NORTHERN CLINICS OF İSTANBUL
VOL 13 • NO 5 • YEAR 2026

P ISSN 2148–4902
E ISSN 2536–4553

Journal Abbreviation:
North Clin Istanb

- WHtR and OGTT dynamics in prediabetes
- Is there a relationship between joint hypermobility and primary lymphedema?
- Mortality in methanol poisoning
- PEPITEM in allergic inflammation
- Set-up accuracy in breast radiotherapy
- SCORE2-DM and inflammatory indices
- Electronic cigarettes and heated tobacco products
- Neutrophil decline and LDL rise
- Behavioral social humanities in medical education

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İSTANBUL KUZEY KLİNİKLERİ

YEAR 2026
VOLUME 13
NUMBER 5

P ISSN 2148 - 4902
E ISSN 2536 - 4553

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Indexed in PubMed, Europe PMC, Web of Science, DOAJ, TRDIZIN, the Turkiye Science Citation Index, TurkMedline, EBSCO, CEEAS, Scopus, CLIO, ProQuest, OUCI, Scilit and Hinari/Research4Life.

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YILDIRIM PRINTING HOUSE
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1. Cad., No: 101, Bagcilar, Istanbul, Turkiye
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Info

Press date: October 2026
Circulation: 50
Type of publication: Periodical

DESIGN

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Northern Clinics of Istanbul (NCI) is a peer-reviewed journal published 6 issues in a year.
This publication is printed on paper that meets the international standard ISO 9706:1994.
National Library of Medicine recommends the use of permanent, acid-free paper in the production of biomedical literature.

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North Clin Istanb 2026;13(5):507–512
doi: 10.14744/nci.2026.03592

ORIGINAL ARTICLE INTERNAL MEDICINE

Association between central obesity assessed by waist-to-height ratio and oral glucose tolerance test glucose dynamics in prediabetes

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ABSTRACT

OBJECTIVE: Prediabetes is a heterogeneous metabolic condition associated with variable risk of progression to type 2 diabetes and cardiovascular complications. Conventional glycemic markers may fail to detect early metabolic deterioration. Recent evidence suggests that oral glucose tolerance test (OGTT)-derived glucose dynamics may provide additional insight into early metabolic alterations. Waist-to-height ratio (WHtR) has emerged as a simple and reliable anthropometric marker of central obesity that may outperform body mass index (BMI) in identifying individuals with increased cardiometabolic risk, including those with prediabetes. However, the relationship between central obesity and early OGTT glucose responses remains insufficiently investigated. To evaluate the association between central obesity assessed by WHtR and OGTT-derived glucose dynamics in individuals with prediabetes.

METHODS: This cross-sectional study included 86 individuals with prediabetes who underwent a standard 75-g OGTT. Anthropometric measurements including BMI and WHtR were recorded. OGTT-derived parameters including peak glucose, time to peak, glucose excursion, early and late glucose exposure (area under the curve), and 1-h glucose levels were analyzed. Associations were evaluated using correlation analyses and group comparisons. Multivariable linear regression adjusted for age and hemoglobin A1c was performed.

RESULTS: WHtR was significantly associated with early OGTT glucose parameters including 30-min glucose (r=0.222, p=0.042), peak glucose (r=0.247, p=0.022), early glucose exposure (r=0.249, p=0.022), and glucose excursion (r=0.245, p=0.023). BMI was not associated with OGTT glucose dynamics. Individuals with elevated 1-h glucose (≥155 mg/dL) had significantly higher WHtR values (p=0.029). In multivariable analysis, WHtR showed a trend toward significance for early glucose exposure.

CONCLUSION: WHtR was associated with early OGTT glucose abnormalities, whereas BMI was not, suggesting that central obesity may be linked to early metabolic alterations before overt dysglycemia develops. WHtR may serve as a simple and practical marker to identify individuals who may benefit from early metabolic risk assessment when evaluated together with OGTT-derived glucose dynamics.

Keywords: Body mass index; central obesity; oral glucose tolerance test; prediabetes; waist-to-height ratio.

Cite this article as: *Sonmezoglu Kutuk I, Yusifova F, Cerit ET, Yalcin MM, Akturk M, Toruner F, et al. Association between central obesity assessed by waist-to-height ratio and oral glucose tolerance test glucose dynamics in prediabetes. North Clin Istanb 2026;13(5):507–512.*

Received: April 27, 2026 *Revised:* June 29, 2026 *Accepted:* August 30, 2026 *Online:* October 15, 2026

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Prediabetes represents an intermediate metabolic state with variable risk of progression to type 2 diabetes and cardiovascular complications [1, 2]. However, conventional glycemic markers may not fully capture early metabolic alterations, as dysglycemic changes often begin before diagnostic thresholds are reached [2–4]. Therefore, there is increasing interest in identifying earlier metabolic markers that may improve risk stratification before the development of overt dysglycemia [4, 5].

Recent evidence has increasingly focused on the role of dynamic glucose responses during the oral glucose tolerance test (OGTT) in identifying early metabolic abnormalities beyond conventional fasting and 2-h measurements [1, 4]. In particular, glucose levels measured at earlier time points have been shown to provide additional prognostic information regarding future metabolic risk [4, 6]. Furthermore, individuals classified as normoglycemic based on standard criteria may still exhibit elevated glucose responses in the early phase of OGTT, which have been linked to underlying metabolic dysfunction [6, 7]. In addition, patterns observed in the glucose response curve, including the timing of peak glucose and early-phase elevations, have been associated with alterations in insulin sensitivity and β-cell function [8–10]. These findings suggest that early-phase glucose exposure and peak glucose levels may reflect metabolic deterioration before overt dysglycemia develops, highlighting the potential value of earlier risk identification and timely preventive interventions [8–10].

Central obesity is a key determinant of insulin resistance and cardiometabolic risk [1, 11, 12]. Among anthropometric indices, waist-to-height ratio (WHtR) has been recently proposed as a simple and reliable marker of central adiposity and metabolic risk that may outperform traditional measures such as body mass index (BMI) [11, 12]. Previous studies have demonstrated associations between WHtR and insulin resistance, dyslipidemia, and increased diabetes risk across different populations [11–13]. However, most of these studies have focused on conventional glycemic outcomes rather than OGTT-derived glucose dynamics [11, 12].

Although both OGTT-derived glucose dynamics and central obesity are independently associated with metabolic risk, the relationship between central adiposity and early glucose response patterns during OGTT remains insufficiently investigated, particularly in individuals with prediabetes [4, 8, 11]. Understanding this relationship may provide additional insight into early metabolic deterioration and help identify high-risk individuals before the development of overt dysglycemia [4, 8]. Based on emerging evidence linking central adiposity with early metabolic dysfunction and insulin resistance, we hypothesized that higher WHtR would be associated with unfavorable OGTT glucose dynamics independent of conventional glycemic classification. These parameters were selected because they capture early-phase glucose dysregulation, which has been shown to reflect β-cell dysfunction and insulin resistance more sensitively than conventional 2-h glucose measurements [8–10]. Therefore, the aim of this study was to investigate the association between central obesity assessed by WHtR and OGTT-derived glucose dynamics including peak glucose, early glucose exposure, and glucose excursion in individuals with prediabetes.

Highlight key points

- WHtR is associated with early OGTT-derived glucose abnormalities in prediabetes.
- WHtR correlates with early glucose exposure and glucose excursion.
- BMI may be delayed or inadequate in reflecting early glucose dynamics.
- Elevated 1-h glucose is associated with higher WHtR.
- WHtR may help identify early metabolic risk beyond conventional markers.

MATERIALS AND METHODS

Study Design and Population

This cross-sectional analysis was conducted using retrospective data from consecutive individuals with prediabetes who had undergone 75-g OGTT as part of routine clinical evaluation at an endocrinology outpatient clinic of Gazi University between June 2025 and February 2026.

Prediabetes was classified based on established diagnostic thresholds for fasting glucose, 2-h OGTT glucose, and hemoglobin A1c (HbA1c) levels [14].

Individuals with previously diagnosed diabetes mellitus, use of glucose-lowering medications, pregnancy, active infection, malignancy, missing clinical data, or current use of systemic corticosteroids were excluded. Demographic characteristics, anthropometric measurements, and laboratory parameters were obtained from medical records.

Table 1. Demographic and metabolic characteristics of the study population

Variables	Total (n=86)	Female (n=61)	Male (n=25)
Age (years)	51±12	50±11	51±13
HbA1c (%)	5.96±0.22	5.99±0.21	5.90±0.25
BMI (kg/m^2)	26.46±3.44	26.61±3.31	26.11±3.77
WHtR	0.57 (0.52–0.61)	0.57 (0.52–0.61)	0.57 (0.52–0.60)

Data are presented as mean±SD or median (IQR). IQR: Interquartile range; BMI: Body mass index; HbA1c: Hemoglobin A1c; SD: Standard deviation; WHtR: Waist-to-height ratio.

Ethical approval for the study was obtained from the Gazi University Ethics Committee (Approval No: 2026-684; Date: March 31, 2026). The study protocol adhered to the ethical standards outlined in the Declaration of Helsinki. Given the retrospective nature of the study, the need for informed consent was waived.

Anthropometric Measurements

Anthropometric measurements were retrieved from patient records and included height, body weight, and waist circumference. BMI was derived by dividing body weight in kilograms by height in meters squared. Waist circumference was assessed at the midpoint between the lower rib margin and the iliac crest at the end of a normal expiration. The WHtR was calculated as the ratio of waist circumference to height, and a value of ≥0.5 was used to define central obesity [15, 16].

Laboratory Measurements and OGTT-derived Glucose Dynamics

Laboratory data including fasting plasma glucose, OGTT glucose measurements, and HbA1c levels were obtained from medical records. Glucose measurements were performed using standard automated laboratory methods, and HbA1c was measured using high-performance liquid chromatography.

OGTT measurements were retrospectively retrieved from patient records. All individuals had undergone a standard 75-g glucose challenge following overnight fasting, as part of routine clinical evaluation. Plasma glucose levels at predefined time points (0, 30, 60, 90, and 120 min) were recorded.

Peak glucose was defined as the highest glucose value during OGTT, and time to peak as the time point at which this value occurred. Glucose excursion was defined as the difference between peak and fasting glucose levels. Glucose exposure was quantified using the area under the curve (AUC), calculated based on serial glucose measurements using the trapezoidal approach. Early and late phases were evaluated separately to reflect temporal glucose dynamics. Early AUC and late AUC were defined as glucose exposure between 0–60 min and 60–120 min, respectively, based on the established distinction between early-phase insulin secretion and late-phase glucose clearance during OGTT, which reflect different pathophysiological processes including hepatic glucose output and peripheral insulin sensitivity [6, 7, 17]. The early-to-late AUC ratio was calculated to reflect the relative contribution of early versus late glucose exposure. Late glucose clearance was defined as the difference between peak glucose and 120-min glucose levels. Elevated 1-h glucose was defined as ≥155 mg/dL [3]. Multivariable linear regression analysis was performed to evaluate the independent association of WHtR with early glucose exposure (early AUC), adjusting for age and HbA1c as potential confounders.

Statistical Analysis

All statistical analyses were carried out using IBM Statistical Package for the Social Sciences Statistics (version 31.0; IBM Corp., Armonk, NY, USA). The final dataset consisted of 86 individuals with complete OGTT data.

Data distribution was evaluated with the Shapiro–Wilk test. Continuous variables are reported as mean±standard deviation or median (interquartile range), depending on distribution, whereas categorical variables are presented as counts and percentages.

The relationships between anthropometric indices and OGTT-derived parameters were examined using Pearson or Spearman correlation coefficients, as appropriate. Group comparisons were performed using inde-

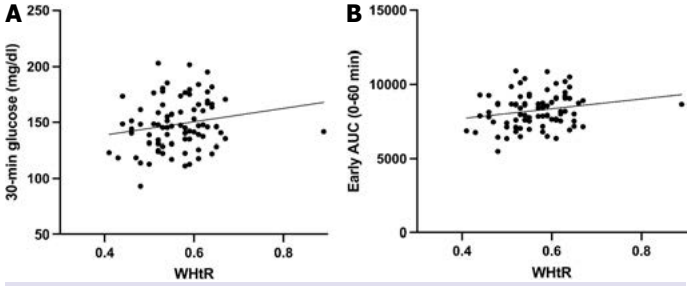

FIGURE 1. Relationship between waist-to-height ratio (WHtR) and early oral glucose tolerance test glucose parameters. **(A)** Scatter plot showing the association between WHtR and 30-min plasma glucose levels. **(B)** Scatter plot showing the association between WHtR and early glucose exposure assessed by the area under the curve (AUC) between 0 and 60 min (Early AUC). Correlations were assessed using Spearman analysis. Solid lines represent linear regression lines.

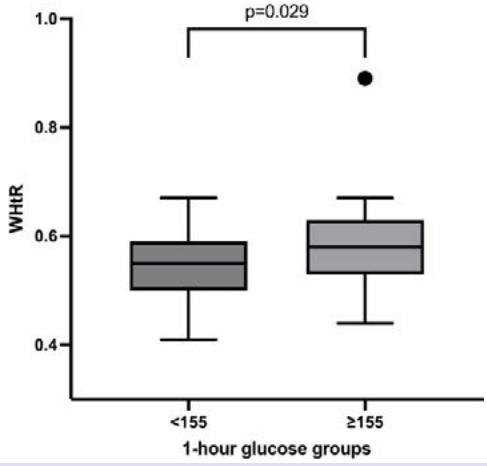

FIGURE 2. Comparison of waist-to-height ratio (WHtR) according to 1-h glucose levels. Individuals with elevated 1-h glucose (≥155 mg/dL) had significantly higher WHtR values compared to those with lower levels (p=0.029). Boxes represent the median and interquartile range; whiskers represent the minimum and maximum values.

pendent samples t-test or Mann–Whitney U test based on data characteristics. A two-sided $p<0.05$ was considered statistically significant.

RESULTS

Study Population Characteristics

A total of 86 individuals with prediabetes were included in the study. The mean age was 51±12 years, and 70.9% of participants were female. The mean HbA1c level was 5.96±0.22%, and the mean BMI was 26.46±3.44 kg/m^2. The median WHtR was 0.57 (0.52–0.61). Baseline demographic and metabolic characteristics are presented in Table 1.

TABLE 2. Correlation of central obesity indices with OGTT-derived glucose dynamics

Variables	WHtR		BMI	
	r	p	r	p
Fasting glucose	0.095	0.383	-0.032	0.769
30-min glucose	**0.222**	**0.042**	0.210	0.054
60-min glucose	0.161	0.140	0.045	0.680
90-min glucose	0.191	0.082	-0.022	0.842
120-min glucose	0.159	0.147	-0.010	0.926
Total glucose AUC	0.184	0.096	0.028	0.798
Peak glucose	**0.247**	**0.022**	0.105	0.335
Time to peak	0.102	0.355	-0.092	0.402
Early AUC	**0.249**	**0.022**	0.142	0.196
Late AUC	0.176	0.112	-0.020	0.854
Early/Late ratio	-0.077	0.487	0.126	0.256
Glucose excursion	**0.245**	**0.023**	0.129	0.237
Late clearance	0.044	0.689	-0.062	0.571

Spearman correlation analysis was used for WHtR and Pearson correlation analysis for BMI. Statistically significant associations are shown in bold. AUC: Area under the curve; WHtR: Waist-to-height ratio; BMI: Body mass index; OGTT: Oral glucose tolerance test.

Correlation between Central Obesity Indices and OGTT Glucose Dynamics

Correlation analysis demonstrated that WHtR was significantly associated with early OGTT glucose parameters including 30-min glucose levels ($r=0.222$, $p=0.042$) (Fig. 1A), peak glucose ($r=0.247$, $p=0.022$), early AUC ($r=0.249$, $p=0.022$) (Fig. 1B), and glucose excursion ($r=0.245$, $p=0.023$). BMI did not show significant correlations with OGTT glucose dynamic parameters (Table 2). In multivariable linear regression analysis including age and HbA1c, WHtR showed a trend toward significance for early AUC ($\beta=0.3385$, $p=0.071$). WHtR remained the strongest predictor among the variables included in the model.

Association between WHtR and Elevated 1-h Glucose

Participants with elevated 1-h glucose levels (≥155 mg/dL) had significantly higher WHtR values compared to those with lower 1-h glucose levels ($p=0.029$) (Fig. 2).

DISCUSSION

In this study, we found that central obesity assessed by WHtR was associated with early OGTT glucose dynamics including 30-min glucose, peak glucose, early glucose exposure, and glucose excursion, whereas BMI was not associated with these parameters. These findings suggest that central adiposity may contribute to early metabolic deterioration before abnormalities in conventional glycemic markers become evident. Similar observations have been reported in previous studies showing that early glucose abnormalities at OGTT may reflect early metabolic dysfunction not detected by traditional glycemic classification [1, 4, 8]. Although the observed correlation coefficients were modest in magnitude, they are consistent with the complexity of metabolic interactions in prediabetes and suggest a clinically meaningful, albeit partial, contribution of central adiposity to early glucose dysregulation.

Another important finding of our study was that WHtR was associated particularly with early glucose exposure rather than late OGTT parameters. This may indicate that central obesity may primarily affect early metabolic defects such as hepatic insulin resistance and early-phase insulin response. Previous studies have demonstrated that early OGTT glucose elevations and delayed peak glucose timing are associated with β-cell dysfunction and progression of dysglycemia [8, 18, 19]. In contrast, late-phase OGTT parameters have been more closely associated with impaired glucose clearance and peripheral insulin resistance, suggesting that early and late glucose dynamics may reflect different pathophysiological processes [6, 7, 17]. In our study, WHtR showed the strongest associations with 30-min glucose, peak glucose levels, and early AUC, highlighting its particular relevance to early-phase glucose dysregulation. Importantly, our findings extend the existing literature by demonstrating that central obesity, as reflected by WHtR, is specifically associated with early glucose abnormalities at OGTT, suggesting a closer link with early β-cell dysfunction rather than later disturbances in glucose metabolism.

We also observed that BMI was not significantly associated with OGTT glucose dynamics, in contrast to WHtR. Notably, while BMI showed only borderline or non-significant correlations, WHtR demonstrated consistent and statistically significant associations with early glucose parameters. This finding is consistent with previous studies suggesting that BMI may not adequately reflect visceral fat distribution or metabolic risk, since individuals with similar BMI values may have substantially different metabolic profiles [11–13]. Studies comparing anthropometric indices have also demonstrated that WHtR may be superior to BMI in identifying individuals at increased metabolic risk [11, 12, 20, 21]. Consistent with this, recent obesity guidelines such as the NICE guideline recommend the use of WHtR alongside BMI to assess central adiposity and stratify cardiometabolic risk [15]. Our results support these findings and suggest that WHtR may be a more sensitive marker of early metabolic abnormalities compared with BMI.

From a clinical perspective, our findings suggest that combining WHtR with OGTT-derived glucose dynamics may improve the early identification of individuals at increased metabolic risk who may otherwise be classified as low risk based on conventional glycemic criteria. WHtR is an inexpensive, easily obtainable, and reproducible anthropometric marker that can be readily implemented in routine clinical practice. Such an approach may allow earlier lifestyle interventions before progression to overt diabetes. Therefore, WHtR may serve as a practical screening tool to identify individuals who may benefit from more detailed metabolic evaluation.

A major strength of this study is the detailed OGTT assessment with multiple time points, allowing a comprehensive evaluation of glucose dynamics. However, this study has several limitations. The cross-sectional design limits causal inference, and the relatively small sample size and single-center setting may affect generalizability. In addition, direct measures of visceral adiposity or body fat distribution were not available, which may limit the interpretation of WHtR as a surrogate marker of central obesity. Furthermore, the absence of direct measures of insulin resistance such as fasting insulin or homeostatic model assessment of insulin resistance, as well as lifestyle-related variables including physical activity and dietary habits, represents an additional limitation that may have influenced the observed associations.

Conclusion

WHtR was associated with early OGTT glucose abnormalities and demonstrated stronger associations with early glucose dynamics than BMI, suggesting that central adiposity may be linked to early metabolic alterations before the development of overt dysglycemia. These findings indicate that WHtR may serve as a simple and practical clinical marker for early metabolic risk assessment, particularly when evaluated together with OGTT-derived glucose dynamics. Our findings also support the integration of anthropometric markers such as WHtR with dynamic metabolic testing to improve early risk stratification.

Ethics Committee Approval: This study was approved by the Gazi University Ethics Committee (Approval No: 2026-684; Date: March 31, 2026).

Informed Consent: The requirement for informed consent was waived due to the retrospective nature of the study.

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

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

Use of AI for Writing Assistance: The authors declared that artificial intelligence was not used in the study.

Authorship Contributions: Concept – ISK, AEA; Design – ISK, AEA; Supervision – AEA; Resource – ISK, AEA; Materials – ISK, AEA; Data Collection and/or Processing – ISK, FY, AEA; Analysis and/or Interpretation – ISK, FY, ETC, MMY, MA, FT, MAK, AEA; Literature Search – ISK, AEA; Writing – ISK, FY, ETC, MMY, MA, FT, MAK, AEA; Critical Reviews – ISK, FY, ETC, MMY, MA, FT, MAK, AEA.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2026;13(5):513–522
doi: 10.14744/nci.2026.64928

Original Article PM & R

Is there a relationship between joint hypermobility and primary lymphedema? A cross-sectional study

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ABSTRACT

OBJECTIVE: This study aimed to evaluate the association between joint hypermobility (JH) and primary lymphedema.

METHODS: This cross-sectional study included 36 patients (30 females and 6 males; age range, 18–65 years) diagnosed with primary lower extremity lymphedema and 32 age-matched healthy controls. Demographic characteristics were recorded for all participants. Generalized joint hypermobility (GJH) was assessed using the Beighton scoring system, and Benign joint hypermobility syndrome (BJHS) was evaluated according to the Brighton criteria.

RESULTS: GJH was observed in 27.8% of patients with lymphedema and 25% of controls, with no statistically significant difference between groups ($p>0.05$). Similarly, the frequency of BJHS was comparable between the lymphedema group (13.9%) and the control group (15.6%; $p>0.05$). Mean Beighton scores did not differ significantly between the lymphedema and control groups ($p>0.05$). Furthermore, neither Beighton scores nor the prevalence of BJHS differed significantly across lymphedema stages ($p>0.05$).

CONCLUSION: JH was not significantly associated with primary lymphedema. Our study may provide a foundation for future research aiming to clarify the relationship between JH and primary lymphedema through objective diagnostic criteria and quantitative connective tissue assessments.

Keywords: Connective tissue; joint hypermobility; lymphedema.

Cite this article as: *Begoglu FA, Ozturk G, Kaysin MY, Karamanlioglu AD, Ozkan FU, Aktas I. Is there a relationship between joint hypermobility and primary lymphedema? A cross-sectional study. North Clin Istanb 2026;13(5):513–522.*

Joint hypermobility (JH) is a clinical condition defined by an excessive range of motion in one or more joints beyond normal physiological limits [1]. The reported prevalence of JH varies widely, ranging from 0.6% to 31.5%, depending on demographic factors such as race, age, and sex. It is more prevalent in women than in men and shows a progressive decline with advancing age [2, 3]. Involvement of multiple joints is generally referred to as generalized joint hypermobility (GJH) or joint laxity [4–6]. JH represents a spectrum ranging from asymptomatic benign hypermobility to symptomatic hypermobility disorders. The Beighton Scoring System – a widely used 9-point scale for assessing articular hypermobility in adults – has been incorporated into both prior and current diagnostic criteria for hypermobility syndromes [7–11]. Despite its recognized limitations [8, 9], the scale remains the most widely accepted assessment tool, with a Beighton score of ≥4 commonly applied as the threshold for defining GJH [12–14].

Received: May 06, 2026 *Revised:* July 29, 2026 *Accepted:* August 06, 2026 *Online:* October 15, 2026

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GJH is widely recognized as a hallmark feature of various hereditary connective tissue disorders; however, its underlying pathophysiological mechanisms remain poorly understood [4, 15]. It has been associated with structural abnormalities in mesoderm-derived connective tissues, particularly muscle, tendon, and venous vascular structures [3, 16–18]. In individuals with JH, alterations in collagen composition, especially an increased ratio of type III to type I collagen, have been documented. These abnormalities in collagen structure contribute to joint laxity and increased fragility of connective tissue [1, 19].

In the last decade, growing attention has been directed toward JH and its related disorders. The literature abounds with case–control studies demonstrating a significant association between GJH – commonly assessed using the Beighton score – and specific extra-articular manifestations [4, 6, 12–15]. In 2017, Castori et al. [4] defined JH and these associated complications as JH-related comorbidities, among which cardiovascular manifestations represent a notable component [15]. JH is associated with venous disorders to varying degrees. Laxity of the vessel walls in patients with underlying connective tissue abnormalities may explain this association [18, 20]. Fundamentally, the lymphatic and venous systems predominantly arise from a shared mesodermal lineage [21–23]. The lymphatic system depends on connective tissue both for structural support and for the proper functioning of its anchoring filament network [24]. Impairment of lymphatic transport capacity leads to interstitial fluid accumulation, triggering progressive remodeling of fibro-adipose connective tissue [25, 26]. Primary lymphedema may occur either as an isolated disorder or as a component of complex genetic syndromes that include vascular anomalies [27, 28].

GJH has not yet been systematically evaluated in patients with primary lymphedema, a disorder of the lymphatic system that shares a common developmental origin with connective tissue. Given the gaps in the current literature and the potential clinical implications of establishing a link between primary lymphedema and hypermobility, the present study aimed to examine the association between GJH and primary lymphedema and to explore its relationship with lymphedema stages.

Highlight key points

- JH was identified in 27.8% of patients with primary lymphedema but showed no significant association with either the presence or severity of lymphedema.
- Age was inversely associated with GJH, consistent with the well-documented decline in JH with advancing age.
- These findings may establish a reference point for future research incorporating objective diagnostic criteria and quantitative connective tissue assessments to further clarify the relationship between JH and primary lymphedema.

MATERIALS AND METHODS

This cross-sectional observational study included 36 patients aged 18–65 years who presented to the Physical Medicine and Rehabilitation (PMR) lymphedema outpatient clinic with lower-extremity swelling and were diagnosed with primary lymphedema, as well as 32 age-matched healthy volunteers. Participants were enrolled between April and October 2024.

All patients were evaluated by an experienced PMR specialist. The diagnosis of primary lymphedema was established in accordance with the diagnostic approach outlined in the 2023 Consensus Document of the International Society of Lymphology [25], based on a combination of medical history and physical examination findings. Diagnostic criteria included lower-extremity swelling, a positive Stemmer sign, the presence or absence of pitting edema, normal Doppler ultrasonography findings, and normal abdominopelvic and inguinal ultrasonography for etiological assessment. Lymphatic insufficiency was defined by lymphoscintigraphic findings, including delayed tracer transit time, dermal backflow, asymmetric nodal uptake, the presence of collateral lymphatic channels, and tracer uptake in the deep lymph nodes [29].

Patients diagnosed with primary lymphedema were classified according to disease severity as Stage I, II, or III. Stage I was defined by pitting edema that improved with limb elevation; Stage II by persistent pitting edema unresponsive to elevation; and Stage III by fibro-adipose tissue deposition accompanied by skin changes [25]. Age-matched healthy volunteers were recruited as controls. Healthy controls were selected from volunteers without a history or clinical signs of lymphedema, lower-extremity edema, connective tissue disorder, or any known musculoskeletal or neurological condition that could affect joint mobility. All controls underwent the same clinical assessment procedure for JH as the lymphedema group.

Exclusion criteria included secondary lymphedema; lower-extremity edema due to cardiac, renal, hepatic, or myxedematous causes; use of medications known to cause edema (including corticosteroids, calcium chan-

nel blockers, gabapentin, and pregabalin); a diagnosis of rheumatologic disease; and the presence of active cellulitis or erysipelas of the lower extremities in patients with primary lymphedema.

Demographic data were recorded for all participants. Joint range of motion was assessed during the evaluation visit using the Beighton scoring system, and the Brighton criteria were applied. Age-dependent cutoff scores of ≥5 for individuals aged 18–50 years and ≥4 for individuals aged >50 years were used in the current study, according to the 2017 updated criteria for the Beighton score [11, 30]. However, to facilitate comparability, prevalence rates of GJH with a cutoff ≥4/9 were presented for all ages [30]. The diagnosis of benign joint hypermobility syndrome (BJHS) was determined according to the revised 1998 Brighton criteria. All clinical assessments were performed by a single experienced PMR specialist to ensure consistency.

The study was approved by the Ethics Committee (approval date: March 14, 2024; approval number: 3/12) and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Statistical Analyses

Statistical analyses were performed using IBM Statistical Package for the Social Sciences (SPSS) Statistics version 22 (IBM SPSS, Turkey). Normality of continuous variables was assessed using the Lilliefors-corrected Kolmogorov–Smirnov test. Age and body mass index (BMI) were normally distributed and were compared between groups using independent-samples Student's t-tests. The Beighton score was not normally distributed in either group (Lilliefors p=0.023 and p=0.002, lymphedema and control groups, respectively) and was therefore compared using the Mann–Whitney U test. Categorical variables (sex, GJH, and BJHS) were compared using Fisher's exact test, which was used in preference to the chi-square test throughout because several expected cell counts were below 5. Odds ratios (OR) with 95% confidence intervals (CIs) are reported alongside p-values for all binary outcomes so that the magnitude and precision of each estimate, not only its statistical significance, can be judged. To account for potential confounders, two additional analyses were performed. First, a baseline-comparability analysis compared the lymphedema and control groups on every recorded sociodemographic and clinical covariate (sex, education, marital status, occupation, smoking/alcohol use, and comorbidity) using Fisher's exact test (with Monte Carlo simulation for sparse r × c tables). Second, multivariable logistic regression models were fitted for GJH and BJHS, each adjusting for group, age, and BMI, to test whether the crude (unadjusted) group comparison was robust to these two continuous confounders.

A pre-specified sensitivity analysis collapsed Stage II and Stage III into a single "Stage II–III (moderate–advanced)" category and compared it against Stage I ("early") using the Mann–Whitney U test (Beighton score) and Fisher's exact test (GJH, BJHS), which yields adequately sized comparison groups (n=13 vs. n=23). Categorical comparisons across the three ordinal stages used the Fisher–Freeman–Halton exact test (the extension of Fisher's exact test to r × c tables), and the Kruskal–Wallis test was used for the Beighton score across the three stages.

Sample Size

The power analysis was performed using G*Power. The effect size of 0.40 (Δ:40%) used in the sample size calculation was derived from the literature [17]. A study of 184 patients with lumbar disc herniation found that BJHS, assessed with the revised (Brighton, 1998) criteria, was present in 68.4% of patients. That study did not include a control group; the prevalence of GJH in the general population ranges from approximately 10% to 30% [17]. Using the upper bound of this range (28.4%) as a conservative estimate of the expected prevalence in a non-hypermobile comparison population, and 68.4% as the expected prevalence in a population enriched for musculoskeletal referral [17], gives an expected between-group prevalence difference of Δ=68.4−28.4%=40.0%, consistent with the Δ=40%. With these two proportions (p1=0.684, p2=0.284), α=0.05 (two-tailed), and 80% power, a two-independent-proportions z test (unpooled variance in the power term) indicates a minimum required sample size of n=24 per group. The present study enrolled 36 patients with primary lymphedema and 32 controls.

RESULTS

Demographic and Clinical Characteristics of the Participants

The average age of the participants was 41.41±12.38 years. Overall, 76.5% of participants were female, and 23.5% were male. Comorbidities were present in 30.9%

Table 1. Demographic and clinical characteristics of the participants

Characteristics	n=68	Percentage
Sex		
Female	52	76.5
Male	16	23.5
Comorbidities		
Present	21	30.9
Absent	47	69.1
Distribution of comorbidities*		
Hypertension	7	33.3
Diabetes mellitus	7	33.3
Thyroid dysfunction	8	38.1
Hyperlipidemia	4	19
Others	5	23.8
Lymphedema		
Present	36	52.9
Absent	32	47.1
Lymphedema side		
Right	6	16.7
Left	11	30.6
Bilateral	19	52.8
Lymphedema stage**		
1	13	36.1
2	21	58.3
3	2	5.6
GJH		
Present	18 (F:17 M:1)	26.5 (F:94.4)***
Absent	50	73.5
BJHS		
Present	10 (F:10 M:0)	14.7 (F:100)***
Absent	58	85.3

BJHS: Benign joint hypermobility syndrome; GJH: Generalized joint hypermobility. *Some patients had more than one comorbidity; percentages do not sum to 100%; **Percentages for lymphedema side and stage are calculated among patients with lymphedema only (n=36), all other percentages are based on the total sample (n=68); ***Sex distribution percentages calculated on subgroup totals.

of participants. The most common comorbid conditions were thyroid disorders (38.1%), followed by hypertension (33.3%), diabetes mellitus (33.3%), hyperlipidemia (19.0%), and other conditions (23.8%; Table 1).

Primary lymphedema was present in 52.9% of participants (n=36). The duration of lymphedema

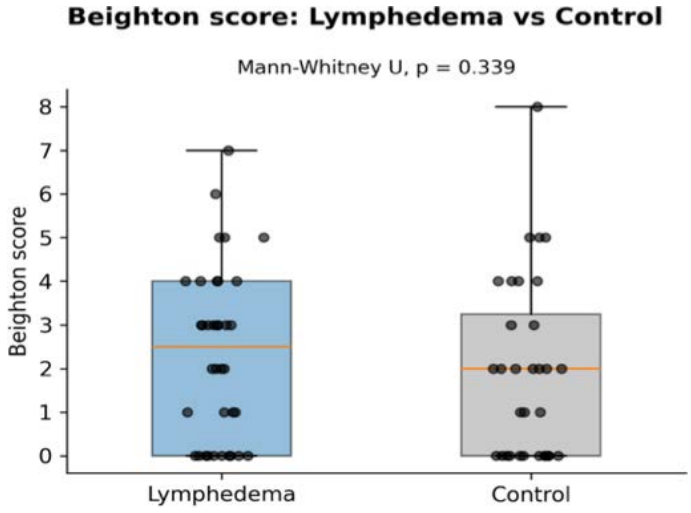

Figure 1. Beighton score by group. Boxplots show median and interquartile range; points represent individual participants (jittered).

ranged from 1 to 34 years, with a mean duration of 10.88±8.35 years. Among patients with lymphedema, 52.8% had bilateral involvement, whereas 47.2% had unilateral involvement (16.7% right-sided and 30.6% left-sided). According to disease stage, 36.1% of patients were classified as Stage I, 58.3% as Stage II, and 5.6% as Stage III (Table 1).

Across the entire study population, 18 participants (26.5%) met the criteria for GJH, and 10 participants (14.7%) were diagnosed with BJHS. GJH was identified in 17 females (94.4%) and one male (6.25%). BJHS was observed exclusively among female participants (100%), with no cases detected in males (Table 1).

Comparisons of Demographic and Hypermobility Outcomes Between Groups

The two groups did not differ significantly on any recorded sociodemographic or clinical covariate, indicating that observed differences in hypermobility outcomes are unlikely to be explained by imbalances in these variables (Table 2).

No statistically significant differences were observed in age, BMI, sex distribution, Beighton score, GJH prevalence, or BJHS prevalence. The ORs were close to 1.0 with wide (CIs) or both GJH (OR=1.15, 95% CI [0.39, 3.41]) and BJHS (OR=0.87, 95% CI [0.23, 3.34]), indicating that the data are compatible with a wide range of true effects and do not provide strong evidence against a modest association in either direction (Fig. 1, 2).

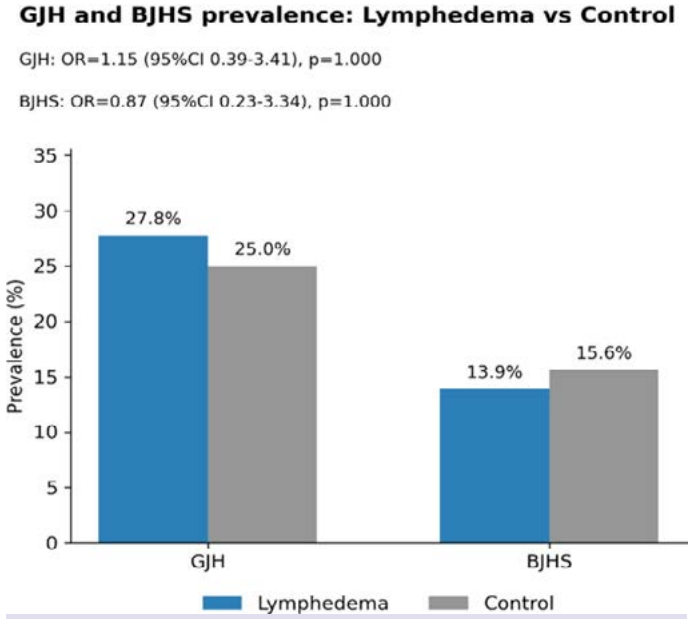

FIGURE 2. Prevalence of generalized joint hypermobility and benign joint hypermobility syndrome by group, with odds ratios (95% confidence interval) and Fisher's exact p-values.

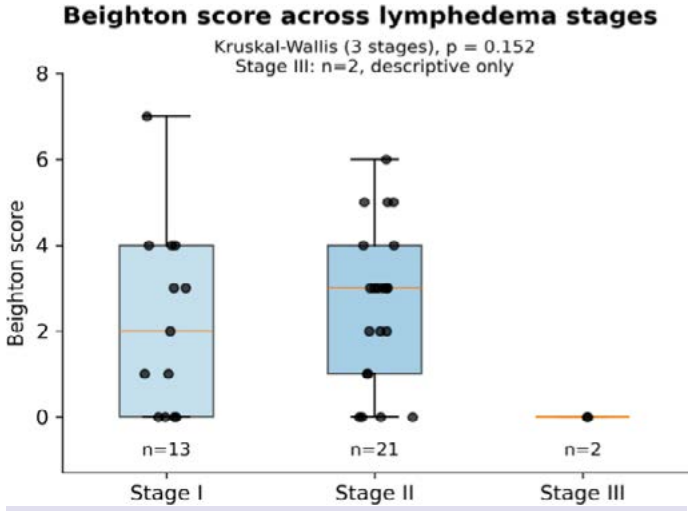

FIGURE 3. Beighton score across lymphedema stages. Stage III (n=2) is labeled for transparency and should be interpreted descriptively only.

Lymphedema Stage-Based Hypermobility Score Comparisons

Among the 36 patients with primary lymphedema, 13 (36.1%) were classified as Stage I, 21 (58.3%) as Stage II, and only 2 (5.6%) as Stage III. This severely limits the reliability of any three-level comparison involving Stage III. The exploratory three-level analysis (Table 3; Fig. 3, 4) found no statistically significant differences in Beighton score, GJH, or BJHS across stages; however, given that both Stage III patients had a Beighton score of 0 and neither met GJH nor BJHS criteria, these Stage III estimates should be regarded as descriptive only and not as evidence of a true stage effect.

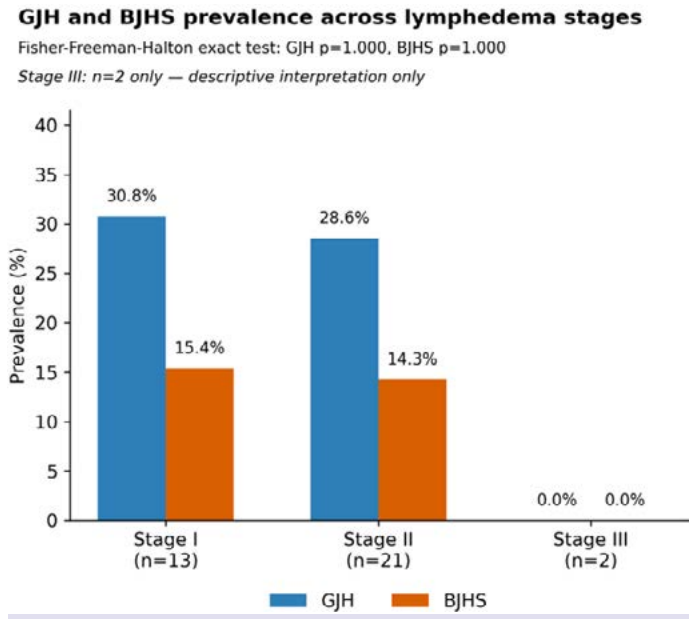

FIGURE 4. Prevalence of GJH and BJHS across lymphedema stages (Fisher–Freeman–Halton exact test).

To provide an adequately powered alternative to the three-level comparison, a pre-specified sensitivity analysis combined Stage II and Stage III into a single "Stage II–III (moderate–advanced)" category (n=23) and compared it with Stage I ("early," n=13; Table 4; Fig. 5, 6). This mirrors the original findings: No statistically significant differences were observed in Beighton score, GJH, or BJHS between early and moderate-to-advanced disease. The three-level comparison was retained as supplementary/exploratory.

Multivariable Logistic Regression Analysis for GJH and BJHS

To evaluate whether the crude group comparison was confounded by age or BMI, multivariable logistic regression models were fitted for GJH and BJHS (Table 5). After adjustment, the association between group (lymphedema vs. control) and GJH remained non-significant, and the point estimate was essentially unchanged (adjusted OR=1.03, 95% CI [0.30, 3.46]), as was the association with BJHS (adjusted OR=0.75, 95% CI [0.17, 3.13]). Age was independently and inversely associated

Table 2. Comparisons of demographic and hypermobility outcomes between groups

Variables	Lymphedema (n=36)	Control (n=32)	Test	OR (95% CI)	p
Age, years, Mean±SD	40.86±12.72	42.03±12.15	Student t	—	0.699
BMI, kg/m², Mean±SD	29.34±6.89	28.71±5.42	Student t	—	0.677
Sex, (%)			Fisher's exact	—	0.252
Female	83.3	68.8			
Male	16.7	31.2			
Education level, (%)			Fisher's exact	—	0.928
Primary	27.8	34.4			
Secondary	22.2	18.8			
Higher education	47.2	46.9			
Illiterate	2.8	0.0			
Marital status, (%)			Fisher's exact	—	0.614
Married	61.1	68.8			
Single	38.9	31.2			
Occupation, (%)			Fisher's exact	—	0.609
Housewife/unemployed	33.3	21.9			
Civil servant	11.1	6.2			
Worker	5.6	12.5			
Retired	2.8	3.1			
Student	2.8	0.0			
Engineer/architect/physician	11.1	9.4			
Education sector	8.3	3.1			
Service sector	25.0	43.8			
Smoking/alcohol use, (%)			Fisher's exact	—	0.612
Neither	75.0	78.1			
Smoker	19.4	21.9			
Alcohol	5.6	0.0			
Comorbidity, (%)			Fisher's exact	—	0.606
Present	27.8	34.4			
Absent	72.2	65.6			
Beighton score, Mean±SD	2.33±1.97	1.94±2.06	Mann–Whitney U	—	0.339
GJH positive, (%)	27.8	25.0	Fisher's exact	1.15 (0.39–3.41)	1.000
BJHS positive, (%)	13.9	15.6	Fisher's exact	0.87 (0.23–3.34)	1.000

BMI: Body mass index; GJH: Generalized joint hypermobility; BJHS: Benign joint hypermobility syndrome; OR: Odds ratio (Lymphedema vs. Control); CI: Confidence interval; Bold rows show the test statistic and p-value for the categorical variable as a whole; indented rows below each bold row show the frequency and percentage within each group for every category level. None of the baseline covariates (sex through comorbidity) differed significantly between groups (all p>0.05), supporting adequate comparability.

with GJH (adjusted OR=0.91, 95% CI [0.85, 0.97], p=0.006), and BMI was independently and positively associated with BJHS (adjusted OR=1.13, 95% CI [1.01, 1.29], p=0.043). These two incidental findings support the validity of the modeling approach (the model detects known/plausible associations) while confirming that the null group-level finding is not attributable to confounding by age or BMI.

Table 3. Beighton score, GJH, and BJHS across lymphedema stages (exploratory, three-level comparison)

Variabless	Stage I (n=13)	Stage II (n=21)	Stage III (n=2)[a]	Test	p
Beighton score	2.23±2.17	2.62±1.83	0.00±0.00	Kruskal–Wallis	0.152
GJH, (%)	30.8	28.6	0.0	Fisher–Freeman–Halton	1.000
BJHS, (%)	15.4	14.3	0.0	Fisher–Freeman–Halton	1.000

GJH: Generalized joint hypermobility; BJHS: Benign joint hypermobility syndrome. a: Stage III results are descriptive only (n=2) and should not be interpreted as a formal statistical comparison.

Table 4. Sensitivity analysis: Stage I versus Stage II–III (Combined)

Variables	Stage I (n=13)	Stage II–III combined (n=23)	Test	p
Beighton score	2.23±2.17	2.39±1.90	Mann–Whitney U	0.775
GJH, (%)	30.8	26.1	Fisher's exact	1.000
BJHS, (%)	15.4	13.0	Fisher's exact	1.000

GJH: Generalized joint hypermobility; BJHS: Benign joint hypermobility syndrome.

Table 5. Multivariable logistic regression for GJH and BJHS, adjusted for age and BMI

Outcome	Predictor	Adjusted OR (95% CI)	p
GJH	Group (Lymphedema vs. Control)	1.03 (0.30–3.46)	0.966
GJH	Age	0.91 (0.85–0.97)	**0.006**
GJH	BMI	0.99 (0.89–1.10)	0.853
BJHS	Group (Lymphedema vs. Control)	0.75 (0.17–3.13)	0.688
BJHS	Age	0.99 (0.92–1.05)	0.640
BJHS	BMI	1.13 (1.01–1.29)	**0.043**

GJH: Generalized joint hypermobility; BJHS: Benign joint hypermobility syndrome; BMI: Body mass index; OR: Odds ratio; CI: Confidence interval.

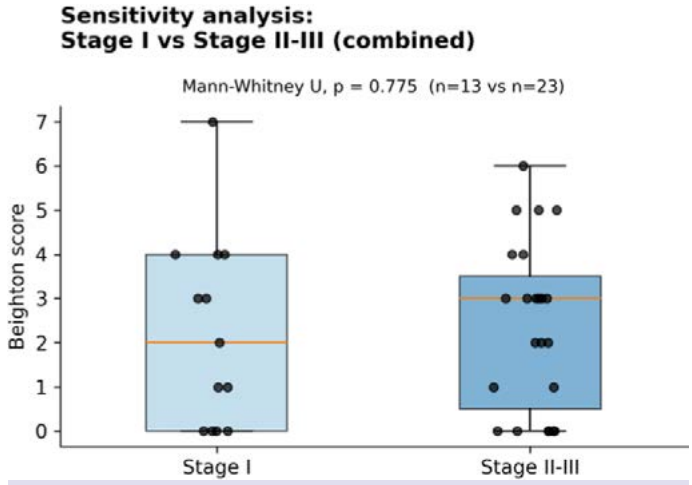

Figure 5. Sensitivity analysis: Beighton score, Stage I versus Stage II–III (combined).

DISCUSSION

In view of the limited evidence available in the current literature, this study aimed to investigate the association between primary lymphedema and JH as well as lymphedema stage. In this study, hypermobility was identified in 27.8% of patients with primary lymphedema. No significant association was found between GJH or BJHS and primary lymphedema or lymphedema severity. BMI was positively associated with BJHS. In addition, consistent with previous literature, this study demonstrated a female predominance of JH and an inverse association between age and JH [2, 3].

In recent years, interest in JH and its related disorders has grown, encompassing a range of uncommon clinical presentations. As noted in the introduction, several hereditary connective tissue disorders share JH

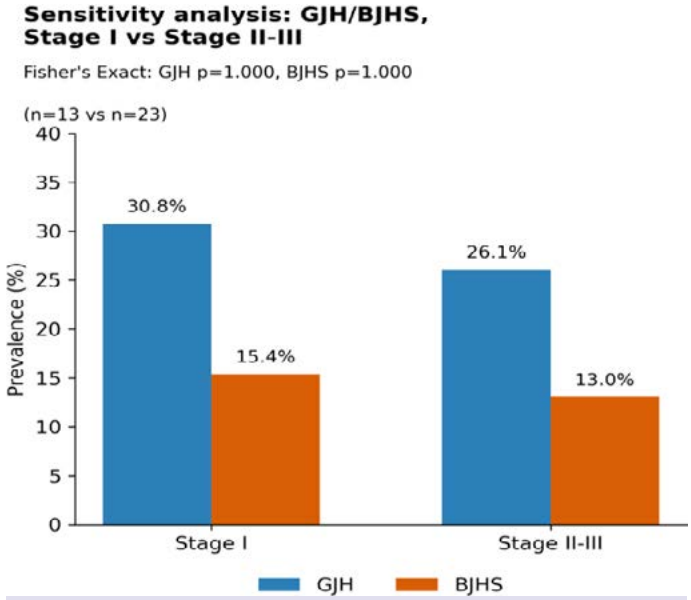

FIGURE 6. Sensitivity analysis: prevalence of GJH and BJHS, Stage I vs. Stage II–III (combined).

as a feature while lacking other characteristic clinical manifestations [3, 4, 15–19]. The literature demonstrates a significant association between GJH – commonly assessed using the Beighton score – and both articular and extra-articular conditions [4]. However, the pathophysiological mechanisms linking hypermobility spectrum disorders (HSDs) to extra-articular manifestations remain even less well understood [10].

Previous studies have investigated the association between lymphovascular disorders and JH [18, 31–33]. Some studies reported variable associations between JH and venous diseases [2, 18], such as venous insufficiency, suggesting it as a potential risk factor. This may be explained by the weakness of supporting connective tissue structures observed in JH patients [2, 34], as connective tissue weakness within venous walls has also been implicated in the pathophysiology of varicose veins [34].

Connective tissue forms a fundamental component of both blood and lymphatic vessel walls, directly affecting vascular compliance and contributing to elevated permeability, particularly under conditions of increased pressure [35]. Lymphedema is a chronic, progressive disorder defined by the buildup of protein-rich interstitial fluid resulting from impaired lymphatic function [26]. This study investigated the potential association between lymphedema and JH, as the lymphatic system consists of mesoderm-derived connective tissue.

Assessment of JH in patients with primary lymphedema may provide valuable insights into diagnostic approaches and treatment strategies. At this point, enhancing physician awareness of JH and associated articular and extra-articular manifestations in primary lymphedema is essential. Given the complexity and severity of these clinical features, careful consideration may be necessary to improve appropriate management strategies. However, contrary to our hypothesis, no significant association was found between JH and primary lymphedema. In this study, the Beighton score was used to screen for hypermobility, as it is best utilized as a screening tool, and a positive score indicates that an individual has GJH. However, low scores on the Beighton do not rule out generalized, mono, pauci, or regional hypermobility [9, 36]. The study offers a reference point to guide future research on affected clinical populations of interest. Therefore, future studies should incorporate objective diagnostic criteria and validated instruments to better characterize JH in patients with primary lymphedema.

Castori et al. [4] stated that the term "syndrome with JH" should be used for genetic conditions characterized by JH. Primary lymphedema represents a diverse spectrum of congenital lymphatic dysplasias leading to progressive lymphatic fluid accumulation and tissue swelling. It may occur either as an isolated condition or as part of a syndromic disorder [29]. For many of the Ehlers–Danlos syndrome subtypes, pathogenic variants of genes have been identified [4]. Syndromic forms of lymphedema accompanied by JH have been described – such as Nevo syndrome and mandibulofacial dysostosis–lymphedema syndrome [27] – these conditions are rare. In a reported case, the coexistence of JH and lymphedema in a patient's sibling suggested a possible shared pathophysiological basis between connective tissue disorders and lymphatic dysfunction [20]. However, the genes implicated in these syndromes do not overlap with the core genes associated with primary lymphatic disorders and lymphovascular malformations [28, 37]. Future studies should explore the pleiotropic effects of genes involved in syndromic forms of primary lymphedema to better elucidate the shared mechanisms underlying lymphatic and connective tissue abnormalities.

In lymphedema, tissue alterations are predominantly driven by chronic inflammation, leading to progressive fibrosis and increased tissue stiffness. No statistically significant differences were observed between lymphedema stages in terms of mean Beighton scores or the prevalence of GJH and BJHS. In this regard, the sensitivity

of clinical hypermobility assessments may be limited in patient populations in which fibrosis predominates, particularly when diagnostic criteria such as the Beighton score, reflecting the number of hypermobile joints rather than the severity of connective tissue laxity, are used [38]. Future studies incorporating quantitative assessments of connective tissue properties – such as elastography or histopathological analysis – may provide further insight.

Study Limitations

Considering the complex range of signs and varying degrees of symptoms of HSDs, as well as the cross-sectional design, causality between JH and primary lymphedema cannot be established. Using a single PMR specialist for all assessments improved standardization, but the lack of assessor blinding may have introduced observer bias, particularly in partially subjective measures such as the Beighton score. Furthermore, potential confounding factors such as BMI, physical activity level, menopausal status, occupational workload, and sports history were not assessed, which may have affected the prevalence of JH. The Stage III subgroup was very small (n=2), limiting meaningful statistical comparisons. In addition, because JH decreases with advancing age [2, 3], the relatively high median age of 41 years in the study population may have led to an underestimation of generalized JH prevalence. Because no prior study has examined JH prevalence in primary lymphedema, the effect size for sample size calculation was derived from a study of BJHS in lumbar disc herniation [17], which may not fully reflect the expected effect in this population.

Conclusion

JH was identified in 27.8% of patients with primary lymphedema; however, no significant association was observed between JH and primary lymphedema or disease severity. Age was inversely associated with JH. A female predominance was observed. These findings may serve as a basis for future studies incorporating objective diagnostic criteria, validated instruments, and quantitative assessments of connective tissue properties to better characterize JH. In addition, exploring the pleiotropic effects of genes involved in syndromic forms of primary lymphedema may further elucidate the underlying mechanisms.

Ethics Committee Approval: This study was approved by The University of Health Sciences Hamidiye Scientific Research Ethics Committee (Approval number: 3/12 – 14.03.2024).

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 received no financial support.

Use of AI for Writing Assistance: The authors declared that artificial intelligence was not used in the study.

Authorship Contributions: Concept – FAB, IA; Design – FAB; Supervision – FUO, GO; Resource – IA, FUO; Materials – MYK, ADSK; Data Collection and/or Processing – FAB; Analysis and/or Interpretation – FAB, GO; Literature Search – FAB, MYK; Writing – FAB, GO, MYK, ADSK, FUO, IA; Critical Reviews – FAB, GO, MYK, ADSK, FUO, IA.

Peer-review: Externally peer-reviewed.

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Appendix 1. Beighton score for joint hypermobility

Beighton test	Definition	Score range
Fifth finger	Passive dorsiflexion >90°	0–2
Thumb	Passive apposition to the forearm	0–2
Elbow	Hyperextension >10°	0–2
Knee	Hyperextension >10°	0–2
Trunk flexion	Palms on the floor with knees extended	0–1
Total score		0–9

A Beighton score ≥4 indicates generalized joint hypermobility.

Appendix 2. Revised Brighton (1998) criteria for benign joint hypermobility syndrome

Category	Criterion
Major	Beighton score ≥4/9 (current or historical)
	Arthralgia >3 months in ≥4 joints
Minor	Beighton score 1–3/9 (0–3 if age ≥50 years)
	Arthralgia in 1–3 joints, back pain, or spondylosis/spondylolysis/spondylolisthesis
	Recurrent dislocation or subluxation in ≥1 joint
	≥3 soft-tissue lesions (epicondylitis, tenosynovitis, and bursitis)
	Marfanoid habitus
	Skin abnormalities (striae, hyperextensibility, thin skin, and papyraceous scars)
	Eye signs (ptosis, myopia, and antimongoloid slant)
	Varicose veins, hernia, or uterine/rectal prolapse

Benign joint hypermobility syndrome is diagnosed if two major, or one major plus two minor, or four minor, or two minor plus an affected first-degree relative are present.

North Clin Istanb 2026;13(5):523–532
doi: 10.14744/nci.2026.59852

Original Article Anesthesiology & Reanimation

Clinical and laboratory characteristics associated with in-hospital mortality in methanol poisoning: A 5-year retrospective cohort study

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ABSTRACT

OBJECTIVE: To compare the demographic, clinical, and laboratory characteristics of survivors and non-survivors presenting to the emergency department with methanol poisoning and to identify variables associated with in-hospital mortality.

METHODS: This single-center retrospective observational cohort study included all consecutive adults (≥18 years) diagnosed with methanol poisoning at the emergency department of Giresun Training and Research Hospital, Türkiye, between September 01, 2021, and May 01, 2026. Patients were categorized as survivors and non-survivors. Demographic characteristics, clinical findings, laboratory parameters, severity scores (Glasgow Coma Scale [GCS], Acute Physiology and Chronic Health Evaluation II [APACHE II], and Sequential Organ Failure Assessment [SOFA]), and treatment variables were compared using univariable analyses.

RESULTS: A total of 26 patients were included, and 9 (34.6%) died. At emergency department presentation, loss of consciousness was more frequent among non-survivors (88.9% vs. 11.8%; p<0.001). Non-survivors also had lower GCS scores (median 4 vs. 15; p<0.001) and admission pH (7.00 vs. 7.11; p=0.011), together with higher lactate (7.1 vs. 2.9 mmol/L; p<0.001), creatinine (1.52 vs. 1.00 mg/dL; p=0.001), glucose (194 vs. 123 mg/dL; p=0.029), and anion gap levels (27.0 vs. 20.9 mmol/L; p=0.018). Within the intensive care unit (ICU) subgroup (n=20), ICU non-survivors (n=9) had higher APACHE II and SOFA scores than ICU survivors (n=11) (p=0.002 and p<0.001, respectively). During the subsequent hospital course, all non-survivors required invasive mechanical ventilation compared with none of the survivors (100% vs. 0%; p<0.001); this finding was considered a marker of greater clinical severity rather than a baseline prognostic characteristic.

CONCLUSION: In this small exploratory cohort, non-survivors showed greater neurological impairment and a general pattern of more severe metabolic derangement at emergency department presentation. Within the selected ICU subgroup, ICU non-survivors had higher APACHE II and SOFA scores than ICU survivors. These findings are hypothesis-generating and may help identify clinical features warranting heightened concern; however, they should not be interpreted as validated prognostic criteria or as a risk-stratification tool.

Keywords: Emergency department; metabolic acidosis; methanol; methanol poisoning; mortality.

Cite this article as: *Sahin Y, Aksoy I. Clinical and laboratory characteristics associated with in-hospital mortality in methanol poisoning: A 5-year retrospective cohort study. North Clin Istanb 2026;13(5):523–532.*

Methyl alcohol (methanol) is a colorless solvent found in glass cleaning solutions, antifreeze, paint thinners, photocopier fluids, and many industrial products. Because methanol is substantially less expensive than ethanol, it may be illegally substituted for ethanol in counterfeit alcoholic beverages [1, 2]. Although methanol itself is not particularly toxic, its metabolites—formaldehyde and formic acid, which are

Received: July 31, 2026 *Revised:* August 11, 2026 *Accepted:* August 26, 2026 *Online:* October 19, 2026

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produced through the action of the alcohol dehydrogenase enzyme—inhibit cellular respiration, leading to metabolic acidosis, vision loss, central nervous system damage, and death [3, 4]. Due to its low cost compared to ethyl alcohol, methanol is frequently used in the production of counterfeit and illicit alcoholic beverages; in Türkiye, it has also occasionally caused individual and mass poisoning outbreaks [5, 6].

The clinical course of methanol poisoning ranges widely, from asymptomatic cases to multiple organ failure and death. Nausea and vomiting, headache, blurred vision, complete vision loss, altered consciousness, tachypnea, and severe metabolic acidosis are the primary symptoms [4, 7]. The inability to measure blood methanol levels at every center and the non-specific nature of presenting symptoms make diagnosis difficult; therefore, the diagnosis is typically made based on the history, the presence of metabolic acidosis with a high anion gap, and the exclusion of other causes [8, 9]. The cornerstone of treatment consists of administering ethanol or fomepizole to inhibit the formation of toxic metabolites, correcting acidosis, providing folic/folinic acid supplementation, and performing hemodialysis to remove toxic metabolites [10, 11]. Despite advances in treatment options, mortality rates remain high due to late hospital presentation and delayed diagnosis [12, 13].

In patients presenting to emergency departments with methanol poisoning, early recognition of clinical and laboratory features associated with poor outcomes may facilitate timely clinical assessment, appropriate monitoring, and management [14]. Although the literature reports that low pH, high anion gap, severe base deficit, high lactate, low Glasgow Coma Scale (GCS), elevated creatinine, and vision loss are associated with mortality [9, 15, 16], regional differences, access to healthcare, and patient profiles limit the generalizability of these findings. In particular, there are a limited number of retrospective analyses based on real-world data that focus on parameters at the time of emergency department presentation.

In this study, we aimed to describe and compare the clinical and laboratory characteristics of survivors and non-survivors among adult patients presenting to the emergency department with methanol poisoning and to evaluate their unadjusted associations with in-hospital mortality. The study further sought to identify exploratory clinical features at emergency department presentation that may warrant heightened clinical concern.

Highlight key points

- Real-world data identified variables associated with mortality in methanol poisoning
- Low GCS, severe acidosis, and elevated lactate were linked to poor outcomes.
- ICU non-survivors had substantially higher APACHE II and SOFA scores.
- Clinical findings at emergency department presentation may support early risk stratification.
- The findings provide practical evidence for the early management of methanol poisoning.

MATERIALS AND METHODS

Study Design and Population

This single-center, retrospective, observational cohort study included all consecutive patients aged ≥18 years who presented to the emergency department of Giresun Training and Research Hospital with a diagnosis of methanol poisoning between September 01, 2021, and May 01, 2026. The study was conducted in accordance with the principles of the Declaration of Helsinki. Due to the retrospective nature of the study and the use of anonymized data, the requirement for informed consent was waived by the Ethics Committee. Patients younger than 18 years, pregnant women, patients with exposure to other toxic alcohols (e.g., ethylene glycol or isopropyl alcohol), and those with incomplete medical records were excluded.

Data Collection and Definitions

Clinical data were retrospectively extracted from the hospital information management system and electronic medical records using a standardized data collection form. The following variables were recorded: age, sex, season of presentation, Charlson Comorbidity Index, presenting symptoms, cause of poisoning (counterfeit/homemade alcohol or cologne), GCS score at presentation, intensive care unit (ICU) admission, requirement for invasive mechanical ventilation, hospital and ICU length of stay, blood gas and biochemical parameters obtained at emergency department presentation, brain imaging findings, treatments administered, and in-hospital mortality. For all patients, the blood gas and biochemical parameters analyzed in this study were derived from the first blood samples obtained at emergency department presentation, before the initiation of specific treatment for suspected methanol poisoning, including sodium bicarbonate administration, ethanol antidote therapy, he-

modialysis, and invasive mechanical ventilation. When multiple laboratory measurements were available, only the first pretreatment values were included in the analysis; subsequent measurements obtained after therapeutic interventions were not analyzed. For patients admitted to the ICU, Acute Physiology and Chronic Health Evaluation II (APACHE II) and Sequential Organ Failure Assessment (SOFA) scores were calculated using data obtained at the time of ICU admission.

Diagnosis and Treatment Protocol

Patients were identified from the hospital information management system and electronic medical records among adult emergency department presentations recorded with a clinical diagnosis of methanol poisoning during the study period. Because serum methanol measurements were unavailable at our institution, biochemical confirmation of methanol exposure was not possible. For inclusion in the study, patients were required to have a documented credible history of suspected methanol-containing alcohol ingestion, compatible clinical manifestations, and high-anion-gap metabolic acidosis, together with a final clinical diagnosis of methanol poisoning established during routine clinical care by the treating emergency physician and/or clinical toxicologist. Alternative causes of high-anion-gap metabolic acidosis were evaluated on the basis of the available medical history, physical examination, routine biochemical and blood gas findings, and the overall clinical context. Specifically, the medical records were reviewed for evidence of diabetic or starvation ketoacidosis, severe lactic acidosis attributable to an alternative critical illness, advanced renal failure/uremia, salicylate toxicity, and exposure to ethylene glycol or other substances capable of producing high-anion-gap metabolic acidosis. These alternative diagnoses were considered unlikely when they were not supported by the available clinical and laboratory findings and the overall presentation was judged by the treating physician and/or clinical toxicologist to be consistent with methanol poisoning. The diagnosis used for study inclusion was therefore the clinical diagnosis documented during the index hospitalization rather than a diagnosis assigned retrospectively solely for study purposes. Nevertheless, because serum methanol concentrations and osmolal gap measurements were unavailable, diagnostic misclassification cannot be completely excluded.

The anion gap at presentation was calculated using the standard formula: anion gap = $Na^+ - (Cl^- + HCO_3^-)$, based on serum electrolyte and bicarbonate values obtained at admission. Serum albumin was not routinely measured, so an albumin-corrected anion gap could not be calculated; serum and urine osmolality were not routinely measured either, so the osmolal gap could not be calculated.

Intravenous ethanol infusion was administered as an antidote when clinically indicated, and sodium bicarbonate was used for the correction of severe metabolic acidosis. Emergency hemodialysis was initiated in patients with severe or refractory metabolic acidosis, visual disturbances, renal impairment, or other clinical indications determined by the treating team. The general institutional approach to the management of methanol poisoning remained unchanged throughout the study period. Folic acid supplementation was administered to only three patients. Due to the retrospective nature of the study, the reasons for its non-administration in the remaining patients could not be reliably determined from the medical records. Treatment decisions were made by the treating clinical team as part of routine care and were not standardized by the study protocol; therefore, treatment variables were evaluated descriptively and were not interpreted as comparative measures of treatment effectiveness.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences version 21.0 (IBM Corp., Armonk, NY, USA). The normality of continuous variables was assessed using the Shapiro–Wilk test. Continuous variables with non-normal distributions are presented as median (interquartile range [IQR]) and were compared using the Mann–Whitney U test. Categorical variables are presented as frequencies (percentages) and were compared using the chi-square test or Fisher's exact test, as appropriate. For continuous variables, between-group effect estimates were additionally reported as Hodges-Lehmann location-shift estimates with 95% confidence intervals (CIs), calculated as non-survivors minus survivors. The 95% CIs were obtained using 10,000 bootstrap resamples. These estimates were reported to provide information on the magnitude and precision of the observed between-group differences rather than relying solely on p-values. A two-sided $p<0.05$ was considered statistically significant. All analyses were univariable (unadjusted). Due to the limited sample size (n=26) and the small number of in-hospital deaths (n=9), multivariable regression analyses were not performed; therefore, the reported variables should be interpreted as factors

associated with in-hospital mortality rather than independent predictors or risk factors. Given the exploratory nature of the study and the limited sample size, no adjustment was made for multiple comparisons. Therefore, individual P values were interpreted cautiously and were not considered confirmatory evidence, particularly in the context of multiple unadjusted comparisons. The reported effect estimates and their 95% CIs should likewise be interpreted as exploratory and unadjusted. Overall, the findings should be regarded as hypothesis-generating and require confirmation in larger prospective studies.

RESULTS

Patient Characteristics

During the study period, 26 adult patients with a clinical diagnosis of methanol poisoning were identified and screened for eligibility. None were excluded due to age <18 years, pregnancy, incomplete medical records, or exposure to another toxic alcohol. Therefore, all 26 eligible patients were included in the final analysis. Of these, 9 patients (34.6%) were non-survivors and 17 (65.4%) were survivors. The vast majority of patients were male (24/26; 92.3%), and the median age of the entire population was similar across groups (non-survivors: 51 [49.5–56.5]; survivors: 54 [48–59]; p=0.387). In terms of seasonal distribution, presentations were most common in spring and fall; no significant difference in seasonal distribution was observed between the groups (p=1.000). The cause of poisoning was counterfeit/homemade alcohol in 61.5% of cases and cologne in 38.5%, with no significant difference between groups (p=1.000) (Table 1).

Clinical Findings at Emergency Department Presentation

Loss of consciousness at emergency department presentation was significantly more frequent among non-survivors (88.9% vs. 11.8%; p<0.001). Confusion and visual disturbances were numerically more frequent among survivors, although these differences did not reach statistical significance (Table 1).

Subsequent Hospital Course and Treatment

During the subsequent hospital course, all non-survivors required invasive mechanical ventilation compared with none of the survivors (100% vs. 0%; p<0.001). All non-survivors were admitted to the ICU, compared with 64.7% of survivors. Hemodialysis and ethanol antidote therapy were also more frequently administered to non-survivors, although these differences did not reach statistical significance (Table 1). Because these variables occurred after emergency department presentation and were influenced by clinical severity and treatment decisions, they should be interpreted as descriptors of the subsequent clinical course and management rather than as baseline prognostic characteristics.

Severity Scores

The GCS score at emergency department presentation was lower among non-survivors than survivors in the overall cohort (median 4.0 [IQR, 3.5–5.5] vs. 15.0 [14.0–15.0]; Hodges-Lehmann difference, -10.0; 95% CI, -11.0 to -9.0; p<0.001). APACHE II and SOFA scores were available only for the 20 patients admitted to the ICU and were therefore analyzed exclusively within this subgroup. ICU non-survivors (n=9) had higher APACHE II scores than ICU survivors (n=11) (25.0 [20.5–25.5] vs. 11.0 [3.0–14.0]; Hodges-Lehmann difference, 14.0; 95% CI, 9.0–20.0; p=0.002) and higher SOFA scores (12.0 [9.0–12.0] vs. 4.0 [4.0–6.0]; Hodges-Lehmann difference, 6.0; 95% CI, 5.0–8.0; p<0.001) (Table 2).

Laboratory Findings

At emergency department presentation, non-survivors had lower pH than survivors (7.00 [6.88–7.08] vs. 7.11 [7.01–7.33]; Hodges-Lehmann difference, -0.17; 95% CI, -0.30–-0.03; p=0.011). Non-survivors also had higher lactate (7.1 [6.8–8.7] vs. 2.9 [1.9–5.1] mmol/L; Hodges-Lehmann difference, 4.3; 95% CI, 3.0–6.0; p<0.001), creatinine (1.52 [1.42–1.85] vs. 1.00 [0.83–1.23] mg/dL; Hodges–Lehmann difference, 0.56; 95% CI, 0.36–0.83; p=0.001), glucose (194 [137–219] vs. 123 [98–151] mg/dL; Hodges-Lehmann difference, 56; 95% CI, 10–88; p=0.029), and anion gap (27.0 [24.6–31.2] vs. 20.9 [14.6–27.8] mmol/L; Hodges-Lehmann difference, 7.3; 95% CI, 2.6–12.7; p=0.018). No statistically significant between-group differences were observed for the remaining laboratory parameters (Table 3). Given the exploratory nature of the analyses and the absence of adjustment for multiple comparisons, these findings should be interpreted cautiously.

Neuroimaging Findings

Cranial imaging was performed in 16 of the 26 patients (61.5%) based on clinical indications. Among the 16 pa-

Table 1. Baseline characteristics, clinical presentation, and subsequent hospital course according to survival status

Variables	Non-survivors (n=9)	Survivors (n=17)	p
A. Baseline demographic and clinical characteristics			
Age	51.0 (49.5–56.5)	54.0 (48.0–59.0)	0.387
Sex			
Female	0 (0.0)	2 (11.8)	0.529
Male	9 (100.0)	15 (88.2)	
Season			
Winter	2 (22.2)	4 (23.5)	1.000
Spring	3 (33.3)	6 (35.3)	
Summer	1 (11.1)	1 (5.9)	
Autumn	3 (33.3)	6 (35.3)	
Charlson Comorbidity Index	1.0 (0.5–2.5)	1.0 (1.0–2.0)	0.936
Hypertension	1 (11.1)	6 (35.3)	0.357
Coronary artery disease	0 (0.0)	2 (11.8)	0.529
COPD	1 (11.1)	1 (5.9)	1.000
Cerebrovascular disease	1 (11.1)	2 (11.8)	1.000
Liver cirrhosis	1 (11.1)	1 (5.9)	1.000
Cause of poisoning			
Counterfeit/homemade alcohol	6 (66.7)	10 (58.8)	1.000
Cologne	3 (33.3)	7 (41.2)	
Use of other substances/Medications			
None	9 (100.0)	14 (82.4)	0.686
Medication	0 (0.0)	2 (11.8)	
Medication+substance	0 (0.0)	1 (5.9)	
B. Clinical findings at emergency department presentation			
Visual disturbance	1 (11.1)	8 (47.1)	0.098
Loss of consciousness	8 (88.9)	2 (11.8)	**<0.001**
Confusion	0 (0.0)	6 (35.3)	0.063
Dyspnea	0 (0.0)	3 (17.6)	0.529
Nausea/vomiting	0 (0.0)	2 (11.8)	0.529
C. Subsequent hospital course and treatment			
ICU admission	9 (100.0)	11 (64.7)	0.063
ICU length of stay (days) (n=9/n=11)	3.0 (1.5–14.0)	3.0 (2.0–5.0)	0.729
Hospital length of stay (days)	3.0 (1.5–14.0)	4.0 (2.0–6.5)	0.913
Need for invasive mechanical ventilation	9 (100.0)	0 (0.0)	**<0.001**
Hemodialysis	9 (100.0)	12 (70.6)	0.129
Ethanol (antidote) therapy	9 (100.0)	10 (58.8)	0.058
Bicarbonate therapy	8 (88.9)	11 (64.7)	0.357
Folic acid	0 (0.0)	3 (17.6)	0.529
Pulse steroid therapy	0 (0.0)	3 (17.6)	0.529
Symptomatic treatment	0 (0.0)	5 (29.4)	0.129

Data are presented as median (interquartile range) and n (%). COPD: Chronic obstructive pulmonary disease; ICU: Intensive care unit. Fisher's exact test (2×2 comparisons) or the Freeman-Halton extension of Fisher's exact test (r×c comparisons: Season; Use of other substances/medications) and Mann-Whitney U test were used, given the small expected cell counts throughout this table. A $p<0.05$ was considered statistically significant.

Table 2. Clinical severity scores according to survival status

Score	Non-survivors (n=9)	Survivors	HL difference (95% CI)	p
GCS (n=9/n=17)	4.0 (3.5–5.5)	15.0 (14.0–15.0)	-10.0 (-11.0– -9.0)	<0.001
APACHE-II (n=9/n=11)	25.0 (20.5–25.5)	11.0 (3.0–14.0)	14.0 (9.0–20.0)	0.002
SOFA (n=9/n=11)	12.0 (9.0–12.0)	4.0 (4.0–6.0)	6.0 (5.0–8.0)	<0.001

Data are presented as median (Interquartile range). GCS was assessed at emergency department presentation in the overall cohort (n=26). APACHE II and SOFA scores were calculated only for patients admitted to the intensive care unit (n=20) and were compared between ICU non-survivors (n=9) and ICU survivors (n=11). Between-group effect estimates are presented as Hodges-Lehmann location-shift estimates with 95% confidence intervals (CIs), calculated as non-survivors minus survivors. The 95% CIs were obtained using 10,000 bootstrap resamples. p-values were calculated using the two-sided Mann–Whitney U test. All analyses were unadjusted and exploratory. GCS: Glasgow Coma Scale; APACHE II: Acute Physiology and Chronic Health Evaluation II; SOFA: Sequential Organ Failure Assessment; CI: Confidence interval.

Table 3. Pretreatment biochemical and blood gas parameters at emergency department presentation according to survival status

Parameter	Non-survivors (n=9)	Survivors (n=17)	HL difference (95% CI)	p
WBC ($\times 10^9$/L)	13.51 (8.56–21.50)	12.67 (10.29–16.26)	-0.19 (-3.93–7.65)	0.978
Hemoglobin (g/dL)	14.3 (11.8–15.2)	15.4 (13.6–16.2)	-1.2 (-2.7–0.8)	0.196
Hematocrit (%)	46.6 (37.7–49.2)	45.7 (41.7–50.2)	-1.8 (-7.9–4.9)	0.608
Platelet count ($\times 10^9$/L)	291 (105–446)	270 (207–328)	3 (-131–171)	0.978
Glucose (mg/dL)	194 (137–219)	123 (98–151)	56 (10–88)	**0.029**
Urea (mg/dL)	30.0 (23.5–66.0)	26.0 (21.5–30.5)	6 (-3–42)	0.215
Creatinine (mg/dL)	1.52 (1.42–1.85)	1.00 (0.83–1.23)	0.56 (0.36–0.83)	**0.001**
ALT (U/L)	48 (24–66)	31 (18–50)	13 (-4–34)	0.118
AST (U/L)	60 (30–180)	39 (30–66)	16 (-6–170)	0.269
Potassium (mmol/L)	5.5 (4.3–6.0)	4.6 (4.0–5.3)	0.8 (-0.6–1.5)	0.160
Sodium (mmol/L)	137 (135–141)	138 (136–140)	0 (-3–4)	0.704
Chloride (mmol/L)	101 (91–106)	102 (98–106)	0 (-8–3)	0.417
CRP (mg/L)	9.87 (3.32–119.48)	4.31 (1.34–9.87)	6.73 (-0.88–182.96)	0.195
pH	7.00 (6.88–7.08)	7.11 (7.01–7.33)	-0.17 (-0.30– -0.03)	**0.011**
pO_2 (mmHg)	42 (34–78)	39 (28–54)	7 (-7–33)	0.434
sO_2 (%)	47 (36–95)	65 (54–78)	-4 (-27–24)	0.808
pCO_2 (mmHg)	40 (36–50)	35 (25–46)	6 (-1–16)	0.124
HCO_3^-	9.5 (9.1–11.9)	11.8 (9.1–22.2)	-2.3 (-9.7–0.4)	0.246
Base deficit (mmol/L)	-19.3 (-21.6–14.0)	-17.9 (-22.8–1.4)	-2.8 (-13.7–3.1)	0.403
Lactate (mmol/L)	7.1 (6.8–8.7)	2.9 (1.9–5.1)	4.3 (3.0–6.0)	**<0.001**
Anion gap (mmol/L)	27.0 (24.6–31.2)	20.9 (14.6–27.8)	7.3 (2.6–12.7)	**0.018**

Data are presented as median (Interquartile range). Effect estimates are Hodges-Lehmann location-shift estimates calculated as non-survivors minus survivors, with 95% CIs obtained using 10,000 bootstrap resamples. p-values were calculated using the two-sided Mann–Whitney U test. All analyses are unadjusted and exploratory, and no correction for multiple comparisons was applied. CI: Confidence interval; WBC: White blood cell; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CRP: C-reactive protein.

tients who underwent imaging, 3 (18.8%) demonstrated bilateral basal ganglia abnormalities considered compatible with methanol toxicity. Because neuroimaging was performed selectively rather than routinely in the entire cohort, these findings were evaluated descriptively, and no between-group statistical comparison was performed.

DISCUSSION

In this small exploratory cohort, several variables available at emergency department presentation differed between survivors and non-survivors. Non-survivors had substantially lower GCS scores and admission pH and higher lactate, creatinine, glucose, and anion gap levels. These findings should be interpreted as unadjusted associations rather than independent prognostic factors. In contrast, ICU admission, invasive mechanical ventilation, hemodialysis, and antidotal or supportive treatments occurred after the initial presentation and were influenced by disease severity and clinical decision-making. Accordingly, these subsequent variables should be considered descriptors of the clinical course and management intensity rather than baseline prognostic characteristics.

In our study, the mortality rate was found to be 34.6%. In the literature, mortality from methanol poisoning ranges from approximately 20–55%, depending on the amount of methanol ingested, time to presentation, concomitant ethanol intake, and geographic region [5, 15, 17]. In an outbreak series in Istanbul, the mortality rate was 45.5%, while in a series of 116 cases reported from Çukurova, it was approximately 23% [16, 17]. The fact that our rate falls within this range can be explained by our status as a tertiary care center, where patients with severe clinical conditions present, and by delayed presentation due to the consumption of counterfeit alcohol.

About 92.3% of our entire population was male, and the median age was around 50 years, which is consistent with studies reporting that methanol poisoning particularly affects middle-aged men [2, 6, 18]. Age and Charlson comorbidity index did not differ significantly between survivors and non-survivors in this cohort; however, given the limited statistical power, the absence of statistically significant differences should not be interpreted as evidence that age or underlying comorbidity was unrelated to mortality.

Regarding clinical presentation, loss of consciousness was substantially more frequent among non-survivors than survivors, whereas confusion and visual disturbances were numerically more frequent among survivors but did not differ significantly between groups. Given the small sample size and multiple unadjusted comparisons, these findings should be interpreted cautiously. Nevertheless, the marked difference in loss of consciousness, together with the substantially lower GCS scores observed among non-survivors, suggests greater neurological impairment in patients with fatal outcomes, consistent with previous reports [7, 9, 19]. Within the selected subgroup of patients admitted to the ICU (n=20), ICU non-survivors had substantially higher APACHE II and SOFA scores than ICU survivors, consistent with previous reports demonstrating associations between higher illness severity scores and poor clinical outcomes in methanol poisoning [13, 20]. However, these findings should be interpreted strictly as an exploratory ICU subgroup comparison. Because APACHE II and SOFA scores were unavailable for patients who were not admitted to the ICU, and because ICU admission itself was related to disease severity, these results cannot be generalized to the overall cohort or interpreted as evidence that these scores independently predicted mortality.

Among the blood gas and biochemical parameters, non-survivors had lower admission pH and higher lactate and anion gap levels, whereas HCO_3^- and base deficit did not differ significantly between groups. These acid-base variables are biologically and mathematically interrelated and should therefore not be interpreted as independent biological or prognostic signals. Rather, the overall pattern suggests greater metabolic derangement among non-survivors, consistent with more severe poisoning. Previous studies have similarly reported associations between greater acid-base disturbance and adverse outcomes in methanol poisoning [15, 16, 21]. Although formate-mediated inhibition of mitochondrial oxidative metabolism may contribute to lactate accumulation, elevated lactate is not specific to methanol metabolism or formate toxicity and may also reflect circulatory compromise, tissue hypoxia, seizures, adrenergic stress, or other concurrent physiological disturbances in critically ill patients [3, 4]. Accordingly, the higher lactate levels observed among non-survivors should be interpreted as part of the overall metabolic and physiological derangement rather than as evidence of a specific pathogenic mechanism or an independent prognostic contribution. The higher anion gap observed among non-survivors should likewise be interpreted cautiously because it was derived from an unadjusted univariable comparison, was not corrected for serum albumin, and osmolal gap data were unavailable. Given the small sample size, multiple unadjusted comparisons, and the potential collinearity among pH, lactate, bicarbonate, base deficit, and anion gap, the present study cannot determine the independent prognostic contribution of any individual acid-base parameter. Creatinine and glucose levels were also higher among non-survivors; however, these findings should similarly be regarded as exploratory and require confirmation in larger studies [12, 22].

From a treatment perspective, all non-survivors required invasive mechanical ventilation, and nearly all underwent hemodialysis and received ethanol antidote therapy; these rates were numerically higher than in the survivors, in which dialysis and ethanol therapy were also administered to the majority of patients (70.6% and 58.8%, respectively), and the between-group differences did not reach statistical significance, likely reflecting the limited sample size. This pattern is consistent with greater overall clinical severity among non-survivors; however, the markedly higher use of invasive mechanical ventilation, hemodialysis, and ethanol antidote therapy should not be interpreted as evidence of adverse prognostic effects of these interventions. These treatments were initiated in response to greater poisoning severity and are therefore inherently susceptible to confounding by indication. In particular, the observation that all non-survivors required invasive mechanical ventilation whereas none of the survivors did most likely reflects profound neurological and physiological deterioration rather than a causal relationship between mechanical ventilation and mortality. The cornerstones of methanol poisoning treatment are alcohol dehydrogenase inhibition (with ethanol or fomepizole), aggressive correction of acidosis, and hemodialysis to remove toxic metabolites [10, 11]. Although fomepizole has a higher enzyme affinity and a lower side-effect profile compared to ethanol, its cost and limited availability mean it is not available at every center; consequently, ethanol treatment is preferred at our center, as is the case in Türkiye [10, 11]. Among the 16 patients who underwent cranial imaging, bilateral basal ganglia abnormalities compatible with methanol toxicity were identified in three patients (18.8%). Because imaging was performed selectively according to clinical indications, this finding should be interpreted descriptively and cannot be used to estimate the prevalence of neuroimaging abnormalities in the overall cohort.

When interpreting these findings in relation to previous studies, several differences in study setting and case mix should be considered. Although the associations of greater neurological impairment and more severe metabolic derangement with mortality are broadly consistent with previous reports [9, 15, 16, 19, 21], direct comparisons should be made cautiously. Our cohort was small, derived from a single tertiary-care center, and included only patients with a clinical diagnosis of methanol poisoning without biochemical confirmation. Differences across studies in referral patterns, severity at presentation, availability and timing of antidotal therapy and hemodialysis, diagnostic criteria, and time from ingestion to hospital presentation may contribute to variation in reported mortality rates and associated clinical or laboratory findings. In addition, the relatively high mortality observed in our cohort may partly reflect the clinical severity and referral characteristics of patients treated at our institution rather than the outcomes of methanol poisoning in the broader population. Therefore, the present findings should be viewed as context-specific exploratory observations and should not be assumed to be directly generalizable to other institutions, regions, or patient populations.

From a clinical perspective, greater neurological impairment and more severe metabolic derangement at presentation may identify patients warranting heightened clinical concern and close monitoring. However, the present findings should not be interpreted as validated prognostic criteria or used to define treatment thresholds, given the small sample size, unadjusted analyses, and absence of predictive-model validation. Treatment decisions, including antidotal therapy, correction of metabolic acidosis, and hemodialysis, should therefore continue to be based on established clinical indications rather than on the associations observed in this study. At the population level, effective policies aimed at preventing the production and consumption of counterfeit or illicit alcohol remain important for reducing methanol poisoning and related mortality [5, 6].

Study Limitations

The major limitations of our study include its retrospective, single-center design, the relatively small sample size, and, importantly, the absence of biochemical confirmation of methanol exposure. Serum methanol concentrations were unavailable, and serum and urine osmolality were not routinely measured; therefore, the osmolal gap could not be calculated. Although study inclusion required a compatible exposure history, clinical presentation, high-anion-gap metabolic acidosis, and a clinical diagnosis of methanol poisoning after consideration of alternative causes, the possibility of diagnostic misclassification cannot be completely excluded.

The small sample size (n=26) and limited number of in-hospital deaths (n=9) precluded reliable multivariable modeling and limited the statistical power and precision of the analyses. Accordingly, multivariable logistic regression and receiver operating characteristic analyses were not performed, and the present study cannot establish

independent prognostic factors, predictive performance, or clinically applicable cutoff values. All reported associations are unadjusted and exploratory. Because multivariable adjustment was not feasible, confounding by differences in baseline clinical severity, comorbidities, presentation delay, and other measured or unmeasured factors cannot be excluded. Consequently, the observed associations should not be interpreted as independent effects of individual variables on mortality. Although effect estimates with 95% CIs were additionally reported to characterize the magnitude and precision of between-group differences, these estimates remain unadjusted and should be interpreted within the same exploratory framework. Moreover, because multiple comparisons were performed without adjustment for multiplicity, individual P values should be interpreted cautiously, and some statistically significant findings may represent chance observations (type I error). The findings should therefore be considered hypothesis-generating and require confirmation in larger, adequately powered prospective studies.

APACHE II and SOFA scores were available only for patients admitted to the ICU and were therefore analyzed exclusively within this selected subgroup. Because ICU admission itself was related to disease severity, these comparisons may be affected by selection bias and cannot be generalized to the overall cohort or interpreted as demonstrating independent prognostic effects of these scores. Similarly, treatment-related variables, including invasive mechanical ventilation, hemodialysis, and antidotal or supportive therapies, occurred after initial presentation and were determined by clinical severity and physician decision-making. These variables are therefore susceptible to confounding by indication and should be interpreted as descriptors of subsequent clinical management rather than baseline prognostic characteristics or comparative treatment-effect data.

Another important limitation was the inability to reliably determine the interval between methanol ingestion and hospital presentation in a substantial proportion of patients. Because many exposures involved counterfeit or homemade alcoholic beverages, the timing and duration of consumption were frequently uncertain or inadequately documented. Presentation delay therefore represents an important unmeasured confounder, as patients presenting later may have had more advanced neurological and metabolic toxicity, potentially contributing simultaneously to lower pH, higher anion gap, greater impairment of consciousness, increased treatment requirements, and higher mortality. Consequently, the observed unadjusted associations cannot be separated from the potential influence of delayed presentation.

Finally, cranial imaging was performed selectively according to clinical indications in only 16 of 26 patients rather than systematically in the entire cohort. Therefore, the observed frequency of bilateral basal ganglia abnormalities among imaged patients should not be interpreted as their prevalence in the overall cohort. Ophthalmological examinations were also not systematically available for all patients, which may have resulted in underestimation of visual complications.

Conclusion

In this small retrospective cohort of patients with clinically diagnosed methanol poisoning, non-survivors showed greater neurological impairment and a general pattern of more severe metabolic derangement at emergency department presentation. These findings represent unadjusted associations and should not be interpreted as independent prognostic effects of individual clinical or laboratory variables. Within the selected ICU subgroup, ICU non-survivors had higher APACHE II and SOFA scores than ICU survivors, but these findings cannot be generalized to the entire cohort. Treatment-related variables reflected subsequent clinical management and disease severity rather than baseline prognostic characteristics. Overall, these exploratory observations may help identify clinical features warranting heightened concern but do not constitute validated prognostic criteria or a risk-stratification tool. Larger prospective studies with multivariable modeling and appropriate validation are required.

Ethics Committee Approval: This study was approved by The Giresun University Medical Research Ethics Committee (Approval No. 15; June 11, 2026).

Informed Consent: The requirement for informed consent was waived by the ethics committee due to the retrospective nature of the study and the use of anonymized data.

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

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

Use of AI for Writing Assistance: The authors used ChatGPT (OpenAI) exclusively to improve the language, grammar, and readability of the manuscript.

Authorship Contributions: Conception – YS, IA; Design – YS, IA; Supervision – YS, IA; Materials – YS; Data Collection and/ or Processing – YS, IA; Analysis and/ or Interpretation – YS, IA; Literature Review – YS; Critical Review – YS, IA.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2026;13(5):533–543
doi: 10.14744/nci.2026.67355

Original Article PHYSIOLOGY

Systemic peptide inhibitor of transendothelial migration administration attenuates microvascular leukocyte adhesion in an ovalbumin-induced mouse model of allergic inflammation

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ABSTRACT

OBJECTIVE: Allergic rhinitis (AR) is driven by type 2 immune polarization and excessive leukocyte trafficking. Current pharmacotherapies act symptomatically rather than at the level of transendothelial migration (TEM). Peptide inhibitor of TEM (PEPITEM) is a B lymphocyte-derived peptide that engages endothelial cadherin-15 to stimulate sphingosine-1-phosphate secretion and inhibit lymphocyte firm adhesion. Although effective in Type 1 autoimmune and metabolic inflammation, PEPITEM has not been examined in Type 2 allergic disease. To evaluate whether systemic PEPITEM administration attenuates systemic leukocyte trafficking in an ovalbumin (OVA)-induced murine AR model, using desloratadine as a clinically established comparator.

METHODS: Thirty-two C57Bl/6 mice (16 males, 16 females; 10–12 weeks; 20–25 g) were randomized to four groups (n=4 per sex per group): control, allergy, allergy+desloratadine (10 mg/kg oral gavage), and allergy+PEPITEM (200 µg/200 µL intraperitoneal). AR was induced by intraperitoneal OVA/alum sensitization (days 1–13) and intranasal 1% OVA challenge (days 22–25). Leukocyte rolling counts, stationary, and adherent leukocytes in mesenteric post-capillary venules were quantified by intravital microscopy (IVM). Peripheral leukocyte differentials were determined by hemogram. One-way analysis of variance was used as the primary analysis.

RESULTS: OVA sensitization and challenge increased peripheral lymphocytosis (p=0.0446–0.0059) and eosinophilia (p=0.0019) and significantly elevated stationary leukocytes and adhesion (p=0.0003) in mesenteric venules, while rolling was statistically unchanged. PEPITEM reduced firmly adherent leukocyte density (p<0.05 vs. allergy group) and normalized blood eosinophilia, with effects comparable to those observed in the desloratadine-treated group. Tissue oxidative and inflammatory marker analysis showed limited organ-specific changes; PEPITEM reduced myeloperoxidase activity in the liver (p=0.0158) and colon (p=0.0288), whereas malondialdehyde and glutathione levels were largely unchanged across tissues.

CONCLUSION: In this study, systemic PEPITEM administration reduces systemic leukocyte adhesion and several allergic phenotypes during OVA-driven Type 2 inflammation, with effects of similar magnitude to desloratadine.

Keywords: Allergic rhinitis; intravital microscopy; leukocyte trafficking; peptide inhibitor of transendothelial migration.

Cite this article as: *Sevim M, Akbaba A, Yerli D, Sulu E, Saglam M, Musayev M, Yayli ZS. Systemic peptide inhibitor of transendothelial migration administration attenuates microvascular leukocyte adhesion in an ovalbumin-induced mouse model of allergic inflammation. North Clin Istanb 2026;13(5):533–543.*

Received: August 03, 2026 *Revised:* August 12, 2026 *Accepted:* August 26, 2026 *Online:* October 16, 2026

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Istanbul Provincial Directorate of Health - Available online at www.northclinist.com

Allergic rhinitis (AR) is one of the most prevalent chronic inflammatory diseases worldwide, affecting 15–25% of adults in Western populations [1] and 11.8–36.4% of the adult population in Turkiye [2]. It is defined by nasal congestion, rhinorrhea, sneezing, and pruritus in response to harmless environmental allergens and is associated with substantial impairment in quality of life, sleep disturbance, fatigue, and cognitive dysfunction [3]. AR is increasingly recognized as a systemic disease. Activated T lymphocytes, elevated circulating cytokines, and altered leukocyte trafficking produce distant organ effects beyond the nasal mucosa [3, 4]. Despite this burden, available pharmacological options – H1-antihistamines, intranasal glucocorticoids, leukotriene receptor antagonists – act on downstream mediators rather than the underlying immune dysregulation, and a substantial proportion of patients achieve only partial benefit [5].

The pathophysiology of AR is dominated by Type 2 immune polarization. Repeated mucosal allergen exposure causes dendritic cells to activate $CD4^+$ T helper 2 (Th2) lymphocytes, which drive immunoglobulin E (IgE) synthesis, mast cell sensitization, and eosinophil recruitment [6, 7]. Allergen cross-linking of IgE on sensitized mast cells releases histamine, tryptase, leukotrienes, and prostaglandins, which together activate endothelial cells, increase vascular permeability, and upregulate adhesion molecule expression to enhance leukocyte infiltration of the nasal mucosa [8]. $CD4^+$ T lymphocytes [9], eosinophils, and basophils accumulate at the inflamed site, and their abundance correlates with symptom severity [10]. Targeting leukocyte trafficking is therefore a mechanistically rational therapeutic strategy.

The leukocyte adhesion cascade encompasses up to seven discrete steps: Margination, tethering (capturing), rolling, activation and firm adhesion, intraluminal crawling, transmigration, and interstitial migration [11]. Rolling is mediated by selectin-ligand bonds; firm adhesion requires integrin activation (principally LFA-1/ICAM-1 and VLA-4/VCAM-1) elicited by chemokines on the activated endothelium [12]. Pharmacological disruption of firm adhesion and transmigration – without impeding the early rolling step – has shown efficacy in multiple inflammatory disease models and is emerging as a strategy for chronic inflammation [13, 14].

Peptide inhibitor of transendothelial migration (PEPITEM) is a 14-amino acid peptide (SVTEQGAELSNEER) proteolytically derived from the B lymphocyte-secreted 14-3-3ζδ protein [15]. PEPITEM binds endothelial cadherin-15, stimulating sphingosine-1-phosphate (S1P) synthesis and release, which in turn inhibits integrin-mediated lymphocyte firm adhesion and transendothelial migration without affecting selectin-mediated rolling [15]. In preclinical models of autoimmune disease – including systemic lupus erythematosus (SLE), rheumatoid arthritis, and Sjögren's syndrome – PEPITEM administration reduced T lymphocyte trafficking and tissue infiltration [15–17]. The PEPITEM/cadherin-15 axis suppresses T lymphocyte infiltration in murine SLE glomerulonephritis [16] and was recently shown to reduce leukocyte trafficking, macrophage accumulation in obese adipose tissue, and regulate the inflammatory response in peritonitis, psoriasis, and inflammatory arthritis [18, 19].

To our knowledge, all published PEPITEM studies have addressed Type 1 autoimmune or metabolic contexts. The peptide's behavior in Type 2 – driven allergic inflammation – where eosinophils and IgE-class B-cell responses dominate the cellular milieu and where leukocyte adhesion is amplified by mast cell mediators – has not been examined. We therefore designed this study to test the hypothesis that systemic PEPITEM administration attenuates systemic leukocyte trafficking in an OVA-induced murine AR model. Desloratadine, a second-generation H1-antihistamine of the class recommended as first-line pharmacotherapy for AR [5], served as the positive comparator. It was selected in preference to other antihistamines for three reasons specific to this design. First, among second-generation H1-antihistamines, its activity is best documented at the endothelial step that constitutes our primary endpoint: Desloratadine suppresses Nuclear factor kappa-B activation and downregulates ICAM-1 and P-selectin expression, together

Highlight key points

- Systemic administration of PEPITEM successfully prevents the rise of circulating eosinophils in an ovalbumin-induced murine model of allergic inflammation, achieving results similar to the antihistamine desloratadine.
- Treatment with PEPITEM significantly reduces the density of firmly adherent leukocytes in mesenteric post-capillary venules without impeding the early leukocyte rolling step.
- Beyond microvascular interactions, PEPITEM effectively lowers myeloperoxidase (MPO) activity in both the liver and colon, demonstrating a reduction in systemic, organ-specific inflammatory responses.
- This research provides the first preclinical evidence that PEPITEM can mitigate Type 2 allergic disease phenotypes, suggesting its potential as a novel therapeutic strategy targeting the leukocyte adhesion cascade.

with interleukin (IL)-6 and IL-8 release, in activated endothelial cells, and reduces inflammatory cell infiltration in OVA-induced AR in rodents [20, 21]. Second, it is non-sedating and essentially devoid of anticholinergic activity [20], thereby minimizing confounding of the microvascular hemodynamic variables measured under anesthesia, in contrast to first-generation agents. Third, a validated oral dosing regimen for desloratadine in a rodent ovalbumin (OVA) allergy model was already available, allowing an established dose to be used.

Sex was included as an independent experimental variable.

MATERIALS AND METHODS

Animals and Ethical Approval

Thirty-two C57Bl/6 mice (16 males, 16 females) aged 10–12 weeks and weighing 20–25 g were obtained from Koç University Animal Research Facility. Animals were housed in HEPA-filtered cages (≥70 cm^2/animal) at 22±2°C and 65–70% relative humidity under a 12-h light/dark cycle, with ad libitum access to standard pelleted chow and water. General health and stress indicators were monitored daily. All procedures were approved by the Marmara University Animal Experiments Local Ethics Committee (Protocol No: 09.2024mar). The experiments were performed in compliance with the Turkish law on the use of animals in experiments, regarding the ARRIVE guidelines, the guidelines of the New York Academy of Sciences, and in accordance with the Helsinki Declaration. Animals were randomly allocated to the four groups. All offline analyses (intravital microscopy [IVM] video review) were performed by observers blinded to group allocation.

Experimental Design and Groups

Mice were allocated, stratified by sex, to four groups (n=4 per sex per group; n=8 per group; total n=32). (1) Control – no intervention beyond vehicle injections, (2) Allergy – OVA sensitization and challenge, (3) Allergy+Desloratadine (Allergy+Des) – OVA model with oral desloratadine, used as the clinically established positive control [5, 20, 21], (4) Allergy+PEPITEM (Allergy+PEP) – OVA model with intraperitoneal PEPITEM.

Female mice were monitored for estrous cycle stage by vaginal cytology at 10× light microscopy. Sensitization and intranasal challenge for each female were timed to the same phase of the estrous cycle to minimize hormonal confounding of immune responses [22]. Sample size was estimated using the resource-equation approach [23, 24].

Induction of AR

The OVA-based AR model was adapted from established murine protocols [25]. OVA adsorbed to aluminum hydroxide was selected as the sensitizing allergen because it is the most extensively characterized and most reproducible allergen–adjuvant combination for inducing Type 2-polarised allergic airway and nasal inflammation in mice, reliably generating allergen-specific IgE, Th2 cytokine production and eosinophilia in a standardized acute sensitization-and-challenge format [26]. OVA is additionally a chemically defined, non-infectious single protein available at standardized purity, which minimizes the batch-to-batch variability in allergenic and endotoxin load associated with crude allergen extracts, because such variability can itself modify endothelial activation and leukocyte adhesion, the endpoints under study. Finally, the C57Bl/6 background was required for comparability with previous in vivo PEPITEM studies [15, 18, 19]. Because the murine allergic phenotype is strongly strain-dependent with respect to airway reactivity, inflammatory cell composition, and cytokine production [27], a robust adjuvanted OVA protocol was the appropriate means of achieving reliable Type 2 polarization in this background.

In the sensitization phase, mice in allergy groups received seven intraperitoneal injections, on days 1, 3, 5, 7, 9, 11, and 13, of 100 μg chicken OVA (OVA; Sigma-Aldrich, Grade V) in phosphate-buffered saline (PBS) adsorbed onto 2 mg aluminum hydroxide (Al(OH)3; alum), total volume 200 μL. Control mice received equivalent volumes of PBS. Following a 7-day rest period (days 14–21), in the challenge phase (days 22–25), sensitized mice received intranasal instillation of 1% OVA in PBS (10 μL per nostril; total 20 μL) on 4 consecutive days. Control mice received intranasal PBS.

Treatment Administration

Treatment was administered once daily on each of the 4 consecutive intranasal challenge days (days 22–25), 1 h before each intranasal OVA challenge, giving four administrations per animal. (1) PEPITEM group: PEPITEM 200 μg in 200 μL PBS intraperitoneally (i.p.) [15]; (2) Desloratadine group: desloratadine 10 mg/kg in 200 μL PBS by oral gavage (i.g.) [28]; (3) Control and allergy groups: equivalent volumes of PBS administered by the same routes.

The PEPITEM dose of 200 μg/animal corresponds to approximately 8–10 mg/kg in the 20–25 g mice used here and lies within the range of intraperitoneal doses previously shown to inhibit leukocyte trafficking in vivo, which include bolus i.p., doses of up to 300 μg/mouse in zymosan-induced peritonitis [29] and continuous subcutaneous delivery of approximately 0.8 mg/week in a diet-induced obesity model [18]. Repeated i.p. bolus dosing timed to the inflammatory stimulus follows the paradigm established in previous PEPITEM peritonitis studies [19, 29]. Administration 1 h before each challenge was intended to align peak peptide exposure with the acute challenge window and to match the exposure timing of the desloratadine comparator. A single dose level was used because this was a first-in-model proof-of-concept experiment.

Intravital Video Microscopy

On the 4th challenge day, IVM was performed. Mice were anesthetized with ketamine hydrochloride (100 mg/kg, i.p.) and xylazine (7.5 mg/kg, i.p.), with maintenance doses of ketamine (20 mg/kg) and xylazine (1.5 mg/kg, i.p.) administered as required. Anesthetic depth was monitored by limb-withdrawal reflex. Circulating leukocytes were fluorescently labeled by retro-orbital injection of rhodamine 6G (0.2 mg/kg in 0.1 mL saline, Sigma-Aldrich) and subsequently visualized within mesenteric postcapillary venules using IVM (Olympus BX53 Fluorescence Microscope and Olympus DP73 camera system). Rhodamine 6G labeling enabled visualization of circulating leukocytes but did not distinguish leukocyte subsets.

Following a midline laparotomy, the mesentery was gently exteriorized onto a temperature-controlled stage and transilluminated; tissue moisture was maintained by superfusion with Krebs–Ringer bicarbonate buffer (1 mL/min, pH 7.3–7.4, 37°C). Venules with 20–60 μm diameters were selected. For each mouse, at least three 100-μm segments or at least one 300-μm segment were recorded for ~120 s at 16 fps. For statistical analysis, values from all recorded venular segments within each animal were averaged. Leukocytes that adhered to the post-capillary venule for more than 2 s but left the venule before 30 s were considered "stationary." The number of "firmly adherent leukocytes" (leukocytes that adhered to the post-capillary venule for more than 30 s) per vessel segment was measured, averaged for time and each animal. Stationary and firmly adherent leukocytes were expressed as numbers/min/mm^2. Leukocyte rolling was quantified by drawing a reference line through the middle of each postcapillary venule and counting the number of leukocytes that crossed this line while rolling, expressed as cells/min [30, 31]. Leukocyte transmigration/extravasation was not directly quantified in this study. To calculate rolling velocity, 5–10 rolling leukocytes were selected from each venule. The distances traveled by these leukocytes in 5 s were measured. The rolling speed was calculated by dividing the measured distance by the time taken and reported as micrometers per second (μm/s). Venular flow was estimated from venular cross-sectional area and mean leukocyte flow velocity. Cross-sectional area was calculated as $\pi(d/2)2$, where d is venular diameter. Flow was calculated as cross-sectional area × mean leukocyte flow velocity. Values were converted from μm^3/s to μL/s [32].

After IVM, mice were sacrificed by cardiac puncture while under anesthesia.

Hematological Analysis

Whole blood obtained by cardiac puncture was immediately analyzed using an automated hematology analyzer (URIT-3300; URIT Medical Electronic, China), with manufacturer-supplied parameters validated for murine samples by reference QC.

A drop of whole blood was used to prepare thin smears for differential counts. Blood was stained with May–Grünwald (2 min) followed by Giemsa (15 min). For each smear, ≥100 leukocytes were classified to obtain neutrophils, lymphocytes, monocytes, and eosinophils proportions.

Measurement of Myeloperoxidase (MPO) Activity

MPO activity, a widely used enzymatic index of granulocyte (predominantly neutrophil) infiltration into tissue, was selected as a tissue-level correlate of the intravascular leukocyte–endothelial interactions quantified by IVM and was quantified by the H2O2-dependent oxidation of o-dianisidine dihydrochloride. Pieces of large intestine (colon), lung, and liver were homogenized at 9× tissue weight in potassium phosphate buffer containing hexadecyltrimethylammonium bromide (HETAB). Homogenates were centrifuged (12,000 rpm, 10 min, 4°C); supernatants were discarded and the pellets re-homogenized in 50 mmol/L K2HPO4 with 0.5% (w/v) HETAB and 10 mmol/L EDTA. MPO activity was read at 460 nm at 37°C and expressed as U/g tissue, where 1 U is the change in absorbance over 1.0 min^{-1}.

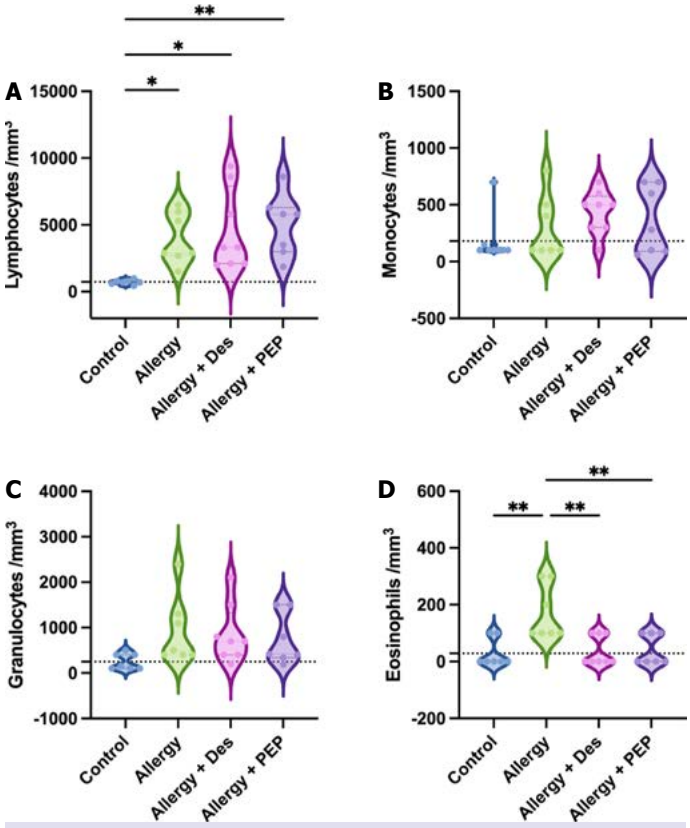

FIGURE 1. Effect of peptide inhibitor of transendothelial migration on peripheral blood leukocyte differentials. Violin plots showing **(A)** lymphocyte, **(B)** monocyte, **(C)** neutrophil (granulocyte), and **(D)** eosinophil counts (cells/mm³) (n=8/group). Groups: Control (blue), Allergy (green), Allergy+Des (pink), Allergy+PEP (purple).

*: P<0.05; **: P<0.01. One-way analysis of variance with *post hoc* Tukey's test.

Measurement of Malondialdehyde (MDA) and Reduced Glutathione (GSH)

The same tissues in which MPO activity was measured were homogenized in 10% trichloroacetic acid up to 10× tissue weight using an Ultra-Turrax, then centrifuged (3,000 rpm, 15 min, 4°C). Supernatants were clarified by a second spin (15,000 rpm, 8 min). MDA was measured at 535 nm and expressed as nmol/g tissue as an index of lipid peroxidation. GSH was quantified by the modified Ellman method at 412 nm and expressed as µmol/g tissue [33].

Statistical Analysis

Data normality was assessed using the Shapiro–Wilk test. Normally distributed variables were compared using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for single-factor comparisons. For sex-based data, two-way ANOVA (sex × treatment) followed by Tukey's post hoc test was used. Statistical significance was defined as $p<0.05$. All analyses were performed using GraphPad Prism 11 (GraphPad Software, USA).

RESULTS

Peripheral Blood Leukocyte Differentials

Peripheral blood leukocyte differentials showed distinct changes in lymphocyte and eosinophil populations after OVA sensitization and challenge. Circulating lymphocyte counts were elevated in all three allergy-receiving groups compared to control (one-way ANOVA, $p=0.0446–0.0059$; Fig. 1A). Lymphocytosis was not normalized by either treatment.

Eosinophil counts were elevated specifically in the untreated allergy group ($p=0.0019$ versus controls; Fig. 1D). Both desloratadine and PEPITEM significantly reduced eosinophil counts compared with untreated allergic mice, bringing values closer to the control range.

Monocyte and neutrophil counts did not differ significantly between groups (Fig. 1B, C). Sex-stratified analyses were broadly consistent with the pooled results, although the statistical significance of eosinophil count differences varied between sexes (Appendix 1).

Leukocyte Trafficking in Mesenteric Post-Capillary Venules

Representative IVM images and videos showed visibly increased leukocyte–endothelial interactions in the allergy group, with fewer vessel-associated leukocytes in the desloratadine- and PEPITEM-treated groups (Fig. 2D). Mean rolling leukocyte counts did not differ significantly between groups (Fig. 2A). Stationary leukocyte densities were significantly elevated in the untreated allergy group versus control while desloratadine significantly reduced this response ($p=0.0001$, $p=0.0103$; Fig. 2B). Stationary leukocyte counts tended to be lower in the PEPITEM-treated group than in the untreated allergy group, but the difference was not statistically significant. Total adherent leukocyte density was markedly higher in the allergy group than in the control ($p=0.0003$; Fig. 2C). Both desloratadine and PEPITEM significantly reduced adhesion compared with the untreated allergy group ($p=0.0310$ and 0.0008, respectively). Sex-stratified analysis showed similar overall patterns (Appendix 2).

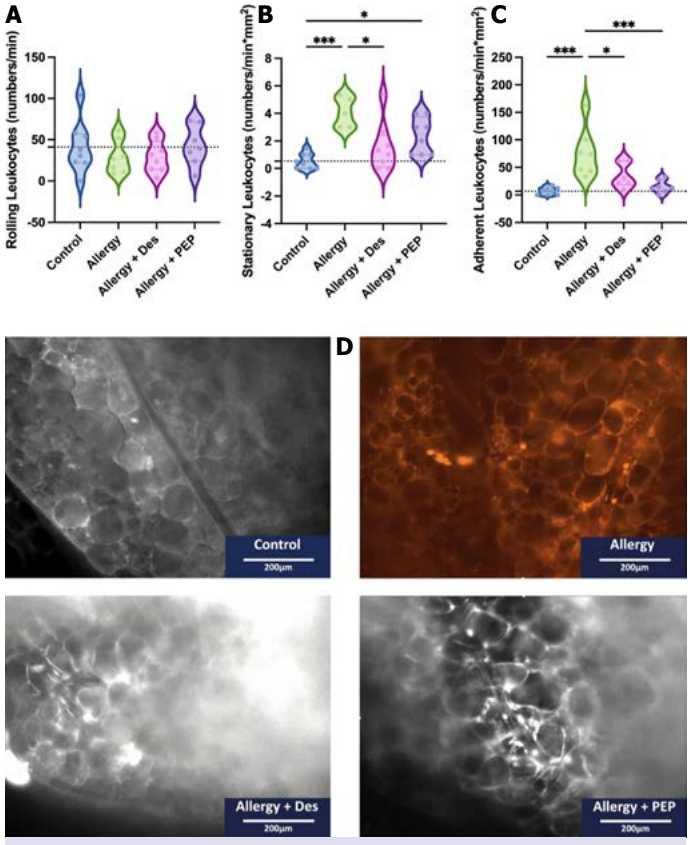

FIGURE 2. Effect of peptide inhibitor of transendothelial migration on leukocyte trafficking in mesenteric post-capillary venules. Violin plots showing **(A)** rolling counts, **(B)** stationary, and **(C)** adherent leukocyte densities; **(D)** representative intravital microscopy images of each group. Groups as in Figure 1.

*: P<0.05; ***: P<0.001. One-way analysis of variance with *post hoc* Tukey's test.

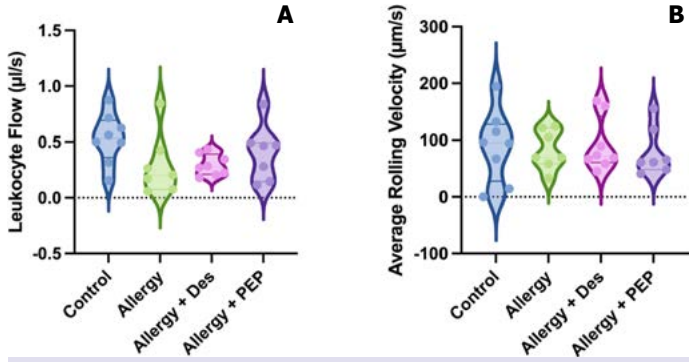

FIGURE 3. Measurements of leukocyte flow and leukocyte rolling velocity in mesenteric post-capillary venules. Violin plots showing **(A)** leukocyte flow, **(B)** average leukocyte rolling velocity. Groups as in Figure 1.

One-way analysis of variance with *post hoc* Tukey's test.

Leukocyte Flow and Rolling Velocity

Leukocyte flow and average rolling velocity were also evaluated to determine whether group differences in leukocyte adhesion could be attributed to changes in microvascular flow dynamics and changes in rolling pattern. Leukocyte flow did not differ significantly between groups (Fig. 3A). Average rolling velocity was likewise comparable across groups, with no statistically significant differences detected (Fig. 3B). Sex-stratified analysis is shown in Supplement (Appendix 3).

Tissue Levels of MDA, MPO Activity, GSH Content in Lung, Liver, and Colon

Tissue MPO activity, MDA levels, and GSH content were assessed in lung, liver, and colon samples. In the lung, allergy significantly increased MDA levels compared with controls (p=0.0184; Fig. 4D), whereas its increase was prevented in both desloratadine- and PEPITEM-treated groups. Lung MPO activity did not differ significantly among groups (Fig. 4A). In the liver, MPO activity was significantly lower in the PEPITEM-treated group than in the allergy group (p=0.0158; Fig. 4B), while MDA levels remained comparable across groups (Fig. 4E). In the colon, MPO activity was significantly reduced by both desloratadine and PEPITEM treatment (p=0.0358 and 0.0288, respectively; Fig. 4C); however, MDA levels showed no statistically significant differences between groups (Fig. 4F). No significant differences in GSH content were observed in any of the examined tissues (Fig. 4G–I). Overall, these findings indicate that OVA-induced allergic inflammation was associated with tissue-specific changes in oxidative and inflammatory markers.

DISCUSSION

In this study, systemic PEPITEM administration reduced peripheral eosinophilia and total leukocyte adhesion in mesenteric post-capillary venules during OVA-induced allergic inflammation, with a non-significant trend toward fewer stationary leukocytes. The magnitude of these reductions was of similar order to that achieved by desloratadine, a clinically established H1-antihistamine. Importantly, leukocyte rolling was not significantly altered, whereas firm adhesion was significantly reduced, a dissociation consistent with the cadherin-15/S1P mechanism described for PEPITEM in Type 1 autoimmune and metabolic inflammatory contexts [15–18].

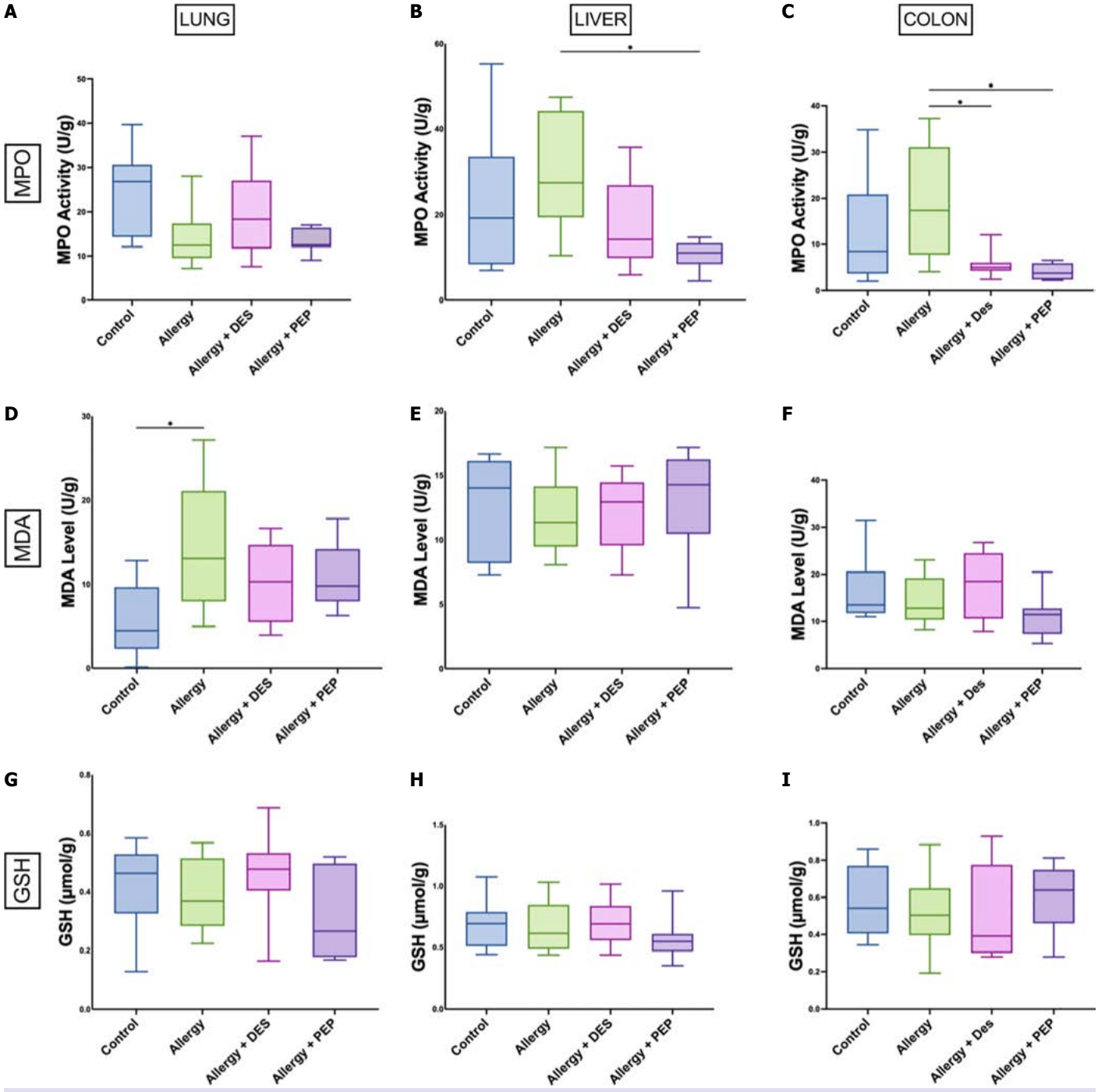

FIGURE 4. Tissue levels of malondialdehyde (MDA), myeloperoxidase (MPO) activity, and glutathione (GSH) content in lung, liver, and colon. Box-and-whisker plots show spectrophotometric assays in lung **(A, D, G)**, liver **(B, E, H)**, and colon **(C, F, I)** tissues. Groups as in Figure 1.

*: P<0.05. Center line indicating the median, boxes representing the interquartile range, and whiskers extending from the 5th to 95th percentiles. One-way analysis of variance with *post hoc* Tukey's test.

Both PEPITEM and desloratadine normalized peripheral blood eosinophil counts in the OVA model. Several mechanisms could produce this effect: (i) reduced eosinophil egress from circulation into tissue, which would be expected to elevate rather than reduce blood eosinophils unless tissue retention is dominant; (ii) reduced eosinophilopoiesis, plausibly through reduced IL-5 signaling; or (iii) accelerated peripheral clearance.

The present blood-only data cannot distinguish between these. We therefore restrict our interpretation to the observation that PEPITEM, like desloratadine, prevents the OVA-driven rise in circulating eosinophils. Mechanistic studies could incorporate IL-5 quantification and tissue eosinophil counts to dissect these possibilities.

Lymphocytosis persisted in allergy+PEPITEM and allergy+desloratadine animals. One possibility, drawn from the natalizumab (anti-VLA-4) literature, is that PEPITEM blocks lymphocyte exit from blood into inflamed tissue and thereby maintains, or elevates circulating counts despite reduced tissue infiltration [34]. However, the present design measured only blood lymphocytes; we did not quantify tissue (nasal, lung, lymph node) lymphocyte counts and therefore cannot confirm this redistribution hypothesis. An equally plausible alternative is that PEPITEM does not affect antigen-driven Th2 clonal expansion, leaving the circulating pool unchanged. Resolving these competing interpretations requires tissue lymphocyte phenotyping.

The dissociation we observe (rolling statistically unchanged, while adhesion reduced) is consistent with prior demonstrations that PEPITEM acts on the integrin-activation step of the leukocyte adhesion cascade [15]. The data are best described as consistent with, rather than confirmatory of, the canonical PEPITEM mechanism, and would benefit from corroboration in larger studies, ideally with cadherin-15 blockade or scrambled-peptide controls to address selectivity directly. Findings in IVM suggest that the observed differences in stationary and firmly adherent leukocytes were not accompanied by major changes in leukocyte flow or rolling velocity, which is also consistent with the reported absence of an effect of PEPITEM on selectin-mediated leukocyte rolling [15].

The IVM data report on mesenteric post-capillary venules, not nasal microvasculature. This choice was deliberate because intravital imaging of nasal mucosal microvessels in OVA-challenged mice is technically infeasible, while the mesenteric venule bed is a well-validated platform for quantifying systemic leukocyte trafficking responses [11, 35]. AR is increasingly recognized as a systemic disease, with circulating activated leukocytes and systemic vascular alterations [3, 4]. Nevertheless, mesenteric and nasal vascular beds differ in adhesion molecule expression patterns (mesenteric venules favor ICAM-1/LFA-1-driven neutrophil traffic; nasal mucosa eosinophil/Th2 recruitment is biased toward VCAM-1/VLA-4 and CCR3-mediated chemotaxis) [10, 36]. Accordingly, we frame the present IVM observations as evidence that systemic leukocyte adhesion is elevated by OVA challenge and reduced by PEPITEM and desloratadine, rather than as a direct measurement of nasal-mucosal leukocyte trafficking. Demonstrating equivalent effects in nasal/airway endothelium requires either airway IVM, leukocyte profiling, or nasal histology with leukocyte counts. Desloratadine acts acutely on H1 receptors expressed on sensory neurons and endothelial cells [37], while PEPITEM is hypothesized to act upstream by limiting leukocyte-derived mediator release at inflamed sites.

Tissue oxidative and inflammatory marker analysis was performed to assess whether OVA-induced allergic inflammation and treatment effects extended beyond circulating leukocyte responses and microvascular leukocyte–endothelial interactions. MPO, MDA, and GSH were selected as a deliberately focused panel rather than as a comprehensive inflammatory profile. MPO was chosen because it provides an enzymatic, tissue-level index of granulocyte accumulation and therefore extends the primary leukocyte-trafficking endpoint from the vascular lumen assessed by IVM into the tissue compartment, whereas MDA and GSH index the lipid peroxidation and thiol antioxidant reserve that accompany allergic tissue inflammation and that have previously been reported to change in OVA-driven AR and food allergy models [38–40]. All three can be assayed in the same tissue homogenate by validated spectrophotometric methods [33], which was a practical requirement because tissue and blood volumes were prioritized for the IVM and hematological endpoints that constituted the primary outcomes.

Allergic inflammation is closely associated with epithelial activation, leukocyte recruitment, and mediator release, all of which may promote local oxidative stress and tissue inflammatory enzyme activity. In the present study, the biochemical changes were tissue-specific rather than uniform across organs. MPO activity, a marker commonly associated with inflammatory activity of granulocytes, tended to increase in the colon and was reduced by both desloratadine and PEPITEM treatment. This could be a result of decreased leukocyte adhesion; however, transmigration counts are not assessed in this study. This finding is consistent with a previous OVA-induced AR study reporting increased colonic MPO activity, supporting the concept that AR may be accompanied by extra-nasal inflammatory alterations [38–40]. PEPITEM also reduced liver MPO activity, whereas

lung MPO activity was not significantly changed. In contrast, MDA levels showed only limited changes, and GSH content did not differ significantly among groups in lung, liver, or colon tissues. Previous experimental studies have reported increased MDA and/or reduced GSH in OVA-induced AR and food allergy models [39, 40], whereas these oxidative stress markers were largely unchanged in the present study. These differences may be related to the biological compartment analyzed, timing of tissue collection, animal strain, allergen exposure protocol, or the number of challenge exposures. Overall, our findings suggest that OVA-induced allergic inflammation was associated with selective tissue inflammatory changes. However, because tissue leukocyte subsets and local cytokine profiles were not assessed, these biochemical findings should be interpreted as supportive evidence of organ-specific inflammatory modulation rather than as direct mechanistic proof.

To our knowledge, this is the first study to evaluate PEPITEM in a type 2 allergic disease model. Previous investigations have focused primarily on Th1-dominant autoimmune disorders, including systemic lupus erythematosus, rheumatoid arthritis, and Sjögren's syndrome, or on metabolic inflammation, whereas the present findings extend the potential anti-inflammatory activity of PEPITEM to allergic inflammation [15–18]. Nevertheless, several limitations should be acknowledged. Validation of the AR model was incomplete, as OVA-specific IgE and Immunoglobulin G (IgG)-1 levels, bronchoalveolar lavage eosinophil counts, and nasal histopathology were not assessed. In addition, mesenteric IVM provided a systemic assessment of leukocyte trafficking but did not directly evaluate cellular behavior within the nasal mucosa, and leukocyte subsets could not be distinguished because cell-type-specific fluorescent labeling was not performed. Furthermore, Type 2 cytokines (IL-4, IL-5, IL-13), interferons, immunoglobulin responses, and bronchoalveolar lavage composition were not measured. The primary endpoint of this study was leukocyte–endothelial interaction, and the canonical PEPITEM mechanism acts at the endothelial cadherin-15/S1P axis to block integrin-mediated firm adhesion downstream of chemokine signaling [15], so leukocyte adhesion rather than cytokine concentration constitutes the proximal readout of the hypothesized mechanism. The MPO, MDA and GSH panel was therefore intended to extend the trafficking readout into tissue and cannot substitute for direct cytokine profiling. Quantification of IL-4, IL-5, IL-13, interferon-gamma, eotaxin/CCL11 and OVA-specific IgE and IgG1 is a priority for mechanistic follow-up, particularly as PEPITEM has been shown to modulate cytokine and chemokine release in other inflammatory models [17, 19]. A further consideration is that, although OVA/alum is the best-characterized murine sensitization protocol, OVA is not a clinically relevant human aeroallergen and repeated mucosal exposure in the absence of adjuvant can induce tolerance. Confirmation in a model driven by a clinically relevant aeroallergen such as house dust mite is therefore an important next step [26]. Only one PEPITEM dose level was tested, so the dose–response relationship, the minimum effective dose, and the duration of effect after the final administration remain undefined, and no conclusion regarding relative potency versus desloratadine can be drawn. Importantly, the present study cannot determine whether PEPITEM directly modulates eosinophil trafficking or acts indirectly through suppression of Th2-associated cytokine signaling and subsequent reductions in IL-5- and eotaxin-mediated recruitment. Despite these limitations, the consistent reduction in leukocyte-endothelial interactions and eosinophilia observed across multiple analyses provides a rationale for further investigation. If confirmed in adequately powered studies incorporating comprehensive immunological, histological, and mechanistic endpoints, PEPITEM may represent a novel therapeutic strategy for AR that targets the endothelial cadherin-15/S1P regulatory axis rather than conventional histamine- or leukotriene-dependent pathways. While the current findings do not support direct clinical conclusions, they establish a foundation for future studies aimed at defining the therapeutic potential and mechanisms of PEPITEM in allergic disease.

Conclusion

This study provides initial evidence that systemic PEPITEM attenuates selected microvascular leukocyte–endothelial interactions associated with OVA-induced allergic inflammation. PEPITEM reduced peripheral eosinophilia and total adhesion in mesenteric post-capillary venules to a magnitude comparable to that of desloratadine, while leukocyte rolling and stationary leukocyte counts were statistically unchanged.

Ethics Committee Approval: This study was approved by The Marmara University Local Ethics Committee For Animal Experiments (09.2024mar – 12.02.2024).

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

Financial Disclosure: This work was supported by TUBITAK under Grant 123S882.

Use of AI for Writing Assistance: During the preparation of this work, the author(s) used Claude solely to improve the English language, grammar, and readability of the manuscript. After using this tool, the author(s) thoroughly reviewed and edited the text and take full responsibility for the final content of the publication. The AI was not used to generate data, draw conclusions, or create original scientific ideas.

Authorship Contributions: Concept – MSe, AA; Design – MSe, AA; Supervision – MSe, AA; Resource – MSe, AA; Materials – MSe, AA; Data Collection and/or Processing – MSe, AA, DY, ES, MS, MM, ZS; Analysis and/or Interpretation – MSe, AA, DY, ES, MS, MM, ZS; Literature Search – MSe, AA, DY, ES, MS, MM, ZS; Writing – MSe, AA, DY, ES, MS, MM, ZS; Critical Reviews – MSe, AA, DY, ES, MS, MM, ZS.

Acknowledgments: The authors gratefully acknowledge Prof. Dr. Ed Rainger and Prof. Dr. Asif Iqbal for generously providing the PEPITEM molecule used in this study.

Peer-review: Externally peer-reviewed.

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Appendix 1. Sex-stratified effects of PEPITEM on peripheral blood leukocyte differentials

Parameters	Groups	Female				Male			
		Mean	SEM	n	Tukey's test (p)	Mean	SEM	n	Tukey's test (p)
Lymphocytes (/mm^3)	Control	1300	70.71	4	–	1100	302.76	4	–
	Allergy	3450	1068.87	4	ns	4666.6	1003.88	4	ns
	Allergy+Des	2700	346.41	4	ns	6450	1672.07	4	0.0016 versus control
	Allergy+PEP	6900	862.1	4	0.0022 versus Control; 0.0242 versus Allergy+Des	3540	827.80	4	ns
Monocytes (/mm^3)	Control	225	25	4	–	200	40.82	4	–
	Allergy	200	100	4	–	375	170.17	4	–
	Allergy+Des	400	57.73	4	–	475	131.49	4	–
	Allergy+PEP	526.6667	126.6	4	–	237.5	154.4	4	–
Granulocytes (/mm^3)	Control	475	47.87	4	–	400	100	4	–
	Allergy	625	225	4	–	1333.333	560.75	4	–
	Allergy+Des	550	86.6	4	–	1150	413.31	4	–
	Allergy+PEP	906.6667	316.2	4	–	607.5	300.75	4	–
Eosinophils (/mm^3)	Control	25	25	4	–	25	25	4	–
	Allergy	166.6667	66.66	4	ns	175	47.87	4	0.0232 versus control
	Allergy+Des	50	28.86	4	ns	25	25	4	0.0232 versus allergy
	Allergy+PEP	66.66667	33.33	4	ns	25	25	4	0.0232 versus allergy

Sex-stratified data showing lymphocyte, monocyte, granulocyte, and eosinophil counts (cells/mm3). Groups: Control, Allergy, Allergy+Des, Allergy+PEP. Lymphocytes two-way analysis of variance (ANOVA): sex effect: $F(1, 22)=0.2929$, $p=0.5938$; treatment effect: $F(3, 22)=7.789$, $p=0.0010$. Monocytes two-way ANOVA: sex effect: $F(1, 25)=0.02424$ $p=0.8775$; treatment effect: $F(3, 25)=1.415$ $p=0.2617$. Granulocytes two-way ANOVA: Sex effect: $F(1, 22)=1.370$ $p=0.2543$; treatment effect: $F(3, 22)=1.385$ $p=0.2736$. Eosinophils two-way ANOVA: sex effect: $F(1, 22)=0.3427$ $p=0.5643$; treatment effect: $F(3, 22)=7.133$ $p=0.0016$. Two-way ANOVA with post hoc Tukey's test. PEPITEM: Peptide inhibitor of transendothelial migration.

Appendix 2. Sex-stratified effects of PEPITEM on leukocyte trafficking in mesenteric post-capillary venules

Parameters	Groups	Female				Male			
		Mean	SEM	n	Tukey's test (p)	Mean	SEM	n	Tukey's test (p)
Rolling (numbers/min)	Control	28.45	3.687	4	–	53.875	21.27	4	–
	Allergy	21	4.490	4	–	40.666	16.04	4	–
	Allergy+Des	18.375	6.132	4	–	40.20	9.866	4	–
	Allergy+PEP	50.166	22.08	4	–	36.75	5.170	4	–
Stationary leukocytes (numbers/min*mm^2)	Control	0.583	0.322	4	–	0.5	0.288	4	–
	Allergy	3.777	0.777	4	0.0192 versus Control	4.5	0.5	4	0.0091 versus Control
	Allergy+Des	0.875	0.381	4	0.0362 versus Allergy	2.25	1.165	4	ns
	Allergy+PEP	2.333	0.666	4	ns	2	0.707	4	ns
Adherent leukocytes (numbers/min*mm^2)	Control	3.23	1.928	4	–	10.20	3.656	4	–
	Allergy	78.40	29.39	4	0.0021 versus Control	74.09	15.70	4	0.0163 versus Control
	Allergy+Des	20.53	4.269	4	0.0191 versus Allergy	70.02	7.81	4	0.0259 versus Control
	Allergy+PEP	15.44	6.199	4	0.0181 versus Allergy	15.07	6.809	4	0.0283 versus Allergy; 0.0443 versus Allergy+Des

Sex-stratified data showing rolling counts, stationary, and adherent leukocyte densities. Groups as in Appendix 1. Rolling two-way analysis of variance (ANOVA): sex effect: F(1, 22)=2.373 p=0.1377; treatment effect: F(3, 22)=0.6811 p=0.5730. Stationary two-way ANOVA: sex effect: F(1, 20)=0.7062 p=0.4106; treatment effect: F(3, 20)=8.095 p=0.0010. Adhesion two-way ANOVA: Sex effect: F(1, 21)=1.865 p=0.1865; treatment effect: F(3, 21)=11.19 p=0.0001.Two-way ANOVA with post hoc Tukey's test. PEPITEM: Peptide inhibitor of transendothelial migration, SEM: Standard error of the mean.

Appendix 3. Sex-stratified measurements of leukocyte flow and leukocyte rolling velocity in mesenteric post-capillary venules

Parameters	Groups	Female				Male			
		Mean	SEM	n	Tukey's test (p)	Mean	SEM	n	Tukey's test (p)
Leukocyte flow (µl/s)	Control	0.3994	0.1038	4	–	0.662374	0.0849	4	–
	Allergy	0.3511	0.1852	4	–	0.212374	0.0261	4	–
	Allergy+Des	0.2753	0.0284	4	–	0.310782	0.0646	4	–
	Allergy+PEP	0.2871	0.1006	4	–	0.48702	0.1413	4	–
Average rolling velocity (µm/s)	Control	77.117	24.828	4	–	101.3724	39.926	4	–
	Allergy	70.336	18.786	4	–	99.70121	16.195	4	–
	Allergy+Des	68.334	9.2079	4	–	114.2602	29.375	4	–
	Allergy+PEP	77.3007	21.335	4	–	79.51546	25.937	4	–

Sex-stratified data showing leukocyte flow and average leukocyte rolling velocity. Groups as in Appendix 1. Leukocyte flow two-way analysis of variance (ANOVA): sex effect: F(1, 22)=1.323 p=0.2625; treatment effect: F(3, 22)=2.282 p=0.1073. Leukocyte rolling velocity two-way ANOVA: Sex effect: F(1, 22)=1.929 p=0.1788; treatment effect: F(3, 22)=0.09518 p=0.9619. Two-way ANOVA with post hoc Tukey's test. SEM: Standard error of the mean.

North Clin Istanb 2026;13(5):544–550
doi: 10.14744/nci.2026.28459

Original Article RADIATION ONCOLOGY

Set-up accuracy in megavoltage computed tomography-guided adjuvant breast radiotherapy: An analysis of differences between automatic registration and human observers

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ABSTRACT

OBJECTIVE: The objective of the study was to evaluate the agreement of set-up shift measurements obtained by the TomoTherapy automatic image-registration system, radiation therapists (RTTs), and physicians during adjuvant breast radiotherapy, and to investigate the effects of RTT number and target volume on positioning accuracy.

METHODS: This retrospective single-center study included 40 female breast cancer patients treated with adjuvant helical TomoTherapy under daily megavoltage computed tomography (MVCT) guidance. Set-up shifts in the lateral, longitudinal, vertical, and roll directions were analyzed over 1,200 treatment fractions. Pairwise comparisons among the TomoTherapy system, RTTs, and physicians were performed using paired t-tests or Wilcoxon signed-rank tests with Bonferroni correction. The effect of RTT number was evaluated using the Wilcoxon signed-rank test within subgroups, and that of clinical target volume (CTV)/boost volume using the Kruskal-Wallis test.

RESULTS: High agreement was observed among the TomoTherapy system, RTTs, and physicians. The largest mean difference between RTT and physician measurements was 1.56 mm and remained within the institutional 5-mm CTV-to-planning target volume margin. Although statistically significant differences were found in selected translational axes, RTT number and CTV/boost volume did not significantly influence set-up shift values.

CONCLUSION: Daily MVCT-guided TomoTherapy provides reliable and reproducible patient positioning. Standardized RTT-led image-guided workflows maintain clinically acceptable set-up accuracy regardless of RTT team size or target volume.

Keywords: Breast neoplasms; helical tomotherapy; image-guided; radiotherapy; radiotherapy setup errors.

Cite this article as: *Tepetam H, Dursun CU, Nasir Can SS, Hanilce MM, Gunalp G, Gedik D, Karabulut Gul S. Set-up accuracy in megavoltage computed tomography guided adjuvant breast radiotherapy: An analysis of differences between automatic registration and human observers. North Clin Istanb 2026;13(5):544–550.*

Breast cancer (BC) is the most prevalent malignancy among women worldwide, accounting for diagnoses in United States and serving as a leading cause of cancer-related mortality. In 2023, an estimated that 297,790 new invasive cases and 43,170 deaths were projected to occur the United States alone, with curative outcomes achievable in approximately 70–80% of patients diagnosed with early-stage, non-metastatic disease [1, 2]. Recent advancements in BC screening techniques, including mammography and genetic testing, have greatly enhanced early detection and contributed to improved survival rates [3]. The standard treatment for BC typical-

Received: July 24, 2026 *Revised:* August 15, 2026 *Accepted:* August 31, 2026 *Online:* October 16, 2026

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ly comprises three main therapeutic modalities: Surgery, radiotherapy (RT), and systemic therapy. Adjuvant RT plays a critical role by targeting and eradicating residual tumor cells that may remain following surgical interventions, whether breast-conserving surgery (BCS) or mastectomy [4]. Over the decades, BC RT has evolved significantly, transitioning from two-dimensional techniques to three-dimensional conformal RT, followed by intensity-modulated RT and volumetric modulated arc therapy. These advancements have improved the precision of tumor targeting while minimizing radiation exposure to surrounding healthy tissues [5, 6]. In recent years, the integration of image-guided RT (IGRT) has further enhanced treatment accuracy by utilizing real-time imaging during therapy sessions. While IGRT has become a routine part of modern RT, this innovative technique also introduces several challenges that must be addressed to optimize its implementation and effectiveness [6, 7].

In the RT process, ensuring that the patient remains in a fixed and consistent position during each treatment session is crucial for accurately delivering radiation to the tumor area and minimizing the impact on surrounding healthy tissues [8]. Set-up refers to the process of positioning the patient correctly before RT to precisely direct the planned radiation dose to the tumor region [9]. During TomoTherapy-based IGRT, pretreatment megavoltage computed tomography (MVCT) images are registered with the planning CT to verify patient positioning and quantify set-up deviations [10, 11]. MVCT provides volumetric anatomical information and enables the calculation and correction of translational shifts and roll before treatment [11]. Given the critical importance of accurate positioning in RT, advancements in imaging technologies have made it possible to achieve even greater precision and reliability in treatment delivery. To the best of our knowledge, few studies have simultaneously compared automated registration, radiation therapist (RTT) assessment, and physician approval in daily MVCT-guided TomoTherapy breast RT.

The aim of our study is to ensure accurate treatment positioning and effective immobilization of female BC patients undergoing adjuvant therapy with a TomoTherapy device in our clinic. To achieve this, we compare the set-up shift values obtained from the TomoTherapy Accuray automatic image alignment system with those recorded by physicians and RTTs. This study includes three key comparisons: the first between the automatic image alignment system and the values determined by physicians, the second between the values recorded by physicians and those recorded by RTTs, and the third between the automatic image alignment system and RTTs. By conducting these detailed comparisons, we aim to gain a comprehensive understanding of the discrepancies or consistencies between the three sources of data. Identifying potential set-up variations through this multi-faceted analysis will allow us to address inaccuracies in positioning and enhance the reliability of our RT practices. This study emphasizes how accurate set-up procedures directly impact treatment accuracy and reduce risks to patient safety.

Highlight key points

- Daily MVCT ensured reproducible patient positioning during adjuvant breast radiotherapy.
- Automated and human set-up measurements showed excellent clinical agreement.
- RTT-led image guidance maintained positioning accuracy within institutional margins.
- Target volume size and RTT number were not associated with set-up accuracy.
- Standardized TomoTherapy workflows supported reliable treatment delivery.

MATERIALS AND METHODS

Study Design and Participants

This retrospective, single-center study included 40 female patients with BC who received adjuvant RT at our institution. Of these, five patients underwent mastectomy, while the remaining 35 received BCS. The average age of the patients was 53.3 years. In addition, 28 patients had cancer in the left breast, and 12 in the right breast. Patients were selected based on the inclusion criteria of receiving adjuvant RT with the TomoTherapy system (Accuray Inc., Sunnyvale, CA, USA). Contouring of the clinical target volume (CTV) and boost volumes was performed using established guidelines: The ESTRO consensus guideline for early-stage BC for BCS cases and the RTOG atlas for postmastectomy cases [12, 13]. This approach ensured precision and consistency in contouring across all patients. All treatments were supervised by physicians and delivered using the TomoTherapy system for each fraction. The study was approved by the Institutional Ethics Committee (Approval No: 2025/010.99/16/17, Date: May 27, 2025) and was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from all patients.

Equipment and Techniques

Daily MVCT imaging was performed before each fraction to verify treatment position and alignment. The TomoTherapy Accuray automatic image alignment system automatically calculated initial set-up shift values, including lateral, longitudinal, vertical, and roll directions, and these values were recorded. The automatic registration parameters used for this process were "Full Image Technique," "Standard Resolution," and "Translations+Roll" settings, ensuring consistency in the evaluation of patient positioning. Subsequently, RTTs reviewed the values suggested by the automatic system, made their adjustments, and recorded these adjustments separately. Following the RTTs, the physician conducted a final review of the patient's position, and the physician's approved shift values were determined. Both RTTs and physicians independently evaluated the MVCT images in three planes: Coronal, sagittal, and vertical (Fig. 1). Finally, patients were treated using the shift values approved by the physician. The study aimed to evaluate the alignment consistency between the measurements obtained by the TomoTherapy system, RTTs, and physicians to enhance treatment accuracy and reliability.

In addition, the number of RTTs involved in treating each patient was documented and analyzed. In this context, the number of RTTs per patient was categorized into two groups: "3 or fewer" and "4 or more," and the potential impact of RTT numbers on set-up shift values was evaluated. Similarly, CTV/boost volumes were categorized, and their potential impact on set-up shift values was also analyzed.

Data Collection

Data were collected for each treatment session, and set-up shift values were categorized by source: TomoTherapy system, RTTs, and physicians. Three comparisons were performed: system versus physician, physician versus RTT, and system versus RTT. All statistical comparisons were performed at the individual fraction level, comprising a total of 1,200 fractions from the 40 patients.

Statistical Analysis

The normality of data distribution was evaluated using the Shapiro–Wilk and Kolmogorov–Smirnov tests. For normally distributed data, paired t-tests were used, while Wilcoxon signed-rank tests were applied to non-normally distributed data. Because three pairwise comparisons were performed within each axis, a Bonferroni correction was applied, and an adjusted threshold of $p<0.017$ (0.05/3) was considered statistically significant for these comparisons. Within each RTT-number subgroup, pairwise comparisons were performed using the Wilcoxon-signed-rank test, and the relationship between CTV/boost volumes and set-up shifts was analyzed using the Kruskal-Wallis test. Statistical analysis was performed using the Statistical Package for the Social Sciences software (version 27; IBM Corp., Armonk, NY, USA), and a significance level of $p<0.05$ was considered.

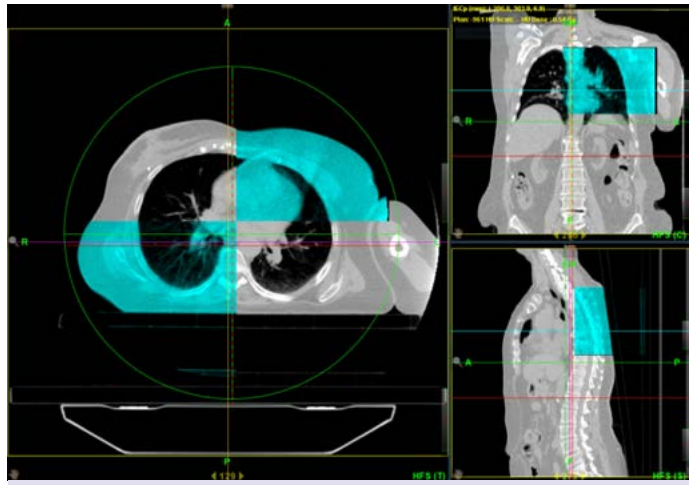

Figure 1. Representative registration of daily pretreatment megavoltage computed tomography (MVCT) with the planning CT during helical TomoTherapy. Axial (left), coronal (upper right), and sagittal (lower right) views are shown; the colored overlay depicts the registered comparison used for set-up verification.

RESULTS

Set-up shift values from all 1,200 treatment fractions were analyzed to provide a comprehensive overview of the data. To further evaluate the statistical properties of these values, the Shapiro–Wilk test was applied to assess the normality of the data distribution. Upon analysis, it was determined that, among the three groups (RTTs, Tomo, and physicians), the roll parameters did not meet the criteria for normal distribution, while the remaining parameters, including lateral, longitudinal, and vertical directions, demonstrated normal distribution. The mean differences between RTT and physician measurements across all four axes were as follows: 0.36 mm for the lateral axis, 0.98 mm for the vertical axis, 1.56 mm for the longitudinal axis, and 0.09° for the roll axis. These observations establish a robust statistical foundation, allowing for meaningful comparisons and detailed analyses to be conducted in the subsequent stages of this study (Table 1).

Table 1. Descriptive statistics and normality results for set-up parameters

Parameter	Mean value#	p	Parameter	Mean value#	p
rtt_lat	0.40	0.656	rtt_vert	8.40	0.328
tomo_lat	1.13	0.602	tomo_vert	8.59	0.393
dr_lat	0.76	0.803	dr_vert	9.38	0.245
rtt_long	-1.30	0.109	rtt_roll	-0.07	0.003*
tomo_long	-1.52	0.261	tomo_roll	-0.10	<0.001*
dr_long	-2.86	0.062	dr_roll	0.02	0.034*

#Mean values for the translational axes (lateral, longitudinal, and vertical) are expressed in millimeters (mm), whereas the roll parameter is a rotational displacement expressed in degrees (°). *Indicates a non-normal distribution (Shapiro-Wilk, p<0.05). RTT: Radiation therapist; tomo: TomoTherapy automatic registration; dr: Physician; lat: Lateral; vert: Vertical; long: Longitudinal.

Table 2. Statistical comparison table of set-up parameters

Comparison	Parameter	p	Comparison	Parameter	p
tomo_lat - dr_lat	Lateral	0.034	tomo_long - dr_long	Longitudinal	0.052
rtt_lat - tomo_lat	Lateral	<0.001*	rtt_long - tomo_long	Longitudinal	0.720
rtt_lat - dr_lat	Lateral	0.081	rtt_long - dr_long	Longitudinal	<0.001*
tomo_vert - dr_vert	Vertical	0.225	tomo_roll - dr_roll	Roll	0.549
rtt_vert - tomo_vert	Vertical	0.756	rtt_roll - tomo_roll	Roll	0.600
rtt_vert - dr_vert	Vertical	0.012*	rtt_roll - dr_roll	Roll	0.545

*Indicates statistical significance at the Bonferroni-adjusted threshold of p<0.017. RTT: Radiation therapist; tomo: TomoTherapy automatic registration; dr: Physician; lat: Lateral; vert: Vertical; long: Longitudinal.

Statistical comparisons revealed significant differences in specific parameters. For instance, in the lateral direction, tomo_lat showed significant differences when compared to rtt_lat but not dr_lat, whereas no significant difference was observed between rtt_lat and dr_lat, indicating consistency between rtt and dr measurements in this axis. In the vertical axis, although no significant differences were observed between tomo_vert and either rtt_vert or dr_vert, a notable difference was found between rtt_vert and dr_vert. Similarly, in the longitudinal axis, while there were no significant differences between tomo_long and either rtt_long or dr_long, rtt_long demonstrated a significant difference compared to dr_long. These findings emphasize the variability in set-up shift values across specific axes depending on the measurement source. The roll axis, however, did not show statistically significant differences between any of the groups. These results are summarized in Table 2, providing a detailed breakdown of the comparisons and their statistical significance.

During the treatment process, patients were grouped based on the number of different RTTs involved in their care. Patients treated by "3 or fewer RTTs" comprised 12 individuals, while those treated by "4 or more RTTs" comprised 28 individuals. Across all patients, the number of different RTTs per treatment ranged from a minimum of 1 to a maximum of 7. This grouping was used to evaluate the potential impact of RTT numbers on set-up accuracy. For each group, comparisons among tomo, rtt, and dr measurements were performed across four parameters (lateral, vertical, longitudinal, and roll). These comparisons were conducted using the non-parametric Wilcoxon signed-rank test. The tests evaluated differences between "tomo-rtt," "tomo-dr," and "rtt-dr" measurements for all axes separately within each group. The results of the analysis showed that, for both groups, no statistically significant differences were found in any of the four parameters (lateral, vertical, longitudinal, and roll). These findings suggest that the number of RTTs involved in the treatment process did not significantly

influence the consistency of measurements across the sources (tomo, rtt, and dr).

Kruskal–Wallis tests were conducted to evaluate the effect of CTV breast/chest wall and CTV boost volumes on set-up shift values. The results showed that these parameters did not have a statistically significant impact on set-up shift values ($p>0.05$). Furthermore, the analysis demonstrated that alignment consistency among tomo, RTT, and physician measurements was maintained across both breast/chest wall and boost volume categories.

DISCUSSION

The accuracy and consistency of set-up shift measurements among TomoTherapy (tomo), RTTs, and physicians in adjuvant BC RT were evaluated using MVCT-based image guidance across 1,200 treatment fractions. In addition, the influence of parameters such as RTT team size and CTV size on set-up accuracy was analyzed. These findings provide a comprehensive real-world understanding of alignment precision and inter-observer variability, highlighting the practical interplay between automated systems and human interpretation in modern IGRT workflows, and contextualizing their clinical significance through comparisons with existing literature. Although the automatic registration provided the initial alignment, the RTTs and physicians independently evaluated the MVCT images in three planes before approving the final shifts; the strong concordance observed among the three sources therefore reflects the reliability and reproducibility of this multi-observer verification workflow rather than a mere propagation of the automated result.

When comparing measurements between RTTs and physicians, the largest mean difference of 1.56 mm was observed in the longitudinal axis, while the smallest mean difference of 0.09° was found in the roll axis. These differences remain within the planning target volume (PTV) boundaries established in our clinic by applying a 5 mm margin around the CTV. In this context, clinical relevance is more important than statistical significance: Although statistically significant differences were observed in selected translational axes, the maximum mean discrepancy remained substantially below the institutional 5-mm planning margin and is therefore unlikely to affect treatment delivery. Previous studies have demonstrated that a 5 mm expansion from CTV to PTV is sufficient to compensate for set-up variability and ensure reliable dosimetry even under challenging conditions [14]. The findings of the current study are consistent with these results; all observed differences, including those in the roll and longitudinal axes, were within these margins. This further reinforces the robustness of the 5 mm PTV margin in compensating for systematic and random errors in BC RT [14, 15]. In addition, the effectiveness of these margins highlights that RTT-led set-up procedures can maintain alignment accuracy without the need for physician intervention. This ensures that the treatment target consistently remains within the planned RT field. The observed alignment precision underscores the importance of standardized protocols and their role in minimizing variability within clinical settings. Another important observation is that the number of RTTs involved in the treatment process did not significantly affect the set-up accuracy. When comparing patients treated by three or fewer RTTs versus those treated by four or more, no statistically significant differences were identified. This finding indicates that RTTs are adequately trained and demonstrate comparable expertise in operating the TomoTherapy device. Furthermore, this result aligns with the literature emphasizing the direct impact of RTT training and experience on RT accuracy [16]. The consistency observed across varying RTT numbers reflects the effectiveness of the clinic's training programs and the reliability of alignment protocols.

Previous breast RT studies have demonstrated that set-up accuracy may vary according to treatment position [17]. Daily orthogonal planar image-guided set-up in free-breathing patients has been shown to provide accurate tangential breast RT with few residual errors exceeding 5 mm [18]. Because residual geometric uncertainties may persist despite image verification, systematic PTV margins remain important for maintaining treatment accuracy [19]. More directly, MVCT-based image guidance in TomoTherapy enables the detection and correction of translational and roll deviations and has demonstrated millimetric set-up assessment with limited interobserver variability [10, 11]. These findings are consistent with the strong agreement observed among the automatic system, RTTs, and physicians in the present study. Despite this overall agreement, the roll measurements were non-normally distributed, although differences among the three measurement sources were not statistically significant. Residual set-up errors remain clinically relevant because translational shifts exceeding 5 mm or rotations exceeding 2° may produce clinically relevant dosimetric changes [20]. Simulated isocenter shifts

may also alter target coverage and doses to surrounding normal tissues, emphasizing the importance of accurate position verification [21]. Rotational set-up deviations can additionally be quantified using six-degree-of-freedom image-guidance approaches [22]. Therefore, careful evaluation of rotational alignment remains important. Standardized training and automated verification workflows may help reduce human error and improve consistency in RT procedures [23].

Particularly after BCS, post-operative seroma may cause clinically relevant changes in the tumor-bed and boost volume. In addition to set-up verification, pretreatment MVCT may provide visual evidence of such anatomical changes and help identify cases in which adaptive replanning should be considered [24]. In the present study, no statistically significant association was detected between CTV/boost volume categories and set-up shift values. However, longitudinal anatomical changes and adaptive replanning were not evaluated.

This study has several limitations that should be considered when interpreting its findings. First, its retrospective, single-center design and relatively small sample size (40 patients) may limit the generalizability of the results to other institutions, patient populations, and treatment platforms. Nevertheless, setup accuracy was evaluated across 1,200 daily treatment fractions, providing a comprehensive assessment of positioning reproducibility in routine clinical practice. Because multiple fractions originated from each patient, however, within-patient correlation was not modeled using repeated-measures approaches such as mixed-effects models; this limitation should be considered when interpreting the statistical findings. Second, all patients were treated with a single delivery technique (helical TomoTherapy) under MVCT-based image guidance, so the findings may not be directly transferable to other treatment units or image-guidance systems. The current statistical analysis focused on paired comparisons of setup shift values; therefore, dedicated agreement methods such as Bland-Altman analysis and intraclass correlation coefficients were not performed. These approaches could provide complementary information on systematic bias, limits of agreement, and interobserver consistency in future studies. Finally, the dosimetric consequences of the observed set-up variations were not quantified. Future prospective, multicenter studies would help confirm and extend these findings.

Conclusion

This study emphasizes the critical importance of accurate patient positioning in adjuvant BC RT to ensure effective treatment delivery and minimize risks to healthy tissues. Comparisons between the TomoTherapy automatic alignment system, RTTs, and physicians revealed high consistency in set-up shift measurements, with differences remaining within clinically acceptable margins. In addition, the findings demonstrated that the number of RTTs involved in the treatment process did not significantly impact measurement accuracy, and neither CTV nor boost volumes influenced positioning accuracy. Advanced imaging techniques such as MVCT effectively addressed volumetric and positional changes, maintaining alignment precision throughout the treatment process. These results underscore the value of integrating advanced technologies and rigorous protocols to optimize RT outcomes for BC patients.

Ethics Committee Approval: This study was approved by The Institutional Ethics Committee of Kartal Dr. Lutfi Kirdar City Hospital (Approval No. 2025/010.99/16/17; May 27, 2025).

Informed Consent: Written informed consent was 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 received no financial support.

Use of AI for Writing Assistance: During the preparation of this manuscript, the authors used ChatGPT (OpenAI) solely to improve the language, grammar, and readability of the manuscript.

Authorship Contributions: Concept – CUD, MMH; Design – CUD, DG; Supervision – CUD, SKG; Resource – SKG; Materials – CUD, SSNC, GG; Data Collection and/or Processing – CUD, SSNC, GG; Analysis and/or Interpretation – CUD, HT; Literature Search – HT, DG, MMH; Writing – CUD, MMH, SSNC; Critical Reviews – HT, DG, SKG.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2026;13(5):551–557
doi: 10.14744/nci.2026.34793

Original Article INTERNAL MEDICINE

Evaluation of the relationship between the SCORE2-diabetes mellitus cardiovascular risk score, degree of obesity, and systemic inflammatory indices in patients with type 2 diabetes mellitus

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ABSTRACT

OBJECTIVE: This study evaluated the relationship between the SCORE2 diabetes mellitus (SCORE2-DM) cardiovascular risk score, obesity status, clinical and laboratory parameters, and hematologic systemic inflammatory indices in patients with type 2 diabetes mellitus (T2DM).

METHODS: This single-center, retrospective cross-sectional study included 100 patients with T2DM aged 40–69 years without established atherosclerotic cardiovascular disease. Patients with type 1 diabetes, active infection or inflammatory disease, malignancy, or hematologic disease were excluded from the study. Ten-year cardiovascular risk was calculated using the SCORE2-Diabetes algorithm. Patients were classified by body mass index (BMI) into normal weight, overweight, and obesity classes. Systemic immune-inflammation index, systemic inflammation response index, neutrophil-to-lymphocyte ratio, monocyte-to-lymphocyte ratio, and platelet-to-lymphocyte ratio were calculated from complete blood count (CBC) parameters. Obesity-class comparisons were performed using the Kruskal–Wallis test, the sex comparison using the Mann–Whitney U-test, and correlations using Spearman analysis.

RESULTS: The median age was 50 (40–67) years, and 50% of participants were women. Obesity class distribution was: Normal weight 13%, overweight 36%, class 1 obesity 35%, class 2 obesity 12%, and class 3 obesity 4%. SCORE2-DM did not differ significantly according to obesity class ($p=0.250$) or sex (Mann–Whitney $U=1289.5$, $p=0.788$). SCORE2-DM correlated significantly with variables directly incorporated into the algorithm, including age ($rho=0.621$, $p<0.001$), smoking status ($rho=0.324$, $p=0.001$), systolic blood pressure ($rho=0.230$, $p=0.021$), total cholesterol ($rho=0.227$, $p=0.023$), glycated hemoglobin ($rho=0.338$, $p<0.001$), age at diabetes diagnosis ($rho=0.338$, $p<0.001$), and estimated glomerular filtration rate ($rho=-0.223$, $p=0.026$); these associations are expected because of mathematical coupling. Diabetes duration also showed a positive correlation with SCORE2-DM ($rho=0.291$, $p=0.003$). BMI showed an exploratory weak inverse correlation with SCORE2-DM ($rho=-0.214$, $p=0.032$). None of the inflammatory indices were significantly correlated with SCORE2-DM.

CONCLUSION: In this retrospective cross-sectional T2DM cohort, SCORE2-DM did not differ significantly according to BMI-defined obesity class or sex, and CBC-derived inflammatory indices showed no significant cross-sectional association with the score. Correlations with variables incorporated into SCORE2-DM are expected because of mathematical coupling and should not be interpreted as independent validation findings. The weak inverse association between BMI and SCORE2-DM should be interpreted cautiously in view of multiple exploratory comparisons. Longitudinal studies with cardiovascular outcomes are required to evaluate the prognostic or predictive value of the inflammatory indices.

Keywords: Biomarkers; cardiovascular diseases; diabetes mellitus type 2; inflammation; obesity; risk assessment.

Cite this article as: *Tayfur M, Emir S. Evaluation of the relationship between the SCORE2-diabetes mellitus cardiovascular risk score, degree of obesity, and systemic inflammatory indices in patients with type 2 diabetes mellitus. North Clin Istanb 2026;13(5):551–557.*

Received: July 21, 2026 *Revised:* August 19, 2026 *Accepted:* September 09, 2026 *Online:* October 16, 2026

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Istanbul Provincial Directorate of Health - Available online at www.northclinist.com

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease whose global prevalence is rising rapidly and which is recognized as one of the leading risk factors for cardiovascular disease (CVD) [1, 2].

The risk of cardiovascular events is markedly higher in individuals with diabetes than in those without diabetes, and CVD constitutes the leading cause of morbidity and mortality in patients with T2DM [3].

Accurate cardiovascular risk estimation is important for planning preventive treatment strategies. The SCORE2 algorithm, developed by the European Society of Cardiology (ESC), is widely used to estimate 10-year cardiovascular risk in the general population [4]. Because the original SCORE2 model does not incorporate diabetes-specific risk information, the SCORE2-diabetes mellitus (SCORE2-DM) algorithm was developed by adding diabetes-specific variables – age at diabetes diagnosis, glycated hemoglobin (HbA1c), and estimated glomerular filtration rate (eGFR) – to conventional risk factors such as age, smoking, systolic blood pressure, and total and high-density lipoprotein (HDL) cholesterol, and it has been validated in European populations [5].

Obesity plays an important role in the pathogenesis of T2DM and in the development of cardiovascular complications, and is traditionally associated with increased cardiovascular risk [6]. Nevertheless, conflicting findings referred to in the literature as the "obesity paradox" have also been reported, indicating that patients with mild-to-moderate obesity in certain chronic disease groups (such as heart failure, diabetes, and acute coronary syndrome) may show better clinical outcomes than normal-weight patients [7, 8]. The validity of this paradox in the T2DM population, and the mechanisms underlying it, remain a subject of ongoing debate.

In recent years, a growing number of publications have suggested that low-cost, readily accessible systemic inflammation markers derived from the routine complete blood count – such as the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR), systemic immune-inflammation index (SII), and systemic inflammation response index (SIRI) – may be associated with cardiovascular risk and mortality [9–11]. However, studies evaluating the relationship between these indices and SCORE2-DM, a current and diabetes-specific risk score, remain limited.

In this study, we aimed to evaluate the relationship between the SCORE2-DM cardiovascular risk score and sex, degree of obesity, demographic, clinical, and laboratory parameters, and hematologic inflammatory indices (SII, SIRI, NLR, MLR, and PLR) in patients with T2DM. We hypothesized that the degree of glycemic control and accompanying metabolic parameters would be more strongly associated with SCORE2-DM than conventional obesity measures, whereas simple hematologic inflammatory indices would not show a significant cross-sectional association with the score in this population.

Highlight key points

- Correlations between SCORE2-DM and variables incorporated into its algorithm were expected because of mathematical coupling.
- SCORE2-DM did not differ significantly according to BMI-defined obesity class or sex in this sample.
- BMI showed an exploratory weak inverse correlation with SCORE2-DM; this marginal finding requires cautious interpretation because no multiplicity adjustment was applied.
- CBC-derived inflammatory indices, including SII, SIRI, NLR, MLR, and PLR, showed no significant cross-sectional correlations with SCORE2-DM.

MATERIALS AND METHODS

Study Design, Setting, and Ethical Approval

This study was designed as a single-center, retrospective cross-sectional study based on existing medical records of patients attending the outpatient internal medicine clinic of a tertiary training and research hospital. Demographic, clinical, anthropometric, and laboratory data used in the analyses corresponded to the same outpatient encounter, and no additional intervention or sample collection was performed. The protocol was approved by the local Scientific Research Ethics Committee as a non-pharmaceutical clinical study on December 18, 2025 (decision no. 472; reference no. B.10.1.TKH.4.34.H.GP.0.01/478). Study-specific written informed consent was not obtained; the retrospective protocol and submitted study documentation were reviewed and approved by the ethics committee. The study followed the principles of the Declaration of Helsinki.

Patient Selection

Inclusion criteria: Patients aged 40–69 years with a diagnosis of T2DM and no established atherosclerotic CVD (ASCVD) were included in the study. This age range and the requirement of being free of ASCVD were chosen

to match the target population, in which the SCORE2-DM algorithm was developed and validated.

Exclusion criteria: Patients with type 1 diabetes, established ASCVD, active infection or inflammatory disease, a history of malignancy, or hematologic disease were excluded from the study.

Eligible patient records were screened according to the inclusion and exclusion criteria. A sex-balanced analytical sample was intentionally constructed, comprising 50 women and 50 men who met the eligibility criteria; therefore, the final sample was not a consecutive sample.

Data Collection

Demographic data (age, sex, and smoking history), anthropometric and clinical data (systolic and diastolic blood pressure, waist circumference, height, weight, and body mass index [BMI]), and age at diabetes diagnosis were recorded from the outpatient evaluation. Laboratory data (total cholesterol, HDL cholesterol, HbA1c, eGFR, and albuminuria) and the neutrophil, lymphocyte, monocyte, and platelet counts used to calculate the inflammatory indices were obtained from laboratory testing performed as part of the same outpatient visit as the clinical and anthropometric assessment.

Obesity Classification

Patients were classified into five categories according to World Health Organization BMI cut-off points [12]: Normal Weight (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), Class 1 obesity (30.0–34.9 kg/m^2), Class 2 obesity (35.0–39.9 kg/m^2), and Class 3 obesity (≥40.0 kg/m^2).

Calculation of the SCORE2-DM Cardiovascular Risk Score

Ten-year CVD risk was calculated using the SCORE2-Diabetes algorithm [5], which is recommended for clinical use in the 2023 ESC Guidelines on the management of CVD in patients with diabetes. In addition to conventional risk factors (age, sex, smoking status, systolic blood pressure, and total and HDL cholesterol), this algorithm incorporates diabetes-specific variables, namely, age at diabetes diagnosis, HbA1c, and eGFR, the latter reflecting the well-established independent contribution of impaired renal function to cardiovascular risk [13]. Risk calculation was performed with all patients assigned to the "high-risk" European risk region. Total cholesterol and HDL-C values were entered into the calculator (MDCalc) in mg/dL, and HbA1c values recorded as percentages (%) were converted to mmol/mol before SCORE2-DM calculation. In the descriptive tables, HbA1c is presented as a percentage (%) for clinical readability.

Calculation of Inflammatory Indices

Systemic inflammatory indices were calculated from complete blood count parameters using the following formulas:

NLR = Neutrophil count/Lymphocyte count

PLR = Platelet count/Lymphocyte count

MLR = Monocyte count/Lymphocyte count

SII = (Platelet count × Neutrophil count)/Lymphocyte count

SIRI = (Neutrophil count × Monocyte count)/Lymphocyte count.

Statistical Analysis

Statistical analyses were performed using Jamovi (v2.7) [14] and R (v4.5) [15]. Normality of continuous variables was assessed using the Shapiro–Wilk test. Normally distributed variables were expressed as mean±standard deviation (mean ± standard deviation), non-normally distributed variables as median (minimum-maximum), and categorical variables as number (%). The Kruskal–Wallis test was used to compare SCORE2-DM scores across the five obesity classes; when the omnibus test was significant, the Dwass-Steel-Critchlow-Fligner test was planned for pairwise *post hoc* comparisons. The Mann–Whitney U-test was used for the two-group comparison between sexes, with rank-biserial correlation reported as the effect-size estimate. Relationships between SCORE2-DM and demographic, clinical, laboratory, and inflammatory index parameters were assessed using Spearman correlation analysis. These correlation analyses were exploratory, and no formal adjustment for multiple comparisons was applied; marginal p-values were therefore interpreted cautiously. No formal a priori sample-size calculation was performed. A sensitivity analysis based on the available sample (n=100), a two-sided $\alpha=0.05$, and 80% power indicated that correlation magnitudes of approximately $|r|\geq 0.28$ could be detected under standard correlation-test assumptions; smaller associations may therefore have remained undetected. A two-sided $p<0.05$ was considered statistically significant for the exploratory analyses.

Table 1. Distribution of patients according to obesity class (n=100)

Obesity class	Percentage
Normal weight	13.0
Overweight	36.0
Class 1 obesity	35.0
Class 2 obesity	12.0
Class 3 obesity	4.0

RESULTS

Descriptive Statistics

A total of 100 participants were included in the study. Of these, 50 (50%) were men and 50 (50%) were women. Active smoking was present in 33% (n=33) of the participants. All patients were classified within the "high-risk" SCORE2 risk region. The distribution of participants according to obesity classification is presented in Table 1.

Baseline demographic, clinical, laboratory, and inflammatory index characteristics are summarized in Table 2.

Comparison of the SCORE2-DM Score between Groups

SCORE2-DM scores did not differ significantly across obesity classes (Normal Weight, Overweight, Class 1, Class 2, and Class 3 Obesity) (χ^2=5.39, df=4, p=0.250, ε^2=0.054). As the omnibus test was not significant, *post hoc* pairwise comparisons were not performed. Similarly, the Mann–Whitney U-test showed no significant difference in SCORE2-DM scores between men and women (U=1289.5, p=0.788), with a negligible rank-biserial effect size (r_rb=0.032).

Correlation Analyses

Exploratory relationships between the SCORE2-DM score and demographic, clinical, laboratory, and inflammatory index findings were assessed using Spearman correlation analysis. The results are summarized in Table 3. Because no formal multiplicity adjustment was applied, marginal p-values should be interpreted cautiously.

Regarding demographic parameters and algorithm components, SCORE2-DM showed a strong positive correlation with age (rho=0.621, p<0.001) and positive correlations with smoking status (rho=0.324, p=0.001) and systolic blood pressure (rho=0.230, p=0.021). These associations are expected because age, smoking status, and systolic blood pressure are direct inputs to the SCORE2-DM algorithm and, therefore, represent mathematical coupling rather than independent validation findings. A positive correlation was also observed with age at diabetes diagnosis (rho=0.338, p<0.001), another direct algorithm input. Diabetes duration,

Table 2. Demographic, clinical, laboratory, and inflammatory index characteristics of the participants (n=100)

Variable	Mean±SD / Median (min–max)
Age (years)†	50 (40–67)
Smoking, n (%)	33 (33.0)
Systolic BP (mmHg)†	130 (90–180)
Diastolic BP (mmHg)†	80 (65–110)
Waist circumference (cm)	104.2±8.8
Height (cm)	165.2±10.1
Weight (kg)	82.5±13.3
BMI (kg/m²)†	30.0 (21.8–45.0)
Diabetes duration (years)†	7 (1–20)
Age at diabetes diagnosis (years)	44.4±7.0
Total cholesterol (mg/dL)†	199 (96–453)
HDL-C (mg/dL)†	42 (20–76)
HbA1c (%)†	7.9 (6.2–14.9)
eGFR (mL/min/1.73 m²)†	97 (47–139)
Albuminuria (mg/g)†	17 (2–278)
Neutrophil (10³/µL)†	4.69 (2.20–10.70)
Platelet (10³/µL)†	262.5 (148.0–726.0)
Lymphocyte (10³/µL)†	2.66 (1.40–5.20)
Monocyte (10³/µL)†	0.48 (0.22–1.05)
SII†	499.2 (196.0–1908.3)
SIRI†	0.84 (0.33–2.24)
NLR†	1.78 (0.62–4.50)
MLR†	0.18 (0.10–0.37)
PLR†	102.6 (40.0-251.0)
SCORE2-DM (%)†	12.1 (2.8–40.2)

†Median (minimum-maximum) values are presented for variables that did not follow a normal distribution according to the Shapiro–Wilk test. BP: Blood pressure; BMI: Body mass index; HDL-C: High-density lipoprotein cholesterol; eGFR: Estimated glomerular filtration rate; SII: Systemic immune-inflammation index; SIRI: Systemic inflammation response index; NLR: Neutrophil-to-lymphocyte ratio; MLR: Monocyte-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SD: Standard deviation; SCORE2-DM: SCORE2-Diabetes mellitus; HbA1c: Glycated hemoglobin.

TABLE 3. Spearman correlation results between the SCORE2-DM score and the parameters examined

Parameter	rho	p
Age	0.621	<0.001*
Smoking	0.324	0.001*
Systolic blood pressure	0.230	0.021*
Diastolic blood pressure	0.089	0.381
Diabetes duration	0.291	0.003*
Age at diabetes diagnosis	0.338	<0.001*
Waist circumference	-0.042	0.679
BMI	-0.214	0.032*
Total cholesterol	0.227	0.023*
HDL-C	-0.174	0.083
HbA1c	0.338	<0.001*
eGFR	-0.223	0.026*
Albuminuria	0.088	0.384
Neutrophil	-0.100	0.322
Platelet	-0.171	0.089
Lymphocyte	-0.017	0.865
Monocyte	-0.028	0.780
SII	-0.109	0.279
SIRI	-0.041	0.684
NLR	-0.051	0.616
MLR	0.020	0.844
PLR	-0.031	0.756

*: P<0.05 (unadjusted). Correlation analyses were exploratory, and no formal correction for multiple comparisons was applied. BMI: Body mass index; HDL-C: High-density lipoprotein cholesterol; eGFR: Estimated glomerular filtration rate; SII: Systemic immune-inflammation index; SIRI: Systemic inflammation response index; NLR: Neutrophil-to-lymphocyte ratio; MLR: Monocyte-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; SCORE2-DM: SCORE2-Diabetes mellitus; eGFR: Estimated glomerular filtration rate; HbA1c: Glycated hemoglobin.

which is not entered separately into SCORE2-DM, was positively correlated with the score (rho=0.291, p=0.003); however, this should be interpreted cautiously because diabetes duration is mathematically related to current age and age at diabetes diagnosis. BMI showed an exploratory weak inverse correlation with SCORE2-DM (rho=-0.214, p=0.032). eGFR was inversely correlated with SCORE2-DM (rho=-0.223, p=0.026), as expected because eGFR is a direct component of the algorithm. No significant relationship was found with diastolic blood pressure (p=0.381) or waist circumference (p=0.679).

Regarding laboratory findings, SCORE2-DM was positively correlated with total cholesterol (rho=0.227, p=0.023) and HbA1c (rho=0.338, p<0.001). Because both variables are directly incorporated into the SCORE2-DM algorithm, these correlations are expected and should not be interpreted as independent validation findings. No statistically significant correlation was found between SCORE2-DM and HDL-C, albuminuria, neutrophil, platelet, lymphocyte, or monocyte values (p>0.05 for all parameters).

Regarding inflammatory indices, no statistically significant correlation was found between the SCORE2-DM score and any of the inflammatory indices studied (SII, SIRI, NLR, MLR, PLR) (p=0.279, p=0.684, p=0.616, p=0.844, and p=0.756, respectively).

DISCUSSION

In this retrospective cross-sectional study of 100 patients with T2DM aged 40–69 years and free of established ASCVD, we evaluated the relationship between the SCORE2-DM cardiovascular risk score and degree of obesity, sex, clinical and laboratory parameters, and hematologic systemic inflammatory indices. The main findings were that SCORE2-DM did not differ significantly according to BMI-defined obesity class or sex; correlations with variables directly incorporated into SCORE2-DM were observed as expected because of mathematical coupling; diabetes duration was positively associated with the score but is mathematically related to age and age at diagnosis; BMI showed an exploratory weak inverse correlation with SCORE2-DM; and none of the inflammatory indices studied showed a significant cross-sectional association with SCORE2-DM.

The absence of statistically significant differences in SCORE2-DM scores between sexes or across BMI-defined obesity classes should not be interpreted as statistical independence. SCORE2-DM is a composite algorithm that incorporates sex, age, smoking status, systolic blood pressure, total and HDL cholesterol, age at diabetes diagnosis, HbA1c, and eGFR [5]; consequently, group-level score distributions reflect the combined distribution of these inputs. A recent retrospective study of 203 individuals with obesity reported that Framingham cardiovascular risk scores were correlated with several anthropometric and metabolic variables, including waist circumference, blood pressure, glycemic measures, and lipid parameters, further illustrating the multifactorial nature of cardiovascular risk estimation in obesity [16].

The observed correlations between SCORE2-DM and HbA1c, total cholesterol, systolic blood pressure, age, smoking status, age at diabetes diagnosis, and eGFR are expected because these variables are direct inputs to the SCORE2-DM calculation [5]. Although these variables are well-established cardiovascular risk factors in T2DM [17–21], their correlations with a score that mathematically incorporates them do not constitute independent validation findings and should not be interpreted as demonstrating independent effects within the present dataset. This distinction is particularly important when interpreting the associations with HbA1c, total cholesterol, and systolic blood pressure.

An exploratory weak inverse correlation was observed between BMI and SCORE2-DM (rho=−0.214, unadjusted p=0.032). This finding requires substantial caution because 22 correlation tests were examined without formal adjustment for multiple comparisons and no cardiovascular outcome data were available. Although inverse associations between BMI and outcomes, often termed the "obesity paradox," have been reported in some chronic disease populations [7, 8], the present cross-sectional association with a calculated risk score does not constitute evidence of an obesity paradox. The result may instead reflect confounding by the distribution of age, age at diabetes diagnosis, smoking status, or other SCORE2-DM inputs, as well as the limitations of BMI as a measure of adiposity [22, 23]. Accordingly, this marginal finding should be regarded as hypothesis-generating and requires confirmation in larger longitudinal cohorts with actual cardiovascular outcomes.

The positive correlation between age at diabetes diagnosis and SCORE2-DM (rho=0.338, p<0.001) is expected because age at diagnosis is a direct input to the SCORE2-DM algorithm [5]. It therefore reflects mathematical coupling rather than an independent discovery. Diabetes duration is not entered as a separate variable in SCORE2-DM; however, it is mathematically related to current age and age at diabetes diagnosis (diabetes duration = current age − age at diagnosis). Thus, although diabetes duration was positively correlated with the score (rho=0.291, p=0.003), this association may partly reflect the mathematical structure of the algorithm and should not be interpreted as an independent validation finding.

Previous studies have suggested that hematologic indices such as NLR, SII, SIRI, MLR, and PLR may be associated with cardiovascular events or mortality in populations with diabetes [9–11]. In the present study, none of these indices showed a significant cross-sectional association with SCORE2-DM. This apparent difference in findings likely reflects the different outcome constructs: Previous studies primarily evaluated actual clinical outcomes, whereas our analysis examined association with a calculated risk score. Importantly, the absence of a cross-sectional association with SCORE2-DM does not establish that these inflammatory indices lack prognostic or predictive value, nor does it determine whether they provide incremental prognostic information beyond SCORE2-DM. Such questions require longitudinal studies incorporating cardiovascular events or mortality. The limited sample size may also have reduced the ability to detect associations of small magnitude.

The strengths of our study include the evaluation of a current, diabetes-specific risk score (SCORE2-DM) together with a comprehensive set of parameters in a clearly defined T2DM population. The study also has several limitations. Its single-center, retrospective cross-sectional design precludes causal or prognostic inference, and actual cardiovascular events or mortality were not assessed. The sample size was relatively limited (n=100), no formal a priori sample-size calculation was performed, and the sensitivity analysis indicates that smaller correlations may have remained undetected. The intentionally sex-balanced sample (50 women and 50 men) does not reflect the natural sex distribution of the source outpatient population and may limit representativeness. In addition, the Class 3 obesity subgroup included only four participants, which substantially limits the power and stability of comparisons across the five BMI categories. All patients were assigned to a single European risk region ("high risk"), and the exploratory correlation analyses were conducted without formal correction for multiple comparisons. Finally, BMI-based obesity classification provides limited information about visceral fat distribution and body composition; although waist circumference was available, more sensitive anthropometric or imaging-based measures of adiposity were not included.

Conclusion

In this retrospective cross-sectional T2DM cohort, SCORE2-DM did not differ significantly according to BMI-defined obesity class or sex, and the CBC-derived inflammatory indices studied (SII, SIRI, NLR, MLR, and PLR) showed no significant cross-sectional association with the score. Correlations between SCORE2-DM and variables incorporated into its calculation are expected because of mathematical coupling and should

not be interpreted as independent validation findings. The exploratory weak inverse association between BMI and SCORE2-DM should be interpreted cautiously because multiple correlations were examined without formal multiplicity adjustment and the smallest obesity subgroup was limited. Because actual cardiovascular outcomes were not assessed, no conclusions can be drawn regarding the prognostic, predictive, or incremental value of the inflammatory indices beyond SCORE2-DM. Larger longitudinal studies incorporating cardiovascular events and mortality are needed to address these questions.

Ethics Committee Approval: This study was approved by The Scientific Research Ethics Committee of the University of Health Sciences, Umraniye Training and Research Hospital, Republic of Turkiye Ministry of Health (Date: 18.12.2025; Decision no. 472).

Informed Consent: Study-specific written informed consent was not obtained because this was a retrospective study based on existing medical records. The study protocol was reviewed and approved by the institutional ethics committe.

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

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

Use of AI for Writing Assistance: During the preparation of this manuscript, the authors used ChatGPT for language editing and academic phrasing support.

Authorship Contributions: Concept – SE; Design – MT, SE; Supervision – MT; Materials – SE; Data Collection and/or Processing – MT; Analysis and/or Interpretation – SE; Literature Search – MT; Writing – MT; Critical Reviews – MT.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2026;13(5):558–567
doi: 10.14744/nci.2026.20280

Original Article PUBLIC HEALTH

Awareness, knowledge, perceptions, and use of electronic cigarettes and heated tobacco products among adults attending a university hospital: A cross-sectional study

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ABSTRACT

OBJECTIVE: The aim of this study was to evaluate awareness, knowledge, perceptions, and use of electronic cigarettes and heated tobacco products (HTPs) among adults attending a university hospital and to identify independent factors associated with ever use of these products.

METHODS: This cross-sectional study included 384 adults aged ≥18 years attending the outpatient clinics of a university hospital in Turkiye. Data were collected using a structured questionnaire assessing sociodemographic characteristics, smoking status, awareness, knowledge, perceptions, and use of electronic cigarettes and HTPs. Descriptive statistics, Chi-square and Mann–Whitney U-tests, and multivariable logistic regression analyses were performed to identify factors independently associated with ever use of electronic cigarettes and HTPs.

RESULTS: Awareness of electronic cigarettes was substantially higher than that of HTPs (90.4% vs. 37.5%), while ever use was reported by 38.0% and 10.4% of participants, respectively. More than half of the participants lacked knowledge regarding the harmfulness, addictiveness, legal status, and smoking cessation role of HTPs. Current smokers were significantly more likely than non-smokers to perceive electronic cigarettes and HTPs as less harmful, less addictive, and useful for smoking cessation (all $p<0.05$). In multivariable logistic regression analyses, increasing age was associated with lower odds of ever use of electronic cigarettes (adjusted odds ratio [AOR]: 0.91,95% confidence interval [CI]: 0.89–0.94) and HTPs (AOR: 0.93, 95% CI: 0.89–0.97). Male sex (electronic cigarettes: AOR: 3.85, 95% CI: 2.18–6.82; HTPs: AOR: 2.65, 95% CI: 1.25–5.62) and current cigarette smoking (electronic cigarettes: AOR: 10.41, 95% CI: 5.57–19.46; HTPs: AOR: 3.88, 95% CI: 1.77–8.49) were independently associated with ever use of both products.

CONCLUSION: Although awareness of electronic cigarettes was high, awareness of HTPs was considerably lower, and substantial knowledge gaps and misconceptions regarding HTPs persisted. Current smokers were more likely to hold favorable perceptions of both products, and younger age, male sex, and current cigarette smoking were independently associated with ever use. These findings underscore the need for evidence-based public health interventions to improve public knowledge, correct misconceptions regarding the harmfulness and smoking cessation role of these products, and support tobacco control strategies aimed at preventing the uptake of emerging tobacco products.

Keywords: Attitudes; electronic cigarettes; health knowledge; heated tobacco products; practice; public health; tobacco use.

Cite this article as: *Arslan Torba T, Onsuz MF, Akbay F, Er O, Sahin B, Agit FB, et al. Awareness, knowledge, perceptions, and use of electronic cigarettes and heated tobacco products among adults attending a university hospital: A cross-sectional study. North Clin Istanb 2026;13(5):558–567.*

Received: August 10, 2026 *Revised:* August 21, 2026 *Accepted:* September 09, 2026 *Online:* October 19, 2026

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Istanbul Provincial Directorate of Health - Available online at www.northclinist.com

In recent years, the use of new-generation tobacco and nicotine products, such as electronic cigarettes and heated tobacco products (HTPs), has increased worldwide, making these products increasingly visible [1, 2]. Electronic cigarettes and HTPs are often promoted and marketed as less harmful alternatives to conventional cigarettes. However, accumulating evidence indicates that these products are not harmless. Electronic cigarette use has been associated with adverse respiratory and cardiovascular effects, including airway inflammation and vascular and endothelial dysfunction. Similarly, HTP use has been associated with inflammatory responses, impaired respiratory function, and adverse cardiovascular effects. Both products can also sustain nicotine dependence. Although uncertainties remain regarding their long-term health effects, current evidence raises important concerns about their potential health risks [1, 3–6].

Electronic cigarettes deliver nicotine through aerosolized liquids and are widely perceived as less harmful alternatives to conventional cigarettes [7]. The main reasons for using electronic cigarettes include the belief that they are less harmful than conventional cigarettes, the desire to quit or reduce smoking, the availability of a variety of flavors, the influence of social circles, and curiosity [8].

HTPs are devices that heat tobacco to approximately 350°C to produce a nicotine-containing aerosol without combustion and are therefore referred to as "heat-not-burn" products [9]. The reasons for HTP use include the desire to quit or reduce smoking, the belief that these products are less harmful than conventional cigarettes, and the preference for their taste and flavor [10]. However, uncertainties remain regarding the long-term health effects of these products, while evidence supporting their effectiveness for smoking cessation remains limited, and discrepancies may exist between public perceptions and the available evidence [1].

Misperceptions regarding the safety of novel tobacco and nicotine products may contribute to experimentation and continued use, particularly among young adults. Therefore, understanding public awareness, knowledge, and perceptions is essential for informing tobacco control policies and public health interventions [1, 4]. In particular, HTPs have been investigated to a more limited extent than electronic cigarettes in terms of public awareness, knowledge, perceptions, and use, as they are relatively newer products.

Although several studies have examined electronic cigarette use in Turkiye, studies simultaneously evaluating awareness, knowledge, perceptions, and use of both electronic cigarettes and HTPs remain limited. This study aimed to evaluate awareness, knowledge, perceptions, and use of these products among adults attending the outpatient clinics of a university hospital and to identify factors associated with ever use.

Highlight key points

- Awareness of electronic cigarettes was substantially higher than awareness of HTPs among adults attending a university hospital.
- Considerable uncertainty and misconceptions regarding the harmfulness, addictiveness, legal status, and smoking cessation role of HTPs were identified.
- Current cigarette smokers had more favorable perceptions of electronic cigarettes and HTPs and reported higher lifetime use than non-smokers.
- Younger age, male sex, and current cigarette smoking were independently associated with ever use of both electronic cigarettes and HTPs.
- Evidence-based public health communication is needed to correct misconceptions and improve knowledge regarding novel tobacco products.

MATERIALS AND METHODS

Study Design and Setting

This cross-sectional study was conducted between April 2025 and June 2025 among adults aged ≥18 years attending the outpatient clinics of a university hospital in Eskisehir, Turkiye. The required sample size was calculated using Epi Info software. Assuming an expected awareness prevalence of 50% regarding electronic cigarettes and HTPs, a 95% confidence level, and a 5% margin of error, the minimum required sample size was determined to be 384 participants.

Individuals waiting in the outpatient clinic waiting areas were informed about the purpose and subject of the study. Those who agreed to participate provided both written and verbal informed consent. Participants completed a structured questionnaire under the supervision of medical students involved in the data collection process. Completion of the questionnaire took approximately 10–15 min.

Ethical Considerations

Ethical approval for the study was obtained from the Non-Interventional Clinical Research Ethics Commit-

tee of Eskisehir Osmangazi University (Approval No. E-25403353-050.04-250201638, dated March 27, 2025). Written permission to collect the study data was also obtained from the Chief Physician's Office of the university hospital where the study was conducted. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki. Written ad verbal informed consent was obtained from all participants before their participation in the study.

Data Collection

A questionnaire was developed based on the literature and used as the data collection instrument in this study [11–15]. The questionnaire consisted of five sections.

The first section included questions assessing the participants' sociodemographic characteristics, including age, sex, marital status, educational level, employment status, household composition, and household income.

The second section included questions on factors that might be associated with electronic cigarette and HTP use, including a history of chronic or psychiatric disease, alcohol consumption, and conventional cigarette smoking status.

The third section assessed participants' awareness, sources of information, and use of electronic cigarettes and HTPs. Participants were asked whether they had previously heard of these products, their sources of information, whether they had ever tried them, and their current use status.

The fourth section consisted of statements regarding electronic cigarettes and HTPs. Participants were asked to evaluate issues related to the perceived harmfulness of these products, their addictiveness, their role in smoking cessation, the risks associated with passive exposure, and their legal status.

The fifth section assessed participants' opinions regarding the reasons for using electronic cigarettes and HTPs. Participants were asked about potential motivations for use, including smoking cessation, the belief that these products are less harmful, social influences, curiosity, flavor characteristics, and the perception that they can be used in indoor environments.

Definitions

Participants who smoked at least one cigarette per day were classified as current smokers [3].

Awareness of electronic cigarettes and HTPs was assessed separately using a single question for each product asking whether the participant had previously heard of the respective product. Participants who responded "yes" were classified as being aware of that product.

Ever use of electronic cigarettes or HTPs was defined as having used the respective product at least once during the participant's lifetime.

Statistical Analysis

The data were analyzed using IBM Statistical Package for the Social Sciences Statistics version 31.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were presented as frequencies, percentages, means, standard deviations, medians, and interquartile ranges, as appropriate. The normality of continuous variables was assessed using the Kolmogorov–Smirnov test. The Mann–Whitney U-test was used to compare continuous variables, and the Pearson Chi-square test was used to analyze categorical variables. When appropriate, Bonferroni-corrected post hoc Z-tests were performed.

Separate multivariable logistic regression models were constructed to identify factors associated with ever use of electronic cigarettes and HTPs. Variables considered clinically and epidemiologically relevant based on the study findings and previous literature were included as independent variables in the models. The results of the logistic regression analyses were reported as adjusted odds ratios (AORs) with 95% confidence intervals (CIs). $p \leq 0.05$ was considered statistically significant.

RESULTS

Sociodemographic and Health Characteristics

A total of 384 individuals participated in the study, of whom 59.6% were women. The mean age of the participants was 36.78±15.78 years (range: 18–79 years). Overall, 40.9% of the participants were current smokers. A history of chronic disease was reported by 34.9% of the participants, while 12.5% reported a history of psychiatric disease. In addition, 69.3% of the participants reported living with their families. The sociodemographic and health characteristics of the study population are presented in Table 1.

Awareness, Sources of Information, and Use of Electronic Cigarettes and HTPs

Awareness of electronic cigarettes was higher than that of HTPs. Overall, 90.4% of the participants reported having previously heard of electronic cigarettes, whereas

Table 1. Sociodemographic and health characteristics of the study participants (n=384)

Variable	Category	Percentage
Sex	Female	59.6
	Male	40.4
Marital status	Married	50.3
	Single	44.0
	Other (divorced/widowed)	5.7
Educational level	University or Higher	54.2
	High school	31.0
	Other (primary/middle school)	14.8
Employment status	Unemployed	55.7
	Employed	44.3
Living arrangement	With family	69.3
	With friends/peers	15.1
	Alone	15.6
Income level	Low	8.6
	Middle	85.4
	High	6.0
History of chronic disease	No	65.1
	Yes	34.9
History of psychiatric disease	No	87.5
	Yes	12.5
Smoking status	Current smoker (≥1 cigarette/day)	40.9
	Former smoker	2.6
	Never smoker	56.5

37.5% had heard of HTPs. The proportion of participants who reported ever using electronic cigarettes was 38.0%, compared with 10.4% for HTPs. Current use was 5.2% for electronic cigarettes and 1.3% for HTPs. Friends and social circles were the primary source of information about electronic cigarettes (60.2%), whereas the internet and social media were the primary source for HTPs (18.2%). Data on awareness, sources of information, and use of electronic cigarettes and HTPs are presented in Table 2.

Awareness and Use According to Smoking Status

Current smokers demonstrated significantly higher awareness and use of electronic cigarettes than non-smokers. Awareness of electronic cigarettes was higher among current smokers than non-smokers (96.2% vs. 86.3%, p=0.001), whereas awareness of HTPs did not differ significantly between the two groups (42.7% vs. 33.9%, p=0.081).

Similarly, ever use of both products was significantly more common among current smokers than non-smokers. Ever use of electronic cigarettes was reported by 61.8% of current smokers compared with 21.6% of non-smokers, while ever use of HTPs was reported by 17.8% and 5.3% of the two groups, respectively (both p<0.001). Detailed findings are presented in Table 3.

Knowledge and Perceptions Regarding Electronic Cigarettes and HTPs

Participants showed substantially greater uncertainty regarding HTPs than electronic cigarettes across all knowledge and perception statements. For HTPs, "don't know" responses predominated for all statements, particularly those regarding legal status, addictiveness, harmfulness, smoking cessation, and passive exposure.

When responses were compared according to smoking status, current smokers demonstrated significantly more favorable perceptions of both electronic cigarettes and HTPs than non-smokers. They were more likely to perceive these products as less addictive, less harmful, associated with lower risks from passive exposure, and useful for smoking cessation (all p<0.05), whereas perceptions regarding their legal status did not differ significantly between the groups. Detailed findings are presented in Table 4.

Perceived Reasons for Using Electronic Cigarettes and HTPs

Participants more frequently associated electronic cigarettes than HTPs with several potential reasons for use. The most commonly reported reasons were taste, flavor, and smell, followed by smoking cessation and the belief that these products are less harmful than conventional cigarettes. In contrast, substantially fewer participants associated HTPs with these reasons. Detailed findings are presented in Table 5.

Factors Associated with Ever Use of Electronic Cigarettes and HTPs

Multivariable logistic regression analyses showed that younger age, male sex, and current cigarette smoking were independently associated with ever use of both electronic cigarettes and HTPs. Each 1-year increase in age was associated with lower odds of ever using electronic cigarettes (AOR=0.91, 95% CI: 0.89–0.94, p<0.001) and HTPs

Table 2. Awareness, sources of information, and use of electronic cigarettes and HTPs (n=384)

Variables	HTPs (%)	Electronic cigarettes (%)
Never heard of	62.5	9.6
Primary source of information	Social media 18.2	Friends and social circles 60.2
Never used	89.6	62.0
Ever used	10.4	38.0
Current users	1.3	5.2

HTPs: Heated tobacco products.

Table 3. Comparison of awareness and use of electronic cigarettes and HTPs according to smoking status (n=384)

Electronic cigarette and HTP status	Current smokers (n=157) (%)	Non-smokers* (n=227) (%)	Total (%)	χ^2/p
Awareness of HTPs				
No	57.3	66.1	62.5	χ^2=3.035
Yes	42.7	33.9	37.5	p=0.081
Awareness of electronic cigarettes				
No	3.8	13.7	9.6	χ^2=10.310
Yes	96.2	86.3	90.4	p=0.001
Ever use of electronic cigarettes				
No	38.2	78.4	62.0	χ^2=63.639
Yes	61.8	21.6	38.0	p<0.001
Ever use of HTPs				
No	82.2	94.7	89.6	χ^2=15.660
Yes	17.8	5.3	10.4	p<0.001

*: Non-smokers included both former smokers and never smokers; HTPs: Heated tobacco products.

(AOR=0.93, 95% CI: 0.89–0.97, p<0.001). Compared with women, men had higher odds of ever using electronic cigarettes (AOR=3.85, 95% CI: 2.18–6.82, p<0.001) and HTPs (AOR=2.65, 95% CI: 1.25–5.62, p=0.011). Current cigarette smokers also had higher odds of ever using electronic cigarettes (AOR=10.41, 95% CI: 5.57–19.46, p<0.001) and HTPs (AOR=3.88, 95% CI: 1.77–8.49, p<0.001) than non-smokers. History of psychiatric disease and living arrangement were not independently associated with ever use of either product (Table 6).

DISCUSSION

In this study, awareness of electronic cigarettes was found to be considerably higher than awareness of HTPs. However, substantial knowledge gaps and uncertainty were identified regarding the addictiveness, harmfulness, role in smoking cessation, and legal status of HTPs. The findings also showed that perceptions that electronic cigarettes and HTPs are less harmful than conventional cigarettes and can be used for smoking cessation remain prevalent among the study population. Furthermore, current smokers had more favorable perceptions of these novel products than non-smokers, while younger age, male sex, and current cigarette smoking were independently associated with ever use of both electronic cigarettes and HTPs.

Our findings showed that electronic cigarettes were substantially more widely recognized than HTPs. While electronic cigarettes have become well-known products among the general population, awareness of HTPs remained limited, with considerable uncertainty regarding their health

Table 4. Knowledge and perceptions regarding electronic cigarettes and HTPs according to smoking status

Statements	Current smokers (n=157) (%)	Non-smokers* (n=227) (%)	Total (n=384) (%)	χ^2/p
"The import and sale of electronic cigarettes are prohibited in Türkiye."				
Agree	35.7	37.0	36.5	
Disagree	28.0	23.8	25.5	χ^2=0.904
Don't know	36.3	39.2	38.0	p=0.636
"The import and sale of HTPs are prohibited in Türkiye."				
Agree	21.7	24.7	23.4	
Disagree	15.3	14.1	14.6	χ^2=0.500
Don't know	63.1	61.3	62.0	p=0.779
"Electronic cigarettes may be less addictive."				
Agree	29.3[b]	15.4[b]	21.1	
Disagree	49.7[a]	52.9[a]	51.6	χ^2=12.545
Don't know	21.0[a]	31.7[a]	27.3	p=0.002
"HTPs may be less addictive."				
Agree	14.0[b]	6.2[b]	9.4	
Disagree	33.1[a]	37.4[a]	35.7	χ^2=6.789
Don't know	52.9[a]	56.4[a]	54.9	p=0.034
"Electronic cigarettes may be less harmful."				
Agree	25.5[b]	12.3[b]	17.7	
Disagree	49.0[a]	58.1[a]	54.4	χ^2=11.010
Don't know	25.5[a]	29.5[a]	27.9	p=0.004
"HTPs may be less harmful."				
Agree	18.5[b]	6.6[b]	11.5	
Disagree	31.2[a]	39.6[a]	36.2	χ^2=13.433
Don't know	50.3[a]	53.7[a]	52.3	p=0.001
"The aerosol from electronic cigarettes is less harmful."				
Agree	30.6[b]	13.2[b]	20.3	
Disagree	40.8[a]	52.4[a]	47.7	χ^2=17.354
Don't know	28.7[a]	34.4[a]	32.0	p<0.001
"The aerosol from HTPs is less harmful."				
Agree	16.6[b]	6.2[b]	10.4	
Disagree	30.6[a]	41.0[a]	36.7	χ^2=12.356
Don't know	52.9[a]	52.9[a]	52.9	p=0.002
"Smoking cessation can be achieved using electronic cigarettes."				
Agree	29.3[b]	14.1[b]	20.3	
Disagree	52.9[a]	58.1[a]	56.0	χ^2=14.876
Don't know	17.8[a]	27.8[a]	23.7	p<0.001
"Smoking cessation can be achieved using HTPs."				
Agree	15.9[b]	7.5[b]	10.9	
Disagree	32.5[a]	41.4[a]	37.8	χ^2=7.999
Don't know	51.6[a,b]	51.1[a,b]	51.3	p=0.018

Within each statement, columns with different superscript letters (a, b) differ significantly according to Bonferroni-adjusted post hoc comparisons of column proportions (Post hoc Z-test) (p<0.05). Columns sharing the same superscript letter do not differ significantly. HTPs: Heated tobacco products; *: Non-smokers included both former smokers and never smokers.

Table 5. Perceived reasons for using electronic cigarettes and HTPs

Reasons for use	Electronic cigarettes n (%)	HTPs n (%)	Both n (%)	Don't know n (%)
Taste/flavor/smell	254 (66.1)	39 (10.2)	40 (10.4)	51 (13.3)
Smoking cessation	253 (65.9)	36 (9.4)	53 (13.8)	42 (10.9)
Belief that they are less harmful than conventional cigarettes	249 (64.8)	48 (12.5)	35 (9.1)	52 (13.5)
Belief that they are less harmful to bystanders	238 (62.0)	46 (12.0)	31 (8.1)	69 (18.0)
Curiosity/social influence/perceived attractiveness	233 (60.7)	41 (10.7)	69 (18.0)	41 (10.7)
Belief that they can be used where cigarette smoking is prohibited	230 (59.9)	37 (9.6)	44 (11.5)	73 (19.0)
Lower cost	188 (49.0)	80 (20.8)	13 (3.4)	103 (26.8)

HTPs: Heated tobacco products.

Table 6. Multivariable logistic regression analysis of factors associated with ever use of electronic cigarettes and HTPs

Variable	Ever use of electronic cigarettesAOR (95% CI)	p	Ever use of HTPs AOR (95% CI)	p
Age (years)	0.91 (0.89–0.94)	<0.001	0.93 (0.89–0.97)	<0.001
Sex				
Female (ref)	1.00	–	1.00	–
Male	3.85 (2.18–6.82)	<0.001	2.65 (1.25–5.62)	0.011
Current cigarette smoking				
No (ref)	1.00	–	1.00	–
Yes	10.41 (5.57–19.46)	<0.001	3.88 (1.77–8.49)	<0.001
History of psychiatric disease				
No (ref)	1.00	–	1.00	–
Yes	1.99 (0.89–4.46)	0.095	2.11 (0.84–5.33)	0.113
Living arrangement				
Family (ref)	1.00	–	1.00	–
Friends/peers	1.51 (0.69–3.28)	0.303	1.37 (0.51–3.65)	0.533
Living alone	1.79 (0.82–3.88)	0.144	1.97 (0.79–4.91)	0.144

HTPs: Heated tobacco products; AOR: Adjusted odds ratio; CI: Confidence interval.

effects, addictive potential, role in smoking cessation, and legal status. A large-scale study conducted across 11 European countries reported that only 27.8% of the population was aware of HTPs [2]. Consistent with these findings, a national study conducted in Pakistan also reported limited awareness and knowledge regarding HTPs [12]. In contrast, in South Korea, where HTPs have been available on the market for a longer period, the use of these products has been reported to be more widespread [16]. These differences may be related to variations in regulatory policies, marketing practices, and product availability across countries. Nevertheless, the inability of a substantial proportion of participants to express an opinion regarding the basic characteristics of HTPs suggests that improving public knowledge about these products should be an important component of tobacco control strategies.

In the present study, novel tobacco products were perceived as being less harmful and less addictive than conventional cigarettes and as potentially helpful for smoking cessation. These perceptions may be related to the tobacco

industry's marketing of electronic cigarettes and HTPs as "alternative" or "harm reduction" products [3]. However, current evidence indicates that HTPs are not harmless products. Their aerosols may contain toxic and carcinogenic substances, they can sustain nicotine dependence, and uncertainties regarding their long-term health effects remain [3, 9, 17]. In addition, evidence supporting the role of HTPs in smoking cessation is limited. A recent systematic review reported that the certainty of the evidence supporting the effectiveness of HTPs in promoting smoking cessation or reducing smoking prevalence was low [4]. The U.S. Centers for Disease Control and Prevention also states that HTPs are not an food and drug administration-approved method for smoking cessation [18]. Furthermore, a recent systematic review and meta-analysis found that more than two-thirds of HTP users continued to smoke conventional cigarettes concurrently, suggesting that HTPs are not supported as an effective smoking cessation aid under real-world conditions [19]. Consistent with these findings, participants in our study most frequently associated novel tobacco products with being less harmful than conventional cigarettes and with helping people quit smoking. Similarly, studies conducted in Europe and Germany have reported that perceived harm reduction and the desire to quit or reduce cigarette smoking are among the primary reasons for HTP use [10, 20]. Therefore, correcting these misconceptions through evidence-based public health communication should be considered a priority for tobacco control.

Current cigarette smokers expressed more favorable views toward electronic cigarettes and HTPs than non-smokers. Compared with non-smokers, current smokers were more likely to agree that both products are less harmful and less addictive than conventional cigarettes and may help with smoking cessation. Consistent with our findings, studies from different countries have reported that electronic cigarettes and HTPs are often perceived as less harmful alternatives to conventional cigarettes and that expectations of harm reduction constitute an important motivation for their use [2, 12, 21, 22]. This may reflect smokers' desire to reduce health risks or quit smoking, leading them to view these products more favorably. Alternatively, it may be associated with tobacco industry marketing strategies specifically targeting current cigarette smokers. Nevertheless, these differences in risk perception suggest that knowledge and perceptions regarding novel tobacco products differ between smokers and non-smokers, with smokers tending to hold more favorable views of these products.

In the multivariable analysis, younger age, male sex, and current cigarette smoking were independently associated with ever use of both electronic cigarettes and HTPs. These findings are consistent with the current literature on the determinants of novel tobacco product use. In a meta-analysis, Scala et al. [19] demonstrated that male sex, younger age, and current cigarette smoking were significantly associated with HTP use, with current smokers having approximately 15-fold higher odds of using HTPs than never smokers. Similarly, in a study conducted across 28 European countries, Laverty et al. [10] reported that HTP use was more common among younger individuals and current cigarette smokers. In a recent study from Germany, Keller et al. [20] found that HTP users were predominantly young men and that a substantial proportion of users continued to smoke conventional cigarettes. Likewise, Miller et al. [23] showed that HTP users in four high-income countries were generally younger individuals, the majority of whom also used cigarettes and/or electronic cigarettes. Younger adults may be more likely to try electronic cigarettes and HTPs due to greater interest in newer tobacco and nicotine products. The higher use observed among men may be related to differences in tobacco-use patterns between men and women. For current smokers, trying these products may be linked to seeking alternatives to conventional cigarettes, particularly when they are perceived as less harmful or as potentially helpful for quitting smoking. This interpretation is also consistent with the more favorable perceptions of these products observed among current smokers in our study.

These findings have practical implications for tobacco control interventions. Healthcare professionals could provide brief, evidence-based counseling, particularly to young adults and current smokers, addressing misconceptions that electronic cigarettes and HTPs are safer alternatives to conventional cigarettes or effective smoking-cessation aids. Individuals who wish to quit smoking could also be referred to smoking-cessation clinics for appropriate cessation support.

Strengths and Limitations

This study has several strengths and limitations. Its main strengths include the comprehensive assessment of awareness, knowledge, perceptions, and ever use of electronic cigarettes and HTPs, as well as being one of the few studies conducted in Turkiye to examine HTPs alongside electronic cigarettes. However, several limita-

tions should be acknowledged. First, the cross-sectional design precludes any inference of causal relationships between the variables. Second, because participants were recruited among individuals attending the outpatient clinics of a single university hospital, the possibility of selection bias cannot be excluded, and the findings may not be generalizable to the broader population. Third, because all information was self-reported, the possibility of information bias, including recall and social desirability bias, cannot be excluded.

Conclusion

This study identified substantial knowledge gaps and uncertainty regarding HTPs despite the high level of awareness of electronic cigarettes. In addition, electronic cigarettes and HTPs were frequently perceived as safer alternatives to conventional cigarettes and as potential smoking cessation aids. These findings highlight a discrepancy between the current scientific evidence and public perceptions of these products.

The findings underscore the need for population-based educational and awareness initiatives addressing novel tobacco products. In particular, disseminating up-to-date, evidence-based information on the health effects, addictive potential, and effectiveness of electronic cigarettes and HTPs for smoking cessation, especially among young people and current cigarette smokers, is essential. Furthermore, population-based longitudinal studies investigating the prevalence, perceptions, and motivations for the use of novel tobacco products are needed to inform and strengthen tobacco control policies. Preventing the marketing of novel tobacco and nicotine products as safe alternatives and correcting public misconceptions through evidence-based risk communication should be prioritized within tobacco control policies.

Ethics Committee Approval: This study was approved by The Non-Interventional Clinical Research Ethics Committee of Eskisehir Osmangazi University (Approval No. E-25403353-050.04-250201638; 27.03.2025).

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 received no financial support.

Use of AI for Writing Assistance: During the preparation of this manuscript, the authors used ChatGPT (OpenAI) solely to assist with translation and language editing.

Authorship Contributions: Concept – MFO; Design – TAT, MFO, FA, OE; Supervision – MFO; Data Collection and/or Processing – FA, DE, BS, FBA, TOB, FS; Analysis and/or Interpretation – TAT, MFO; Literature Search – TAT, FA; Writing – TAT, OE; Critical Reviews – TAT, MFO, FA, OE, BS, FBA, TOB, FS.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2026;13(5):568–576
doi: 10.14744/nci.2026.71419

Original Article PHYSIOLOGY

Periovulatory decline in circulating neutrophils coincides with a low-density lipoprotein-cholesterol rise in healthy young women: A paired within-subject study

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ABSTRACT

OBJECTIVE: Prior experimental work indicated that neutropenia selectively elevates circulating low-density lipoprotein (LDL) cholesterol in male but not female mice, suggesting that estrogen may attenuate a neutrophil-associated mechanism of LDL regulation. Whether a comparable phase- and sex-dependent association between neutrophils and LDL is detectable in healthy humans has not been examined.

METHODS: Eleven healthy women (age 21.5 years, interquartile range 20.0–23.5) were sampled during menstruation and again at the luteinizing hormone-confirmed pre-ovulatory/ovulatory phase. Thirteen healthy men were sampled on matched dates. Serum LDL-cholesterol (LDL-C), a five-part leukocyte differential, and reproductive hormones were measured at each time point. Paired phase comparisons used the Wilcoxon signed-rank test, and within-subject deltas (Δ=Ovu − Mens) were analyzed by Spearman correlation.

RESULTS: In women, the neutrophil percentage fell from menstruation to ovulation (p=0.005), the absolute neutrophil count declined in parallel without reaching significance (p=0.183), and serum LDL-C rose (p=0.002). The lymphocyte count rose reciprocally (p=0.024). The within-subject decrease in neutrophils tended to accompany the LDL rise inversely, although this association did not reach statistical significance at the available sample size. Among abundant subsets, the inverse trend was neutrophil-selective. Δlymphocyte, Δmonocyte, and Δeosinophil counts showed no relation to ΔLDL. Δestradiol did not predict ΔLDL (ρ=-0.22, p=0.542), indicating the neutrophil–LDL trend is not an estradiol proxy. No phase-linked LDL or neutrophil change occurred in men, and the menstrual-phase sex difference in LDL-C (women lower; p=0.038) was no longer significant at ovulation (p=0.622).

CONCLUSION: In healthy young women, a periovulatory decline in circulating neutrophils coincides with a rise in LDL cholesterol, and the two within-subject changes tend to move in opposite directions, although this inverse association did not reach statistical significance in a sample of this size. The trend is neutrophil-selective among abundant leukocytes, is not explained by estradiol, and is absent in non-cycling men. These observational data are compatible with but do not establish a contribution of peripheral neutrophils to LDL turnover that is attenuated during the high-estradiol ovulatory window. Causal and mechanistic inferences require interventional and functional studies in larger cohorts.

Keywords: Estrogen; low-density lipoprotein cholesterol; neutrophils.

Cite this article as: *Sevim M, Altinoluk T, Kahraman MM, Caglayan Yegen B. Periovulatory decline in circulating neutrophils coincides with a low-density lipoprotein cholesterol rise in healthy young women: A paired within-subject study. North Clin Istanb 2026;13(5):568–576.*

Received: August 03, 2026 *Revised:* September 18, 2026 *Accepted:* September 23, 2026 *Online:* October 16, 2026

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The bidirectional relationship between lipid metabolism and innate immune cell trafficking has attracted considerable interest [1, 2]. It is well established that dyslipidemia drives neutrophilia by expanding granulopoiesis through cholesterol-mediated modulation of CXCR4 surface expression on hematopoietic stem cells and by potentiating pro-inflammatory CXCR2 ligand signaling [3, 4]. This unidirectional model, in which altered cholesterol reshapes neutrophil kinetics, has been extensively validated. Far less explored is the reverse direction: whether changes in the circulating neutrophil pool are associated with plasma low-density lipoprotein (LDL) levels. The daily turnover of approximately 1–2×1011 neutrophils [5] represents a substantial lipid flux, and clinical observations suggest a neutrophil–cholesterol coupling. Fraunberger et al. [6] reported reduced serum cholesterol in neutropenic febrile patients. Our own pre-clinical investigation [7] extended this by experimentally manipulating neutrophil counts in C57BL/6 mice. In this study, anti-neutrophil-serum-induced neutropenia and filgrastim-induced neutrophilia both altered total blood cholesterol, with the LDL fraction implicated. Notably, while both manipulations elevated total cholesterol in males, in females only neutrophilia – not neutropenia – produced a significant rise, a sex dissociation that prompted the present question: what protects females from the neutropenia-associated LDL elevation seen in males?

Estrogen is a biologically plausible candidate. Premenopausal women exhibit systematically lower circulating LDL than age-matched men, a disparity that narrows after menopause [8, 9]. Estradiol exerts well-characterized, though context-dependent, effects on neutrophil biology. Estrogen can suppress bone-marrow leukocyte production and promote redistribution of neutrophils out of the circulating pool [10], and, via estrogen receptor alpha (ERα) signaling, it blocks neutrophil adhesion to the vascular endothelium [11]. These actions are not uniform. In tumor-bearing settings, estradiol has instead been reported to augment neutrophil production via ERα [12], so the net direction of estrogenic action on the circulating neutrophil pool is tissue- and context-specific. In parallel, estrogen enhances hepatic LDL-receptor expression, upregulates CYP7A1, and augments reverse cholesterol transport, which has effects tending to lower LDL and raise high-density lipoprotein (HDL) [13, 14].

The menstrual cycle provides a within-subject model in which estradiol rises physiologically from a menstrual low to a pre-ovulatory peak. Circulating neutrophil numbers are themselves under rhythmic (circadian) control [15], and leukocyte counts have been reported to vary across the menstrual cycle, though not consistently in direction [16].

We therefore tested whether the pre-ovulatory estradiol rise is accompanied by a within-subject change in circulating neutrophils that is associated with LDL, whether any such association is specific to neutrophils and independent of estradiol, and whether it diverges from the pattern in non-cycling men.

Highlight key points

- During the menstrual-to-ovulatory transition in healthy young women, the circulating neutrophil percentage significantly decreases, whereas serum LDL cholesterol levels rise, with a parallel decline in absolute neutrophil count.
- This decline shows a moderate within-subject inverse correlation with the rise in LDL cholesterol that is specific to neutrophils and absent in other abundant leukocyte subsets, though it does not reach statistical significance at this sample size.
- The concurrent estradiol rise does not itself predict the LDL change, indicating the neutrophil–LDL relationship is not simply a proxy for estradiol.
- These phase-linked changes are absent in non-cycling men, leading to a temporary convergence of male and female LDL cholesterol levels during the ovulatory window.

MATERIALS AND METHODS

Study Design and Participants

This observational paired repeated-measures study followed the Declaration of Helsinki and was approved by the Marmara University Clinical Research Ethics Committee (protocol 09.2020.1051). All participants gave written informed consent.

Eighteen healthy women aged 18–25 years with regular cycles (25–35 days), not using hormonal contraception, with no metabolic, cardiovascular, immunological, or gynecological disease were recruited. Seven were subsequently excluded because of pre-analytical or assay-related sample-quality problems that rendered one or both of their paired measurements unusable, leaving 11 women in the analyzed dataset. Thirteen healthy men of comparable age served as a non-cycling reference group; one man did not provide a usable second sample, so 12 men contributed paired data. Among women, 10 had paired LDL-cholesterol (LDL-C) measurements; among men, 11 had paired estradiol measurements, and 10 had paired follicle-stimulating hormone (FSH) measurements. Ex-

Table 1. Paired phase comparisons within sex (primary parameters)

Variable	Mens	Ovu	Δ	Test	p	dz
Women (n=11; n=10 for LDL-C)						
Estradiol (pg/mL)	32.3±15.6	88.1±77.4	+55.8	W=3	**0.005**	0.74
LH (IU/L)	6.4±5.3	11.3±9.2	+4.8	W=6	**0.014**	0.76
Neutrophil count (×10⁹/L)	4.37±1.00	3.61±1.16	-0.76	W=17.5	0.183	-0.48
Neutrophil %	63.0±8.4	54.6±8.8	-8.5	W=3	**0.005**	-1.29
LDL-C (mg/dL)	77.4±17.3	89.5±19.0	+12.1	W=0	**0.002**	1.81
CRP (mg/L)	1.86±2.92	0.39±0.26	-1.47	W=15	0.123	-0.50
WBC (×10⁹/L)	6.90±0.96	6.48±1.35	-0.42	W=25	0.504	-0.22
HDL-C (mg/dL)	65.3±14.1	68.5±10.8	+3.2	W=14	0.102	0.55
Men (n=12; n=11 for oestradiol; n=10 for FSH)						
Estradiol (pg/mL)	24.8±10.3	27.3±13.7	+2.5	W=26	0.577	0.21
FSH (IU/L)	5.60±4.67	5.44±4.05	-0.16	W=24	0.770	-0.16
Neutrophil count (×10⁹/L)	3.54±1.08	4.15±1.38	+0.61	W=19	0.129	0.58
Neutrophil %	56.0±6.9	57.7±9.1	+1.7	W=33	0.666	0.24
LDL-C (mg/dL)	99.2±26.6	96.8±25.0	-2.3	W=27	0.621	-0.17
WBC (×10⁹/L)	6.27±1.56	7.09±1.81	+0.83	W=13	**0.040**	0.62

Mean±SD; Wilcoxon signed-rank test; dz=within-subject Cohen's d; bold p<0.05. LDL-C: Low-density lipoprotein cholesterol; WBC: White blood cell; HDL-C: High-density lipoprotein cholesterol; CRP: C-reactive protein; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone.

clusion criteria (both sexes) were body mass index (BMI) >30 kg/m^2, current medication, acute/chronic inflammation, smoking, and blood donation within 3 months.

Sampling and Phase Confirmation

Women were sampled twice: (1) the menstrual phase (days 1–4, confirmed by onset of bleeding; "Mens"); and (2) the pre-ovulatory/ovulatory phase ("Ovu"). Men were sampled on matched dates approximately 14 days apart (Time-1/Time-2) to balance any time-related environmental factors. Adequacy of phase capture was verified post hoc by the significant within-subject estradiol and luteinizing hormone (LH) rise in women.

Measurements

Anthropometry and bioelectrical-impedance body composition were obtained by standard protocols. Fasting (≥10 h) venous blood was drawn at 08:00–10:00 h. A complete blood count with a five-part differential was performed on an automatic hematology analyzer, using a combination of physical, electrical, and optical methods. Total leukocyte count and the percentages and absolute counts of neutrophils, lymphocytes, monocytes, eosinophils, and basophils were recorded. LDL-C, HDL-cholesterol (HDL-C), total cholesterol, triglycerides, and C-reactive protein (CRP) were determined via colorimetric assay using a biochemistry autoanalyzer.

Estradiol, LH, and FSH were measured using a biochemistry autoanalyzer. Estradiol was reported in pg/mL, and LH and FSH in IU/L.

Statistical Analysis

All analyses were conducted in Python 3.10 (NumPy, SciPy, and pandas). Paired within-subject comparisons between the menstrual and ovulatory phases were analyzed with the Wilcoxon signed-rank test, reported as W with the p-value. Descriptive summaries are specified in each table or figure (mean±standard deviation [SD], median [interquartile range (IQR)], or median [range]), together with the mean paired change where reported. Table 1 uses complete pairs for each analyte. Effect sizes for paired tests were computed as Cohen's dz, defined as the mean of the Ovu-minus-Mens difference scores divided by their SD. Comparisons between sexes at a given phase used the Mann–Whitney U test with rank-biserial correlation (r_rb) as effect size, signed so that positive

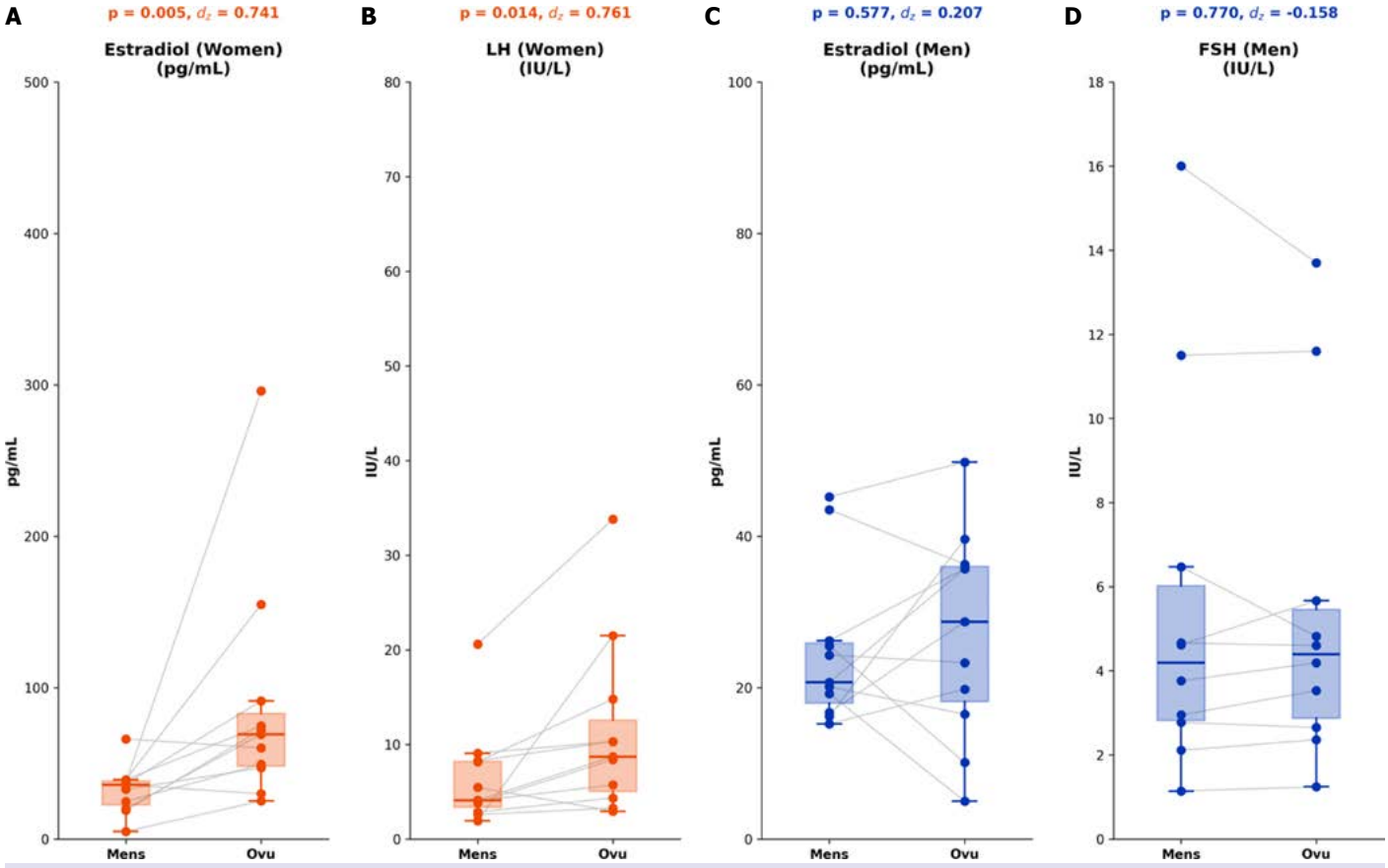

FIGURE 1. Phase-specific hormonal profiles. **(A)** Women's estradiol rose (Δ+55.8 pg/mL; p=0.005, dz=0.74). **(B)** Women's LH rose (Δ +4.8 IU/L; p=0.014, dz=0.76). **(C)** Men's estradiol unchanged (p=0.577). **(D)** Men: follicle-stimulating hormone unchanged (p=0.770). Paired individual trajectories over box plots (median, interquartile range, whiskers); Wilcoxon signed-rank test.

values indicate higher values in women. Spearman rank correlation was used for association analyses, including within-subject delta (Δ=Ovu−Mens) correlations. The pooled male plot in Figure 3C is descriptive: Its 25 observations arise from 13 participants, and no ordinary Spearman inferential p-value is reported because observations within participants are not independent. Each comparison was performed on all participants with complete data for the variables concerned, and the corresponding n is reported throughout. The significance threshold was set at p<0.05. All tests were two-sided.

RESULTS

Participant Characteristics

The study dataset comprised 24 participants (11 women and 13 men; Table 2). Paired analyses included up to 11 women and 12 men because one man did not provide a usable second sample. The available paired sample size varied by analyte: LDL-C analyses included 10 women, and male estradiol and FSH analyses included 11 and 10 men, respectively. Each analysis used participants with complete data for the variables concerned. Age was similar in women (21.5 [IQR 20.0–23.5]) and men (20.0 [IQR 19–23]; p=0.447). As expected, men had higher BMI (24.1 vs. 21.1 kg/m^2, p=0.003), lower body fat (16.5% vs. 24.1%, p=0.002), and greater waist circumference (85.5 vs. 71.0 cm, p<0.001).

Hormonal Validation of Phase

Phase capture was confirmed by within-subject hormonal change in women (Fig. 1A, B): estradiol rose from 32.3±15.6 to 88.1±77.4 pg/mL (Wilcoxon W=3, p=0.005, dz=0.74) and LH from 6.4±5.3 to 11.3±9.2 IU/L (W=6, p=0.014, dz=0.76). The wide ovulatory estradiol distribution (median 69.2 pg/mL) reflects interindividual variation in the precise sampling point relative to the surge. In men, estradiol did not change between matched visits (Fig. 1C, D; Table 1).

Table 2. Participant characteristics (menstrual/time-1 phase)

Variables	Women (n=11)	Men (n=13)	p
Age (years)	21.5 (19–24)	20.0 (18–24)	0.447
BMI (kg/m^2)	21.1 (18.9–23.5)	24.1 (19.5–29.9)	0.003
Body fat (%)	24.1 (15.9–33.4)	16.5 (9.2–24.6)	0.002
Waist (cm)	71.0 (62–77)	85.5 (75–98)	<0.001
LDL-C (mg/dL)	73.5 (58–104)	105.0 (60–137)	0.038
HDL-C (mg/dL)	61.8 (43–91)	51.1 (40–69)	0.049
Total cholesterol (mg/dL)	159.0 (123–208)	163.0 (111–229)	0.602
Triglycerides (mg/dL)	68.2 (28–124)	73.3 (26–128)	0.862
WBC (×10^9/L)	6.7 (5.7–9.2)	5.9 (4.1–9.4)	0.202
Neutrophil count (×10^9/L)	4.2 (3.1–6.6)	3.6 (2.0–5.5)	0.059
Neutrophil %	63.2 (45.5–75.3)	57.5 (42.3–65.2)	0.034
CRP (mg/L)	0.52 (0.17–9.77)	0.49 (0.13–3.10)	0.862

Median (range), Mann–Whitney U. Anthropometric variables were available for 10 of the 11 women, and waist circumference for 12 of the 13 men; menstrual-phase LDL-C was available for 10 of the 11 women. LDL-C: Low-density lipoprotein cholesterol; WBC: White blood cell; HDL-C: High-density lipoprotein cholesterol; BMI: Body mass index; CRP: C-reactive protein.

Phase-Dependent Changes in the Leukocyte Differential and LDL-C in Women

Across the menstrual-to-ovulatory transition (Fig. 2 and Table 1), the neutrophil percentage fell (63.0±8.4→54.6±8.8%; W=3, p=0.005, dz=-1.29), and the absolute neutrophil count declined in parallel, although this change did not reach significance (4.37±1.00→3.61±1.16×10^9/L; W=17.5, p=0.183, dz=-0.48). Reciprocally, the absolute lymphocyte count rose (1.85→2.22×10^9/L; W=8, p=0.024, dz=+0.80), whereas monocyte, eosinophil, and basophil counts were unchanged (Table 3). Serum LDL-C rose (median 73.5→90.0 mg/dL; W=0, p=0.002, mean Δ=+12.1 mg/dL), whereas the fall in CRP (1.86→0.39 mg/L; W=15, p=0.123) did not reach significance. Neither total white blood cell (WBC) nor HDL-C changed.

In men, neutrophil percentage, neutrophil count, and LDL-C were unchanged. Total WBC showed a nominal rise (p=0.040). No change was observed in estradiol levels (Fig. 2D, F, Table 1).

Table 3. Full leukocyte differential, women, paired phase change (absolute counts ×10^9/L; n=11)

Subsets	Mens	Ovu	Δ	p	dz
Neutrophil	4.37	3.61	-0.76	0.183	-0.48
Lymphocyte	1.85	2.22	+0.36	0.024	+0.80
Monocyte	0.48	0.46	-0.02	0.844	-0.17
Eosinophil	0.16	0.17	+0.01	0.922	+0.13
Basophil	0.04	0.05	+0.01	0.230	+0.36

Wilcoxon signed-rank test.

Sex Differences in LDL-C: Phase-Dependent Convergence

At the menstrual/time-1 phase, LDL-C was lower in women than men (median 73.5 vs. 105.0 mg/dL; U=31, p=0.038, r_rb=-0.52). By ovulation, the sex difference was no longer significant (91.0 vs. 95.0 mg/dL; U=57.5, p=0.622, r_rb=-0.13). This convergence was specific to LDL. HDL-C remained higher in women at both phases (p=0.049; p=0.006), and the menstrual-phase female excess in neutrophil percentage (p=0.034), with a comparable trend in neutrophil count (p=0.059), disappeared by ovulation (Table 4).

Within-Subject Neutrophil–LDL Association, Specificity, and Estradiol-Independence

The primary analysis (change in absolute neutrophil count vs. change in LDL-C in women) showed a moderate inverse association that did not reach statistical sig-

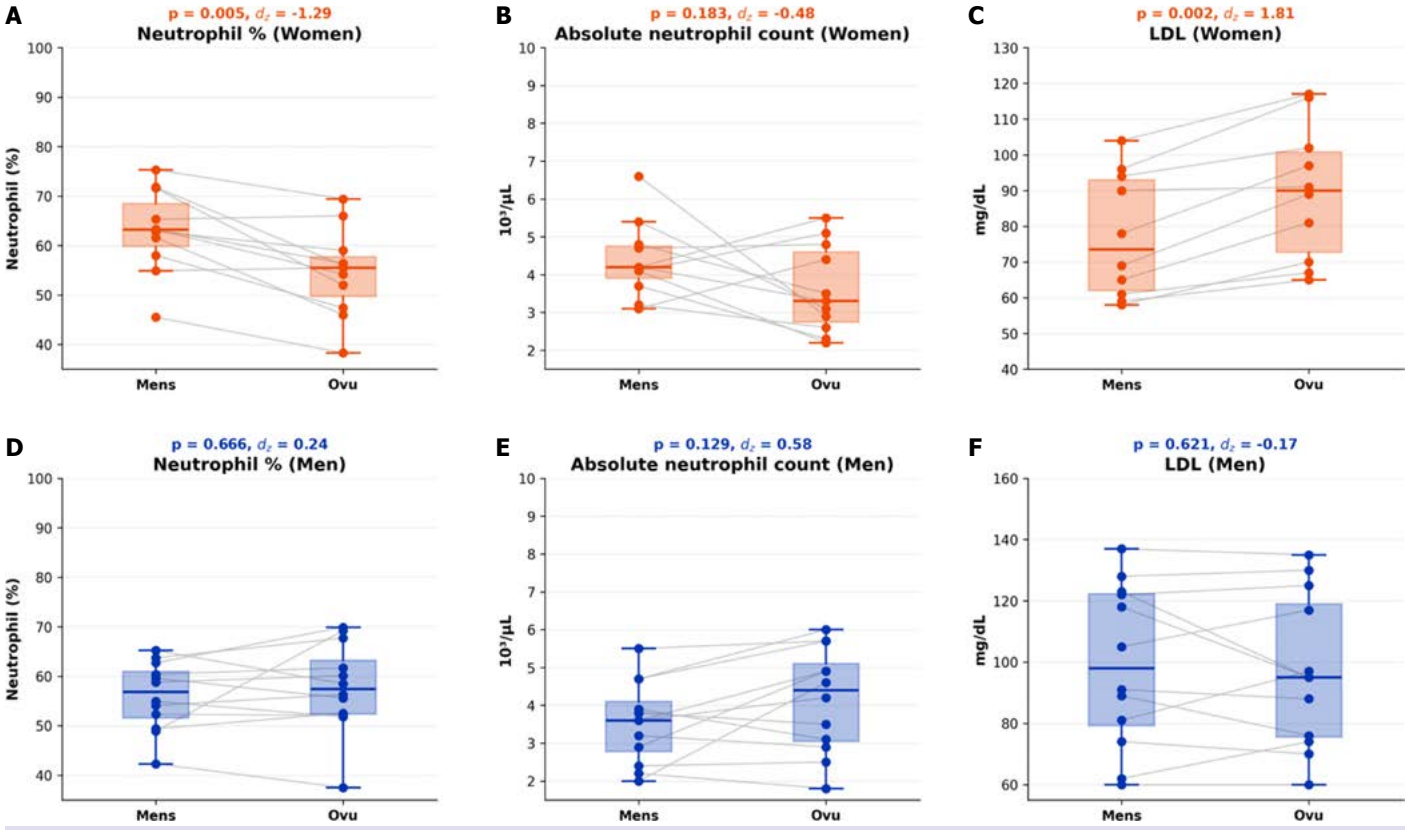

FIGURE 2. Phase-dependent changes in neutrophils and low-density lipoprotein cholesterol (LDL-C). Women **(A–C)**: neutrophil % (A, p=0.005), absolute neutrophil count (B, p=0.183), LDL-C (C, p=0.002). Men **(D–F)**: neutrophil % (D, p=0.666), neutrophil count (E, p=0.129), LDL-C (F, p=0.621). Connected lines=individual participants; boxes show median and interquartile range. Wilcoxon signed-rank test with Cohen's dz.

nificance (Spearman ρ=-0.40, p=0.249, n=10). The secondary neutrophil-percentage analysis was concordant and somewhat stronger (ρ=-0.52, p=0.120, n=10; Fig. 3D). Cross-sectional correlations between neutrophil count and LDL-C within each phase were likewise non-significant (Fig. 3A–C).

Among abundant leukocyte subsets, the inverse trend with ΔLDL was confined to neutrophils. Δlymphocyte count (ρ=+0.01, p=0.980), Δmonocyte count (ρ=-0.09, p=0.806), and Δeosinophil count (ρ=-0.16, p=0.667) showed no association with ΔLDL (Table 5). Δbasophil count showed a positive but non-significant association (ρ=+0.41, p=0.234); basophil counts in any case lie near the analyzer's lower precision limit (≈0.04–0.05×10^9/L).

Δestradiol itself did not predict ΔLDL (ρ=-0.22, p=0.542; Fig. 3E), so the ovulatory estradiol rise was not accompanied by a proportionate LDL change. The neutrophil–LDL trend is therefore unlikely to be a simple proxy for estradiol level.

In men, the Δneutrophil%–ΔLDL association was not significant and was directionally opposite (ρ=+0.51, p=0.089, n=12; Fig. 3F).

DISCUSSION

The principal observation is that a periovulatory decline in circulating neutrophils occurs alongside a concurrent rise in LDL-C in healthy young women, and that these two within-subject changes tend to move in opposite directions – an inverse trend that is specific to neutrophils among abundant leukocytes, is not explained by estradiol level, and is not reproduced in men.

These findings contextualize our experimental work [7], in which neutropenia elevated LDL-C in male but not in female mice. In that study, the absence of an LDL-elevating effect in females was speculated as one of the pieces of evidence that estrogen, either through direct hepatic LDLR upregulation or through buffering of neutrophil-dependent lipid clearance, maintains LDL homeostasis even when the

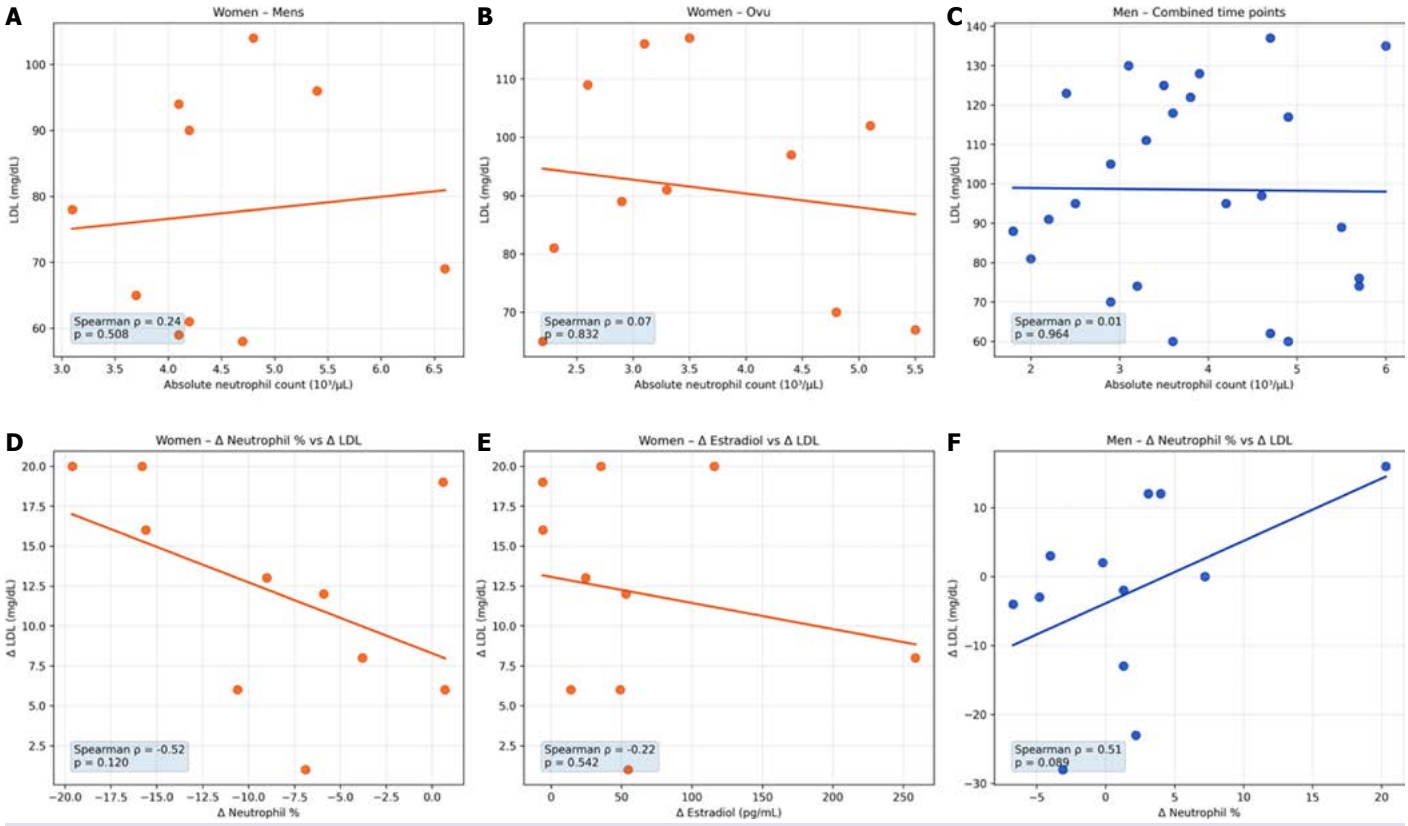

FIGURE 3. Within-subject neutrophil-low-density lipoprotein (LDL) associations. **(A–C)** Cross-sectional Spearman correlations between absolute neutrophil count and LDL-C: women at menstruation (a, ρ=+0.24, p=0.508, n=10), women at ovulation (b, ρ=+0.07, p=0.832, n=11), and men with both time points pooled (c, ρ=+0.01, p=0.964, n=25) – all non-significant. **(D)** Women: Δneutrophil % versus ΔLDL-C (ρ=-0.52, p=0.120, n=10). **(E)** Women: Δestradiol versus ΔLDL-C (ρ=-0.22, p=0.542, n=10). **(F)** Men: Δneutrophil % versus ΔLDL-C (ρ=+0.51, p=0.089, n=12). Least-squares regression lines are shown for visualization only.

neutrophil pool is diminished [7]. In the present human study, reduced neutrophils coinciding with elevated LDL specifically in the high-estradiol ovulatory window is consistent with a speculation in which a peripheral, neutrophil-associated route of LDL handling operates at homeostatic neutrophil counts and is transiently diminished when neutrophils fall. The absence of any relationship between Δestradiol and ΔLDL argues against the assumption that estradiol independently drives both variables.

The co-occurrence of falling neutrophil counts and rising LDL at ovulation is consistent with several mechanistic hypotheses. Neutrophils internalize native and oxidized LDL particles, participate in reverse cholesterol transport through myeloperoxidase-dependent pathways, and their apoptotic clearance by hepatic Kupffer cells and liver sinusoidal macrophages may feedback on hepatic lipid handling [3, 17]. The periovulatory recruitment of peripheral neutrophils to ovarian tissue, where neutrophilic granulocytes accumulate 8-fold in the thecal layer during ovulation [18], may reduce the peripheral neutrophil pool and thereby diminish the rate of peripheral LDL uptake. Pernes et al. [4] have further shown that lipid availability reciprocally regulates hematopoietic output.

Our finding that neutrophil percentage decreased at ovulation, with a parallel but non-significant decline in absolute count, contrasts with a study by Nowak et al. [16], who reported that WBC count and neutrophil count increased around ovulation in 37 healthy cycling women. This discrepancy may reflect differences in the phase-confirmation process, timing of blood sampling relative to the LH peak, or the phase-specific inflammatory microenvironment. The post-LH-surge period is characterized by complex immune remodeling in which circulating neutrophils are actively recruited to the follicle, potentially depleting the peripheral pool transiently even as bone marrow output increases. The net change in peripheral blood will depend on which of these processes dominates at the exact sampling moment. Our protocol,

Table 4. Sex comparisons at each sampling phase (Mann-Whitney U)

Variable	Women	Men	U	p	r_rb
Menstrual/Time-1					
LDL-C (mg/dL)	73.5	105.0	31	0.038	-0.52
HDL-C (mg/dL)	61.8	51.1	106	0.049	+0.48
Neutrophil %	63.2	57.5	108.5	0.034	+0.52
Neutrophil count ($\times 10^9$/L)	4.2	3.6	104.5	0.059	+0.46
WBC ($\times 10^9$/L)	6.7	5.9	94	0.202	+0.31
Ovulatory/Time-2					
LDL-C (mg/dL)	91.0	95.0	57.5	0.622	-0.13
HDL-C (mg/dL)	68.3	55.1	111	0.006	+0.68
Neutrophil %	55.5	57.4	51	0.372	-0.23
Neutrophil count ($\times 10^9$/L)	3.3	4.4	49.5	0.324	-0.25
WBC ($\times 10^9$/L)	6.4	6.8	52	0.406	-0.21

Medians; r_rb=rank-biserial (positive=higher in women). LDL-C: Low-density lipoprotein cholesterol; WBC: White blood cell; HDL-C: High-density lipoprotein cholesterol.

Table 5. Within-subject correlation of leukocyte-subset change with ΔLDL-C, women (n=10)

Δ metric	ρ versus Δ LDL	p
Neutrophil count (primary)	-0.40	0.249
Neutrophil % (secondary)	-0.52	0.120
Lymphocyte count	+0.01	0.980
Monocyte count	-0.09	0.806
Eosinophil count	-0.16	0.667
Basophil count (caution)	+0.41	0.234
Estradiol	-0.22	0.542

Spearman ρ. Neutrophil (absolute) is primary, percentage secondary. LDL-C: Low-density lipoprotein cholesterol.

which used LH surge detection and aimed to sample within 24–48 h of the surge, may have captured an earlier peri-ovulatory window than some prior studies. This biological uncertainty underscores the importance of within-subject designs, which mitigate inter-individual variability and isolate the paired change in each participant.

The increase in mean absolute lymphocyte count from 1.85 to 2.22×10^9/L (p=0.024) may reflect cycle-dependent changes in leukocyte trafficking or hormonal modulation, although the present measurements cannot establish these mechanisms. An increased lymphocyte contribution also complicates interpretation of the fall in neutrophil percentage, particularly because the decline in absolute neutrophil count was not statistically significant. Cycle-related lymphocyte changes are not consistent across studies. Nowak et al. [16] reported stable lymphocyte counts across menstruation, ovulation, and the mid-luteal phase. In our cohort, changes in lymphocyte count and LDL-C were not significantly correlated (Spearman ρ=+0.01, p=0.980; n=10), so the concurrent lymphocyte increase does not itself demonstrate a relationship with LDL-C.

The sex-specific convergence of LDL levels is another noteworthy finding. In the complete paired LDL-C samples, 10 women and 12 men (Table 1), group mean LDL-C concentrations were 77.4 mg/dL in women and 99.2 mg/dL in men at menstruation/Time-1 and 89.5 and 96.8 mg/dL, respectively, at ovulation/Time-2. These correspond to descriptive differences between group means of approximately 21.8 and 7.3 mg/dL. This convergence was specific to LDL (HDL-C remained significantly higher in women at both phases) and supports the interpretation that the ovulatory phase represents a window in which the sex advantage in LDL-C is transiently attenuated. From an evolutionary perspective, the transient LDL rise at ovulation may serve to mobilize cholesterol substrates necessary for steroidogenesis, a well-recognized physiological demand of the periovulatory period [8]. Sex-biased lipid-handling gene expression [19] may provide the substrate on which cyclic hormonal change acts.

Several limitations of this study require explicit acknowledgment. First, the sample size (11 women, of whom 10 contributed paired LDL measurements, and 13 men) provides adequate power only for large effect sizes. The moderate inverse neutrophil–LDL correlation observed here ($\rho \approx -0.40$–-0.52) did not reach statistical significance at this n, so it must be treated as a trend requiring confirmation, and the sample also limits the precision of all correlation estimates and precludes subgroup analyses by BMI, body fat, or CRP tertile. Second, the observational design precludes causal inference; common upstream signals (e.g., ovarian cytokines, LH) could independently affect neutrophils and lipoproteins. Third, peripheral counts cannot distinguish true clearance changes from compartmental redistribution. Fourth, LDL subfraction and neutrophil functional assays were not performed.

Conclusion

In healthy young women, a periovulatory neutrophil decline coincides with an LDL-C rise, and the two changes tend to vary inversely and selectively within participants, a trend that is not explained by estradiol and is absent in men. The pattern is compatible with a neutrophil-associated contribution to LDL turnover modulated across the cycle. Interventional and functional studies in larger cohorts are required to test causality and mechanism.

Ethics Committee Approval: This study was approved by the Marmara University Clinical Research Ethics Committee (09.2020.1051 – 16.10.2020).

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 received no financial support.

Use of AI for Writing Assistance: During the preparation of this work, the author(s) used Gemini solely to improve the English language, grammar, and readability of the manuscript.

Authorship Contributions: Concept – MS, BCY; Design – MS, BCY; Supervision – MS, BCY; Resource – MS, BCY; Materials – MS, BCY; Data Collection and/or Processing – MS, TA, MMK, BCY; Analysis and/or Interpretation – MS, TA, MMK, BCY; Literature Search – MS, TA, MMK, BCY; Writing – MS, TA, MMK, BCY; Critical Reviews – MS, TA, MMK, BCY.

Acknowledgments: This study is derived from the medical specialisation thesis of Mustafa Sevim, titled "The Effect of the Number of Circulating Neutrophils on Blood LDL Level", accepted by Marmara University Faculty of Medicine. The authors thank Alper Yildirim, Yelda Toy, Cagla Unlu, Emine Nur Avci, Yagmur Islek and Akay Ekmekci for their support in the initial phase of the study.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2026;13(5):577–588
doi: 10.14744/nci.2026.67760

Original Article FAMILY MEDICINE

Integration of behavioral, social and human sciences into Turkish undergraduate medical curricula: A descriptive curriculum document analysis

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ABSTRACT

OBJECTIVE: The objective of the study was to examine the representation of the Behavioral, Social and Human Sciences (BSSH) topics defined in the National Core Curriculum 2020 (NCC-2020) within undergraduate medical curricula in Turkiye, evaluate the documented educational characteristics of their integration, and explore differences according to selected institutional characteristics.

METHODS: Medical faculties providing undergraduate medical education during the 2019–2020 academic year were identified through the official website of the Council of Higher Education. Publicly available undergraduate curriculum documents from 56 medical faculties were retrieved from institutional websites. The NCC-2020 BSSH framework consisted of 35 main categories and 217 subcategories (252 items), which were consolidated into 208 BSSH topics after removing duplicate or conceptually overlapping items. The presence of these topics in course titles was examined. Documented teaching methods, assessment methods, and responsible academic departments were also analyzed. Differences in BSSH representation were examined according to accreditation status, institutional type, geographical region, and the year in which faculties admitted their first students.

RESULTS: The documented representation of BSSH topics in undergraduate medical curricula was limited and uneven. Only 14 of the 208 BSSH topics were represented in at least 80% of curricula, whereas 130 topics (62.5%) appeared in fewer than 20% of faculties. Accredited medical faculties incorporated significantly more BSSH topics into their curricula than non-accredited faculties ($p<0.05$). Lecture-based teaching and written examinations predominated in the curriculum documents, while learner-centered teaching methods, performance-based assessment approaches, and interdisciplinary participation from the social sciences and humanities were documented only rarely. No significant differences were found according to institutional type or the year in which faculties admitted their first students, whereas significant regional differences were observed.

CONCLUSION: The documented integration of BSSH into undergraduate medical curricula in Turkiye remains limited and uneven. Although a common curricular core centered on preventive medicine, public health, and routine professional practice was consistently represented, broader humanistic, structural, and systems-oriented dimensions of BSSH were substantially less visible. These findings support the need for more comprehensive and longitudinal integration of BSSH through learner-centered educational approaches, competency-based assessment, and interdisciplinary collaboration

Keywords: Accreditation; behavioral; social and human sciences; curriculum integration; medical education; undergraduate medical curriculum.

Cite this article as: *Layik ME, Saracoglu M, Korkmaz Yalcin D, Yildiz S. Integration of behavioral, social and human sciences into Turkish undergraduate medical curricula: A descriptive curriculum document analysis. North Clin Istanb 2026;13(5):577–588.*

Received: May 07, 2026 *Revised:* September 20, 2026 *Accepted:* September 23, 2026 *Online:* October 15, 2026

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Recent conceptualizations increasingly define health and healthcare from a holistic perspective grounded in complex systems thinking [1]. This shift has redefined the competencies expected of physicians and requires undergraduate medical education to move beyond a predominantly biomedical focus by incorporating the psychological, behavioral, sociocultural, environmental, and genetic dimensions of health [2]. Preparing physicians who are able to understand patients not only through their biological conditions but also through their behavioral, social, and humanistic contexts has therefore become one of the principal rationales for integrating Behavioral, Social and Human Sciences (BSSH) into medical education [3]. In line with this perspective, the Institute of Medicine recommended the systematic integration of BSSH into undergraduate medical education to improve patient care, physician-patient relationships, and healthcare systems [4]. This position was subsequently reinforced by the Association of American Medical Colleges (AAMC), which emphasized that scientific knowledge and biomedical competence alone are insufficient for effective medical practice [2]. Likewise, competency frameworks such as CanMEDS and the World Federation for Medical Education Global Standards define competencies closely aligned with BSSH, including communication, professionalism, ethics, cultural responsiveness, and patient-centered care [4, 5].

Following these international developments, several initiatives have been undertaken to strengthen the role of BSSH in undergraduate medical education in Turkiye. Although the 2014 National Core Curriculum (NCC) did not identify BSSH as a separate curricular component, it emphasized professionalism and recommended the integration of behavioral, social, and human sciences within the curriculum [6]. This approach was substantially expanded in the revised NCC-2020, in which BSSH was formally established for the 1st time as a distinct curricular component with explicitly defined behavioral, social, and humanistic competencies [7]. Against this background, the present study aimed to establish a national baseline for the integration of BSSH into undergraduate medical education in Turkiye immediately before the implementation of the NCC-2020. Specifically, the study examined the extent to which the BSSH topics subsequently included in the NCC-2020 had already been incorporated into undergraduate medical curricula during the 2019–2020 academic year. In addition, it investigated whether the level of BSSH integration differed according to institutional characteristics, including medical faculty type (public/foundation), accreditation status, year of establishment, and geographical region.

Highlight key points

- BSSH representation was limited and uneven across medical curricula in Turkiye.
- Broader humanistic, structural, and systems-oriented domains were underrepresented.
- Accredited faculties showed greater representation of BSSH topics.
- Documented teaching and assessment relied mainly on conventional approaches.
- Interdisciplinary participation from non-medical disciplines was limited.

MATERIALS AND METHODS

This study employed a descriptive web-based document analysis of publicly available undergraduate medical curricula to evaluate the integration of BSSH. Medical faculties providing undergraduate medical education during the 2019–2020 academic year were identified through the official website of the Council of Higher Education (YÖK). Faculties that had not yet graduated their first cohort, foundation universities located outside Turkiye, and faculties whose curriculum documents were not publicly accessible were excluded. The faculty selection process is summarized in Figure 1. The final sample consisted of 56 medical faculties, of which 23 were accredited by the National Medical Education Accreditation Board (UTEAK) [8] (Fig. 1).

The BSSH section of the NCC-2020 comprised 35 main topics and 217 subtopics (252 topics in total). Before analysis, the list was reviewed to identify duplicate and conceptually overlapping topics. Topics with identical titles appearing under different main topics were counted only once. Topics with different wording were merged only when the differences were considered terminological rather than conceptual, that is, when they referred to the same educational concept despite differences in wording. The list was refined through iterative discussions among the research team and the doctoral Thesis Monitoring Committee, which consisted of the authors and two medical education experts, one of whom had participated in the development of the NCC-2020. Any uncertainties regarding conceptual overlap were resolved through consensus. Following this process, a final

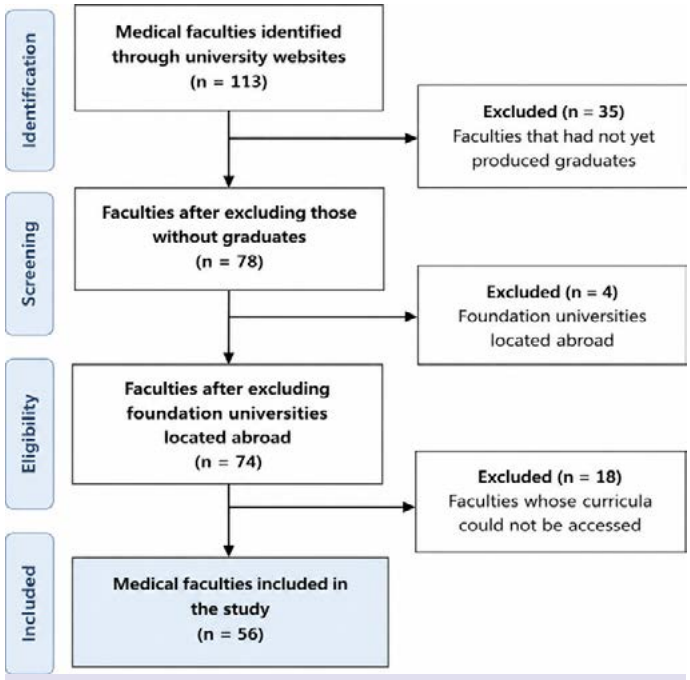


FIGURE 1. Flow diagram of faculty selection process.

list of 208 unique BSSH topics was retained as the unit of analysis. The complete list of these 208 topics is provided in Appendix 1.

The curricula of the included medical faculties were systematically reviewed using the final list of 208 BSSH topics. Identification of BSSH topics was based solely on course titles explicitly reported in the publicly accessible undergraduate curriculum documents published on the official websites of the medical faculties. Learning objectives, detailed course content, committee or module descriptions, internship documents, program outcomes, and detailed assessment documents were not used to identify BSSH topics because these materials were not consistently available across institutions. Attempting to infer BSSH topics from inconsistently available documents would have increased subjective interpretation and reduced the reproducibility of the analysis. Consequently, BSSH topics addressed within course content but not explicitly reflected in course titles may not have been captured.

In this study, "integration" was operationally defined as the explicit representation and documented distribution of BSSH topics across the undergraduate curriculum, as identified through course titles. The term refers to document-based curricular integration and does not imply verified implementation, educational effectiveness, or achieved student competence.

For each of the 208 BSSH topics, it was determined whether the topic was explicitly represented in a course title. When a topic was identified, additional descriptive information reported in the available curriculum documents was extracted, including the curricular phase (preclinical, clinical, or internship), course hours, educational delivery methods, curriculum component (e.g., committee, module, or internship), responsible discipline(s), assessment methods, and the academic department(s) responsible for teaching. These additional variables were used to describe the curricular placement and characteristics of the identified topics; they were not used to infer the presence of a BSSH topic when it was not explicitly stated in a course title. All extracted data were recorded in a Microsoft Excel spreadsheet.

This study was approved by the Van Yuzuncu Yil University Non-Interventional Clinical Research Ethics Committee (2021/06-02, 14 May 2021) and was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical Analysis

The 208 BSSH topics identified from the NCC-2020 were searched in the undergraduate medical curricula of the included medical faculties and summarized as frequencies and percentages. Statistical analyses were performed using IBM Statistical Package for the Social Sciences Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA).

The assumptions of normality and homogeneity of variance were assessed using the Shapiro-Wilk test and Levene's test, respectively. As all comparison groups satisfied the assumptions (all $p>0.05$), parametric tests were considered appropriate. Comparisons of the mean number of BSSH topics between two-group variables (year of establishment of the medical faculty, university status, and accreditation status) were performed using Student's t-test. Differences among geographical regions were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test for post hoc pairwise comparisons. Duncan's multiple range test was selected for post hoc comparisons because its stepwise procedure provides relatively high statistical power for detecting differences among group means. Compared with more conservative procedures such as Tukey's test, Duncan's test offers greater sensitivity for detecting smaller group differences, although with less stringent control of Type I error. In addition to p-values, effect sizes were calculated to evaluate the practical significance of

the findings. The authors used Cohen's d for Student's t-tests and eta squared (η^2) for one-way ANOVA.

Multiple correspondence analysis (MCA) was performed to explore the relationships among categorical variables, reduce data complexity, and visualize their configuration in a two-dimensional space.

RESULTS

A total of 56 medical faculties met the inclusion criteria and were included in the analysis. Of these, 49 (87.5%) were public universities and 23 (41.1%) were accredited. Nearly all accredited faculties (95.7%, n=22) were public universities.

The included medical faculties were distributed across all geographical regions of Turkiye. Fourteen faculties were located in the Marmara Region, 12 in Central Anatolia, 9 in the Black Sea Region, 6 each in Eastern Anatolia and the Aegean Region, 5 in the Mediterranean Region, and 4 in Southeastern Anatolia.

The representation of the 208 BSSH topics was examined across the curricula of the included medical faculties. The highest proportion of BSSH topics represented in a single curriculum was 34.1% (n=71), indicating that no curriculum represented more than approximately one-third of the BSSH topics. Only five faculties represented 30% or more of the BSSH topics (n>62), whereas 26 faculties represented between 20% (n=42) and 25% (n=52). The lowest proportion of BSSH topics represented in a single curriculum was 10.1% (n=21), and only three faculties represented fewer than 15% (n=31) of the BSSH topics.

Representation of BSSH topics was compared according to the geographical region of the university, the year in which the institution admitted its first students (before 2000 vs. 2000 or later), accreditation status during the 2019–2020 academic year, and institutional type (public or foundation). A statistically significant difference was found among geographical regions in the mean number of BSSH topics represented in the curricula (p=0.004, η^2=0.31). Faculties located in the Mediterranean Region had the highest mean number of represented BSSH topics, whereas those in the Southeastern Anatolia Region had the lowest. The mean numbers of BSSH topics represented in the remaining geographical regions were generally similar.

No statistically significant difference was found in the mean number of BSSH topics represented in the curricula between faculties established before 2000 and those established in 2000 or later (p=0.150, Cohen's d=0.39). Nevertheless, faculties established before 2000 had a higher mean number of BSSH topics represented in their curricula.

Table 1. Descriptive statistics and comparison of the mean number of BSSH topics according to medical faculty characteristics

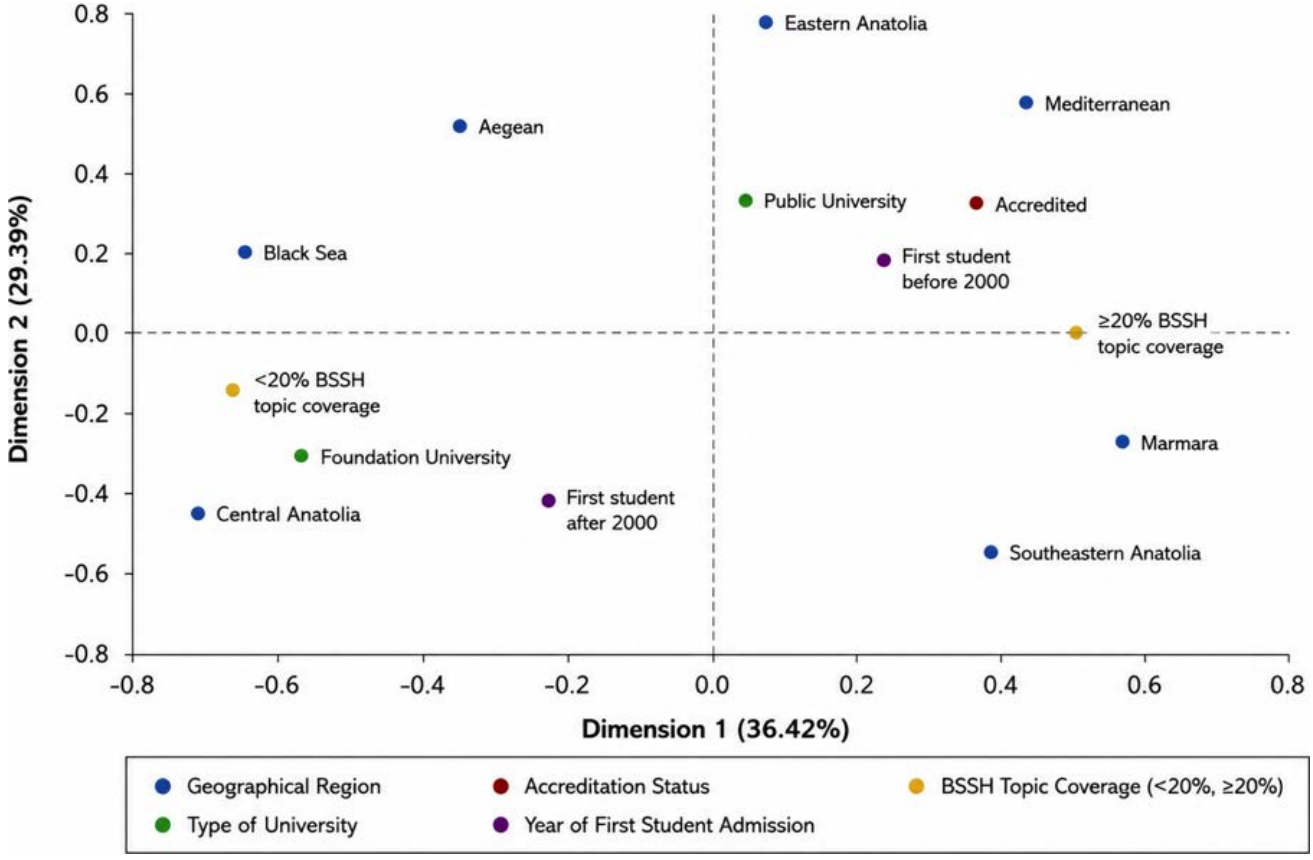
Characteristics	n	Mean number of BSSH topics±SD	p
Geographical region			
Mediterranean	5	55.20±11.48[a]	0.004
Eastern Anatolia	6	48.83±13.76[ab]	
Aegean	6	52.50±8.78[ab]	
Southeast Anatolia	4	33.25±8.85[c]	
Central Anatolia	12	42.33±8.48[bc]	
Black Sea	9	45.22±6.36[ab]	
Marmara	14	52.07±9.11[ab]	
Year of first student admission			
Before 2000	28	49.57±10.22	0.150
After 2000	28	45.46±10.81	
Type of university			
Public	49	48.14±10.43	0.248
Foundation	7	43.14±11.84	
Accreditation status (2019–2020)			
Accredited	23	52.48±11.22	0.003
Not accredited	33	44.06±8.81	

a, b, c: Different superscript letters indicate statistically significant differences between groups according to Duncan's multiple range test (p<0.05). Groups sharing at least one common letter are not significantly different. BSSH: Behavioral, Social and Human Sciences; SD: Standard deviations

Although public medical faculties had a higher mean number of BSSH topics represented in their curricula than foundation medical faculties, the difference was not statistically significant (p=0.248, Cohen's d=0.47).

The mean number of BSSH topics represented in the curricula was significantly higher in accredited medical faculties than in non-accredited faculties (p=0.003, Cohen's d=0.85) (Table 1).

The results of the MCA are presented in Figure 2. Figure 2A shows the category plot, in which categories located closer together have more similar profiles and therefore tend to be more closely associated, whereas categories located farther apart indicate weaker associations. Dimension 1 explained 36.42%, and Dimension 2 explained 29.39% of the total inertia, together accounting for 65.81% of the total variation.



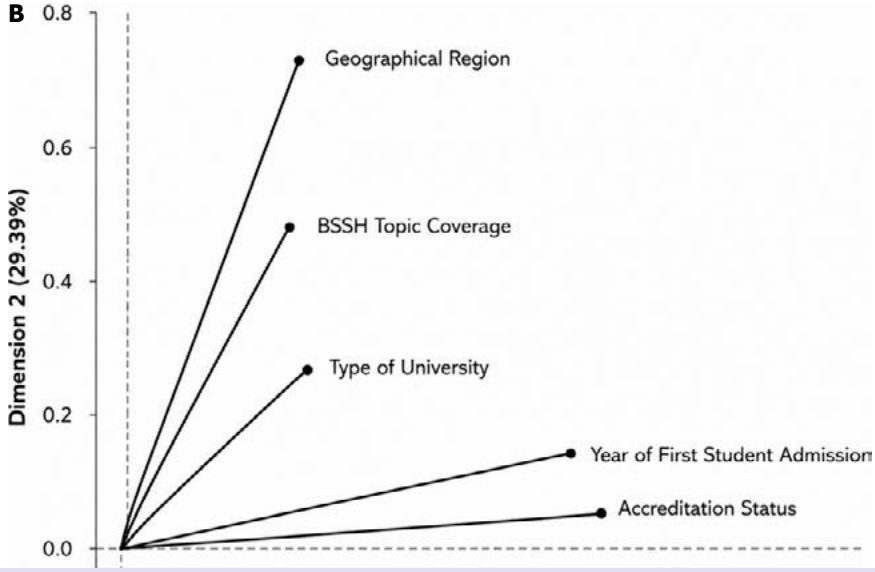


FIGURE 2. **(A)** Multiple correspondence analysis (MCA) category plot illustrating the relationships among geographical region, university type, accreditation status, year of first student admission, and Behavioral, Social and Human Sciences (BSSH) topic coverage. Categories located closer together have more similar profiles and therefore tend to be more closely associated, whereas categories located farther apart show weaker associations. The categories "<20%" and "≥20%" represent medical faculties with BSSH topic coverage below and at least 20%, respectively. Dimension 1 and Dimension 2 explain 36.42% and 29.39% of the total inertia, respectively. **(B)** Discrimination measures of the variables included in the MCA. Variables located farther from the origin contribute more to the discrimination of the two dimensions, whereas variables located closer to the origin contribute less. Dimension 1 and Dimension 2 explain 36.42% and 29.39% of the total inertia, respectively.

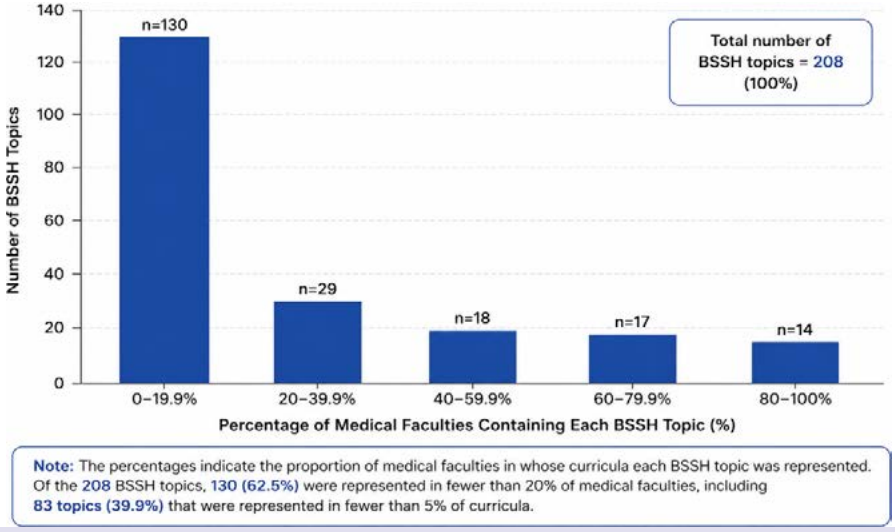


Figure 3. Distribution of the 208 Behavioral, Social and Human Sciences (BSSH) topics according to the percentage of medical faculties in which they were represented. The bars indicate the number of BSSH topics included within each percentage range. Of the 208 topics, 130 were represented in fewer than 20% of medical faculties; this group includes 83 topics that were represented in fewer than 5% of curricula.

The category plot showed that public universities, accredited medical faculties, admission of the first students before 2000, and representation of ≥20% of the BSSH topics tended to be positioned close to one another. Similarly, the Mediterranean, Eastern Anatolia, and Aegean regions were located closer to these categories than the remaining geographical regions.

Figure 2B presents the discrimination measures for the variables included in the MCA. Geographical region and the representation of BSSH topics contributed relatively more to Dimension 2, whereas accreditation status and year of first student admission contributed mainly to Dimension 1. University type also contributed to the discrimination of the dimensions, indicating that all variables included in the analysis contributed to the overall MCA solution.

To provide an overview of how the 208 BSSH topics were distributed according to their level of representation across curricula, topics were grouped into five 20-percentage-point intervals based on the proportion of medical faculties in which they were represented (Fig. 3). This categorization was intended to facilitate visualization of the overall distribution and to identify highly represented (80.0–100%) and underrepresented (0–19.9%) topics for further description in the subsequent tables.

Only 14 of the 208 BSSH topics were represented in at least 80% of the curricula of the included medical faculties (Table 2). These topics were predominantly related to preventive medicine and public health, including immunization, communicable disease control, reproductive and sexual health, substance use, occupational health, child protection, patient rights, and emergency preparedness. Together, they represent the common curricular core of BSSH education across the included medical faculties.

Table 2. BSSH topics represented in 80–100% of medical faculty curricula

Name of topic	n	%
Immunization	56	100.00
Sexually transmitted infections and their prevention	56	100.00
Substance use	53	94.64
Alcohol use	51	91.07
Child neglect and abuse	50	89.29
Managerial processes, occupational health and safety	50	89.29
Control of communicable diseases in the community	48	85.71
Occupational diseases	47	83.93
Chronic (non-communicable) diseases	47	83.93
Abuse (physical, emotional, and sexual)	46	82.14
Patients' rights and responsibilities	46	82.14
Emergencies and disasters	46	82.14
School health	46	82.14
Reproductive and sexual health	45	80.36

BSSH: Behavioral, Social and Human Sciences.

A total of 130 of the 208 BSSH topics (62.5%) were represented in fewer than 20% of the curricula. Because of the large number of topics in this group, they were organized into broad conceptual themes using the terminology and conceptual framework of the NCC-2020 BSSH list to facilitate presentation rather than to provide an additional level of analysis (Table 3). The underrepresented topics spanned a wide range of domains, including health systems and governance, social determinants of health, professional identity and physician well-being, professionalism and ethics, communication and teamwork, behavioral and mental health, culture and humanistic perspectives, vulnerable populations and the life-course approach, environment, disasters and planetary health, and digital transformation and emerging technologies.

To provide an overview of the distribution of BSSH topics across curricula, the 208 topics were grouped into five 20-percentage-point intervals according to the proportion of medical faculties in which they were represented (Fig. 3). The highest (80.0–100%) and lowest (0–19.9%) representation groups were subsequently examined in greater detail.

Since a single BSSH topic could be represented in multiple course titles and across different curricular phases, each representation (hereafter referred to as a BSSH topic occurrence) was analyzed separately according to its educational characteristics. Furthermore, when a course title involved more than one teaching method, assessment method, academic discipline, or responsible academic department, each characteristic was coded independently (multiple coding). Consequently, the total number of observations varies across the categories presented in Table 4 and may exceed the number of unique BSSH topic representations.

Most BSSH topic occurrences were identified during the preclinical phase (58.65%, n=1,891) and within committee-based curricula (56.65%, n=1,659). Classroom-based lectures were the predominant teaching method (79.71%, n=2,279), followed by seminars (5.91%, n=169) and workplace or clinical training (5.04%, n=144).

The most frequently used assessment methods were theoretical examinations (61.37%, n=2,250) and structured oral examinations (20.76%, n=761), whereas competency-based assessment accounted for only 6.52% (n=239) of all assessment methods and was used primarily during the internship phase.

Most BSSH topic occurrences (96.46%, n=2,344) were assigned to a single academic discipline. The academic departments responsible for teaching these BSSH topic occurrences were predominantly affiliated with faculties of medicine (95.46%, n=2,313), whereas only 1.49% (n=36) were affiliated with faculties of social sciences or other non-medical faculties (Table 4).

DISCUSSION

This study provides a baseline assessment of the representation of BSSH in undergraduate medical curricula immediately before the implementation of the BSSH recommendations in the NCC-2020. Overall, the findings indicate that the documented representation of BSSH in undergraduate medical curricula in Turkiye was limited and varied substantially across medical faculties. The following sections discuss the study's principal findings regarding the scope of BSSH representation, accreditation, teaching and assessment approaches, and interdisciplinary participation.

In the present study, only a limited proportion of the BSSH framework was reflected in publicly available undergraduate medical curriculum documents, indicating that substantial areas of BSSH remain insufficiently represented. More importantly, the distribution of topics followed a clear curricular gradient rather than a random pattern. Only a limited common curricular core, represented in at least 80% of medical faculties, was identified, consisting primarily of topics related to preventive medicine, public health priorities, patient rights, occupational health, and routine clinical practice. In contrast, nearly two-thirds of the BSSH topics were represented in fewer than 20% of curricula and encompassed a broad range of domains, including health systems and governance, social determinants of health, professional identity and physician well-being, professionalism and ethics, communication and teamwork, behavioral and mental health, culture and humanistic perspectives, vulnerable populations, planetary health, and digital transformation. Taken together, these findings suggest that undergraduate medical curricula continue to prioritize clinically oriented, biomedical, and public health-related BSSH content, whereas broader relational, societal, humanistic, structural, and systems-oriented dimensions of BSSH remain substantially underrepresented. This pattern is consistent with international literature demonstrating persistent challenges in the comprehensive integration of BSSH

Table 3. Themes of BSSH topics represented in 0–19.9% of medical faculty curricula

Theme	Representative BSSH topics
Health systems, health policy, and governance	Globalization and health; Population changes and policies; Multi-stakeholder health approach (one health, international health regulations, national and international non-governmental organizations); Public policies that promote health; In health services (demand, presentation, access, etc.); Biopolitics and globalization; Disruption/prevention of health service delivery; Social accountability; Health and social structures, discourse communities, knowledge-power relationship; Physician payment methods and performance-based system; The impact of privatization and related policies on health; Integration of door retention and step health care.
Social determinants of health and health equity	Unemployment and Poverty; Violations of the right to health; Social justice and welfare; Child labor; Social environment and social well-being; Social/human capital, social support; Space and health; Internal migration; Migration for economic reasons; Economic well-being; The psychosocial burden created by violations of rights in social life; Indicators related to chronic diseases and inequalities arising from their distribution in society; Access to education and educational processes (including medical education).
Professional identity, physician well-being, and professional life	Workload and burnout; Loss of personal rights; Work and health interaction; Well-being of the Physician; Work-related diseases; The effect of the disease on work and returning to work after illness (rehabilitation); Stress in work environments; Alienation from the profession; Job satisfaction; Migration of health workers and free movement of labor; Professional values, professional identity development and historical perspective, being constantly open to professional and individual development; Defending health and profession; Organizational and managerial processes and working life.
Professionalism, ethics, and legal issues	Medical errors and unprofessional behavior; Legal and ethical divergences and dilemmas in medical practice; National and international documents, legal regulations and ethical codes related to medicine and health; Use of advanced technology and ethical issues; Ethical situations in medical record, prescription and report writing; Legal and ethical situations in forced treatment; Organizational and ethical issues; Reproductive health and ethics; Aspects of health practices that require self-criticism; Defensive medicine; Conflicts of interest, self-exploitation; Extreme medical applications; Abuse of power in health and education processes; Consent; Autonomy.
Communication, teamwork, and conflict management	Violence against healthcare workers; Communication problems; Conflict/intimidation (mobbing) in health service delivery processes; Intra-team and inter-team conflict; Intercultural interaction and problems (communication, ethics, etc.); Overly demanding patient; Manipulative patient; Resilient patient; Frequently admitted patient; Power relationship, control, power sharing; Conflict between disciplines and professions; Conflict between institutions/units.
Behavioral and mental health	Behavioral addiction (gambling, shopping, technological, internet addiction); Stress management; Exposure (use of health services by pretending to be a patient to gain benefits); Patient in age; Psycho-social aspects of symptoms and conditions such as chronic pain, dementia, sleep problems, and depression; Flexibility/resilience, embracing uncertainties/dilemmas; Stress-related physical and mental complaints; Loneliness and the need for social support; Familial and social burden due to addiction; Non-compliance with preventive and therapeutic practices/refusal (e.g. vaccine hesitancy); Physical and mental health; Major life events; Psychological and social well-being; Social isolation; Sedentary life.
Culture, society, and humanistic perspectives	Gender-based discrimination; Community and family health; Wellness states; Culture and health beliefs/behaviors; Body-mind dilemma and reductionist approaches; Cultural diversity in health perception, behaviors and practices; Commercialization of healthy living habits; In the processes of socialization and participation in social life; Scientific paradigm and paradigm shift; Consumer society and health; A world of belonging, purpose and meaning; Power and control; Involvement/influence in processes related to oneself; Medicalization of everyday life (medicalization); Participation in social life; Social and cultural diversity.

Table 3 (cont). Themes of BSSH topics represented in 0–19.9% of medical faculty curricula

Theme	Representative BSSH topics
Vulnerable populations and life-course approach	Violence against children; Elder neglect and abuse; Children with special needs (chronic health problems, neuro-developmental disorders)/disabilities; Priority/disadvantaged groups; Health at different stages of life; War/terrorism and migration, refugee; Children (unaccompanied, protected and street children); Peer bullying; Migrants/refugees, asylum seekers; Individuals stigmatized due to illness; Fragility; Child marriage; The burden of the caregiver and the giver; Cyber violence; Adolescent pregnancy; Maintaining functionality.
Environment, disasters, and planetary health	Water and food security; Accidents at home; Urbanization; Health problems related to nuclear accidents; Climate and energy policies; Ecological destruction and health impacts; Environmental and ecological ethics; Man-made disasters; Climate migration.
Digital transformation and emerging technologies	Artificial intelligence; Technology and health interaction; Media and management of excessive/noisy information; Current diagnosis-treatment tools; Digitalization, personal health data and management of big data; Online health apps; Wearable Technologies; Technological communication tools and channels.

BSSH: Behavioral, Social and Human Sciences.

into undergraduate medical education. Previous studies have identified structural and curricular barriers to BSSH integration and considerable variation in the incorporation of social determinants of health across medical schools [9–11]. Similar challenges have been reported across several of the domains that were underrepresented in the present study. Medical humanities education remains heterogeneous and often lacks longitudinal integration,[18] while emerging areas such as artificial intelligence and health systems science continue to require more systematic curricular integration [9]. More broadly, previous work has emphasized that meaningful BSSH education should extend beyond the inclusion of individual topics to incorporate humanistic, social, cultural, and structural perspectives throughout medical training [12–14]. Taken together, the present findings and international literature suggest that the challenge is not simply to increase the number of BSSH topics in medical curricula, but to achieve a more comprehensive and coherent integration of the broader domains of BSSH.

Another important finding of this study was that accredited medical faculties incorporated significantly more BSSH topics into their curricula than non-accredited faculties, with a large effect size. One possible explanation for this association is that accreditation processes may encourage closer alignment with NCC standards, including expectations related to BSSH. This finding is consistent with previous reports from other countries. In the United States, accredited medical schools have been shown to incorporate BSSH-related content into their curricula more systematically [10]. Accreditation has been widely recognized as a mechanism for promoting educational quality, supporting continuous curriculum improvement, and enhancing institutional recognition [11]. Supporting this perspective, a recently published international guide on BSSH integration emphasized the importance of developing a national framework to facilitate the systematic incorporation of BSSH into undergraduate medical education [12]. Overall, these findings suggest that accreditation and national curriculum standards may play complementary roles in promoting the integration of BSSH into medical education.

According to the curriculum documents analyzed, BSSH topics were most commonly planned as classroom lectures, indicating that their documented delivery continues to rely predominantly on conventional teaching approaches. This reliance on lecture-based teaching suggests a strong emphasis on content-oriented approaches in the documented curricula, with relatively limited documentation of interactive, experiential, or learner-centered methods. This observation is consistent with previous literature suggesting that effective BSSH education requires more than the inclusion of additional curricular content. Rather, the development of competencies such as communication, professionalism, ethical reasoning, collaboration, and reflective practice depends on educational approaches that are contextual, interdis-

Table 4. Features of BSSH topics

	Features	n	%
Curricular phases of course titles containing BSSH topics	Preclinical	1891	58.65
	Clinical	983	30.49
	Internship	350	10.86
Curriculum containing course title	Committee	1659	56.65
	Internship	1065	36.37
	Other (elective, course, special study module, block etc.)	204	6.97
Training delivery methods	Trainer presentation (lecture/classroom)	2279	79.71
	Skill training practices	137	4.79
	On-the-job/Clinical training	144	5.04
	Seminar	169	5.91
	Other (lab practice, community-based training, case-based training, problem-based learning, team-based learning, etc.)	130	4.49
Measurement and evaluation methods	Theoretical exams	2250	61.37
	Practical exams	201	5.48
	Structured oral	761	20.76
	Competency exam	239	6.52
	Other (mini clinical exams, presentation, simulated patient assessment, project assignments, CORE, OSCE, homework etc.)	154	4.20
	No assessment	61	1.66
Discipline(s) offering the course	Single discipline	2344	96.46
	Multi-disciplinary	86	3.54
Faculty members' faculties	Faculty of medicine	2313	95.46
	Social sciences and others	36	1.49
	Undetermined	74	3.05

BSSH: Behavioral, Social and Human Sciences.

ciplinary, and actively engage learners. A 2016 international consensus report highlighted the importance of contextual and interdisciplinary learning for BSSH education [13], while Cuff and Vanselow similarly recommended interactive educational strategies that integrate BSSH throughout medical training [10]. Bleakley et al. [3] further argued that attributes such as ethical sensitivity, empathy, collaboration, critical reflection, and professional judgment are best developed through educational approaches extending beyond traditional biomedical teaching and incorporating perspectives from the humanities and the arts. Likewise, international experience suggests that BSSH should be introduced early in medical education and reinforced through longitudinal integration across the curriculum rather than being confined to isolated courses [3, 10, 13, 14].

The documented assessment of BSSH topics relied primarily on written examinations and structured oral examinations, whereas performance-based, reflective, and workplace-oriented assessment methods were only rarely reported. This pattern suggests a greater emphasis on knowledge-oriented assessment than on approaches designed to capture broader BSSH-related competencies, such as professionalism, ethical reasoning, reflective capacity, interpersonal skills, and understanding of the social dimensions of health. Previous studies have similarly argued that assessment in BSSH should extend beyond factual knowledge to encompass systems thinking, sociopolitical awareness, and ecological perspectives [12]. A recent systematic review likewise identified reflective writing, self-assessment, structured observation, and written assignments as commonly recommended

approaches for assessing BSSH proficiency, while also highlighting the need for greater standardization and clearer competency frameworks [15]. Consistent with these observations, Carr et al. [16] reported that none of the reviewed studies employed an assessment framework specifically designed for medical humanities education, underscoring the continuing absence of theory-informed and internationally accepted assessment models. These observations highlight the need for competency-based assessment frameworks capable of evaluating the reflective, ethical, interpersonal, and social competencies expected of future physicians.

According to the curriculum documents, responsibility for teaching BSSH-related content was assigned predominantly to departments within faculties of medicine, whereas the involvement of educators from the social sciences and humanities appeared to be very limited. Although physicians play a central role in BSSH education, limited participation of experts from departments such as psychology, sociology, anthropology, philosophy, ethics, and the humanities may constrain students' exposure to the diverse perspectives needed to understand the social, cultural, behavioral, and ethical dimensions of health and illness. Previous literature has consistently emphasized that meaningful BSSH education depends on genuine interdisciplinary collaboration. Collett et al. [13] argued that the active involvement of social scientists is essential for effective BSSH teaching, while the AAMC recommended that BSSH should be delivered through interdisciplinary teams including both physician and non-physician educators [2]. Similarly, Batistatou et al. and Bleakley et al. [3, 17] highlighted that perspectives from the humanities and social sciences – including literature, philosophy, psychology, sociology, and the arts – contribute to the development of empathy, ethical reasoning, communication skills, reflective practice, and professional identity, and therefore should be integrated longitudinally throughout undergraduate medical education rather than confined to isolated courses. Collectively, these findings suggest that strengthening BSSH education requires interdisciplinary collaboration in curriculum design and teaching, enabling students to engage with the multiple perspectives that shape contemporary medical practice.

Overall, the findings suggest that undergraduate medical curricula in Turkiye have established an identifiable curricular core for BSSH education, particularly in areas closely aligned with preventive medicine and public health. However, broader humanistic, structural, and systems-oriented dimensions remain less visible, while teaching, assessment, and faculty composition continue to reflect predominantly traditional educational approaches. Together, these findings provide a baseline against which the implementation of NCC-2020 can be evaluated and may serve to inform future curriculum development aimed at achieving a more comprehensive integration of BSSH into undergraduate medical education.

Study Limitations

This study has several limitations. First, the analysis was restricted to publicly available undergraduate curriculum documents and was based solely on course titles. Because learning objectives, detailed syllabi, committee or internship documents, program outcomes, and assessment materials were not consistently available across institutions, these sources were not included in the analysis. Consequently, some BSSH content embedded within these documents may not have been captured, potentially leading to an underestimation of the extent of BSSH representation in undergraduate medical curricula. Nevertheless, restricting the analysis to publicly available and consistently available data sources ensured a transparent, reproducible, and comparable evaluation across all participating medical faculties. Furthermore, curriculum documents represent the documented rather than the implemented curriculum. Therefore, the term "integration," as used in this study, refers to documented curricular integration and should not be interpreted as evidence of implementation fidelity, educational effectiveness, or achieved student competence.

Conclusion

The present study demonstrates that the documented representation of BSSH in undergraduate medical curricula in Turkiye remains both limited and uneven. Although a common curricular core centered on preventive medicine, public health, and routine professional practice was consistently represented, broader humanistic, structural, and systems-oriented dimensions of BSSH were substantially less visible across curricula. Accredited medical faculties incorporated significantly more BSSH topics into their curricula than non-accredited faculties; however, conventional teaching and assessment approaches predominated, and interdisciplinary participation from the social sciences and humanities remained limited. Taken together, these findings suggest that strengthening BSSH integration requires more than

expanding curricular content alone. Future curriculum development should promote longitudinal integration of BSSH topics, learner-centered teaching, competency-based assessment, and interdisciplinary collaboration. As the findings reflect the period immediately preceding the implementation of NCC-2020, this study provides a baseline against which future developments in the documented integration of BSSH into undergraduate medical education in Turkiye can be evaluated. However, this document-based analysis does not establish the extent to which the documented curriculum was implemented in practice or translated into student learning and competence. Therefore, the findings should be interpreted as evidence of documented curricular integration rather than verified educational implementation or effectiveness.

Appendix Online Link: https://jag.journalagent.com/nci/abs_files/NCI-67760/NCI-67760_(0)_Appendix1-NCI-67760.pdf

Ethics Committee Approval: This study was approved by the Van Yuzuncu Yil University Non-Interventional Clinical Research Ethics Committee (2021/06-02 – 14/05/2021).

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 received no financial support.

Use of AI for Writing Assistance: The authors declared that artificial intelligence was not used in the study.

Authorship Contributions: Concept – SY, MEL; Design – SY; Supervision – SY; Resource – MEL; Materials – MEL; Data Collection and/or Processing – MEL, DKY; Analysis and/or Interpretation – DKY; Literature Search – MEL, MS; Writing – MEL; Critical Reviews – SY, MS.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2026;13(5):589–598
doi: 10.14744/nci.2026.59458

Original Article PEDIATRIC GENETICS

Reduced penetrance and phenotypic heterogeneity of 16p13.11 copy number variations: Insights from a Turkish cohort

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ABSTRACT

OBJECTIVE: Copy number variations (CNVs) involving the 16p13.11 locus have been increasingly recognized as important contributors to the etiology of neurodevelopmental and multisystem disorders. In this study, we characterized the clinical and molecular spectrum of 16p13.11 CNVs in a Turkish cohort and evaluated the associated phenotypic variability.

METHODS: 16p13.11 CNVs identified in 10 unrelated families were evaluated. Clinical, neurodevelopmental, and dysmorphic features were systematically assessed.

RESULTS: Among cases with microdeletions (1 female, 3 males), the most frequent findings included language delay, motor delay, learning difficulties, and epilepsy, accompanied by autism spectrum disorder, anxiety, and structural brain anomalies. In the microduplication group (9 females, 4 males, and 1 fetus), motor and language delay, intellectual disability, and epilepsy were observed, together with behavioral abnormalities such as hyperactivity and self-injurious behavior. Segregation analysis showed that one patient with a 16p13.11 deletion inherited the CNV from an affected parent, whereas the remaining patients were *de novo*. In contrast, all patients with 16p13.11 duplications inherited the rearrangement from asymptomatic parents.

CONCLUSION: Our findings highlight reduced penetrance in 16p13.11 duplications, supporting the concept of this region as a susceptibility locus for neurodevelopmental disorders. In contrast, the predominantly *de novo* occurrence of deletions and the presence of subtle findings in inherited cases emphasize the need for detailed familial evaluation.

Keywords: 16p13.11 region; chromosomal microarray analysis; copy number variations; reduced penetrance; variable expressivity.

Cite this article as: *Yuksel Ulker A, Usluer E, Kaplan I. Reduced penetrance and phenotypic heterogeneity of 16p13.11 copy number variations: Insights from a Turkish cohort. North Clin Istanb 2026;13(5):589–598.*

Chromosome 16p13.11 is a genomic region prone to structural rearrangements, including both microdeletions and microduplications, due to the presence of low copy repeats (LCR16s) that mediate non-allelic homologous recombination (NAHR). As a result, recurrent copy number variants (CNVs) are frequently observed in this locus. These CNVs have been increasingly associated with neurodevelopmental and neuropsychiatric disorders, as well as various congenital anomalies [1, 2].

Microdeletions of 16p13.11, typically spanning 1.5–1.65 Mb, have been associated with developmental delay, intellectual disability, microcephaly, epilepsy, behavioral abnormalities, and facial dysmorphism [3, 4]. Possible critical genes within this region include *NDE1* and *NTAN1*,

Received: December 19, 2025 *Revised:* July 04, 2026 *Accepted:* October 22, 2026 *Online:* November 10, 2026

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which are implicated in neurodevelopmental phenotypes, while *MYH11* and *ABCC6* are associated with cardiovascular and neuropsychiatric abnormalities, respectively [5–8]. In addition, *ABCC1* has been linked to non-syndromic hearing loss [9]. Microduplications of this region are associated with a similar spectrum of neurodevelopmental and neuropsychiatric features; however, their phenotypic expression tends to be more variable and often milder compared to deletions [2, 10–12]. Both deletions and duplications demonstrate incomplete penetrance and variable expressivity, complicating genotype–phenotype correlations.

Despite the growing number of studies, including large cohort analyses and evidence from curated databases, the phenotypic spectrum of 16p13.11 CNVs remains highly heterogeneous, particularly with respect to penetrance and clinical variability. Studies from Türkiye have reported 16p13.11 CNVs within broader chromosomal microarray cohorts; however, these studies have not primarily focused on the detailed clinical characterization of this region [13, 14]. Therefore, additional data from Turkish cohorts may increase the representation of Turkish patients in the literature and support clinical evaluation and genetic counseling.

In this study, we present 10 unrelated families carrying 16p13.11 CNVs, including three with microdeletions and seven with microduplications. Through clinical and neurodevelopmental assessments, we aimed to characterize 16p13.11 CNVs in a Turkish cohort, with particular emphasis on phenotypic heterogeneity, inheritance patterns, and their relevance to clinical evaluation and genetic counseling.

Highlight key points

- 16p13.11 duplications were consistently inherited from clinically asymptomatic parents in our cohort, indicating reduced penetrance.
- 16p13.11 deletions occurred predominantly de novo; in the inherited case, the transmitting parent exhibited subtle neuropsychiatric features.
- Distinct differences in inheritance patterns and phenotypic presentation were observed between duplication and deletion carriers.

MATERIALS AND METHODS

Data Collection

Patients who underwent chromosomal microarray analysis at the Department of Medical Genetics, Adana City Education and Research Hospital, a tertiary care center located in the Çukurova region of Türkiye, between 2023 and 2026 were evaluated. Patients were referred for suspected genetic etiologies, including neurodevelopmental delay, intellectual disability, epilepsy, behavioral abnormalities, and congenital malformations. Cases in whom a 16p13.11 microdeletion or microduplication was identified by chromosomal microarray analysis were included in the study.

Data were collected retrospectively from patient records maintained by the genetics department. The collected data included demographic characteristics, pregnancy history, microarray breakpoints, and clinical phenotypes. The study was conducted in accordance with the principles of the Declaration of Helsinki. Ethics approval was obtained from the Ethics Committee (Date: November 20, 2025, No: 882).

Analysis and Classification

Peripheral blood samples collected in EDTA tubes were used for genomic DNA extraction following standard laboratory protocols. CMA was performed using the CytoScan™ HT-CMA Assay (Thermo Fisher Scientific, Waltham, MA, USA), which is based on SNP microarray technology. After wet-lab procedures, hybridized chips were scanned using the GeneTitan™ MC Fast Scan system (Thermo Fisher Scientific).

Raw data were analyzed using RHAS, MSV, and CHAS software, aligned to the GRCh38 human reference genome. The analysis was performed using B Allele Frequency and Log R Ratio plots to detect copy number changes, mosaicism, uniparental disomy, and regions of homozygosity.

Annotation of identified CNVs was carried out using multiple public databases, including DECIPHER, ClinGen, database of genomic variants, dbVar, PubMed, and OMIM. Classification of CNVs followed the technical standards and guidelines of the American College of Medical Genetics and Genomics and ClinGen [15]. Reporting nomenclature complied with the International System for Human Cytogenomic Nomenclature (ISCN 2020).

RESULTS

Clinical Findings

Patients with 16p13.11 Deletion

Three pediatric patients and one affected parent with 16p13.11 microdeletions were evaluated. The ages of

Table 1. Clinical, neurological, and ımaging features of patients with 16p13.11 deletions

	Patient 1	Patient 2	Patient 3	Patient 4
Presenting complaint	Hypotonia	Autism	Neuromotor developmental delay	Anxiety disorder
Sex	Male	Male	Female	Male
Inheritance	De novo	De novo	Father inherited (Patient 4)	Not available
Age	7 months	13 years	13 years	50 years
Language delay	-	+	+	-
Motor delay	+	-	+	-
Learning difficulties	Not available	+	+	-
Intellectual disability	Not available	+	+	-
Behavioral / Psychiatric findings		Autism, stereotypic movements	-	Anxiety disorder
Epilepsy	-	+	+	-
Other neurological findings	Hypotonia		Cerebral palsy	
Brain structural findings	Mildly prominent lateral and third ventricles	-	Periventricular leukomalacia	-
Facial/Dysmorphic features	Hypertelorism	Non-syndromic	Microcephaly	Non-syndromic
Skeletal system	Clubfoot	-	Operated scoliosis, thoracic kyphosis, limited bilateral knee extension	Operated disk hernia
Other		Phimosis	Strabismus	

the pediatric patients ranged from 7 months to 13 years. Clinical features of patients with 16p13.11 duplications are shown in Table 1.

Developmental delay and learning difficulties were identified in two patients; the youngest patient (7 months old) could not be reliably assessed for learning difficulties.

Epilepsy was identified in two patients, with seizure onset at approximately 1 year of age. Regarding behavioral characteristics, autistic features were observed in one patient.

Cranial imaging findings included mildly prominent lateral and third ventricles in one patient and periventricular leukomalacia in another.

Ophthalmologic evaluation revealed strabismus in one patient, while hearing assessments were normal in all patients.

Among dysmorphic features, hypertelorism was observed in one patient, whereas the others were non-syndromic. Bilateral clubfoot and hypotonia during infancy were noted in one patient. Phimosis was detected in one patient.

Patient 3 with microcephaly and neurodevelopmental delay inherited the 16p13.11 deletion from her father (Patient 4). Although the father was non-syndromic and had no history of neurodevelopmental delay, he had a long-standing anxiety disorder of unknown etiology. He was under psychiatric follow-up and was unable to maintain employment.

Patients with 16p13.11 Duplication

Fourteen individuals from seven families carrying the 16p13.11 microduplication were evaluated, including six postnatal patients, seven asymptomatic parental carriers, and one fetus. All duplications were inherited from clinically evaluated, unaffected parents. The ages of the postnatal patients ranged from 5 to 13.5 years (median: 7 years). Clinical features are summarized in Table 2.

For frequency-based analyses, only postnatal patients were included, excluding parental carriers, the prenatal case, and Patient 10 due to the presence of an additional CNV. Accordingly, clinical feature analyses were based on five postnatal patients.

TABLE 2. Clinical, neurological, and ımaging features of patients with 16p13.11 duplications

Findings	Patient 5	Patient 6	Patient 7	Patient 8 (Fetus)	Patient 9	Patient 10	Patient 11
Presenting complaint	Seizure, developmental delay	Speech delay	Speech delay, motor delay	Fetal findings	Short stature	Neuromotor developmental delay	Hearing loss
Sex	Female	Female	Male	Not available	Female	Female	Female
Inheritance	Inherited from asymptomatic father	Inherited from asymptomatic mother	Inherited from asymptomatic mother	Inherited from asymptomatic mother	Inherited from asymptomatic father	Inherited from asymptomatic father	Inherited from asymptomatic mother
Age	5 years	6 years	7 years		13.5 years	5 years	7.5 years
Neurodevelopmental, behavioral, and neurological findings	Language delay, motor delay, epilepsy	Language delay, motor delay, learning difficulties, intellectual disability, self-mutilation	Language delay, motor delay, intellectual disability, hyperactivity, epilepsy		-	Language delay, motor delay, intellectual disability	-
Brain structural findings	-	Mild cerebral atrophy	Thin and short corpus callosum, mildly prominent optic nerve sheath		-	Thin corpus callosum, prominent subarachnoid space in frontal region	-
Other findings	Hemangioma on back	Operated bilateral clubfoot (PEV), strabismus	Coxa valga, phimosis, bilateral cryptorchidism, atopic dermatitis	Prenatal USG: Acrania, dextrocardia, omphalocele	Myopia	Additional CNV on chromosome 8	Transient hearing loss

USG: Ultrasonography, CNV: Copy number variations.

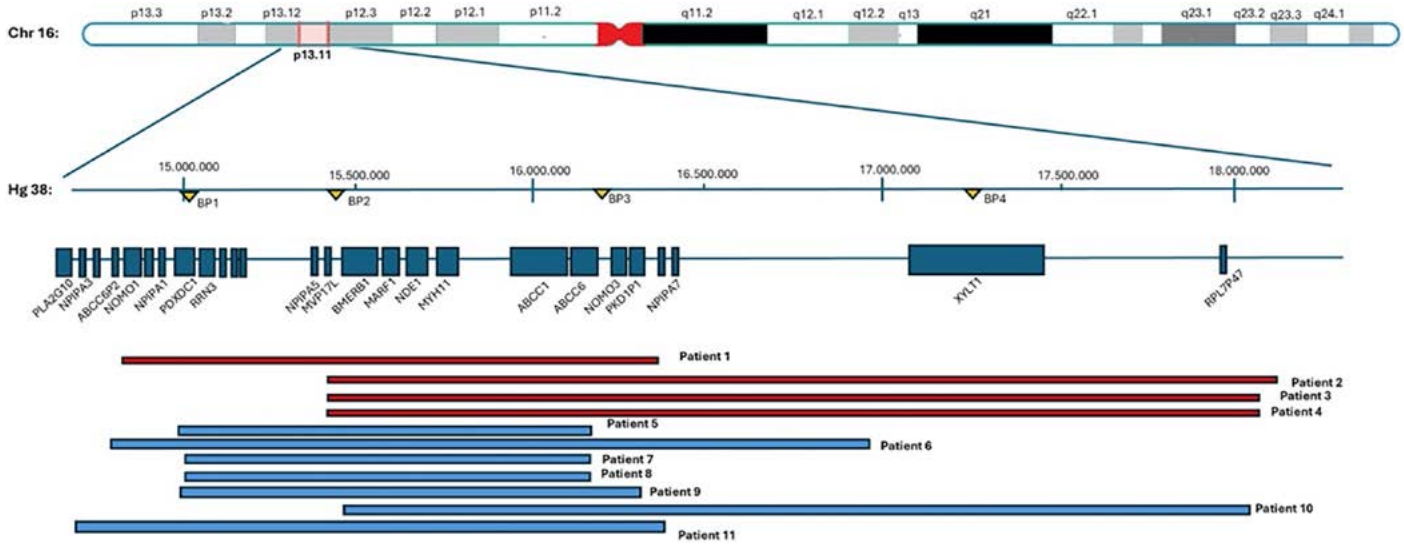

FIGURE 1. Chromosomal ideogram of the 16p13.11 deletion/duplication region and patient distribution. The upper panel shows the 16p13.11 locus (hg38), with genes located within the recurrent rearrangement region. The positions of the recurrent breakpoint regions (BP1–BP4) are indicated. The lower panel illustrates the deletion intervals (red bars) and duplication intervals (blue bars) for patients.

Among the postnatal patients, 3 patients (3/5) were admitted due to neurodevelopmental delay, 1 (1/5) due to short stature, and 1 (1/5) due to hearing loss. Language delay and motor delay were identified in 3 patients (3/5), while intellectual disability and epilepsy were each observed in 2 patients (2/5). Regarding behavioral characteristics, 1 patient (1/5) exhibited self-mutilation and another (1/5) displayed hyperactivity.

Cranial magnetic resonance imaging revealed a short and thin corpus callosum in 1 patient (1/5) and mild cerebral atrophy in another (1/5). Ophthalmologic evaluation showed strabismus in 1 patient (1/5) and moderate myopia in another (1/5). One patient (1/5) underwent surgery for bilateral pes equinovarus, and 1 patient (1/5) had coxa valga.

Three patients were examined with echocardiography, and no structural heart anomalies were detected.

Prenatal evaluation of the fetus revealed multiple congenital anomalies, including acrania, dextrocardia, and omphalocele.

Molecular Findings

Chromosomal microarray analysis identified recurrent CNVs involving the 16p13.11 locus in our cohort. In the deletion group, rearrangement sizes ranged from 1.53 Mb to 2.82 Mb (mean ~2.37 Mb). In the duplication group, sizes ranged from 1.15 Mb to 2.14 Mb (mean ~1.37 Mb).

Genomic characterization revealed that most duplications originated within the *PDXDC1* gene and extended to *ABCC6*, whereas deletions typically spanned from *MPV17L* to *RPL7P47*. The detailed breakpoints and CNV intervals for all cases are shown in Figure 1 and Table 3.

DISCUSSION

This study presents the clinical and molecular features of 16p13.11 CNVs in 10 unrelated Turkish families and examines their inheritance patterns. Previous reports have shown that CNVs at 16p13.11 are associated with developmental delay, intellectual disability, epilepsy, behavioral abnormalities, and multisystem involvement, with especially cardiac manifestations [11, 16] In previous studies reported from Türkiye, 16p13.11 CNVs have been described within larger chromosomal microarray cohorts in which this region was not the primary focus [13, 14]. Consistent with these reports, our findings also demonstrate variable expressivity, reduced penetrance and a high rate of inherited duplications. However, our study provides clinical and molecular data from a Turkish cohort.

Chromosome 16 is characterized by several regions with a high density of LCRs, particularly at 16p11.2, 16p12.1, 16p13.11, and 16p13.3, which predispose these loci to recurrent structural rearrangements through NAHR [17, 18]. Consistent with this genomic archi-

TABLE 3. Molecular findings of patients with 16p13.11 CNVs

			Length	ACMG
Molecular findings of patients with 16p13.11 deletions				
Patient 1	arr[GRCh38] 16p13.11(14833608_16369679)x1	*NOMO1, MIR3179-1, MIR3670-1, …, PKD1P2, MIR6511A3* (41 genes) OMIM/Morbid Genes: *ABCC1, ABCC6, MYH11, NDE1*	1.5Mb	Pathogenic
Patient 2	arr[GRCh38] 16p13.11(15391011_18215736)x1	*MPV17L, BMERB1, MARF1, …., XYLT1, RPL7P47* (29 genes) OMIM / Morbid Genes: *ABCC1, ABCC6, MYH11, NDE1, XYLT1*	2.8 Mb	Pathogenic
Patient 3	arr[GRCh38] 16p13.11(15391011_18162067)x1	*MPV17L, BMERB1, MARF1, …, XYLT1, RPL7P47* (29 genes) OMIM / Morbid Genes: *ABCC1, ABCC6, MYH11, NDE1, XYLT1*	2.8 Mb	Pathogenic
Patient 4 (Father of patient 3)	arr [GRCh38]16p13.11 (15391011_18162067)x1	*MPV17L, BMERB1, MARF1, …, XYLT1, RPL7P47* (29 genes) OMIM / Morbid Genes: *ABCC1, ABCC6, MYH11, NDE1, XYLT1*	2.8 Mb	Pathogenic
Molecular findings of patients with 16p13.11 duplications				
Patient 5	arr[GRCh38] 16p13.11(14958889_16195675)x3	*PDXDC1, MIR1972-1, NTAN1, …, RPL17P40, ABCC6* (23 genes) OMIM / Morbid Genes: *ABCC1, ABCC6, MYH11, NDE1*	1.2 Mb	VUS (inherited)
Father of Patient 5	arr[GRCh38] 16p13.11(14820784_16369679)x3	*ABCC6P2, NOMO1, MIR3179-1, …, PKD1P2, MIR6511A3* (42 genes) OMIM / Morbid Genes: *ABCC1, ABCC6, MYH11, NDE1*	1.5 Mb	
Patient 6	arr[GRCh38] 16p13.11(14824820_16965478)x3	*ABCC6P2, NOMO1, MIR3179-1, …, PLA2G10FP, SPRING1P3* (45 genes) OMIM / Morbid Genes: *ABCC1, ABCC6, MYH11, NDE1*	2.1 Mb	VUS (inherited)
Mother of Patient 6	arr[GRCh38] 16p13.11(14960949_16425931)x3	*PDXDC1, MIR1972-1, NTAN1, …, NPIPA7, PLA2G10FP* (35 genes) OMIM / Morbid Genes: *ABCC1, ABCC6, MYH11, NDE1*	1.5 Mb	
Patient 7	arr[GRCh38] 16p13.11(15033033_16195675)x3	*PDXDC1, NTAN1, RRN3, …, RPL17P40, ABCC6* (22 genes) OMIM / Morbid Genes: *ABCC1, ABCC6, MYH11, NDE1*	1.2 Mb	VUS (inherited)
Mother of Patiet 7	arr[GRCh38] 16p13.11(15033033_16195675)x3	*PDXDC1, NTAN1, RRN3, …, RPL17P40, ABCC6* (22 genes) OMIM / Morbid Genes: *ABCC1, ABCC6, MYH11, NDE1*	1.2 Mb	
Patient 8	arr[GRCh38] 16p13.11(15036113_16185426)x3	*PDXDC1, NTAN1, RRN3, …, RPL17P40, ABCC6* (22 genes) OMIM / Morbid Genes: *ABCC1, ABCC6, MYH11, NDE1*	1.1 Mb	VUS (inherited)
Mother of Patient 8	arr[GRCh38] 16p13.11(15036113_16195675)x3	*PDXDC1, NTAN1, RRN3, …, RPL17P40, ABCC6* (22 genes) OMIM / Morbid Genes: *ABCC1, ABCC6, MYH11, NDE1*	1.2 Mb	
Patient 9	arr[GRCh38] 16p13.11(15125441_16317633)x3	*PDXDC1, PKD1P6, MIR6511B2, …, PKD1P1, NPIPA6* (25 genes) OMIM / Morbid Genes: *ABCC1, ABCC6, MYH11, NDE1*	1.2 Mb	VUS (inherited)
Father of Patient 9	arr[GRCh38] 16p13.11(15125441_16317633)x3	*PDXDC1, PKD1P6, MIR6511B2, …, PKD1P1, NPIPA6* (25 genes) OMIM / Morbid Genes: *ABCC1, ABCC6, MYH11, NDE1*	1.2 Mb	

Table 3 (cont.). Molecular findings of patients with 16p13.11 CNVs

Molecular findings of patients with 16p13.11 duplications			Length	ACMG
Patient 10	arr[GRCh38] 8p23.3(214984_7179163)x1, 16p13.11 (15446044_18123956)x3	*BMERB1, MARF1, MIR6506, ..., XYLT1, RPL7P47* (28 genes) OMIM / Morbid Genes: *ABCC1, ABCC6, MYH11, NDE1, XYLT1*	7 Mb/ 2.7Mb	VUS (inherited)
Father of Patient 10	arr[GRCh38] 16p13.11(15396616_18131856)x3	*MPV17L, BMERB1, MARF1, ..., XYLT1, RPL7P47* (29 genes) OMIM / Morbid Genes: *ABCC1, ABCC6, MYH11, NDE1, XYLT1*	2.7Mb	
Patient 11	arr[GRCh38] 16p13.11(14683916_16369679)x3	*PLA2G10, NPIPA3, PLAG2G10BP, ..., PKD1P2, MIR6511A3* (47 genes) OMIM/Morbid Genes: *ABCC1, ABCC6, MYH11, NDE1*	1.7Mb	VUS (inherited)
Mother of Patient 11	arr[GRCh38] 16p13.11(14833608_16207329)x3	*NOMO1, MIR3179-1, ..., RPL17P40, ABCC6* (31 genes) OMIM/Morbid Genes: *ABCC1, ABCC6, MYH11, NDE1*	1.4Mb	

ACMG: American College of Medical Genetics and Genomics; classification per ACMG/ClinGen guidelines; Mb: megabase pair; VUS: Variant of uncertain significance.

tecture, breakpoint analysis in our cohort showed that most duplications spanned from *PDXDC1* to *ABCC6*, whereas deletions typically extended from *MPV17L* to *RPL7P47*. These findings support the notion that CNV formation at 16p13.11 is not random but mediated by recurrent recombination hotspots within LCR16 blocks. The CNV region identified in all patients encompassed *interval II*, which represents the recurrent rearrangement hotspot within the 16p13.11 locus [19]. Consistent with previous reports, we were unable to establish a clear genotype–phenotype correlation based on the specific interval affected by the CNVs in our cohort. Although cardiac manifestations have been previously linked to alterations involving the *MYH11* gene, no cardiac abnormalities were observed in our patients, except one fetus with dextrocardia, despite all CNV regions encompassing *MYH11* [7]. The involvement of NDE1 in cortical development has been previously established. In our cohort, all CNVs included *NDE1*; however, neurodevelopmental abnormalities were observed in only two patients with duplications restricted to this locus [20].

Significant differences were observed between patients with deletions and those with duplications in terms of inheritance patterns and clinical expression. Consistent with previous studies, patients with deletions in our cohort predominantly presented with neurodevelopmental features, including developmental delay, epilepsy, and behavioral abnormalities. The majority of deletions occurred *de novo*. Notably, in the patient with an inherited deletion, the parent had long-standing psychiatric symptoms and was receiving medical treatment. This observation suggests that parents may exhibit mild or atypical features that can be easily overlooked. Indeed, deletions involving 16p13.11 have been reported to be associated with a broad spectrum of neuropsychiatric phenotypes [21]. These findings underscore the importance of comprehensive family evaluations, including a detailed psychiatric history, when assessing the inheritance patterns and clinical impact of 16p13.11 CNVs.

In our cohort, cases with 16p13.11 duplications demonstrated more pronounced reduced penetrance and greater phenotypic variability compared to deletion cases. These features complicate clinical interpretation and necessitate more detailed evaluation of duplication cases. In the literature, developmental delay (71–78%), speech delay (8–57%), intellectual disability (35%), behavioral problems (31%), and epilepsy (19%) are among the most commonly reported features in cases with 16p13.11 duplications [11, 22]. When only isolated postnatal

16p13.11 duplication cases (n=5) were evaluated, the neurodevelopmental features observed in our cohort were consistent with this distribution. In contrast, systemic findings such as auditory disorders (10%), cardiovascular anomalies (20%), and renal anomalies (24%), which have been reported in the literature, were either not observed or were present in only a limited number of cases in our cohort. Consistent with previous studies, our patients did not exhibit any specific or recognizable dysmorphic features, supporting the notion that dysmorphism is not a defining characteristic of the 16p13.11 duplication phenotype [23]. In addition, brain MRI findings identified in a limited number of patients were consistent with the frequencies reported in the literature. However, it should be taken into consideration that these rates may be lower if asymptomatic cases are also included in the evaluation. Notably, acrania identified in a fetus may represent a potential phenotypic feature not previously associated with 16p13.11 duplication; however, this observation should be interpreted with caution given the multifactorial etiology of acrania.

In our cohort, the frequent identification of asymptomatic parents suggests the presence of additional factors that modulate the phenotypic expression of 16p13.11 CNVs. In addition, accumulating evidence indicates that the overall "genetic burden," defined as the combined effect of multiple rare variants, may influence clinical outcomes in neurodevelopmental disorders [24, 25]. Furthermore, modifier genes located outside the 16p13.11 region may influence phenotypic outcomes by altering neurodevelopmental pathways. Environmental factors, including prenatal exposures and perinatal complications, may also contribute to the variability observed among cases. Together, these factors likely interact to shape the highly heterogeneous clinical presentation associated with 16p13.11 CNVs.

Supporting this concept, one patient in our cohort carried an additional large de novo deletion at 8p23.3 (~7 Mb) in addition to the 16p13.11 duplication. Given its size and gene content, this deletion is likely to have a significant impact on the clinical phenotype. Notably, this patient showed a more severe clinical presentation compared to other cases with 16p13.11 duplication in our cohort. Previous studies have reported that *de novo* deletions involving the distal 8p23 region are associated with neurodevelopmental phenotypes, including developmental delay, intellectual disability, microcephaly, and neurobehavioral disorders, supporting its clinical relevance [26]. Therefore, the observed features may represent a blended phenotype resulting from the combined effect of both CNVs rather than the 16p13.11 duplication alone. This finding supports a potential combined effect of additional genetic variants and highlights the importance of considering additional CNVs in genotype–phenotype interpretation.

These findings also have important implications for genetic counseling. The presence of reduced penetrance and variable expressivity in 16p13.11 CNVs poses significant challenges, particularly in prenatal settings. The identification of a CNV in a fetus may lead to uncertainty regarding prognosis, as the clinical outcome can range from normal development to significant neurodevelopmental impairment [27]. This uncertainty complicates decision-making processes for families and requires careful, non-directive counseling. In addition, the detection of the same CNV in asymptomatic parents further complicates risk assessment, as it raises questions regarding penetrance, expressivity, and the likelihood of clinical manifestation in the offspring. Therefore, comprehensive genetic counseling should include detailed discussion of phenotypic variability, incomplete penetrance, and the potential contribution of additional genetic or environmental factors. These aspects are essential to support informed decision-making and appropriate clinical management.

Nonetheless, our study had several limitations. The relatively small sample size may restrict the generalizability of our findings and limit the ability to identify less frequent clinical features. Although parental testing was performed in our cohort, the retrospective study design limited uniform access to standardized phenotypic data for all asymptomatic carriers. In addition, exome sequencing or other sequence-level analyses were not systematically performed in our cohort. Therefore, the potential contribution of additional genetic variants to the observed phenotypes cannot be excluded. This limitation is particularly relevant for the prenatal case presenting with multiple congenital anomalies, in which additional sequence-level variants could not be evaluated. For these reasons, our data primarily illustrate the possible phenotypic spectrum of 16p13.11 deletions and duplications rather than defining the frequency of specific clinical features. Future population-based or prospective family-wide studies will be essential to address these limitations.

CONCLUSION

In conclusion, our findings add to the existing clinical and molecular knowledge of 16p13.11 CNVs and highlight

the complexity of their interpretation, particularly in the context of reduced penetrance and variable expressivity. Asymptomatic carriers were more frequently identified among cases with duplications compared to those with deletions. Neurodevelopmental findings in patients with duplications were consistent with previous studies, whereas systemic anomalies were observed at a lower frequency. Importantly, the inclusion of parental testing for all patients represents a strength of our study, enabling accurate determination of inheritance patterns. This comprehensive familial evaluation further highlighted the variable expressivity and incomplete penetrance characteristic of 16p13.11 CNVs.

Ethics Committee Approval: The Adana City Education and Research Hospital Ethics Committee granted approval for this study (date: 20.11.2025, number: 882).

Informed Consent: This retrospective study used anonymized patient data; therefore, the requirement for written informed consent was waived by the Ethics Committee.

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

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

Use of AI for Writing Assistance: The authors declared that artificial intelligence was not used in the study.

Authorship Contributions: Concept – AYU, EU, IK; Design – AYU, EU, IK; Supervision – AYU, EU, IK; Data collection and/or processing – AYU, IK; Analysis and/or interpretation – AYU, IK; Literature review – AYU, IK; Writing – AYU, IK; Critical review – AYU, IK, EU.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2026;13(5):599–605
doi: 10.14744/nci.2026.53503

Original Article Ophthalmology

Investigation of factors influencing intraocular lens implantation strategy following capsular tension ring implantation

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ABSTRACT

OBJECTIVE: The aim of this study was to evaluate the determinants of post-operative visual acuity and the factors influencing surgeons' intraocular lens (IOL) implantation strategy in eyes undergoing capsular tension ring (CTR) implantation during cataract surgery.

METHODS: This retrospective observational cohort study evaluated the outcomes of phacoemulsification with CTR implantation performed between 2023 and 2025. Pre-operative variables included age, cataract stage, and pseudoexfoliation (PEX); intraoperative parameters comprised the extent of zonular dialysis and vitreous prolapse. Multivariable regression, with effect sizes reported as odds ratios or β coefficients and 95% confidence intervals (CIs), was used to identify factors associated with post-operative visual outcome and with IOL implantation strategy.

RESULTS: Of 89 eyes (of 89 patients), 23 received a three-piece sulcus IOL and 66 an in-the-bag foldable IOL. Best-corrected visual acuity (BCVA) improved from a median of 1.0 (interquartile range [IQR] 0.5–1.9) to 0.2 (IQR 0.1–0.4) logMAR (mean 0.37±0.49; p<0.001). Sulcus implantation was more frequent in eyes with greater zonular dialysis (median 90° vs. 0°; p=0.014), vitreous prolapse (p=0.017), and PEX (p=0.035). No significant difference in post-operative BCVA was detected between the two groups (median 0.2 in both; p=0.98). In the multivariable model, greater zonular dialysis (odds ratio [OR] 1.10/10°, 95% CI 1.01–1.20) and vitreous prolapse (OR 5.84, 95% CI 1.27–26.9) were independently associated with sulcus implantation. Post-operative BCVA was associated with pre-operative visual acuity rather than with IOL position; cataract stage was associated with the outcome in univariable analysis (p=0.035) but not after adjustment for pre-operative BCVA (β=-0.19, p=0.065).

CONCLUSION: In this CTR-treated cohort, favorable 12-month visual and lens-related outcomes were observed across a wide range of zonular compromise. Greater zonular dialysis and vitreous prolapse were independently associated with sulcus IOL implantation. No difference in 12-month BCVA was detected between the two strategies; given the sample size, this indicates the absence of a detected difference rather than equivalence of the two approaches. The findings should be considered hypothesis generating rather than confirmatory and warrant validation in larger prospective, multicenter studies incorporating objective assessments.

Keywords: Capsular tension ring; cataract surgery; intraocular lens; surgical outcomes; zonular weakness.

Cite this article as: *Celik Yaprak D, Dogan Gokce G. Investigation of factors influencing intraocular lens implantation strategy following capsular tension ring implantation. North Clin Istanb 2026;13(5):599–605.*

Zonular fibers connect the ciliary body to the equator of the lens, maintaining both lens position and curvature. Laxity, attenuation, or structural disruption of these fibers is defined as zonular weakness. In such cases, lens instability becomes evident, complicating intraocular procedures such as cataract surgery and increasing

Received: August 03, 2026 *Revised:* September 07, 2026 *Accepted:* September 18, 2026 *Online:* October 15, 2026

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the risk of intraocular lens (IOL) decentration or dislocation [1]. In the general population, the prevalence of zonular weakness ranges from 0.46% to 2.6%. Several etiologic risk factors are associated with zonular instability, including advanced age, pseudoexfoliation (PEX) syndrome, Marfan syndrome, homocystinuria, retinitis pigmentosa, mature cataracts, high axial myopia, and a history of ocular trauma or previous surgery [2]. The clinical presentation of zonular deficiency spans a broad spectrum: Mild cases may exhibit only subtle zonular laxity, whereas severe cases can demonstrate pronounced phacodonesis or frank lens subluxation. Intraoperatively, zonulopathy may manifest at various stages of cataract surgery; anterior capsular wrinkling during continuous curvilinear capsulorhexis, capsular bag instability during intraocular manipulation, or progressive imbalance of the capsulozonular complex serve as critical warning signs for the surgeon [3].

To address these challenges, capsular tension rings (CTRs) were developed to stabilize the capsular bag in the presence of zonular weakness or dialysis, redistribute zonular forces, and reduce the intraoperative and post-operative risks of IOL decentration and tilt [4]. However, the literature remains conflicting regarding their efficacy. An intraindividual randomized study reported that simultaneous implantation of a CTR and a plate-haptic IOL in eyes without zonular weakness did not improve rotational stability and was associated with greater decentration, tilt, and axial displacement [5]. Other investigations found that CTR implantation does not significantly alter refractive outcomes compared with routine phacoemulsification, indicating that standard IOL power formulas can be used safely even in eyes receiving a CTR [6].

The first CTR designed for human use was introduced in 1993 [7]. Since then, the device has undergone multiple modifications to improve its stability, flexibility, and clinical applicability, and various designs continue to be used in clinical practice, with surgical and visual outcomes extensively reported [8].

Although CTRs are widely used to enable in-the-bag IOL fixation in zonulopathic eyes, the factors that lead surgeons to choose sulcus over in-the-bag fixation after CTR implantation – and whether that choice affects the visual outcome – have rarely been examined directly. In the present study, we describe post-operative visual outcomes in eyes that received a CTR and examine the clinical and intraoperative factors associated with IOL implantation strategy. The design is descriptive and does not permit any assessment of the efficacy of CTR implantation relative to surgery without a CTR.

Highlight key points

- Capsular tension ring (CTR) implantation provided effective capsular stabilization in eyes with varying degrees of zonular weakness.
- At 12 months, visual acuity improved substantially after CTR-assisted cataract surgery, and no lens-related complications were detected on slit-lamp examination.
- Greater zonular dialysis and intraoperative vitreous prolapse were independently associated with sulcus intraocular lens (IOL) implantation.
- Post-operative visual acuity was primarily associated with pre-operative best-corrected visual acuity rather than IOL position.

MATERIALS AND METHODS

This retrospective observational cohort study included patients who underwent phacoemulsification with CTR implantation at our institution between 2023 and 2025. The study adhered to the tenets of the Declaration of Helsinki, and Institutional Ethics Committee approval was obtained before data collection. Consecutive eyes in which an 11-mm or 12-mm CTR was used during phacoemulsification were evaluated. Patients aged 18–90 years were included; one eye per patient was analyzed. Exclusion criteria were corneal opacities compromising imaging quality, retinal disorders that could affect post-operative outcomes, previous ocular surgery, pre-operative spherical refractive error ≥6.0 D, and cylindrical refractive error ≥3.0 D by autorefractometry.

No a priori sample-size or power calculation was performed. The study included all consecutive eligible eyes that underwent phacoemulsification with CTR implantation at our center between 2023 and 2025; therefore, the sample size was determined by the number of eligible procedures performed during the study period. All procedures were performed at a single tertiary referral center by surgeons with varying levels of surgical experience, using a standardized phacoemulsification technique and the same CTR models (11 mm or 12 mm). Surgeon identity and individual level of surgical experience were not recorded in the surgical documentation system and could therefore not be included as covariates, clustering variables, or random effects in the statistical models.

Pre-operative assessment included best-corrected visual acuity (BCVA) (Snellen, converted to logMAR), intraocular pressure, and slit-lamp biomicroscopy of the anterior and posterior segments. Intraoperative parameters – PEX status, pupil dilation, cataract morphology and grade, anterior capsulorhexis complications, degree of zonular dialysis, evidence of zonular insufficiency (PEX, shallow anterior chamber, high myopia, or phacodonesis), intraoperative vitreous prolapse, posterior capsule integrity, and IOL location (bag vs. sulcus) – were recorded from the integrated surgical documentation system. Cataracts were graded clinically at the slit lamp by the operating surgeon before surgery, in line with the descriptive framework of Lam et al. [9], as mild (early lens opacity with a clearly visible red reflex and a discernible fundus view), moderate (denser nuclear and/or cortical opacity with a diminished red reflex and a partially obscured fundus view), or mature (complete lens opacification with an absent red reflex and no fundus view). Photographic grading (for example, Lens Opacities Classification System III) was not performed; the classification is therefore clinical and categorical and is subject to a degree of inter-observer variability. Lens-related complications were defined as any of the following, documented at slit-lamp examination during follow-up: Clinically evident IOL decentration or tilt; dislocation of the IOL or of the IOL–capsular bag complex; pupillary optic capture; clinically significant capsular contraction; or any reoperation for IOL repositioning, exchange or explantation. Post-operative results at 12 months were analyzed.

Statistical Analysis

Analyses were performed with Jamovi (v2.7; The Jamovi Project, Sydney, Australia). Normality was assessed with the Shapiro–Wilk test and Q-Q plots. Normally distributed variables are presented as mean±standard deviation and non-normally distributed variables as median (interquartile range, IQR). Because the degree of zonular dialysis and pre-operative BCVA were right-skewed, they are summarized and compared non-parametrically. Two-group comparisons used the independent-samples t-test or the Mann–Whitney U-test as appropriate; categorical variables used the Chi-square or Fisher exact test. Factors associated with IOL implantation strategy were examined with multivariable logistic regression, and determinants of post-operative BCVA with multivariable linear regression. Covariates were selected a priori based on their clinical relevance to zonular instability and IOL implantation strategy. The degree of zonular dialysis and intraoperative vitreous prolapse were included as direct indicators of compromised capsular support, while PEX was included as an important underlying cause of zonular weakness. All three variables were also significantly associated with implantation site in the univariable analysis. Given the 23 sulcus cases, the logistic model was limited to these three covariates to reduce the risk of overfitting.

Effect sizes are reported as odds ratios (logistic) or β coefficients (linear) with 95% CIs, and model fit as McFadden pseudo-R^2 (logistic) or adjusted R^2 (linear). A two-sided $p<0.05$ was considered statistically significant.

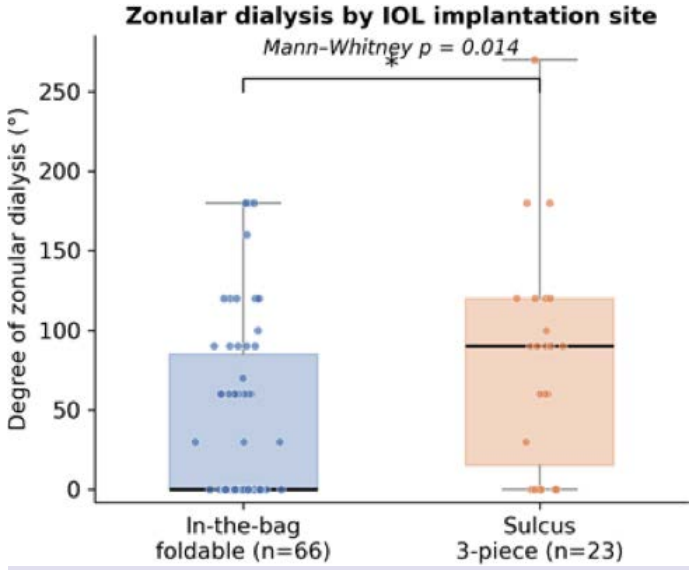

FIGURE 1. Degree of zonular dialysis by intraocular lens implantation site. The sulcus group had a higher degree of dialysis (median 90° vs. 0°; Mann–Whitney p=0.014). Boxes show median and interquartile range; points are individual eyes.

RESULTS

A total of 89 eyes of 89 patients were included. Mean age was 67.5±12.1 years. BCVA improved significantly, from a median of 1.0 (IQR 0.5–1.9) to 0.2 (IQR 0.1–0.4) logMAR (mean 0.37±0.49; Wilcoxon $p<0.001$). Zonular pathology was present in all cases: Clinically evident zonular dialysis in 49 eyes (55%) and zonular weakness without overt dialysis in 40 eyes (45%). The degree of zonular dialysis was median 30° (IQR 0°–90°; mean 53.7°±61.8°; range 0°–120°), reflecting a right-skewed distribution.

When stratified by cataract stage, 26 eyes had mature, 40 moderate, and 23 mild cataract. Pre-opera-

Table 1. Pre-operative and intraoperative characteristics by IOL position following CTR implantation

Parameters	Foldable IOL, in-the-bag (n=66)	3-piece IOL, ciliary sulcus (n=23)	p
Age (years, mean±standard deviation)	66.2±13.0	71.3±7.5	0.08
Cataract stage, n (%)			0.1
Mature	23 (35)	3 (13)	
Moderate	27 (41)	13 (57)	
Mild	16 (24)	7 (30)	
Pseudoexfoliation, n (%)	9 (14)	8 (35)	0.035
Pre-operative BCVA, logMAR, median (IQR)	1.00 (0.50–1.90)	1.30 (0.70–1.90)	0.57
Intraoperative vitreous prolapse, n (%)	4 (6)	6 (26)	0.017
Zonular dialysis, n (%)	32 (48)	17 (74)	0.05
Zonular weakness, n (%)	34 (52)	6 (26)	
Degree of dialysis (°), median (IQR)	0 (0–85)	90 (15–120)	0.014
Post-operative 12-month BCVA, logMAR, median (IQR)	0.20 (0.10–0.45)	0.20 (0.10–0.30)	0.98

BCVA: Best-corrected visual acuity; CTR: Capsular tension ring; IOL: Intraocular lens; IQR: Interquartile range. Right-skewed variables are summarized as median (IQR) and compared with the Mann–Whitney U test; categorical variables with the Chi-square or Fisher exact test. The p-value for the zonular dialysis/weakness split.

Table 2. Multivariable regression models: Effect sizes with 95% CIs

Predictor	Effect size	95% CI	p
Logistic – outcome: Sulcus versus in-the-bag (McFadden pseudo-R^2=0.146)			
Degree of zonular dialysis (per 10°)	OR 1.10	1.01–1.20	**0.029**
Vitreous prolapse	OR 5.84	1.27–26.9	**0.023**
Pseudoexfoliation	OR 3.13	0.91–10.7	0.070
Linear – outcome: Post-operative BCVA (adjusted R^2=0.13)			
Pre-operative BCVA	β +0.27	+0.08 to +0.46	**0.005**
Cataract stage	β -0.19	-0.39 to +0.01	0.065
Degree of zonular dialysis (per °)	β +0.001	-0.001 to +0.003	0.23
Vitreous prolapse	β -0.10	-0.47 to +0.27	0.58
IOL implantation site (sulcus)	β -0.04	-0.31 to +0.23	0.74

OR: Odds ratio; CI: Confidence interval; IOL: Intraocular lens; β: Unstandardized linear-regression coefficient; BCVA: Best-corrected visual acuity (logMAR). The logistic model was kept parsimonious to respect the events-per-variable limit set by the 23 sulcus cases. Cataract stage was coded 1=Mature, 2=Moderate, and 3=Mild.

tive BCVA differed significantly across cataract stages (p<0.001), whereas age did not (p=0.1). Eyes with mature cataract had worse baseline acuity than lower-grade eyes. In univariable analysis, cataract stage was associated with 12-month post-operative BCVA (p=0.035), but this association did not persist after adjustment for pre-operative BCVA. Group characteristics are shown in Table 1.

IOL implantation site was in-the-bag foldable IOL in 66 eyes and three-piece ciliary sulcus IOL in 23 eyes. Implantation site was not significantly associated with age (66.2±13.0 vs. 71.3±7.5 years; p=0.08) or with post-operative BCVA (median 0.2 in both groups; p=0.98). It was significantly associated with the degree of zonular dialysis (median 90° [IQR 15°–120°] in the sulcus group vs. 0° [IQR 0°–85°] in the in-the-bag group; p=0.014;

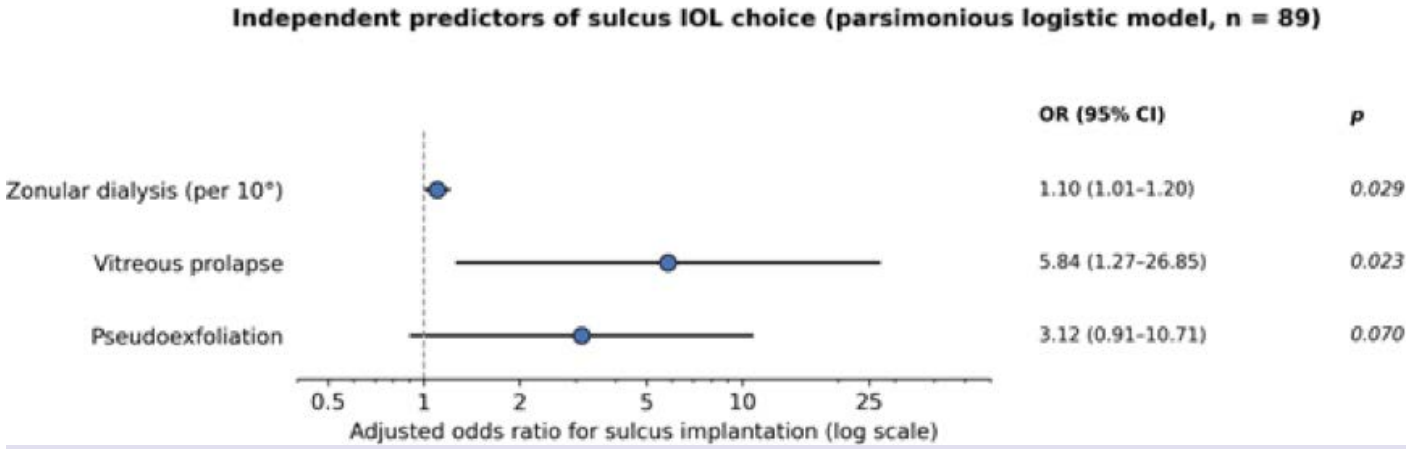

FIGURE 2. Adjusted odds ratios (95% confidence interval) for sulcus intraocular lens implantation from the parsimonious logistic model. A greater degree of zonular dialysis and intraoperative vitreous prolapse were independently associated with sulcus implantation.

Fig. 1), intraoperative vitreous prolapse (26% vs. 6%; p=0.017), and PEX (8/23 vs. 9/66; Fisher p=0.035). Cataract stage was not significantly associated with implantation strategy (p=0.1). In multivariable logistic regression, a greater degree of zonular dialysis (adjusted OR 1.10/10°, 95% CI 1.01–1.20; p=0.029) and intraoperative vitreous prolapse (adjusted OR 5.84, 95% CI 1.27–26.9; p=0.023) were independently associated with sulcus implantation, whereas PEX was not (OR 3.13, 95% CI 0.91–10.7; p=0.070). The model showed modest fit (McFadden pseudo-R^2=0.146). Effect estimates are shown in Table 2 and Figure 2.

The linear regression was performed as a complete-case analysis and included 69 eyes with complete data for all model variables. Twenty eyes were excluded because at least one variable required for the linear regression model was not documented. The complete-case approach assumes that these data were missing at random; this assumption cannot be verified retrospectively, and a selective loss of eyes with poorer outcomes cannot be excluded.

In multivariable linear regression for post-operative BCVA, IOL implantation site was not associated with the outcome (β=-0.04, 95% CI -0.31 to +0.23; p=0.74), nor were the degree of zonular dialysis (β=+0.001, 95% CI -0.001 to +0.003; p=0.23), vitreous prolapse (β=-0.10, 95% CI -0.47 to +0.27; p=0.58), or cataract stage (β=-0.19, 95% CI -0.39 to +0.01; p=0.065). Post-operative BCVA was significantly associated with pre-operative BCVA (β=+0.27, 95% CI +0.08 to +0.46; p=0.005). The overall model showed low explanatory power (adjusted R^2=0.13).

No lens-related complications, as defined above, were observed at the 12-month follow-up. No eye had a spherical refractive error ≥6.0 D or astigmatism ≥3.0 D.

DISCUSSION

In this cohort, favorable 12-month visual and lens-related outcomes were observed following CTR-assisted cataract surgery across a broad spectrum of zonular compromise. Surgeons more frequently chose sulcus IOL implantation in eyes with greater zonular dialysis and vitreous prolapse, yet post-operative BCVA did not differ by IOL position. Post-operative visual outcome was primarily associated with pre-operative visual acuity. It should be emphasized that a non-significant between group comparison of BCVA (p=0.98) in a sample of this size indicates that no difference was detected, not that the two strategies are equivalent; the study was not powered for a formal equivalence test, and the comparison should be interpreted together with the confidence interval of the adjusted estimate (β=-0.04 95% CI -0.31 to +0.23), which does not exclude clinically relevant differences in either direction.

These findings suggest that once adequate capsular support is achieved with a CTR, the choice of IOL position may have a limited impact on functional outcome. This is consistent with reports that CTRs redistribute zonular tension and enhance capsular stability, reducing the influence of zonular pathology on lens positioning and visual performance [4]. In our cohort, neither a difference in 12-month BCVA nor any lens-related complication was detected between the in-the-bag and sulcus

groups; given the sample size, the observational design and the limited follow-up, this should not be interpreted as evidence that CTR implantation neutralizes the biomechanical consequences of zonular loss, but only that no such difference was detectable in this series. Literature suggests that when zonular dehiscence involves fewer than three clock-hours, conventional CTR implantation generally allows safe in-the-bag placement, whereas more extensive loss may require capsular tension segments or modified rings; in the absence of adequate support, sulcus placement is a reasonable alternative, particularly in severe PEX or traumatic zonular compromise [10]. In our cohort (median dialysis 30°; range 0°–120°), outcomes were achieved safely across this range, suggesting that factors beyond the absolute degree of dialysis inform the optimal strategy.

Notably, the sulcus implantation rate in our series (26%) was considerably higher than the 7% reported by Tribus et al. [11] following CTR implantation. This difference may reflect variation in surgical decision thresholds, case mix and case complexity, or in the frequency of intraoperative events such as vitreous prolapse, rather than a true difference in capsular support. Because surgeon identity and experience were not recorded in our dataset, we were unable to examine surgeon-level variation directly, and this explanation remains a hypothesis rather than a demonstrated finding. Among the available options – sulcus posterior chamber IOL (PCIOL), anterior chamber IOL, iris-fixated IOL, or in-the-bag PCIOL with CTR – in-the-bag PCIOL supported by a CTR is generally preferred for its biomechanical and optical advantages [12].

When the multivariable model was restricted to a parsimonious set of covariates appropriate for the 23 sulcus events, a greater degree of zonular dialysis (adjusted OR 1.10/10°) and intraoperative vitreous prolapse (adjusted OR 5.84) remained independently associated with sulcus implantation. A saturated model containing all candidate variables abolished these associations – an expected consequence of overfitting when the number of predictors approaches the number of events – so the parsimonious estimates are more credible. The wide confidence interval around the odds ratio for vitreous prolapse (OR 5.84, 95% CI 1.27–26.9) likely reflects the small number of eyes in which vitreous prolapse occurred (n=10, including six eyes in the sulcus group). Accordingly, the effect estimate should be interpreted with caution, as the true magnitude of the association remains imprecisely estimated.

Unlike a Guyana cohort in which intraoperative zonular abnormalities and posterior capsule rupture independently predicted poor visual outcomes [13], our study included only CTR-stabilized eyes, which may have mitigated the negative impact of zonular compromise on vision. Consistent with reports that modified CTRs provide favorable outcomes in extensive zonular insufficiency [14], no lens-related complications occurred at 12 months even though standard CTRs were used.

Several limitations should be acknowledged. First, the retrospective design carries risks of data incompleteness and selection bias; in particular, the decision between in-the-bag and sulcus fixation was made intraoperatively and was not protocol-driven, so residual confounding by unrecorded intraoperative findings cannot be excluded. Second, the sulcus group was small (23 eyes); the multivariable models were therefore at risk of overfitting, the study was underpowered to detect between-group differences in BCVA, and the non-significant regression results should be interpreted with corresponding caution. Third, this was a multi-surgeon series and the choice between in-the-bag and sulcus fixation is plausibly correlated within surgeons; because surgeon identifiers were not recorded, we could not fit a mixed-effects model or compute cluster-robust standard errors. Unaccounted clustering of this kind would tend to make our standard errors optimistic and our confidence intervals narrower than they should be, so the reported precision is best regarded as an upper bound. For the same reason, the contribution of surgical judgment could not be quantified and should be regarded as an untested hypothesis rather than a demonstrated effect; prospective recording of surgeon identity and experience is required to address this properly. Fourth, post-operative IOL position, tilt, and decentration were not assessed with objective imaging (for example, anterior-segment OCT or Scheimpflug photography); positional outcomes therefore rest on slit-lamp examination alone, and subtle malposition may have gone undetected. Fifth, no lens-related complications were observed. However, given the sample size and 12-month follow-up, the study was underpowered to detect rare or late events. In particular, late IOL-capsular bag dislocation may occur beyond the first post-operative year and may therefore not have been captured. In addition, because the study did not include a non-CTR comparator group, the observed visual and lens-related outcomes cannot be attributed specifically to CTR implantation, and no conclusions regarding CTR efficacy can be drawn from these data. Finally, follow-up was

limited to 12 months, so long-term visual and positional stability could not be assessed. The main strength of the study is that, to our knowledge, it directly examines the drivers of the in-the-bag-versus-sulcus decision after CTR implantation and their relationship to visual outcome in a real-world tertiary-center setting. These findings should be regarded as hypothesis-generating and require confirmation in larger prospective studies.

Conclusion

Visual acuity improved substantially at 12 months in eyes undergoing CTR-assisted cataract surgery for zonular dialysis or weakness, and no lens-related complications were recorded on slit-lamp examination. Although in-the-bag implantation was more common, three-piece sulcus IOL placement was more frequently selected in the presence of greater zonular dialysis and vitreous prolapse, both of which were independently associated with implantation strategy. These findings indicate that measurable intraoperative features are associated with the choice of IOL position. IOL position was not associated with post-operative BCVA in this cohort; however, the width of the corresponding confidence interval means that a clinically meaningful difference between the two strategies cannot be excluded. Visual outcome was associated primarily with pre-operative BCVA; cataract stage was associated with the outcome in univariable analysis but not after adjustment for baseline vision. Larger prospective studies incorporating objective assessments of post-operative IOL position, capsular stability, refractive outcomes, and long-term complications are needed to validate these findings.

Ethics Committee Approval: This study was approved by The Ethics Committee of Kartal Dr. Lutfi Kirdar City Hospital (Decision No: 2026/010.99/24/54, January 27, 2026).

Informed Consent: Written informed consent was 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 received no financial support.

Use of AI for Writing Assistance: Artificial intelligence (AI)–assisted technologies were used exclusively for language editing and manuscript improvement.

Authorship Contributions: Concept – DCY, GDG; Design – DCY; Supervision – DCY, GDG; Data Collection and/or Processing – DCY, GDG; Analysis and/or Interpretation – GDG; Literature Search – DCY; Writing – DCY, GDG; Critical Reviews – DCY, GDG.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2026;13(5):606–614
doi: 10.14744/nci.2026.99223

Original Article DERMATOLOGY AND VENEREOLOGY

Convergence and divergence in the burden of skin diseases across organisation for economic co-operation and development countries, 2000–2023

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ABSTRACT

OBJECTIVE: The aim of the study was to examine temporal trends and cross-country convergence in age-standardized disability-adjusted life-year (DALY) rates for 15 non-neoplastic skin and subcutaneous diseases across 38 Organisation for Economic Co-Operation and Development (OECD) countries between 2000 and 2023, and to assess whether sex differences followed a common trajectory.

METHODS: This ecological longitudinal panel study used estimates from the Global Burden of Disease 2023 study. Sigma convergence was assessed using annual cross-country dispersion, whereas unconditional beta convergence was evaluated based on the relationship between baseline disease burden and subsequent logarithmic change. Overall and club convergence were examined using the Phillips-Sul log-t framework. Female-to-male DALY rate ratios were analyzed separately.

RESULTS: The balanced dataset comprised 41,040 observations. The largest increases in median DALY rates were observed for pyoderma (+134.2%), cellulitis (+107.2%), and acne vulgaris (+51.6%). Sigma convergence was identified for eight diseases, whereas beta convergence was observed for only three. No disease showed a single common trajectory across countries; instead, two to seven disease-specific country clusters emerged. For both atopic dermatitis and urticaria, 15 countries were classified as divergent and did not belong to any convergence club. Female-to-male DALY rate ratios also failed to exhibit a common cross-country trajectory for all 15 diseases.

CONCLUSION: The burden of skin diseases across OECD countries did not converge toward a single common trajectory. Instead, disease-specific convergence patterns, divergent countries, and cross-country variation in sex differences were observed. These findings highlight substantial heterogeneity beneath median trends and may support disease-, country-, and sex-sensitive surveillance rather than a uniform OECD-wide approach.

Keywords: Basic medical sciences; dermatology; disability-adjusted life years; health status disparities; longitudinal studies; skin diseases.

Cite this article as: *Tumturk M. Convergence and divergence in the burden of skin diseases across organisation for economic co-operation and development countries, 2000–2023. North Clin Istanb 2026;13(5):606–614.*

Skin and subcutaneous diseases constitute a substantial component of the global disease burden because of their high prevalence and considerable effects on quality of life [1, 2]. Although their overall burden has increased markedly over the past three decades, trends vary across countries and disease groups [2, 3]. Differences remain evident even among Organisation for Economic Co-Operation and Development (OECD) countries, despite their broad similarities in economic development and health system capacity. This raises an important question: Are national skin disease burdens moving toward a common level over time, or are disparities persisting?

Received: September 15, 2026 *Revised:* October 09, 2026 *Accepted:* October 18, 2026 *Online:* November 10, 2026

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Comparisons based on selected years provide only a partial picture of cross-country inequalities. Examining how these differences change over time may offer more useful evidence for health policy. Convergence analysis provides a framework for this purpose. Sigma convergence indicates whether the dispersion of disease burden across countries decreases over time, whereas beta convergence examines whether countries with a higher initial burden subsequently experience different rates of change [4]. These methods have been applied to mortality, health expenditure, and other population health indicators [5, 6]. However, countries may not follow a single common pattern. The Phillips-Sul method first tests whether all countries converge. If overall convergence is rejected, it identifies groups of countries with similar trajectories, termed convergence clubs, as well as countries that do not belong to any club, termed divergent countries [7]. This approach can reveal persistent cross-country differences that may be obscured in analyses of the full country sample [8].

This study examines temporal trends and cross-country convergence in age-standardized disability-adjusted life year (DALY) rates for 15 non-neoplastic skin and subcutaneous diseases across 38 OECD countries from 2000 to 2023. Sigma convergence, unconditional beta convergence, and Phillips-Sul overall and club convergence are used to assess whether countries are moving toward a common trajectory for each disease. Female-to-male DALY rate ratios are analyzed separately to determine whether sex differences follow similar cross-country patterns. By integrating these complementary approaches, the study evaluates whether OECD countries are converging toward a shared burden trajectory or retaining disease- and sex-specific heterogeneity. Beyond OECD-wide median trends, the analysis provides dermatologists and public health authorities with a disease- and country-specific basis for surveillance and priority setting.

Highlight key points

- Median disability-adjusted life-year rates increased most markedly for pyoderma (+134.2%), cellulitis (+107.2%), and acne vulgaris (+51.6%) between 2000 and 2023.
- Sigma convergence was observed for eight diseases and beta convergence for three, indicating limited reductions in cross-country inequalities.
- Phillips-Sul analysis rejected a common convergence trajectory for all 15 diseases and identified two to seven disease-specific convergence clubs.
- Pronounced heterogeneity in disease burden and female-to-male disparities supports disease-specific, sex-sensitive, and country-tailored surveillance across OECD countries.

MATERIALS AND METHODS

Study Design and Data Source

This ecological longitudinal panel study used estimates from the Global Burden of Disease Study 2023 (GBD 2023), obtained through the GBD Results Tool maintained by the Institute for Health Metrics and Evaluation (IHME). A fixed panel of the 38 current member countries of the OECD was examined over the period 2000–2023. Fifteen non-neoplastic skin and subcutaneous disease categories were included. The conditions included in the residual category "other skin and subcutaneous diseases" are listed in Supplementary Table S1 according to the GBD 2023 cause hierarchy. All analyses were based on age-standardized rates derived from aggregated data. Data source: Institute for IHME, GBD Study 2023, GBD Results Tool. Accessed in September 2026. Neoplastic skin conditions were excluded because melanoma and non-melanoma skin cancers are classified within the neoplasm framework in GBD and differ substantially from non-neoplastic dermatologic conditions in their mortality contribution, disease course, and burden profile. The analysis therefore focused on non-neoplastic skin and subcutaneous diseases to maintain a clinically and epidemiologically coherent disease set.

Outcomes

The primary outcome was the age-standardized DALY rate/100,000 population. Sex-specific DALY rates for females and males were additionally analyzed, and sex differences analysis were summarized using the female-to-male rate ratio. DALYs were selected as the primary analytical measure because they capture overall health loss rather than disease occurrence alone. DALYs combine years of life lost due to premature death and years lived with disability, the latter calculated by multiplying disease prevalence by the severity weight of the associated disability [9].

Descriptive Analyses

For each disease, country-level distributions of age-standardized DALY rates were summarized for 2000 and

2023 using median values. Relative changes in median rates between 2000 and 2023 were calculated. Point estimates from GBD 2023 were used in the primary analyses. The corresponding lower and upper uncertainty bounds provided by GBD were retained in the source dataset but were not propagated through the primary convergence models.

Sigma and Beta Convergence

Sigma convergence was evaluated using the annual cross-country standard deviation of the natural logarithm of age-standardized rates. A negative temporal slope indicated declining cross-country dispersion and was interpreted as evidence of sigma convergence, whereas a positive slope indicated divergence. Temporal trends were estimated using heteroskedasticity- and autocorrelation-consistent Newey-West standard errors. Beta convergence was evaluated by relating the logarithmic change in disease burden over 2000–2023 to the logarithm of the baseline rate in 2000. A negative coefficient was interpreted as evidence that countries with higher initial burden experienced relatively lower subsequent growth or greater decline. Heteroskedasticity-consistent HC3 standard errors were used.

Phillips-Sul Convergence Analysis

For each disease, overall convergence was first evaluated using the Phillips-Sul log-t regression with a trimming parameter of r=0.30 (i.e., the first 30% of the time series was discarded) and an Andrews fixed bandwidth Quadratic Spectral heteroskedasticity and autocorrelation consistent estimator. The null hypothesis of convergence was rejected when the one-sided log-t statistic was below -1.645. When overall convergence was rejected, the Phillips-Sul club clustering algorithm was applied to identify groups of countries exhibiting similar relative transition dynamics, using a sieve threshold initialized at $c^*=0$ and increased in increments of 0.1, when necessary, up to a maximum of 3. The procedure included identification of a core group, sequential membership testing, formation of subsequent clubs, and tests for possible club merging. Adjacent clubs were merged when the combined log-t test did not reject convergence, and countries not assigned to a valid club were classified as divergent.

Sex Disparity Analysis

Sex disparity was assessed using country-specific female-to-male ratios of age-standardized DALY rates. Ratios >1 indicated a higher burden among females, whereas ratios below 1 indicated a higher burden among males. For each disease, the median country-specific ratio across OECD countries was summarized for 2000 and 2023. The Phillips-Sul framework was additionally applied to determine whether these sex disparity trajectories converged across OECD countries.

Statistical Analysis and Software

Analyses were conducted in R version 4.5.0 within RStudio version 2026.08.2 Build 200. Phillips-Sul analyses were implemented following the algorithm used in Convergence Clubs version 2.2.5. Sensitivity analyses were performed using r=0.20 and r=0.40. For the sigma and beta convergence analyses, p-values were adjusted separately across the 15 disease-specific tests using the Benjamini-Hochberg false discovery rate procedure. Statistical significance after correction was defined as $q<0.05$. The Phillips-Sul log-t tests were not subjected to multiplicity correction.

RESULTS

Data Coverage

The balanced DALY dataset comprised 41,040 observations, corresponding to 38 OECD countries ×15 skin and subcutaneous disease categories ×24 years (2000–2023) ×sex groups (both sexes combined, females, and males). The primary both sex convergence panel comprised 13,680 observations.

Changes in Age-Standardized DALY Rates

Across OECD countries, the largest increases in the median age-standardized DALY rate occurred for pyoderma (+134.2%), cellulitis (+107.2%), and acne vulgaris (+51.6%). The largest reductions occurred for fungal skin diseases (-17.1%) and scabies (-6.4%). In 2023, atopic dermatitis had the highest median DALY rate, followed by urticaria, psoriasis, and contact dermatitis (Table 1).

Sigma Convergence

After Benjamini-Hochberg correction, cross-country dispersion in DALY rates declined significantly for eight conditions: Psoriasis, viral skin diseases, seborrhoeic dermatitis, urticaria, decubitus ulcer, alopecia areata, scabies, and fungal skin diseases. Dispersion increased for pruritus, contact dermatitis, and other skin and subcutaneous

TABLE 1. Disability-adjusted life year trends and convergence results

Disease	Median, 2000	Median, 2023	Change, percentage	Sigma coefficient, (q-value)	Beta coefficient, (q-value)	Overall, log-t	Final, clubs	Divergent, countries
Pyoderma	4.99	11.68	134.2	0.001768 (0.176)	-0.1926 (0.198)	-10.76	4	0
Cellulitis	2.89	5.99	107.2	-0.000216 (0.586)	-0.0888 (0.373)	-23.63	4	1
Acne vulgaris	15.93	24.15	51.6	-0.000257 (0.120)	-0.1686 (0.202)	-54.94	4	1
Psoriasis	36.32	41.49	14.2	-0.000505 (<0.001)	-0.1350 (0.049)	-28.69	5	0
Pruritus	1.23	1.39	12.6	0.001960 (<0.001)	0.1390 (0.217)	-9.71	5	1
Other skin and subcutaneous diseases	14.18	14.66	3.4	0.001736 (<0.001)	0.2211 (0.279)	-64.4	3	1
Viral skin diseases	12.36	12.46	0.8	-0.000095 (<0.001)	-0.0058 (0.110)	-45.73	5	8
Seborrhoeic dermatitis	4.11	4.11	0.1	-0.000053 (<0.001)	-0.0165 (0.110)	-33.15	4	3
Atopic dermatitis	111.71	111.53	-0.2	-0.000011 (0.554)	0.0002 (0.967)	-44.31	6	15
Contact dermatitis	41.51	41.41	-0.2	0.000052 (<0.001)	0.0132 (0.194)	-57.88	6	9
Urticaria	55.46	55.28	-0.3	-0.000037 (0.019)	-0.0020 (0.439)	-45.2	7	15
Decubitus ulcer	5.21	5.19	-0.5	-0.009710 (<0.001)	-0.4373 (<0.001)	-1.74	2	0
Alopecia areata	1.89	1.88	-0.6	-0.000112 (<0.001)	-0.0106 (0.014)	-38.86	6	4
Scabies	3.45	3.22	-6.4	-0.004959 (<0.001)	-0.0756 (0.198)	-68.45	4	1
Fungal skin diseases	11.31	9.37	-17.1	-0.001515 (<0.001)	-0.1565 (0.194)	-89.09	3	2

diseases. No clear change was identified for pyoderma, cellulitis, acne vulgaris, or atopic dermatitis.

Beta Convergence

After multiplicity correction, significant unconditional beta convergence in DALY rates was identified for psoriasis, decubitus ulcer, and alopecia areata. The remaining conditions showed no statistically robust evidence of beta convergence or divergence. Thus, reductions in cross-country dispersion were not consistently accompanied by slower growth or faster decline among countries with a higher initial burden.

Phillips-Sul Convergence and Convergence Clubs

Under the primary Phillips-Sul specification, the full panel log-t test rejected overall convergence for all 15 conditions, with all test statistics below the -1.645 critical value. Log-t statistics ranged from -1.74 for decubitus ulcer to -89.09 for fungal skin diseases. The club classification and merging procedure identified between two and seven disease-specific convergence clubs. Decubitus ulcer formed two clubs, whereas urticaria formed seven. The largest numbers of countries classified as divergent were observed for atopic dermatitis and urticaria (15 countries each), followed by contact dermatitis (9 countries) and viral skin diseases (8 countries). No country was classified as divergent for decubitus ulcer, psoriasis, or pyoderma (Fig. 1). All retained clubs satisfied the convergence criterion. Disease-specific club memberships are presented in Supplementary Table S2, and convergence heterogeneity is summarized in Figure 2.

The rejection of full panel convergence was robust to alternative trimming fractions for all diseases at r=0.20 and for 14 of the 15 diseases at r=0.40; decubitus ulcer was the only exception at r=0.40 (t=1.09) (Supplementary Table S3).

Sex Differences

In 2023, the median female-to-male DALY rate ratio exceeded 1.00 for nine conditions. The greatest relative burden among females was observed for urticaria (1.48), pruritus (1.46), and alopecia areata (1.41). Conversely, the lowest female-to-male ratios, indicating a relatively higher burden among males, were observed for cellulitis (0.68), pyoderma (0.84), and decubitus ulcer (0.87). Full panel convergence of the female-to-male ratio was rejected for all 15 conditions, indicating that sex differences did not follow a common cross-country transition path (Table 2).

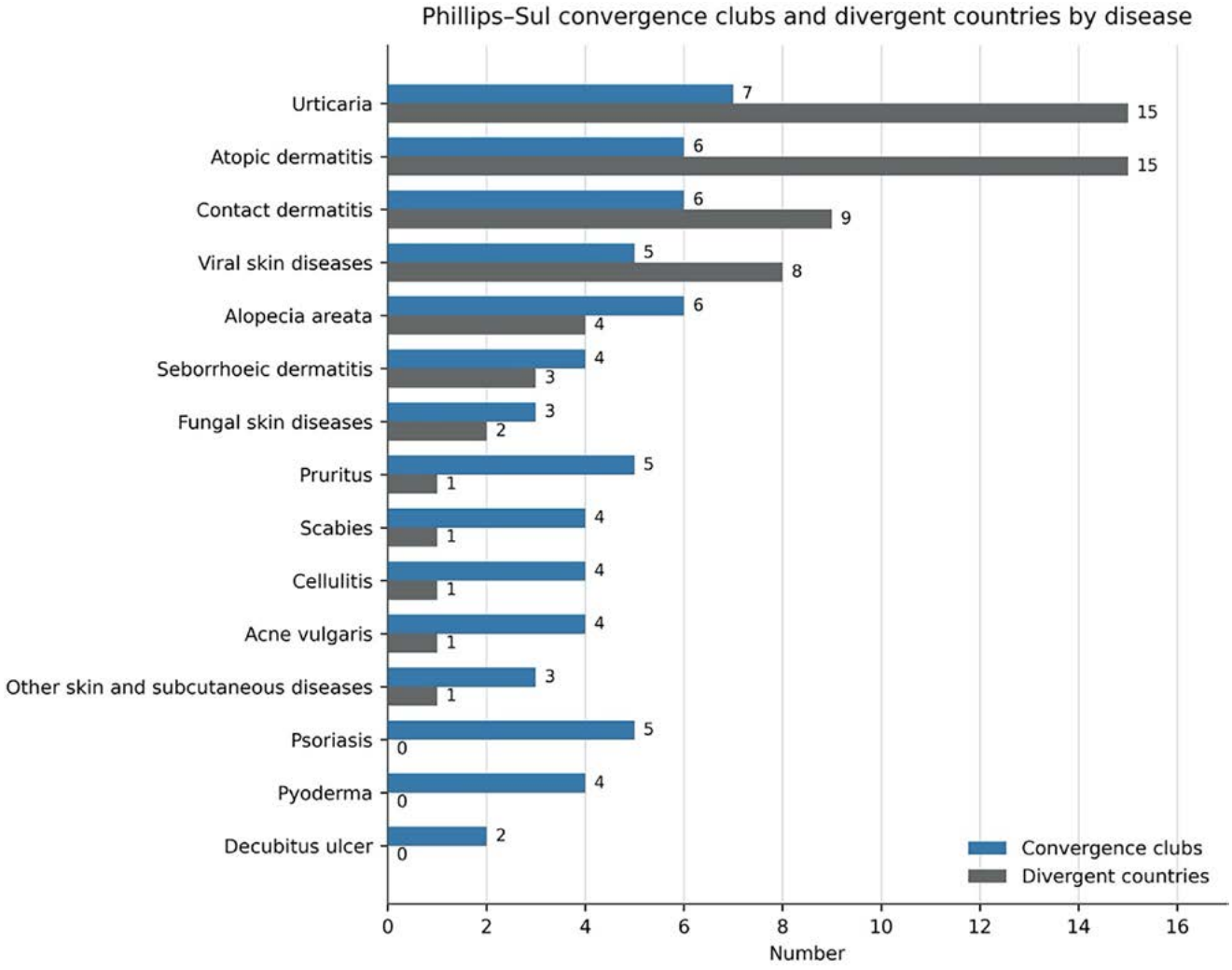

FIGURE 1. Disease-specific numbers of convergence clubs and divergent countries across Organisation for Economic Co-operation and Development countries, 2000–2023.

DISCUSSION

This study examined changes and convergence in the burden of 15 skin and subcutaneous diseases across 38 OECD countries from 2000 to 2023. Three main findings emerged. First, changes in median age-standardized DALY rates varied substantially across diseases. Second, sigma and beta convergence were both observed in only three conditions. Third, the Phillips-Sul analysis rejected a single transition path for every disease. Together, these findings suggest that skin disease burdens have remained heterogeneous rather than converging toward a common OECD-wide pattern. Decubitus ulcer showed the weakest evidence against full convergence (log-t=-1.74, just below the −1.645 critical value; one-sided p=0.041), and its rejection was not robust at r=0.40 (Supplementary Table S3).

The largest relative increases in median DALY rates were observed for pyoderma (134.2%), cellulitis (107.2%), and acne vulgaris (51.6%). These conditions differ clinically, which is relevant for interpreting the magnitude of change. Pyoderma and cellulitis are bacterial infections usually managed in primary or emergency care; most episodes respond to antibiotics, but severe cases can progress to bacteremia and deep tissue infection requiring hospitalization [10]. Their estimated burden is therefore closely linked to how such infections are detected, coded, and treated. In the United States, skin and soft tissue infection incidence increased by 24% between 2000 and 2012 and cellulitis hospitalizations doubled between 1998 and 2013 [11, 12]. Recurrence is also frequent, reported in 13–47% of patients within a year, so successful treatment of an individual episode does

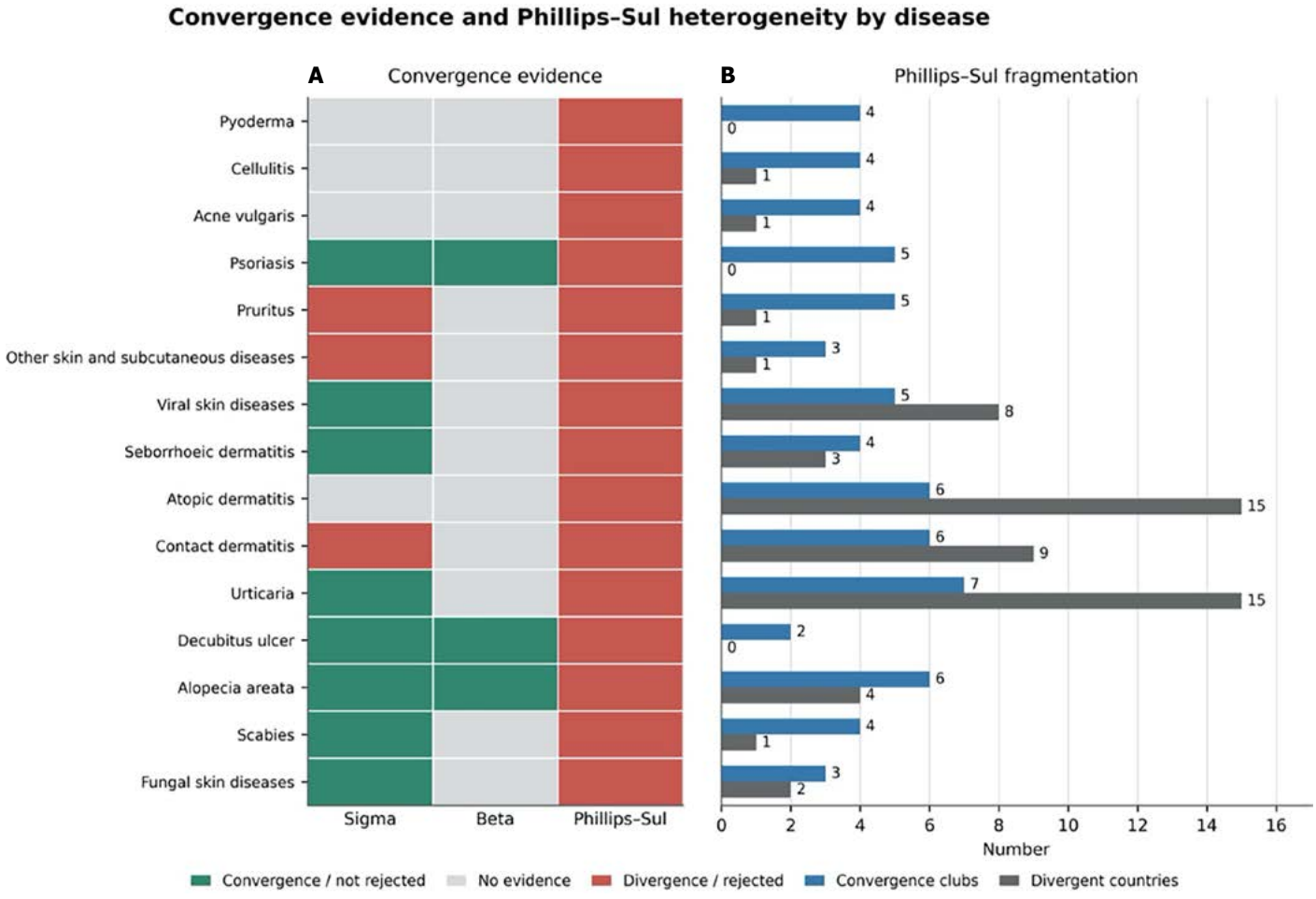

FIGURE 2. Convergence evidence and Phillips-Sul heterogeneity by disease. Panel A summarizes disease-specific evidence from sigma, unconditional beta, and full panel Phillips-Sul analyses. Green indicates convergence or failure to reject convergence, grey indicates no statistically significant evidence of convergence or divergence, and red indicates divergence or rejection of full panel convergence. Panel B presents the numbers of final convergence clubs and countries classified as divergent after the Phillips-Sul classification and merging procedure.

not necessarily prevent further health loss. Estimates for this group also depend on where they are measured: the pooled prevalence of impetigo was 27.9% in community settings but 10.6% when diagnosed in healthcare settings [12, 13]. Acne vulgaris, by contrast, is a chronic, rarely fatal disorder managed mainly in outpatient settings; it affects approximately 9.4% of the world population, and 85% of cases begin between the ages of 12 and 24 years [14]. These increases may partly reflect changes in disease recognition, diagnostic and coding practices, healthcare access, treatment patterns, and GBD modeling inputs; the present ecological design cannot distinguish these influences from true changes in disease occurrence. These findings are consistent with previous evidence of an increasing global burden of skin diseases and acne. Fungal skin diseases showed the largest decline (17.1%), followed by scabies (6.4%) [2, 15].

Atopic dermatitis had the highest median DALY rate in 2023, consistent with GBD 1990–2017, where it ranked first among skin diseases in DALYs [16]. It affects 10–30% of children and 2–10% of adults, and approximately 80% of cases begin in infancy or childhood with unpredictable individual trajectories [17, 18]. Because the disease is chronic and relapsing and has no curative treatment, its burden is almost entirely morbidity-based, as is the case for skin diseases more broadly; in China, 98.83% of DALYs lost to skin disease in 2019 were years lived with disability [19]. Its early onset and frequently persistent course mean that this disability may accumulate over many years of life, contributing to a sustained

Table 2. Sex disparities in disability-adjusted life-year rates

Disease	Female-to-male ratio 2000	Female-to-male ratio 2023	Change, percentage	Ratio log-t statistic
Urticaria	1.484	1.484	-0.0	-37.14
Pruritus	1.524	1.460	-4.2	-24.27
Alopecia areata	1.405	1.409	+0.3	-26.63
Contact dermatitis	1.288	1.285	-0.3	-45.31
Other skin, Subcutaneous diseases	1.245	1.239	-0.4	-33.95
Acne vulgaris	1.230	1.221	-0.7	-11.17
Seborrhoeic dermatitis	1.133	1.130	-0.3	-24.92
Scabies	1.048	1.049	+0.1	-13.25
Psoriasis	1.049	1.045	-0.3	-8.75
Atopic dermatitis	0.989	0.978	-1.1	-41.82
Fungal skin diseases	0.951	0.950	-0.1	-28.81
Viral skin diseases	0.945	0.944	-0.1	-42.40
Decubitus ulcer	0.897	0.868	-3.2	-19.10
Pyoderma	0.879	0.838	-4.6	-18.59
Cellulitis	0.756	0.679	-10.2	-7.48

population-level burden. This position is also consistent with its quality-of-life consequences: Among adults with moderate-to-severe atopic dermatitis, 44% reported a high quality-of-life burden, and activity impairment was its strongest predictor [20].

Sigma convergence was observed for eight diseases, whereas beta convergence was found for only three; psoriasis, decubitus ulcer, and alopecia areata satisfied both criteria. This limited overlap is not contradictory, because sigma convergence reflects reduced dispersion across countries, whereas beta convergence examines whether countries with a higher initial burden experience a different subsequent rate of change. Cross-country variation may therefore narrow without being driven by initially high-burden countries. Similar differences between convergence processes have been reported for other health outcomes [7].

The Phillips-Sul analysis rejected overall convergence for all 15 diseases, identifying two to seven convergence clubs per disease. Similar club structures have been reported for life expectancy, infant mortality, and sustainable development indicators [8, 21]. Moreover, institutional similarity does not necessarily translate into uniform health trajectories [5, 6].

Club membership provides a more nuanced view of cross-country patterns than the OECD median alone. Countries within the same club followed broadly similar trajectories over time, even when their absolute disease burden differed. Thus, countries with comparable DALY rates may still require different surveillance priorities if their burden is evolving in different directions. These trajectory-based patterns may help inform condition- and country-specific monitoring priorities. However, club membership is sensitive to the study period and modeling choices and should therefore be interpreted as a descriptive pattern rather than evidence of shared underlying causes [7].

Divergence was most extensive for atopic dermatitis and urticaria (15 countries each), followed by contact dermatitis (nine) and viral skin diseases (eight); these results indicate heterogeneity but do not explain it. Decubitus ulcer showed the weakest evidence against full convergence (log-t=-1.74; one-sided p=0.041, just below the -1.645 critical value), was partitioned into two clubs without divergent countries, and was one of three conditions with both sigma and beta convergence. The residual category of other skin and subcutaneous diseases aggregates conditions not modeled separately in GBD across several ICD-10 groups (Supplementary Table S1). It should not be read as a homogeneous clinical entity, which makes its increased cross-country dispersion difficult to interpret.

In 2023, women had higher DALY rates than men for nine of the 15 conditions. The female excess was largest for urticaria, pruritus, and alopecia areata, whereas the male excess was largest for cellulitis, pyoderma, and decubitus ulcer. Overall convergence in the female-to-male ratio was rejected for every condition.

These patterns are broadly consistent with previous reports: women are more often affected by nonfatal, disabling conditions such as dermatitis, pruritus, urticaria, and alopecia areata, and men by potentially fatal infections such as cellulitis [17, 22, 23]. The female-predominant conditions are largely chronic, symptomatic, and visible, so differences in symptom reporting, consultation, and diagnosis may contribute to the recorded burden, whereas exposure, injury, and comorbidity profiles may be more relevant for the male-predominant infections and pressure-related wounds. Not all patterns are reproduced elsewhere, however: GBD 2019 found no significant sex difference for decubitus ulcer [24].

Sex differences should therefore be interpreted condition by condition. They do not allow inference about biological mechanisms and may partly reflect differences in healthcare-seeking behavior and diagnosis.

Overall, these findings are descriptive rather than causal. A uniform OECD-wide approach may overlook differences between conditions and countries. The rising estimated burden of pyoderma, cellulitis, and acne may merit closer surveillance, although our results do not identify the underlying causes or indicate where policy intervention would be most effective.

This study has several limitations. It relied on GBD model-based estimates and inherits their assumptions and data gaps. This is particularly relevant for skin diseases, because diagnosis, healthcare-seeking behavior, and access to dermatological care vary considerably between countries, and models must rely on out-of-sample prediction where original data are unavailable, with additional risk of misclassification from administrative coding [19]. The ecological design does not permit individual-level or causal inference. Uncertainty intervals were not incorporated into the convergence models; club assignments may be sensitive to specification and study period, and sex-related analyses were secondary. The study period included the COVID-19 pandemic, which may have affected healthcare access, diagnosis, and burden estimates. Finally, socioeconomic, demographic, and health system characteristics were not examined.

CONCLUSION

The age-standardized burden of skin and subcutaneous diseases did not converge toward a single pattern across OECD countries between 2000 and 2023. Trends, convergence classifications, and relative sex differences varied among diseases and countries. These patterns may support disease-specific and country-sensitive surveillance, although the present design describes trajectories rather than their determinants. Future research could validate the identified clubs and examine whether their formation is associated with socioeconomic conditions, demographic structure, health system capacity, access to care, or diagnostic practices.

Supplementary Tables Link: https://jag.journalagent.com/nci/abs_files/NCI-99223/NCI-99223_(0)_NCI-99223_(0)_Supplementary_Tables.pdf

Ethics Committee Approval: This study used publicly available, aggregated, and de-identified data from the GBD 2023 study and involved no human participants or identifiable personal data. Therefore, ethics committee approval was not required.

Informed Consent: Informed consent was not required because this study used publicly available, aggregated, and de-identified data and did not involve human participants.

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

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

Use of AI for Writing Assistance: During the preparation of this manuscript, the author used ChatGPT (OpenAI) to assist with English-language editing and clarity.

Peer-review: Externally peer-reviewed.

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North Clin Istanb 2026;13(5):615–617

Letter to the Editor

Questioning causal attribution between ozone therapy, pneumocephalus, and meningitis: A critical appraisal of the case report by Elmas Dal

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To the Editor,

The case report by Elmas Dal describes meningitis associated with pneumocephalus shortly after oxygen–ozone therapy for back pain [1]. Although the temporal relationship justifies considering the intervention as a possible contributing factor, the evidence does not establish the proposed mechanism: inadvertent dural penetration by a contaminated needle with introduction of gas and bacteria into the subarachnoid space.

The report states that ozone injections were administered to paravertebral regions and the anterior neck, but essential procedural information is absent, including needle type, gauge and length, insertion depth and angle, injected volume, ozone concentration, anatomical landmarks, imaging guidance, and exact technique [1]. This is particularly relevant because the procedure is described as intramuscular or paravertebral. Such an injection is anatomically distinct from intraforaminal, epidural, or intrathecal procedures [1]. For gas to enter the subarachnoid space directly, the needle would have to advance substantially beyond the muscular target and penetrate deeper structures and the dura [2, 3]. No imaging, procedural record, cerebrospinal fluid (CSF) return, or other objective evidence documents such penetration. The proposed trajectory therefore remains hypothetical.

Pneumocephalus on CT is objective and clinically important [4], but it does not demonstrate that ozone was injected into the subarachnoid space. Intracranial gas may have different causes, and although the temporal proximity of ozone treatment makes the procedure a reasonable suspect, it does not establish the route by which gas entered the intracranial compartment.

Microbiological evidence raises similar concerns. Clinical presentation and CSF findings strongly support an acute meningitic syndrome, including 4000 leukocytes/mm3, protein of 483 mg/dL, and glucose of 40 mg/dL [1, 3]. However, Gram staining, acid-fast staining, CSF culture, blood culture, and subsequent CSF culture were negative, and no microorganism was identified [1]. Prior meropenem and vancomycin treatment could have reduced culture sensitivity [3, 5], but this cannot establish bacterial etiology or procedural inoculation.

Moreover, no evidence demonstrates contamination of the needle, syringe, ozone delivery system, or environment. No pathogen isolated from the patient could be matched to a procedural source. Thus, contamination remains a hypothesis rather than a demonstrated event. Clinical improvement following antibiotics and dexamethasone is compatible with bacterial meningitis but is not pathogen-specific evidence, while the absence of molecular testing further limits etiological attribution [3, 5].

The case therefore documents an acute meningitic syndrome with pneumocephalus occurring shortly after ozone therapy, representing a legitimate safety signal. However, the available evidence does not demonstrate dural penetration, subarachnoid ozone administration, bacterial inoculation by contaminated equipment, or a microbiological link between meningitis and the procedure. Accordingly, describing the event as pneumocephalus and culture-negative meningitis occurring after, or temporally associated with, ozone therapy would more accurately distinguish temporal association from demonstrated causation. The proposed mechanism is plausible, but the available procedural, anatomical, and microbiological evidence does not establish it.

Cite this article as: *Franzini M, Vaiano F, Tirelli U, Ricevuti G, Valdenassi L, Chirumbolo S. Questioning causal attribution between ozone therapy, pneumocephalus, and meningitis: A critical appraisal of the case report by Elmas Dal. North Clin Istanb 2026;13(5):615–617.*

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

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

Use of AI for Writing Assistance: The authors declared that artificial intelligence was not used in the study.

Authorship Contributions: Concept – SC, FV; Design – MF, LV, FV; Supervision – MF, LV, GR; Resource – FV, LV, MF; Materials – UT, SC; Data Collection and/or Processing – SC, FV; Analysis and/or Interpretation – FV, SC; Literature Search – SC; Writing – SC; Critical Reviews – FV, MF, LV.

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Received: August 10, 2026 *Revised:* October 14, 2026
Accepted: October 19, 2026 *Online:* April 00, 2026

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doi: 10.14744/nci.2026.58740
Istanbul Provincial Directorate of Health - Available online at www.northclinist.com

Authors' Reply

To the Editor,

The patient was admitted to our hospital with a history of receiving ozone injections for a herniated disc in the paravertebral and anterior cervical regions at an external facility two days prior. On physical examination, ecchymoses were present in the paravertebral and anterior cervical regions.

Since I was not the person performing the procedure and was not present with the patient at the time, I cannot provide information about the type of needle used, its thickness, length, insertion depth, approach angle, number of injections, volume administered, ozone concentration, anatomical landmarks, whether imaging guidance was used, or the technique employed. During my brief conversation with the person who performed the procedure, I was only able to confirm that he had administered these injections himself. Furthermore, no clinic retains the materials it has used that have become medical waste following a procedure. There is no way to access these materials retroactively.

The individual mentioned in the case presentation is a hospitalized patient, not a subject or test subject. Fluoroscopic or computed tomography (CT)-guided documentation intended to clarify the exact course of the event to provide data for a potential case presentation would not contribute to the patient's treatment; furthermore, it would constitute an unethical procedure performed unnecessarily for the purposes of an academic study. Furthermore, the procedure record or report regarding the procedure is not available because it was not provided by the person who performed the procedure.

The absence of any trauma, surgery, infection with gas-producing organisms, or other cause that could lead to pneumocephaly in the patient calls this procedure into question. Therefore, the presence of pneumocephaly on cranial imaging, combined with the prior paravertebral ozone injection, establishes a near-certain cause-and-effect relationship.

In a patient presenting with headache, neck stiffness, fever, leukocytosis, and altered mental status, and with cerebrospinal fluid (CSF) findings consistent with meningitis, the primary diagnosis to consider is meningitis. Based on the patient's history, physical examination, laboratory tests,

and imaging studies, no etiology other than this procedure – which could lead to meningitis – has been identified.

A lumbar puncture was performed approximately 10 h after treatment for meningitis was initiated. It was concluded that the negative results of the Gram stain and CSF culture were due to this time lag. Antibiotic pretreatment administered before the lumbar puncture significantly reduces the likelihood of a positive CSF culture and accelerates CSF sterilization [1, 2].

Antibiotics administered to patients before a CSF sample is collected can potentially affect not only the culture results but also the diagnostic accuracy of Gram staining [3].

Since the procedure was performed at an off-site facility and the patient presented to the emergency department 2 days after the incident, it was not possible to obtain cultures from the syringe, the ozone administration system, the clinical environment, skin preparation materials, the injection site, or other procedural equipment. The fact that no other etiological cause capable of leading to meningitis was identified through the patient's history, physical examination, laboratory tests, and imaging studies indicates a near-certain cause-and-effect relationship between the procedure and the resulting meningitis.

In conclusion, to confirm the etiology – that is, to turn 99 into 100 – I believe it is inappropriate to pursue procedures that are impossible to perform (such as obtaining a culture from a syringe used 2 days earlier at an external facility) or to resort to practices that are unethical from the author's perspective and offer no benefit to the patient's treatment (such as fluoroscopic or CT-guided documentation).

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

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

Use of AI for Writing Assistance: The author declared that artificial intelligence was not used in the study.

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Received: September 01, 2026 *Accepted:* September 22, 2026
Online: October 19, 2026

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doi: 10.14744/nci.2026.03443
Istanbul Provincial Directorate of Health - Available online at www.northclinist.com