constraints for treatment, limitations on corticosteroid use, and the need to sustain tuberculosis treatment. This study aimed to present our clinical experience with SCARs related to antituberculosis drugs and their management.

MATERIALS AND METHODS

Patients hospitalized in the tuberculosis ward of a tertiary care reference hospital between January 1, 2015, and September 1, 2023, were retrospectively reviewed. Patients who consulted the immunology and allergy clinic were included in the study. Those who were consulted and diagnosed with SCARs were documented. Demographic data, diagnosis, anti-tuberculosis drugs used, diagnosis of SCARs, Registry of Severe Cutaneous Adverse Reactions (RegiSCAR) scores of patients with DRESS, medications used in the treatment of SCARs, and anti-tuberculosis drug treatment after SCARs were recorded. A skin patch test was performed in the early period immediately after the clinical picture improved. This tests were prepared with 10% and 30% petrolatum Sureyyapasa Chest Diseases and Thoracic Surgery Training and Research Hospital ethics committee approval was obtained for the study (07-09-2023/116.2017.R-311). This study was conducted in accordance with the Declaration of Helsinki.

Highlight key points

- Severe cutaneous adverse reactions may cause serious problems during treatment with antituberculosis drugs.
- An early indicator for DRESS may be an increase in peripheral blood eosinophils.
- The use of anti-IL-5 treatments (mepolizumab) may help accelerate the recovery process in DRESS treatment.

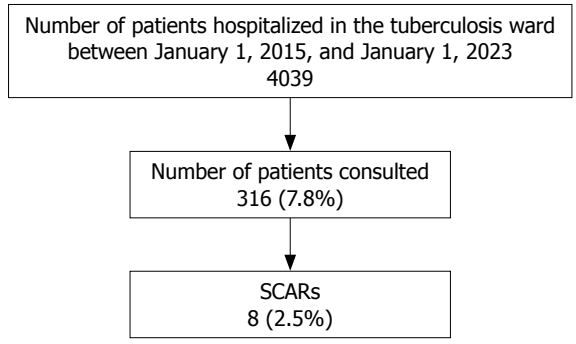

Figure 1. Working flowchart.

RESULTS

During the study period, 4,039 patients were hospitalized in the tuberculosis ward for inpatient examination and treatment. A total of 316 (7.8%) patients were consulted, and out of these, eight (2.5%) patients were evaluated as SCARs. The working flowchart is shown in Figure 1.

Of the eight patients diagnosed with SCARs, four were male (50%) and four were female (50%). The patients were between 30 and 70 years of age. Of the eight patients, seven (87.5%) were diagnosed with DRESS, and one was diagnosed with SJS (12.5%). The RegiSCAR (Registry of Severe Cutaneous Adverse Reactions) score of cases with DRESS was four (probable DRESS) in six cases and three (possible DRESS) in one case. Elevated liver enzymes were observed in four out of seven DRESS cases. Demographic and clinical characteristics of patients are presented in Table 1. In six cases, SCARs developed during the treatment of drug-resistant tuberculosis with HRZE (Isoniazid, Rifampicin, Pyrazinamide, Ethambutol). In two cases, SCARs developed during Multidrug Resistant Tuberculosis (MDR-TB) treatment.

TABLE 1. Characteristics and demographic information of the cases

PN	Age	G	Tuberculosis diagnosis	Culpirit drugs	SCARs diagonosis	Reaction time (day)	RegiSCAR score for DRESS	Eosinophil count cells/µl	Treatment of reaction	Diagnostic patch test	New treatment protocol
1	70	F	Pleural	HRZE	DRESS	12	4	1670	Methylprednisolone Fexofenadine	Negative	Amikacin PAS Protionamid Cycloserine
2	52	M	Pulmonary	HRZE	DRESS	18	4	1620	Methylprednisolone Fexofenadine Mepolizumab	Negative	Amikacin PAS Protionamid Cycloserine Moxifloxacin
3	30	F	Pulmonary MDR+	Ethambutol Amikacin PAS Moxifloxacin Protionamid Cycloserine	DRESS	29	4	3140	Methylprednisolone Fexofenadine	Protionamid 30% Sikloserin 10%+	Ethambutol Levofloxacin Bedaqualine Clofazamine
4	67	M	Pulmonary	HRZE	DRESS	17	4	2510	Methylprednisolone Fexofenadine Mepolizumab	Negative	Amikacin PAS Protionamid Cycloserine Levofloxacin
5	57	M	Pulmonary	HRZE	DRESS	58	4	1240	Methylprednisolone Fexofenadine	Negative	Amikacin PAS Protionamid Cycloserine Moxiflosacin
6	62	M	Meningitis	HRZE	SJS	60	–	470	Methylprednisolone Fexofenadine	Negative	Amikacin Protionamid Cycloserine Moxifloxacin Linezolid

Table 1 (cont). Characteristics and demographic information of the cases

PN	Age	G	Tuberculosis diagnosis	Culpirit drugs	SCARs diagonosis	Reaction time (day)	RegiSCAR score for DRESS	Eosinophil count cells/µl	Treatment of reaction	Diagnostic patch test	New treatment protocol
7	33	F	Pulmonary MDR+	Moxifloxacin Streptomycin Ethambutol Pyrazinamide Amikacin PAS	DRESS	34	3	740	Methylprednisolone Fexofenadine Mepolizumab	Protionamid 30%+ Sikloserin 10%+ Pyrazinamide 30%	Levofloxacin Bedaqualine Clofazamine Linezolid Delamanide PAS
8	65	F	Pulmonary	HRZE	DRESS	25	4	8960	Methylprednisolone Fexofenadine	Negative	Protionamid Cycloserine Levofloxacin

[a]MDR: Multi drug resistant; [b]HRZE: Isoniazid, rifampicin, pyrazinamid, ethambutol; [c]PAS: Para-aminosalicylic acid; [d]DRESS: Drug reaction with eosinophilia and systemic symptoms; [e]SJS: Stevens-Johnson syndrome; G: Gender; F: Female; M: Male; PN: Patient no

The peripheral blood eosinophil count of patients with DRESS ranged between 740 and 8,690cells/µL. Skin patch tests with HRZE were negative. In cases of MDR-TB, skin patch tests were positive for cycloserine and protionamide in one case, and for cycloserine, protionamide, and pyrazinamide in the other case. Methylprednisolone intravenous (IV) 1 mg/kg and Fexofenadine 180 mg/day per oral (PO) were used in the treatment of SCARs in all cases. Additionally, in three patients, the anti-IL-5 drug mepolizumab was administered at 100 mg for the treatment of DRESS. All patients were switched to an alternative treatment protocol and tolerated the new regimen well. In a patient diagnosed with MDR-TB, ethambutol was included in both the old and new treatment protocols.

DISCUSSION

Overall, the primary approach to managing all SCARs is to identify and eliminate the culprit drug [6]. DRESS typically occurs two to eight weeks after starting the medication [9]. In our cases, the reaction time ranged between 12 and 60 days. Eosinophilia can be observed only in 60–70% of cases with DRESS [10]. All of our DRESS patients had eosinophilia. Except for one case, all patients had hypereosinophilia (>1500 cells/µL). Basal eosinophil counts of the patients before the initiation of tuberculosis treatment were normal (<500 cells/µL).

The culprit drug is generally not identified in 10-20% of DRESS cases [11]. Identifying the culprit drug is challenging due to the simultaneous use of multiple drugs in tuberculosis treatment. Skin patch tests to identify the culprit drug in DRESS should be conducted six months after the reaction symptoms recover, with positivity rates ranging from 32% to 64% depending on the specific drug [12]. In a review, patch tests were recommended to be performed four weeks after steroid treatment and four to six weeks after the reaction in patients with DRESS [13]. However, the patients were diagnosed with tuberculosis, and immediate treatment initiation was necessary. Therefore, in our cases, a skin patch test was performed in the early period immediately after the clinical picture improved. Since these tests were not standardized, they were prepared with 10% and 30% petrolatum. Except for two cases of MDR, patch tests were negative. The negative results of these tests can be attributed to their early application.

DRESS cases involving all first-line antituberculosis drugs (HRZE and streptomycin) have been reported in the literature. Rifampicin has been reported as the

most common antituberculosis drug causing DRESS [13, 14]. In one case, both ethambutol and isoniazid-induced DRESS were reported [15]. Skin patch tests with cycloserine and protionamide were positive in two MDR-TB cases. Furthermore, one MDR-TB patient had a positive skin patch test with pyrazinamide, in addition to cycloserine and protionamide. In the literature, there is no standardization of irritant concentrations, and there is a lack of data on the specificity and sensitivity of skin patch tests with these drugs. Nevertheless, these drugs that showed positive patch tests were not included in the new treatment protocol and were not used.

Methylprednisolone (IV) and fexofenadine (PO) (7–14 days) were used in the treatment of all patients with SCARs. In three pulmonary tuberculosis patients, one of whom was MDR-TB, a rapid increase in peripheral blood eosinophil count was observed when the steroid dose was reduced. There was also concern that steroids may cause tuberculosis progression due to their immunosuppressant effect. In these three patients, mepolizumab 100 mg every four weeks subcutaneously (with off-label approval) was administered to expedite the DRESS treatment process and promptly initiate new antituberculosis treatment. These patients are the first cases in which mepolizumab was utilized in the treatment of DRESS caused by antituberculosis drugs. There are case reports in the literature on the use of mepolizumab in the treatment of DRESS related to various drugs [16, 17]. Two patients received three doses at 28-day intervals, while one patient received a single dose of mepolizumab. After the first administration of Mepolizumab, eosinophil counts decreased rapidly, and steroid treatment was not required.

Upon recovery of DRESS, a new antituberculosis treatment protocol was planned for the patients. This basic strategy was implemented to prevent SCAR recurrence and to initiate tuberculosis treatment promptly. The treatment regimen administered to patients is detailed in Table 1.

One patient developed SJS after receiving HRZE. This patient tested positive for Human Immunodeficiency Virus (HIV). However, the patient was not on any antiviral treatment. A skin biopsy was also performed on this patient, confirming the diagnosis of SJS. When the patient's symptoms improved, a treatment protocol involving new antituberculosis drugs was initiated, and the patient tolerated it well. On the other hand, desensitization to the same antituberculosis drugs was reported in an HIV-positive patient who developed SJS due to the anti-tuberculosis drug [18].

In the database of the Korean Registry of SCAR, it was observed that the frequency of DRESS in SCARs due to antituberculosis drugs was higher than in SCARs due to non-antituberculosis drugs (77.4% vs. 45.8%, $p<0.001$) [7]. In our cases, DRESS was observed in seven patients (87.5%) and SJS in one patient (12.5%). Consistent with the literature, DRESS was the most common presentation of SCARs related to antituberculosis drugs.

The overall mortality rate for DRESS is reported to be between 3.8% and 10%, and between 19.4% and 29% in SJS [13, 19]. No mortality was observed in our cases.

Information on genetic predisposition and viral reactivation in the pathogenesis of DRESS is available in the literature. Human Herpesvirus-6 (HHV-6) is most commonly associated with DRESS, although other human herpesviruses have also been reported, including HHV-7, Cytomegalovirus (CMV), Epstein-Barr virus (EBV), and Herpes Simplex virus [20, 21]. No tests for viral reactivation were performed in our DRESS cases, which is a limitation of our study. There are two in vitro tests, the lymphocyte transformation test (LTT) and the enzyme-linked immunospot (ELISpot) assay, which can be used to help identify the culprit drug [22]. High sensitivity and specificity have been reported for anti-TB drugs with LTT (sensitivity: 87.5%, specificity: 100%; $p=0.004$) [22]. Due to the difficulty in accessing the test, the LTT for moxifloxacin and streptomycin could only be performed in MDR case number 7, resulting in a negative outcome. In this case, another quinolone group antibiotic (levofloxacin) was safely used in the new treatment protocol. The inability to perform LTT and ELISpot assays in all of our patients is another limitation of our study.

There were some limitations in the present study. First, it was a retrospective, single-center study. Nonetheless, it provides crucial clinical information due to the sample size and specific patient groups. Besides, study is performed in the tertiary care hospital which is the largest reference center. Data were collected electronically from the hospital online database to prevent incorrect and missed data entry. Second the results were not generalized to whole population. These results may be helpful for physicians managing patients who had SCARs due to antituberculosis drugs.

Conclusion

The most common SCAR caused by anti-tuberculosis drugs is DRESS. The diagnostic approach is difficult due to the use of many drugs in treatment. Monitoring eosinophil count may serve as an early warning laboratory result in symptomatic patients. Especially in the treatment of DRESS, mepolizumab can be used for early recovery in addition to systemic steroids and antihistamines with off-label approval. When symptoms improve, implementing a new treatment protocol that excludes the use of old medications may enable treatment to continue without interruption.

Ethics Committee Approval: The Sureyyapasa Chest Diseases and Thoracic Surgery Training and Research Hospital Ethics Committee granted approval for this study (date: 07.09.2023, number: 116.2017.R-311).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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

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Original Article ORTHOPEDICS & TRAUMATOLOGY

Relationship between Hill-Sachs lesions and morphometry of glenoid, acromion and coracoid process

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ABSTRACT

OBJECTIVE: Hill-Sachs lesions are considered as an a priori risk factor for glenohumeral instability. Determination of glenoid and coracoacromial arch morphometric properties of patients with Hill-Sachs lesions may aid in the diagnosis, identification of optimal treatment strategies and prevention of recurrence.

METHODS: Computed tomography (CT) images of individuals between the ages of 20–40 were examined and the morphometric characteristics of the 39 patients with Hill-Sachs lesions and 71 control patients with healthy shoulder structures were compared. Glenoid inclination (GI), acromio-humeral distance (AHD), glenoid height (GH), glenoid superior width (GWs), glenoid inferior width (GWi), glenoid surface (GS), coraco-acromial distance (CAD), coraco-glenoid distance (CGD) and acromio-glenoid distance (AGD) were determined as morphometric features.

RESULTS: The average values of the measurements in Hill-Sachs and control groups, respectively, were; glenoid inclination (1.06±6.61°; 4.33±6.20°), acromio-humeral distance (6.66±1.26; 7.67±1.64 mm), glenoid height (33.90±2.34; 35.03±3.29 mm), glenoid superior width (19.89±5.89; 19.83±2.37 mm), glenoid inferior width (24.28±2.53; 26.04±3.06 mm), glenoid surface (647.93±91.26; 721.58±136.86 mm^2), coraco-acromial distance (40.24±3.99; 38.10±3.80 mm), coraco-glenoid distance (29.39±3.64; 29.96±4.44 mm) and acromio-glenoid distance (31.44±3.45; 31.64–33.56 mm). Glenoid inclination (p=0.011), acromio-humeral distance (p=0.001), glenoid height (p=0.039), glenoid inferior width (p=0.001), glenoid surface area (p=0.002) and coraco-acromial distance (p=0.008) were significantly different between the groups.

CONCLUSION: The glenoid and coracoacromial arch morphometry showed significant differences in patients with Hill-Sachs lesions. Recognizing these differences can reduce recurrence rates by reducing risk factors in the treatment of glenohumeral instability and providing the closest anatomical integrity to normal.

Keywords: Coracoacromial arch; glenoid; glenohumeral instability; Hill-Sachs lesions.

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Hill-Sachs lesions, first described in detail by H.A. Hill and M.D. Sachs in 1940, are postero-lateral defects of the humeral head usually occurring with anterior glenohumeral dislocations [1]. The prevalence has been reported in previous studies to vary between 31% and 94% for anterior shoulder dislocations, and this rate increases as the number of dislocations increases and may reach 100% [2–7]. This variability in the incidence

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is due to the difficulty of diagnosing a Hill-Sachs lesion with plain radiographs. Currently, three dimensional computed tomography (3D CT) it is seen as the gold standard for detecting these lesions [8].

The glenohumeral joint is the most commonly dislocated joint in the body and 90% of dislocations are anterior dislocations [9]. The etiology of anterior glenohumeral instability is multifactorial. However, anatomical features are important for instability. It is important to know the risk factors and features of the anatomical structure for the diagnosis and treatment of this common pathology [10]. Glenoid, acromion and coracoid process morphologies are important bone structures for glenohumeral joint stability. In patients with Hill-Sachs lesion, understanding the morphemetric properties of these structures may increase the chance of success in treatment by helping identify the risk factors for this lesion. In addition, it is necessary to know the anatomy and morphometry of the region very well to treat a region [11].

Although many treatment methods have been described for recurrent glenohumeral dislocations, post-treatment recurrence rates are still high [12]. Although Hill-Sachs lesions are a defect that develop as a result of recurrent anterior dislocations, they also appear as a risk factor in the success of the treatment. Previous studies mostly aimed to determine the relationship between the size and location of the Hill-Sachs lesion and glenohumeral instability. Very few studies have aimed to determine the risk factors and the effect of anatomical structure for Hill-Sachs lesions. The aim of the present study was to determine the relationship between glenoid, acromion and coracoid processes and Hill-Sachs lesions, which leads to recurrent pathology and decreases quality of life.

Highlight key points

- Glenoid, acromion, and coracoid process morphometry differed significantly in patients with Hill-Sachs lesions.
- In patients with Hill-Sachs lesions, acromio-humeral distance, glenoid inclination, glenoid height, glenoid width, and glenoid surface area decreased significantly.
- In patients with Hill-Sachs lesions coraco-acromial distance increased significantly.

MATERIALS AND METHODS

Cohort's Selection

Our comparative study was planned retrospectively. Computed tomography (CT) images which included the shoulder area, obtained for any reason by the Department of Radiology of The Kocaeli University Hospital between 2010 and 2019 were used. Exclusion criteria included any image showing dislocation, fracture, degeneration, advanced osteoarthritis, and tumor or images that were not suitable for the measurement of any of the parameters for technical reasons. The age-range of subjects was also limited to 20–40 years of age to exclude possibility of bone development and/or osteoarthritis. The diagnoses of the patients were discussed by the radiologists of our institution. A senior radiologist was consulted in case of uncertainty.

CT Scan Modalities

CT images were obtained with a Toshiba Aquilion 64 or a Toshiba Activion 16 Multislice CT (Toshiba Medical Systems, Otawara, Japan). Images were evaluated using the Kocaeli University hospital Picture Archiving and communication system (PACS) and Sectra Workstation IDS7 (Sectra AB, Linköping, Sweden) software was used for measurements.

Measurements

Measurements were made of the following features: glenoid inclination (GI); acromio-humeral distance (AHD); glenoid height (GH); glenoid superior width (GWs); glenoid inferior width (GWi); glenoid surface area (GS); coraco-acromial distance (CAD); coraco-glenoid distance (CGD); and acromio-glenoid distance (AGD). Patient group consisted of those images which included Hill-Sachs lesions while the control group consisted of images containing consensus healthy bone structure. All images were evaluated in two separate groups and measurements were compared using appropriate statistical tests. Each measurement was made by 2 investigators and averaged. Senior radiologist made the final decision in case of any disagreement. An example of a patient with Hill-Sachs lesion is shown in Figure 1.

Definitions of Measurement Parameters (Fig. 2)

Glenoid angle (°): Measured using the angle β, between the glenoid fossa and the cortical edge of the fossa supraspitanus in the coronal plane [13].

Acromio-humeral distance (mm): The section with the shortest distance between caput humeri and acromion in the coronal plan was determined and the distance between them was measured in this section [14].

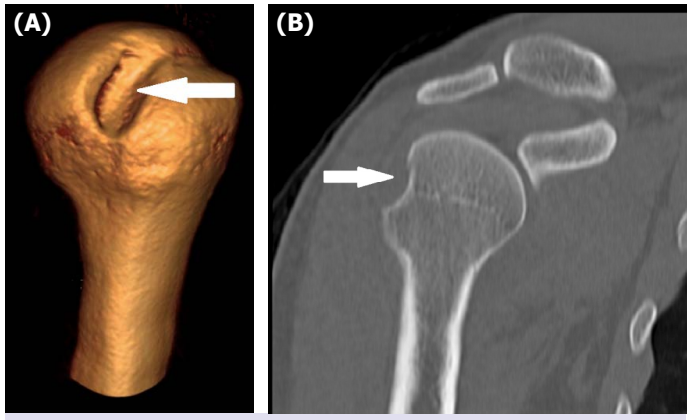

FIGURE 1. Patient with Hill-Sachs lesion on 3D CT (right) and CT (left). Hill-Sachs lesion view of a 27-year-old male patient. **(A)** Reconstructed 3D CT image; **(B)** CT image in the coronal plan.

Glenoid height (mm): The distance between the supraglenoid tubercle and the lowest point of the glenoid surface was measured in the 3D reconstructed image [15].

Glenoid superior width (mm): Calculated by measuring the anteroposterior width of glenoid in the upper half of the glenoid in the 3D reconstructed image.

Glenoid inferior width (mm): Calculated using the anteroposterior widest distance of the glenoid surface in the 3D reconstructed image [15].

Glenoid surface area (mm^2): The glenoid surface is regarded as an ellipse. In the 3D reconstructed image, an elliptical area was calculated using the distance between the supraglenoid tubercle and the infraglenoid and the widest anteroposterior distance [16].

Coraco-acromial distance (mm): The distance between the extreme point of the acromion and the extreme point of the coracoid process was measured in the 3D reconstructed image [17].

Acromio-glenoid distance (mm): The distance between the extreme point of acromion and supraglenoid tubercle was measured in the 3D reconstructed image.

Coraco-glenoid distance (mm): The distance between the extreme point of the coracoid process and the supraglenoid tubercle was measured in the 3D reconstructed image.

Statistical Analysis

Descriptive statistics were assessed. For continuous variables mean, standard deviation, confidence interval are shown. For categorical data, numbers and per-

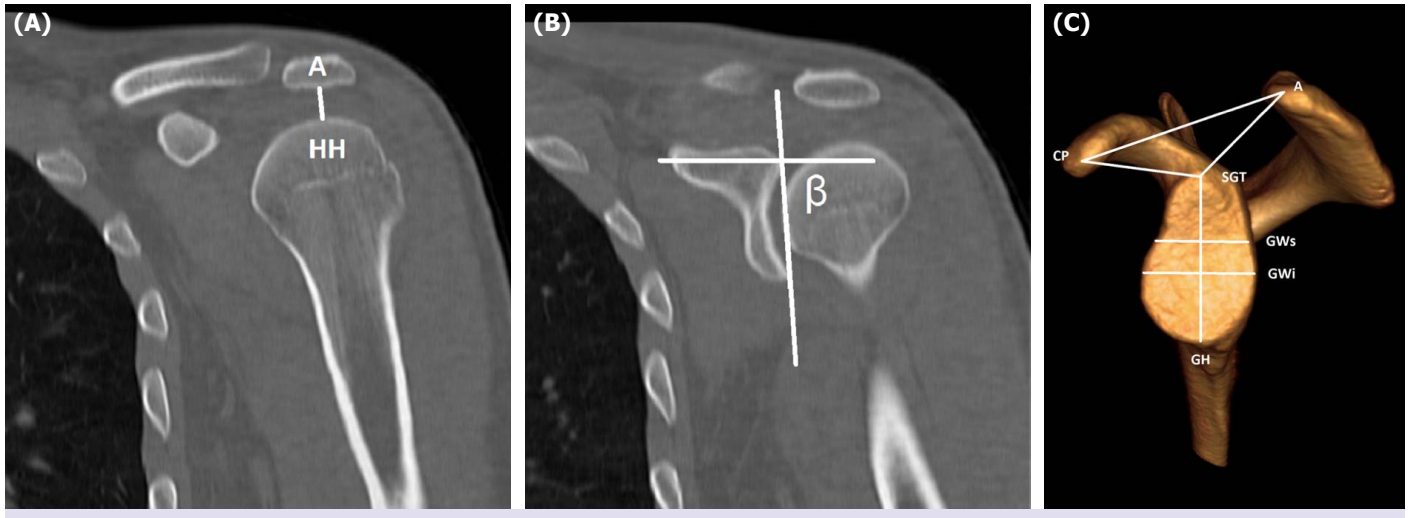

FIGURE 2. Images showing dimensions used to obtain measurements. **(A)** Acromio-humeral distance: The shortest distance between acromion and humeral head in the coronal plane. **(B)** Glenoid inclication: The angle β between the line drawn from the deepest point of the supraspinatus fossa and the line between the most superior and the most inferior of glenoid fossa in the coronal plane. The β angle was obtained using the following (90-x) = β where x = the measured angle. **(C)** Coraco-acromial distance: the line between acromion and coracoid process; acromio-glenoid distance: the line between acromion and supraglenoid tubercle; coraco-glenoid distance: the line between coracoid process and supraglenoid tubercle; glenoid hight: the line between supraglenoid tubercle and the lowest point of the glenoid; glenoid superior width: the widest anteroposterior distance of superior half of glenoid; glenoid inferior width: widest anteroposterior distance of the glenoid.

A: Acromion; HH: humeral head; CP: Coracoid process; SGT: Supraglenoid tubercle; GH: Glenoid height; GWs: glenoid superior width; GWi: Glenoid inferior width.

Table 1. Demographic characteristics of the patient and control groups

Groups	Hill-Sachs n=39	Controls n=71	p
Gender			0.387
Male	79.5	71.8	
Female	20.5	28.2	
Side			0.825
Right	64.1	61.9	
Left	35.9	38.1	
Age (years)			0.223*
Mean±SD	26.26±5.82	27.43±5.48	
Range	20-40	20-40	

SD: Standard deviation; Chi-square test was used; *: Mann-Whitney U test was used.

centages are presented. The compliance of continuous data to normal distribution was evaluated by Kolmogorov-Smirnov and Shapiro-Wilk tests. The t-test was used to compare two independent groups with parametric distribution and the Mann-Whitney U test was used to compare two independent groups with non-parametric features. Chi-square test was used for comparison of categorical data. For statistical significance, $p<0.05$ in 95% Confidence Interval were considered significant. Intraclass correlation coefficient (ICC) was used to determine the level of agreement between observers. A values between 0.50 and 0.75 was accepted as moderate agreement, values between 0.75 and 0.90 was considered as good agreement and values between 0.90 and 1.00 was considered excelent agreement level [18].

For statistical analyses, 21.0 version of the Statistical Package for the Social Sciences (SPSS), version 21.0, (IBM Inc., Chicago, IL, USA) was used.

Ethics Committee Approval

The study was approved by Kocaeli University Non-Interventional Ethics Committee with the decision number GOKAEK 2020/20.30 and project number 2020/347. The study was performed according to Helsinki Declaration.

RESULTS

Study images were obtained from 39 patients (31 males; 79.5%) which were compared with 71 controls (51 males; 71.8%). Demographic features of these two groups are compared in Table 1.

In the Hill-Sachs group nearly two-thirds of the affected shoulders were on the right (n=25, 64.1%) while 14 (35.9%) left shoulder views were obtained. In the control group there were 44 (61.9%) right and 27 (38.1%)

Table 2. The comparison of glenohumeral morphological parameters between Hill-Sachs and control groups

	Hill-Sachs n=39		Normal n=71		p
	Mean±SD	95% CI	Mean±SD	95% CI	
GI (°)	1.06±6.61	-1.08–3.20	4.33±6.20	2.84–5.72	0.011*
AHD (mm)	6.66±1.26	6.25–7.07	7.67±1.64	7.27–8.06	0.001*
AGD (mm)	31.44±3.45	30.32–32.56	32.63±3.88	31.64–33.56	0.114
CAD (mm)	40.24±3.99	38.95–41.53	38.10±3.80	37.28–39.07	0.008*
CGD (mm)	29.39±3.64	28.21–30.57	29.96±4.44	28.98–30.96	0.496
GH (mm)	33.90±2.34	33.14–34.66	35.03±3.29	34.26–35.81	0.039
GWs (mm)	19.89±5.89	17.98–21.80	19.83±2.37	19.27–20.40	0.042*
GWi (mm)	24.28±2.53	23.46–25.11	26.04±3.06	25.37–26.78	0.001*
GS (mm^2)	647.93±91.26	618.34–677.51	721.58±136.86	690.96–753.96	0.002*

GI: Glenoid inclication; AHD: Acromio-humeral distance; AGD: acromio-glenoid distance; CAD: Coraco-acromial distance; CGD: coraco-glenoid distance; GH: glenoid hight; GWs: glenoid superior width; GWi: Glenoid inferior width; GS: Glenoid surface; SD: Standard deviation; CI: Confidence interval; *: Mann Whitney U test was used.

left shoulder views. There was no significant difference between the groups according to the image laterality (p=0.825) and there was no significant difference between the groups according to age (p=0.223)

Intraclass correlation coefficent values between two observers were found as (0.93) for glenoid inclination, (0.89) for acromio-humeral distance, (0.78) for glenoid height, (0.80) for superior glenoid width, (0.90) for inferior glenoid width, (0.94) for coraco-acromial distance, (0.96) for coraco-glenoid distance and (0.96) for acromio-glenoid distance.

Measurements of a total of nine morphometric properties were made in both patient and control groups and compared statistically. These results and comparisons are shown in Table 2.

Glenoid inclication (β angle) was significantly smaller in the Hills-Sachs group compared to the control group (p=0.011). Acromio-humeral distance was significantly larger in the control group compared to the Hill-Sachs group (p=0.001). Acromio-glenoid distance tended to be smaller in the Hill-Sachs group than in the controls but this was not significant (p=0.114). Distance measurement between acromion and coracoid was significantly greater in the Hill-Sachs group (p=0.008). Coraco-glenoid distance tended to be larger in the control group but not significantly so (p=0.496). There was a significant difference between the groups for glenoid height (p=0.039), glenoid inferior width (p=0.001) and glenoid superior width (p=0.042) with the Hill-Sachs patients having lower values. The glenoid surface area was found to be significantly larger in the control group compared to the Hill-Sachs group (p=0.002).

DSCUSSION

Although there are different measurement methods and definitions of glenoid inclination, the most widely accepted definition is the angle between the vertical axis of the glenoid and the transverse axis of the scapula [19]. According to the method described by Mauerer et al. [13] it is calculated by subtracting the β angle from 90 degrees. Previous studies have reported this angle in healthy individuals to be 4.63°±4.86° [20], while Churcill et al. [21] reported a gender difference with the angle varying from 4.0°±3.4° in men to 4.5°±3.8° in women. The glenoid inclination has been evaluated as a risk factor for rotator cuff pathologies in several previous studies [19, 22, 23]. There are few studies investigating the relationship between glenohumeral instability and glenoid inclination. Hohmann et al. [24] determined the mean glenoid inclination to be 1.6°±5.9° in patients with glenohumeral instability and 4.0°±6.8° in control groups in their magnetic resonance imaging (MRI) study. Control group values and Hill-Sachs group values in our study are in agreement with these previous studies and in our cohort, as the value decreases, the glenoid tends towards the inferior. Our results suggest that an inferior change in glenoid inclination appears to be a risk factor for Hill-Sachs lesions.

The acromio-humeral distance has also often been associated with rotator cuff pathologies [25–27]. According to Goutallier et al. [28], distances below 6 mm are associated with rotator cuff tears, while values above 6 mm do not have diagnostic value for rotator cuff tears. In healthy shoulders without rotator cuff pathology, the acromio-humeral distance ranged between 6–14 mm [29]. Saygi et al. [30] using MRI reported that there was a significant difference in acromio-humeral distance between patients with glenohumeral instability and control groups. Although there are very few studies determining the relationship between acromio-humeral distance and glenohumeral instability, our study supports the study of Saygi et al. [30] In our study, the acromio-humeral distance was significantly smaller in the Hill-Sachs group. However in order to clarify the relationship of acromio-humeral distance measurement to Hill-Sachs, we think that further MRI studies are needed to exclude rotator cuff lesions since soft tissue pathologies cannot be ruled out in the CT studies.

Glenoid, as a combined morphology, has an important function in glenohumeral joint stability. Matsumura et al. [31] assumed that glenoid bone loss would be greater in recurrent glenohumeral dislocations. These authors therefore examined the contra-lateral shoulder and the dislocated shoulder, and confirmed that there was loss of glenoid bone in the shoulder with significant dislocation. Peltz et al. [32] reported that there was a significant difference in glenoid height and width between the contralateral glenohumeral joints of patients with glenohumeral instability. In contrast to previous studies, the width of the glenoid was measured in two places, superior and inferior, in the present sudy. In addition, we assumed that the glenoid was an ellipse, and calculated the surface area. Our results showed a significant difference in both glenoid height, superior glenoid with, inferior glenoid width and surface area in

Hill-Sachs patients. Thus, the present study demonstrates that glenoid bone loss is both supero-inferior and antero-lateral in patients with a Hill-Sachs lesion.

The effect of coraco-acromial arc morphometry on glenohumeral instability has been investigated by 3D reconstruction methods in recent years. Jacxsens et al. [17], compared the shoulders with instability and control groups and showed that the coracoid process was significantly shorter and the coraco-acromial distance increased in the group with instability. Lopez et al. [33] reported that the reduced coraco-acromial arch angle was associated with glenohumeral instability in a radiography study performed on 100 patients. There are few studies examining the glenohumeral relationship with coraco-acromial arch morphometry. In our study, it was found that the coraco-acromial distance significantly increased in patients with Hill-Sachs lesions, in agreement with the results of Jacxsens et al. [17] In addition, there was no significant relationship between coraco-glenoid and acromio-glenoid distances.

Conclusion

In conclusion, it was shown that glenoid, acromion, and coracoid process morphometry differed significantly in patients with Hill-Sachs lesions. In patients with Hill-Sachs lesions, acromio-humeral distance, glenoid inclination, glenoid height, glenoid width, and glenoid surface area decreased significantly, and coraco-acromial distance increased significantly. By identifying the differences in shoulder structure between individuals with Hill-Sachs lesion and healthy individuals the diagnosis, treatment and follow-up of these patients may be better understood by orthopedic specialists.

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Original Article EMERGENCY MEDICINE

The role of red cell distribution width in predicting 7-day in-hospital mortality in patients with acute ischemic stroke

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ABSTRACT

OBJECTIVE: Red cell distribution width (RDW) is a hematological parameter that is routinely obtained as part of the complete blood count analysis. An increased RDW level, which helps identify erythrocyte anisocytosis, is also a good indicator of the severity and outcome of some other diseases. The aim of this study was to investigate the predictive power of the serum RDW level for determining (seven-days mortality) of patients with acute ischemic stroke (AIS).

METHODS: A total of 527 adult patients with AIS presenting to our emergency department of tertiary-care hospital were retrospectively enrolled. The baseline characteristics, National Institute of Health Stroke Scale (NIHSS) and modified Rankin Scale (mRS) scores, reflecting stroke severity and neurological outcome, and serum RDW values were recorded retrospectively. An univariate and multivariate logistic regression analyses were performed to identify independent predictors of seven-days mortality, including serum levels of RDW, age, NIHSS and mRS values.

RESULTS: Serum RDW levels were significantly lower in patients who survived longer than seven-day compared with those who died within the first seven-days (15.36±1.04% v.s 16.61±1.28%, p=0.001). Furthermore, the mean NIHSS score was significantly lower in patients who survived longer than seven-days compared with those who died within the first seven-days. Additionally, the mean mRS score was significantly lower in patients who survived longer than seven-day compared with those who died within the first seven-day (p=0.001). Receiving operator characteristic analysis revealed a cut-off RDW level of 16.2%, with 66.1% sensitivity and 78% specificity, for distinguishing patients who survived longer than seven-days from who died within the first seven-day.

CONCLUSION: The serum RDW level may serve as a guide in diagnostic decision-making to predict neurological outcome and determine the short-term survival of patients with AIS.

Keywords: Acute ischemic stroke; red cell distribution width; hort-term survival.

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In 2015, 24.9 million patients worldwide were diagnosed with acute ischemic stroke (AIS), and there were 3.0 million deaths due to this disease [1]. According to the projections for 2030, an additional 3.4 million adults in the USA, representing 3.88% of the adult population, will have had a stroke. This means a 20.5% increase in prevalence from 2012 [2]. An ideal marker for the prediction of mortality and poor prognosis in this disease should be inexpensive, sensitive, specific, and easily accessible. It is an active research field to seek a parameter through blood tests to identify prognostic biomarkers for the mortality prediction in stroke cases. Red cell distribution width (RDW) is a measure of changes in the size of the erythrocyte life span of approximately 120 days [3]. Oxidative and inflammatory processes that have an effect on erythrocytes are considered to be the biological pathways that alter RDW [4]. This parameter is measured automatically as part of a complete blood count (CBC) analysis using Beckman Coulter electrical impedance method. The normal range of RDW can be a very useful clinical marker to demonstrate a healthy state. In-hospital mortality and morbidity are associated with the severity of diseases at admission, although it is also related to duration [5]. In patients with AIS, the timely detection and intervention of the severity of the disease is critical to delay its progression. AIS preserves its position as a paramount cause of in-hospital mortality and morbidity despite all advances in treatment. The determination of early and reliable estimation of functional loss after ischemic stroke should be seen as a precious approach to achieve accurate clinical and treatment management. The National Institutes of Health Stroke Scale (NIHSS) and the Modified Rankin Scale (mRS) scores are used to determine the severity of AIS. These scores have been improved to predict the mortality and degree of neurologic disability in AIS patients [6]. The uncertainty of biochemical parameters which can be used as an objective in the determination of the severity of AIS patients and the prognosis is one of the most important clinical issue. Therefore, we aimed to investigate the predictive power of the serum RDW level for determing the neurological outcome and the short-term prognosis (seven-day mortality) of patients with AIS.

Highlight key points

- Serum RDW level is an independent predictor of 7-day in-hospital mortality in patients with acute ischemic stroke (AIS); non-survivors had significantly higher RDW values compared with survivors (16.61±1.28% vs. 15.36±1.04%).
- An RDW cut-off value of 16.2% demonstrated strong discriminative ability for early mortality, with an AUC of 0.772, 66.1% sensitivity, and 78% specificity; patients with RDW ≥16.2% had nearly a 7-fold increased risk of 7-day mortality (OR: 6.912).
- Age, NIHSS score, mRS score, and RDW were identified as independent prognostic factors in multivariate analysis, indicating that incorporating RDW into early risk stratification may enhance clinical decision-making in AIS.

MATERIALS AND METHODS

Study Design and Setting

This study was conducted in accordance with the 1989 Declaration of Helsinki and was approved by the Ethics Committee of the Haydarpasa Numune Training and Research Hospital Faculty of Medicine, University of Health Sciences, HNEAH- KAEK (trial registration no. 2019/71-884). The study population in this retrospective, cross-sectional study consisted of 527 adult patients admitted to the emergency department (ED) of our tertiary care university hospital between January 2015 and June 2018, where they were diagnosed with AIS.

Using the medical record database, we retrospectively reviewed the baseline demographic characteristics, related clinical information, and CBC results (including RDW as a parameter). The baseline demographic characteristics and related clinical information had been obtained from each patient at the time of ED admission. Inclusion criteria; being over 18 years old and diagnosed as AIS in the emergency department. Patients with missing data, those with cancer or anemia (hematocrit <30% or hemoglobin <12 mg/dl for women, hemoglobin <13 mg/dl for men), those receiving ongoing anemia treatment at the time of admission, and those with a previous ischemic stroke within the last year were excluded in order to provide standardization and test the effect of the RDW level for in-hospital mortality. All CBC analyses were conducted using Mindray equipment (model BC-6800). Artificial intelligence–based computer programs were not used in this study.

Statistical Analysis

The Number SPSS 25 Cruncher Statistical System (Stanford University, USA) was used for statistical analysis. Numerical data (e.g. stroke scores; NIHSS; mRS value; and RDW level) are expressed as means±standard deviations (SD), and categorical variables (gender and age) as numbers (n). The suitability of the quan-

Table 1. Distribution of 527 adult patients according to the descriptive characteristics

Variables (%)	Value
Female	43.3 (n=228)
Male	56.7 (n=299)
Age (mean±SD)	68.21±13.51
7-day mortality	22.4 (n=118)
Hypertension	69.3 (n=365)
Atrial fibrillation	11
Coronary artery disease	25.4 (n=134)
Diabetes mellitus	41.7 (n=220)
Smoking	19.5 (n=103)

SD: Standard deviation.

Table 2. Distribution of RDW values, NIHSS score at admission – discharge, Modified Rankin Score and Modified Rankin prognosis

RDW	
Min–Max (Median)	12.3–18.9 (15.6)
Mean±SD	15.65±1.22
NIHSS entry score	
Min-Max (Median)	0-19 (4)
Mean±SD	5.36±4.07
Entry NIHSS ≤ exit NIHSS, n=527	
Good prognosis (%)	82.4
Poor prognosis (%)	17.6
Modifiye rankin score	
Min–Max (Median)	0–6 (2)
Modifiye rankin prognosis, n=527	
Good prognosis (%)	63.8
Poor prognosis (%)	36.2

RDW: Red cell distribution width; NIHSS: The National Institutes of Health Stroke Scale; mRS: Modified Rankin Scale; SD: Standard deviation; Min: Minimum; Max: Maximum.

titative data for normal distribution was tested using the Shapiro-Wilk test. Student-t test was used to compare normally distributed age, cholesterol, hematocrit, leukocyte, platelet, and RDW variables between two groups with and without mortality. The Mann-Whitney U test was conducted to compare NIHSS score and mRS at admission between the two groups. The Pearson chi-square test was used to compare the qualitative data. The Spearman correlation analysis was performed to evaluate the relationship between the NIHSS and mRS scores at admission and RDW, which were not normally distributed. Diagnostic screening tests (sensitivity, specificity) and the receiver operating characteristic (ROC) analysis were used to determine the predictive value for these parameters. The backward logistic regression analysis was undertaken to determine the risk factors affecting seven-day mortality. Confidence intervals were given at the 95% level and a value of $p<0.05$ was considered to indicate significance.

RESULTS

This study was conducted with 527 patients with AIS, of whom 43.3% (n=228) were female and 56.7% (n=299) were male. The ages of the subjects included in the study ranged between 23 and 98, and the mean age was 68.21±13.51 years. It was observed that 22.4% (n=118) of the cases died within seven days of admission. Hypertension was observed in 69.3% (n=365), atrial fibrillation (AF) in 11%, chronic artery disease in 25.4% (n=134), and diabetes mellitus in 41.7% (n=220). It was determined that 19.5% (n=103) of the subjects were smokers. The length of hospital stay ranged from 1 to 120 days with a median value of 8 as shown in Table 1. The RDW measurements of the subjects ranged from 12.3 to 18.9, with an average of 15.65±1.22. The NIHSS scores at entry range from 0 to 19, with a median value of 4. According to NIHSS the prognosis was good for 82.4% (n=434) of cases and poor for 17.6% (n=93). The mRS score of the cases ranged from 0 to 6, with a median value of 2. According to mRS a good prognosis was observed in 63.8% (n=336) and a poor prognosis in 36.2% (n=191) (Table 2). The in-hospital mortality group was significantly older and had a significantly higher frequency of diabetes mellitus ($p<0.001$ and $p=0.023$, respectively). The NIHSS and mRS scores of the patients with seven-day mortality were significantly higher than those without mortality ($p<0.001$ and $p<0.001$, respectively) (Table 3). The RDW values of seven-day mortality were significantly higher than those without mortality ($p<0.001$) as shown in Figure 1. The RDW values of the patients with a poor prognosis were significantly higher than those with a good prognosis according to the NIHSS scores ($p<0.001$). A weak significant correlation was found between the RDW values and the NIHSS scores at admission (the NIHSS score

Table 3. Evaluation of seven-day mortality according to descriptive characteristics

	7-day mortality (-) (n=409)	7-day mortality (+) (n=118)	P
Age, Mean±SD	65.41±12.80	77.91±11.29	[a]0.001**
Gender (%)			[b]0.210
Female	75.0	25.0	
Male	79.6	20.4	
Smoking (%)			[b]0.420
-	76.9	23.1	
+	80.6	19.4	
HT (%)			[b]0.537
-	75.9	24.1	
+	78.4	21.6	
AF (%)			[b]0.507
-	77.2	22.8	
+	81.0	19.0	
CAD (%)			[b]0.472
-	78.4	21.6	
+	75.4	24.6	
DM (%)			[b]0.023*
-	81.1	18.9	
+	72.7	27.3	
Hematocrit, Mean±SD	40.57±4.67	40.41±5.17	[a]0.738
Leukocyte, Mean±SD	8929.07±2916.97	9312.20±2893.39	[a]0.209
Platelets, Mean±SD	239792.91±71686.20	244092.37±76902.82	[a]0.573
NIHSS entry score, Median (Q1–Q3)	4 (2–6)	7 (4–12)	[c]0.001**
Modified rankin score, Median (Q1–Q3)	1 (1–3)	5 (2–6)	[c]0.001**
RDW, Mean±SD	15.36±1.04	16.61±1.28	[a]0.001**

SD: Standard deviation; HT: Hypertension; AF: Atrial fibrillation; CAD: Coronary artery diseases; DM: Diabetes mellitus; NIHSS: The National Institutes of Health Stroke Scale; RDW: Red cell distribution width; mRS: Modified Rankin Scale.

at admission increased as RDW increased) (r=0.194; p<0.001). The RDW values of the patients with a poor prognosis were statistically higher than those with modified prognosis with a good prognosis according to the mRS scores (p<0.001). A positive significant correlation was determined between the RDW values and the mRS scores, in which the latter increased in parallel to the former (r=0.254; p<0.001; p<0.001) (Table 4). When the variables that were considered to have an effect on seven-day mortality are evaluated by the backward (conditional) logistic regression analysis, it was seen that the model provided significant results, and the explanatory

Table 4. The correlation of RDW with NIHSS and Modified Rankin Scale scores

Variable	Correlation coefficient (r)	p
RDW vs NIHSS	0.194	<0.001
RDW vs mRS	0.254	<0.001

RDW: red cell distribution width; NIHSS: National Institutes of Health Stroke Scale; mRS: Modified Rankin Scale; Student's t-test; r = Spearman's correlation coefficient; P<0.01.

Table 5. Independent predictors of seven-day mortality in patients with AIS identified in univariate and multivariate logistic regression analysis

Variable	OR	95% CI	p
Age	1.074	1.045–1.103	<0.001
RDW	2.203	1.677–2.893	<0.001
mRS	2.021	1.704–2.396	<0.001

AIS: Acute ischemic stroke; OR: Odds ratio; CI: Confidence interval; RDW: Red cell distribution width; DM: Diabetes mellitus; RDW: Red cell distribution width; mRS: Modified Rankin Scale. The values of the last observed step are given in the model.

coefficient of the model (88.4%) was good (p<0.001; p<0.01). At the end of the fourth step, the effects of age, RDW and mRS scores on seven-day mortality were statistically significant (p<0.001; p<0.001; p<0.001). The remaining variables that were analyzed in the model were found to have non-significant effects although they provided significant or close to significant results in univariate evaluations (p>0.05). According to the model, the seven-day mortality risk was increased 1.074 times by one unit increase in age (95% confidence interval (CI): 1.045–1.103], 2.203 times by one unit increase in RDW (95% CI: 1.677–2.893), and 2.021 times by one unit increase in mRS (95% CI: 1.704–2.396) (Table 5). The cut-off point for RDW was calculated as 16.2, at which the sensitivity of this parameter was 66.1%, specificity was 78%, positive predictive value was 46.4%, and negative predictive value was 88.9%. The ROC curve obtained for RDW revealed 77.2%. The risk of seven-day mortality was 6.912 times greater (95% CI: 4.419–10.810) in patients with an RDW of 16.2 or above (Fig. 2).

DISCUSSION

In the present study, RDW emerged as a reliable biomarker for predicting seven-day mortality among patients with acute ischemic stroke (AIS). While previous research has demonstrated the prognostic value of RDW in estimating 28-day and one-year mortality in AIS populations [7, 8], our study is the first, to our knowledge, to establish its utility in forecasting early (within seven days) mortality. Accurate early prediction of stroke-related mortality holds significant clinical importance for optimizing patient management and resource allocation. Given its low cost and widespread availability, incorporating RDW

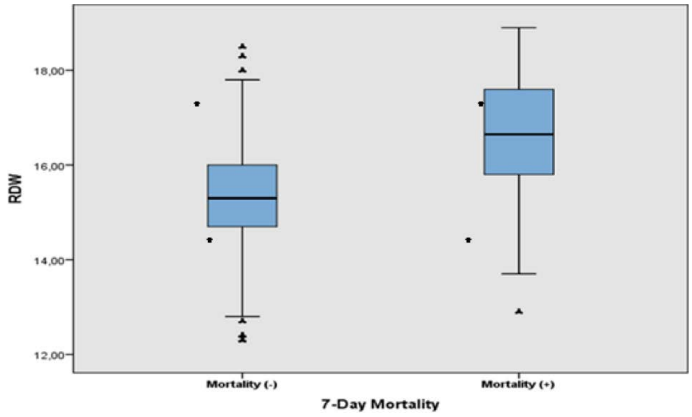

Figure 1. Distribution of RDW levels in patients who survived longer than seven-day (mortality-) and those who died within the first seven-day (mortality+).

RDW: Red cell distribution width.

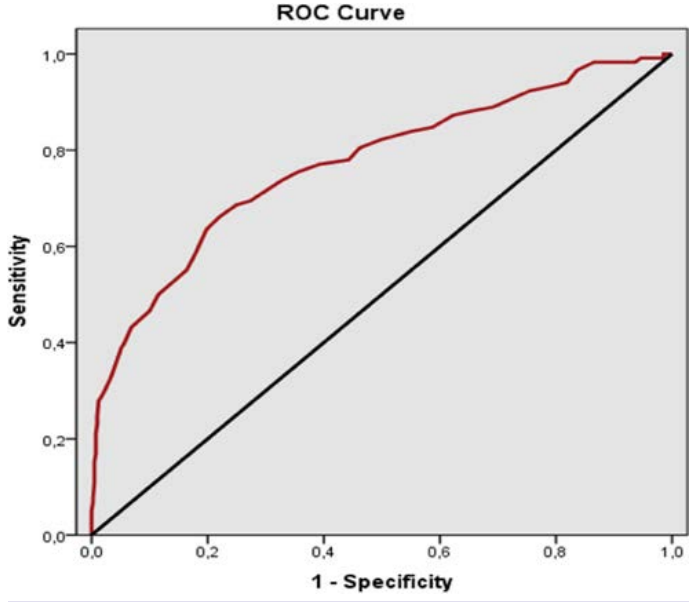

Figure 2. Receiver operating characteristic (ROC) curve of RDW for predicting 7-day mortality. Area under the curve (AUC)=0.772. Patients with RDW ≥16.2% had an odds ratio (OR) of 6.912 (95% CI: 4.419–10.810) for 7-day mortality.

measurement into the initial evaluation of AIS patients may offer a practical tool for risk stratification and potentially inform more tailored therapeutic strategies.

The RDW level has been shown to be effective in predicting cardiovascular diseases [9], acute kidney injury [10], trauma [11], and cancer [12]. However, the literature contains limited data on the relationship between the RDW level and in-hospital mortality in patients with AIS. At the same time, most available studies an-

alyzed RDW as a predictor of long-term mortality. In the long-term follow-up of patients, various factors, such as the quality of hospital care, uncontrolled chronic diseases, and post-stroke infections can have an effect on the mortal course of the disease, which interferes with the ability of RDW to predict AIS mortality [13]. Furthermore, RDW is severely affected by conditions, such as anemia and cancer, and less affected by environmental factors [14]. Elevated RDW has long been used in the classification of anemia or the diagnosis of iron deficiency anemia [15]. In a study examining cases with malignant and benign breast cancer, the RDW value was shown to be significantly increased in the former compared to the latter [16]. Similarly, another study evaluating cases with malignant and benign uterine lesions found RDW to be higher in patients with malignant lesions, although this parameter was increased in both groups [17]. In addition, malignancy is frequently accompanied by gastrointestinal dysfunction and malnutrition. This coexistence is considered to cause iron, folic acid and vitamin B12 deficiencies, which may lead to anemia and ultimately an increase in RDW [18]. In the light of these findings reported in the literature, in the current study, we excluded AIS cases with a diagnosis of anemia or cancer, which are known to alter RDW levels. In the literature, however, in studies investigating the efficacy of RDW in predicting 28-day and one-year mortality in AIS cases, diseases such as anemia and cancer that alter the level of RDW were not excluded [7–13]. In this context, we consider that by eliminating the effect of other diseases on the RDW level, we consider that our study provides clearer data about the isolated mortality outcomes. While several previous studies have explored RDW in predicting 28-day or long-term mortality in AIS patients [7–8, 13], there remains a gap in literature concerning its predictive utility for early-period mortality. This study uniquely addresses that gap by focusing on 7-day outcomes. From a clinical perspective, early identification of high-risk AIS patients based on RDW levels may assist emergency physicians in triage decisions, determining intensive monitoring needs, and possibly guiding early ICU admission.

Although the underlying mechanisms linking elevated RDW to poor outcomes in AIS patients remain incompletely understood, several hypotheses have been proposed to clarify its prognostic relevance [4]. One prevailing theory centers on the role of systemic inflammation, which may contribute to increased RDW levels and heightened mortality risk in AIS. Inflammatory processes are known to disrupt iron metabolism and suppress bone marrow function, thereby impairing erythropoiesis. Furthermore, proinflammatory cytokines may interfere with normal erythrocyte maturation, resulting in a heterogeneous population of red blood cells with diminished deformability [19, 20]. These structurally compromised erythrocytes exhibit impaired flow dynamics, potentially promoting thrombotic events and contributing to the pathophysiology of acute ischemic stroke [21]. In most laboratories, the normal reference range of RDW is 11–15% [9]. In a study of ischemic stroke, the cut-off value of RDW in predicting stroke was determined as 14% [AUC: 0.760; CI: 0.676–0.844] [22]. Another previous study demonstrated the efficacy of an RDW of greater than 15% in predicting adverse neurological outcomes in patients with subarachnoid hemorrhage [23]. In another study conducted with acute pancreatitis cases, the RDW cut-off value for 30-day mortality was reported to be 14% (AUC: 0.842) [24]. A baseline RDW of >15.5% was also independently associated with mortality in patients undergoing aortic valve implantation (1.83, CI: 1.44–2.32) [25]. However, only few studies have been conducted to reveal the relationship between stroke-related mortality and RDW, and no data is available on the cut-off value of RDW in predicting mortality among the existing studies. Thus, our study is the first in the literature to specify a cut-off value for the RDW prediction of seven-day mortality in AIS patients. In this study, we calculated this value as 16.2 (AUC: 0.772) and found that the seven-day mortality risk was 6.912 times greater in patients with an RDW of ≥16.2 (95% CI: 4.419–10.810). In our study, in addition to RDW, age and NIHSS score, the mRS score was found to be an independent prognostic factor in mortality.

In this study, we investigated the link between poor prognosis and RDW in patients with high mRS and NIHSS scores, and found that elevated RDW was significantly correlated with cerebral ischemic complications. The present data are consistent with the results of a previous study in subarachnoid hemorrhage patients that showed a significant relationship between mRS and NIHSS scores and RDW in the univariate analysis [26]. Furthermore, Turcato et al. [27] revealed RDW, age and NIHSS score as the main determinants according to the nomogram. This was confirmed by our results and found to be important and independent determinants of unfavorable outcome. The measurement of RDW, which is a routinely obtained hematologic parameter in emergency department, together with the above mentioned scores can be more efficient than using the scores alone in evaluating the prognosis of patients with AIS.

Limitations

This study has several limitations, the foremost being its retrospective nature and single-center setting, which may limit the generalizability of the findings. Additionally, RDW levels were measured at a single time point—upon patient admission to the emergency department—which restricts the ability to assess dynamic changes in this parameter over time. These methodological considerations should be addressed in future prospective, multicenter studies involving AIS populations. Nevertheless, a notable strength of this study lies in the exclusion of patients with acute anemia and malignancies, which helped to minimize potential confounding factors and enhance the internal validity of the results.

Conclusion

The results of logistic regression analysis revealed that serum RDW level was independently associated with seven-day mortality in patients with AIS. Furthermore, the receiver operating characteristic analysis showed that the best cut-off value of RDW for the prediction of seven-day mortality was 16.2%, at which its sensitivity and specificity were 66.1% and 78%, respectively. For rapid and accurate clinical decision-making, the serum RDW level might help predict neurological outcomes and determine the short-term survival of patients with AIS. Further controlled clinical trials involving larger numbers of subjects are warranted.

Furthermore, our study did not include inflammatory biomarkers such as C-reactive protein (CRP) or ferritin, which could act as unmeasured confounders influencing RDW levels. Future studies should consider these factors to enhance model accuracy.

Ethics Committee Approval: The Haydarpasa Numune Training and Resarch Hospital granted approval for this study (date: 16.07.2019, number: 2019/71).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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

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Use of AI for Writing Assistance: The authors declared that artificial intelligence-supported technologies (such as Large Language Models [LLMs], chatbots or image generators, ChatGPT) were not used in the production of the study.

Authorship Contributions: Concept – MAA, KY, IT, AA; Design – BGY, OT, MAA; Supervision – SD, AA, BGY; Fundings – MAA, BGY, SD; Materials – MAA, IT, OT; Data collection and/or processing – MAA, AA, BB; Analysis and/or interpretation – MAA, BGY, KY; Literature search – MAA, IT, SD; Writing – MAA, OT, BB; Critical review – KY, MAA, AA.

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Original Article PULMONOLOGY

The relationship of impulse oscillometry results with respiratory muscle strength and exercise capacity in patients with emphysema

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ABSTRACT

OBJECTIVE: Assessment of small airway function, respiratory mechanics, respiratory muscle strength, and exercise capacity plays a crucial role in both pharmacological and non-pharmacological treatment planning, as well as in evaluating treatment efficacy in patients with emphysema. Impulse oscillometry (IOS) allows for the evaluation of the physiological properties of both large and small airways, along with lung parenchyma involvement. This study aimed to in*vestigate small airway function and respiratory mechanics related to lung parenchyma using IOS and to examine their relationship with respiratory muscle strength and exercise capacity in emphysema patients.

METHODS: Thirty patients diagnosed with emphysema by high-resolution computed tomography (HRCT) and airway obstruction based on GOLD (Global Initiative for Chronic Obstructive Lung Diseases) spirometric criteria were included in this study. Demographic characteristics, IOS parameters, respiratory muscle strength (MIP, MEP), and exercise capacity (Endurance Shuttle Walk Test [ESWT], VO_2 peak) were analyzed. The study was approved by the Ankara Kecioren Training and Research Hospital Ethics Committee (approval number: 2012-KAEK-15/2210) and conducted in accordance with the Declaration of Helsinki.

RESULTS: There was a statistically significant positive correlation between GOLD spirometric stages and IOS parameters, particularly the resistance difference at 5 and 20 Hz (R5-R20) and reactance area (AX). A significant negative correlation was observed between GOLD stages and reactance at 5 Hz (X5), maximal inspiratory pressure (MIP), ESWT, and VO_2 peak. Additionally, MIP was negatively correlated with R5, R5-R20, and AX, but positively correlated with X5. VO_2 peak was negatively correlated with R5, R20, R5-R20, and AX, while it was positively correlated with X5, MIP, and maximal expiratory pressure (MEP). ESWT was negatively correlated with R5, R5-R20, and AX, and positively correlated with X5.

CONCLUSION: This study demonstrated that emphysema involves impairment of small airways and lung parenchyma, which can be effectively assessed using IOS. The impaired respiratory mechanics significantly decrease exercise capacity. Prioritizing therapies that target small airways may enhance pharmacological outcomes, and the observed correlation between IOS parameters and respiratory muscle strength may provide guidance for inhaler selection in clinical practice.

Keywords: Emphysema; exercise capacity; muscle strength; oscillometry.

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Chronic Obstructive Pulmonary Disease (COPD) is a common, preventable, and treatable disease characterized by persistent airflow limitation and respiratory symptoms, related to structural changes in the airways and alveoli resulting from long-term exposure to harmful gases and particles [1]. The primary patho-

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physiological feature of COPD, namely airflow limitation, arises from small airway disease and lung parenchymal destruction (emphysema), both of which can vary between individuals [1].

Emphysema, a clinical and pathological phenotype of COPD, is characterized by the irreversible enlargement of airspaces distal to the terminal bronchioles and destruction of the surrounding lung parenchyma [2]. In such patients, pathophysiological changes including decreased elastic recoil, mechanical disadvantage of the diaphragm due to hyperinflation, reduced dynamic compliance, and peripheral muscle weakness due to chronic inflammation are commonly observed. These mechanisms contribute to the development of clinical manifestations such as dyspnea, reduced exercise capacity, and impaired quality of life [3, 4].

Impulse oscillometry (IOS) is a technique that evaluates respiratory function using sound waves, allowing measurement of the physiological properties of both large and small airways, as well as lung parenchyma. Although applicable to all age groups due to its independence from patient effort and cooperation, the literature regarding its use in adult pulmonary diseases remains limited [5, 6].

Studies have shown that, in addition to alveolar wall damage, there is a loss of elastic fibers in the small conducting airways. This suggests that structural damage is not limited to the alveoli but also affects the small airways [7]. In emphysema, evaluating small airway function, respiratory mechanics, respiratory muscle strength, and exercise capacity is crucial for determining both pharmacological and non-pharmacological treatment needs, as well as for monitoring treatment effectiveness.

This prospective observational study aims to assess small airway functions and lung parenchyma-related respiratory mechanics using IOS in individuals diagnosed with emphysema, and to investigate the relationship between these results and respiratory muscle strength and exercise capacity.

Highlight key points

- IOS effectively reflects peripheral airway dysfunction in emphysema.
- Key parameters (X5, AX, R5–R20) correlate with inspiratory muscle strength and exercise capacity.
- Reactance values (X5, AX) are superior to resistance values for assessing disease severity.

MATERIALS AND METHODS

Study Population

This study was conducted with 30 patients over the age of 18 who presented to the Pulmonary Rehabilitation Clinic of the University of Health Sciences, Ankara Ataturk Sanatorium Training and Research Hospital between January 28, 2021, and March 2021, and were diagnosed with emphysema by high-resolution computed tomography (HRCT).

Patients were excluded if they were undergoing treatment for malignancy, had contraindications for exercise testing or respiratory muscle strength measurement [8, 9], exhibited significant parenchymal pathology other than emphysema on HRCT, or were unable to provide informed consent.

For each participant, data were collected on age, sex, comorbidities, smoking history (pack-years), and the use of long-term oxygen therapy. Pulmonary function test (PFT) results were achieved from patient records, and values for forced expiratory volume in one second (FEV1), forced vital capacity (FVC), and the FEV1/FVC ratio were recorded. Based on these results, each patient was staged according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) and documented in the study form.

The activity level causing dyspnea was assessed for all patients, and the results were evaluated using the Modified Medical Research Council (mMRC) dyspnea scale. Each patient's score on the scale was recorded in the study form [10].

Throughout the study, infection control measures were rigorously implemented. A minimum interval of 15 minutes was maintained between each patient, and the testing room was regularly ventilated. Single-use mouthpieces and filters were used for each test and were disposed of in medical waste bins immediately afterward. Devices used during testing were disinfected in accordance with the manufacturer's instructions.

This study was approved by the Clinical Research Ethics Committee of Ankara Kecioren Training and Research Hospital on 26 January 2021 (approval number: 2012-KAEK-15/2210). The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all participants in compliance with institutional and national ethical guidelines.

Impulse Oscillometry

IOS is a method used to assess respiratory function by measuring airway resistance (R) and reactance (X) parameters through sound waves. IOS measurements are performed during tidal breathing [5]. The test is conducted using a device system comprising a loudspeaker, adapter, pneumotachograph, transducer, and computer. Low-pressure sound waves of various frequencies, generated by the loudspeaker, are delivered to the airways via the mouth. These sound waves superimpose onto the patient's spontaneous breathing, and the signals reflected from the airways are analyzed using software linked to pressure and flow transducers. The frequency of sound waves used in IOS typically ranges from 5 to 35 Hz (Hertz).

Impedance (Z), which reflects the overall mechanical response of the respiratory system, represents the total force required to transmit a pressure wave and consists of R and X components [11].

Resistance is defined as the energy required to maintain airflow in the area through which the sound wave is transmitted. Total airway resistance is evaluated using the resistance at 5 Hz (R5), while proximal airway resistance is represented by resistance at 20 Hz (R20). Peripheral airway resistance is calculated as the difference between R5 and R20 Hz (R5–R20) [11].

The area of reactance (AX) represents the area under the reactance curve between 5 Hz and the resonant frequency (Fres), providing information about the elastic properties of the respiratory system.

In this study, respiratory function was assessed using the Carefusion Vyntus Jaeger IOS device (Germany). The IOS device was checked and calibrated daily. For each patient, values for R5, R20, R5–R20, reactance at 5 Hz (X5) and AX were obtained and recorded in the study form [5].

To avoid influencing patient performance, IOS measurements were conducted prior to exercise testing. All tests were performed in accordance with the guidelines of the European Respiratory Society (ERS) [12]. During the test, patients were seated in an upright and comfortable position with their head in a neutral position. The mouthpiece was fully sealed with the lips to prevent air leakage. To compensate for the compliance of the cheeks, the patients placed their hands firmly over them, and the nose was occluded using a nasal clip.

Respiratory Muscle Strength

Respiratory muscle strength was assessed using a portable electronic mouth pressure measurement device (Micro RPM, CareFusion, UK). For the measurement of maximal inspiratory pressure (MIP), patients were instructed to perform a maximal inspiratory effort lasting 1–3 seconds following a full expiration, while wearing a nose clip to occlude nasal airflow and sealing the mouthpiece tightly with their lips. For the measurement of maximal expiratory pressure (MEP), after a full inspiration, patients were similarly asked to perform a maximal expiratory effort lasting 1–3 seconds, using a nose clip and ensuring proper sealing of the mouthpiece.

Assessment Of Exercise Capacity

The Incremental Shuttle Walk Test (ISWT) and the Endurance Shuttle Walk Test (ESWT) were used to assess the patients' exercise capacity. Both tests were performed in accordance with the 2014 guidelines of the ERS [13]. The tests were conducted indoors on a flat, hard surface. Two cones were placed 9 meters apart.

Patients were instructed to start walking upon hearing an audio signal from a speaker, walk around the cone, wait for the next signal, and continue walking back and forth between the cones at progressively increasing speeds according to the audible signals.

Before the tests, patients were given a minimum of 15 minutes to rest. Baseline measurements were taken, including resting blood pressure, heart rate, oxygen saturation, dyspnea, and fatigue scores.

At the end of the test, the patient was seated and post-exercise measurements were recorded, including oxygen saturation, heart rate, blood pressure, and the severity of dyspnea and fatigue. The total distance walked and the estimated predicted peak oxygen uptake (VO2 peak) value calculated from this distance are recorded. Based on 85% of the estimated VO_2 peak, the target walking speed was determined. The patient was then instructed to walk between the cones at this fixed speed, guided by audio signals. The total walking duration was recorded in the study form.

Statistical Analysis

The demographic and clinical characteristics of patients who met the study criteria were evaluated using descriptive statistical values such as frequency (n), percentage (%), mean, and standard deviation. The relationship between IOS results and respiratory muscle strength and

Table 1. Results of patients

	Mean±SD	Median	Minimum	Maximum
Age (year)	59.17±8.26	58.50	46	75
Smoking history (p/y)	43.43±26.61	40	0	120
mMRC score	1.70±0.53	2	1	3
FEV_1 %	59.03±24.55	62.00	25	115
FVC %	77.90±20.34	80.50	30	125
FEV_1/FVC	58.43±15.65	61.10	26.9	87.76
GOLD stage	2.30±1.05	2	1	4
R5 (kPa/L/s)	0.45±0.17	0.44	0.20	1.00
R20 (kPa/L/s)	0.28±0.8	0.27	0.16	0.45
R5-R20 (kPa/L/s)	0.16±0.14	0.14	0.01	0.72
X5 (kPa/L/s)	-0.17±0.10	-0.17	-0.39	0.02
MIP (cmH_2O)	78.27±16.07	81	50	103
MEP (cmH_2O)	116.40±26.71	113	60	166
ISWT (meter)	371.33±119.42	365	180	600
ESWT (minute)	9.33±6.87	6	2.15	20
VO_2 peak (ml/kg/min)	13.47±2.99	13.31	8.69	19.19

p/y: Package/year; mMRC: Modified Medical Research Council; FEV_1: Forced expiratory volume in one second; FVC: Forced vital capacity; GOLD: Global Initiative for Chronic Obstructive Lung Disease; IOS: Impulse oscillometry; R5; Resistance at 5 Hz; R20: Resistance at 20 Hz; R5-R20:Difference between R5 and R20; X5: Reactance at 5 Hz; AX: The area of reactance; MIP: Maximal inspiratory pressure; MEP: Maximal expiratory pressure; ISWT: Incremental Shuttle Walk Test; ESWT: Endurance Shuttle Walk Test; VO_2 peak: Predict peak oxygen uptake; SD: Standard deviation.

exercise capacity was analyzed using Pearson correlation analysis. In addition, the assumptions of normal distribution were verified using the Shapiro-Wilk test prior to performing Pearson correlation and regression analyses. To assess whether IOS parameters could predict respiratory muscle strength and exercise capacity scores, simple linear regression analysis was performed.

Given the limited number of studies directly focusing on the subject of this research, no reliable reference for the expected effect size could be identified. Consequently, it was not feasible to define a hypothetical effect size for conducting a formal power analysis. The study was therefore designed based on available resources and field conditions. Due to time and financial constraints, the analysis was planned to include data from a minimum of 30 participants. This sample size was determined by considering the minimum number of participants required to allow for the valid application of parametric statistical methods under the study conditions. This limitation confers a pilot character to the study and is intended to provide preliminary insights that may guide future research involving larger sample sizes.

A significance level of $p<0.05$ was considered statistically significant for all analyses. Statistical analyses were performed using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY, USA).

RESULTS

A total of 30 patients (29 males, 1 female) who presented to the pulmonary rehabilitation clinic and met the study criteria were included. The average smoking history was 43.43±26.61 pack-years, with a median value of 40 pack-years (Table 1).

According to GOLD spirometric staging based on FEV_1%, 8 patients (26.7%) were in stage 1, 10 (33.3%) in stage 2,7 (23.3%) in stage 3, and 5 (16.7%) in stage 4 (Table 1).

Among the identified comorbidities were congestive heart failure in 2 patients (6.7%), hyperlipidemia in 2 (6.7%), diabetes mellitus (DM) in 4 (13.3%), benign prostatic hyperplasia (BPH) in 3 (10%), hypertension in 9 (30%), and atherosclerotic heart disease (ASHD) in 6 (20%). Four patients were receiving long-term oxygen therapy.

Table 2. Correlation between impulse oscillometry parameterswith mMRC, GOLD spirometric stages, respiratory muscle strength and exercise capacity results of patients

	R5 (kPa/L/s)	R20 (kPa/L/s)	R5-R20 (kPa/L/s)	X5 (kPa/L/s)	AX
mMRC score					
r	0.130	0.027	0.149	-0.270	0.198
p	0.492	0.887	0.432	0.149	0.294
GOLD stage					
r	0.244	-0.100	0.401	-0.443	0.500
p	0.193	0.597	**0.028**	**0.014**	**0.005**
MIP (cmH_2O)					
r	-0.426	-0.277	-0.389	0.606	-0.526
p	**0.019**	0.138	**0.034**	**<0.001**	**0.003**
MEP (cmH_2O)					
r	-0.305	-0.335	-0.255	0.342	-0.305
p	0.101	0.070	0.174	0.064	0.101
ISWT (meter)					
r	-0.584	-0.372	-0.406	0.454	-0.502
p	**<0.001**	**0.043**	**0.026**	**0.012**	**0.005**
ESWT (minute)					
r	-0.502	-0.162	-0.391	0.379	-0.472
p	**0.005**	0.391	**0.032**	**0.039**	**0.008**
VO_2 peak (ml/kg/min)					
r	-0.584	-0.372	-0.406	0.454	-0.502
p	**<0.001**	**0.043**	**0.026**	**0.012**	**0.005**

mMRC: Modified Medical Research Council; GOLD: Global Initiative for Chronic Obstructive Lung Disease; MIP: Maximal inspiratory pressure; MEP: Maximal expiratory pressure; ISWT: Incremental Shuttle Walk Test; ESWT: Endurance Shuttle Walk Test; VO_2 peak: Predict peak oxygen uptake; IOS: Impulse oscillometry; R5; Resistance at 5 Hz; R20: Resistance at 20 Hz; R5-R20: Difference between R5 and R20; X5: Reactance at 5 Hz; AX: The area of reactance; r: Pearson correlation. Statistically significant p-values were given as bold.

The mean R5–R20 value was 0.16±0.14 kPa/L/s, and in 80% of the patients this value was above 0.07. The mean AX value was 1.84±1.36, with 86.6% of patients having an AX value above 0.33 (Table 1).

The mean MIP was 78.27±16.07 cmH_2O; no patients had a value below 30 cmH_2O. The mean MEP was 116.40±26.71 cmH_2O, with no patients scoring below 40 cmH_2O (Table 1).

According to the statistical analysis results (Table 2).

A significant negative correlation was found only between smoking history and the X5 parameter (p=0.046, r=-0.367).

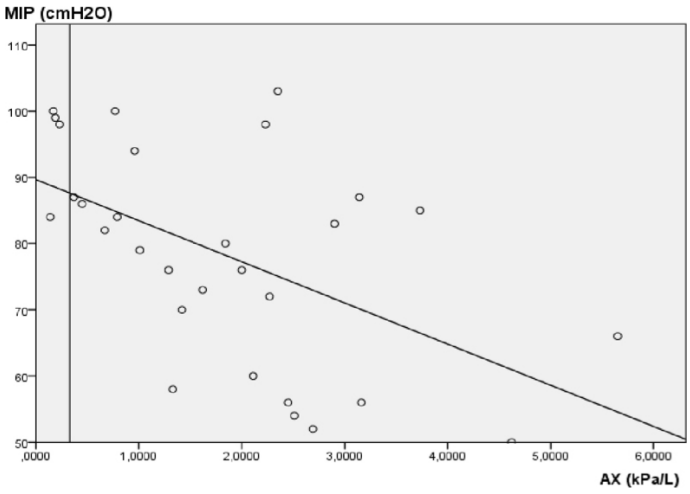
Although observational associations were noted between dyspnea severity and increased airway resistance, decreased elastic recoil, and compliance, no statistically significant relationship was found between mMRC dyspnea score and IOS parameters (p>0.05).

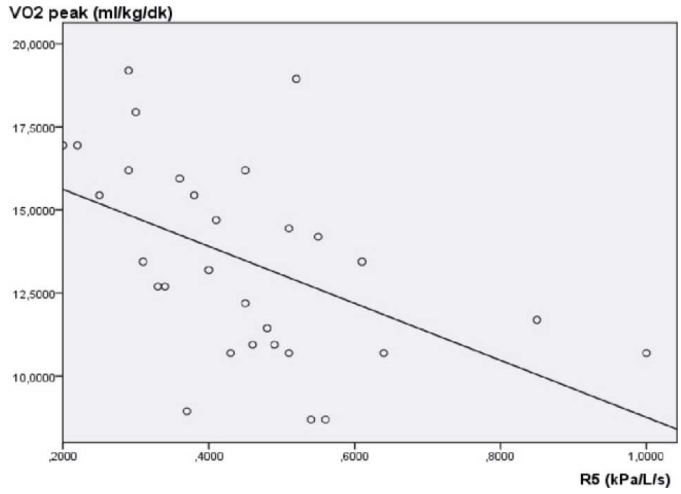
Significant positive correlations were identified between GOLD stage and both R5–R20 (p=0.028, r=0.401) and AX (p=0.005, r=0.500), while a significant negative correlation was found with X5 (p=0.014, r=-0.443).

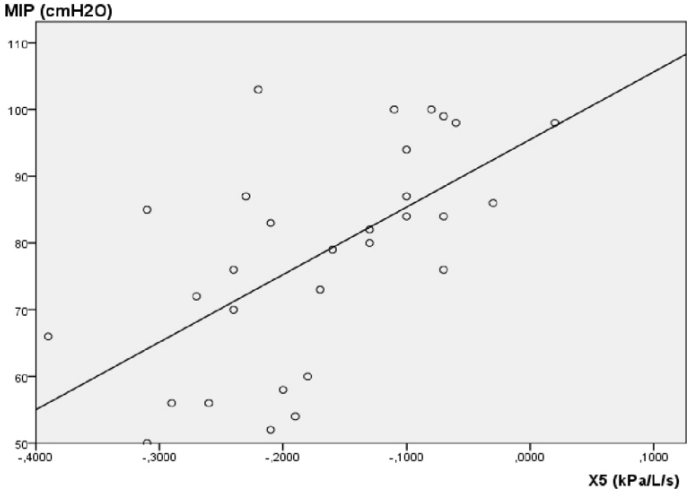
A significant negative correlation was observed between GOLD stage and MIP (p=0.045, r=-0.369).

Significant negative correlations were found between GOLD stage and ISWT distance (p=0.025, r=-0.409), ESWT duration (p=0.015, r=-0.441), and VO_2 peak (p=0.025, r=-0.409).

MIP showed significant negative correlations with R5 (p=0.019, r=-0.426), R5–R20 (p=0.034, r=-0.389),







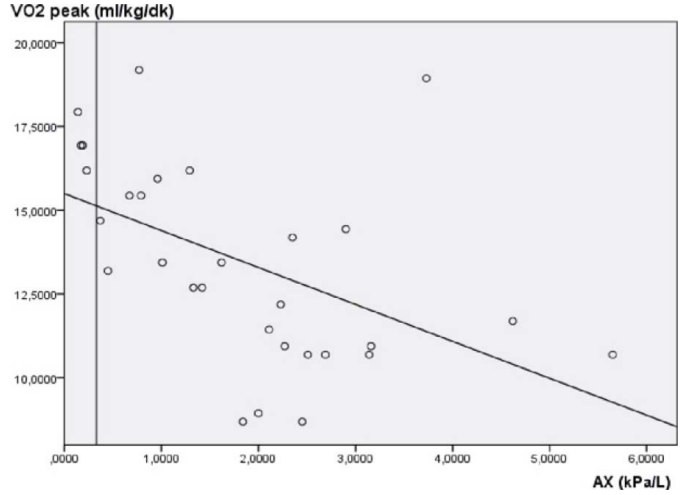


FIGURE 1. Correlation between X5 and AX with MIP. Scatter plot.

MIP: Maximal inspiratory pressure; X5: Reactance at 5 Hz; AX: Reactance area.

FIGURE 2. Correlation between R5 and AX with VO2 peak. Scatter plot.

R5: Resistance difference at 5; AX: Reactance area; VO2 peak: Predict peak oxygen uptake.

and AX (p=0.003, r=-0.526), and a significant positive correlation with X5 (p<0.001, r=0.606) (Fig. 1).

No statistically significant relationship was found between IOS parameters and MEP (p>0.05).

Significant positive correlations were observed between both MIP and MEP with VO_2 peak (p<0.001, r=0.637; p=0.039, r=0.379, respectively).

VO_2 peak showed significant negative correlations with R5 (p<0.001, r=-0.584), R20 (p=0.043, r=-0.372), R5–R20 (p=0.026, r=-0.406), and AX (p=0.005, r=-0.502); and a significant positive correlation with X5 (p=0.012, r=0.454) (Fig. 2).

ESWT duration was significantly negatively correlated with R5 (p=0.005, r=-0.502), R5–R20 (p=0.032, r=-0.391), and AX (p=0.008, r=-0.472); and positively correlated with X5 (p=0.039, r=0.379).

DISCUSSION

This study investigated the relationship between IOS parameters, respiratory muscle strength and exercise capacity in patients with emphysema. The greatest changes were observed in X5, AX, and R5–R20, respectively. These results suggest that small airways, elastic recoil, and compliance are more prominently affected than total airway resistance. It was also demonstrated that the increase in resistance is more pronounced in small airways compared to large airways.

The need for evaluating small airways and respiratory mechanics in patients with emphysema has brought attention to the use of the IOS, which allows measurement during tidal breathing with minimal patient cooperation. Although studies on the use of IOS in COPD have increased in recent years, the number of studies focusing specifically on IOS-based assessments of respiratory function and mechanics in emphysematous patients remains limited in the literature [11].

Li et al. [14] reported no significant correlation between mMRC dyspnea scores and pulmonary function test results; however, correlations were reported for IOS parameters R5, R5–R20, and AX. A significant relationship was also identified between CAT (COPD Assessment Test) scores and R5. In the present study, although increased airway resistance, reduced elastic recoil, and decreased compliance were associated with greater dyspnea perception, no statistically significant correlation was found between mMRC scores and IOS parameters. This may be explained by the unidimensional nature of the mMRC scale and the influence of extrapulmonary factors on dyspnea perception.

Gong et al. [15] have suggested that changes in X5 may be useful for monitoring COPD progression. Several studies have reported that reactance measurements are more strongly correlated with pulmonary function than resistance measurements [15]. Consistent with this, our study identified significant correlations between GOLD spirometric stages and X5, AX, and R5–R20, with higher correlation coefficients for reactance parameters compared to resistance parameters. These findings indicate that as disease severity increases, small airway resistance increases while elastic recoil decreases.

A negative association was observed between GOLD stage and exercise capacity. Notably, the correlation coefficient was higher for endurance test duration compared to VO_2 peak, supporting the hypothesis that dynamic hyperinflation is a major mechanism of dyspnea in emphysematous patients [7].

In the study by Kim et al. [16], reductions in MIP and MEP values were reported in COPD patients, with a significant negative correlation between MIP and GOLD stage, while no significant correlation was found for MEP. Similarly, in our study, MIP decreased with advancing GOLD stage, while MEP remained unchanged.

Frantz et al. [17] observed increased pulmonary resistance and reduced reactance in symptomatic COPD patients despite normal spirometric findings. In our study, respiratory muscle strength and exercise capacity were compared with both IOS and spirometry data, and IOS parameters showed higher correlation coefficients.

In the study by Khalil et al. [18], no significant correlation was found between spirometry and MIP; however, a significant correlation was identified between MIP and six-minute walking distance. In our study, MIP was correlated with all IOS parameters except R20, with the strongest correlation observed with X5. This suggests that respiratory muscle strength may be more closely associated with the elastic properties of the lungs and air trapping.

It is well-established that expiratory muscle weakness in COPD is associated with systemic inflammation and has limited correlation with pulmonary function, being more closely linked to cough effectiveness. Consistent with this, our study found no statistically significant correlation between MEP and IOS parameters.

As the disease progresses, functional impairment and exercise limitation are known to increase [19]. The associations found between VO_2 peak and IOS parameters in this study indicate that impaired respiratory mechanics play a key role in reduced exercise capacity in emphysema.

This study has several limitations that should be acknowledged. Being conducted at a single center may limit the generalizability of the findings. In addition, time and financial constraints restricted both the sample size and the overall scope of the study. The predominance of male participants, which reflects the demographic characteristics commonly seen in COPD populations, may have introduced a gender-based sampling bias. The lack of longitudinal follow-up data hinders the ability to evaluate changes in IOS parameters or clinical outcomes over time. Moreover, the study was conducted during the COVID-19 pandemic, which could have influenced patient access, routine clinical workflows, and overall participation.

Conclusion

These results indicate that, in patients with emphysema, not only parenchymal destruction but also small airways are affected, and impairments in respiratory mechanics involving peripheral airways can be assessed using IOS. Additionally, the findings support the notion that impaired respiratory mechanics significantly contribute to reduced exercise capacity in emphysema, highlighting the importance of targeting small airways in pharmacological treatment strategies. The correlation between IOS

parameters and inspiratory muscle strength may serve as a guide in selecting inhaler therapies. IOS can be utilized to identify ventilatory limitations indicating pulmonary rehabilitation and to evaluate treatment efficacy in emphysema patients. Furthermore, IOS parameters such as X5 and AX may potentially be used in combination with plethysmographic methods for patient selection and assessing changes in respiratory mechanics following bronchoscopic lung volume reduction procedures.

Ethics Committee Approval: The Ankara Kecioren Training and Research Hospital Clinical Research Ethics Committee granted approval for this study (date: 26.01.2021, number: 22012-KAEK-15/2210).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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

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Use of AI for Writing Assistance: The authors declared that artificial intelligence–assisted technologies (such as large language models, chatbots, or image generators) were not used in the preparation of this study.

Authorship Contributions: Concept – HK, SS, PE; Design – HK, SS, PE; Supervision – HK, SS, PE; Fundings – HK, SS, PE; Materials – HK, SS, PE; Data collection and/or processing – HK, SS, PE; Analysis and/or interpretation – HK, SS, PE; Literature search – HK, SS, PE; Writing – HK, SS, PE; Critical review – HK, SS, PE.

Peer-review: Externally peer-reviewed.

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Original Article PHARMACOLOGY

Drug intoxications in the emergency department: Our 1-year experience

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ABSTRACT

OBJECTIVE: Drug intoxication (DRI), which occurs due to accidental or suicidal use of high doses of drugs, has a high mortality and morbidity rate. emergency departments of hospitals are the units where these patients first come to the hospital, where the first interventions are performed and where the mortality rate is higher than other departments. therefore, DRI is a more important health problem for the emergency department (ED) than other departments of hospitals. In this study, we aimed to retrospectively investigate the demographic characteristics, drugs used and clinical course of patients admitted to the ED of our hospital with the diagnosis of DRI.

METHODS: Patients admitted to the ED of Malatya Training and Research Hospital with the diagnosis of DRI were retrospectively screened from the hospital database. The patients' demographic characteristics, medications and clinical conditions were recorded. Patients over the age of 18 years who were diagnosed with DRI and whose medicationuse was identified were included in this study. Patients under the of 18 and those poisoned by other substances such as alcohol, illegal chemicals, or agricultural pesticides were excluded.

RESULTS: Demographic characteristics, type of intoxication and clinical course of the patients included in the study are shown in Table 1. In 2023, 282 patients were admitted to the emergency department of our hospital with the diagnosis of DRI. Of these, 60% were women and 40% were men (n=170, n=112, respectively) and there was no statistically significant difference between the numbers of men and women (p=0.156). Fifteen percent of the women were over 50 years of age and 85% were under 50 years of age. this ratio was 55% to 45% in men. there was no statistically significant difference in the age distribution between men and women (p=0.246, p=0.260, respectively), (Table 1). 77% of the patients used overdose drugs for suicidal purposes and 23% accidentally (n=217, n=65, respectively). There was no statistically significant difference in the type of DRI, total number of males and females and age distribution (p=0.26). While 60% of the patients were hospitalized, 25% were admitted to intensive care unit and 35% to service (n=70, n=100, respectively). There were statistically significantly more patients admitted to service (p=0.03).

CONCLUSION: The results of our study show that the number of female patients admitted to the ED of our hospital with the diagnosis of DRI, patients under 50 years of age and suicidal use are higher, the most commonly used drugs in these patients are NSAIDs, SSRIs and antipyschotics as a drug group and drugs used in psychiatric disorders. Our study has shown that frequently used and easily accessible drugs are more likely to be abused, and that questioning such drugs, especially in patients presenting to the emergency service with drug intoxication in this region, may be valuable for diagnosis and treatment.

Keywords: Drug intoxication; emergency department; nonsteroidal anti-inflammatory drug; suicide.

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Drug intoxication (DRI), which occurs due to accidental or suicidal overdose of drugs, has a high mortality and morbidity rate. It is an important health problem with an increasing incidence in recent years [1]. Worldwide, the second leading cause of death in individuals under 30 years of age is suicidal DRI after death by traffic accidents [2]. In particular, emergency departments of hospitals are the units where these patients first come to the hospital, where the first interventions are made and where the mortality rate is higher than other departments, so for the emergency department (ED), DRI is a more important health problem than other departments of hospitals. In the majority of Europe, approximately 30% of the cases between the ages of 15–64 admitted to the ED with intoxication are due to illicit substance use [3]. In a study conducted in Spain in patients admitted to the ED with intoxication, it was found that although there was no difference in the number of sexes, there was a difference in the drugs used [4]. The majority of patients admitted to the ED with intoxication are DRI cases. In a study conducted in our country, it was found that 0.2% of the cases admitted to the ED were DRI cases and 71% of these were single drug and 29% were DRI due to multiple drug use [4]. Therefore, it is very important for ED physicians to intervene early in patients presenting with DRI and to implement rapid and effective treatment methods. In addition, knowing the type of drug addiction in the region and the drug use tendency of the patients who come with suspicion of DRI will increase the chance of early diagnosis and treatment and reduce morbidity and mortality. At the same time, conducting local studies in which the demographic characteristics, clinical conditions and medications used by patients presenting with ED and DRI will make a significant contribution to more effective diagnosis and treatment of such patients.

In this study, we aimed to retrospectively investigate the demographic characteristics, medications used and clinical course of patients admitted to the emergency department of our hospital with the diagnosis of DRI.

MATERIALS AND METHODS

Ethical Approval

Approval for the study was obtained from the Scientific Research Committee of Malatya Training and Research Hospital (date:13.06.2024 no: 2446255678). This study was conducted in accordance with the Declaration of Helsinki.

Highlight key points

- The majority of patients presenting to theemergency department with drug intoxication are women.
- NSAIDs are the most frequently used drugs, and antidepressants and antipsychotic agents used in psychiatric disorders constitute the most common drug groups involved.

Study Population

Patients admitted to Malatya Training and Research Hospital with the diagnosis of ED in January 2023–2024 were retrospectively screened from the hospital database. Demographic characteristics, medications and clinical conditions of the patients were recorded. The study included DRI patients over the age of 18 who were identified to have used the medication; cases of poisoning with other substances such as alcohol, illegal substances, chemicals, pesticides and patients under the age of 18 were excluded.

Statistical Analysis

Data analysis was performed using SPSS (version 20.0, Inc., Chicago, USA). The Kolmogorov-Smirnov test was used to check whether the distribution of continuous variables was normal. Descriptive statistics for continuous variables were presented as mean±standard deviation (SD) or median, while categorical variables were presented as number of cases (n) and percentage (%). Categorical variables were evaluated using the Chi-square test. Median values and non-normally distributed data were analyzed using the Mann-Whitney U test. P values <0.05 were considered statistically significant.

RESULTS

Demographic characteristics, type of intoxication, and clinical course of the patients included in the study are shown in Table 1. In 2023, 282 patients were admitted to the emergency department of our hospital with the diagnosis of DRI. Of these, 60% were women and 40% were men (n=170, n=112, respectively) and there was no statistically significant difference between the numbers of men and women (p=0.156). Fifteen percent of the women were over 50 years of age and 85% were under 50 years of age. this ratio was 55% vs. 45% in men. there was no statistically significant difference between men and women in terms of age distribution (p=0.246, p=0.260, respectively), (Table 1). 77% of the patients used overdose drugs for suicidal purposes and 23% accidentally

Table 1. Demographic characteristics of patients and type of intoxication

	Woman n=170 (%)	Male n=112 (%)	p	Total n=282 (%)	p
≥50	15	0	0.246	29.5	0.246
<50	85	55	0.246	70.5	0.246
Suicide	79	73	0.26	77	0.26
Overdose	21	17	0.26	23	0.26
Intensive care	25	25	**0.003**	25	**0.003**
Service	39	30	**0.003**	100	**0.003**

(n=217, n=65, respectively). There was no statistically significant difference in the type of DRI, total number of males and females and age distribution (p=0.26). While 60% of the patients were hospitalized, 25% were admitted to intensive care unit and 35% to ward (n=70, n=100, respectively). There were statistically significantly more patients admitted to ward (p=0.03).

Drugs causing DRI are shown in Table 2. Nonsteroidal anti-inflammatory drugs (NSAIDs) were the most commonly used drugs (n=63). The second and third most frequently used drugs were selective serotonin reuptake inhibitors (SSRIs) and drugs used in psychiatric diseases such as atypiscotic drugs (APDs) (n=47, n=28, respectively).Drugs used at 10 and below were not shown separately in the table, but were combined and given under the name of other drugs (OD) (n=26). 16 patients were told by their relatives that they were taking medication, but the medication they were taking could not be identified and is shown as undetected drug (TED) in the table (Table 2).

DISCUSSION

The most common cause of intoxication in patients admitted to ED is DRI [4]. the majority of patients admitted to ED with a diagnosis of DRI are women [4].in a study conducted in our country, it was found that 70% of patients admitted to ED with a diagnosis of DRI were women [4].in another study conducted in our country, this rate was found to be 67% [5]. In a study conducted abroad, it was found that the rate of female patients was 50% [6]. The findings of our study were consistent with these studies, the rate of female patients was 60%.

Table 2. Drugs that cause drugs intoxication

Drug	%
PPI	4
AP	10
Antiepileptic	6
SSRI	16
NSAID	22
UDD	5
Antibiotics	5
BDZ	6
Other	9

PPI: Proton pump inhibitors; AP: Antipsychotics; SSRI: Selective serotonin reuptake inhibitors; NSAID: Nonsteroidal anti-inflammatory drugs; UDD: Undetectable drugs; BDZ: Benzodiazepines.

In a study conducted in Turkiye, it was found that 30% of the patients admitted to hospital with the diagnosis of DRI were hospitalized in intensive care and 67% were hospitalized in service [7]. In another study conducted in our country, it was shown that 70% of the patients admitted to ED with the diagnosis of DRI were discharged from ED after treatment, 30% were hospitalized in the service and very few patients were hospitalized in intensive care. In the same study, 82% of the patients were admitted to ED with the diagnosis of DRI due to suicidal intent and 18% due to accidental high dose drug use [4]. In our study, 25% of the patients were admitted to the intensive care unit and 35% to the service. these results in our study are generally compatible with the results of other studies. the higher number of intensive care unit admissions in some studies may be due to the type and amount of the drug used, the time taken for patient to present to the ED and the poor general condition of the patients due to the presence of additional diseases.

In a retrospective study conducted in Turkiye, antidepressants and analgesics were found to be the most commonly used drugs in patients admitted to the ED with a diagnosis of DRI [8]. Another study conducted in Turkiye showed that the most commonly used drugs in patients admitted to the ED with a diagnosis of DRI were NSAIDs and drugs used in psychiatric patients [5]. In another study conducted in Turkiye, antidepressants were found to be the most commonly used drugs causing DRI [9]. In a study conducted abroad, similar to the studies conducted in Turkiye, it was shown that the

most commonly used drugs in cases of DRI were psychiatric drugs [10]. In another study conducted abroad, bezodiazepines and opioids were found to be the most commonly used drugs in DRI patients [11]. The results of our study generally supported the studies conducted both in Turkiye and abroad. When evaluated as a single drug type, NSAIDs were the most commonly used drug in cases of DRI, but when evaluated as a drug group, the most commonly used drug group was drugs used in psychiatric diseases such as SSRIs, antipsychotic drugs and benzodiazepines. It is considered as an expected result that the most commonly used drugs in cases of accidental overdose and suicidal DRI are psychiatric drugs used in the treatment of these patients.

Conclusion

The results of our study show that the number of female patients admitted to the ED department of our hospital with a diagnosis of DRI, patients under the age of 50 and suicidal use are higher, and the most commonly used medication in these patients are NSAIDs, SSRIs and antipsychotics as a drug group and drugs used in psychiatric disorders.

Ethics Committee Approval: The Malatya Training and Research Education Planning Ethics Committee granted approval for this study (date: 13.06.2024, number: 244625678).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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

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

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Original Article Ophthalmology

Evaluation of sociodemographic and clinical characteristics of dermatochalasis patients: A retrospective epidemiologic study in a tertiary care eye clinic

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ABSTRACT

OBJECTIVE: The aim of this study was to evaluate systemic and environmental risk factors, clinical findings and surgical trends in patients admitted to our clinic with the diagnosis of dermatochalasis.

METHODS: Between January and March 2024, 114 patients with upper eyelid dermatochalasis were retrospectively analyzed cross-sectionally. Demographic data, history of systemic disease, surgical history, presenting complaints, tear tests (Schirmer, tear breakup time (TBUT)) and dermatochalasis grades were recorded.

RESULTS: In the patient cohort, females constituted 66.66%, with a mean age of 53.08±6.35 years. The predominant complaint was a sensation of heaviness or fatigue in the upper eyelid, as reported by 41.22% of the patients. A substantial 70.17% of the participants presented with at least one systemic illness, with diabetes mellitus, hypertension, thyroid disorders, and rheumatologic diseases being the most common. Symptoms of dry eye were observed in 31.57% of the patients, with an average Schirmer test result of 11.03±3.07 mm and a TBUT of 5.87±1.86 seconds. No significant correlation was found between Schirmer and TBUT values and the severity of dermatochalasis (p>0.05). Among the patients, 37.71% were considering surgery, with 65.11% seeking it for cosmetic reasons and 34.88% for functional purposes.

CONCLUSION: Dermatochalasis is a complex condition associated with functional and systemic effects and aesthetic problems. A history of systemic disease, dry eye symptoms and visual field limitation should be considered in managing the disease. Therefore, a multidisciplinary approach should be adopted, and patient expectations should be considered in treatment planning. Prospective studies with a large sample size may better elucidate the etiopathogenesis and patient management.

Keywords: Blepharoplasty; dermatochalasis; eyelid; oculoplasty.

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Dermatochalasis, characterized by the laxity and drooping of bilateral eyelids, predominantly manifests in middle and advanced age, with a higher incidence in the upper eyelid [1]. It is often observed in conjunction with blepharochalasis, a condition marked by recurrent eyelid edema, although it may also present independently. Dermatochalasis can be mistaken for blepharochalasis and steatoblepharon, the latter referring to septal laxity and adipose tissue prolapse [2, 3]. This condition, characterized by facial wrinkles or folds, can negatively affect an individual's physical and psychological well-being [4].

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Associated symptoms may include visual field defects, ocular surface irritation, and headaches. Dermatochalasis is considered a component of the aging process, with its pathophysiology linked to structural changes in the eyelid skin's elastic fibers and collagen bonds [5, 6].

Numerous risk factors for the development of dermatochalasis have been documented. A particular study investigated genetic background, smoking habits, hormonal status in female patients, and body mass index (BMI) in patients with dermatochalasis. The study's findings indicated that genetic predisposition, smoking habits, and high BMI are risk factors for dermatochalasis [7]. Research has also shown that connective tissue diseases and conditions that accumulate in connective tissue, such as amyloidosis, may increase the risk of dermatochalasis [8, 9]. A study of anterior segment findings in patients with type 2 diabetes identified dermatochalasis as the most prevalent finding after blepharitis. The development of dermatochalasis has also been documented in patients with thyroid orbitopathy [7–10].

Patients with dermatochalasis seek treatment for aesthetic purposes or for symptoms such as heaviness and visual field limitation. Blepharoplasty, the most common treatment for dermatochalasis worldwide, involves the surgical removal of excess skin from the eyelids. This procedure addresses ocular ptosis and can be performed on both the upper and lower eyelids [10, 11].

This study aimed to investigate the systemic and environmental risk factors associated with dermatochalasis in patients presenting at our clinic.

MATERIALS AND METHODS

In this retrospective cross-sectional study, we analyzed 114 patients diagnosed with dermatochalasis who presented to our institution's general ophthalmology outpatient clinic between January and March 2024. All patients aged 18 years or older who were identified as having upper eyelid dermatochalasis during routine clinical examination—regardless of their initial presenting complaint—were included. Exclusion criteria comprised a history of previous eyelid surgery, congenital or neurologic ptosis, and active ocular surface infection or inflammation. The study was approved by Selcuk University Faculty of Medicine Ethics Committee (date: 26.03.2024, number: 2024/181). This study was conducted in accordance with the principles outlined in the Helsinki Declaration.

Highlight key points

- Dermatochalasis is not only an aesthetic problem; it is also associated with systemic diseases and functional symptoms such as dry eye, visual field limitation, and periorbital pain.
- The rate of systemic comorbidity was higher in female patients, especially diabetes, hypertension, thyroid dysfunction, and rheumatologic diseases, which were frequently associated with dermatochalasis.
- The reasons for surgery are not only cosmetic concerns; many patients also request blepharoplasty for functional complaints.

Each patient's age, gender, and history of systemic diseases were documented. Anterior segment findings were assessed during ophthalmologic examination. The condition of the upper eyelids was recorded, the severity of drooping was graded, and brow ptosis (displacement of the eyebrows from their normal position) was documented. Patients' presenting complaints were detailed, and the presence of symptoms such as a sensation of heaviness or fatigue in the upper eyelid, cosmetic discomfort, dry eyes (burning, stinging, sanding), watery eyes (epiphora), visual field limitation, and forehead pain due to eye strain was investigated. Objective tear function tests, including the Schirmer test and tear breakup time (TBUT), and ocular surface evaluation were conducted in patients reporting dry eye symptoms. All Schirmer and TBUT measurements were performed under standardized conditions in a controlled environment (ambient temperature: 22–24°C) and within the same time window (09:00–12:00 a.m.) to minimize the influence of diurnal variation. No topical treatments were administered before the testing phase.

Requests for blepharoplasty and the reasons for surgical requests were evaluated. Dermatochalasis grading was conducted according to the classification by Leonie C. Jacobs et al. [7] According to this classification, the severity of dermatochalasis was graded as usual (A) if the upper eyelid skin does not touch the eyelashes, mild (B) if the upper eyelid touches the eyelashes, moderate (C) if the upper eyelid extends over the eyelashes, and severe (D) if the upper eyelid skin extends over the eye.

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 21.0 (IBM Corp., Armonk, USA); a p-value of less than 0.05 was considered statistically significant. All quantitative data were presented as mean±standard deviation, while categorical data were expressed as numbers and percentages. Nor-

Table 1. Demographic data of patients with dermatochalasis (n=114)

Variables	
Gender, (%)	
Female	66.66
Male	33.33
Place of residence, (%)	
City center	75.43
Village	24.56
Mean age, (mean years±SD)	
Female	53.639±6.55
Male	53.33±5.96
Total	53.08±6.35

SD: Standard deviation.

mality of continuous variables was assessed using the Shapiro-Wilk test to determine the appropriate statistical approach.

RESULTS

A total of 114 patients diagnosed with dermatochalasis were included in the study, of whom 66.66% (n=76) were female. The overall mean age was 53.08±6.35 years. The majority of participants (75.43%) resided in urban areas, with a smaller proportion living in rural or peripheral districts (Table 1).

The most frequently reported symptom was a sensation of heaviness or fatigue in the upper eyelid, followed by aesthetic dissatisfaction, ocular irritation symptoms (burning, stinging), visual field constriction, and frontal headache due to compensatory muscle overuse (Table 2). These findings reflect the multifaceted burden of dermatochalasis beyond cosmetic concern.

A systemic comorbidity was present in 70.17% of patients, with a predominance among females. Diabetes mellitus and hypertension were the most frequently encountered conditions, often occurring concomitantly. Other common systemic diseases included thyroid dysfunction and rheumatologic disorders (Table 2), all of which may contribute to structural weakening of periorbital connective tissues.

Surgical history revealed that 65.78% of patients had previously undergone at least one operation, with gynecological procedures being predominant among female participants. These findings may point toward a potential interplay between cumulative surgical exposure and connective tissue degeneration, though causal inference requires further investigation.

Table 2. Systemic diseases and background characteristics in patients with dermatochalasis

	%
Application complaint	
Feeling of heaviness or fatigue in the upper eyelid	41.22
Cosmetic discomfort	28.94
Stinging and burning sensations in the eyes, and watery eyes (epiphora)	24.56
Visual field limitation	20.17
Pain in the forehead due to eye strain	14.91
Systemic diseases	
Diabetes	21.92
Hypertension	20.17
Thyroid dysfunction	13.15
Rheumatology diseases	16.66
Have at least two systemic diseases	30.70
Surgical history	
No history of surgery	33.33
History of at least one surgery	65.78
History of two or more surgeries	22.80

Table 3. Patient's evaluation of dermatochalasis and their thoughts about surgery (n=114)

	%
Dermatochalasis evaluation	
Group A	12.28
Group B	35.96
Group C	35.96
Group D	15.78
Surgical motivation	
Requesting surgery	37.71
Who doesn't want to have surgery	62.28
Reason for requesting surgery	
Functional purpose	34.88
Aesthetic purpose	65.11

In terms of clinical grading, most patients were categorized within mild to moderate severity groups (Groups B and C), while severe dermatochalasis (Group D) was less frequently observed (Table 3). Despite reported symptomatology, only 37.71% of patients expressed willingness to undergo blepharoplasty. Among those, cosmetic motivation was predominant (65.11%), whereas the remainder cited functional limitations as their primary concern.

Dry eye symptoms were reported in 31.57% of the cohort. Objective tear function testing revealed a mean Schirmer score of 11.03±3.07 mm and a TBUT of 5.87±1.86 seconds. Epiphora was present in 21% of cases. Despite the frequency of symptoms, statistical analysis demonstrated no significant association between tear function parameters and dermatochalasis severity (Schirmer: p=0.681; TBUT: p=0.755). Additionally, brow ptosis was identified in 11.4% of the patients and was considered in surgical planning when present.

DISCUSSION

In this study, we comprehensively evaluated the demographic and clinical characteristics and presenting complaints and symptoms of patients with dermatochalasis who were admitted to our outpatient clinic. Our findings indicate that dermatochalasis predominantly affects individuals in the advanced age group. It is well-established that the ligaments within skin tissue weaken with age, leading to increased skin laxity. The prevalence of dermatochalasis is anticipated to rise with age [7]. Although the condition affects both sexes, most patients in our clinic were female. This higher prevalence in women may be attributed to hormonal factors, differences in skin structure, and biological changes associated with aging [12, 13].

Previous studies have suggested that residing in rural areas may increase the risk of ultraviolet sunlight exposure, potentially leading to changes in skin tissue [14]. Our analysis, however, revealed that most patients resided in urban centers. While our study did not directly support this relationship, comparative studies between rural and urban populations may elucidate the causal link between living environment and dermatochalasis. Nonetheless, our study's limited sample size may restrict the generalizability of our findings.

A significant proportion of our patients had at least one systemic disease, with a statistically higher prevalence in women compared to men. The most common combination of systemic diseases observed was diabetes and hypertension. Existing literature suggests a potential association between dermatochalasis and vascular and metabolic disorders. Although it has been reported that diabetes may alter the structure and function of connective tissue components, data on the effects of hypertension on the skin and periorbital region remain limited [15]. In our study, thyroid dysfunction and rheumatologic diseases were also prevalent among dermatochalasis patients. Connective tissue involvement in thyroid orbitopathy may predispose individuals to dermatochalasis [16]. Similarly, impaired connective tissue metabolism and reduced skin hemodynamics in hypothyroidism may contribute to the development of dermatochalasis [17]. The chronic inflammatory nature of rheumatologic diseases may adversely affect dermal structures' integrity and regenerative capacity [18]. In this context, a thorough assessment of existing systemic diseases in patients with dermatochalasis is clinically essential for surgical planning, intraoperative bleeding control, and postoperative wound healing.

Although the study provided detailed documentation of systemic comorbidities observed in patients with dermatochalasis, a statistical analysis was not conducted to explore the relationship between specific systemic diseases and the severity of dermatochalasis. This decision was based on the limited sample size, particularly within certain stratified subgroups, which would have undermined the validity of any statistical inference. Specifically, small cell counts in multiple contingency tables would violate the assumptions required for robust chi-square testing. Nevertheless, we recognize the clinical relevance of this association and recommend that future studies with larger and more balanced sample distributions investigate this potential relationship using prospective designs.

A subsequent analysis of the patients' surgical histories indicated that most had undergone at least one surgical procedure. Gynecologic surgeries, such as cesarean sections and hysterectomies, were frequently recorded, particularly among female patients. Surgical interventions and the administration of general anesthesia have been shown to elicit a systemic stress response in the body [19]. It has been hypothesized that this stress response may lead to alterations in skin structure, particularly by affecting connective tissue homeostasis. Whether repeated surgical exposure contributes to the development of dermatochalasis through chronic systemic inflammation, oxidative stress, or vascular dysfunction warrants further investigation. Consequently, the potential impact of surgical history on the pathophysiology of dermatochalasis should be prospectively evaluated in future studies.

In our study, the most common complaint among patients was a sensation of heaviness and fatigue in the upper eyelid. This suggests that dermatochalasis should be considered a cosmetic issue and a functional disorder. Aesthetic discomfort was also frequently reported, highlighting the importance of the psychosocial aspect affecting patients' quality of life. Additionally, irritative symptoms such as stinging and burning suggest indirect involvement of the ocular surface. Visual field limitation and forehead pain due to eye strain indicate secondary effects of dermatochalasis on visual performance and peripheral muscle groups [12]. These findings underscore the necessity of a multidisciplinary approach in evaluating dermatochalasis and emphasize the importance of considering functional and symptomatic effects beyond aesthetic concerns.

One of our study's notable findings was the standard reporting of dry eye symptoms in patients with dermatochalasis. Various studies have previously emphasized the relationship between dermatochalasis and ocular surface health, rendering this observation significant. Dermatochalasis is known to contribute to the pathogenesis of dry eye, particularly by disrupting meibomian gland function and decreasing the stability of the tear lipid layer. Maintaining lid hygiene and employing appropriate surgical approaches may alleviate symptoms in this context [12]. However, our study found no statistically significant relationship between dermatochalasis groups and dry eye severity. This result may be attributed to the limited sample size or the sensitivity of subjective symptom assessment, but it may also be due to heterogeneity in the patient population. Therefore, it is essential to include dry eye evaluation in the routine examination process for patients with dermatochalasis and to address its management with a multidisciplinary approach. Further studies with larger samples and prospective and parametric assessments are needed to clarify this issue.

Upon analyzing the distribution of patients according to dermatochalasis groups, it was observed that the majority were classified within Groups B and C, indicating mild to moderate dermatochalasis. This finding suggests that these levels of dermatochalasis are more prevalent clinically, whereas severe forms are less frequently encountered. Our findings indicate that most patients did not pursue surgical intervention, likely due to the minimal functional impairment caused by dermatochalasis and the relatively low priority of aesthetic concerns among patients. Nonetheless, the fact that some patients seek surgical intervention for both aesthetic and functional reasons underscores the importance of addressing dermatochalasis based on individual complaint profiles. In this context, clinical classification, patient subjective perception, impact on quality of life, and personal expectations should be integrated into the treatment decision-making process. Additionally, factors such as cultural values, sociocultural status, aesthetic sensitivity, and access to healthcare services are hypothesized to be critical variables influencing the motivation for surgery [20]. This multidimensional approach supports the necessity for a more personalized framework in treatment planning for individuals with dermatochalasis.

This study has certain limitations. Firstly, the study's retrospective design rendered the data quality dependent on the accuracy and completeness of patient records, with potential information gaps or biases possibly limiting the generalizability of the results. A prospective study design could facilitate a more standardized and systematic evaluation of symptoms and clinical findings, thereby establishing more robust causal links associated with dermatochalasis. Secondly, the study's conduct within a single center and with a limited sample size implies that the rates and clinical associations observed may differ in other geographical or demographic populations. Finally, although meibomian gland dysfunction (MGD) has been proposed in the literature as a contributing factor to ocular surface instability in patients with dermatochalasis, it was not directly assessed in our study. Therefore, any reference to MGD in the present discussion remains theoretical and is based on existing evidence from prior studies rather than direct clinical observation. Future investigations incorporating clinical or imaging-based assessment of MGD would offer a more comprehensive understanding of the dry eye component associated with dermatochalasis. Despite these limitations, our study contributes to the existing literature by providing a detailed clinical profile of patients with dermatochalasis in a tertiary healthcare institution.

Conclusion

Dermatochalasis is a medical condition marked by the sagging of skin and diminished elasticity in the upper eyelids, often linked to aging. While it is frequently seen as a cosmetic issue, it can lead to functional problems, such as restricted visual fields and disorders of the ocular surface, especially in more severe cases. The patients studied here demonstrate that dermatochalasis goes beyond mere aesthetic concerns, causing functional issues

like dry eye symptoms, excessive tearing, and discomfort around the eyes. The condition is further aggravated by meibomian gland dysfunction and changes in the tear lipid layer, which negatively affect ocular surface health. Addressing and treating this condition, which involves the skin's collagen and elastic fibers, necessitates a multidisciplinary approach. Future multicenter, prospective controlled studies with larger participant groups are crucial to gain more detailed and widely applicable insights into the causes and progression of dermatochalasis, as well as patient preferences for surgical intervention.

Ethics Committee Approval: The Selcuk University Clinical Research Ethics Committee granted approval for this study (date: 26.03.2024, number: 2024/181).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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

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Original Article RADIOLOGY

Microwave ablation for hepatocellular carcinoma: A single-center experience and outcomes

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ABSTRACT

OBJECTIVE: Microwave ablation (MWA) has emerged as a minimally invasive treatment for hepatocellular carcinoma (HCC), offering a promising alternative for patients ineligible for surgical resection. This study aims to evaluate the efficacy, safety, and clinical outcomes of MWA in a single-center cohort, focusing on treatment success, recurrence rates, and long-term survival.

METHODS: A retrospective analysis was conducted on 54 patients who underwent percutaneous ultrasound-guided MWA for HCC between January 2019 and December 2023. Patient demographics, tumor characteristics, and procedural outcomes were assessed. Treatment success was defined as complete ablation confirmed by contrast-enhanced imaging one month post-procedure. Local tumor progression (LTP) and recurrence rates were evaluated during follow-up. Survival outcomes were analyzed based on follow-up data, considering overall survival and recurrence-free survival rate.

RESULTS: A total of 71 tumors were treated in 54 patients. Complete ablation was achieved in 91.5% of tumors following the initial or secondary procedure. LTP was observed in 16.6% of patients during follow-up. No major complications were reported. The median follow-up period was 36 months, and survival outcomes were comparable to those reported for other ablative techniques. No statistically significant correlation was found between tumor size and ablation success ($p>0.05$).

CONCLUSION: MWA is an effective and safe treatment modality for HCC, demonstrating high technical success and acceptable recurrence rates. These findings support the continued integration of MWA in multidisciplinary HCC management, particularly for patients ineligible for surgery. Further research with larger cohorts and longer follow-up is needed to optimize patient selection and refine ablation strategies.

Keywords: Hepatocellular carcinoma; image-guided ablation; liver neoplasms; microwave ablation; tumor recur-rence.

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Hepatocellular Carcinoma (HCC) remains one of the most common and deadly forms of liver cancer. Globally, particularly in regions with high prevalence of hepatitis B and C infections [1]. Traditional surgical resection, while effective, is not always feasible due to factors such as underlying liver disease, tumor location, or patient comorbidities [2].

In the last few decades, radiofrequency ablation (RFA) and microwave ablation (MWA), two new local thermal ablation techniques, have gotten more and more attention as ways to treat HCC [3].

Randomised controlled trials have demonstrated that, for HCC patients with tumors smaller than 3 cm, the overall survival (OS) rates of those undergoing ra-

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diofrequency ablation are comparable to those receiving hepatectomy [4]. Seki et al. [5] first reported the use of MWA for treating HCC in 1994.

MWA utilises electromagnetic waves to generate heat, leading to coagulative necrosis of tumor tissue. Compared to RFA, MWA offers several advantages, including faster heating, larger ablation zones, and less susceptibility to the "heat-sink" effect, where nearby blood vessels can cool the tissue and reduce the effectiveness of the ablation [5, 6].

Recent studies, including those conducted at single centres, have demonstrated the efficacy of MWA in treating HCC, especially for tumors less than 5 cm in diameter. These studies show promising overall survival and disease-free survival rates, positioning MWA as a viable option for patients who are not candidates for surgery [3, 6, 7].

Despite the growing body of evidence supporting MWA, further research is necessary to refine patient selection criteria, optimise procedural techniques, and compare long-term outcomes with other treatment modalities.

This study aims to contribute to the existing literature by providing a comprehensive analysis of MWA for HCC based on data from a single centre. The main goal is to find out how well MWA works in terms of overall survival, recurrence rates, and procedure safety. This will help us understand its role in modern management of hepatocellular carcinoma.

Highlight key points

- Microwave ablation (MWA) achieved a high technical success rate (91.5%) in treating hepatocellular carcinoma (HCC).
- Local tumor progression occurred in 16.6% of patients during the 36-month median follow-up period.
- Treatment success was consistently high across tumors of varying sizes, with no significant differences.
- MWA proved safe, with no major complications observed during or after procedures.
- Results support MWA as an effective, minimally invasive option for patients unsuitable for surgical resection.

MATERIALS AND METHODS

Patient Selection

The retrospective study protocol was approved by the institutional review board (decision no: 273425506/132; approval date:10.04.2025) and conducted in compliance with the principles outlined in the Declaration of Helsinki. The review included all patients who underwent MWA for HCC between January 2019 and December 2023. We assessed patient demographics, risk factors associated with liver disease and HCC, and pre-operative alpha-fetoprotein (AFP) levels. We evaluated the severity of liver disease using the Child-Pugh score [8]. Imaging findings consistent with LI-RADS criteria and elevated AFP levels established the diagnosis of HCC [9]. For lesions that did not exhibit radiological features typical of HCC, a pre-operative biopsy was performed to confirm the diagnosis, but tissue diagnosis of HCC was not mandatory for treatment. Pre-operative imaging assessments were performed using contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI) when possible, with tumor size, location, and number being documented. Tumor size was recorded as the maximum tumor diameter observed on cross-sectional imaging or ultrasound.

A multidisciplinary team evaluated all patients with HCC at a weekly conference. This team included specialists from interventional radiology, medical oncology, radiation oncology, gastroenterology, and transplant surgery. Multi-modality treatment options, including resection, ablation, transplantation, and transarterial and systemic therapies, were considered for all patients. Patients were often sent for a possible transplant evaluation early on in their treatment. Ablation therapy was used as a bridge to transplant, especially for people with unifocal or multifocal disease, especially if they were going to have to wait a long time for transplantation or if their disease burden would make them ineligible for transplant.

Operative Technique

We positioned the patient supine for the procedure. To confirm the puncture site, angle, and depth of needle insertion, conventional colour Doppler and ultrasound were performed. Using the predetermined puncture path and depth, an MWA needle was guided into the tumor's centre under ultrasound. The optimal electrode placement was determined based on the tumor's size, ensuring that the thermal coagulation and necrosis affected both the tumor and at least 1 cm of surrounding healthy tissue. Depending on the tumor's dimensions, different microwave settings and power levels were chosen for effective thermal coagulation necrosis. Ablation durations varied between 1 to 5 minutes based on lesion dimensions and proximity to vascular structures. We activated the microwave device once the MWA needle reached the target area. For tumors smaller than 3.0 cm in diameter,

a single needle with multiple ablation points was utilised, whereas tumors larger than 3.0 cm required a multi-needle, multi-point approach. Real-time monitoring of the tumor's response during MWA was conducted. Immediately after the ablation period was accomplished, the procedure was completed by performing tract ablation for the section up to the liver capsule.

All procedures were conducted at a single center by three interventional radiologists using a percutaneous approach while the patient was under general anaesthesia. In cases where TACE was also performed, it was carried out before general anaesthesia was administered, followed by MWA under general anaesthesia. All thermal ablation procedures were performed with percutaneous MWA using only US guidance under general anesthesia. A water-cooled MWA (Microwave Ablation) antenna (15G-16G, Vison-China Medical Devices R&D Center; Canyon Medical Inc.), connected to a microwave platform, was inserted into the tumor under ultrasonographic guidance. We systematically included the procedural details, specifying the microwave device's power settings (60–100 Watts), duration (1–5 minutes depending on lesion size), and ablation points used during treatment. After the procedure, patients were closely monitored for complications during an overnight hospital stay, and follow-up checks were conducted 5–7 days later. We carefully documented any adverse events reported by the patients in their medical records.

Follow-up and Recurrence

Patients underwent follow-up imaging at 3, 6, and 12 months post-surgery, provided there were no complications. Contrast-enhanced CT or MRI was used to assess local tumor progression (LTP) at the ablation site and to detect any delayed complications. The LI-RADS treatment response (LR-TR) criteria were used to check for recurrence, and any radiologically suspicious lesion at or near the previous ablation site was checked for viability according to standard procedures. We calculated the time to recurrence from the date of ablation to the date of radiologic detection. Follow-up imaging was interpreted by abdominal imaging specialists with subspecialty training.

Statistical Analysis

All statistical analyses were performed using SPSS for Windows (version 16.0; SPSS Inc., Chicago, IL, USA). Descriptive statistics were utilised to calculate quantitative data, including mean values, standard deviations, and proportions, which were used to analyse patient and operative outcomes. Univariate analyses were conducted with systemic and local recurrence as the dependent variables. A significance level of $p<0.05$ was considered statistically significant.

Survival rates were assessed with the Kaplan-Meier approach, covering the period from tumor diagnosis to death or the last follow-up appointment.

Table 1. Patient demographics and tumor size

Features	No. of patient (%)
N	54
Median age, y (range)	65
Male	76
Female	24
History of smoking	59
Liver cirrhosis (%)	81
Child-pugh A	63
Child-pugh B	37
AFP (ng/mL) ≤ 20	31
AFP (ng/mL) >20	69
HBV positive	81
Post-ablation TACE (%)	7

N: Number; AFP: Alpha-fetoprotein; HBV: Hepatitis B virus; TACE: Transarterial chemoembolization.

RESULTS

Patient and Tumor Characteristics

A total of 63 patients with hepatocellular carcinoma were treated with percutaneous ultrasound-guided microwave ablation at our institution between January 2019 and December 2023. Of these, 9 patients were excluded due to incomplete data, leaving a final cohort of 54 patients for analysis. In our cohort of 54 patients with a total of 71 tumors, 38 patients (70.4%) had a single HCC lesion, and 30 patients (55.6%) had HCC lesions ≤3.0 cm in size. No patients presented with portal vein thrombosis or extrahepatic metastases. Table 1 outlines the demographic and clinical characteristics of the patients, including age, sex, liver function status, underlying liver disease etiology, Child-Pugh scores, AFP levels, and comorbidities.

Table 2. Succes and LTP rate of different-size tumors

	<1 cm	1–3 cm	>3	p (<1 vs 1–3 cm)	p (1–3 vs >3 cm)
Complete ablation	13 (100%; CI: 77–100)	20 (95%; CI: 75–99)	32 (86%; CI: 70–95)	1	0.226
Partial ablation	0	1 (5%)	5 (14%)		
LTP	1 (7.6%; CI: 1–35)	4 (19%; CI: 8–39)	4 (11%; CI: 4–25)	0.62	0.44

LTP: Local tumor progression; CI: Confidence interval.

Regarding the radiological characteristics of the HCC lesions, 13 lesions (24%) were smaller than 1 cm, 21 lesions (39%) were between 1 and 3 cm, and 37 lesions (68.5%) were larger than 3 cm (Table 2).

MWA Success Rate and Complications

All patients underwent triphasic CT or dynamic MRI prior to the MWA procedure. Based on post-procedure imaging (triphasic CT) performed 3 to 4 weeks after treatment, complete ablation, indicative of technical success, was achieved in 65 out of 71 lesions (91.5%) (Fig. 1). However, six tumors (8.5%) demonstrated incomplete ablation one month after the initial procedure. Each of these six tumors was identified in a different patient. Among these patients, five had tumors larger than 3 cm, while one had a tumor smaller than 3 cm. The patient with the tumor smaller than 3 cm underwent a secondary ablation, which successfully achieved complete ablation. Of the remaining five patients, four were treated with transarterial chemoembolization (TACE), and one received conservative management.

Overall, 49 out of 54 patients (91.0%) achieved treatment success, defined as complete ablation one month following the initial or secondary ablation. When stratifying the lesions by size (<3 cm, 3–5 cm, and >5 cm), the complete ablation rates were not statistically significant (Table 2).

Local tumor progression was observed in 9 out of 54 patients (16.6%) during follow-up (Table 3). Microwave ablation was the preferred modality for treating local recurrences, provided that the patients remained eligible for the procedure. Four patients received additional MWA treatments. However, four others were excluded from further MWA due to extrahepatic metastases or the presence of more than five nodules.

Table 3. Summary of treatment and follow-up outcomes for patients undergoing microwave ablation

Microwave ablation, n	54
Ablated tumors per patient, n (range)	1.3 (1–4)
LTP, n (%)	9 (16)
Median follow-up (month)	36

LTP: Local tumor progression.

Minor complications were observed in approximately 12% of patients. These primarily included mild post-procedural pain, localized hematoma at the needle insertion site, and transient fever.

DISCUSSION

MWA has emerged as a minimally invasive and effective option for the treatment of HCC. Its ability to achieve high intratumoral temperatures and larger ablation volumes in shorter durations (<10 min) highlights its potential as a reliable therapeutic modality [10]. Additionally, advancements in high-powered generators and internal cooling systems have enhanced the safety and efficacy of MWA, particularly in challenging anatomical locations [11]. These developments underscore its suitability for treating a broad spectrum of HCC cases, including those previously considered difficult to manage due to proximity to critical structures or poor hepatic reserve.

While previous studies have compared MWA to other ablative techniques, such as RF [6]. The primary aim of this study is not to establish superiority over alternative modalities but to evaluate the clinical outcomes and feasibility of MWA in a specific patient population. In

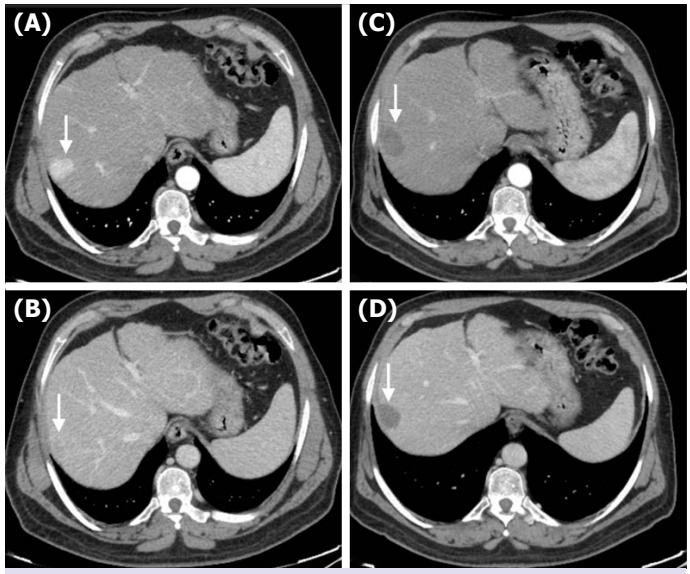

Figure 1. Dynamic contrast-enhanced CT images of the liver before and one month after microwave ablation (MWA). Hepatocellular carcinoma (HCC) lesion located in segment 7 (arrow) demonstrates arterial phase enhancement and venous phase washout on pre-ablation images. Pre-ablation arterial phase **(B)** and pre-ablation venous phase **(D)** images show lesion enhancement. Post-ablation arterial phase **(A)** and post-ablation venous phase **(C)** images at one-month follow-up demonstrate the absence of lesion enhancement, indicating successful ablation.

particular, MWA's ability to manage localised disease effectively and its repeatability make it a promising option for both curative and palliative intents.

Previous studies have reported a complete ablation rate of 89.0%–94.9% for HCCs [9, 12, 13]. In the current study, treatment success—defined as complete ablation one month after the initial or secondary procedure—was achieved in 49 out of 54 patients (91.0%), consistent with the outcomes of earlier research.

Furthermore, our findings were similar to those observed in surgical resection and radiofrequency ablation studies [14, 15].

Our results were somewhat inferior to those reported by Thamtorawat et al. [16] who retrospectively reviewed 173 MWA-treated HCCs up to 5 cm in 129 patients. In their study, the technical success rate was 96.5%, compared to 91% in our study [16].

The results of our study highlight the effectiveness of MWA as a feasible treatment alternative for patients with HCC, especially for those ineligible for surgical resection. Our investigation revealed no statistically significant correlation between tumor size and the efficacy of achieving complete ablation with microwave ablation (MWA). For instance, Thamtorawat et al. [16] observed significantly higher technical success rates and lower recurrence rates in HCC lesions smaller than 3 cm compared to larger lesions. Similarly, Liang et al. [17] demonstrated that patients with smaller HCC tumors experienced improved long-term survival and reduced local recurrence rates after MWA.

The apparent discrepancy between our findings and those previously published may primarily be attributed to the limited statistical power associated with our relatively small sample size and the inherent heterogeneity of tumor characteristics within the patient population. Additionally, factors such as tumor location, proximity to major vascular structures, and variability in procedural techniques could also influence the effectiveness of ablation irrespective of size alone, although these parameters were not systematically analyzed in our study.

While MWA effectively controls local disease, recurrence remains a significant challenge, as observed in our study. Approximately 16% of patients exhibited either local tumor progression (LTP) or the emergence of new lesions over the follow-up period, a result that aligns with findings from prior studies on MWA [17–19].

This recurrence rate highlights the aggressive characteristics of HCC and the necessity for continuous monitoring following ablation. It is essential to acknowledge that the majority of recurrences in our sample were controllable through further MWA sessions or alternative therapeutic measures, demonstrating the efficacy of MWA not only as a main treatment but also as part of a multimodal strategy [20]. Our findings emphasise the significance of thorough post-procedural monitoring in enhancing long-term oucomes. Consistent imaging and evaluations of AFP levels are essential in detecting recurrences early, allowing for timely interventions that can mitigate disease progression [21].

This proactive approach to monitoring, coupled with the ability to perform repeat ablations, positions MWA as a versatile and sustainable treatment option for HCC, particularly in the context of bridging patients to transplantation or in managing recurrences [18].

Nevertheless, it is crucial to acknowledge the constraints of MWA. The technique may be less effective for treating larger tumors (>5 cm) or those with complex vascular anatomy, where achieving complete abla-

tion in a single session may be difficult. In some cases, MWA may require augmentation with additional treatments, such as TACE, or incorporation into a sequential therapeutic strategy to enhance efficacy [22, 23].

This study has several limitations that warrant consideration. The retrospective design inherently restricts the ability to establish causality, while the single-center setting may limit the generalizability of the findings. Although the sample size was adequate for initial observations, it may not encompass the full spectrum of patient variability encountered in broader clinical practice. An important limitation of our study is the lack of systematic analysis regarding anatomical factors such as proximity to major vascular structures and regions of high perfusion, both of which may significantly influence ablation efficacy. It is well-established in the literature that tumors adjacent to large vessels or located in highly perfused liver regions pose a challenge for thermal ablation techniques due to the heat-sink effect, potentially leading to incomplete ablation or higher recurrence rates

Additionally, the follow-up period, while sufficient for evaluating short- to medium-term outcomes, does not provide insights into the long-term efficacy and safety of MWA. To better understand the durability of MWA in preventing recurrence and improving survival, longer follow-up studies are essential.

Moreover, this study did not evaluate parameters such as vessel proximity and high perfusion rates, which are recognized as critical factors influencing ablation efficacy. Existing literature indicates that MWA outperforms other modalities, such as RFA, in high-perfusion regions and near large vessels by creating larger and more consistent ablation zones. Future research should include detailed assessments of variables like vessel proximity and perfusion rates to refine ablation techniques and optimize therapeutic outcomes.

Conclusion

MWA is an efficacious therapeutic option for HCC, providing a minimally invasive alternative to surgery with similar results in suitably chosen individuals. The results of this study support the continued use of MWA, particularly for patients with early-stage disease and good liver function. As technology and techniques continue to evolve, MWA is likely to play an increasingly prominent role in the management of HCC, especially in the context of a multidisciplinary approach to treatment.

Ethics Committee Approval: The Umraniye Training and Reseasrch Hospital Clinical Research Ethics Committee granted approval for this study (date: 10.04.2025, number: B.10.1.TKH.4.34.H.G .P.0.01/132).

Informed Consent: Informed consent was not required due to the retrospective nature of this study.

Conflict of Interest: The authors declare that they have no conflicts of interest related to this study.

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ORIGINAL ARTICLE PEDIATRICS

Effects of levetiracetam monotherapy on thyroid function in pediatric epilepsy patients

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ABSTRACT

OBJECTIVE: Childhood epilepsy requires long-term antiepileptic drug (AED) therapy, and its potential endocrine effects are a critical consideration. While the impact of AEDs on thyroid function is well documented in adults, data on pediatric populations remain scarce. Levetiracetam (LEV), a broad-spectrum AED widely prescribed for children, has an unclear influence on thyroid function. This study aims to evaluate the effects of LEV on thyroid function in children with epilepsy.

METHODS: A retrospective observational study was conducted at the Pediatric Neurology Clinic of Umraniye Hospital between 2020 and 2022. One hundred pediatric patients, aged 2.3 to 18 years, diagnosed with epilepsy and using LEV monotherapy for at least 12 months were included. Thyroid-stimulating hormone (TSH) and free thyroxine (fT4) levels were measured at baseline and at 3, 6, and 12 months. Statistical analyses were performed using paired t-tests, with a p-value <0.05 considered statistically significant.

RESULTS: The mean TSH levels increased from 1.46±0.3 mIU/L at baseline to 1.60±0.3 mIU/L at 12 months (p=0.0017). Despite the statistically significant rise, TSH levels remained within normal reference ranges, and no clinically significant hypothyroidism was observed. Changes in fT4 levels were not statistically significant, except for a borderline increase at 12 months (p=0.05). Four patients exhibited transient TSH elevations but did not require thyroid hormone replacement therapy.

CONCLUSION: LEV monotherapy does not appear to significantly impact thyroid function in pediatric epilepsy patients. While a slight but statistically significant increase in TSH levels was observed, these changes were clinically insignificant. These findings suggest that LEV is a safe option regarding thyroid function in children with epilepsy. Further long-term prospective studies are warranted to confirm these results.

Keywords: Antiepileptic drugs; levetiracetam; pediatric epilepsy; thyroid function.

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Epilepsy is one of the most common chronic neurological disorders in children, characterized by recurrent unprovoked seizures. It affects approximately 0.5–1% of the pediatric population worldwide, significantly impacting the quality of life and development of affected individuals [1]. Antiepileptic drugs (AEDs) are the cornerstone of epilepsy management, aiming to achieve seizure control and prevent neurological complications. While older generation AEDs like valproate and carbamazepine are effective, they are often associated with adverse effects, including potential impacts on endocrine function, particularly thyroid hormones [2, 3].

Thyroid hormones play a critical role in growth, brain development, and metabolic regulation in children. Disruptions in thyroid function can lead to growth retardation, cognitive impairments, and other developmental issues [4]. Previous studies have reported that AEDs such

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as valproate and carbamazepine can reduce free thyroxine (fT4) levels and induce subclinical hypothyroidism in pediatric patients [5, 6]. These effects are thought to be due to hepatic enzyme induction or interference with thyroid hormone synthesis and metabolism.

Levetiracetam (LEV) is a newer, broad-spectrum AED widely used in the treatment of pediatric epilepsy due to its favorable pharmacokinetic properties, efficacy, and tolerability [7]. Unlike many other AEDs, LEV does not undergo significant hepatic metabolism and has minimal drug–drug interactions, making it a convenient option for long-term therapy [8]. However, the impact of LEV on thyroid function in children remains unclear, with limited and inconclusive data available.

Understanding the potential endocrine effects of LEV is essential for optimizing treatment strategies and ensuring the safety of pediatric patients on long-term AED therapy. This study aims to investigate the effects of LEV monotherapy on thyroid function in children with epilepsy over a 12-month period.

Highlight key points

- The effects of levetiracetam (LEV) monotherapy on thyroid function were evaluated in children with epilepsy.
- A statistically significant increase in TSH levels was observed during follow-up; however, values remained within normal reference ranges.
- Free thyroxine (fT4) levels showed no clinically meaningful changes over the 12-month treatment period.
- No patients developed clinical hypothyroidism or required thyroid hormone replacement therapy.
- LEV appears to be a safe antiepileptic option with minimal impact on thyroid function in pediatric patients.

Table 1. Epilepsy classification

Epilepsy type	Number of patients (n)
Childhood rolandic epilepsy	46
Childhood occipital lobe epilepsy	28
Frontal lobe epilepsy	8
Juvenile myoclonic epilepsy	18

MATERIALS AND METHODS

Study Design and Setting

A retrospective observational study was conducted at the Pediatric Neurology Clinic of Umraniye Training and Research Hospital between January 2020 and December 2022. Ethical Approval: This study was approved by the Clinical Research Ethics Committee of Umraniye Training and Research Hospital on September 7, 2023 (ethics committee no:22432288), and all procedures adhered to the principles of the Declaration of Helsinki.

Patient Selection

Inclusion Criteria

- Pediatric patients under 18 years of age diagnosed with epilepsy according to the International League Against Epilepsy (ILAE) classification [9].
- Patients who had been on LEV monotherapy for at least 12 months.
- Availability of thyroid function tests (TSH and fT4) at baseline, 3 months, 6 months, and 12 months.

Exclusion Criteria

- Patients with pre-existing thyroid disease or endocrine disorders.
- Use of medications known to affect thyroid function other than LEV.
- Incomplete medical records or missing thyroid function tests.

Treatment Protocol

LEV therapy was initiated at a dose of 10 mg/kg/day and titrated up to 30–40 mg/kg/day over one month, based on clinical response and tolerability. All patients received LEV as monotherapy throughout the study period.

Data Collection

Demographic data, epilepsy classification, seizure frequency, and LEV dosage were recorded from medical records. Thyroid function tests, including thyroid-stimulating hormone (TSH) and free thyroxine (fT4) levels, were obtained at baseline (prior to initiation of LEV) and at 3, 6, and 12 months of therapy. Blood samples were collected in the morning after an overnight fast and analyzed using chemiluminescent immunoassay methods.

Statistical Analysis

Statistical analyses were performed using SPSS software version 25.0 (IBM Corp., Armonk, NY, USA). Data

TABLE 2. Thyroid function tests over time and p-values

Time point	TSH (mIU/L) (mean±SD)	fT4 (ng/dL) (mean±SD)	p-value for TSH	p-value for fT4
Baseline	1.46±0.3	1.05±0.2	–	–
3 months	1.50±0.4	1.07±0.2	0.26	0.48
6 months	1.55±0.4	1.09±0.3	0.013	0.26
12 months	1.60±0.3	1.12±0.3	0.0017	0.05

SD: Standard deviation; TSH: Thyroid stimulating hormone; fT4: Free thyroxine; p-values were calculated using paired t-tests comparing each time point to the baseline values. Statistical significance: $p<0.05$ was considered statistically significant.

are expressed as mean±standard deviation (SD). Paired t-tests were used to compare thyroid function at each time point with baseline values. A p-value less than 0.05 was considered statistically significant.

RESULTS

Patient Characteristics

A total of 100 patients met the inclusion criteria, comprising 56 females and 44 males, with a mean age of 10.6±3.4 years (range: 2.3–18 years). The types of epilepsy diagnosed in the patients are presented in Table 1. The mean TSH and fT4 levels at each time point are detailed in Table 2.

At 6 months, TSH levels showed a statistically significant increase from baseline ($p=0.013$), and this increase remained significant at 12 months ($p=0.0017$). Despite these statistical differences, TSH levels remained within normal reference ranges (0.5–4.5 mIU/L). Changes in fT4 levels were not statistically significant at 3 and 6 months. At 12 months, there was a borderline significant increase in fT4 levels compared to baseline ($p=0.05$), but values remained within normal limits (0.8–1.8 ng/dL).

Clinical Outcomes

Four patients exhibited transient elevations in TSH levels above the upper normal limit at either 6 or 12 months. These patients were asymptomatic and were referred to pediatric endocrinology for further assessment. None required thyroid hormone replacement therapy, and TSH levels normalized upon subsequent follow-up.

No patients displayed clinical symptoms indicative of hypothyroidism, such as fatigue, weight gain, or growth retardation, throughout the study period.

DISCUSSION

The present study evaluated the impact of levetiracetam (LEV) monotherapy on thyroid function in pediatric patients with epilepsy over a one-year period. Our findings indicate that while there are slight but statistically significant increases in thyroid-stimulating hormone (TSH) levels during LEV therapy, these changes are clinically insignificant and do not result in thyroid dysfunction. Our findings align with limited existing research on LEV's impact on thyroid function. Cebeci et al. [9] conducted a study comparing LEV and valproate monotherapy in children and found no significant changes in thyroid function tests in the LEV group over six months. Similarly, Adhimoolam et al. [8] reported that chronic LEV treatment did not significantly alter thyroid hormone levels in adult patients. These studies, along with our findings, support the notion that LEV has a minimal effect on thyroid function. In contrast, several studies have documented that older generation AEDs, such as carbamazepine and valproate, can significantly affect thyroid function. Verrotti et al. [2] and Elshorbagy et al. [3] demonstrated reductions in fT4 levels and increases in TSH levels in pediatric patients on these medications, leading to subclinical hypothyroidism. The mechanisms proposed include hepatic enzyme induction enhancing the metabolism of thyroid hormones and interference with hormone synthesis. LEV's favorable thyroid profile may be attributed to its unique pharmacokinetic and pharmacodynamic properties. LEV is not significantly metabolized by hepatic enzymes and does not induce or inhibit cytochrome P450 enzymes [1, 7]. This reduces the likelihood of interactions that could accelerate the metabolism of thyroid hormones or alter their synthesis. Additionally, LEV's primary mechanism of action involves binding to the synaptic vesicle protein 2A (SV2A), which is not directly associated with endocrine function [10]. This contrasts with other AEDs that may impact hormonal pathways.

Maintaining normal thyroid function is crucial in the pediatric population due to the essential role of thyroid hormones in growth, neurodevelopment, and metabolism [4]. Hypothyroidism in children can lead to serious complications, including cognitive delays, growth retardation, and developmental abnormalities. Therefore, the selection of AEDs that do not adversely affect thyroid function is of paramount importance.

Our study suggests that LEV is a safe and effective option for treating epilepsy in children without significant risk to thyroid health. This has practical implications for clinicians when choosing an AED, particularly for long-term management. The minimal impact on thyroid function reduces the need for frequent endocrine monitoring and potential interventions, thereby decreasing the burden on patients and healthcare systems.

Strengths of the Study

- Comprehensive Follow-Up: The study evaluated thyroid function at multiple time points over a one-year period, providing insight into both short-term and longer-term effects of LEV therapy.
- Monotherapy Focus: Including only patients on LEV monotherapy eliminates confounding effects from other AEDs, allowing for a clearer assessment of LEV's impact.
- Clinical Correlation: Monitoring for clinical signs of hypothyroidism reinforces the clinical relevance of the findings.

Limitations

This study has limitations, including its retrospective design and relatively small sample size, which may not detect rare adverse effects. The follow-up period of one year may not capture long-term changes in thyroid function. Additionally, other thyroid parameters, such as triiodothyronine (T3) levels and thyroid antibody status, were not evaluated, which could provide a more comprehensive understanding of thyroid function dynamics.

Conclusion

Levetiracetam appears to be a safe antiepileptic drug option concerning thyroid function in children with epilepsy. The slight increases in TSH levels observed were statistically significant but clinically insignificant, with no cases of hypothyroidism detected. These results support the continued use of LEV in the pediatric population without heightened concern for thyroid dysfunction. Further prospective studies with larger cohorts and extended follow-up periods are recommended to confirm these findings and evaluate long-term thyroid health, including a broader range of thyroid parameters. Investigating the underlying mechanisms of LEV's minimal impact on thyroid function may also contribute to the development of AEDs with favorable endocrine profiles.

Ethics Committee Approval: The Umraniye Training and Research Hospital Clinical Research Ethics Committee granted approval for this study (date: 07.09.2023, number: B.10.1.TKH.4.34.H.G.P.0.01/310).

Informed Consent: Informed consent was obtained from all patients and/or their legal guardians where applicable.

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Original Article PEDIATRICS

Fetal echocardiography: A nine-year experience at a single tertiary center

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ABSTRACT

OBJECTIVE: Fetal echocardiography is a vital diagnostic tool used during pregnancy to evaluate the structure and function of the fetal heart. It allows for the early detection of congenital heart diseases (CHDs) and fetal arrhythmias, enabling timely prenatal counseling, perinatal planning, and therapeutic interventions when necessary. While routine obstetric ultrasounds may detect basic structural anomalies, fetal echocardiography provides more detailed anatomical and functional information, particularly in high-risk pregnancies. This study presents a nine-year experience of fetal echocardiography at a tertiary center, focusing on diagnostic accuracy, clinical contribution, and postnatal outcomes.

METHODS: We retrospectively analyzed 405 fetuses who underwent fetal echocardiography between March 2016 and March 2025. All evaluations were performed by an experienced pediatric cardiologist using standard imaging protocols. Fetal cardiac findings and postnatal confirmation data were assessed.

RESULTS: Major structural cardiac anomalies were identified in 48 fetuses (11.9%), with the most common being Tetralogy of Fallot and Transposition of the Great Arteries. Minor findings such as echogenic intracardiac focus, premature atrial contractions, and small ventricular septal defects (VSDs) were seen in 104 cases (25.7%). Significant arrhythmias, including supraventricular tachycardia and complete heart block, were detected in 14 cases (3.5%).

CONCLUSION: Fetal echocardiography is a reliable, non-invasive method for the early and accurate diagnosis of fetal cardiac anomalies. It facilitates appropriate prenatal referral and postnatal care planning, ultimately improving neonatal outcomes.

Keywords: Congenital heart disease; fetal arrhythmia; fetal echocardiography.

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Congenital heart diseases (CHDs) are observed in approximately 1% of all live births and are the most common cause of death in the early neonatal period [1, 2]. Timely diagnosis is essential for planning the appropriate medical and/or surgical interventions, and fetal echocardiography plays a central role in the early detection of these conditions [3].

Fetal echocardiography is primarily recommended for pregnancies classified as high-risk due to fetal, maternal, or genetic indications. However, it may also be performed in low-risk pregnancies based on various clinical concerns [4, 5]. Although the optimal time for fetal echocardiography is between 18 and 22 weeks of gestation, it can be performed earlier or later when necessary [4]. The transition from fetal to neonatal circulation is a critical period for postnatal adaptation. Prenatal diagnosis of CHDs is crucial to prevent hemodynamic instability during this transitional phase and to facilitate the planning of specialized postnatal care [6]. Furthermore, timely diagnosis aids in making critical decisions regarding the timing, location, and mode of delivery.

Also, fetal arrhythmias can occur even in fetuses with structurally normal hearts. While most fetal arrhythmias are benign, persistent arrhythmias and conduction blocks

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can lead to decreased cardiac output, resulting in non-immune hydrops fetalis and fetal loss. Early detection and appropriate management of fetal arrhythmias are essential for preventing intrauterine and neonatal mortality [7].

The aim of this study is to emphasize the role of fetal echocardiography in detecting CHDs and major fetal arrhythmias, and its role in guiding prenatal counseling and perinatal management.

MATERIAL AND METHODS

Study Design and Data Collection

This retrospective study was conducted using fetal echocardiographic evaluations performed between March 2016 and March 2025 in the Pediatric Cardiology Outpatient Clinic. All fetal echocardiographic assessments included in the study were performed and reported by the same pediatric cardiologist (Oyku Tosun, the author). The data were obtained from clinical records and fetal echocardiography reports and were analyzed retrospectively. Cardiac anomalies detected during fetal echocardiography were categorized based on the anticipated need for postnatal management. Cardiac anomalies that were likely to require urgent intervention or treatment during the early postnatal period were defined as complex CHDs. In contrast, anomalies that were unlikely to necessitate immediate postnatal intervention and required only routine follow-up were defined as minor CHDs.

The following variables were reviewed from the medical records at the time of referral:

- Gestational age at the time of examination
- Maternal age
- Gravida, parity, and history of abortion
- Presence of multiple pregnancy
- Use of assisted reproductive technologies (ART)
- Referral indication for fetal echocardiography

As this was a retrospective study based on medical records, informed consent was not required. All patient data were handled in accordance with Helsinki Declaration.

Inclusion and Exclusion Criteria

Inclusion Criteria

Patients who underwent detailed fetal echocardiographic evaluation in the Pediatric Cardiology Outpatient Clinic and had examinations that were fully reported by the same physician.

Highlight key points

- Complex congenital heart disease was detected in 11.9% of fetuses, with all diagnoses confirmed postnatally by the same pediatric cardiologist.
- Tetralogy of Fallot and D-transposition of the great arteries were the most frequently identified complex congenital heart diseases.
- Minor cardiac findings were common (25.7%), predominantly echogenic intracardiac focus, premature atrial contractions, and small ventricular septal defects.
- Significant fetal arrhythmias occurred in 3.5% of cases; timely transplacental and/or direct fetal therapy restored sinus rhythm and resolved hydrops when present.
- Abnormal cardiac findings on second-trimester ultrasound independently constituted the leading referral indication for complex congenital heart disease detection.

Exclusion Criteria

- Patients who had fetal echocardiography performed only in other departments and had no reports documented by the author.
- Patients with incomplete or missing detailed reports for any reason.
- Cases in which postnatal diagnosis could not be confirmed due to loss to follow-up.
- Patients in whom reports indicated that adequate imaging quality could not be performed.

Fetal Echocardiography Procedure

All examinations were carried out by the same pediatric cardiologist using a Samsung H60 Ultrasound System (Samsung Healthcare Systems) with a convex transducer. All examinations were performed in accordance with the American Heart Association (AHA) fetal echocardiography guidelines [8]. The patient was placed in the supine position, with the upper body slightly elevated to optimize the imaging quality. The fetal position and left/right and anterior/posterior orientation were first identified in order to determine the abdominal situs, cardiac position and axis. Standard imaging planes, including the four-chamber view, outflow tracts, great arteries, three-vessel and trachea view, aortic arch, and ductal arch view were assessed in all cases. Relevant findings from these standard views were recorded for each case. Color Doppler and pulsed-wave (PW) Doppler imaging were used to assess blood flow velocities and direction, and obtained data were documented. Fetal heart rate was assessed and recorded using both M-mode imaging and PW Doppler techniques. The

Table 1. Demographic characteristics of the study population (n=405)

Characteristic	Value
Mean maternal age (years)	29.80±5.87
Mean gestational age (weeks)	22±1.6
Nulliparous patients (%)	56.8
Multiparous patients (%)	43.2
Twin pregnancies (%)	5.9
Pregnancies via ART (%)	8.9

ART: Assisted reproductive technologies.

presence and type of any arrhythmia detected during the examination were also documented.

Only examinations that resulted in optimal imaging quality, without limitations caused by maternal body habitus or fetal position and movement, were recorded and included in the analysis.

Statistical Analysis

Descriptive statistical analyses were performed for all variables included in the study. The frequency and percentage distributions of categorical variables were analyzed using the SPSS software program (version 21.0. Armonk, New York: IBM Corp.).

RESULTS

Study Population

A total of 427 pregnant women who underwent fetal echocardiographic evaluation between March 2016 and March 2025 were retrospectively reviewed. Of these, 10 patients were excluded due to suboptimal image quality, and 12 patients were excluded due to the lack of postnatal confirmation of findings. The final study population consisted of 405 patients who met the inclusion criteria.

Demographic Characteristics

The mean maternal age was 29.80±5.87 years (range: 16–48 years), and the mean gestational age at the time of examination was 22±1.6 weeks. Among the included patients, 230 (56.8%) were nulliparous and 175 (43.2%) were multiparous. There were 24 twin pregnancies (5.9%), and 36 pregnancies (8.9%) were conceived using assisted reproductive technologies (ART) (Table 1).

Table 2. Referral indications for fetal echocardiography

Referral indication	Number of patients (n)	Percentage (%)
Abnormal cardiac findings on second-trimester USG	80	19.8
Suboptimal cardiac imaging on routine screening	60	14.8
Self-referral	53	13.1
Fetal arrhythmia	50	12.3
GDM	40	9.9
ART pregnancy	36	8.9
Previous child with CHD	22	5.4
Multiple gestation	24	5.9
Pregestational DM	20	4.9
Advanced maternal age	20	4.9
Total	405	100

ART: Assisted reproductive technologies; CHD: Congenital heart disease; DM: Diabetes mellitus; GDM: Gestational diabetes mellitus; USG: Ultrasonography.

Referral Indications

Referral indications were recorded for all patients. The most common referral reason was abnormal cardiac findings on second-trimester ultrasound (19.8%), followed by suboptimal cardiac visualization on routine screening (14.8%) and self-referral (13.1%) (Table 2).

Fetal Echocardiographic Findings

Among the 405 patients who underwent fetal echocardiographic evaluation, 239 fetuses (59.0%) had normal cardiac anatomy and rhythm. In 48 fetuses (11.9%), complex CHD was detected and all diagnoses were postnatally confirmed by the same pediatric cardiologist. Among fetuses diagnosed with complex CHDs, the most common referral indication was the detection of abnormal cardiac findings during routine second-trimester ultrasound screening (80%). The most frequently identified anomalies were Tetralogy of Fallot (TOF) and D-Transposition of the Great Arteries (D-TGA), each observed in 9 cases (18.8%). Other notable diagnoses included double outlet right ventricle (DORV), complete atrioventricular septal defect (AVSD), Ebstein anomaly, truncus arteriosus (TA), hypoplastic left heart syndrome (HLHS), and pulmonary atresia with intact ventricular septum (PA-IVS). Detailed distribution of the complex fetal cardiac CHDs is pre-

Table 3. Distribution of fetal complex congenital heart diseases

Diagnosis	Number of cases (n)	Percentage (%)
BLV	1	2.1
DORV	5	10.4
TOF	9	18.8
TOF-APVs	1	2.1
cAVSD	3	6.3
D-TGA	9	18.8
Ebstein's anomaly	3	6.3
Critical PS	2	4.2
DCRV	1	2.1
TA	2	4.2
HLHS	2	4.2
DAA/VR	1	2.1
IVS-PA	3	6.3
CMP	2	4.2
Taussig-Bing anomaly	3	6.3
Severe CoA	1	2.1
IAA	1	2.1
Total	48	100

APVs: Absent pulmonary valve syndrome; BLV: Borderline left ventricle; cAVSD: Complete atrioventricular septal defect; CMP: Cardiomyopathy; CoA: Coarctation of the Aorta; PS: Pulmonary stenosis; DAA/VR: Double aortic arch / vascular ring; DCRV: Double-Chambered right ventricle; DORV: Double outlet right ventricle; D-TGA: Transposition of the great arteries; HLHS: Hypoplastic left heart syndrome; IAA: Interrupted aortic arch; TA: Truncus arteriosus; TOF: Tetralogy of fallot.

Table 4. Distribution of fetal minor heart diseases

Cardiac finding	Number of patients (n)	Percentage (%)
EIF	40	38.5
PAC	30	28.8
VSD	21	20.2
-Small perimembranous VSD	11	—
-Small muscular VSD	10	—
AS/BAV	5	4.8
C-TGA	2	1.9
Left atrial isomerism (isolated)	1	1.0
Left pulmonary artery sling	2	1.9
Isolated right aortic arch	2	1.9
PAPVR+ASD	1	1.0
Total	104	100

EIF: Echogenic intracardiac focus; PAC: Premature atrial contractions; VSD: Ventricular septal defect; AS/BAV: Mild aortic stenosis/ bicuspid aortic valve; C-TGA: Corrected transposition of the great arteries; PAPVR+ASD: Partial anomalous pulmonary venous return+ atrial septal defect.

sented in Table 3. For all fetuses diagnosed with complex CHD, families were counseled in detail regarding postnatal prognosis and the expected clinical course. In cases where urgent postnatal intervention or surgical treatment was expected, prenatal consultations were arranged with appropriate tertiary care centers. Delivery planning was coordinated through a multidisciplinary approach to ensure optimal perinatal and postnatal management.

Among the 405 patients who underwent fetal echocardiographic evaluation, 104 (25.7%) had minor cardiac anomalies, including benign rhythm disorders and mild structural anomalies. All diagnoses were postnatally confirmed by the same pediatric cardiologist through standardized echocardiographic and electrocardiographic evaluations. The distribution of these findings is presented in Table 4. The most frequently observed minor cardiac finding was isolated echogenic intracardiac focus (EIF) (38.5%), followed by premature atrial contractions (PAC) (28.8%) which disappeared in the third trimester. The most commonly detected mild structural congenital heart defect was ventricular septal defect (VSD) (20.2%). Among the 24 fetuses diagnosed with VSD, 11 had small perimembranous defects and 10 had small muscular defects requiring postnatal follow-up.

Among the 405 fetuses, significant rhythm disturbances were identified in 14 cases (3.5%) (Table 5). Sustained tachyarrhythmia was diagnosed in 10 of these 14 fetuses. Hydrops fetalis was present in five of these fetuses, atrial fibro-flutter (AF) was detected in one, while the remaining four had long ventriculoatrial (VA) tachyarrhythmia. All cases with hydrops were treated with a combination of direct fetal therapy and transplacental antiarrhythmic treatment. Sinus rhythm was successfully restored in all, and hydrops regressed. In the other five fetuses without hydrops, short VA tachyarrhythmia was observed and sinus rhythm was successfully achieved with transplacental therapy alone in all.

Complete atrioventricular block (CAVB) accompanied by fetal bradycardia was detected in three of 14 fetuses. Maternal serologic evaluation revealed the presence of anti-Ro/SSA and anti-La/SSB antibodies. To prevent the progression to cardiac failure and the de-

Table 5. Summary of significant fetal arrhythmias and management

Type of arrhythmia	Number of cases (n)	Hydrops fetalis present	Treatment approach	Sinus rhythm achieved
Sustained tachyarrhythmia (total)	10	5	Direct fetal+ transplacental therapy	Yes
— AF	1	Yes	Direct fetal+ transplacental therapy	Yes
— Long VA tachycardia	4	Yes	Direct fetal+ transplacental therapy	Yes
— Short VA tachycardia	5	No	Transplacental therapy only	Yes
CAVB + bradycardia	3	No	Maternal treatment; postnatal pacemaker implanted	CAVB
2:1 AVB+ suspected LOTS	1	No	Transplacental therapy; Long QT confirmed postnatally	LQTs

AF: Atrial flutter; AV: Atrioventricular; AVB: Antrioventricular block; CAVB: Complete atrioventricular block; LQTS: Long QT syndrome; VA: Ventriculoatrial.

velopment of hydrops fetalis transplacental treatment, including corticosteroids and/or β-agonists, was administered in all cases. Postnatally, all 3 infants required permanent pacemaker implantation and were referred to a specialized arrhythmia center.

One fetus referred due to bradycardia was found to have a 2:1 atrioventricular block (AVB). Based on these findings, long QT syndrome (LQTS) was suspected, and close prenatal follow-up was initiated. Genetic counseling was provided, and LQTS was also diagnosed in the mother. The diagnosis was confirmed postnatally by electrocardiography (ECG), which revealed a prolonged QT interval of 650 milliseconds.

DISCUSSION

In this retrospective study, we evaluated the diagnostic efficacy and clinical impact of fetal echocardiography in detecting CHDs and major arrhythmias.

In line with previous studies [9, 10], our findings indicate that the most common reason for referral to fetal echocardiographic evaluatin was the detection of abnormal cardiac findings during the second-trimester detailed ultrasonography ultrasoun (19.8%), followed by suboptimal cardiac visualization during routine obstetric ultrasound (14.8%). The high referral rate based on detailed ultrasound findings demonstrate the importance of systematic cardiac screening protocols and adequate training of sonographers. Furthermore, the considerable proportion of referrals due to suboptimal imaging suggests the ongoing need for improved imaging conditions and techniques, particularly in low-resource settings or in cases with maternal or fetal factors limiting visualization.

Among 405 fetuses, complex CHDs were identified in 11.9%, which is comparable to rates reported by Makkar et al. [11] (15%) in a single center study. It is slightly higher than those observed in the previous studies reported by Alp et al. [12] and Ozbarlas et al. [13], where the CHDs detection rate was approximately 5.6% and 7.8%. respectively. This discrepancy may be attributable to differences in referral criteria, as our study group included a high proportion of patients referred due to abnormal ultrasound findings (19.8%) or suboptimal cardiac visualization (14.8%).

In our study, the most common complex CHDs were TOF and D-TGA, each identified in 2.2% of cases. Similar results were reported in the systematic review and meta-analysis by Van der Linde et al. [14], where TOF was the most frequent complex CHD, followed by D-TGA. Early and accurate prenatal diagnosis of these conditions is essential for appropriate counseling, postnatal planning, and timely surgical intervention.

Minor cardiac findings, including isolated VSD and EIF, were present in 25.7% of cases in our study. This is consistent with prior literature, where rates of incidental or minor findings have ranged from 20% to 30% depending on population characteristics and imaging protocols. The clinical significance of these findings varies; while EIF is typically considered a benign variant, isolated small VSDs require follow-up for potential hemodynamic relevance [15–17].

In previous studies, fetal arrhythmias have been reported to occur in 1–3% of pregnancies and to account for approximately 20% of referrals for fetal cardiological evaluation [18, 19]. Consistent with the literature, fetal arrhythmias constituted 12.3% of the indications for fetal echocardiographic evaluation in our study.

Benign PACs were observed in 28.8% of our cases, consistent with previously reported rates [20, 21].

Notably, we identified significant arrhythmias in 3.5% of fetuses, including sustained tachyarrhythmias with hydrops fetalis present in 5 cases. These findings are higher than the previous studies [22, 23]. In cases of persistent fetal tachyarrhythmia, heart failure is an unavoidable consequence of hydrops fetalis and is associated with a high mortality rate [24]. Therefore, timely restoration of a normal fetal heart rate through appropriate treatment is crucial for fetal survival and the achievement of term delivery. The favorable response to transplacental antiarrhythmic therapy in our cohort, including complete rhythm normalization and hydrops resolution, underscores the importance of early detection and specialized management, as also emphasized by Demirci et al. [25].

Additionally, we identified one case of LQTS, which was confirmed postnatally. Similarly, in the case series by Zidere et al. [26], LQTS was suspected in four fetuses based on the presence of fetal bradycardia. In our case, suspicion was also raised due to bradycardia, and the concurrent finding of second-degree AV block further supported the diagnosis. Although rare, such cases are increasingly recognized through advanced fetal rhythm assessment and highlight the importance of early detection for appropriate neonatal management and genetic counseling.

In our study, CAVB was detected in three fetuses, all of whom tested positive for maternal anti-Ro/SSA and anti-La/SSB antibodies, indicating an immune-mediated etiology. Transplacental treatment, including corticosteroids and/or β-agonists, was administered in all cases. Similarly, in the study by Jaeggi et al. [27] involving 37 fetuses with CAVB, combined transplacental therapy was applied and shown to significantly reduce both fetal morbidity and mortality [26]. These findings, in line with our results, indicate the importance of early detection and effective transplacental management to improve fetal survival and reduce complications associated with this potentially life-threatening condition.

Limitations

The retrospective and single-center nature of this study limits its generalizability to the broader population. Additionaly, our institution serves as a referral center for high-risk pregnancies, which may have resulted in a higher observed prevalence of cardiac anomalies compared to the general obstetric population. Future prospective, multicenter studies with larger cohorts are needed to validate these findings and provide more generalizable insights into the impact and outcomes of fetal echocardiography.

Conclusion

When performed under appropriate conditions by experienced specialists, fetal echocardiography plays a critical role in the diagnosis of CHDs and significant fetal arrhythmias. Detecting congenital heart anomalies during the fetal period not only enables effective multidisciplinary planning for postnatal care but also allows early counseling of families regarding prognosis and management options. Additionally, the early detection of life-threatening arrhythmias facilitates early initiation of transplacental or direct fetal therapy, markedly improving perinatal outcomes and survival rates. These findings underscore the importance of fetal echocardiography in optimizing outcomes for both the fetus and the family. Our study reinforces the high diagnostic utility of fetal echocardiography in both structural and rhythm-related cardiac anomalies. The results further support the integration of fetal cardiology services into perinatal care, particularly in tertiary referral centers.

Ethics Committee Approval: The Istanbul Medeniyet University Non-Interventional Clinical Research Ethics Committee granted approval for this study (date: 22.04.2025, number: 2025/07-17).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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Original Article PEDIATRICS

Endoscopic and histopathological findings in children with growth retardation

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ABSTRACT

OBJECTIVE: The study aimed to describe the clinical characteristics, endoscopic and histopathological findings of children admitted to the paediatric gastroenterology outpatient clinic for growth retardation.

METHODS: The study was carried out with 90 patients aged 2–18 years who presented to our paediatric gastroenterology outpatient clinic with complaints of growth retardation and underwent oesophagogastroduodenoscopy (EGD) because growth retardation persisted despite adequate caloric support (at least 3 months).

RESULTS: The mean age of the patients was 11.11±4.27 years, 56 (62.2%) were female and 52 (57.8%) were under 12 years of age. Of the patients, 36.7% had mild, 47.8% moderate and 15.6% severe acute malnutrition, 22.2% stunting and 7.8% severe stunting. Associated dyspeptic symptoms were present in 34 (37.8%) patients and celiac antibodies were positive in 20 (22.2%) patients. Iron deficiency was present in 28.9% and B12 deficiency in 41.1% of the patients. The most common endoscopic findings were pangastritis (88.9%), duodenitis (61.1%), bile reflux (35.6%) and bulbitis (31.1%). Chronic Helicobacter pylori (*H. pylori*) gastritis was found in 72.2%, celiac disease in 20.0% and reflux oesophagitis in 17.8% of the patients. No association was found between chronic *H. pylori* gastritis and iron deficiency anaemia and vitamin B12 deficiency (p=0.90, p=0.89). There was no association between chronic *H. pylori* gastritis and height-for-age, weight-for-height and BMI z scores (0.31, 0.57, 0.43).

CONCLUSION: In this study, positive endoscopic findings were found in all children with persistent growth retardation despite adequate caloric support, even in the absence of accompanying dyspeptic symptoms. More studies evaluating the usefulness of endoscopy in children with growth retardation are needed.

Keywords: Child; endoscopy; growth retardation.

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Growth retardation is a state of malnutrition caused by inadequate caloric intake, malabsorption or excessive caloric expenditure. It may be a sign of underlying systemic disease and is a common clinical problem, accounting for 5–10% of patients seen in outpatient clinics [1, 2]. The anthropometric criteria recommended by the World Health Organisation (WHO) for the diagnosis of growth retardation are weight for height z scores in the first 5 years of life and body mass index (BMI) z scores in children aged >5 years (Weight for height/ BMI z scores: -1 to -1.9: mild acute malnutrition, -2 to -2.9: moderate acute malnutrition, ≤-3: severe acute malnutrition). The height for age z score is used to assess chronic malnutrition (Height for age z-score <-2: stunting, <-3: severe stunting) [3].

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The causes of growth retardation can be organic (medical) or non-organic (social or environmental). Organic gastrointestinal (GIS) causes include gastrointestinal obstruction, gastroesophageal reflux disease, eosinophilic oesophagitis and gastroenteritis, food allergy, celiac disease, intestinal lymphangiectasia and inflammatory bowel disease [1, 2, 4]. The effect of Helicobacter pylori (*H. pylori*) infection on growth retardation remains controversial. Some studies have shown that *H. pylori* has a negative effect on children's growth (both height and weight or only height growth) [5–11], while other reports suggest that *H. pylori* infection has no negative effect on growth [12–16].

Oesophagogastroduodenoscopy (EGD) is an invasive procedure that is widely used in the diagnosis and management of upper gastrointestinal disorders [17]. Currently, the most common indication for endoscopy in children is abdominal/epigastric pain, followed by vomiting, growth retardation or weight loss [18].

The aim of this study is to investigate endoscopic and histopathological findings in pediatric patients who have had unexplained and persistent nutritional problems despite adequate caloric support for at least 3 months and who underwent EGD due to suspected underlying gastrointestinal disease.

Highlight key points

- All children with persistent growth retardation showed abnormal endoscopic findings, even without dyspeptic symptoms.
- Pangastritis, duodenitis, bile reflux, and bulbitis were the most common endoscopic findings.
- Chronic Helicobacter pylori gastritis and celiac disease were frequent histopathological diagnoses in these patients.

MATERIALS AND METHODS

This study was carried out with 90 patients aged 2–18 years who were admitted to the Paediatric Gastroenterology Outpatient Clinic of Kahramanmaras Sutcu Imam University Hospital between June 2023 and September 2024 with the complaint of growth retardation and who underwent EGD because unexplained nutritional problems persisted despite adequate caloric support (at least 3 months). The approval of Kahramanmaras Sutcu Imam University Medical Research Ethics Committee numbered 22/01 and dated 02.09.2024 was obtained and this study was conducted according to the Declaration of Helsinki.

Demographic characteristics, complaints, clinical findings, laboratory results, endoscopic and histopathological findings were obtained from patient records. Upper GI endoscopy was performed by the same paediatric gastroenterologist using an Olympus GIF-H180J endoscope under deep sedation provided by the anaesthetist. Biopsy specimens were transported to the pathology laboratory in formaldehyde solution. A rapid urease test was also used to establish the diagnosis of *H. pylori*, and the presence and colonisation of *H. pylori* in biopsy specimens was assessed by histopathology. The presence and severity of *H. pylori* gastritis was determined using the modified Sidney scoring system, and the presence of celiac disease was determined using the Marsh scoring system.

To evaluate growth retardation, weight-for-height *z*-scores in the first 5 years of age and BMI *z*-scores at >5 years of age were used as anthropometric criteria as recommended by WHO. The height-for-age *z*-score was used to assess chronic malnutrition. Patients were classified as preterm (<37 weeks of gestation) and term (≥37 weeks of gestation) according to gestational age, SGA (birth weight <10th percentile for gestational age) and normal (birth weight 10th–90th percentile for gestational age) according to birth weight.

Descriptive statistics were presented as numbers and percentages for categorical variables and as mean standard deviation for numerical variables. The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to assess whether continuous variables were normally distributed. The Chi-square test was used to analyse categorical variables. The Mann-Whitney U test was used to analyse continuous variables in independent groups that did not have parametric characteristics. Analyses were performed using the SPSS software program, version 21.0 (Armonk, New York: IBM Corp.). The level of statistical significance was set at $p<0.05$.

RESULTS

The mean age of the patients was 11.11±4.27 years, 56 (62.2%) were female and 52 (57.8%) were under 12 years of age. Of the patients, 36.7% had mild, 47.8% moderate and 15.6% severe acute malnutrition, 22.2% stunting and 7.8% severe stunting. Associated dyspeptic symptoms were present in 34 (37.8%) patients and celiac antibodies were positive in 20(22.2%) patients. Iron deficiency was present in 28.9% and B12 deficiency in 41.1%

of the patients (Table 1). The most common endoscopic findings were pangastritis (88.9%), duodenitis (61.1%), bile reflux (35.6%) and bulbitis (31.1%). Chronic Helicobacter pylori (*H. pylori*) gastritis was found in 72.2%, celiac disease in 20.0% and reflux oesophagitis in 17.8% of the patients (Table 2).

No association was found between the presence of accompanying dyspeptic symptoms and the degree of malnutrition (p=0.12) (Table 3). No association was found between chronic *H. pylori* gastritis and iron deficiency anaemia and vitamin B12 deficiency (p=0.90, p=0.89). There was no association between chronic *H. pylori* gastritis and height-for-age, weight-for-height and BMI z scores (p=0.31, p=0.57, p=0.43) (Table 4). There was no association between the degree of malnutrition and birth week, birth weight and consanguineous marriage (p=0.81, p=0.83, p=0.64).

DISCUSSION

Growth retardation may affect patients of any gender, race and age, but it is most commonly observed in infants and young children. Anthropometric assessment is essential to determine the severity of nutritional deficiency [2]. In this study, the majority of patients were under 12 years of age [2–6 years (17.8%) and 6–12 years (40%)] and 47.8% were acutely moderately malnourished. 30% were chronically malnourished (stunted).

Micronutrient deficiencies, including vitamins and trace minerals (e.g. copper, zinc, iron, iodine), are associated with impaired cognitive function, increased risk of infection, poor growth, developmental delay and increased risk of morbidity and mortality [14]. Therefore, children with growth retardation should be screened for these nutrient deficiencies and supplemented if necessary [15]. Iron deficiency is the most common micronutrient deficiency worldwide and its prevalence in children and adolescents in Turkiye has been reported to vary from 6.5% to 42% in different regions [16]. Epidemiological studies have highlighted that the rate of B12 deficiency varies between 5% and 60% [17]. In this study, iron deficiency was found in 28.9% and B12 deficiency in 41.1% of the patients.

Endoscopic evaluation in children with growth retardation is not well defined. In a prospective study performed in children with growth retardation referred to a paediatric gastroenterology clinic, the diagnostic value of endoscopy was found to be higher than imaging and lab-

Table 1. Demographic and clinical characteristics of the patients (n=90)

	%
Gender	
Female	62.2
Male	37.8
Age groups	
2–6 years	17.8
6–12 years	40
>12 years	42.2
Birth week	
Preterm	4.4
Term	95.6
Birth weight	
Normal	87.8
SGA	12.2
Consanguineous marriage	
No	72.2
Yes	27.8
Degree of malnutrition	
Mild	36.7
Moderate	47.8
Severe	15.6
Stunting	
No	70.0
Yes	22.2
Severe	7.8
Accompanying dyspeptic symptom	
No	62.2
Yes	37.8
Antitissue trans Ig A	
Negative	77.8
>3–7 times positive	2.2
>x7 times positive	2.2
>10 times positive	17.8
Iron deficiency anaemia	
Yes	28.9
No	71.1
Vitamin B12 deficiency	
Yes	41.1
No	58.9

SGA: Small for gestational age; Antitissue trans Ig A: Antitissue transglutaminase immunoglobulin A.

Table 2. Endoscopic and histopathological findings of the patients

	%
Endoscopic diagnosis	
Pangastritis	88.9
Duodenitis	61.1
Bile reflux	35.6
Bulbitis	31.1
Oesophagitis	16.7
Antral gastritis	10.0
Lower esophageal sphincter insufficiency	7.8
Hiatal hernia	1.1
Oesophageal ulcer	1.1
Antral ulcer	1.1
81.1% of patients had more than one abnormal finding.	
Histopathological findings	
Chronic *Helicobacter pylori* gastritis	72.2
Chronic gastritis	27.8
Mild chronic duodenal inflammation	56.7
Reflux oesophagitis	17.8
Celiac disease	20.0
Potential celiac disease	2.2
88.8% of patients had more than one abnormal finding.	

Table 3. Relationship between dyspeptic symptoms and malnutrition

	Accompanying dyspeptic symptoms				p
	No		Yes		
	n	%	n	%	
Degree of malnutrition					0.12
Mild	25	44.6	8	23.5	
Moderate	23	41.1	20	58.8	
Severe	8	14.3	6	17.6	

Table 4. The relationship between chronic *Helicobacter pylori* gastritis and anthropometric measurements

	Chronic *Helicobacter pylori* gastritis				p
	No		Yes		
	M	SD	M	SD	
Height for age z score	-1.69	1.25	-1.42	1.26	0.31*
Weight for height z score	-2.08	0.58	-1.94	0.52	0.57*
BMI z score	-2.37	0.95	-2.48	0.95	0.43*

M: Mean; SD: Standard deviation; BMI: Body mass index; *: Non parametric test.

oratory evaluation. No positive endoscopy result was obtained in any of the children who underwent endoscopy for growth retardation without accompanying symptoms [18]. All of the patients in our study had positive endoscopic findings and accompanying dyspeptic symptoms were present in 37.8%. The most common endoscopic findings in our patients were pangastritis (88.9%), duodenitis (61.1%), bile reflux (35.6%) and bulbit (31.1%) and 81.1% of the patients had more than one abnormal endoscopic finding.

Celiac disease (CD) is a chronic autoimmune enteropathy caused by the ingestion of gluten in genetically predisposed individuals and its prevalence varies between 8% and 24% in children with unexplained growth retardation [19]. Gastroesophageal reflux disease is also one of the organic causes of growth retardation in children [20]. In this study, celiac antibodies were positive in 22.2% of the patients and CD was found in 20.0% and reflux oesophagitis in 17.8%.

H. pylori infection is common in both developed and developing countries. In Turkiye, the prevalence of *H. pylori* was found to be 82.5% in adults, 75.8% in children aged 13–18 years and 40% in children aged 4–6 years [21, 22]. *H. pylori* has been shown to cause acute and chronic gastritis, peptic ulcer disease and stomach cancer. It has been suggested that it may also be associated with extraintestinal findings including iron deficiency anaemia, immune thrombocytopenic purpura, growth retardation and vitamin B12 deficiency, but the findings are contradictory [23]. Three different pathophysiologies explaining the negative effect of *H. pylori* infection on growth have been defended. Firstly, malabsorption which develops due to suppression of gastric acid secretion, secondly, dyspepsia-related decreased appetite caused by *H. pylori* infection. Finally, iron deficiency anaemia due to *H. pylori* infection is also thought to impair growth [24]. In our study, chronic *H. pylori* gastritis was found in 72.2% of

the patients and no relation was found between chronic *H. pylori* gastritis and iron deficiency anaemia and vitamin B12 deficiency. There was no relationship between *H. pylori* gastritis and height-for-age, weight-for-height and BMI z scores of the patients.

Conclusion

In this study, positive endoscopic findings were detected in all children with growth retardation and unexplained nutritional problems despite adequate caloric support, even in the absence of accompanying dyspeptic symptoms, and their treatments were reorganized according to these findings. In children with unexplained growth retardation, performing an EGD to detect organic gastrointestinal diseases will contribute to patient follow-up. More studies evaluating the usefulness of endoscopy in children with growth retardation are needed.

Ethics Committee Approval: The Kahramanmaras Sutcu Imam University Clinical Research Ethics Committee granted approval for this study (date: 02.09.2024, number: 22/01).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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

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Use of AI for Writing Assistance: The author declared that artificial intelligence-supported technologies (such as Large Language Models [LLMs], chatbots or image generators, ChatGPT) were not used in the production of the study.

Peer-review: Externally peer-reviewed.

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Original Article PEDIATRICS

Efficacy and safety of magnesium prophylaxis in children with migraine without aura

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ABSTRACT

OBJECTIVE: Migraine is one of the prevalent types of primary headache disorders in children and significantly affects their quality of life. Although pharmacological prophylaxis is often necessary, conventional treatments are frequently limited by adverse effects and modest efficacy. Magnesium, a vital intracellular cation involved in numerous neuronal and vascular functions, has been proposed as a safer alternative. However, data on its use in pediatric migraine remain limited. To investigate the effectiveness and safety profile of magnesium oxide prophylaxis in children diagnosed with migraine without aura.

METHODS: This retrospective study included pediatric patients aged 7–18 years with a diagnosis of migraine without aura, treated exclusively with magnesium oxide at a dosage of 6–9 mg/kg/day or a fixed dose of 365 mg/day for four months. Headache frequency, migraine-related disability (assessed by PedMIDAS), and quality of life (measured by HIT-6) were evaluated before and after four months of prophylaxis.

RESULTS: Following magnesium prophylaxis, a statistically significant reduction was observed in headache frequency, PedMIDAS scores, and HIT-6 scores ($p<0.001$ for all). Additionally, disability levels according to PedMIDAS grading improved significantly. No participants reported side effects during the study.

CONCLUSION: Magnesium oxide appears to be a well-tolerated, safe, and potentially effective prophylactic option for children with migraine without aura. It was associated with a significant reduction in attack frequency, disability scores, and improved quality of life. Despite promising results, the absence of a control group and serum magnesium data are notable limitations. Further prospective, randomized controlled studies are required to confirm these findings and establish the most effective dosage, duration, and formulation of magnesium for pediatric use.

Keywords: Children; magnesium; migraine; nutraceuticals; prophylaxis.

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Migraine is the most frequent primary headache in children, with a reported prevalence of approximately 10% [1]. It typically presents with recurrent episodes of headache accompanied by symptoms such as nausea, vomiting, photophobia, and/or phonophobia. It is one of the most important disease-causing conditions associated with disability and has a negative impact on the child's quality of life. Therefore, effective management is critical. The treatment of pediatric migraine consists of the acute treatment of attacks and prophylactic therapy.

It is estimated that about 30% of children with migraine require prophylactic treatment [2]. The primary

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aim of prophylaxis is to decrease the frequency, severity, and duration of migraine attacks, thereby minimizing their disruptive effects on the child's life. Prophylactic treatment includes antidepressants, antiseizure medications, antihistamine drugs, beta-blockers, and calcium channel antagonists. Some studies have demonstrated limited efficacy of these agents in children, with a relatively high incidence of adverse effects [3]. In recent years, this has led to growing interest in alternative treatment modalities—particularly nutraceuticals—among clinicians, patients, and their families, due to their perceived safety and tolerability. Nutraceutical approaches include vitamins (e.g., riboflavin), trace elements such as magnesium, coenzyme Q10, and various herbal compounds [4]. Although several studies support the use of these agents in adults, evidence in the pediatric population remains limited [5–7].

Among these options, magnesium has garnered particular attention. It is the second most common cation inside the cell and is involved in many enzymatic reactions. Magnesium plays a key role in neuronal excitability, neurotransmitter release, and vascular tone regulation—all processes involved in migraine pathophysiology [8, 9]. Magnesium deficiency is hypothesized to contribute to migraine through multiple mechanisms, including promoting cortical spreading depression, enhancing platelet aggregation, modulating serotonin receptor function, and altering the synthesis and release of neurotransmitters such as nitric oxide. It has been proposed that magnesium may be a key factor in determining individual susceptibility to migraine attacks. Its potential effects include neuroinflammation suppression, calcium channel antagonism, N-methyl-D-aspartate (NMDA) receptor inhibition, and regulation of hormonal and serotonergic systems. Additionally, magnesium deficiency may facilitate neurogenic inflammation, partly mediated by substance P, further implicating its role in migraine pathogenesis [8, 9].

Given the limited evidence available in children, the present study was conducted to assess the safety and effectiveness of magnesium prophylaxis in pediatric patients diagnosed with migraine without aura.

MATERIALS AND METHODS

This retrospective study evaluated the medical records of children diagnosed with migraine without aura who received magnesium prophylaxis and were followed at the Pediatric Neurology Outpatient Clinic of Istanbul Medeniyet University Goztepe Prof. Dr. Suleyman Yalcin State Hospital between January 2022 and July 2023.

Highlight key points

- Magnesium prophylaxis significantly reduced headache frequency in children with migraine without aura.
- Prophylaxis with magnesium also improved quality of life and reduced migraine-related disability, as assessed by HIT-6 and PedMIDAS scores.
- No serious side effects were documented, supporting the safety and tolerability of magnesium in the pediatric population.

Data collected included demographic information (age, sex), family history of migraine, duration of migraine, and the frequency of attacks before and after magnesium prophylaxis.

Inclusion Criteria were as Follows

- Age between 7 and 18 years,
- Diagnosis of migraine without aura according to the International Classification of Headache Disorders, 3rd edition (ICHD-3) [10],
- Not receiving any other prophylactic treatment for migraine aside from magnesium.

Exclusion Criteria Included

- Presence of a chronic systemic illness or psychiatric disorder,
- Discontinuation of magnesium treatment before study completion,
- Headaches not fulfilling diagnostic criteria for migraine,
- Non-compliance with follow-up visits,
- Concurrent use of other migraine prophylactic medications or vitamin/mineral supplements.

Magnesium prophylaxis was initiated in patients experiencing frequent migraine attacks (defined as ≥4 attacks per month) or significant impairment in quality of life. All participants received oral magnesium oxide at a dosage of 6–9 mg/kg/day or a fixed dose of 365 mg/day, administered for a period of four months [5–7].

Patients were evaluated at baseline, after one month, and at the end of the fourth month. At each visit, data were collected on headache frequency and severity, functional disability due to migraine, and any reported side effects.

Table 1. Characteristics of headache before and after treatment (n=50)

	Before treatment (Mean±SD)	After treatment (Mean±SD)	p
Headache frequency (day/month)	11.3±8.3 (2–30)	5.6±7 (0–30)	<0.001
HIT-6 scores	65.4±6.6 (50–78)	59±10 (31–76)	<0.001
PedMIDAS scores	28.9±24.2 (4–130)	16.1±21.5 (0–120)	<0.001

HIT-6: Headache Impact Test; PedMIDAS: Pediatric migraine disability assessment tool; SD: standard deviation.

Migraine-related disability was measured using the Pediatric Migraine Disability Assessment (PedMIDAS) tool [11]. PedMIDAS scores were categorized into four grades: Grade 1, 0–10 (little to no disability); Grade 2, 11–30 (mild disability); Grade 3, 31–50 (moderate disability); and Grade 4, >50 (severe disability) [12].

Quality of life was assessed using the Headache Impact Test (HIT-6), a validated six-item self-report questionnaire widely used in headache research and clinical practice [13].

Both the PedMIDAS and HIT-6 assessments were administered before treatment (baseline) and at the fourth month of magnesium prophylaxis. The primary outcomes were changes in headache frequency (average days of headache per month), PedMIDAS scores, and HIT-6 scores before and after prophylaxis.

The study protocol was approved by the Istanbul Medeniyet University Ethics Committee under decision number 2023/0627 and was conducted in accordance with the Helsinki Declaration.

Statistical Analysis

All statistical analyses were performed using SPSS for Windows, version 15.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were presented as mean±standard deviation. Student's t-test was employed for comparisons between two groups, while the chi-square test was used to analyze categorical variables. The Wilcoxon signed-rank test was used to assess differences in PedMIDAS disability grades before and after treatment. A p-value<0.05 was considered statistically significant.

RESULTS

The study consisted of 50 children (16 males, 34 females; mean age: 13.4±2.75 years) diagnosed with migraine without aura. A positive family history of migraine was present in 19 patients (38%). The average duration of migraine symptoms was 36±12 months.

Table 2. Comparison of migraine-related disability based on the PedMIDAS grading system before and after treatment (n=50)

Disability grade	Before treatment (%)	After treatment (%)
Grade 1 (little/none)	14	60
Grade 2 (mild)	52	26
Grade 3 (moderate)	22	8
Grade 4 (severe)	12	6

PedMIDAS: Pediatric migraine disability assessment tool.

Characteristics of headache before and after treatment are presented in Table 1. After magnesium prophylaxis, there was a statistically significant decrease in headache frequency, PedMIDAS scores, and HIT-6 scores (p<0.001) (Table 1). None of the children reported any side effects related to magnesium prophylaxis.

Furthermore, analysis of migraine-related disability using the PedMIDAS grading system revealed a significant improvement in disability levels after treatment compared to baseline (p<0.001) (Table 2).

DISCUSSION

In this study, we evaluated the effect of magnesium oxide prophylaxis on the frequency and disability of migraine in children without aura. Our findings demonstrated that a 4-month magnesium prophylaxis significantly reduced both headache frequency and associated disability, while also improving the quality of life in affected children.

Pediatric migraine management often presents unique challenges. Conventional prophylactic medications such as amitriptyline and topiramate have shown limited benefit in children and are frequently associated with adverse effects [3]. Additionally, the high placebo response observed in pediatric trials complicates clinical decision-making [14]. These factors have led to increased interest in nutraceuticals, including magnesium, as safer and more acceptable alternatives for children [15].

Magnesium is an important intracellular cation involved in many enzymatic and physiological reactions, including neurotransmitter release, vasomotor tone regulation, and energy metabolism [8, 9]. It also acts as a natural NMDA receptor antagonist, a mechanism believed to play a role in migraine pathogenesis. Some studies have reported lower magnesium levels in serum, intracellular compartments, blood cells, saliva, and brain tissue of patients with migraine, indicating a potential link between magnesium deficiency and migraine development [16–19].

While the American Academy of Neurology and the American Headache Society support magnesium use for adult migraine prophylaxis according to evidence classified as Level B [20], its effectiveness in children remains controversial and not yet well established. The current literature on this topic is sparse, with a limited number of studies evaluating magnesium's role in pediatric migraine prophylaxis [5–7, 21, 22].

In a double-blind, randomized, placebo-controlled study, Wang and colleagues administered magnesium oxide at a dosage of 9 mg/kg/day for a duration of 12–16 weeks to a group of 42 pediatric migraine patients. The study observed a notable reduction in the severity of headaches among those receiving magnesium. However, the impact on headache frequency was less definitive. While there was a decrease in the number of headache days within the magnesium group, the difference in the rate of reduction between the magnesium and placebo groups was not statistically significant [5]. Gastrointestinal symptoms, such as diarrhea and soft stools, were reported more frequently in the magnesium group, though no other major adverse events were noted. Overall, the findings suggested that magnesium oxide, at doses up to 9 mg/kg/day, is well tolerated in children [5].

In a cohort study including 34 children who were given either magnesium oxide or magnesium glycinate at a dosage of 4–6 mg/kg/day for six months, the authors reported a decrease in the frequency of migraine attacks, as well as improvements in quality of life and anxiety and depressive symptoms. Additionally, migraine-related disability, assessed using PedMIDAS scores, decreased after three months of magnesium prophylaxis, with the most significant improvement observed at six months. No adverse effects were reported [7].

Gallelli et al. [6] used magnesium at a dosage of 400 mg/day for 18 months in 160 children with migraine without aura. The children were divided into four treatment groups based on whether they received paracetamol or ibuprofen for acute attacks, with or without magnesium as prophylactic treatment. The authors found that magnesium prophylaxis reduced both the intensity and frequency of headache attacks. In addition, no side effects related to the treatment were reported.

Castelli et al. [21] studied 41 children with periodic syndromes, 25 of whom were diagnosed with migraine and received magnesium pidolate at a dosage of 1.5–4.5 g/day (corresponding to 122–366 mg of elemental magnesium). They reported a decrease in headache frequency in 72.5% of the patients.

In our study, consistent with the existing literature, magnesium prophylaxis was found to decrease the frequency of migraine attacks [5–7, 21].

A significant reduction was observed in migraine-related disability, as assessed by PedMIDAS scores, and in quality of life scores, as measured by HIT-6, following magnesium treatment. Furthermore, when patients were classified according to the PedMIDAS grading system, a marked improvement in disability grades was noted after treatment. To date, only one study in the literature has addressed this issue: Kovacevic et al. [7] reported a decrease in migraine-related disability, assessed by PedMIDAS, and an improvement in quality of life, assessed by the KIDSCREEN-27 questionnaire, following magnesium prophylaxis.

The optimal dosage, duration of treatment, and most effective formulation of magnesium vary between studies. Wang et al. [5] administered magnesium oxide at a dosage of 9 mg/kg/day for 12–16 weeks. Kovacevic et al. [7] used both magnesium oxide and magnesium glycinate at doses of 4–6 mg/kg/day for 3–6 months. Gallelli et al. [6] administered 400 mg/day of magnesium for 18 months, although the specific formulation was not reported. Castelli et al. [21] used magnesium pidolate at a dosage of 1.5–4.5 g/day (equivalent to 122–366 mg of elemental magnesium) for 1–2 months. In our study, magnesium oxide was used at a dosage of 6–9 mg/kg/

day or 365 mg/day for a duration of four months. Consistent with previous studies, no serious side effects were observed in our cohort. Although the ideal dosage and duration of magnesium prophylaxis in pediatric patients have yet to be clarified, existing data indicate that daily doses up to 9 mg/kg and treatment periods of 3–6 months are both safe and tolerable.

One of the major limitations in evaluating the effectiveness of magnesium in pediatric migraine is the high placebo response rate reported in this population [3, 14]. This raises uncertainty as to whether the observed improvement is due to the pharmacological effect of magnesium itself or to a placebo effect. In our study, the absence of a control group and the lack of comparison with a placebo arm prevent definitive conclusions regarding the specific efficacy of magnesium. Additionally, serum magnesium levels could not be assessed, as this was a retrospective study and such data were not available in the medical records. However, it is important to emphasize that previous studies have reported that only about 2% of total body magnesium is present in the extracellular compartment [8]. Therefore, routine serum magnesium measurements do not accurately reflect total body magnesium status.

Conclusion

Despite the impact of migraine on children's daily quality of life and disability, the majority of children with migraine who present to healthcare providers do not receive prophylactic treatment. Magnesium appears to be a cost-effective, accessible, and safe supplement that may help reduce attack frequency and improve both disability and quality of life in the pediatric population. Further prospective, randomized controlled studies are warranted to validate the effectiveness of magnesium prophylaxis, determine the most appropriate dosage and formulation, and establish standardized treatment protocols for children with migraine.

Ethics Committee Approval: The Istanbul Medeniyet University Clinical Research Ethics Committee granted approval for this study (date: 27.09.2023, number: 2023/0627).

Informed Consent: Written informed consents were obtained from patients who participated in this study.

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

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Original Article ONCOLOGY

In vivo antioxidant and anti-inflammatory effects of *Myrtus communis* against ionizing radiation-induced gastrointestinal injury: TROD-GROG-002 study

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ABSTRACT

OBJECTIVE: This study aimed to investigate the *in vivo* radioprotective effects of *Myrtus communis* (MC) on the gastrointestinal system.

METHODS: A total of 30 female rats were divided into four groups: i) Control; ii) irradiation (IR) only; iii) MC-pretreated; and iv) MC-treated. The rats received oral MC extract (100 mg/kg/day) for 4 days before exposure to 10 Gy IR or continued until sacrifice. On the fourth day of IR exposure, the rats were sacrificed, and histopathological and biochemical analyses were performed on the ileum, pancreas, and liver tissues.

RESULTS: Malondialdehyde and myeloperoxidase levels decreased in both MC-treated groups, while glutathione levels and Na^+-K^+-ATPase activity increased ($p<0.01$), with significant histopathological improvements compared to the IR-only group.

CONCLUSION: The results of this study demonstrate that MC significantly decreases ionizing radiation-induced oxidative and inflammatory damage in the gastrointestinal systems of rats. Therefore, it may be regarded as a new candidate with radioprotective potential for future clinical application.

Keywords: Ionizing radiation; ileum; liver; Myrtus communis; pancreas; rats.

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Radiotherapy has been a key therapeutic intervention in gastrointestinal cancer treatment, and new treatment techniques have improved its efficacy [1, 2]. Modern techniques reduce the radiation dose received and the volume of surrounding organs irradiated. However, the effectiveness of radiation therapy has been associated with harmful side effects due to the damage to healthy tissues [3–5]. Different regions of the gastrointestinal system may be directly affected by the primary tumor location or by organs at risk [4, 5]. Radiation-induced late effects such as ulcers, bleeding, enteritis, and proctitis reduce the quality of life of cancer survivors [5–7]. Moreover, the side effects of radiotherapy often negatively affect tumor control, which may lead to incomplete treatment [8]. In abdominal radiotherapy, protecting normal tissues with medical and pharmacological agents and technical advancements still require further investigation [6, 8].

Ionizing radiation directly and indirectly causes cellular damage through reactive oxygen species, leading to cellular dysfunction and triggering inflammatory responses. These effects can cause either repairable damage to essential cellular structures or permanent damage, which may result in cell death [9, 10]. Several agents and interventions have been studied to decrease normal tissue injury due to radiotherapy on gastrointestinal organs [8, 11, 12]. Preclinical studies have shown that plant-derived products and their phenolic compounds may be a potential solution for suppressing radiation-induced gastrointestinal damage. These compounds were targeted due to their antioxidant and anti-inflammatory activities [13–20]. Hence, phenolic compounds such as phenolic acids and flavonoids may be potential radioprotectors for clinical usage.

Myrtus communis (MC) is a species of flowering plant in the myrtle family, Myrtaceae, with leaves that contain phenolic acids, flavonoids, terpenoids, tannins, coumarins, and oligomeric nonprenylated acyl phloroglucinol compounds. The leaves, berries, flowers, and essential oils are already used for medicinal properties [21, 22], and the anti-inflammatory and anti-antioxidant effects of MC have been extensively studied. However, to date, no studies have investigated the effects of MC on radioprotection [23–27]. Therefore, the present study aimed to investigate the potential protective effect of MC on abdominal organ damage, including the ileum, liver, and pancreas, as a novel radioprotection intervention.

Highlight key points

- This experimental study investigates the *in vivo* antioxidant and anti-inflammatory (radioprotective) effects of *Myrtus communis* against ionizing radiation–induced gastrointestinal damage.
- MC reduced oxidative stress markers, as indicated by lower levels of malondialdehyde (MDA) and myeloperoxidase (MPO).
- MC enhances antioxidant and cellular defenses, increases glutathione levels, and Na^+-K^+-ATPase activity.
- Histopathological improvements in gastrointestinal tissues compared to the IR-only group.
- MC may be a potential new radioprotective agent for future clinical applications.

MATERIALS AND METHODS

Experimental Animals

The present study received approval from the Marmara University Animal Care and Use Committee (approval no: 07.2022). A total of 30 female Rattus norvegicus rats (age, 3 months; weight, 250-300 g) were obtained from Istanbul Health Sciences University. The animals were housed at 22-24°C with a 12-hour light/dark cycle at Marmara University Experimental Animals Application and Research Center. Each cage housed four animals, and the rats received food and water regularly before and during the experiment, with no restrictions imposed during the study. An adaptation period was provided for the animals to acclimate to the environment before the experiment. Throughout the experiment, the animals were kept in a low-stress, clean, and quiet environment where they could maintain their natural behaviors and were protected from stressful factors such as noise and light. The oral gavage administration of MC and irradiation (IR) was painless during the experiment; therefore, no pain-relieving treatment was administered. This experimental study was conducted in accordance with the principles outlined in the Helsinki Declaration.

Collection of MC leaves and obtaining the extract. *Myrtus communis* L. subsp. communis leaves were collected from the Turgutlu district of Manisa province, Turkiye, by Dr. Gizem Bulut, an expert botanist. Voucher specimens were stored in the Herbarium in the Faculty of Pharmacy, Marmara University (MARE No: 13006). MC extract was prepared according to a previously described procedure [26]. Dried powdered leaves (100 g) were extracted with 96% ethanol using a Soxhlet

extractor. After filtration, the extract was dried using a rotary evaporator. The MC extract powder, with a yield of 28.56%, was kept in a dark glass bottle in a refrigerator (4°C) until subsequent use.

Experimental groups and procedures: Animals were divided into four groups: i) The control group (n=6), which received only oral saline (SF) for the duration of the experiment (8 days); ii) the IR group (n=8), which received oral SF for 4 days, commencing on the day of IR exposure; iii) the MC pretreated group (n=8), which received oral MC for 8 days, commencing 4 days before IR exposure; and iv) the MC treatment group (n=8), which received oral MC for 4 days, commencing on the day of IR exposure.

A previous study described the preparation of the MC extract, and in the present study, the same conditions were followed, demonstrating its efficacy [26]. A total of 100 mg/kg MC extract was administered daily in the morning to the MC treatment and MC pretreated groups using an oral gavage tube, as previously described [26, 27].

Anesthesia (60 mg/kg ketamine, 10 mg/kg xylazine) was injected intraperitoneally to immobilize the rats immediately before the IR procedure. After this, the anesthetized rats were positioned face down on a board with their tails and legs taped. Computed tomography images with 1-mm slices were obtained for radiotherapy planning simulation, and 3D planning was performed. Following completion of 3D planning, a single fraction of 10 Gy IR was delivered with a linear accelerator using 6 MV photons from two anteroposterior directions, at a dose rate of 500 MU/min. After administering with ether anesthesia, all animals were decapitated by guillotine on the fourth day after IR exposure. After the rats were sacrificed, appropriate methods were used to remove the ileum, pancreas, and liver tissues.

Biochemical analysis: Tissue samples were analyzed to determine malondialdehyde (MDA) and glutathione (GSH) levels, and myeloperoxidase (MPO) and Na^+-K^+-ATPase activity as previously described [28–33].

Histopathological evaluation: Histopathological analysis with hematoxylin and eosin was performed as previously described [34]. Samples were fixed in a 10% formaldehyde solution, dehydrated in an ascending alcohol series, cleared in toluene, and embedded in paraffin. For histopathological evaluation, paraffin sections (5 μm) were stained with hematoxylin and eosin and examined under a photomicroscope (Olympus BX51; Olympus Corporation). Histopathological evaluation was performed by histologists who were blind to the study groups. Both histologists evaluated and scored the samples independently, and the results were the same. In cases that were not compatible, the results were averaged after discussion between the two researchers. Modified semiquantitative histopathological criteria are summarized in Table 1. Histopathological scores were given as 0, none; 1, mild; 2, moderate; and 3, severe for each criterion. Each criterion was calculated as the sum of its scores, and in each specimen, at least 5 microscopic areas were examined. The maximum score calculated was 3 for the ileum, 6 for the liver, and 12 for the pancreas [35–37].

Table 1. Histopathological evaluation criteria

Tissue	Histopathological appearance
Ileum	• Normal • Degeneration of surface epithelium and mild inflammatory cell infiltration • Degeneration of surface and crypt epithelium, a moderate decrease in the number of goblet cells with moderate inflammatory cell infiltration • Flattened mucosal villi with a severe decrease in the number of goblet cells and severe inflammatory cell infiltration
Liver	• Hepatocyte degeneration • Congested and dilated sinusoids
Pancreas	• Acinar cell degeneration • Edema • Vascular congestion • Langerhans islet degeneration

Statistical Analysis

The statistical analysis were performed using GraphPad Prism 6.0 (GraphPad Software, San Diego, CA). Groups of data were compared using a one-way analysis of variance followed by Tukey's post hoc test. Data were presented as the mean±S.E.M. at the 95% confidence level, and $p<0.05$ was considered statistically significant. The resource equation method was used to calculate the sample size [38]. Therefore, in the present study, for the total sample size (n=30) and an effect size of 0.25, the statistical power for independent samples was calculated at 60.7% at the 5% significance level [39].

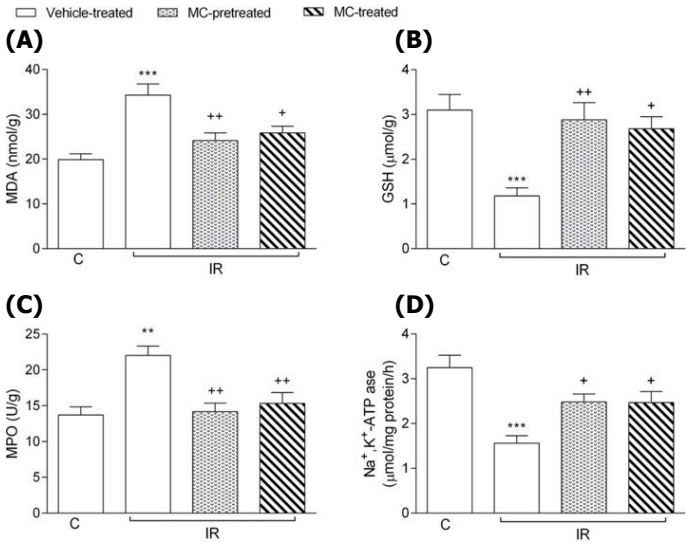

FIGURE 1. Ileum tissue: **(A)** MDA and **(B)** GSH levels, and **(C)** MPO and **(D)** Na^+-K^+-ATPase activity in ileum tissues in the control **(C)**, vehicle, MC-pretreated, and MC-treated groups.

**: P<0.01; ***: P<0.001 vs. control group; +: P<0.05; ++: P<0.01 vs. saline treated-IR group. Statistical analysis was performed using ANOVA (the mean values with 95% confidence level). MDA: Malondialdehyde; GSH: Glutathione; MPO: Myeloperoxidase; MC: *Myrtus communis*; IR: Irradiation.

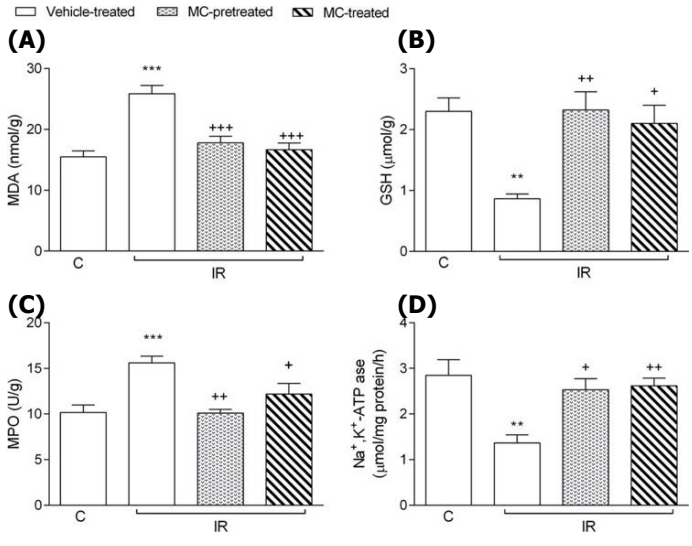

FIGURE 2. Pancreatic tissue: **(A)** MDA and **(B)** GSH levels, and **(C)** MPO and **(D)** Na^+-K^+-ATPase activity in pancreatic tissues in the control **(C)**, vehicle, MC-pretreated, and MC-treated groups.

**: P<0.01; ***: P<0.001 vs. control group; +: P<0.05; ++: P<0.01, +++: P<0.001 vs. saline treated-IR group. Statistical analysis was performed using ANOVA (the mean values with 95% confidence level). MDA: Malondialdehyde; GSH: Glutathione; MPO: Myeloperoxidase; MC: *Myrtus communis*; IR: Irradiation.

RESULTS

Biochemical analysis. IR groups showed increased MDA and MPO levels and decreased GSH and Na^+-K^+-ATPase activity in the ileum, pancreas, and liver tissues ($p<0.001$ vs. control). MC-treated and pretreated groups showed reversed effects ($p<0.01$ vs. IR; Fig. 1–3).

Histopathological Analysis

The histological scores among tissues of the experimental groups are shown in Table 2. Regular ileum morphology of the mucosa with villus formation and normal liver and pancreas parenchyma morphologies were observed in the control group. By contrast, blunted villus damage and dilated glandular structures in the ileum were observed in the IR group. This difference was significant from the control ($p<0.001$). The damage notably decreased in the ileum tissues in the MC pretreated ($p=0.02$) and treated ($p=0.01$) groups (Fig. 4). In the pancreas, irregularities and cellular damage in pancreatic acini, as well as irregularities in cellular organization and dilatation between cells in the islets of Langerhans, were observed in the IR group ($p<0.001$). However, these effects were reduced in the pretreated and MC-treatment

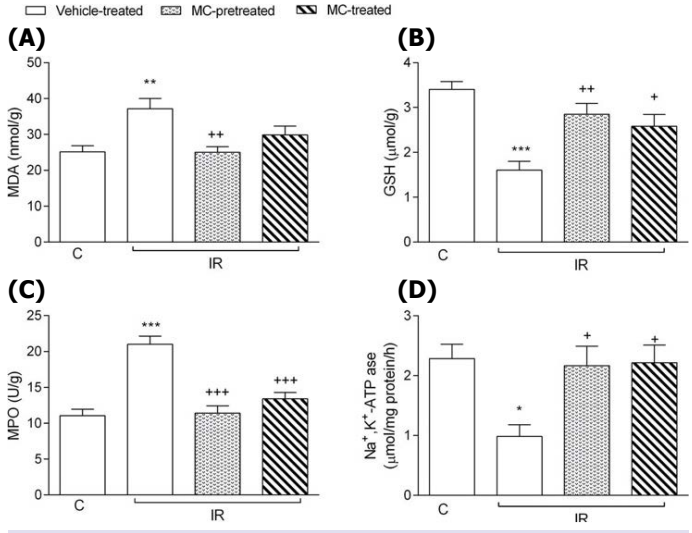

FIGURE 3. Liver tissue: **(A)** MDA and **(B)** GSH levels, and **(C)** MPO and **(D)** Na^+-K^+-ATPase activity in liver tissues in the control **(C)**, vehicle, MC-pretreated, and MC-treated groups.

*: P<0.05; **: P<0.01; ***: P<0.001 vs. control group; +: P<0.05, ++: P<0.01; +++: P<0.001 vs. saline treated-IR group. Statistical analysis was performed using ANOVA (the mean values with 95% confidence level). MDA: Malondialdehyde; GSH: Glutathione; MPO: Myeloperoxidase; MC: *Myrtus communis*; IR: Irradiation.

Table 2. Histological scores among tissues of the experimental groups

	Control	IR	MC-Ptx	MC-tx
Ileum	0	2.87±0.12*	2.33±0.33•**	1.37±0.23¶***
	*p<0.001, •p=0.002 and ¶p=0.01 compared to control; **p=0.02 compared to IR; ***p=0.01 compared to MC-Ptx			
Pancreas	0	8.16±0.16*	5.66±0.33•**	4.25±0.25¶±
	*p<0.001, •p<0.001 and ¶p<0.001 compared to control; **p=0.002 and ±p=0.002 compared to IR			
Liver	0	5.83±0.16*	4.5±0.22•**	4.0±0.36¶±
	*p<0.001, •p<0.001 and ¶p<0.001 compared to control; **p=0.001 and ±p=0.002 compared to IR			

IR: Irradiation; MC-PTx: MC-pretreatment; MC-tx: MC treatment.

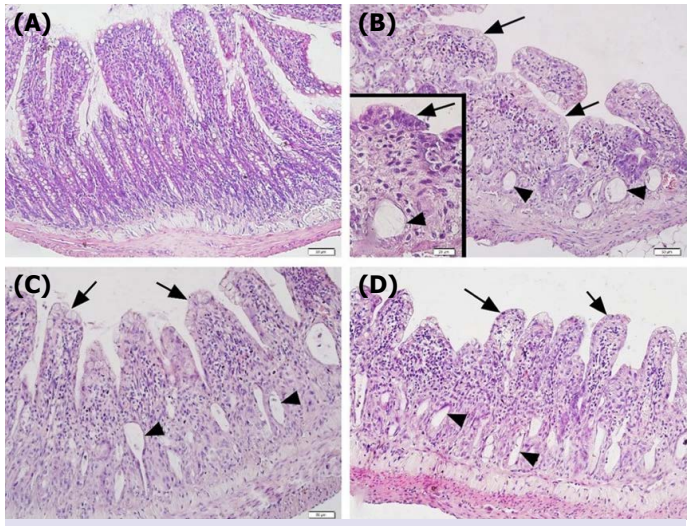

Figure 4. Histopathological results for ileum samples: Representative photomicrographs of the ileum samples in the control, IR, MC-pretreated, and MC-treated groups. **(A)** Regular villus morphology in the control group; **(B)** blunted villus morphology (arrow) and highly dilated glandular structures (arrowhead) in the IR group; **(C)** lengthened villus structures (arrow) and a small number of dilated mucosal glands (arrowhead) in the MC-pretreated and **(D)** MC-treated groups were observed through hematoxylin and eosin staining (scale bar, 50 μm; inset in B, 20 μm).

MC: *Myrtus communis*; IR: Irradiation.

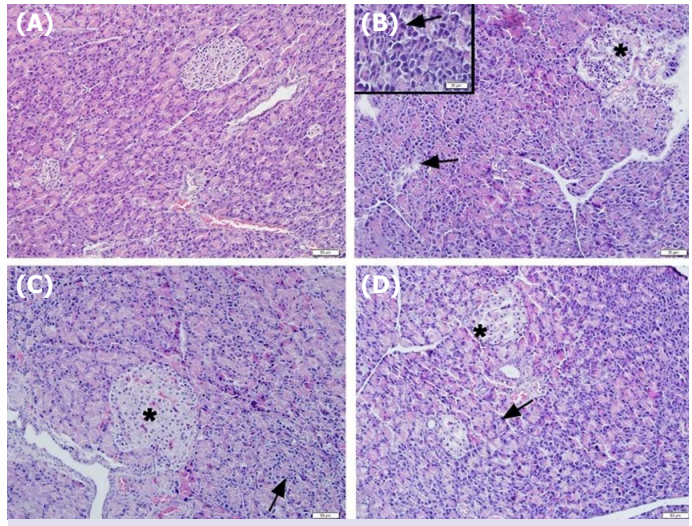

Figure 5. Histopathological results for pancreas samples: Representative photomicrographs of the pancreas samples in the control, IR, MC-pretreated, and MC-treated groups. **(A)** Regular pancreas parenchyma in the control group; **(B)** irregularity and cellular degeneration in the pancreatic acini (arrowhead) and irregularity and cellular dilatation in the Langerhans islet (*) in the IR group; **(C)** cellular degeneration in the pancreatic acini (arrowhead) in some areas and regular morphology of the Langerhans islets in the MC-pretreated and **(D)** MC-treated groups were observed through hematoxylin and eosin staining (scale bar, 50 μm; inset in B, 20 μm).

MC: *Myrtus communis*; IR: Irradiation.

groups (p=0.002) (Fig. 5). In the liver, sinusoidal congestion, deterioration in the organization of the hepatic cords, and hepatocyte damage were observed in the IR group (p<0.001). A reduction in sinusoidal congestion and a decreased number of damaged hepatocytes were observed in the MC-pretreatment (p=0.001) and MC-treatment (p=0.002) groups (Fig. 6).

DISCUSSION

Ionizing radiation generates free radicals, causes cellular damage through oxidizing molecules, and disrupts antioxidant balance, increasing oxidative and inflammatory markers [9, 10]. MC is a medicinal plant that has been used

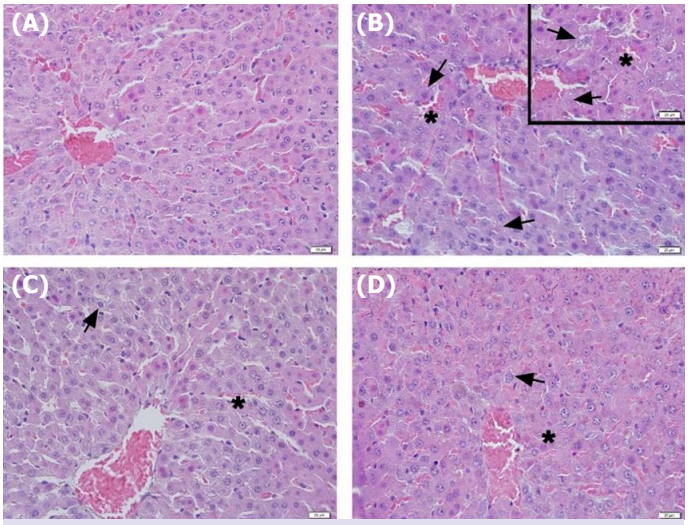

FIGURE 6. Histopathological results for liver samples: Representative photomicrographs of the liver samples in the control, IR, MC-pretreated, and MC-treated groups. **(A)** Regular liver parenchyma in the control group; **(B)** sinusoidal congestion (*) and hepatocyte damage (arrowhead) in the IR group; **(C)** decreased sinusoidal congestion (*) and hepatocyte damage (arrow) in MC-pretreated and **(D)** MC-treated groups were observed through hematoxylin and eosin staining (scale bar, 20 μm).

MC: *Myrtus communis*; IR: Irradiation.

for centuries due to its antioxidant and anti-inflammatory properties, which protect against several toxins [21–27]. Previous studies have shown that MC contains high levels of phenolics (368.68 mg/g) and flavonoids (111.35 mg/g), including caffeic acid derivatives, ellagic acid, and various forms of myricetin and quercetin [24, 26]. Its antioxidant and anti-inflammatory effects have been confirmed by *in vitro* and *in vivo* studies [24–27, 37]. In the present study, both the pretreated and treated groups of MC decreased ionizing radiation-induced early injury in gastrointestinal tissues in the experimental model. It was hypothesized that MC may protect against radiation-induced oxidative damage by reducing lipid peroxidation and boosting tissue GSH levels through its phenolic compounds.

MPO is a product of an increased inflammatory response and is synthesized in neutrophils and macrophages. It is an essential enzyme for normal neutrophil function, released from stimulated neutrophils and other tissue-damaging substances [30]. MPO is released by activated neutrophils in irradiated tissues, as previously described [40, 41]. In the present study, MC reduced MPO activity, especially in the pretreatment group, suggesting its anti-inflammatory and radioprotective effects. Previous experimental studies have also demonstrated that MC reduces serum IL-6 and TNF-α, which play a role in inflammation and MPO activity [23].

Na^+-K^+-ATPase is an enzyme that maintains the homeostasis of sodium and potassium ions in cells and operates energy-dependently. Na^+-K^+-ATPase also demonstrates intracellular catabolism due to its energy-dependent nature [42]. Following IR exposure in rats, there is impairment in the function of the Na^+-K^+-ATPase enzyme, manifested as decreased sodium binding capacity and a reduced number of active enzyme molecules [43]. In the present study, IR was shown to decrease Na^+-K^+-ATPase activity. In both the MC pretreated and treated groups, there was a similar increase in Na^+-K^+-ATPase activity in all tissues compared with the control group. In addition, decreased Na^+-K^+-ATPase activity is associated with endothelial damage, and MC plant extract is protective [44].

In the present study, radiation-induced damage to villus and glandular structures was observed in the ileum, as previously described [35, 40, 41]. Treatment with MC decreased oxidative damage and histopathological alterations via its strong antioxidative and anti-inflammatory effects. The radioprotective effect of MC may be due to its high content of phenolic compounds, which confer significant antioxidant and anti-inflammatory properties. Furthermore, as a photochemical, the mechanisms underlying MC's effects may involve direct interactions with the MAPK and PI-3K pathways [45].

The cerulein-induced pancreatitis model is the most commonly used experimental model for acute pancreatitis. Ceruelin increases inflammatory factors and cellular and oxidative damage in the pancreas [37]. In the present study, radiation-induced irregularities and cell damage in the exocrine pancreatic acinar structures are similar to those in previous inflammatory models. The pathogenesis of radiation-induced pancreas damage involves endothelial cell injury in the microcirculation supplying acinar cells, and ischemia contributes to this injury, in addition to radiation-induced cell injury [46]. In the present study, MC demonstrated anti-inflammatory and protective effects against histopathological damage in the irradiated pancreatic tissue.

In the present study, IR induced morphological and histopathological changes in the liver. Furthermore, previous studies have indicated that a liver biopsy may reveal endothelial damage, sinusoidal congestion, and parenchymal atrophy due to IR [47]. It has been shown that MC is protective against damage induced by toxins such

as aflatoxin and carbon tetrachloride toxicity [48]. The results of the present study have also demonstrated that MC applications may exhibit hepatoprotective effects against oxidative stress.

Modern techniques allow for reducing side effects by limiting the in-field tissues that receive doses during radiotherapy. Despite that, new agents with radioprotective properties still need to be investigated. Our research group is currently studying potential radioprotective agents for this purpose. A previous report from our group showed that MC ameliorates ionizing radiation-induced oxidative and inflammatory effects in brain tissue, as assessed biochemically and histopathologically [49]. Hence, these are the suggestions that MC may be a suitable candidate for the radioprotection clinic of radiotherapy.

Limitations

The authors chose a single large-fraction model, which researchers have long preferred for *in vivo* studies to investigate radiation-induced normal tissue injury. This unfractionated procedure offers several advantages, including easy set-up, shorter commitment, lower maintenance and housing costs, and the avoidance of anesthesia requirements before each fraction. However, this model may not fully mimic fractionated radiotherapy. Another limitation of the present study was its preclinical nature; the drug development process must be completed before MC can enter radiotherapy practice.

Conclusion

The present study demonstrated that MC has radioprotective potential for prophylactic or therapeutic purposes against damage caused by the inflammatory effects of ionizing radiation in different gastrointestinal tissues. The results may provide key information regarding the clinical use of MC as a novel radioprotective agent in the future.

No study has examined the *in vitro* effects of MC on irradiated gastrointestinal cells. Similarly, the effects of MC on critical intracellular molecules in irradiated cells warrant exploration. Moreover, investigating chronic effects that require several weeks for procedure via fractionated models may be another subject. The study includes only a few biochemical markers, so it could also be expanded to include additional inflammatory markers such as TNF-α, IL-6, IL-1β, and CCL4. Extending the studies to other critical tissues affected by radiation injury, such as bone marrow, lungs, or skin, may help find the potential protective effects of MC.

The study does not explore the effects of different doses of *Myrtus communis* extract, which is critical for establishing a dose-response relationship. Since this is the first study of the protective effects of MC against ionizing radiation, the main previous dose reported in the literature was used. Future studies may examine the radioprotective effects of using MC at different doses. Our study can be a reference for these studies.

Ethics Committee Approval: This experimental study was approved by the Marmara University Animal Experiments Local Ethics Committee (date: 11.01.2022, number: 07.2022).

Conflict of Interest: The authors declare no conflicts of interest.

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REVIEW BIOPHYSICS

Different chromatographic techniques and recent advancements for biomedical and pharmaceutical applications

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ABSTRACT

Chromatography remains a cornerstone analytical technique in pharmaceutical and biomedical sciences, with recent innovations significantly expanding its capabilities. Advances such as fast chromatography, two-dimensional liquid chromatography (2D-LC), supercritical fluid chromatography (SFC), and hyphenated techniques, including liquid chromatography-mass spectrometry (LC-MS) and gas chromatography-mass spectrometry (GC-MS), have broadened the scope of its applications. These developments enhance resolution, sensitivity, and efficiency, enabling more robust analysis of complex biological and pharmaceutical samples. These methods address complex analytical challenges, improving precision, speed, and efficiency in separating and analyzing biomolecules. Emerging technologies, including miniaturized liquid chromatography, shear flow chromatography, column arrays, and microfluidic chip-based systems, present exciting opportunities for the future. These developments enhance the capability of chromatography to analyze trace compounds, optimize drug formulations, and ensure the quality control of pharmaceuticals. Chromatography is also increasingly integrated with cutting-edge techniques like metabolomics and proteomics, furthering its impact on biomarker discovery and personalized medicine. This paper reviews recent advancements in chromatographic methods and their practical applications in the pharmaceutical and biomedical fields. It highlights the critical role of chromatography in drug discovery, purification of therapeutic compounds, and metabolite profiling.

Keywords: Biomedical analysis; biomarker discovery; chromatography; pharmaceutical applications; protein.

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Chromatography is a versatile biophysical technique used for the separation, identification, and purification of compounds from complex mixtures, enabling both qualitative and quantitative analysis. In this technique, the separation of a compound into the desired shape, size, charge, and groups according to the binding specificity [1].

Chromatography distinguishes itself from classical separation techniques, such as crystallization, extraction, or distillation, by its ability to be effectively applied even when the exact composition of a mixture is unknown. However, selecting an appropriate chromatographic method necessitates a comprehensive understanding of the physicochemical properties of the compounds involved. The versatility

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and superior resolving power of chromatographic systems have solidified its status as a premier analytical technique, extensively utilized in scientific, industrial, and medical contexts. It plays an integral role in the methodologies of numerous scientific studies and serves as a vital tool for environmental monitoring and the production of high-purity compounds in the pharmaceutical and chemical industries. Moreover, beyond its critical function in protein analysis, chromatography is essential for the separation and analysis of small-molecule drugs, which constitute a significant class of pharmaceutical compounds [2, 3].

The advantages of new chromatography methods are high efficiency, faster separation speed, high flow rate, increased detection sensitivity, and cost savings. Chromatography has many medical applications, such as pharmaceutical analysis, the food and beverage industry, the chemical industry, forensic medicine, and environmental science [4].

Chromatography's versatility and high resolving power have established it as a premier analytical technique, widely employed across scientific, industrial, and medical domains. It serves as a cornerstone in the methodologies of numerous scientific studies and is instrumental in environmental monitoring and the production of high-purity compounds in the pharmaceutical and chemical industries. In pharmaceutical applications, particularly during preclinical and clinical studies, various chromatographic methods are utilized to evaluate the bioactivity of pharmaceuticals—defined as the effect of a substance on living tissues or biological systems, encompassing efficacy, receptor binding, and metabolic impact. These methods include high-performance affinity chromatography (HPAC), cell membrane chromatography (CMC), mixed-mode chromatography (MMC), and high-performance liquid chromatography (HPLC), each offering unique advantages in analyzing bioactive compounds [5, 6].

The success of chromatography in developing fast and accurate analytical techniques offers greater specificity and sensitivity in drug research. In recent years, chromatography has attracted attention as a valid method for examining clinical or pharmaceutical samples and analyzing drug-protein binding [3, 5].

In recent years, chromatography has become one of the most important techniques used in clinical analysis to separate drugs according to their properties and interaction patterns, identify their metabolites, and determine their amount. Chromatographic methods are essential for early diagnosis of disease, evaluation of the course of the disease, monitoring of drug effects, and therapeutic intervention, where biological materials such as whole blood, serum, plasma, urine, stool, and tissue are tested during the diagnosis and treatment process in research and routine settings [7].

Highlight key points

- Chromatography, electrophoresis, and microfluidic chips enable the separation of specific analytes and the analysis of chemical compounds with high purification sensitivity.
- Techniques such as fast LC, 2D-LC, SFC, LC-MS, and GC-MS provide more information with increased productivity, reliability, robustness, resolution, speed, and sensitivity.
- Chromatographic methods play a fundamental role in biotechnology and biopharmaceutical analysis by in-depth investigations of protein structure and function, enabling the design of separation methods tailored to the target protein.
- Microfluidic chips offer a new research platform for the miniaturization, integration, and portability of analytical devices, simplifying analysis processes.
- Three-dimensional chromatographic separation has the potential to revolutionize various industries by offering high efficiency and resolution for the separation of complex samples.

The adverse effects of environmental chemical pollutants on quality of life and their contribution to the incidence and development of life-threatening chronic diseases are well established. However, the routes of exposure to these chemicals and their roles in chronic diseases are still unclear. Environmental pollutants enter the human body through various routes and are transported to target organs through blood. The risks of human exposure to these substances and the relationship between chemical pollutants and chronic diseases are extensively studied by monitoring them with biomarkers in plasma or serum samples. Chromatography is a widely utilized technique in clinical laboratories for diagnosing inborn errors of carbohydrate, protein, and lipid metabolism. It facilitates the measurement of key parameters, including vitamins, hormones, metabolites, tumor markers, and drugs in biological fluids. Additionally, chromatography enables the analysis of environmental samples for drugs, toxins, and pollutants, contributing to the discovery of novel biomolecules and providing valuable insights into disease mechanisms and biomarker identification [8, 9].

In this review, we aim to provide information about the basic principles of chromatography and emphasize the importance of new chromatographic techniques applied in pharmaceutical and clinical analysis based on current literature.

Table 1. Classification of chromatography

Classification basis	Type	Subtype/description
Based on the interaction of solute to the stationary phase	Adsorption Chromatography	Based on surface adsorption (e.g., silica gel in TLC)
	Ion Exchange Chromatography (IEC)	Ion-exchange chromatography facilitates the separation of analytes by leveraging their electrostatic interactions with a charged stationary phase.
	Partition Chromatography	Separation via solute partitioning between two phases
	Size Exclusion Chromatography (SEC)	SEC separates molecules, such as proteins and polymers, by size using porous beads as the stationary phase.
Based on chromatographic bed shape	Two Dimensional	Thin Layer Chromatography (TLC), Paper Chromatography
	Three Dimensional	Column Chromatography (used in most HPLC applications)
Chromatographic methods are classified by the mobile phase's physical state	Liquid Chromatography (LC)	Widely used in HPLC and bio-separations
	Super Critical Fluid Chromatography (SFC)	Combines properties of gas and liquid phases for efficient separations
	Gas Chromatography (GC)	Used for volatile compounds and environmental samples

CLASSIFICATION OF CHROMATOGRAPHY

The separation of proteins, which are fundamental to vital biological processes, from biomaterials is critical for investigating their structures and functions. Biochemical transitions from a normal to a pathological state in living systems are often associated with health issues, particularly through molecular, structural, and functional alterations in proteins that drive the onset and progression of various diseases. Owing to these alterations, proteins represent valuable biomarkers for disease screening, early diagnosis, and the determination of disease type and stage, as well as for elucidating the molecular mechanisms underlying the transition from physiological to pathological states. Protein separation and purification are generally accomplished through one or more chromatographic steps. These processes rely on the differential retention times of proteins within the chromatographic column, which result from distinct interactions between proteins and the column's stationary phase materials. Chromatographic techniques are classified based on various principles, including the nature of solute-stationary phase interactions, the physical state of the mobile phase, and the configuration of the chromatographic bed (Table 1) [10, 11].

Specifically, chromatography methods can be classified into several modes based on their separation mechanisms. HPLC has various subtypes, such as Ion Exchange Chromatography (IEC), Hydrophobic Interaction Chromatography (HIC), Affinity Chromatography, Size Exclusion Chromatography (SEC), and Reverse Phase Chromatography (RP-HPLC), each employing distinct physicochemical interactions between the analyte and stationary phase. Additionally, non-HPLC-based techniques such as Gel Chromatography, Hydroxyapatite Chromatography (HAC), and Supercritical Fluid Chromatography (SFC) are used in specific contexts. Beyond these established chromatographic techniques, modern and specialized chromatography methods such as Paper Chromatography Hybridization Assay (PACHA) [12], Optical force chromatography (OFC) [13], High-performance and Immunoaffinity chromatography [14], Mixed mode chromatography [15], and Dye ligand chromatography [16] are gaining importance. With the further development of biotechnology and in-depth studies of the structure and function of proteins, protein separation and purification technology is also developing rapidly.

HIGH-PRESSURE LIQUID CHROMATOGRAPHY (HPLC)

HPLC is the most accurate analytical method and is widely used for both quantitative and qualitative analysis of drug products and for determining drug product stability. HPLC method is important for separating and measuring the parent drug and reaction impurities [17].

HPLC is highly practical for both laboratory and clinical applications, delivering accurate and precise results with enhanced specificity. Extensive research and numerous patents have validated the versatility of HPLC across various domains within the healthcare sector, opening up numerous promising opportunities for this analytical technique [18].

The use of biological samples such as serum, plasma, cerebrospinal fluid (CSF), breast aspirate, saliva, tear samples, tissue homogenates, and cellular lysates in the HPLC-protein separation method provides a great opportunity for comparison and correlation of markers observed in these different systems for a single disease. It also helps provide additional information about various biochemical processes in the biological system. Using the "Reverse Phase" HPLC technique is generally advantageous in separating minimal amounts of proteins. In any abnormal condition, there is a change in the expression levels of proteins depending on the ongoing biochemical processes [19].

In the field of medicine, HPLC has various applications, such as the analysis of antibiotics, endogenous neuropeptide detection in brain extracellular fluids, drug stability studies, quality control, and the evaluation of pharmaceutical products' shelf life [20].

HPLC analysis of pharmaceutical products is used to determine the amount of drug in biological samples, confirm the drug's identity, and provide quantitative results [21].

Various chromatographic methods, notably reverse-phase (RP) chromatography, are under investigation for the separation of neurotransmitters (NTs) and their metabolites. High-Performance Liquid Chromatography (HPLC) coupled with intracerebral microdialysis and electrochemical detection (ECD) is widely employed to study brain serotonergic and dopaminergic systems. As a prominent separation technique in NT analysis, HPLC, particularly under reverse-phase conditions, delivers rapid and selective results. The integration of HPLC with ECD offers a robust approach for elucidating the dynamics of NTs and their metabolites, advancing neuroscience research and facilitating potential clinical applications. Ongoing research into HPLC-ECD systems provides promising opportunities for enhancing the sensitivity and specificity of neurotransmitter detection [22].

Significant advancements in classical High-Performance Liquid Chromatography (HPLC) have led to the development of innovative techniques, including Rapid Resolution Liquid Chromatography (RRLC), Ultra Performance Liquid Chromatography (UHPLC), Ultra Fast Liquid Chromatography (UFLC), and Nano Liquid Chromatography (Nano LC). These modern approaches enhance analytical performance by improving resolution, speed, and sensitivity, thereby expanding the applicability of HPLC in various scientific and industrial contexts.

Rapid Resolution Liquid Chromatography (RRLC)

RRLC is not a distinct type of chromatography but rather a refinement of the classical HPLC technique. It employs shorter chromatographic columns and stationary phases packed with smaller particle sizes (typically 1.8–3 μm) to allow for faster separation without compromising resolution. These modifications improve efficiency, reproducibility, and peak capacity while significantly reducing analysis time and solvent consumption [23].

This system has become widely adopted in the pharmaceutical industry to provide maximum resolution and rapidity of testing. Increased reproducibility and sensitivity are achieved during analysis with the RRLC method. High detection speed and reduced analysis cost make this method valuable in the quality control of herbal medicines. RRLC has the advantage of being faster chromatography and having high resolving power compared to HPLC [24].

Nano Liquid Chromatography (NLC)

Nano-liquid chromatography can be defined in various ways depending on the flow rate of the mobile phase and the column diameter. Here, the flow rate of the mobile phase is nano ml/min. NLC is a faster and cheaper technique than HPLC, producing less waste due to reduced mobile phase consumption. It helps reduce the sample requirement and increase the sensitivity. It has been found that the resolution power increases when analyzing complex samples. It also has higher efficiency [25]. Although NLC is particularly useful for small molecules due to its high sensitivity and low sample volume, it is also widely applied in the separation and quantification of peptides and proteins, especially in proteomics and biomarker research [26].

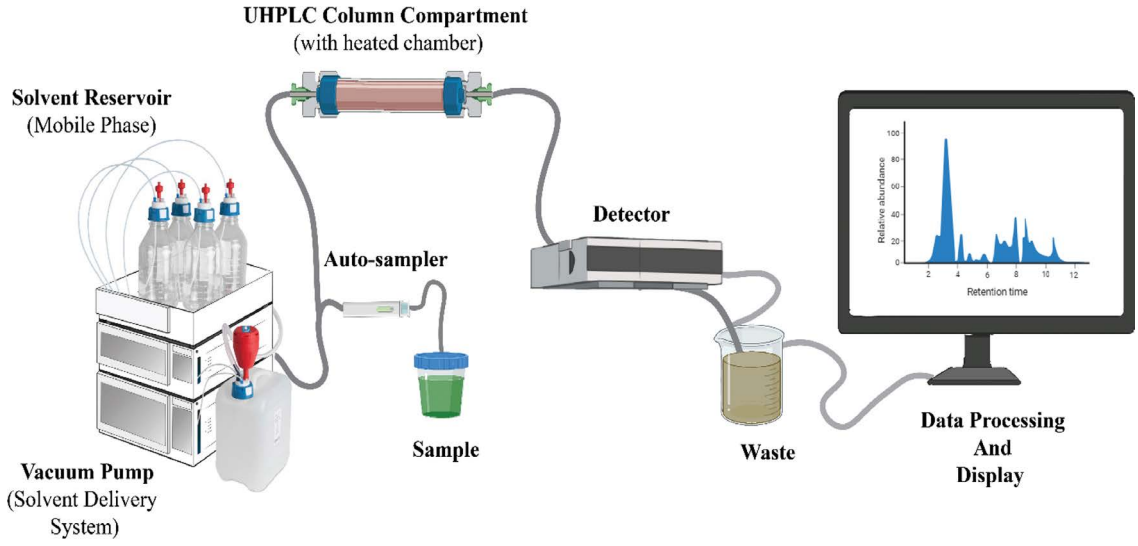

FIGURE 1. Schematic representation of UHPLC [31].

UHPLC: Ultra performance liquid chromatography.

Ultra-High Performance Liquid Chromatography (UHPLC)

Ultra-High-Performance Liquid Chromatography (UHPLC) has gained significant traction in recent years due to its ability to achieve rapid separation of small molecules with high resolution. This technique is widely employed for the analytical separation of components in multicomponent mixtures, finding applications across diverse fields, including chemistry, pharmaceuticals, food science, and biochemistry. UHPLC serves as a critical tool in both research and industrial settings, enabling high-quality separations without compromising analytical performance. Its superior resolution has facilitated the development of novel analytical procedures, building on existing methods and reducing the need for reanalysis, thereby marking a significant advancement in liquid chromatography. In the pharmaceutical domain, UHPLC is particularly vital for the analysis of drugs, which play a central role in medical practice. Rational prescribing, grounded in clinical pharmacology, is essential to ensure that the appropriate therapeutic agents are administered to patients at the optimal time, enhancing treatment efficacy and safety [27]. Recent advancements in analytical tools have significantly enhanced the discovery and monitoring of new pharmaceuticals. The development of rapid chromatographic methods has become increasingly vital for analytical laboratories. Notable technological progress has been achieved through improvements in detector design, optimization of data processing systems, and refinements in chromatographic techniques. Ultra-High-Performance Liquid Chromatography (UHPLC), which builds upon the foundational principles of High-Performance Liquid Chromatography (HPLC), delivers exceptional performance, offering enhanced resolution, speed, and sensitivity for pharmaceutical analysis (Fig. 1). While UHPLC shows a dramatic increase in speed, resolution, and analysis sensitivity as well as analysis sensitivity by having a particle size of less than 2 pm and operating the system at higher pressure, the mobile phase can operate at higher linear speeds compared to HPLC. In addition, the analysis time and solvent consumed in UHPLC are less than those of all previously used chromatographic techniques. This technique is considered a new focus in liquid chromatographic studies. Therefore, it has been shown in the literature that an existing HPLC method can be developed and replaced by the UHPLC method in the pharmaceutical industry. It has been demonstrated that UHPLC is an important and meaningful tool for enhancing the quality of pharmaceutical analyses and the efficiency of researchers [28–30].

The only disadvantage of UHPLC may be the high back pressure, which can be reduced by increasing the column temperature. The UHPLC technique is generally acceptable and significantly improves speed, sensitivity, and resolution compared to conventional HPLC [32]. In conclusion, the UHPLC technique is a technique that provides ultra-fast analysis without affecting analytical reliability and sensitivity, with a speed approximately ten times higher than conventional liquid chromatographic methods applied in the analysis of pharmaceutical substances [33].

The literature reports a wide range of applications for Ultra-High-Performance Liquid Chromatography coupled with Tandem Mass Spectrometry (UHPLC-MS/MS) in the analysis of various drug classes, including antidiabetic, anticancer, antibiotic, cardiovascular, antiviral, nonsteroidal anti-inflammatory drugs (NSAIDs), and others. Recent studies demonstrate that UHPLC-MS/MS effectively separates drugs and their metabolites from impurities and degradation products. This technique provides a robust platform for the isolation, characterization, and identification of degradation products and impurities, yielding critical insights into drug properties. Such information supports optimized storage conditions, enhances quality control methodologies, and contributes to the development of safer therapeutic treatments [34].

SUPERCRITICAL FLUID CHROMATOGRAPHY (SFC)

SFC provides a cost-effective and rapid approach for the purification of small proteins and pharmaceutical compounds. Thavendran Govender and colleagues developed an SFC-based method for purifying biosynthesized human insulin, thereby establishing a scalable process suitable for large-scale production. Their findings demonstrated that insulin samples retained full biological activity following SFC purification, confirming the method's effectiveness in producing high-purity, biologically active compounds [35–37]. SFC offers distinct advantages in kinetic performance and serves as a complementary technique to liquid chromatography (LC), making it highly effective for pharmaceutical analysis. Preparative chromatography represents a pivotal separation approach in the pharmaceutical industry, particularly during the early stages of drug development when larger quantities of material are required. Selecting the most cost-efficient method to obtain sufficient amounts of target compounds is therefore essential. In this context, SFC has emerged as a promising strategy for the analysis of drugs within formulations and biological matrices [38, 39]. Modern SFC instruments, complementary to HPLC, are a widely applicable technique with robust and high-resolution properties [40, 41]. Recent technological advances have resolved the known limitations of SFC, such as poor UV sensitivity, limited reliability, and poor quantitative performance, with the introduction of new generation instruments and columns from major chromatographic instrumentation providers. Ultra-high Performance Supercritical Fluid Chromatography (UHPSFC) has emerged as an advanced form of SFC, offering enhanced resolution, faster analysis, and improved reproducibility due to the use of smaller particle columns under higher pressures [42].

HYDROPHOBIC INTERACTION CHROMATOGRAPHY (HIC)

HIC is a separation technique employed to isolate proteins and polypeptides based on differences in surface hydrophobicity. This method enables the purification of biologically active proteins without compromising their native conformation. In HIC, the sample is applied to a hydrophobic stationary phase in the presence of a high-concentration salt solution, promoting adsorption through hydrophobic interactions under conditions of elevated ionic strength. Subsequently, selective desorption is achieved by gradually or linearly decreasing the ionic strength of the mobile phase. Weakly hydrophobic molecules are eluted at higher ionic strengths, whereas strongly hydrophobic molecules are eluted as the ionic strength diminishes [43, 44].

TWO-DIMENSIONAL LIQUID CHROMATOGRAPHY (2D-LC)

Comprehensive two-dimensional liquid chromatography (LC×LC) is a highly effective technique for the separation of complex samples, offering significant advantages over traditional one-dimensional liquid chromatography (1D-LC). By leveraging orthogonal selectivities in two separation dimensions, LC×LC, particularly when coupled with mass spectrometry (LC×LC-MS), achieves high throughput and enhanced resolution. The increasing popularity of two-dimensional liquid chromatography (2D-LC) stems from its successful applications, such as the discovery of novel biopharmaceuticals. LC×LC is particularly valuable for analyzing complex samples containing non-volatile analytes or matrix compounds, providing superior capacity and selectivity compared to 1D-LC. When the two separation dimensions are carefully selected, LC×LC delivers reliable and reproducible results, enabling rapid and efficient comparative analysis, for instance, in the characterization of natural drugs. Despite its complexity, ongoing improvements in robustness and reliability are expected to further enhance the utility of 2D-LC in analytical applications [45, 46].

TABLE 2. Applications of multidimensional chromatography in pharmaceutical and clinical fields

Field	Sample types	Applications	Techniques	Ref
Clinical pharmacology	Human plasma, serum, CSF	Quantification of drugs and metabolites; therapeutic drug monitoring	LC-MS/MS, 2D-LC	[51]
Pharmaceutical research	Cell lysates, tissues	Drug discovery; identification of active metabolites	LC-MS, MMC, 3D-LC	[52]
Toxicology	Plasma, urine	Detection of toxins and pharmaceuticals in overdose or exposure	LC-GC, LC-MS/MS	[53]
Endocrinology	Serum, plasma	Analysis of hormones and hormone-like drugs	LC-MS/MS, 2D-HPLC	[54]
Biomarker discovery	Biological fluids, tissues	Identification and quantification of disease-specific markers	2D-LC, LC-MS, GC-MS	[55, 56]
Oncology	Tumor tissue, blood,	Targeted therapy development; pharmacodynamics and proteomics	LC-MS/MS, 3D-LC	[57]

LC-MS/MS: Liquid chromatography–mass spectrometry/mass spectrometry; 2D-LC: 2-Dimensional liquid chromatography; 3D-LC: 3-Dimensional liquid chromatography; LC-GC: Liquid chromatography–gas chromatography; MMC: Mixed-mode chromatography; 2D-HPLC: 2-Dimensional high-performance liquid chromatography; GC-MS: Gas chromatography–mass spectrometry.

MULTIDIMENSIONAL CHROMATOGRAPHY

Given the complexity of samples comprising diverse components and the inherent limitations in the separation efficiency of single-column chromatography, multidimensional chromatography—integrating multiple techniques with orthogonal separation principles and varying affinities—has garnered substantial interest as a solution. This approach synergistically combines chromatographic methods, thereby reducing analysis time while enhancing overall efficiency, sensitivity, and accuracy. However, solvent system incompatibility remains a primary challenge in the implementation of two-dimensional (2D) chromatography. Due to the intricate nature of analytes and their low concentrations within biological matrices, multidimensional chromatography finds extensive application in the biomedical and pharmaceutical industries (Table 2). The analysis of complex compounds requires analytical systems that are highly reliable, selective, and precise. Recent studies indicate that mixed-mode chromatography (MMC), which provides superior separation efficiency for proteins compared to single-mode techniques and alleviates mobile phase compatibility issues, holds substantial promise for improving analytical performance and achieving more robust separation outcomes [47–49].

RECENT DEVELOPMENTS IN THE QUANTITATIVE ANALYSIS OF THERAPEUTIC PROTEINS IN BIOLOGICAL MATRICES

The inherent complexity of therapeutic proteins, characterized by their intricate molecular structures and the presence of diverse active substances within complex biological matrices, presents substantial challenges to the development of analytical methods that achieve high specificity, sensitivity, accuracy, and robustness. The creation of reliable and precise quantitative assays for these sophisticated protein-based therapeutics in biological fluids, such as plasma or serum, continues to be a pivotal and formidable obstacle in advancing biopharmaceutical research and development [50].

Pharmaceutical applications of therapeutic proteins in treating many diseases are continuously being developed. Although various analytical methods are available for quantifying biofluid drugs, no ideal or universal method can be applied to various therapeutic proteins or all conditions. Efficient and reliable bioanalytical methods are required to accelerate therapeutic protein identification and successful clinical development. Selective quantitative analyses, especially in high-throughput formats, are critical to meet regulatory requirements for pharmacokinetic and pharmacodynamic evaluation

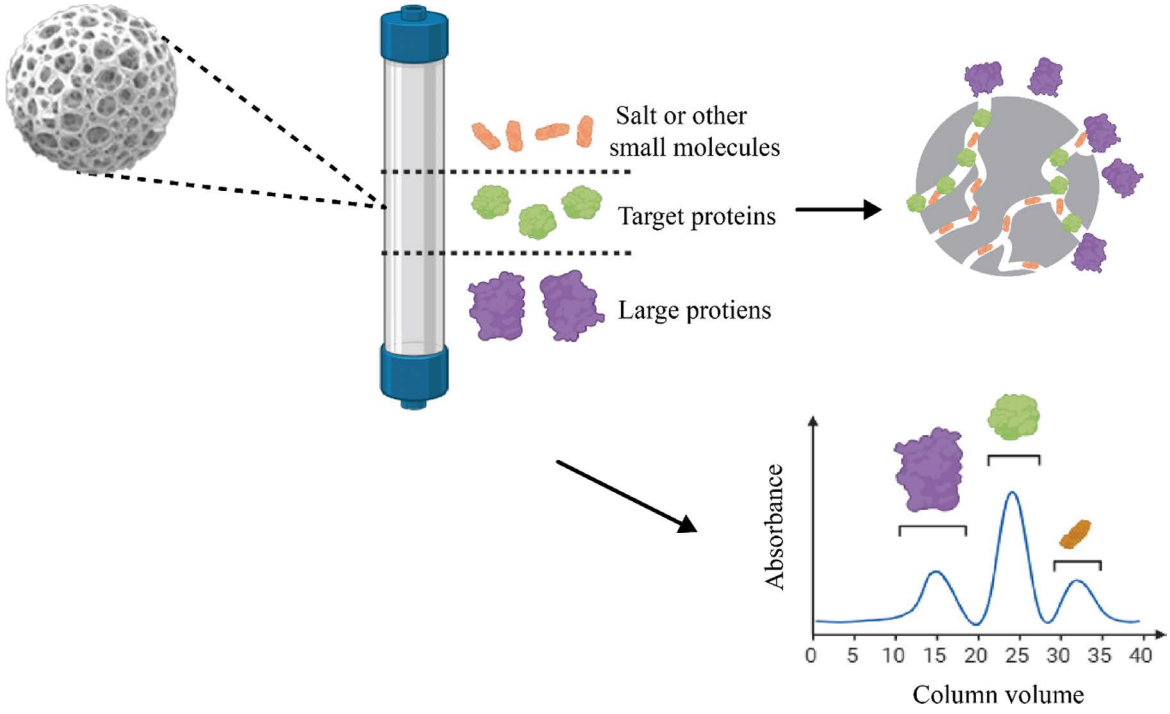

FIGURE 2. Size exclusion chromatography (SEC) process [63].

of drugs for new drug approval. However, although it is not a standard approach, Liquid Chromatography-Mass Spectrometry (LC-MS/MS) is often the preferred method for identifying and quantitatively analyzing therapeutic proteins in complex biological samples due to its high sensitivity, specificity, and efficiency. In recent years, the devices have become highly developed, automated, and equipped with high-level information technology, allowing high throughput, automatic sample analysis, data processing, and storage [58].

Routine measurement of cancer biomarkers contributes significantly to better clinical outcomes for patients regarding early diagnosis, risk stratification, and treatment monitoring, among other applications. Protein tumor markers are important because proteins carry out most of the biological processes and thus dynamically reflect changes in cancer pathophysiology. Mass spectrometry-based targeted proteomics is a powerful tool for absolute peptide and protein quantification in biological matrices and has numerous advantages in clinical applications in oncology. Using liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based methodologies allows laboratories to overcome the difficulties associated with more commonly used immunoassays for tumor marker measurement [59, 60].

SIZE EXCLUSION CHROMATOGRAPHY (SEC)

Size Exclusion Chromatography (SEC) is widely used in the qualitative and quantitative characterization of therapeutic proteins (Fig. 2). The primary advantage of this technique lies in the suitability of its mobile phase conditions for characterizing proteins. Due to their distinct physicochemical properties, therapeutic proteins are susceptible to various modifications—such as oxidation, deamidation, glycosylation, aggregation, misfolding, and adsorption—during preparation, formulation, or storage. The clinical application of therapeutic proteins has enabled the treatment of numerous life-threatening conditions. Hundreds of therapeutic proteins are currently under clinical evaluation for the treatment of various diseases, including genetic disorders, cancer, and infectious diseases [61].

In this context, the characterization of protein modifications requires a range of analytical methods because a single technique does not allow the evaluation of all necessary parameters. Protein aggregates are usually analyzed by light scattering-based methods such as sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), size exclusion liquid chromatography (SEC-HPLC), asymmetric area flow fractionation (AF4), fluorescence spectroscopy, circu-

lar dichroism (CD) or multi-angle laser light scattering (MALLS). Among these techniques, SEC-HPLC is currently used as the standard separation technique for quantifying protein dimers, trimers, and oligomers. The main advantage of this approach is the mild elution conditions that allow the characterization of the protein by identifying the conformational structure. As a result, significant advances have been made through SEC approaches that provide a new level of chromatographic performance or efficiency [62].

AFFINITY CHROMATOGRAPHY (AC)

Affinity Chromatography (AC) is a specialized form of liquid chromatography that exploits the specific and reversible binding interactions between biological macromolecules and their corresponding ligands. In this technique, a ligand is immobilized on a stationary matrix, enabling the selective separation of a target protein based on the specificity of its interaction with the ligand. For instance, Immobilized Metal Affinity Chromatography (IMAC) has been effectively utilized to purify angiotensin-converting enzyme (ACE) inhibitor peptides from casein hydrolysates. AC offers several advantages, including high selectivity, robust stability, cost-effectiveness, and excellent reproducibility. However, protein isolation methods, which rely on subtle differences in the physicochemical properties of macromolecules, often face challenges such as prolonged processing times and low yields, resulting in difficulties achieving high-purity proteins [64–66].

AC is an increasingly vital separation technique in the analysis of biological samples and pharmaceutical agents. By employing a biologically relevant stationary phase, AC enables the selective retention of analytes and the investigation of biomolecular interactions. This method can be applied independently or in combination with complementary techniques, such as reversed-phase High-Performance Liquid Chromatography (HPLC) or mass spectrometry. Given its numerous recent applications, AC is poised to gain further prominence as a critical tool for the separation and analysis of biological and pharmaceutical agents in complex matrices [67, 68]. AC is a versatile analytical technique widely applied across diverse fields, from investigating drug–protein binding interactions to improving the detection and quantification of low-abundance proteins. It plays a pivotal role in elucidating the kinetic properties of drug–protein interactions and in identifying specific drug-binding sites, as reported in numerous studies. Moreover, AC has been extensively utilized in omics research—including proteomics, metabolomics, and genomics—where it contributes to the advancement of high-throughput screening platforms for potential therapeutic agents, particularly when integrated with complementary analytical methodologies [69, 70].

MICROFLUIDICS-BASED CHROMATOGRAPHY

Liquid chromatographic chips are increasingly gaining prominence in research and applications due to their substantial advantages over conventional, larger-scale analytical systems. These advantages include minimal requirements for sample and reagent volumes, rapid and cost-efficient analysis, elimination of dead volume in connections, and the capacity for multiplex measurements, making them a highly effective tool for advanced analytical applications (Fig. 3). Liquid chromatographic (LC) chips and conventional chromatographic systems, such as High-Performance Liquid Chromatography (HPLC) and Ultra-High-Performance Liquid Chromatography (UHPLC), share theoretical applicability across similar domains. However, as LC chip technology continues to evolve, research primarily focuses on validating methodological concepts, including particle design, packaging processes, chip architecture, pumping systems, hyphenation techniques, and detection methods. The advent of microfluidic chip-based LC-MS systems has significantly advanced proteomics research by enabling high-throughput analysis of biological macromolecules, such as peptides, proteins, and glycans, in diverse biological matrices, as well as small molecules, including pharmaceutical compounds, drugs, and their metabolites, in applications such as tumor and disease analysis. LC chip-MS systems are poised for widespread adoption due to their efficiency in analyzing small-volume samples. Ongoing development efforts focus on critical areas such as micropackaging preparation, sample injection performance, detection sensitivity, parallelization, and hyphenation with other analytical platforms. Despite the diverse developmental trajectories of LC chip technology, it is evident that future efforts will prioritize significant miniaturization of analytical devices, further enhancing their utility in analytical sciences [71–73].

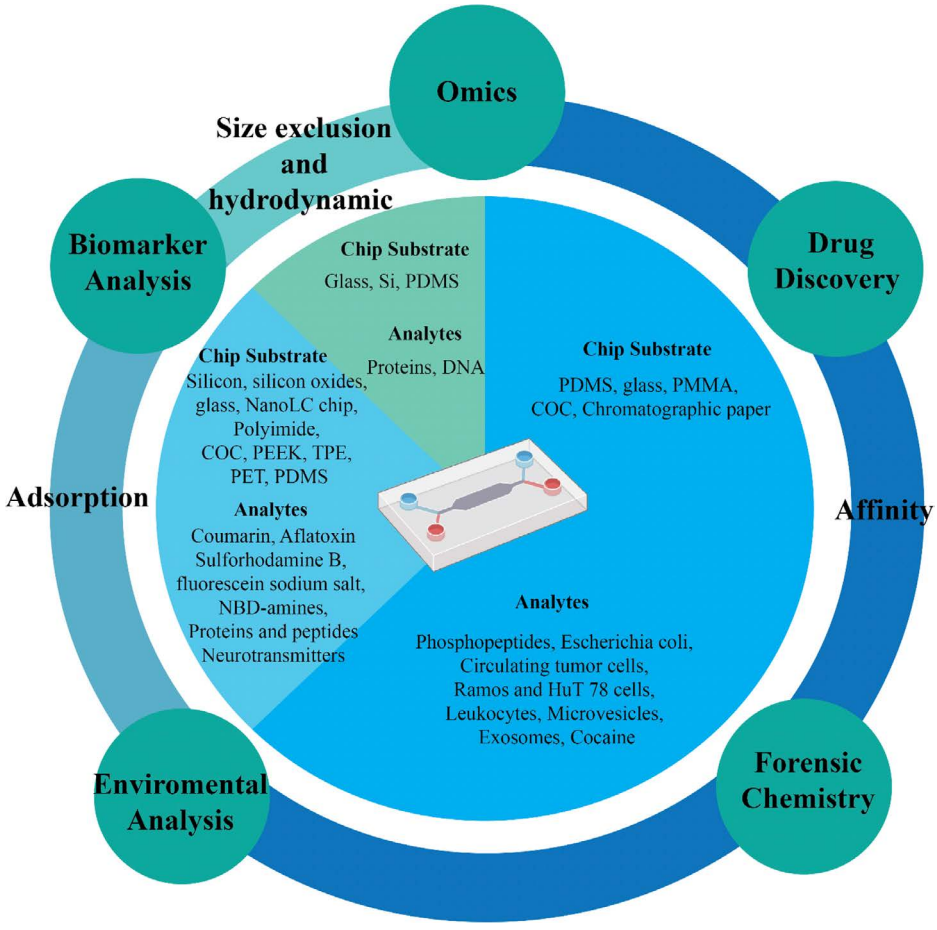

FIGURE 3. The applications and separation mechanisms of microfluidics-based liquid chromatography [74].

PDMS: Polydimethylsiloxane; PMMA: Polymethyl methacrylate; COC: Cyclic olefin copolymer; PEEK: Polyether ether ketone; PET: Polyethylene terephthalate; TPE: Thermoplastic Elastomer; PDMS: Polydimethylsiloxane; Si: Silicon.

HIGH-PERFORMANCE THIN-LAYER CHROMATOGRAPHY (HPTLC)

High-performance thin-layer chromatography (HPTLC) is one of the most widely applied methods for analysis in pharmaceutical industries, clinical chemistry, forensic chemistry, biochemistry, cosmetology, food and drug analysis, environmental analysis, etc. Due to its many advantages, it is the only chromatographic method that offers the option of presenting the results as images, for example. HPTLC is one of the advanced instrumental techniques based on all the features of thin-layer chromatography (TLC). Its advantages, such as automation, screening, complete optimization, selective detection principle, minimum sample preparation, etc., make it a powerful analytical tool for chromatographic information of complex mixtures of pharmaceuticals, natural products, clinical samples, foodstuffs, and other substances. Other advantages include simplicity, low cost, parallel analysis of samples, high sample capacity, fast results, and multiple detection. Many reports on clinical medicine studies have already been published in many journals. The use of HPTLC is now highly recommended for the analysis of drugs in serum and other tissues [75–77].

IMMOBILIZED ARTIFICIAL MEMBRANE CHROMATOGRAPHY (IAM)

The development of immobilized artificial membrane (IAM) chromatography has opened up new perspectives for using chromatographic techniques in drug discovery by combining the simulation of the cell membrane environment with rapid measurements. Studies are being conducted on the potential of IAM chromatography to model permeation through significant physiological barriers and drug-membrane interactions. Other applications are also

being investigated to calculate complex pharmacokinetic properties related to tissue binding and to predict cell accumulation/retention. The unstable nature of IAM chromatography as a boundary condition between passive diffusion and binding defines its potential applications. However, despite its successful performance in many drug-membrane interaction studies, IAM is still used as a supporting technique rather than a stand-alone technique. To have a more focused and consistent application in drug discovery, further studies considering different biological processes are still needed to examine IAM chromatography efficiency [78]. The primary goal is to purify and extract one or more components of a sample for the analytical purpose of chromatography to determine the sample's qualitative and quantitative chemical structure [79].

MAGNETO CHROMATOGRAPHY (MC)

The advent and progress of nanotechnology have driven remarkable advancements across various scientific disciplines. In contrast to conventional magnetic separation techniques, magnetic or magneto-chromatography (MC) systems have emerged as a powerful method, garnering significant attention. MC employs an external magnetic field to separate particles based on their size or to distinguish cations, anions, and various compounds with differing magnetic susceptibilities. Additionally, MC can be adapted to separate diamagnetic compounds through strategies such as complexation with metal ions, reactions with metal complexes, or binding to magnetic or magnetized particles. The external magnetic field is critical to the separation process. MC systems offer distinct advantages, enabling the effective separation of nanoparticles and diverse compounds with varying magnetic susceptibilities [80, 81].

CHROMATOGRAPHIC TECHNIQUES USED SIMULTANEOUSLY IN DIAGNOSIS

The application of chromatographic techniques in clinical diagnosis is highly successful. Bioanalytical techniques such as GC-MS and gas chromatography-thermal conductivity are widely used to diagnose diseases. All these techniques are noninvasive and have great potential in the medical field and the development of medical biomedical analytical methods. Bioanalytical tools such as chromatography, spectroscopy, and immunological analysis are used in the diagnosis of diseases such as AIDS, hepatitis, and acute respiratory disorders. Furthermore, hybrid techniques such as LC-NMR, LC-MS/MS, GC-MS/MS, etc., can be used in medicine and forensic sciences in the near future [82, 83]. Due to new tools and improved bioinformatics, screening complex biological samples such as exhaled air, tissue, blood, and urine has become increasingly popular in identifying biomarkers in different types of cancer. However, despite some progress, identifying biomarkers has proven to be challenging due to the small number of new biomarkers (except for recent genetic markers) considered for introduction into clinical analysis. Recent developments in gas chromatographic methods for identifying biomarkers in cancer detection, diagnosis, and treatment offer scope for the Gas Chromatography (GC) method. Future studies may focus on different types of body fluids, such as ascitic fluid in ovarian cancer, secretions in pancreatic cancer, or pleural fluid in lung cancer. So far, two multidimensional analytical tools, GC-MS, have been predominantly used in cancer studies. Advancements in high-resolution gas chromatography (HRGC), coupled with developments in mass spectrometry (MS), have enabled the application of advanced techniques such as GC-MS-MS (tandem mass spectrometry), MDGC-MS (multidimensional gas chromatography with mass spectrometry), and GC×GC-ToFMS (comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry) for monitoring metabolite changes in complex biochemical processes associated with cancer cells. These multidimensional gas chromatography (MDGC) separation techniques, leveraging enhanced separation capabilities through coupled GC column methods, facilitate improved detection and identification of metabolites, thereby contributing to the discovery of novel cancer biomarkers [84, 85].

CONCLUSION

Chromatographic methods are indispensable for researchers, enabling the selective separation, identification, and analysis of specific analytes, including elements and chemical compounds. Techniques such as chromatography, electrophoresis, and microfluidic chips exhibit exceptional purification sensitivity. Advances in biotechnology, coupled with in-depth studies of protein structure and function, have driven rapid progress in separation and purification technologies. Chromatography is widely recognized as a highly sensitive and effective separation method, enhancing analytical efficiency through improved productivity, reliability, robustness, resolution, speed, and sensitivity. Its continued prominence in bio-

medical and biopharmaceutical analysis is assured, yet achieving optimal purification often necessitates the integration of multiple techniques, as a single method is typically insufficient. A variety of chromatographic techniques—including method development software, fast liquid chromatography (LC), two-dimensional liquid chromatography (2D-LC), supercritical fluid chromatography (SFC), liquid chromatography–mass spectrometry (LC–MS), gas chromatography (GC), and gas chromatography–mass spectrometry (GC–MS)—are routinely employed to overcome analytical challenges associated with complex biomolecules. Moreover, the design of customized separation strategies based on the physicochemical characteristics of the target protein is of critical importance. Microfluidic chip technology has recently emerged as a promising platform, offering substantial potential for the miniaturization, integration, and portability of advanced analytical systems.

The new chromatographic approaches have high efficiency and sensitivity, increased productivity and resolution speed, and less time consumption. In the future, three-dimensional chromatographic separation will be possible, which will be helpful in various industries. Therefore, multidimensional chromatography is quite interesting for separating complex samples.

Furthermore, in the field of medical sciences, there has been increasing interest in molecular-level identification to improve rapid and sensitive detection in early diagnosis. The gradual application of chromatography technology in clinical applications has led to remarkable advances in health and medicine, a vital part of systems biology.

To achieve tremendous success in the pharmaceutical industry and biomedical sciences, advances in these techniques are necessary to drive major and modern developments in impurity analysis.

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