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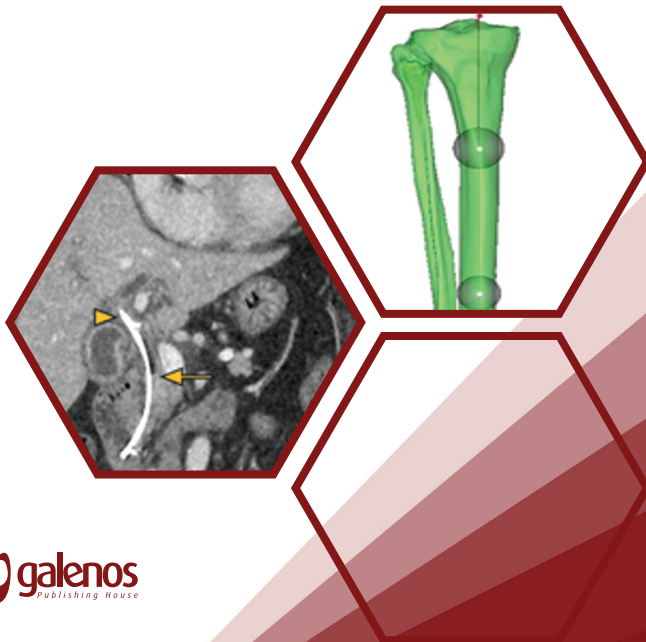
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Prognostic Value of Log Odds of Positive Lymph Nodes (LODDS) in Gastric Cancer: A Comparative Analysis with Lymph Node Ratio and N Stage

 Vural Argın¹,  Muhammed İkbāl Akın²,  Ömer Faruk Kandaz²,  Ahmet Orhan Sunar²,  Nurdan Bezir²,
 Ömer Özduman²,  Selçuk Gülmez²,  Aziz Serkan Senger²,  Erdal Polat²,  Mustafa Duman²

¹Department of General Surgery, Marmara University Faculty of Medicine, İstanbul, Türkiye

²Clinic of Gastroenterological Surgery, Kartal Koşuyolu High Specialization Training and Research Hospital, İstanbul, Türkiye

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ORCID IDs of the authors: V.A. 0000-0002-6526-1821, M.İ.A. 0000-0002-9638-4031, Ö.F.K. 0000-0001-6527-9354, A.O.S. 0000-0001-5564-6923, N.B. 0000-0001-8733-7279, Ö.Ö. 0000-0002-6527-506X, S.G. 0000-0001-9719-1904, A.S.S. 0000-0003-0981-0141, E.P. 0000-0002-9463-9846, M.D. 0000-0002-0276-0543.

ABSTRACT

Objective: The present analysis sought to compare the prognostic relevance of log odds of positive lymph nodes (LODDS), lymph node ratio (LNR), and standard N-based staging in patients who received curative-intent surgical treatment for stomach cancer. **Methods:** Medical records of patients who underwent curative gastrectomy between March 2019 and March 2025 were examined retrospectively. Clinicopathological characteristics, together with lymph node-related parameters, were analyzed. The Kaplan–Meier method was used to estimate survival, and differences across groups were evaluated using the log-rank test. Prognostic factors were investigated through univariate and multivariate Cox regression analyses. Separate multivariate models were constructed for LNR, LODDS, and N stage to account for potential collinearity among nodal variables. Discriminative performance was additionally examined by constructing receiver operating characteristic (ROC) plots, and findings yielding *P* values under 0.05 were taken to be statistically meaningful. **Results:** The analytical cohort comprised 55 cases observed over a median surveillance duration of 29 months. Univariate analysis identified five factors significantly associated with survival: tumor (T) category, node (N) classification, tumor differentiation grade, LNR, and LODDS. Once multivariate correction was applied, the prognostic value of LODDS was preserved as an independent prognostic marker (hazard ratio: 3.159; 95% confidence interval: 1.484-6.722; *P* = 0.003), whereas LNR no longer reached significance. Although N stage retained prognostic relevance, its predictive strength was lower than that observed for LODDS. ROC analysis demonstrated favorable predictive performance for all nodal parameters, with LODDS showing the highest AUC value (0.876). **Conclusion:** In gastric cancer, LODDS appears to retain prognostic relevance for overall survival and may provide superior discriminative ability compared with conventional nodal staging systems.

Keywords: Gastric cancer, lymph node ratio, LODDS, N stage, overall survival, prognosis, ROC analysis

INTRODUCTION

Despite continuous advances in diagnostic modalities and therapeutic strategies, gastric cancer remains a major global health burden and a leading cause of cancer-related mortality worldwide, particularly in patients with advanced disease.¹

Surgical resection remains the primary curative treatment; however, long-term survival is largely influenced by tumor stage and biological behavior.² Among prognostic factors, lymph node involvement plays a central role in determining oncological outcomes.



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The tumor-node-metastasis (TNM) staging system developed by the American Joint Committee on Cancer (AJCC) and the Union for International Cancer Control (UICC) is currently the most widely adopted classification system for gastric cancer. Within this framework, nodal status is determined according to the number of metastatic lymph nodes identified in the surgical specimen.³ Accurate nodal staging requires the examination of at least 16 lymph nodes, as recommended by current guidelines.⁴ Nevertheless, adequate lymph node retrieval may not always be achieved in routine clinical practice, potentially resulting in stage migration and reduced prognostic accuracy. To overcome these limitations, alternative lymph node assessment methods have been proposed. Among these, the lymph node ratio (LNR), defined as the ratio of metastatic to examined lymph nodes, and the log odds of positive lymph nodes (LODDS), which incorporates both positive and negative lymph node counts, have attracted increasing attention.⁵ Several studies have demonstrated that these metrics may provide more refined prognostic information than conventional pN staging, particularly in situations where lymph node harvest is suboptimal. Previous investigations have suggested that LODDS may offer superior prognostic discrimination compared with LNR, especially among patients with limited nodal involvement or node-negative disease.⁶ Unlike LNR, which assigns all node-negative patients to the same category, LODDS incorporates information from both positive and negative lymph nodes, potentially allowing for more precise risk stratification. Furthermore, studies involving large cohorts of patients with gastrointestinal malignancies, including gastric cancer, have reported that LODDS may outperform both conventional nodal staging and LNR in predicting long-term survival outcomes.^{7,8}

The present study aimed to evaluate the prognostic significance of LNR and LODDS in patients undergoing curative surgery for gastric cancer and to compare their predictive performance with that of the conventional pN classification of the AJCC/UICC/TNM staging system.

MAIN POINTS

- Log odds of positive lymph nodes (LODDS) is an independent prognostic marker for overall survival after curative gastrectomy for gastric cancer.
- In multivariate analysis, LODDS retained its independent prognostic significance, whereas lymph node ratio (LNR) lost statistical significance.
- LODDS demonstrated the highest discriminative performance (AUC: 0.876) among LODDS, LNR, and N stage on ROC analysis.
- LODDS may serve as a valuable complementary parameter to conventional nodal staging systems, particularly when lymph node harvest is suboptimal.
- These findings support further evaluation of LODDS in prognostic nomograms and risk stratification models for gastric cancer.

MATERIALS AND METHODS

Study Design and Patient Selection

Records of individuals who received operative management of stomach cancer at a tertiary referral institution from March 2019 through March 2025 were examined in this retrospective study. Among the 140 cases reviewed during the study period, 70 were excluded because of missing clinical data, and 15 were excluded due to peritoneal carcinomatosis detected intraoperatively. The study included 55 patients with complete clinical and pathological data.

Ethics Approval

Authorization for the present work was obtained from the Koşuyolu High Specialization Training and Research Hospital Institutional Review Board prior to initiation (approval date: 03.06.2025, decision no: 2025/109/11/43). All phases of this work adhered closely to the ethical standards outlined in the Helsinki Declaration. Written informed consent for the surgical procedure was obtained from all patients at the time of hospital admission. As this was a retrospective review of existing medical records, separate informed consent specific to the study was not obtained; the study protocol was approved by the Institutional Review Board.

Data Collection

Patient demographic information and clinicopathological characteristics were retrospectively reviewed. Variables evaluated included age, gender, tumor stage, histological grade, histopathological subtype, gastrectomy type, neoadjuvant treatment status, and lymph node-related parameters. Indices related to nodal status included both the total number of harvested nodes and the number found to harbor metastases. Patient outcomes, including follow-up duration, mortality rate, recurrence, and site of recurrence, were evaluated retrospectively.

Definitions

Overall survival was defined as the interval between the date of surgery and either death or the last documented follow-up. The LNR was calculated by dividing the number of metastatic lymph nodes by the total number of examined lymph nodes; patients were categorized into three groups: LNR1 (0), LNR2 ($>0-0.1$), and LNR3 (>0.1). The LODDS was calculated as the natural logarithm of the ratio between positive and negative lymph nodes, and patients were further stratified into five categories: LODDS1 (≤ -4), LODDS2 (-4 to -2.5), LODDS3 (-2.5 to -2), LODDS4 (-2 to -0.5), and LODDS5 (>-0.5). The cut-off values used for both the LNR and LODDS classifications were adopted from previously published and validated studies in the gastric cancer literature to facilitate comparison with existing evidence.⁹

Statistical Analysis

Data processing for all statistical procedures was carried out with SPSS (version 27). For continuous data, results were reported using either mean $\pm$ standard deviation or median (with min

and max), depending on whether the variable followed a normal distribution. Qualitative variables were expressed as counts and percentages. Survival curves were constructed using the Kaplan-Meier method, and subgroup comparisons were performed using the log-rank test. To identify variables associated with overall survival, Cox proportional hazards modeling was subsequently performed using both single-variable and multivariable models. Given the risk of collinearity among the nodal parameters, the N category, LNR, and LODDS were each assessed in separate multivariate models. The results of the survival analysis were expressed as hazard ratios (HRs) with 95% confidence intervals. The discriminative capacity of nodal indicators for overall survival was examined by plotting receiver operating characteristic (ROC) curves, and the corresponding area under the curves (AUCs) were computed. A *P* value below 0.05 was accepted as the threshold for statistical significance.

RESULTS

The cohort ultimately included in the present study consisted of fifty-five individuals. Their mean age stood at 64.60 ± 12.01 years, with male subjects accounting for 60% of the sample. Total gastrectomy was performed in 54.5% of cases, whereas subtotal gastrectomy was performed in the remaining 45.5%, 43.6% of the patients received neoadjuvant therapy. Overall, 49.1% of the patients had grade 3 tumors, and the most common stage was T3(43.6%). Among the N-stage cases, 43.6% were N0, while the remainder had varying degrees of lymph node involvement. Histopathological evaluation revealed that the vast majority of cases (83.6%) were adenocarcinomas (NOS) (Table 1).

Table 1. Clinicopathological Characteristics of Patients with Gastric Cancer (n = 55)

Variable	Overall (n = 55)
Age (years), mean $\pm$ SD	64.60 $\pm$ 12.01
Sex, n (%)	
Male	33 (60.0)
Female	22 (40.0)
Type of surgery, n (%)	
Total gastrectomy	30 (54.5)
Subtotal gastrectomy	25 (45.5)
Neoadjuvant therapy, n (%)	
Yes	24 (43.6)
No	31 (56.4)
Tumor grade, n (%)	
G1	9 (16.3)
G2	19 (34.5)
G3	27 (49.1)

Table 1. Continued

Variable	Overall (n = 55)
T stage, n (%)	
1a	1 (1.8)
1b	11 (20.0)
2	5 (9.1)
3	24 (43.6)
4a	11 (20.0)
4b	3 (5.5)
N stage, n (%)	
0	24 (43.6)
1	10 (18.2)
2	8 (14.5)
3a	7 (12.7)
3b	6 (10.9)
Final pathology, n (%)	
Adenocarcinoma (NOS)	46 (83.6)
Adenocarcinoma with mucinous component	2 (3.6)
Signet ring cell carcinoma	5 (9.1)
Gastric carcinoma with lymphoid stroma	2 (3.6)
Total lymph nodes retrieved, median (min-max)	28 (7-61)
Positive lymph nodes, median (min-max)	1 (0-41)
LNR stage, n (%)	
LNR1	36 (65.4)
LNR2	15 (27.3)
LNR3	4 (7.3)
LODDS stage, n (%)	
LODDS1	6 (10.9)
LODDS2	27 (49.1)
LODDS3	3 (5.5)
LODDS4	12 (21.8)
LODDS5	7 (12.7)
Distant metastasis, n (%)	
Yes	13 (23.6)
No	42 (76.4)
Site of recurrence, n	
Lung	2
Peritoneum	8
Liver	2
Anastomotic site	1
Follow-up (months), median (min-max)	29 (2-75)
Overall survival (months), mean (95% CI)	52.2 (44.1-60.3)

SD, standard deviation; NOS, not otherwise specified; LNR, lymph node ratio; LODDS, log odds of positive lymph nodes; CI, confidence interval; Min, minimum; Max, maximum.

Lymph node evaluation demonstrated a median of 28 retrieved lymph nodes (range, 7-61) and a median of 1 metastatic lymph node (range, 0-41). According to the LNR classification, 65.4% of patients were LNR1. In the LODDS system, LODDS2 represented the largest subgroup, accounting for 49% of the cohort. Median surveillance was 29 months (range, 2-75 months); distant metastatic disease developed in 23.6% of patients during this interval. Peritoneal dissemination, observed in 8 cases, was the most common recurrence pattern, followed by pulmonary and systemic metastases. A median survival of 52.2 months was estimated for the cohort (95% CI: 44.1-60.3) (Table 1). Variables potentially affecting overall survival were assessed by univariate Cox proportional hazards regression. On univariate analysis, several variables were significantly associated with overall survival: T category (HR 1.689; 95% CI 1.172–2.434; $P=0.005$), nodal category (HR 1.624; 95% CI 1.236–2.134; $P<0.001$), and tumor grade (HR 5.711; 95% CI 1.382–23.608; $P=0.016$). Likewise, both LNR (HR 3.262; 95% CI 1.735–6.135; $P<0.001$) and LODDS (HR 2.409; 95% CI 1.516–3.827; $P<0.001$) showed strong prognostic associations with survival. No meaningful association between overall survival and age ($P=0.172$), sex ($P=0.991$), or end outcome status ($p=0.998$) was detected (Table 2).

To minimize the potential impact of multicollinearity among lymph node parameters, separate multivariate Cox regression models were constructed for LNR, LODDS, and N stage.

Table 2. Univariate Cox Regression Analysis for Overall Survival			
Variable	HR	95% CI	P value
Age	0.978	0.947-1.010	0.172
Sex	1.006	0.342-2.958	0.991
T stage	1.689	1.172-2.434	0.005
N stage	1.624	1.236-2.134	< 0.001
Tumor grade	5.711	1.382-23.608	0.016
Final pathology	0.999	0.592-1.688	0.998
LNR stage	3.262	1.735-6.135	< 0.001
LODDS stage	2.409	1.516-3.827	< 0.001

HR, hazard ratio; CI, confidence interval; LNR, lymph node ratio; LODDS, log odds of positive lymph nodes.

Following multivariable adjustment, the association between LNR and overall survival approached but did not reach statistical significance (HR 3.491; 95% CI 0.967-12.593; $p=0.056$), whereas LODDS remained an independent predictor (HR 3.159; 95% CI 1.484-6.722; $p=0.003$). After adjustment, the N category likewise emerged as a significant correlate of survival (HR 1.694; 95% CI 1.011-2.840; $p=0.045$); nonetheless, the magnitude of its prognostic effect remained below that demonstrated by LODDS (Table 3).

The capacity of each nodal measure to differentiate survival outcomes was investigated by means of ROC curve analysis. High AUC values were obtained for all three measures, including LODDS (AUC: 0.876), LNR (AUC: 0.874), and N category (AUC: 0.861), indicating strong predictive ability for overall survival. Among these parameters, LODDS had the highest AUC, indicating superior discriminatory capacity compared with other lymph-node parameters (Figure 1).

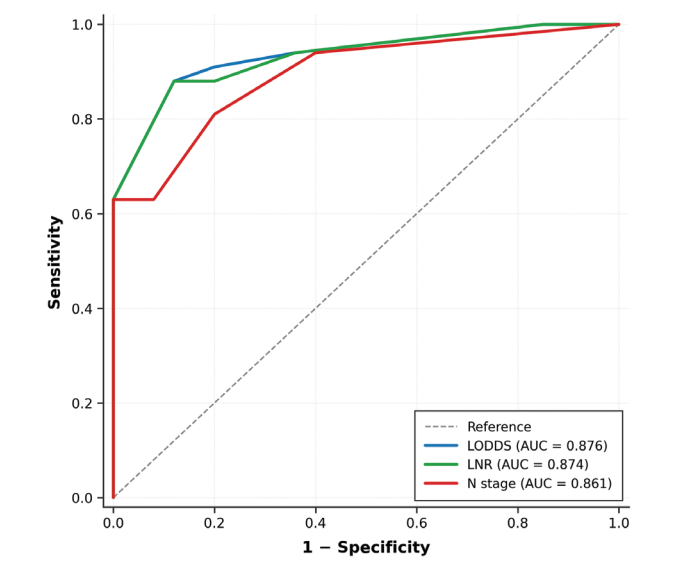


Figure 1. ROC curves for LODDS, LNR, and N stage in predicting overall survival in gastric cancer. AUC values: LODDS = 0.876, LNR = 0.874, N stage = 0.861. LODDS demonstrated the highest discriminative capacity among the three nodal staging systems. LODDS, log odds of positive lymph nodes; LNR, lymph node ratio; ROC, receiver operating characteristic.

Table 3. Multivariate Cox Regression Analysis for Overall Survival

Variable	Model 1 (LNR)		Model 2 (LODDS)		Model 3 (N stage)	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Age	1.002 (0.966-1.040)	0.909	0.996 (0.964-1.030)	0.820	1.012 (0.974-1.051)	0.546
T stage	0.768 (0.378-1.563)	0.467	0.581 (0.300-1.126)	0.108	0.801 (0.421-1.527)	0.501
Tumor grade	3.628 (0.725-18.169)	0.117	3.834 (0.779-18.865)	0.098	3.487 (0.725-16.775)	0.119
LNR	3.491 (0.967-12.593)	0.056	-	-	-	-
LODDS	-	-	3.159 (1.484-6.722)	0.003	-	-
N stage	-	-	-	-	1.694 (1.011-2.840)	0.045

Model 1 includes LNR; Model 2 includes LODDS; Model 3 includes N stage. Each model was adjusted for age, T stage, and tumor grade. Statistically significant P values are shown in bold. HR, hazard ratio; CI, confidence interval; LNR, lymph node ratio (ratio of metastatic to examined lymph nodes); LODDS, log odds of positive lymph nodes.

DISCUSSION

The results of our analysis show that LODDS retains prognostic significance for survival, while the conventional N classification maintains prognostic value. LNR was significant in univariate analysis, but this effect was not preserved after multivariate adjustment. No lymph node parameter demonstrated significant superiority, and LODDS could be used as a complementary approach for prognostic assessment.

Over the past decade, LODDS has attracted increasing attention in gastric cancer research, and findings from large patient cohorts have particularly emphasized its clinical relevance. Drawing on a sample of 10,748 individuals, Xia et al.¹⁰ found that prognostic models built around LODDS outperformed conventional nodal staging schemes in predicting overall survival. In a comparable vein, Gu et al.⁹ reported, drawing on a multi-institutional cohort of 7,620 individuals, that LODDS-driven stratification outperformed both standard pN staging and LNR in prognostic terms. Although our study demonstrates that LODDS is independently associated with overall survival, LODDS did not show a significant advantage compared with other lymph node parameters. Such findings imply that the prognostic utility of LODDS may vary depending on patient characteristics, the size of the analyzed cohort, and the degree to which surgical procedures are standardized. In particular, it may be difficult to demonstrate significant differences among lymph node parameters in case series comprising a limited number of patients reflecting real-world data. Therefore, we believe that, rather than replacing current lymph node assessment systems, LODDS can be used in clinical practice as a complementary parameter.

LNR is widely used as a marker of nodal tumor burden in stomach cancer, and several studies have demonstrated its prognostic relevance. Earlier work has documented a significant association between elevated LNR and poorer survival. Miciak et al.¹¹, in a cohort of 194 patients, identified LNR as an important prognostic indicator associated with tumor stage and survival. Similarly, Peng et al.¹² demonstrated that LNR retains its prognostic significance, particularly when combined with factors related to the tumor microenvironment. However, Liu et al.¹³ demonstrated that LNR-based classification may provide more homogeneous prognostic stratification than both LODDS and conventional N stage in patients with extensive lymph node dissection. Within our cohort, a link between LNR and overall survival emerged on univariate testing, yet this association did not hold once multivariate adjustment was applied. This observation might imply that LNR's predictive power is shaped by factors such as the number of nodes obtained, the extent of nodal dissection, and patient-specific attributes. Moreover, classifying all node-negative patients within the LNR0 category may limit the ability of LNR to adequately reflect prognostic heterogeneity in this subgroup. Although LNR reflects lymph node burden, its partial overlap with N stage and LODDS may have reduced its independent effect in multivariate analysis. Even so, the LNR-derived AUC of 0.874 from ROC analysis suggests that this metric retains robust discriminative power for predicting overall survival. It slightly exceeds the AUC for the N category and aligns closely with the value observed for

LODDS. This value is slightly higher than that for the N stage, and is quite similar to that for LODDS. These findings suggest that LNR is a clinically valuable prognostic parameter, but it may not be sufficient on its own, particularly in situations requiring a more detailed lymph node assessment.

Beyond its prognostic significance, LODDS may have applications in future prognostic models and risk stratification systems for gastric cancer. By incorporating information from both positive and negative lymph nodes, LODDS may provide a more comprehensive assessment of nodal status than conventional pN staging alone, particularly in patients with limited lymph node retrieval. Integration of LODDS into prognostic nomograms and clinical decision-making algorithms may contribute to more individualized postoperative surveillance and treatment planning. Nevertheless, further large-scale prospective studies are required before routine clinical implementation can be recommended.

Study Limitations

This study has several limitations. First, the retrospective, single-center design of the study limits the generalizability of the findings. Second, the relatively small sample size ($n=55$) and limited number of events (mortality) may have affected the stability of the multivariate Cox regression models and led to wide confidence intervals for the calculated HR; indeed, the result obtained for LNR remained at the threshold of statistical significance. Another factor that could lead to selection bias is the exclusion of 70 of the 140 patients initially included in the study due to missing clinical data, and 15 due to peritoneal carcinomatosis detected during surgery. Furthermore, the cut-off values used for the LNR and LODDS classifications were adapted from previously published literature rather than being specifically determined for this study; this means that the results may not reflect the optimal cut-off values for the patient population in question. Patient-specific variations in the extent of lymph node dissection and the number of lymph nodes removed may also have influenced the performance of the LNR and LODDS classifications. Finally, the fact that the findings have not been validated in an external dataset limits the assessment of the results' reproducibility across different patient groups. Therefore, the findings need to be validated in larger, multicenter, and prospective cohorts.

CONCLUSION

In gastric cancer, LODDS appears to function as an independent prognostic indicator and may provide additional value in nodal assessment when combined with established staging systems.

Ethics

Ethics Committee Approval: This retrospective study was approved by the Institutional Ethics Committee of Koşuyolu High Specialization Training and Research Hospital (decision no: 2025/109/11/43, date: 03.06.2025). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Informed Consent: Written informed consent for the surgical procedure was obtained from all patients at the time of hospital admission. As this was a retrospective review of existing medical records, separate informed consent specific to the study was not obtained; the study protocol was approved by the Institutional Review Board.

Footnotes

Author Contributions: Concept Design – V.A., M.İ.A., A.O.S.; Data Collection or Processing – V.A., M.İ.A., A.O.S., N.B.; Analysis or Interpretation – V.A., M.İ.A., Ö.F.K., Ö.Ö., S.G., A.S.S., E.P.; Literature Review – V.A., M.İ.A., M.D.; Writing, Reviewing and Editing – V.A., M.İ.A., A.O.S., E.P., M.D.

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The Relationship Between Orthorexic Tendencies and Hematological Inflammatory Indices

 Mehmet Celal Kefeli¹,  Mustafa Akkuş¹,  Burak Yeşilalan²,  Güler Çelikel¹,  Rıdvan Mehmet Göktaş²

¹Department of Psychiatry, Erzincan Binali Yıldırım University Faculty of Medicine, Erzincan, Türkiye

²Clinic of Psychiatry, Mengücek Gazi Training and Research Hospital, Erzincan, Türkiye

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ORCID IDs of the authors: M.C.K. 0000-0002-0928-4746, M.A. 0000-0002-5674-6632, B.Y. 0009-0007-3096-4440, G.Ç. 0000-0003-4203-2273, R.M.G. 0009-0002-8253-9327.

ABSTRACT

Objective: To investigate the relationship between orthorexia dimensions, specifically healthy orthorexia (HeOr) and orthorexia nervosa (OrNe), and hematological inflammatory markers.

Methods: This cross-sectional, analytical study included 218 healthy volunteers (73.9% male, median age: 25.0 years) who were evaluated between February 2026 and April 2026. Sociodemographic profiles and the Teruel Orthorexia Scale scores were obtained. Complete blood count parameters and inflammatory indices, including the monocyte-to-lymphocyte ratio (MLR) and systemic inflammation response index (SIRI), were analyzed.

Results: HeOr scores demonstrated significant negative correlations with white blood cell count ($r = -0.163$, $P = 0.016$), neutrophil count ($r = -0.160$, $P = 0.019$), monocyte count ($r = -0.244$, $P < 0.001$), platelet count ($r = -0.168$, $P = 0.013$), MLR ($r = -0.134$, $P = 0.049$), and SIRI ($r = -0.185$, $P = 0.006$). Conversely, OrNe scores exhibited a significant negative correlation only with monocyte count ($r = -0.171$, $P = 0.012$) and showed no significant associations with other hematological parameters or systemic inflammatory indices.

Conclusion: Individuals with higher HeOr tendencies display a healthier systemic inflammatory profile, likely reflecting beneficial dietary choices. Despite being characterized by more rigid cognitions and behaviors toward healthy eating, OrNe tendencies do not manifest a healthier inflammatory profile.

Keywords: Healthy orthorexia, orthorexia nervosa, inflammation, hematological markers, SIRI, eating disorders

INTRODUCTION

Dr. Steven Bratman introduced the concept of orthorexia in 1997 to describe a psychological state characterized by an unhealthy preoccupation with clean and wholesome nutrition. Such behavior manifests as an unrelenting pursuit of foods free from artificial additives, alongside obsessive scrutiny regarding how items are sourced, processed, and packaged.¹ Affected individuals typically dedicate substantial portions of their day to procuring foods they consider wholesome while rigorously avoiding perceived impurities. Additionally, breaking these self-imposed dietary regulations frequently triggers intense

episodes of guilt, shame, and anxiety, which in turn drive further dietary restrictions.²⁻⁴ Consequently, these individuals may experience challenges across various biopsychosocial domains, including nutritional deficiencies.³ Maintaining that a focus on wholesome nutrition is not inherently pathological, Barrada and Roncero⁵ examined orthorexic behaviors through a dual framework comprising healthy orthorexia (HeOr) and orthorexia nervosa (OrNe). The fact that these two conditions possess divergent psychological profiles and motivations indicates that OrNe and HeOr are not extensions of one another or opposite poles of a single spectrum, but are instead two discrete constructs yielding completely separate



Corresponding author: Mehmet Celal Kefeli, **E-mail:** mehmet.kefeli@erzincan.edu.tr

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psychological and behavioral consequences.⁶ For individuals with HeOr, The primary motivation underlying food choices is the health content of the food. Findings indicate that the fundamental drives behind OrNe do not invariably revolve around health; instead, they are predominantly characterized by weight management, appearance concerns, and regulation of negative emotions. The clinical characteristics of OrNe exhibit a significant overlap with the symptomatology of eating disorders—specifically anorexia nervosa and bulimia nervosa—as well as obsessive-compulsive disorder (OCD).^{7–9} Existing literature features extensive documentation linking hematological inflammatory markers to clinical conditions that share overlapping features with orthorexia, most notably eating disorders and OCD.^{10–12} Hematological inflammatory indices function as practical, economical indicators of systemic inflammation, derived simply by calculating proportions among distinct leukocyte and platelet counts obtained during standard complete blood evaluations.¹³ Increased metrics—including the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and systemic inflammation response index (SIRI)—signal a cellular shift where innate immunity outweighs adaptive components, thereby pointing to an active systemic inflammatory process.¹⁴ Prior research indicates that individuals with OCD display higher monocyte-to-lymphocyte ratio (MLR), NLR, and PLR figures than healthy population cohorts.^{15,16} Existing literature demonstrates that individuals with eating disorders exhibit significantly higher NLR and PLR values relative to healthy control subjects.¹¹ Furthermore, elevated levels of the systemic immune-inflammation index (SII), SIRI, NLR, and MLR metrics have emerged as unfavorable prognostic markers regarding therapeutic response among patients with eating disorders.¹² On the other hand, higher healthy eating index (HEI-2015/2020) scores have been reported to be associated with lower white blood cell (WBC) counts, neutrophils, NLR, and SII, indicating that a higher-quality diet reduces these markers, which reflect systemic inflammation via blood cells.^{17,18}

MAIN POINTS

- Healthy orthorexia (HeOr) tendencies are strongly associated with a healthier systemic inflammatory profile, characterized by lower levels of leukocytes, neutrophils, monocytes, platelets, monocyte-to-lymphocyte ratio, and systemic inflammation response index.
- Despite rigid cognitions and restrictive behaviors regarding healthy eating, orthorexia nervosa (OrNe) tendencies do not translate into a healthier inflammatory profile, with the exception of decreased monocyte counts.
- HeOr and OrNe exhibit distinct biological manifestations in systemic inflammatory markers, confirming that they are independent constructs rather than opposite poles of a single spectrum.
- The pathological obsessions and stress-related psychological burden observed in OrNe may biologically negate the potential anti-inflammatory benefits of a clean diet.

To the best of our knowledge, our literature review revealed no studies directly examining the relationship between orthorexia and hematological indices that serve as markers of chronic inflammation. Whether orthorectic tendencies lead to an elevation in inflammatory indices—similar to those observed in OCD and eating disorders—or are associated with lower inflammatory parameters, as observed in individuals with healthy eating habits, constitutes the primary research question of our study. Specifically, we hypothesized that higher HeOr tendencies would be significantly associated with lower levels of hematological inflammatory markers—reflecting potential anti-inflammatory benefits of wholesome dietary choices—whereas OrNe tendencies, driven by psychological stress and pathological fixations, would not exhibit such a protective profile.

MATERIALS AND METHODS

Study Population and Data Collection

This project was carried out as a cross-sectional analytical investigation Mengücek Gazi Training and Research Hospital, a tertiary care medical center from February 15, 2026, to April 15, 2026. All research procedures strictly adhered to the ethical guidelines set forth in the Declaration of Helsinki. This study was specifically designed to recruit a homogeneous sample of healthy volunteers, aged 18–60 years, who presented to the hospital medical board to obtain the mandatory pre-employment medical clearance required for private security guard licensing. Our objective was to construct, through the targeted recruitment of this specific applicant pool, a homogeneous, non-clinical sample consisting predominantly of healthy young adults pursuing employment. The effect size was set at $f^2(V) = 0.3$, the alpha (α) level was set at 0.05, and the statistical power ($1-\beta$ error probability) was set at 0.95. Using G*Power (v3.1.9.4), the initial computation indicated that 184 subjects were required. Factoring in an estimated 20% allowance for prospective dropouts or incomplete submissions, the recruitment goal was ultimately expanded to 221 individuals. After applying all selection criteria, 218 eligible subjects constituted the final cohort. A total of three individuals were excluded: one diagnosed with a systemic inflammatory condition and two with incomplete data forms. Volunteers who had at least a primary school education, had the cognitive capacity to complete the questionnaires and comprehend the scope of the study, and had undergone a complete blood count (CBC) for their medical report were targeted for enrollment. To minimize the confounding effects of lifestyle and clinical factors on inflammatory markers, strict inclusion criteria were established. The study recruited only healthy volunteers who were strictly non-smokers and had no history of psychoactive substance use. Furthermore, eligible participants had to be free of a history of psychiatric illness or active psychiatric complaints, severe chronic medical conditions (e.g., diabetes, celiac disease), autoimmune disorders, malignancies, and diagnosed neurological, endocrine, or systemic disorders. Additionally, women who were pregnant or breastfeeding were excluded from participation. Following these defined screening rules, 221 volunteers underwent formal eligibility evaluations. During the final clinical evaluation, one individual was excluded owing to an active systemic inflammatory disease (elevated CRP

levels), and two additional individuals were excluded because of incomplete data collection forms. The final analytical cohort comprised 218 eligible individuals.

A detailed clinical history was obtained from the participants. Systemic physical and mental status examinations were performed. Regarding the blood sampling protocol, all participants were required to provide their venous blood samples following an overnight fast, at a uniform time between 08.30 and 09.30, prior to their outpatient clinical examinations. All hematological measurements were processed in a standardized manner at the hospital's central biochemistry laboratory using the same automated hematology analyzer to prevent inter-assay variability. While C-reactive protein (CRP) was not measured routinely for all participants, it was specifically tested in individuals presenting with clinical suspicion of an inflammatory condition, and those with elevated levels were subsequently excluded. Following anthropometric evaluations, including height and weight measurements to compute each subject's body mass index (BMI), the study instruments—comprising the Data Collection Form and the Teruel Orthorexia Scale (TOS)—were distributed to all participants.

Evaluation of the Patients

To gather demographic and background information, the research team designed a questionnaire tailored to this project. This form, administered during the initial admission, gathered general information and sociodemographic data, including age, educational level, gender, marital status, current medications, and history of comorbidities. Barrada and Roncero⁵ introduced the TOS as a 17-item self-administered measurement tool. The framework divides orthorexic tendencies into two distinct subscales: "Healthy Orthorexia", denoting a preoccupation with clean nutrition and "Orthorexia Nervosa", representing a clinically pathological fixation. The adaptation, validity, and reliability processes for the Turkish version of the scale were originally carried out by Asarkaya.¹⁹ This adapted 16-item version excludes the fourth item because it demonstrated inadequate discriminative capacity. Items are rated on a 4-point Likert scale ranging from 0 to 3, yielding possible score ranges of 0-27 for the HeOr subscale and 0-21 for the OrNe subscale.

Ethics Approval and Consent

Authorization for this research was granted by Erzincan Binali Yıldırım University Clinical Research Ethics Committee (approval no.: 03/01, date: 05.02.2026). Furthermore, written informed consent was secured from all participating individuals.

Artificial Intelligence Statement

Artificial intelligence software was used solely to create the graphical abstract of this manuscript. Nonetheless, the authors meticulously evaluated, edited, and finalized all material post-generation.

Statistical Analysis

We conducted all statistical computations using IBM SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA). Baseline demographic and clinical profiles were summarized using counts and percentages for categorical items. Meanwhile, continuous metrics were detailed as means ± standard deviations (SDs) or medians alongside their minimum-maximum ranges. To determine the normality of the data distribution, the Shapiro-Wilk test was applied, and skewness and kurtosis coefficients were examined (values between -2 and +2 were considered indicative of a normal distribution). Bivariate associations among continuous variables were evaluated using either Pearson's or Spearman's rank coefficients, depending on the normality of the data. A P value below 0.05 was deemed statistically significant.

RESULTS

Table 1 outlines the demographic profiles of the 218 subjects comprising the final analytical sample; the median age was 25 years [interquartile range (IQR): 21-29]. The sample comprised 73.9% males (n=161) and 26.1% females (n=57). Regarding educational background, the cohort consisted primarily of high school graduates (51.8%) and individuals holding a university degree or higher (45.0%), with primary school graduates comprising 3.2%. Single individuals predominated (76.6%), compared with married individuals (23.4%). Additionally, the participants presented an average BMI of 24.88 ± 3.89 kg/m². Regarding occupational status, the

Table 1. Sociodemographic and clinical characteristics of the study participants

Characteristic	Count (n)/percentage (%)	Mean ± SD/median (IQR)
Age (years)	-	25 (21-29)
Gender (female/male)	57 (26.1%)/161 (73.9%)	-
Educational status (primary/high school/university)	7 (3.2%)/113 (51.8%)/98 (45.0%)	-
Marital status (married/single)	51 (23.4%)/167 (76.6%)	-
BMI (kg/m ²)	-	24.88 ± 3.89
Occupational status (private security guard applicant)	218 (100%)	-
Current smoking	0 (0%)	-
History of psychiatric disorders	0 (0%)	-
Chronic medical diseases	0 (0%)	-
Current medication use	0 (0%)	-

Data are presented as n (%) or mean ± SD. For non-normally distributed data, results are expressed as the median (Q1-Q3). SD, standard deviation; IQR, interquartile range; BMI, body mass index; SD, standard deviation.

study sample was composed entirely of individuals applying for medical clearance to obtain a private security guard license. Consequently, at the time of data collection, a substantial proportion of the participants were presumed to be unemployed or actively seeking employment in the security sector. The participants' hematological and clinical profiles are outlined in Table 2. Regarding the orthorexia scale components, the cohort recorded average scores of 12.03 ± 4.32 for the HeOr dimension and 7.96 ± 3.89 for the OrNe dimension. Regarding the CBC profiles, participants exhibited mean values of $7.93 \pm 2.10 \times 10^3/\mu\text{L}$ for WBC, $4.65 \pm 1.75 \times 10^3/\mu\text{L}$ for neutrophils, and $2.51 \pm 0.73 \times 10^3/\mu\text{L}$ for lymphocytes, alongside an average platelet count of $260.87 \pm 62.95 \times 10^3/\mu\text{L}$. Analysis of the inflammatory indices revealed a mean PLR of 111.15 ± 37.63 . For asymmetrically distributed

metrics, the median values were 1.79 (IQR: 1.29-2.29) for NLR and 0.23 (IQR: 0.18-0.28) for MLR. Additionally, the calculated medians for the SII and SIRI stood at 457.98 (IQR: 313.51-602.45) and 0.96 (IQR: 0.63-1.29), respectively. Table 3 details the correlational findings among orthorexia components, BMI, and hematological indices. The analysis yielded a robust positive association between OrNe and HeOr ($r = 0.767, P < 0.001$). In contrast, BMI did not correlate significantly with either of the orthorexic dimensions ($p > 0.05$). In terms of clinical metrics, a weak yet significant positive association was detected between BMI and both WBC ($r = 0.169, P = 0.013$) and lymphocyte counts ($r = 0.215, P = 0.002$). Analysis of HeOr tendencies revealed inverse relationships with multiple blood markers: WBC ($r = -0.163, P = 0.016$), neutrophils ($r = -0.160, P = 0.019$), monocytes ($r = -0.244, p < 0.001$), and

Table 2. Distribution of clinical and hematological parameters of the participants

Parameter	Mean $\pm$ SD/median (IQR)
Healthy orthorexia (HeOr) score	12.03 ± 4.32
Orthorexia nervosa (OrNe) score	7.96 ± 3.89
White Blood Cell (WBC) count ($10^3/\mu\text{L}$)	7.93 ± 2.10
Neutrophil count ($10^3/\mu\text{L}$)	4.65 ± 1.75
Lymphocyte count ($10^3/\mu\text{L}$)	2.51 ± 0.73
Monocyte count ($10^3/\mu\text{L}$)	0.58 ± 0.18
Platelet count ($10^3/\mu\text{L}$)	260.87 ± 62.95
NLR (neutrophil-to-lymphocyte ratio)	1.79 (1.29-2.29)
PLR (platelet-to-lymphocyte ratio)	111.15 ± 37.63
MLR (monocytes / lymphocytes)	0.23 (0.18-0.28)
SII (systemic immune-inflammation index)	457.98 (313.51-602.45)
SIRI (systemic inflammation response index)	0.96 (0.63-1.29)

SII = (platelet $\times$ neutrophil)/lymphocyte; SIRI = (neutrophil $\times$ monocyte)/lymphocyte. Descriptive statistics: Mean (SD) for parametric, median (Q1-Q3) for non-parametric continuous variables. SD, standard deviation; IQR, interquartile range.

Table 3. Correlation matrix between orthorexia scores, BMI, and hematological parameters

Parameter	BMI	HeOr score	OrNe score
BMI (kg/m^2)	-	$r = 0.084, P = 0.217$	$r = -0.014, P = 0.842$
HeOr	$r = 0.084, P = 0.217$	-	$r = 0.767, P < 0.001$
OrNe	$r = -0.014, P = 0.842$	$r = 0.767, P < 0.001$	-
WBC	$r = 0.169, P = 0.013$	$r_s = -0.163, P = 0.016$	$r_s = -0.086, P = 0.207$
Neutrophil	$r = 0.091, P = 0.185$	$r_s = -0.160, P = 0.019$	$r_s = -0.064, P = 0.353$
Lymphocyte	$r = 0.215, P = 0.002$	$r_s = -0.085, P = 0.212$	$r_s = -0.109, P = 0.110$
Monocyte	$r = 0.101, P = 0.138$	$r_s = -0.244, P < 0.001$	$r_s = -0.171, P = 0.012$
Platelet	$r = 0.076, P = 0.266$	$r_s = -0.168, P = 0.013$	$r_s = -0.120, P = 0.078$
NLR	$r_s = -0.077, P = 0.261$	$r_s = -0.053, P = 0.442$	$r_s = 0.037, P = 0.586$
PLR	$r_s = -0.116, P = 0.890$	$r_s = -0.079, P = 0.250$	$r_s = -0.020, P = 0.774$
MLR	$r_s = -0.107, P = 0.118$	$r_s = -0.134, P = 0.049$	$r_s = -0.064, P = 0.346$
SII	$r_s = -0.013, P = 0.850$	$r_s = -0.111, P = 0.104$	$r_s = -0.016, P = 0.817$
SIRI	$r_s = -0.019, P = 0.785$	$r_s = -0.185, P = 0.006$	$r_s = -0.071, P = 0.299$

r , pearson correlation coefficients for normally distributed variables (BMI and scale scores); r_s , spearman's rank correlation coefficients for non-normally distributed variables (hematological parameters and inflammatory indices). Significance level: $P < 0.05$. OrNe, orthorexia nervosa; HeOr, healthy orthorexia; BMI, body mass index; WBC, white blood cell; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; MLR, monocytes/lymphocytes; SII, systemic immune-inflammation index; SIRI, systemic inflammation response index.

platelets ($r = -0.168$, $P = 0.013$). Moreover, increased HeOr was linked to decreased values of both the MLR ($r = -0.134$, $P = 0.049$) and the SIRI ($r = -0.185$, $P = 0.006$). Conversely, the OrNe dimension correlated negatively only with monocytes ($r = -0.171$, $P = 0.012$) and lacked any other significant links to the tested inflammatory indices.

DISCUSSION

Descriptive statistics indicated that the participants' age distribution was heterogeneous, with a notable concentration around 25 years. This situation represents a limitation in terms of its representativeness of the general population. An increase in inflammatory markers with age is well-documented in the literature.²⁰ The potential confounding effect of age on our results was likely reduced by age heterogeneity among participants during assessment of the association between orthorexia and inflammation. Males accounted for 73.9% of the study sample. While this demographic characteristic might be viewed as a drawback regarding the generalizability of the findings, considering the well-established impact of the menstrual cycle on inflammatory pathways, this high male proportion may have minimized menstrual-cycle-related variance in the data.²¹ In our dataset, participants' educational attainment is primarily at the high school and university graduate levels. In the literature, research regarding orthorexia is observed to predominantly focus on individuals within this educational bracket.²² Although this condition might be viewed as a limitation in terms of population representativeness, it could provide a distinct advantage when benchmarking our results against the existing literature. Studies investigating education-related factors have demonstrated that an increase in years of education exerts a protective effect against OrNe.²³ A robust and well-documented positive correlation exists between high BMI, body fat mass, and elevated inflammation.²⁴ Existing literature demonstrates that as BMI escalates—especially among obese populations—there is a parallel elevation in crucial inflammatory indices like CRP.²⁵ Evidence focusing specifically on hematological cellular components and ratios demonstrated that BMI was positively correlated with leukocytes, neutrophils, lymphocytes, monocytes, and MLR in heterogeneous samples. In contrast, BMI exhibits a negative correlation with platelet count, the NLR, and the PLR.¹¹ Consistent with prior literature, the current data demonstrate a direct association between BMI and both leukocyte and lymphocyte counts. Our inability to detect significant correlations across other parameters might be attributed to the lack of obese subjects within our sample, which exhibited a mean value and SD of 24.88 ± 3.89 . HeOr has been defined as a process independent of OrNe.⁵ Although our data demonstrate a strong positive correlation between OrNe and HeOr, the impact of these two constructs on hematological inflammatory pathways is surprisingly distinct. That the biological implications of the two orthorexia dimensions differ to such an extent underscores the validity of this distinction. A negative correlation is observed between HeOr scores and leukocyte, neutrophil, and monocyte levels. This inverse correlation represents a positive clinical marker, signaling a comparatively attenuated state of systemic inflammation. While

elevated HeOr scores do not necessarily equate to actual healthy dietary practices, this inclination has been demonstrated to correlate with healthy eating behaviors.⁶ Daily dietary patterns of these individuals revealed an increased proportion of fruit and vegetable consumption, contrasted by a concomitant decline in the intake of unhealthy items colloquially referred to as junk food.⁷ Therefore, the inverse correlation between HeOr scores and hematological inflammatory indices could be attributed to the healthy dietary practices of this population. Indeed, as the HEI-2015 score—a measure of diet quality—increases, reflecting a healthier diet, total leukocyte counts, neutrophil counts, and NLR values have been found to decrease significantly.²⁰ Serving as a refined metric of chronic systemic inflammation, the MLR illustrates the equilibrium between adaptive and innate immune pathways. Notably, we found this ratio was negatively correlated with HeOr tendencies. Research demonstrates that individuals who closely follow the dietary approaches to stop hypertension (DASH) protocol—formulated to manage blood pressure through high intake of fruits, vegetables, and grains—have significantly lower monocyte levels. Similarly, as the dietary inflammatory index (DII) decreases, monocyte levels decline. In fully adjusted models that simultaneously controlled for age, sex, and BMI, both elevated DASH scores and diminished DII scores demonstrated an association with lower monocyte concentrations.^{17,26} Changes in platelet count, which plays a regulatory role in the immune system, are significant because they are also effective in calculating PLR and SII, which indicate chronic inflammation processes.¹⁶ Although we detected a negative correlation between HeOr scores and platelet count, we observed no significant correlations with PLR and SII. The SIRI is a composite metric that contrasts innate immune responders (neutrophils and monocytes) against adaptive immune cells (lymphocytes).^{27,28} It captures the balance between systemic inflammatory activity and the immune system's regulatory mechanisms. Research demonstrates that SIRI reflects the intensity and severity of systemic inflammation far more effectively and clearly than conventional WBC (leukocyte) counts.^{29,30} Higher SIRI values suggest that a systemic inflammatory state prevails within the organism. SIRI was initially investigated to predict the prognosis and clinical course in cancer cases; currently, it has been increasingly adopted as a risk predictor in conditions such as cardiovascular diseases, atherosclerosis, infections, rheumatoid arthritis, and stroke.^{31,32} Although it has been less extensively investigated in psychiatry compared to other inflammatory biomarkers, elevated SIRI values have been shown to be associated with conditions such as depression and bipolar disorder.^{33,34} It has been reported that each one-unit increase in SIRI values is associated with an approximately 26-fold increase in the probability of a Generalized Anxiety Disorder diagnosis.¹⁶ Among inpatients with anorexia nervosa, SIRI is a highly significant predictive biomarker of response to nutritional rehabilitation. Patients exhibiting a positive clinical response to therapy presented with reduced initial SIRI values.²⁵ Our findings revealed a notable inverse association between HeOr scores and SIRI levels. This finding may be attributed to the healthy dietary habits of individuals strongly inclined toward HeOr. Indeed, existing literature

indicates that an improvement in dietary quality is associated with a concurrent reduction in SIRI levels.¹⁸ Our analysis of hematological markers revealed that OrNe was significantly and inversely associated solely with monocytes. This finding diverges from a prior investigation that found a positive correlation linking OrNe to the MLR.³⁵ Stress-induced harm and dietary restrictions are thought to mediate this link. The variation between our data and the existing literature might be explained by the use of distinct measurement instruments or additional confounding variables affecting the MLR.

Study Limitations

Certain methodological constraints of the present research must be considered. Primarily, the cross-sectional framework and correlational nature of our data make it impossible to deduce cause-and-effect relationships or predict outcomes linking orthorexia components with blood-based inflammatory markers. While our statistical approach demonstrates significant associations, it cannot determine whether these dietary tendencies predict changes in inflammatory markers over time. These preliminary correlations provide vital groundwork; our investigation appears to be the first to explore the association between orthorexic traits and this specific set of inflammatory indices. Second, our study population consisted exclusively of healthy young adult volunteers applying for private security guard licensing, and was predominantly male (73.9%) with an age distribution clustered around 25 years (IQR 21.00-29.00 years). Although this skewed gender distribution limits the generalizability of our results, it served as a methodological advantage by minimizing potential cycle-dependent physiological fluctuations in inflammatory pathways. The uniformity of the sample successfully controlled for potential confounders, such as age, chronic illnesses, and menstrual phases. However, it also limits the extrapolation of our results to general or clinical cohorts. Third, dietary habits and precise nutritional intake were not objectively quantified; rather, eating behaviors were assessed solely with the self-reported TOS. Consequently, potential recall bias or subjective misinterpretation cannot be entirely ruled out. Lastly, although participants with overt infections were not included, unassessed variables such as sleep hygiene and daily exercise routines might still have affected the inflammatory indices. Therefore, prospective, longitudinal studies employing advanced predictive modeling are warranted to build upon this preliminary evidence and to further elucidate the underlying causal mechanisms.

CONCLUSION

Individuals with a HeOr tendency demonstrated lower levels of several hematological markers of inflammation, including leukocytes, neutrophils, monocytes, platelets, MLR, and SIRI, indicating a healthier systemic inflammatory status. Although OrNe scores correlate strongly with HeOr scores, a similar decline is not observed in hematological inflammatory markers among individuals with a high tendency toward OrNe, except for monocyte counts. Despite more rigid cognitions and behaviors concerning healthy eating in OrNe, this approach

did not translate into a healthier systemic inflammation profile in individuals. The detrimental effects of stress and restrictive dieting may play a role in this phenomenon. However, prospective, more comprehensive studies are warranted to further elucidate the underlying mechanism.

Ethics

Ethics Committee Approval: Ethical committee approval was received from the Erzincan Binali Yıldırım University Clinical Research Ethics Committee (approval no.: 2026-03/01, date: 05.02.2026).

Informed Consent: Written informed consent was obtained from the individual participants who agreed to take part in the study.

Footnotes

Author Contributions: Concept Design – M.C.K.; Data Collection or Processing – M.C.K., M.A., B.Y., G.Ç., R.M.G.; Analysis or Interpretation – M.C.K., M.A.; Literature Review – M.C.K.; Writing, Reviewing and Editing – M.C.K.

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Fluoroscopy-Guided Percutaneous Endobiliary Forceps Biopsy for Tissue Diagnosis in Complex Biliary Obstruction: A Consecutive Single-Center Study

 Ayca Uysal  Emre Ünal

Department of Radiology, Hacettepe University Faculty of Medicine, Ankara, Türkiye

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ORCID IDs of the authors: A.U. 0000-0001-6348-9403, E.Ü. 0000-0002-1520-2487.

ABSTRACT

Objective: This study evaluated fluoroscopy-guided percutaneous endobiliary forceps biopsy (PEFB) for tissue diagnosis in complex biliary obstruction when no safe extraluminal biopsy target was available or when prior conventional tissue acquisition had been non-diagnostic.

Methods: This retrospective single-center study included consecutive patients who underwent PEFB between January 2024 and December 2025. PEFB was considered after multidisciplinary evaluation, when tissue diagnosis was required and conventional image-guided or endoscopic tissue acquisition was unavailable, unsuitable, or non-diagnostic. Procedures were performed under fluoroscopic guidance using an 8 French introducer sheath and disposable biopsy forceps. Outcomes included technical success, clinically interpretable tissue acquisition, final diagnosis, biopsy-guided management, and adverse events.

Results: Thirteen patients were included (median age 62 years; 9 women). A safe extraluminal biopsy target was absent in 12 patients; the remaining patient underwent rescue PEFB after two non-diagnostic core biopsies of a desmoplastic pancreatic/peribiliary mass. Five patients had prior endoscopic retrograde cholangiopancreatography-based evaluation, plastic stent placement, or non-definitive endoscopic sampling. PEFB was performed in 13 sessions, with 49 biopsy bites across 16 access-site sampling locations. Technical success and clinically interpretable tissue acquisition were achieved in all patients. Final diagnoses were malignant in eight patients, benign/inflammatory in four, and an intraductal papillary neoplastic lesion in one. PEFB findings guided subsequent management in all patients. No biopsy-related major adverse event occurred.

Conclusion: Fluoroscopy-guided PEFB may provide clinically actionable tissue in selected patients with complex biliary obstruction when conventional image-guided or endoscopic tissue acquisition is unavailable, unsuitable, or non-diagnostic.

Keywords: Bile duct obstruction, biopsy, cholangiocarcinoma, cholangiography, drainage, radiology, interventional

INTRODUCTION

Biliary strictures and complex biliary obstructions remain diagnostically challenging because benign inflammatory, postoperative, ischemic, autoimmune, and stone-related processes may mimic malignancy, whereas malignant obstruction may be infiltrative, intraductal, desmoplastic, or too small to form a discrete target for conventional percutaneous core biopsy.¹ This diagnostic overlap has direct clinical

consequences. In the absence of tissue diagnosis, decisions regarding surgery, systemic therapy, metal-stent-based palliation, steroid or anti-inflammatory treatments, therapy for benign strictures, or surveillance may be delayed, uncertain, or potentially misdirected. Although computed tomography (CT), magnetic resonance imaging (MRI)/magnetic resonance cholangiopancreatography (MRCP), positron emission tomography (PET)/CT, tumor markers, and multidisciplinary



Corresponding author: Ayca Uysal, **E-mail:** aycanuysal@hotmail.com

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assessment are essential for defining disease extent and likelihood, they may not provide definitive benign-malignant discrimination in selected complex cases.¹⁻⁴

Several tissue acquisition strategies are available, including endoscopic retrograde cholangiopancreatography (ERCP)-guided brush cytology or forceps biopsy, endoscopic ultrasonography (EUS)-guided sampling, cholangioscopy-guided biopsy, conventional image-guided biopsy of an extraluminal lesion, and percutaneous transluminal/endobiliary forceps biopsy. Each approach has limitations. ERCP may be limited by altered anatomy, failed cannulation, proximal or hilar strictures, prior stenting, or non-diagnostic cytology. EUS-guided sampling is valuable for pancreatic, distal extrahepatic, nodal, or metastatic targets, but may be less suitable when the disease is duct-centered, hilar, postoperative, or not associated with a discrete extraluminal mass. Cholangioscopy-guided biopsy provides direct intraductal visualization and targeted sampling; however, it may not be available in all centers, may require additional equipment and expertise, and may be impractical in selected patients with altered anatomy, failed endoscopic access, complex biliary drainage needs, cholangitis requiring percutaneous decompression, or established percutaneous biliary access.⁴

Percutaneous transluminal forceps biopsy has shown favorable performance for suspected malignant biliary strictures, with high technical success and specificity in prior series and meta-analyses.⁵⁻¹⁰ Its practical value is most apparent when a patient already requires percutaneous biliary drainage or cholangiographic access and the suspected disease is intraductal, infiltrative, postoperative, stent-obscured, or radiologically occult. In that setting, the existing or clinically required percutaneous biliary access can be used as a direct intraluminal sampling pathway, avoiding the need for a separate extraluminal biopsy target such as a mass, lymph node, parenchymal lesion, or metastasis. Therefore, this study evaluated technical success, clinically interpretable tissue acquisition, diagnostic contribution, management impact, and adverse events associated with fluoroscopy-guided percutaneous endobiliary forceps biopsy (PEFB) in consecutive

patients with complex biliary obstruction when conventional image-guided or endoscopic tissue acquisition was unavailable, unsuitable, or non-diagnostic.

MATERIALS AND METHODS

Study Design and Patient Population

The study was approved by the Hacettepe University Health Sciences Research Ethics Committee (approval no.: SBA 26/195, decision no.: 2026/05-24, date: 03.03.2026), and the requirement for study-specific informed consent was waived because of the retrospective, archive-review design. Written procedural consent had been obtained for all clinically indicated biliary interventions.

This retrospective single-center study included all consecutive patients who underwent fluoroscopy-guided PEFB for complex biliary obstruction between January 2024 and December 2025. All completed PEFB procedures were included. One additional patient was referred for a planned PEFB, but biopsy was not performed because interval cholangiographic, imaging, and tumor-marker findings supported regression of the stricture; this patient was excluded from the analytic cohort.

PEFB was considered after multidisciplinary evaluation, when a tissue diagnosis was required and at least one of the following applied: absence of a safe extraluminal biopsy target on cross-sectional imaging; unavailable, failed, non-diagnostic, or unsuitable endoscopic sampling; altered postoperative anatomy limiting endoscopic access; need for biliary drainage or percutaneous cholangiographic access; or non-diagnostic conventional core biopsy despite persistent clinical suspicion.

A safe extraluminal biopsy target was defined as a mass-forming parenchymal lesion, nodal lesion, distant metastasis, or extraductal soft-tissue component that is visible on CT, MRI/MRCP, US, or PET/CT and suitable for conventional image-guided core biopsy. The absence of such a target did not indicate normal imaging, but rather the absence of a discrete lesion safely accessible for percutaneous core biopsy.

Preprocedural Assessment

Preprocedural assessment included clinical history (including prior malignancy or hepatopancreatobiliary surgery), previous endoscopic or percutaneous tissue sampling, laboratory parameters, tumor markers, and all available CT, MRI/MRCP, and PET/CT examinations. Imaging studies were reviewed by an abdominal radiologist with more than 15 years' experience to determine the level and morphology of obstruction, suspicious ductal or periductal findings, stent-related or postoperative anatomic considerations, vascular relationships, and the presence or absence of a safe extraluminal biopsy target.

All procedures were performed under sedation assisted by anesthesia after intravenous prophylaxis with ceftriaxone. A platelet count greater than 80,000/ μ L and an INR less than 1.5 were required; no patient required correction of coagulopathy before biopsy.

MAIN POINTS

- Complex biliary obstruction may lack a safe extraluminal biopsy target despite suspicious imaging, cholangiographic, laboratory, or clinical findings.
- Fluoroscopy-guided percutaneous endobiliary forceps biopsy provided clinically interpretable tissue in all 13 patients in this consecutive cohort.
- Biopsy findings guided subsequent management, including surgery, systemic therapy, stent-based palliation, management of benign or inflammatory strictures, modification of drainage strategy, and avoidance of oncologic escalation.
- PEFB may be useful as a complementary tissue acquisition strategy when percutaneous biliary access is already required or when conventional sampling is unsuitable or non-diagnostic.

PEFB Technique

All PEFB procedures were performed by an interventional radiologist with more than 10 years of experience in hepatobiliary interventions. The access route and sampling site were selected based on ductal anatomy, existing drainage access, cholangiographic findings, and correlation with cross-sectional imaging. Under fluoroscopic guidance, the target stricture or ductal segment was crossed with a hydrophilic guidewire (RADIFOCUS Guide Wire M; Terumo Corp., Tokyo, Japan). A 0.035-inch Amplatz Super Stiff guidewire (Boston Scientific, Marlborough, MA, USA) was positioned to secure biliary access. A second guidewire was advanced across or adjacent to the target segment, and an 8 French (8-F) Super Arrow-Flex introducer sheath (11- or 24-cm lengths; Arrow International, Inc., Reading, PA, USA) was introduced over this wire and advanced to the stricture or lesion site. The Amplatz wire was maintained alongside the sheath as a safety wire. After removal of the intraluminal guidewire from the sheath, disposable biopsy forceps (Micro-Tech, Nanjing, China) were advanced directly through the sheath lumen to the target segment.

Biopsy bites were obtained from the most suspicious portion of the ductal lesion based on cholangiographic morphology and preprocedural imaging. In patients with multifocal or multi-duct involvement, sampling was directed to the ductal sector or sectors considered most suspicious or clinically relevant. After biopsy, biliary drainage was maintained with either an internal-external or external drainage catheter according to the clinical drainage strategy. Definitive or adjunctive biliary interventions, including metal stent placement, endobiliary radiofrequency ablation, balloon dilatation, or further stent/drainage management, were performed in subsequent sessions when indicated after histopathologic review and multidisciplinary decision-making. This sheath-supported, safety-wire-preserved technique is conceptually related to the previously described percutaneous "cross-and-push" approach, in which the stricture is crossed and the sheath supports forceps advancement into the target lesion.¹⁰

Histopathologic and Cytologic Evaluation

Forceps-derived tissue fragments were fixed in formalin and submitted for histopathologic evaluation by a dedicated gastrointestinal/hepatobiliary pathologist with more than 15 years of experience. When feasible, additional material obtained with the forceps was smeared onto glass slides for cytologic evaluation, according to institutional practice. Cytologic assessment was used as a supportive adjunct and was not analyzed as a separate diagnostic test. Immunohistochemistry was performed when necessary to support a diagnosis, determine tumor origin, or evaluate inflammatory mimics, including IgG4-related disease. No rapid on-site cytopathologic or histopathologic adequacy assessment was performed during PEFB. Clinically interpretable tissue acquisition was defined as retrieval of sufficient tissue for the pathologist to issue a histopathologic interpretation, rather than a report of insufficient or absent tissue. PEFB histopathologic results were categorized as: malignant; suspicious/consistent with

malignancy; benign (inflammatory or fibrotic); neoplastic/premalignant intraductal papillary lesion; inconclusive; or non-diagnostic. A non-diagnostic result was defined as insufficient tissue or absence of interpretable lesional material.

Final Diagnosis, Clinical Impact, and Outcomes

The final diagnosis was determined using a composite reference standard that included surgical pathology when available, PEFB with immunohistochemistry, additional cytologic or histologic sampling, adjudication by a multidisciplinary tumor board, clinical course, tumor marker behavior, treatment response, and imaging follow-up. A Benign final diagnosis required surgical or pathologic confirmation, or clinical or imaging evidence of stability, regression, or absence of malignant progression during follow-up. For patients followed outside the institution, availability of local follow-up data was recorded. Clinical management impact was defined as any biopsy-guided confirmation or change in the treatment pathway, including surgery, systemic therapy, metal stent placement, benign stricture therapy, drainage, stent-strategy modification, steroid or anti-inflammatory treatment, avoidance of oncologic escalation, or follow-up-based management.

The primary outcomes were technical success and clinically interpretable tissue acquisition. Technical success was defined as the successful advancement of the forceps into the target biliary segment with acquisition of tissue. Secondary outcomes included final diagnostic category, number of biopsy bites, access-site sampling locations, biopsy-guided management, and adverse events. Adverse events were identified from procedural reports, inpatient records, laboratory data, imaging follow-up, and clinical notes and were attributed, when possible, to the biopsy maneuver, biliary access or drainage, catheter dysfunction, underlying disease, or unrelated causes. Events were classified according to the 2017 Society of Interventional Radiology adverse event reporting framework.¹¹ Major adverse events were defined as events requiring major therapy or unplanned escalation of care and resulting in permanent sequelae or death; these included clinically significant bleeding, hemobilia requiring intervention or transfusion, bile peritonitis requiring intervention, septic shock, or unplanned intensive care admission. Non-major events included self-limited or medically managed infectious, pain-related, or catheter-related events that did not require escalation of care.

Statistical Analysis

Analyses were descriptive because of the small sample size and the selected clinical characteristics of the cohort. Continuous variables are reported as median (range). Categorical variables are reported as counts and percentages. No formal comparative testing was planned. Sensitivity, specificity, and predictive values were not calculated because of the small cohort size, clinical heterogeneity, non-uniform composite reference standard, and the study's focus on feasibility, tissue acquisition, safety, and clinical actionability rather than on diagnostic accuracy testing. Descriptive statistics were generated using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, NY, USA).

Use of Artificial Intelligence (AI)-Assisted Technologies

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) for English-language editing and clarity. The authors reviewed and edited the manuscript as needed and took full responsibility for its content.

RESULTS

Patient Cohort and Diagnostic Context

Thirteen consecutive patients who underwent technically completed PEFB were included in the analysis. The median age was 62 years (range, 46-78 years), and nine patients were women. No completed PEFB procedures were excluded. One additional patient was referred for planned PEFB, but a biopsy was not performed because interval cholangiographic, imaging, and tumor marker findings supported regression of the stricture. Baseline clinical, imaging, procedural, and outcome data are summarized in Table 1. In 12 of 13 patients, no safe extraluminal percutaneous biopsy target was identified. The remaining patient had a large desmoplastic pancreatic or peribiliary mass, but two prior conventional core biopsies were

non-diagnostic, prompting PEFB as a rescue tissue acquisition strategy.

Five patients had prior ERCP-based evaluation, plastic stent placement, or endoscopic sampling without definitive tissue diagnosis. A representative case illustrating PEFB after non-definitive ERCP-based management is shown in Figure 1. Preprocedural MR/MRCP demonstrated suspicious or indeterminate ductal/periductal abnormalities in 10 patients. Diffusion restriction was present in five patients, all with final malignant diagnoses; however, three malignant cases lacked a diffusion-restricted target suitable for conventional core biopsy. PET/CT was available in five patients; in two of these the scans were negative or interpreted as inflammatory despite a final diagnosis of malignancy. Cancer antigen 19-9 (CA19-9) and cancer antigen 125 (CA125) were elevated or borderline elevated in nine of 12 patients with available tumor marker data. Imaging, PET/CT, and tumor markers were used to guide the indication for biopsy rather than to serve as independent predictors of malignancy. Biopsy targets were hilar, proximal hepatic duct, or hepaticojejunostomy/anastomotic in seven patients; distal or mid common bile duct/perampullary in five patients; and segmental intrahepatic in one patient.

Table 1. Cohort-level summary of indications, procedural characteristics, histopathologic outcomes, management impact, and safety

Domain	Variable	Result
Study cohort	Study design and period	Retrospective, single-center, consecutive study; January 2024-December 2025
	Patients included	13
	Completed PEFB procedures excluded	0
	Planned PEFB not performed	1; canceled because interval cholangiographic, imaging, and tumor marker findings supported regression
	Age	Median 62 years; range, 46-78
	Sex	9 women/4 men
Diagnostic indication	No safe extraluminal percutaneous biopsy target	12/13
	Rescue PEFB after non-diagnostic conventional core biopsy despite a mass	1/13
	Prior ERCP, plastic stent placement, or non-definitive endoscopic sampling	5/13
Preprocedural diagnostic context	MR/MRCP suspicious or indeterminate ductal/periductal abnormality	10/13
	Diffusion restriction on MRI	5/13; all had final malignant diagnoses
	Malignancy despite no diffusion-restricted target suitable for conventional core biopsy	3 patients
	PET/CT performed	5/13
	Focal or indeterminate peribiliary/hilar PET uptake	3/5
	PET/CT negative or interpreted as inflammatory despite final malignant diagnosis	2 patients
	Tumor marker data available	12/13
Target and access	CA19-9 and/or CA125 elevated or borderline elevated	9/12
	Biopsy target level	Hilar/proximal/HJ-anastomotic, 7; distal/mid CBD or perampullary, 5; segmental intrahepatic duct, 1
	Patient-level access route	Right-sided, 10; left-sided, 3
	Access-site sampling locations	16 total; right-sided, 13; left-sided, 3
	Multi-duct or multi-access sampling	2 patients

Table 1. Continued

Domain	Variable	Result
Procedure	Biopsy sessions	13
	Total forceps biopsy bites	49
	Bites per patient	Median 3; range, 3-6
	Biopsy during index biliary access session	1/13
	Delayed biopsy after biliary access/drainage	12/13
	Interval from initial biliary access/drainage to biopsy	Median 7 days; range, 0 days-6 months
	Interval among delayed biopsies only	Median 11 days; range, 5 days-6 months
	Technique	8-F Super Arrow-Flex introducer sheath and disposable Micro-Tech biopsy forceps
	Drainage after biopsy	Internal-external catheter, 10; external catheter, 3
Technical and histopathologic outcomes	Technical success	13/13
	Clinically interpretable tissue acquisition	13/13
	Immunohistochemistry	Performed when required for tumor characterization, tumor-origin confirmation, or inflammatory mimics
	Cytologic smear assessment	Performed when feasible as a supportive adjunct; not analyzed separately
	Final malignant diagnosis	8/13
	Final benign/inflammatory diagnosis	4/13
	Intraductal papillary neoplastic lesion	1/13
	Surgical pathology reference standard	2 patients
	Local imaging, clinical, or surgical outcome data available	11/13
Clinical impact	Outside follow-up after tissue diagnosis	2/13
	Management guided by PEFB findings	13/13
	Malignancy-directed management	Surgery, 2; systemic therapy and/or metal stent-based palliation, 6
	Benign/inflammatory management	Steroid or anti-inflammatory treatment, benign stricture therapy, drainage/stent strategy modification, or follow-up-based avoidance of oncologic escalation, 4
	Neoplastic/premalignant lesion management	Tissue diagnosis enabled continuation of management at an outside center, 1
Safety	Biopsy-related major adverse event	0
	Clinically significant bleeding or hemobilia requiring treatment	0
	Transfusion requirement	0
	Septic shock or unplanned ICU admission	0
	Procedure-related death	0
	Non-major access-, drainage-, or catheter-related events	4 non-major events; all managed without ICU admission, major intervention, or permanent sequelae

Cohort-level summary of diagnostic context, procedural characteristics, histopathologic outcomes, management impact, and safety. Data are presented as number of patients unless otherwise specified. A safe extraluminal biopsy target was defined as any of the following, each suitable for conventional image-guided core biopsy: a mass-forming parenchymal lesion, a nodal lesion, a distant metastatic lesion, or an extraductal soft-tissue component. Imaging findings, PET/CT uptake, and tumor markers were considered clinical triggers for PEFB rather than independent predictors of malignancy. Adverse events were, when possible, attributed to biopsy-related, biliary access/drainage-related, catheter-related, disease-related, or unrelated causes. No biopsy-related major adverse event occurred. CBD, common bile duct; ERCP, endoscopic retrograde cholangiopancreatography; HJ, hepaticojejunostomy; ICU, intensive care unit; MR/MRCP, magnetic resonance imaging/magnetic resonance cholangiopancreatography; PEFB, percutaneous endobiliary forceps biopsy; PET/CT, positron emission tomography/computed tomography.

Procedural Characteristics

PEFB was performed in 13 biopsy sessions, including 16 access-site sampling locations, because two patients underwent multi-duct or multi-access sampling. A total of 49 forceps biopsy

bites were obtained, with a median of 3 bites per patient and a range of 3-6.

Biopsy was performed during the index biliary access session in one patient and as a delayed procedure in 12 patients. The

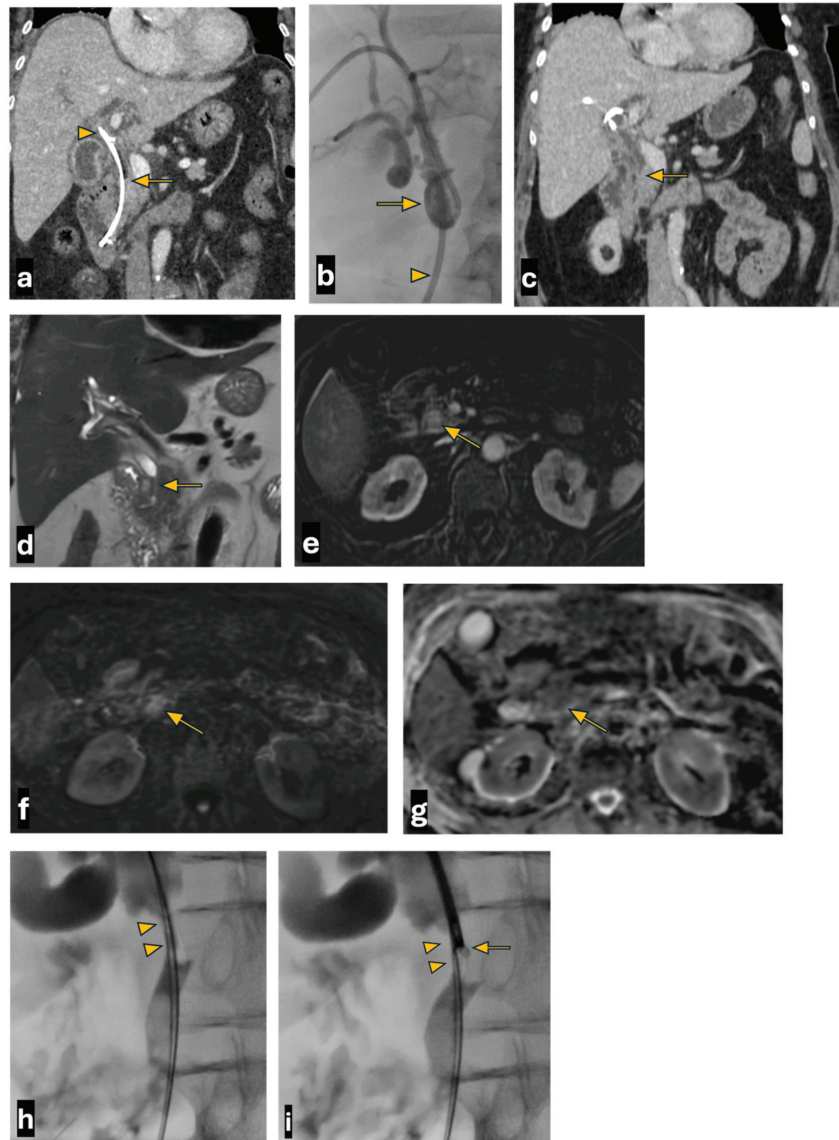


Figure 1. Representative PEFB case after non-definitive ERCP-based management.

A 78-year-old woman presented with cholangitis after ERCP with plastic biliary stent placement, but without a definitive tissue diagnosis. (a) Coronal CT shows the plastic biliary stent (arrowhead) and an abrupt distal common bile duct luminal cut-off with focal mural thickening (arrow). (b) Percutaneous cholangiography shows the external biliary drain (arrow) and plastic stent (arrowhead). (c) CT performed after biliary drainage and stent removal demonstrates persistent distal common bile duct narrowing or occlusion and wall thickening (arrow). (d) Coronal T2-weighted image shows narrowing/occlusion of the distal common bile duct. (e-g) Subtraction, diffusion-weighted, and ADC images demonstrate pathologic mural enhancement and restricted diffusion in the distal common bile duct lesion (arrows). (h) Cholangiography shows the target distal common bile duct stricture or occlusion. (i) Sheath-supported PEFB was performed from the stenotic segment; the arrow indicates the biopsy forceps, and the arrowheads mark the stricture. PEFB yielded histopathologic findings consistent with adenocarcinoma; Whipple pathology confirmed a biliary-type distal common bile duct adenocarcinoma.

ADC, apparent diffusion coefficient; CBD, common bile duct; CT, computed tomography; ERCP, endoscopic retrograde cholangiopancreatography; PEFB, percutaneous endobiliary forceps biopsy.

median interval from initial biliary access or drainage to biopsy was 7 days (range, 0 days to 6 months). Among delayed biopsies, the median interval was 11 days (range, 5 days to 6 months). All procedures were completed using an 8-F introducer sheath and disposable biopsy forceps. After the biopsy, biliary drainage was maintained with an internal-external catheter in 10

patients and an external catheter in three patients. Definitive or adjunctive biliary interventions were performed in subsequent sessions, when indicated, following histopathologic review and multidisciplinary decision-making. Technical success was achieved in all patients.

Histopathologic Findings and Clinical Outcomes

All 13 biopsy procedures yielded clinically interpretable tissue. Immunohistochemistry was performed when required.

Final diagnostic categories were malignant disease in eight patients, benign or inflammatory disease in four, and an intraductal papillary neoplastic lesion of the bile duct in one. Among malignant cases, PEFB demonstrated adenocarcinoma or malignant tissue consistent with cholangiocarcinoma in seven patients. In one patient with a large desmoplastic pancreatic/peribiliary mass and two prior non-diagnostic core biopsies, PEFB showed dysplasia and a dense desmoplastic inflammatory reaction; after immunohistochemical assessment and multidisciplinary tumor board review, the findings were interpreted as favoring adenocarcinoma in the clinical and imaging context. Accordingly, one of the eight final malignant diagnoses was established by multidisciplinary adjudication rather than by unequivocal malignant histopathology obtained by PEFB.

Two malignant diagnoses were confirmed by surgical pathology after the Whipple procedure. Benign or inflammatory diagnoses were supported by pathology, clinical course, tumor marker behavior, treatment response, and follow-up imaging that showed no new or progressive malignant lesions. Local imaging, clinical, or surgical outcome data were available for 11 patients; two patients continued follow-up outside the institution.

PEFB findings guided subsequent clinical management in all patients. Tissue diagnosis supported decisions regarding surgery, systemic therapy, metal stent placement, combined oncologic and biliary palliation, steroid-based therapy or benign stricture therapy, drainage/stent strategy modification, or avoidance of oncologic escalation during follow-up.

Safety

No biopsy-related major adverse event occurred. There were no clinically significant bleeding events, hemobilia requiring treatment, transfusion, bile peritonitis requiring intervention, septic shock, unplanned intensive care admission, or procedure-related death. Four non-major access-related, drainage-related, or catheter-related events occurred: early cholangitis after initial biliary access/drainage, mild postprocedural leukocytosis treated with antibiotics, non-abscessed tract/cutaneous cellulitis, and catheter dislodgement-related segmental cholangitic changes. All events were managed without intensive care admission, major intervention, or permanent sequelae.

DISCUSSION

The principal finding of this consecutive single-center study was that fluoroscopy-guided PEFB provided technically successful and clinically interpretable tissue acquisition in all patients with complex biliary obstruction requiring tissue diagnosis. Most patients lacked a safe extraluminal target for conventional image-guided core biopsy; one patient underwent PEFB after two non-diagnostic core biopsies, despite the presence of a desmoplastic pancreatic/peribiliary mass. PEFB findings guided subsequent clinical management in all patients, and no major biopsy-related adverse events occurred.

The practical value of PEFB in this cohort was related to the diagnostic setting rather than to technical novelty. These were not routine mass-forming biliary tumors with straightforward biopsy targets. Most patients had duct-centered, infiltrative, postoperative, stent-associated, or radiologically occult disease; several had undergone prior ERCP, plastic stent placement, non-definitive endoscopic sampling, or non-diagnostic conventional core biopsy. In this context, tissue-related uncertainty directly affected multidisciplinary decisions regarding surgery, systemic therapy, metal stenting, therapy for benign strictures, steroid-based treatment, drainage strategy, or surveillance. PEFB provided histologic tissue from the ductal abnormality via the existing or clinically required percutaneous biliary route.

These findings are consistent with prior literature supporting percutaneous transluminal forceps biopsy as a feasible and highly specific technique for malignant biliary strictures. Jeon et al.⁷ reported pooled sensitivity of 81%, specificity of 100%, and technical success of 100% in a meta-analysis of 14 studies including 1762 patients. Fohlen et al.⁶ reported successful drainage and tissue sampling in all 50 patients undergoing percutaneous transluminal forceps biopsy during biliary drainage, with 100% specificity. Augustin et al.,¹² in a 13-biopsy cohort similar in size to the present study, reported 100% technical success and adequate histopathologic material in 12 of 13 biopsies. Liu et al.¹³ reported 100% technical success, 82.35% sensitivity, 100% specificity, and 86.05% accuracy for percutaneous intraluminal forceps biopsy after ERCP failure. The present study differs from these broader suspected malignancy cohorts by focusing on a selected clinical scenario: tissue acquisition when imaging did not show a safe extraluminal biopsy target or when prior tissue sampling was non-diagnostic.

This problem remains clinically relevant despite the increasing role of cholangioscopy-guided biopsy. Cholangioscopy permits direct intraductal visualization and targeted biopsy, but it may not be available in all centers and may be impractical in selected patients with altered postoperative anatomy, failed or non-diagnostic ERCP, complex hilar drainage requirements, cholangitis requiring percutaneous decompression, or established percutaneous biliary access.⁴ This role should therefore not be interpreted as competing with cholangioscopy-guided biopsy, but rather as complementary in situations where percutaneous biliary access is already required, or where endoscopic/cholangioscopic sampling is not feasible or has not provided a definitive diagnosis.

The clinical contribution of PEFB in this cohort extended beyond confirming malignancy. Benign and inflammatory tissue diagnoses also guided management by supporting steroid or anti-inflammatory treatment, providing benign stricture therapy, modifying drainage or stent strategies, and avoiding unnecessary oncologic escalation. Similarly, in malignant cases, PEFB guided surgery, systemic therapy, metal stent-based palliation, or combined oncologic and biliary management. Because PEFB provides forceps-derived tissue fragments suitable for routine histopathology and immunohistochemistry, it may provide information beyond cytology alone, including confirmation of tumor origin and evaluation of inflammatory mimics.

Most biopsies in this cohort were performed as delayed procedures after biliary access or drainage. Prior studies have reported greater diagnostic yield when bilirubin levels are lower and sepsis is absent.^{14,15} The present observations are consistent with a selective, staged approach in clinically complex patients, but they do not establish superiority of delayed biopsy over same-session biopsy because timing was not assigned or compared.

Technical factors may have contributed to the high tissue acquisition rate. The procedure employed an 8-F sheath-supported approach, incorporating disposable forceps, direct cholangiographic targeting, imaging correlation, and preservation of biliary access with a safety wire. This approach is related to the "cross-and-push" technique described by Patel et al.,¹⁰ which was associated with improved sensitivity and accuracy in suspected malignant biliary strictures. Inchingolo et al.¹⁶ described a dedicated forceps system using a sheath-supported cross-and-push technique, reporting high diagnostic accuracy and no major complications. Because a small sample size and crush artifacts are recognized limitations of forceps biopsy, adjunctive cytologic assessment may be useful in selected cases. Boos et al.¹⁷ reported that tandem brush cytology and forceps biopsy improved sensitivity compared with either method alone, with preserved specificity. In the present cohort, cytologic smears from forceps-derived material were used only as an adjunct and were not analyzed as a separate diagnostic test.

No biopsy-related major adverse event occurred. The non-major events observed in this cohort were attributable to biliary access or drainage, catheter dysfunction, or the access tract, rather than to the biopsy maneuver itself. This distinction is important because patients undergoing biliary intervention frequently have cholangitis, severe cholestasis, prior instrumentation, complex drainage needs, or tumor-related obstruction. Published series report low but non-negligible rates of hemobilia, bile leak, infection, and other adverse events after percutaneous biliary biopsy.^{6,7} Careful event attribution is necessary when evaluating the safety of PEFB in this population.

Study Limitations

This study has limitations. It was a retrospective, single-center study that included a small, highly selected cohort. The population was heterogeneous, including patients with primary biliary malignancy, metastatic disease, postoperative strictures, inflammatory mimics, intraductal tumor progression, and one desmoplastic pancreatic/peribiliary mass with prior non-diagnostic core biopsies. Sensitivity, specificity, and predictive values were not calculated because the study was not designed as a diagnostic accuracy trial and final diagnoses were based on a composite reference standard rather than uniform surgical pathology. One malignant diagnosis required multidisciplinary adjudication because of dysplasia and a dense desmoplastic inflammatory reaction on PEFB. Two patients continued follow-up outside the institution. Finally, forceps biopsy has intrinsic limitations, including small sample size, crush artifact, and lack of direct visual targeting compared with cholangioscopy-guided biopsy. Emerging techniques such as percutaneous cholangioscopy-guided biopsy and endobiliary

cryobiopsy may improve targeting or sample quality but require additional equipment, expertise, and validation.^{18,19}

CONCLUSION

Fluoroscopy-guided PEFB may provide clinically actionable tissue in selected patients with complex biliary obstruction when conventional image-guided or endoscopic tissue acquisition is unavailable, unsuitable, or non-diagnostic. Its most defensible role is as a complementary problem-solving technique in patients who already require percutaneous biliary access or who lack a safe extraluminal biopsy target.

Data Availability

The data underlying this article cannot be shared publicly because of patient privacy and institutional restrictions. De-identified data may be made available from the corresponding author upon reasonable request and subject to institutional approval.

Ethics

Ethics Committee Approval: This study was approved by the Hacettepe University Health Sciences Research Ethics Committee (approval number: SBA 26/195, decision no.: 2026/05-24, date: 03.03.2026).

Informed Consent: The requirement for study-specific informed consent was waived owing to the retrospective archive-review design. Written procedural consent had been obtained for all clinically indicated biliary interventions.

Footnotes

Author Contributions

Concept Design – A.U., E.Ü.; Data Collection or Processing – A.U.; Analysis or Interpretation – A.U.; Literature Review – A.U.; Writing, Reviewing and Editing – A.U., E.Ü.

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Vertebral Artery Doppler Reference Values in Healthy Turkish Adults: Influence of Age, Sex, and BMI

 Ahmet Tuğrul Akkuş¹,  Muhammed Furkan Barutçigil²,  Said Ali Atalay³

¹Clinic of Radiology, Kağızman State Hospital, Kars, Türkiye

²Clinic of Radiology, Tekirdağ İsmail Fehmi Cumaloğlu City Hospital, Tekirdağ, Türkiye

³Clinic of Radiology, Bayburt State Hospital, Bayburt, Türkiye

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ORCID IDs of the authors: A.T.A. 0000-0001-5269-2502, M.F.B. 0000-0003-4345-0442, S.A.A. 0000-0001-8225-3463.

ABSTRACT

Objective: To establish population-specific bilateral reference values for vertebral artery (VA) diameter, peak systolic velocity (PSV), end-diastolic velocity (EDV), resistive index (RI), pulsatility index (PI), and flow volume in healthy Turkish adults, and to assess the effects of age, sex, laterality, and body mass index (BMI) on these parameters.

Methods: This prospective, multicenter study enrolled 223 healthy Turkish adults from three centers. VA Doppler ultrasonography was performed using a standardized protocol. The measured parameters included VA diameter, PSV, EDV, RI, PI, and flow volume.

Results: Left vertebral arteries exhibited significantly larger diameter (3.73 ± 0.57 mm vs. 3.52 ± 0.61 mm), PSV, EDV, and flow volume compared to the right side ($P < 0.05$). Males had a larger right VA diameter and higher RI and PI bilaterally, whereas females had higher right VA EDV ($P < 0.05$). Age was negatively correlated with PSV, RI, and PI, but positively correlated with left VA diameter. BMI was negatively correlated with PSV, RI, and PI, but did not significantly affect flow volume. Reference percentiles were established based on sex and age.

Conclusion: This study investigated normal reference values for six VA Doppler parameters in 223 healthy Turkish adults, stratified by sex and age, with BMI evaluated as a covariate. It is the first study to include BMI as a covariate and to derive percentile-based norms from a multicenter Turkish cohort. The normal range (5th-95th percentile) for total VA flow volume was 124.7-391.8 mL/min. Because this lower limit lies well below the widely quoted 200 mL/min threshold for vertebrobasilar insufficiency, applying that threshold would misclassify a considerable proportion of healthy adults as abnormal. These findings support improved clinical interpretation of VA Doppler ultrasonography.

Keywords: Vertebral artery, ultrasonography, doppler, body mass index

INTRODUCTION

The vertebral arteries (VAs) arise from the subclavian arteries, course through the transverse foramina of the cervical vertebrae, and form the basilar artery at the pontomedullary junction. The VAs supply the brainstem, cerebellum, thalamus, occipital lobes, and medial temporal lobes.¹ vertebral artery (VA) occlusion, stenosis, or hemodynamic alterations may result in significant neurological conditions ranging from transient ischemic attacks to posterior circulation infarcts.

Digital subtraction angiography remains the gold standard imaging modality for vascular pathologies above the aortic arch. However, it is not a suitable screening tool because it is invasive. Thus, color Doppler ultrasonography (CDU) and computed tomography angiography (CTA) are used for screening imaging modalities for vascular pathologies.² CDU is cost-effective, radiation-free, and repeatable, and has therefore been widely used in the diagnosis of atherosclerotic obstructive diseases of the carotid system; however, data on VA are scarce compared with carotid arteries.



Corresponding author: Ahmet Tuğrul Akkuş, **E-mail:** akkustugrul@gmail.com

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Despite multiple studies, no universally accepted reference values exist for VA Doppler parameters, and inter-study variability [e.g., peak systolic velocity (PSV) ranging from 37 to 53 cm/s across populations] complicates the diagnosis of vertebrobasilar stenosis in clinical practice. This variability likely reflects a combination of population-specific biological differences, inconsistent measurement protocols, and the absence of anthropometric covariates such as body mass index (BMI). Several factors contribute to the variability in VA Doppler parameters. Prior studies were single-center and therefore prone to selection bias. Many studies had limited sample sizes. No prior study has incorporated BMI into VA Doppler evaluation, despite evidence that BMI influences peripheral vascular resistance and carotid artery hemodynamics, suggesting it may also modulate VA flow dynamics. Tarnoki et al.³ demonstrated moderate genetic heritability of VA diameter in a European twin cohort, suggesting that population-specific genetic variation may contribute to inter-study differences in Doppler parameters. Given the distinct genetic and anthropometric profiles of Turkish populations compared with Western European cohorts, population-specific reference values are needed to improve diagnostic accuracy in these populations.

To address the absence of population-specific, BMI-informed, and multicenter-validated reference values for Turkish adults, we designed the present prospective study. By enrolling a large cohort of healthy Turkish adults across geographically diverse centers, using a standardized protocol, we aimed to establish population-specific bilateral reference values for VA diameter, PSV, end-diastolic velocity (EDV), resistive index (RI), pulsatility index (PI), and flow volume and to systematically evaluate the effects of age, sex, laterality, and BMI on these parameters. We aimed to provide normal reference values for the Turkish population to improve the clinical interpretation of VA Doppler examinations. Future validation studies are needed to assess whether population-specific norms reduce diagnostic misclassification compared with existing European reference values.

MAIN POINTS

- Our study is the first in the literature to include body mass index (BMI) in vertebral artery (VA) Doppler evaluation. While BMI was negatively correlated with peak systolic velocity (PSV), resistive index (RI), and pulsatility index (PI), it was not correlated with flow volume.
- The normal range (5th-95th percentile) for total VA flow volume was 124.7-391.8 mL/min; the 5th- percentile value (124.7 mL/min) was well below the commonly used 200 mL/min threshold for vertebrobasilar insufficiency.
- Male participants exhibited a significantly larger right VA diameter (median 3.70 mm vs. 3.50 mm, $P = 0.004$) and higher RI and PI bilaterally, whereas female participants demonstrated a higher right VA end-diastolic velocity ($p = 0.037$).
- No statistically significant differences were observed in the PSV and flow volume between the sexes.

MATERIALS AND METHODS

This prospective, observational, multicenter study was conducted at three centers between March 1, 2026, and June 1, 2026. Ethics committee approval was obtained from Tekirdağ İsmail Fehmi Cumaloğlu City Hospital Clinical Research Ethics Committee (approval no.: AN-262105-45, date: 21.05.2026) and conducted in accordance with the current principles of the Declaration of Helsinki. Written informed consent was obtained from all participants.

A total of 223 volunteers participated in the study. Participants were selected from individuals who applied to outpatient services at the relevant centers or responded to study announcements, and the plan was designed to ensure equal distribution of applications from healthy volunteers across all three centers.

The inclusion criteria for the study are as follows: be older than 18 years of age; have no neurological symptoms or complaints (including dizziness, vertigo, syncope, transient ischemic attack, stroke, ataxia, diplopia, dysphagia, and gait disturbance); agree to participate voluntarily and provide written informed consent; and have sufficient acoustic windows in which VA Doppler ultrasonography can be technically performed.

The exclusion criteria for the study are as follows: history of known neurological disease (stroke, transient ischemic attack, multiple sclerosis, Parkinson's disease, epilepsy, etc.), cervical vertebral pathology (severe osteoarthritis, disc herniation, cervical stenosis, instability, etc.), history of carotid or VA disease (atherosclerosis, dissection, occlusion, stenosis), history of previous surgery or radiation therapy in the head and neck region, uncontrolled hypertension ($\geq 160/100$ mmHg) or inadequate regulation with antihypertensive therapy, atrial fibrillation or other cardiac arrhythmias significantly affecting hemodynamics, congestive heart failure or severe valvular disease, known coagulopathy or active anticoagulant/thrombolytic therapy, active infection, wound, or skin disease affecting the skin in the neck region, morbid obesity (BMI > 40 kg/m²) or abnormal neck anatomy Short neck/thickness that makes examination impossible, inability to measure due to known VA hypoplasia (diameter < 2 mm) or anatomical variant, pregnancy or breastfeeding period, severe difficulty in cooperation that prevents reliable measurement (dementia, psychiatric disorder, etc.).

VA Doppler ultrasonography examinations were performed at all three centers using real-time ultrasonography devices with B-mode, color Doppler, and spectral Doppler (pulsed-wave Doppler) capabilities. The Toshiba Aplio 400 ultrasound system (Toshiba Medical Systems, Otawara, Japan) was used at all three centers. Examinations were performed using a high-frequency linear probe (7-15 MHz). To ensure that device settings and measurement protocols remained consistent throughout the study, examinations at each center were performed by radiology specialists with 5 years' experience in carotid-VA Doppler ultrasound. An inter-center technical standardization meeting was held prior to commencing measurements.

Participants were instructed to avoid consuming caffeinated beverages and smoking for at least 2 hours before the

examination. The examination was performed in a quiet environment at room temperature (20-24 °C), after the participant had rested in the supine position for at least 10 min. The participant was placed in the supine position with the head slightly turned to the opposite side and the neck slightly extended.

The common carotid artery was visualized in the sagittal plane 1-2 cm proximal to the bifurcation in B-mode imaging. The probe was tilted slightly laterally without exerting pressure. The V2 segment of the VA was visualized between the transverse foramina; all Doppler, diameter, and cross-sectional area measurements were obtained in the intertransverse segments at the mid-cervical level (C3-C5). This segment was chosen because it is readily accessible with ultrasound, has a relatively straight course that minimizes angle-related measurement error, and is the standard site for detecting hemodynamically significant stenosis in clinical practice. Spectral Doppler sampling was performed with a Doppler angle of 45°-60°, and all measurements used this angle correction (Figure 1).

PSV and EDV were measured three times and the averages were calculated. The VA diameter (intima-to-intima distance) was measured in millimeters (mm) at the end-diastolic stage of the cardiac cycle. The measurement was taken at a point where the vessel walls were clearly visible in a section perpendicular to the insonation angle (Figure 2). Flow volume (mL/min) was calculated using the device's automatic calculation function as the product of time-averaged mean velocity (TAMV, cm/s) $\times$ cross-sectional area (cm²) $\times$ 60. TAMV was obtained from the angle-corrected spectral Doppler waveform, averaged over three consecutive cardiac cycles. The cross-sectional area was measured directly by manually tracing a circular region of interest along the vessel lumen on the transverse (cross-sectional) image at the same level (Figure 3), and it was not derived from the longitudinally measured diameter.

Statistical Analysis

All statistical analyses were performed using SPSS version 24.0 (IBM Corp., Armonk, NY, USA). Continuous variables are presented as mean $\pm$ standard deviation and median with interquartile range (IQR). The normality of the distribution was assessed using the Kolmogorov-Smirnov test. The Kruskal-Wallis H test was used to assess inter-center homogeneity prior to data pooling. The Mann-Whitney U test was used to compare parameters between sexes, and the Wilcoxon signed-rank test was used to compare left and right sides within the same subject. Spearman's rank correlation coefficient (ρ) was used to assess associations between age, BMI and Doppler parameters. The sex distribution across the centers was assessed using the chi-square test. Percentiles and stratification by sex and age were provided to support the development of the nomogram. Reference limits (5th and 95th percentiles) were derived nonparametrically as empirical percentiles of the observed data, using linear interpolation between adjacent order statistics and without assuming any underlying distribution. In line with the CLSI EP28-A3c guideline, 90% confidence intervals for the overall and sex-specific 5th and 95th percentiles were estimated with the nonparametric rank-based (binomial order-statistic) method (Supplementary Table S1).¹² IQRs were calculated in

the same way. Two implausible values that were considered data-entry errors (a right VA diameter of 0.38 mm and a right VA RI of 7.00) were treated as missing. To assess the robustness of the pooled estimates, the main analyses were repeated after excluding the center with the oldest participants, as a sensitivity analysis (Supplementary Table S2). Statistical significance was set at $p < 0.05$.

RESULTS

Of 283 volunteers assessed for eligibility, 60 were excluded: 20 for uncontrolled hypertension, 10 for cardiac arrhythmia, 8 for atherosclerotic changes in the common or internal carotid artery, 6 for severe difficulty cooperating, 5 for previous stroke, 4 for VA hypoplasia, 3 for pregnancy, 3 for previous head and

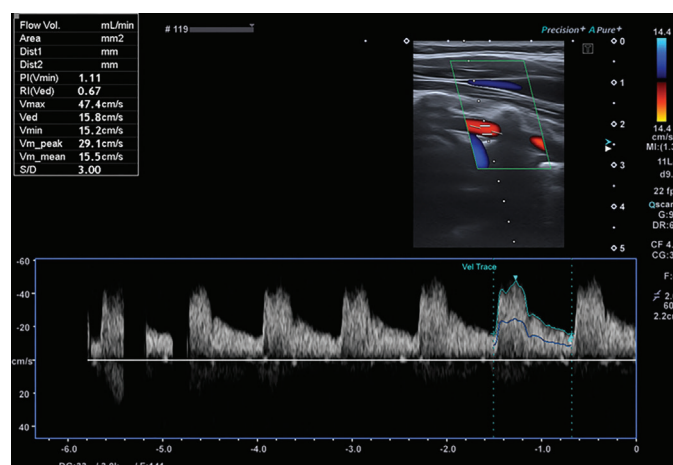


Figure 1. Angle corrected spectral doppler sampling of left vertebral artery with calculation of RI, PI, PSV and EDV.

RI, resistive index; PI, pulsatility index; PSV, peak systolic velocity; EDV, end-diastolic velocity.

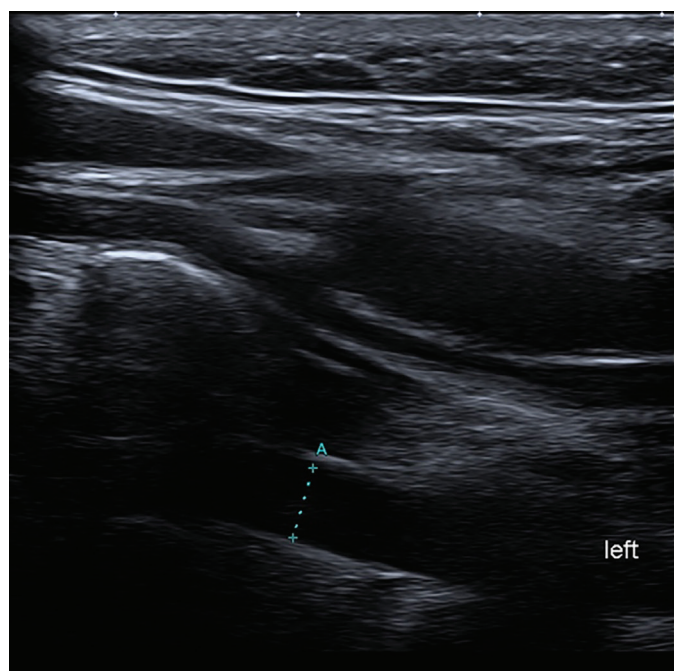


Figure 2. Left vertebral artery diameter measurement.

neck surgery, and 1 for morbid obesity ($\text{BMI} > 40 \text{ kg/m}^2$). A total of 223 healthy adults (140 women, 62.8%; 83 men, 37.2%) from three independent centers were included in the study. The mean ages were 39.4 ± 12.2 years (range 18-72) and mean BMI was $26.9 \pm 4.5 \text{ kg/m}^2$. For one female participant, only age and sex were recorded, and no Doppler or BMI data were available because of a technical problem; therefore, the

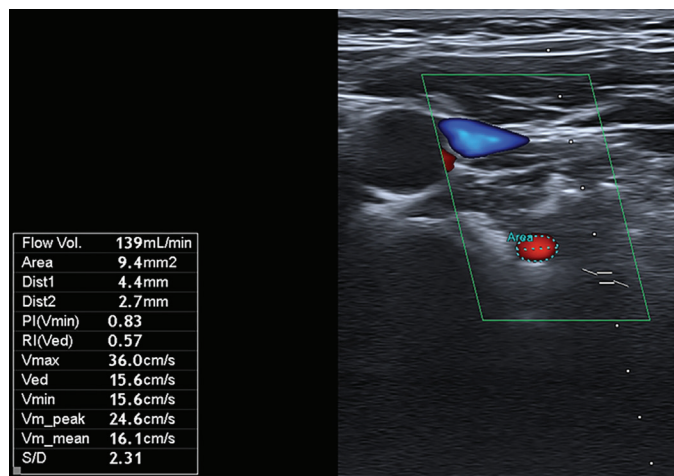


Figure 3. Cross section area (mm^2) measurement of left vertebral artery.

Doppler analyses and reference percentiles were based on 222 participants (139 women, 83 men). In addition, bilateral RI was not recorded in one participant, and right VA diameter was not recorded in another; the two implausible values described in the Methods were set to missing. As a result, left VA RI was available in 221 participants, while right VA diameter and right VA RI were each available in 220 participants. Missing values were not imputed. Participant flow is shown in Figure 4.

There were no statistically significant differences among the three centers in sex distribution ($p = 0.96$) or BMI ($p = 0.78$). A statistically significant difference in age was observed ($p = 0.02$), with an older subgroup at one of the three centers (mean age 42.8 versus 38.0-38.3 years). To assess whether this difference biased the pooled estimates, we performed a sensitivity analysis excluding the older center. The left-sided predominance of all six parameters; the higher right VA diameter and bilateral PI in men; the higher right VA EDV in women; the negative correlations of age with PSV, PI, and left RI; and the negative correlations of BMI with PSV, RI, and left PI remained statistically significant, with direction unchanged. In contrast, the sex difference in RI (left $p = 0.22$, right $p = 0.09$), the correlations of age with right RI ($p = 0.058$) and with EDV (left $p = 0.08$, right $p = 0.15$), and the correlation of BMI with right PI ($p = 0.11$) were no longer significant, although the direction of these associations remained unchanged. The positive correlation between age and left VA diameter was

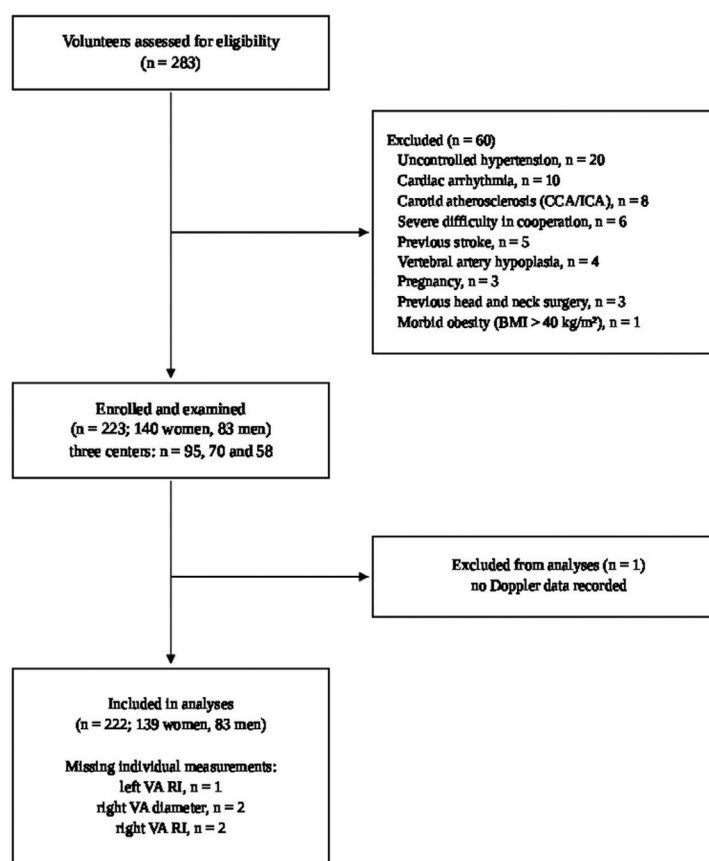


Figure 4. Flow diagram of participant screening, exclusion and inclusion, showing the number of participants available for each analysis.

BMI, body mass index; RI, resistive index; VA, vertebral arterie.

no longer observed after this exclusion ($p = 0.06$, $p = 0.48$) (Supplementary Table S1). The main findings were therefore robust to pooling; however, these weaker associations should be interpreted with caution.

The descriptive statistics for all the Doppler parameters are summarized in Table 1. The left VA had significantly greater diameter, PSV, EDV, and flow volume than the right VA (Table 1). The right VA exhibited significantly higher RI and PI values than the left (Table 1). On average, the left VA diameter was 0.21 mm larger than the right VA diameter (3.73 vs. 3.52 mm), consistent with left-sided predominance.

Right VA diameter differed significantly between male and female participants (median 3.70 mm vs. 3.50 mm, $p = 0.004$; Table 2). There were statistically significant differences between males and females in bilateral RIs and PIs, with higher values in males (Table 2). Female participants had higher right VA EDV than male participants (median 14.25 vs. 13.11 cm/s, $p = 0.037$; Table 2).

Age was negatively correlated with PSV, PI, and RI on both sides (p between -0.13 and -0.30 , all $p < 0.05$), and positively correlated with left VA diameter ($p = 0.19$, $p = 0.006$). No significant correlation was observed between flow volume

and age. BMI correlated negatively with RI, PI, and PSV on both sides, but not with bilateral flow volume (Table 3).

Table 4 shows normal reference percentiles for each parameter, separately for males and females. Fiveth percentile for total flow volume is 124.7 mL/min. Fiveth percentile flow volume values for males and females are 134.96 mL/min and 122 mL/min respectively. Table 5 shows normal reference percentiles for left and right VA by age group.

DISCUSSION

In this prospective study, 223 healthy Turkish adults were examined using VA Doppler ultrasonography to establish a population-specific nomogram. The mean diameters of the VAs were 3.73 ± 0.57 mm for the left VA and 3.52 ± 0.61 mm for the right VA, respectively. Scheel et al.⁴ reported VA diameters ranging from 3.3 ± 0.3 mm to 3.6 ± 0.4 mm. Our measurements of VA diameters are consistent with those found in a study by Yilmaz et al.² which investigated VA Doppler parameters and their correlation with CTA in a Turkish population (right VA diameter 3.4 ± 0.6 mm, left VA diameter 3.7 ± 0.7 mm). In contrast, an Italian twin cohort study by Tarnoki et al.³ reported slightly smaller VA diameters (right VA diameter 3.16 ± 0.77 mm, left VA diameter 3.23 ± 0.77 mm) (Table 6).

Table 1. Vertebral artery Doppler parameters

Parameter	Left VA, mean $\pm$ SD	Right VA, mean $\pm$ SD	P (left vs. right)
Diameter (mm)	3.73 $\pm$ 0.57	3.52 $\pm$ 0.61	<0.001
RI	0.67 $\pm$ 0.08	0.69 $\pm$ 0.08	0.001
PI	1.29 $\pm$ 0.36	1.40 $\pm$ 0.40	0.001
PSV (cm/s)	49.6 $\pm$ 13.9	47.4 $\pm$ 14.2	0.03
EDV (cm/s)	16.4 $\pm$ 6.0	14.5 $\pm$ 6.3	<0.001
FV (mL/min)	126.8 $\pm$ 61.0	107.6 $\pm$ 51.3	<0.001

RI, resistive index; PI, pulsatility index; PSV, peak systolic velocity; EDV, end-diastolic velocity; FV, flow volume; SD, standard deviation; Min, minimum; Max, maximum.

Table 2. Comparison of Doppler parameters between sexes

Parameter	Male, median (IQR)	Female, median (IQR)	P value
BMI (kg/m ²)	25.66 (24.15-27.80)	26.70 (23.40-30.02)	0.20
Left diameter (mm)	3.80 (3.50-4.20)	3.60 (3.30-4.00)	0.08
Left RI	0.69 (0.63-0.74)	0.66 (0.61-0.72)	0.044
Left PI	1.36 (1.08-1.60)	1.21 (1.01-1.38)	0.003
Left PSV (cm/s)	46.10 (41.81-55.78)	46.90 (39.63-57.38)	0.77
Left EDV (cm/s)	15.50 (12.43-18.47)	15.65 (12.60-19.90)	0.25
Left FV (mL/min)	109.00 (79.50-147.50)	117.00 (86.52-162.15)	0.27
Right diameter (mm)	3.70 (3.30-4.20)	3.50 (3.06-3.80)	0.004
Right RI	0.70 (0.67-0.75)	0.69 (0.63-0.73)	0.046
Right PI	1.43 (1.20-1.73)	1.26 (1.10-1.47)	<0.001
Right PSV (cm/s)	45.47 (36.20-51.80)	45.70 (38.15-56.42)	0.34
Right EDV (cm/s)	13.11 (10.62-15.73)	14.25 (11.84-17.76)	0.037
Right FV (mL/min)	100.30 (72.92-124.20)	101.54 (68.71-135.22)	0.62
Total FV (mL/min)	215.60 (174.08-256.50)	222.10 (170.85-297.25)	0.28

Values are medians (interquartile range); P values were obtained with the Mann-Whitney U test. BMI, body mass index; RI, resistive index; PI, pulsatility index; PSV, peak systolic velocity; EDV, end-diastolic velocity; FV, flow volume; IQR, interquartile range; Min, minimum; Max, maximum.

Our RI values for both VAs (left: 0.67 ± 0.08 , right: 0.69 ± 0.08) closely align with those reported by Schöning et al.⁵ (0.66 ± 0.07), Scheel et al.⁴ (0.68 - 0.72), Nemati et al.⁶ (0.69 - 0.71), and Kovacic et al.⁷ (0.70 bilaterally) (Table 6). In contrast, Yılmaz et al.² documented lower RI values of 0.63 bilaterally in a Turkish cohort, which comprised patients exhibiting symptoms of vertebrobasilar insufficiency or anterior circulation issues. The PI values in our study (left: 1.29 ± 0.36 , right: 1.40 ± 0.40) were comparable to those of Schöning et al.⁵ (1.35) and fell within the age-stratified range reported by Scheel et al.⁴ (1.37 - 1.50). Kovacic et al.⁷ reported higher PI values of 1.5 and 1.6 for the left and right VAs, respectively, potentially due to an older study population.⁷ The PSV in our study was 49.6 ± 13.9 cm/s on the left and 47.4 ± 14.2 cm/s on the right side. These PSV values were consistent with those of Kovacic et al.⁷ ($47.2/46.7$ cm/s in women and $44.9/42.3$ cm/s in men) and Scheel et al.⁴ (45 - 52 cm/s across age groups)⁷, but exceeded the values reported by Nemati et al.⁶ (37 - 42 cm/s) and Tarnoki et al.³ (40 - 44 cm/s) (Table 6)^{3,6}. An age-related decline in PSV, as reported by Yazıcı et al.⁸ (from 53 to 48 cm/s), aligns with our correlation analysis ($\rho = -0.20$ to -0.30 , $p < 0.05$).

In our study, the EDV was recorded at 16.4 ± 6.0 cm/s for the left VA and 14.5 ± 6.3 cm/s for the right VA. These results align with those reported by Schöning et al.⁵ (16 cm/s bilaterally), Tarnoki et al.³ (16.5 cm/s for the left and 15.5 cm/s for the right), Scheel et al.⁴ (12 - 17 cm/s), and Yazıcı et al.⁸ (15 - 18 cm/s across various age groups). Conversely, Kovacic et al.⁷ documented lower EDV values, which corresponded with the older population demographic in their study, differing from both our findings and those in the existing literature.⁷ A consistent left-sided predominance has been reported (Table 6). In our study, the VA diameter exhibited a marked left-sided predominance (left 3.73 ± 0.57 mm, right 3.52 ± 0.61 mm), consistent with literature data. Yılmaz et al.² reported left VA dominance (3.7 vs. 3.4 mm) in a Turkish study population, while Kovacic et al.⁷ noted wider left VA diameters in both sexes.⁷ Yazıcı et al.⁸ found no significant side-to-side difference in VA diameter (3.6 ± 0.4 mm in men and 3.5 ± 0.4 mm in women). The right VA

diameter in our study was similar to that reported by Yazıcı et al.,⁸ at 3.52 mm. The left-side predominance observed in our study likely reflects the larger sample size, providing sufficient power to detect this difference compared to that in Yazıcı et al.⁸ The bilateral average VA diameter was 3.16 - 3.23 mm in the twin study by Tarnoki et al.³ which was smaller than that in our study. The authors reported moderate heritability for variation in VA diameter. Our VA diameters were also slightly larger than those reported by Nemati et al.⁶ (3.25 - 3.42 mm, Iranian study population, Table 6).⁶ These results may indicate genetic and anthropometric differences between populations. Scheel et al.⁴ and Yazıcı et al.⁸ reported age-related VA dilation. We observed a positive correlation between age and left VA diameter ($\rho = 0.19$, $p = 0.006$), but not between age and right VA diameter ($\rho = 0.04$, $p = 0.57$). This asymmetry may reflect the left VAs larger baseline diameter and greater hemodynamic load, leading to more pronounced age-related remodeling. Alternatively, it may be a chance finding, given the modest correlation coefficient. Further studies are required to confirm this side-specific effect. Notably, the absence of this correlation after excluding the center with older participants ($\rho = 0.06$, $p = 0.48$; Supplementary Table S1) supports the possibility of a chance or center-related finding.

In our study, the flow volume (left: 126.8 ± 61.0 mL/min, right: 107.6 ± 51.3 mL/min; total: 234.4 mL/min) was significantly higher than the values reported by Schöning et al.⁵ (approximately 85 mL/min per side; total: 171 mL/min), Scheel et al.⁴ (74 - 87 mL/min per side), and Yazıcı et al.⁸ (approximately 85.8 mL/min per side; total: 171 mL/min). Yılmaz et al.² reported a left VA flow volume of 97.6 mL/min and a right VA flow volume of 78.6 mL/min, which are also lower than our findings (Table 6). Nemati et al.⁶ observed a flow volume of 117 - 130 mL/min per side without significant age-related differences, which aligns with our results, as we also found no significant correlation between flow volume and age on either side.⁶ Yazıcı et al.⁸ compared participants aged 20 - 50 years with those aged 51 - 80 years and identified an age-related decrease in VA flow volume (86 vs. 77 mL/min per side); however, this difference

Table 3. Spearman correlations with age and BMI

Parameter	ρ (Age)	p (Age)	ρ (BMI)	p (BMI)
Left diameter (mm)	0.19	0.006	0.08	0.23
Left RI	-0.15	0.02	-0.18	0.006
Left PI	-0.18	0.006	-0.19	0.005
Left PSV (cm/s)	-0.30	<0.001	-0.30	<0.001
Left EDV (cm/s)	-0.15	0.03	-0.11	0.10
Left FV (mL/min)	-0.05	0.43	-0.02	0.80
Right diameter (mm)	0.04	0.57	0.09	0.18
Right RI	-0.13	0.049	-0.18	0.008
Right PI	-0.18	0.008	-0.14	0.03
Right PSV (cm/s)	-0.20	0.002	-0.24	<0.001
Right EDV (cm/s)	-0.14	0.038	-0.12	0.07
Right FV (mL/min)	-0.04	0.56	-0.04	0.55
Total FV (mL/min)	-0.04	0.57	-0.03	0.67

RI, resistive index; PI, pulsatility index; PSV, peak systolic velocity; EDV, end-diastolic velocity; FV, flow volume; Min, minimum; Max, maximum.

is numerically small. The relative stability of VA flow volume is attributed to the preserved metabolic demand of the brainstem compared with that of the cerebral cortex.

Males exhibited a significantly larger right VA diameter (median 3.70 mm vs. 3.50 mm, $p = 0.004$) and RI and PI indices bilaterally, as shown in Table 2. In contrast, female participants exhibited a higher right VA EDV ($P = 0.037$). No statistically significant differences were observed in the PSV and flow volume between the sexes. Consistent with our findings, Scheel et al.⁴ and Schöning et al.⁵ reported no sex differences in flow volume. Similarly, Yazıcı et al.⁸ found no differences in VA diameter or flow volume between males and females, although they did report higher EDV in females, aligning with our results. Kovacic et al.⁷ identified higher velocities in females up to 60 years of age and suggested a correlation between increased velocity and reduced arterial stiffness in premenopausal women.⁷ Kovacic et al.⁷ found higher PSV and EDV in premenopausal women.

The elevated EDV values in our female participants support Kovacic et al.'s⁷ finding of higher velocities in premenopausal women, possibly reflecting reduced arterial stiffness. However, our study did not consider the menopausal status or hormonal factors of female participants; therefore, this interpretation remains speculative. Future studies should stratify participants by menopausal status to test this hypothesis.

Our study is the first in the literature to include BMI as a possible covariate in VA Doppler evaluation.

Interestingly, while BMI was negatively correlated with PSV, RI, and PI, it was not correlated with flow volume. This suggests that BMI affects vascular resistance and pulsatility without altering overall perfusion, possibly due to compensatory changes in vessel diameter or collateral flow. Another reason for the lack of correlation can be the relatively narrow BMI range in our sample (mean 26.9 ± 4.5 kg/m², with morbid obesity excluded). This finding requires further investigation to confirm its validity.

Table 4. Reference percentiles by sex

Parameter	Sex	n	Mean $\pm$ SD	Median	P5	P95
Left diameter (mm)	Female	139	3.68 ± 0.57	3.60	2.88	4.60
	Male	83	3.80 ± 0.57	3.80	2.90	4.60
Left RI	Female	139	0.67 ± 0.07	0.66	0.55	0.80
	Male	82	0.69 ± 0.09	0.69	0.53	0.81
Left PI	Female	139	1.23 ± 0.32	1.21	0.82	1.89
	Male	83	1.39 ± 0.41	1.36	0.81	2.12
Left PSV (cm/s)	Female	139	49.30 ± 13.54	46.90	33.36	71.03
	Male	83	50.13 ± 14.59	46.10	32.59	75.82
Left EDV (cm/s)	Female	139	16.64 ± 5.56	15.65	9.69	25.58
	Male	83	15.91 ± 6.56	15.50	9.33	24.29
Left FV (mL/min)	Female	139	130.50 ± 62.79	117.00	54.31	230.10
	Male	83	120.59 ± 57.69	109.00	56.99	235.00
Right diameter (mm)	Female	137	3.42 ± 0.56	3.50	2.50	4.23
	Male	83	3.68 ± 0.65	3.70	2.52	4.69
Right RI	Female	138	0.68 ± 0.08	0.69	0.55	0.80
	Male	82	0.71 ± 0.08	0.70	0.60	0.84
Right PI	Female	139	1.33 ± 0.37	1.26	0.85	1.98
	Male	83	1.51 ± 0.43	1.43	1.01	2.14
Right PSV (cm/s)	Female	139	48.17 ± 14.44	45.70	27.44	76.58
	Male	83	46.18 ± 13.72	45.47	27.32	69.12
Right EDV (cm/s)	Female	139	15.21 ± 7.04	14.25	7.57	24.57
	Male	83	13.39 ± 4.63	13.11	5.76	19.90
Right FV (mL/min)	Female	139	109.46 ± 54.04	101.54	32.80	202.10
	Male	83	104.39 ± 46.47	100.30	46.47	178.90
Total FV (mL/min)	Female	139	239.96 ± 89.67	222.10	122.00	407.20
	Male	83	224.98 ± 72.37	215.60	134.96	360.70
Total FV (mL/min)	Overall	222	234.4 ± 83.6	218.2	124.7	391.8

P5 and P95 are non-parametric (empirical) percentiles calculated with linear interpolation; 90% confidence intervals for the overall and sex-specific limits are given in Supplementary Table S2. N is the number of participants with a valid measurement (see Results). RI, resistive index; PI, pulsatility index; PSV, peak systolic velocity; EDV, end-diastolic velocity; FV, flow volume; Min, minimum; Max, maximum.

Table 5. Reference percentiles by age group

Parameter	Age group	n	Mean $\pm$ SD	Median	P5	P95
Left diameter (mm)	18-29	59	3.53 $\pm$ 0.50	3.50	2.78	4.23
	30-39	63	3.84 $\pm$ 0.52	3.90	3.00	4.60
	40-49	53	3.67 $\pm$ 0.63	3.60	2.62	4.55
	50-59	30	3.84 $\pm$ 0.64	3.86	3.00	4.72
	60+	17	3.96 $\pm$ 0.55	3.90	3.20	4.91
Left RI	18-29	59	0.70 $\pm$ 0.07	0.70	0.60	0.80
	30-39	63	0.66 $\pm$ 0.08	0.67	0.54	0.79
	40-49	52	0.65 $\pm$ 0.08	0.64	0.55	0.79
	50-59	30	0.67 $\pm$ 0.08	0.67	0.55	0.81
	60+	17	0.70 $\pm$ 0.08	0.70	0.60	0.82
Left PI	18-29	59	1.44 $\pm$ 0.34	1.37	1.00	2.07
	30-39	63	1.27 $\pm$ 0.35	1.27	0.83	1.84
	40-49	53	1.13 $\pm$ 0.33	1.06	0.80	1.76
	50-59	30	1.25 $\pm$ 0.35	1.18	0.81	1.88
	60+	17	1.43 $\pm$ 0.31	1.41	0.94	1.87
Left PSV (cm/s)	18-29	59	55.70 $\pm$ 16.12	52.30	37.73	90.88
	30-39	63	49.10 $\pm$ 10.96	46.80	35.43	66.79
	40-49	53	46.91 $\pm$ 13.01	43.00	32.58	71.65
	50-59	30	45.84 $\pm$ 11.18	42.77	32.85	67.78
	60+	17	45.47 $\pm$ 16.82	44.76	26.29	81.89
Left EDV (cm/s)	18-29	59	16.20 $\pm$ 4.69	15.80	10.66	22.94
	30-39	63	17.54 $\pm$ 7.36	16.40	10.13	25.47
	40-49	53	16.40 $\pm$ 5.01	15.65	9.22	24.73
	50-59	30	15.21 $\pm$ 5.15	14.80	8.82	25.51
	60+	17	14.54 $\pm$ 7.65	12.08	9.65	25.16
Left FV (mL/min)	18-29	59	129.29 $\pm$ 69.65	109.00	54.58	245.40
	30-39	63	125.22 $\pm$ 55.99	113.00	54.93	216.20
	40-49	53	129.14 $\pm$ 53.89	122.00	69.32	228.60
	50-59	30	126.96 $\pm$ 67.86	113.00	53.74	236.65
	60+	17	116.36 $\pm$ 60.86	103.50	60.60	220.60
Right diameter (mm)	18-29	59	3.53 $\pm$ 0.50	3.50	2.59	4.31
	30-39	62	3.50 $\pm$ 0.69	3.60	2.31	4.57
	40-49	53	3.46 $\pm$ 0.64	3.50	2.44	4.59
	50-59	30	3.49 $\pm$ 0.56	3.59	2.54	4.17
	60+	16	3.82 $\pm$ 0.62	3.88	2.95	4.62
Right RI	18-29	59	0.70 $\pm$ 0.06	0.71	0.61	0.80
	30-39	63	0.71 $\pm$ 0.10	0.72	0.56	0.82
	40-49	52	0.68 $\pm$ 0.08	0.67	0.55	0.82
	50-59	29	0.68 $\pm$ 0.08	0.67	0.57	0.81
	60+	17	0.69 $\pm$ 0.07	0.70	0.56	0.77
Right PI	18-29	59	1.44 $\pm$ 0.31	1.40	1.01	2.12
	30-39	63	1.47 $\pm$ 0.47	1.37	0.90	2.04
	40-49	53	1.35 $\pm$ 0.43	1.21	0.85	2.15
	50-59	30	1.26 $\pm$ 0.33	1.21	0.86	1.87
	60+	17	1.38 $\pm$ 0.36	1.28	1.01	1.88

Table 5. Continued

Parameter	Age group	n	Mean ± SD	Median	P5	P95
Right PSV (cm/s)	18-29	59	51.05 ± 13.66	48.22	35.33	80.17
	30-39	63	49.06 ± 15.61	45.70	27.31	80.78
	40-49	53	44.85 ± 13.47	44.76	24.53	69.43
	50-59	30	42.66 ± 13.06	38.60	26.94	67.05
	60+	17	45.21 ± 11.36	41.92	32.69	65.63
Right EDV (cm/s)	18-29	59	15.99 ± 8.47	14.25	9.74	21.84
	30-39	63	14.17 ± 5.60	13.50	5.70	22.71
	40-49	53	14.35 ± 5.39	13.90	6.32	24.39
	50-59	30	13.74 ± 5.33	12.60	6.45	21.23
	60+	17	12.78 ± 2.71	12.08	9.31	16.75
Right FV (mL/min)	18-29	59	109.99 ± 44.11	107.00	46.11	185.40
	30-39	63	103.03 ± 47.10	98.43	32.88	187.81
	40-49	53	108.29 ± 55.38	102.00	30.28	215.00
	50-59	30	109.82 ± 57.72	98.30	42.10	212.45
	60+	17	109.70 ± 67.55	88.00	43.40	208.20
Total FV (mL/min)	18-29	59	239.29 ± 93.48	218.44	120.85	399.10
	30-39	63	228.26 ± 73.67	227.00	133.68	355.40
	40-49	53	237.43 ± 76.87	219.00	139.32	395.40
	50-59	30	236.78 ± 84.48	224.70	134.28	377.85
	60+	17	226.05 ± 108.24	207.67	111.10	404.80

Percentile estimates for the 50-59 (n=29-30) and ≥60 (n=16-17) age groups are based on small samples and are imprecise, particularly at the 5th and 95th percentiles (with n < 20, P5 and P95 approximate the sample minimum and maximum); they should be interpreted with caution.
RI, resistive index; PI, pulsatility index; PSV, peak systolic velocity; EDV, end-diastolic velocity; FV, flow volume.

An important finding of our study is 5th percentile overall and sex-specific flow volume (124.7 mL/min overall, 134.96 mL/min males, 122.0 mL/min females). An arbitrary threshold value of 200 mL/min has been proposed for VA volume in evaluating vertebrobasilar insufficiency.⁹ However 5th- 95th percentiles of VA flow volume in healthy individuals were reported as 102.4-301.0 mL/min in the study by Seidel et al.¹⁰ This study questions the utility of the 200 mL/min threshold for the diagnosis of vertebrobasilar insufficiency. The lower bound for normal VA flow volume in our study is higher than that reported by Seidel et al.¹⁰ This difference is most likely due to our younger cohort compared to Seidel et al.¹⁰ (mean age 39.4 versus 55.8). In a study by Lee et al.,¹¹ the association between VA flow volume and stroke risk was investigated. Almost all the patients (93.6%) in the control group (n=235) had a VA flow volume larger than 100 mL/min. The mean age for the control group was 63.5, which was significantly older than that in our study. This difference can be explained by an age-related decline in VA flow volume. Studies, including ours, that aim to determine the lower limit of normal for VA flow volume have clearly shown values below 200 mL/min, the proposed threshold used to diagnose vertebrobasilar insufficiency. Although there are differences, literature data indicate that the 5th percentile value for VA flow is between 100-125 mL/min.⁹⁻¹¹

Study Limitations

This study has several limitations. First, one center enrolled an older subgroup (mean age, 42.8 vs. 38.0-38.3 years), which may have introduced heterogeneity. A sensitivity analysis excluding this center confirmed the main findings; however, the weaker associations (the sex difference in RI and the correlation between age and left VA diameter) were no longer significant. Second, we excluded individuals with BMI >40 kg/m², limiting the generalizability of our BMI findings across the full obesity spectrum. Third, we did not validate our Doppler measurements against an independent imaging modality (e.g., CTA or MRA); therefore, the measurement accuracy relative to the true vessel dimensions is unknown. Fourth, inter-rater and intra-rater reliability were not formally assessed, although all examiners were experienced and followed a standardized protocol. Fifth, the cross-sectional design precludes causal inference about the effects of age or BMI on VA hemodynamics; longitudinal studies are needed to confirm these associations. Sixth, heart rate and blood pressure were not recorded at the time of the examination. Because heart rate strongly influences RI and PI, differences in heart rate may have contributed to the observed sex- and age-related differences in these indices. Seventh, some age strata were small (n=16-17 in the ≥60 group and

Table 6. Comparison of vertebral artery reference values across published studies

Study	Population	Diameter L/R (mm)	RI L/R	PI L/R	PSV L/R (cm/s)	EDV L/R (cm/s)	Flow volume L/R (mL/min)
Present study	Turkish, n=223 healthy, 3 centers pooled, mean age 39.4y	3.73 / 3.52	0.67 / 0.69	1.29 / 1.40	49.6 / 47.4	16.4 / 14.5	126.8 / 107.6 (total 234.4)
Schöning et al. ⁵ 1994	German, n=48 healthy, mean age 35y	NR / NR	0.66 / 0.66	1.35 / 1.35	48 / 48	16 / 16	85 / 85 (total 171)
Scheel et al. ⁴ 2000	German, n=78 healthy, age 20–85y, 3 age groups	3.3–3.6 / 3.3–3.6	0.68–0.72 / 0.68–0.72	1.37–1.50 / 1.37–1.50	45–52 / 45–52	12–17 / 12–17	74–87 / 74–87
Nemati et al. ⁶ 2009	Iranian, n=96 healthy, age 23–95y	3.25–3.42 / 3.25–3.42	0.69–0.71 / 0.69–0.71	NR	37–42 / 37–42	NR	117–119 / 101–130
Yılmaz et al. ² 2021	Turkish, n=77, mean age 53.6y	3.7 / 3.4	0.63 / 0.63	NR	24.2 / 24.3	8.9 / 8.9	97.6 / 78.6
Tarnoki et al. ³ 2017	Italian twins, n=172, mean age 50y	3.23 / 3.16	NR	NR	44.0 / 40.9	16.5 / 15.5	NR
Kovacic et al. ⁷ 2022	Croatian, n=227 healthy, age 30–86y	3.6 / 3.4 (F); 3.9 / 3.6 (M)	0.7 / 0.8	1.5 / 1.6	47.2 / 46.7 (F); 44.9 / 42.3 (M)	12.0 / 11.5 (F); 10.9 / 10.4 (M)	NR
Yazıcı et al. ⁸ 2005	Turkish, n=96 healthy, age 20–80y, 2 age groups	3.5–3.6/3.5–3.6	0.65–0.68/ 0.65–0.68	NR	48–53 / 48–53	15–18 / 15–18	85.8 / 85.8 (total 171.6)

L/R, left / right; NR, not reported; RI, resistive index; PI, pulsatility index; PSV, peak systolic velocity; EDV, end-diastolic velocity; Min, minimum.

n=29–30 in the 50–59 group), so the 5th and 95th percentile estimates for these groups are imprecise and should be applied with caution until confirmed in larger samples. Our findings are specific to a Turkish population and may not generalize to other ethnic or geographic groups.

CONCLUSION

Our reference values for VA Doppler measurements broadly align with existing literature for diameter, PI, RI, and EDV. However, our values for PSV and flow volume are at the higher end of the published range, likely due to the younger mean age and the community-based, healthy-volunteer study design. Compared to prior Turkish studies (e.g., Yazıcı et al.,⁸ n=96), our multicenter sample of 223 participants provides greater statistical power to detect sex and side differences and enables the derivation of age- and sex-stratified percentile norms, which were not available in earlier single-center datasets. This study provides population-specific normative reference values for six VA Doppler parameters in 223 healthy Turkish adults, stratified by sex and age—the first such dataset to incorporate BMI as a covariate and to derive percentile-based norms from a multicenter Turkish cohort. Notably, the lower limit of normal for total VA flow volume (124.7 mL/min) is well below the commonly cited 200 mL/min threshold, which should therefore not be used on its own to diagnose vertebrobasilar insufficiency.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained from Tekirdağ İsmail Fehmi Cumalıoğlu City Hospital Clinical Research Ethics Committee (approval no.: AN-262105-45, date: 21.05.2026).

Informed Consent: Written informed consent was obtained from the participants.

Footnotes

Author Contributions

Concept Design – A.T.A., Data Collection or Processing – A.T.A., M.F.B., S.A.A., Analysis or Interpretation – A.T.A., Literature Review – M.F.B., S.A.A., Writing, Reviewing and Editing – A.T.A.

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Longitudinal Optical Coherence Tomography Angiography Assessment of Retinal Microvascular Changes in Patients Receiving Oral Isotretinoin

Ahmet Mehmet Somuncu¹, Erel İçel², Müge Göre Karaali³

¹Clinic of Ophthalmology, University of Health Sciences Türkiye, Trabzon Kanuni Training and Research Hospital, Trabzon, Türkiye

²Clinic of Ophthalmology, University of Health Sciences Türkiye, İzmir City Hospital, İzmir, Türkiye

³Dermatology Specialist, Private Practice, Tekirdağ, Türkiye

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ORCID IDs of the authors: A.M.S. 0009-0008-0304-5278, E.İ. 0000-0001-7150-5581, M.G.K. 0000-0003-0645-0662.

ABSTRACT

Objective: To determine whether oral isotretinoin therapy is associated with longitudinal changes in retinal structure and microvasculature measured by optical coherence tomography angiography (OCTA).

Methods: This prospective, longitudinal, bilateral-eye study was conducted at an anonymized Erzincan Binali Yıldırım University Mengücek Gazi Training and Research Hospital between 01 June 2021 and 01 June 2022. Fifty-four patients receiving oral isotretinoin for acne were enrolled; of these, 34 patients (68 eyes) completed baseline, month 1, month 3, and month 6 visits, forming the complete-case sample. All quantitative optical coherence tomography (OCT)/OCTA examinations were performed on the Nidek retinopathy study (RS)-3000 advance platform after topical tropicamide-induced dilation. Acquisitions were repeated until the image-quality index was at least 0.70; scans that did not reach this threshold or had clinically relevant artifacts were excluded. Longitudinal change was analyzed with patient-clustered Gaussian generalized estimating equations, adjusted for eye laterality, age, and sex. Time was modeled categorically. Endpoint-level multiplicity was controlled using the Benjamini–Hochberg false-discovery rate (FDR) procedure, and pairwise contrasts were assessed using the Holm procedure and a global FDR correction.

Results: The analytic dataset included 34 patients, 68 eyes, and 272 eye-time observations. Mean age was 20.71 ± 3.33 years; 22 patients (64.7%) were women and 12 (35.3%) were men. No OCT or OCTA endpoint showed an FDR-confirmed time effect; no significant change was detected in any parameter. The lowest omnibus time-effect P value was observed for inner-nasal Early Treatment Diabetic Retinopathy Study 9 (ETDRS9) perfusion (Wald $\chi^2 = 9.786$, $df = 3$, $p = 0.0205$), but this finding did not survive FDR adjustment ($q = 0.5284$). The smallest pairwise contrast was the inner-temporal ETDRS9 perfusion between months 6 and 1 (difference = 1.338, SE = 0.532, $p = 0.0119$), which was not significant after the within-endpoint Holm correction ($p = 0.0715$) or the global FDR correction ($q = 0.5934$). Patient-averaged models, non-parametric repeated-measures tests, and time-by-eye interaction models were consistent with the primary analysis.

Conclusion: Over 6 months of oral isotretinoin therapy, no retinal structural or OCTA microvascular parameters exhibited changes that remained significant after correction for multiple testing. Small nominal fluctuations should be interpreted as exploratory signals rather than confirmatory evidence of retinal microvascular toxicity.

Keywords: Acne vulgaris, isotretinoin, optical coherence tomography angiography, OCTA, retinal microvasculature, foveal avascular zone, generalized estimating equations



Corresponding author: Ahmet Mehmet Somuncu, **E-mail:** mehmetmehmet89@hotmail.com

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INTRODUCTION

Oral isotretinoin remains a mainstay of systemic treatment for severe, scarring, or treatment-resistant acne vulgaris.¹ Its mucocutaneous and anterior-segment ocular adverse effects are well recognized, but retinal and microvascular effects are less clearly defined.² Quantitative optical coherence tomography (OCT) and OCT angiography (OCTA) allow non-invasive assessment of retinal structure and perfusion that may not be evident on routine clinical examination.^{3,4}

OCTA can quantify the retinal and choroidal microvasculature without dye injection. However, OCTA metrics are sensitive to acquisition quality, eye movement, segmentation errors, projection artifacts, and device-specific processing algorithms.^{5,6} For longitudinal studies of systemic medications, strict image-quality control and statistical modeling of repeated bilateral-eye data are essential.

Published OCT studies of isotretinoin have been inconsistent. Several studies reported no meaningful short-term change in central macular thickness, choroidal thickness, or retinal nerve fiber layer measurements,^{7–9} whereas other work described choroidal vascular changes in acne patients under isotretinoin therapy.¹⁰ Dedicated longitudinal OCTA data during treatment remain limited.¹¹ We therefore evaluated retinal structural outcomes and OCTA perfusion outcomes over 6 months of oral isotretinoin therapy using patient-clustered longitudinal models and multiplicity-adjusted interpretation.

MATERIALS AND METHODS

Study Design and Setting

This prospective longitudinal bilateral-eye study was conducted at Erzincan Binali Yıldırım University Mengücek Gazi Training and Research Hospital between 01 June 2021 and 01 June 2022. Patients receiving oral isotretinoin for acne vulgaris underwent bilateral OCT/OCTA at baseline and at months 1, 3, and 6 of treatment. Visits were scheduled relative to the first isotretinoin dose and accepted within a window of ± 7 days for

month 1 and ± 14 days for months 3 and 6. Of the 54 enrolled patients, 20 did not complete all scheduled visits and were excluded from the complete-case analysis. The final analytic dataset therefore comprised 34 patients, 68 eyes, and 272 eye-time observations. Reporting followed STROBE principles where applicable.¹²

Eligibility Criteria

Eligible patients were aged 16 years or older, had acne vulgaris for which oral isotretinoin had been prescribed by the dermatology department as part of routine care, and were able to undergo repeated dilated imaging. Patients were excluded if they had any prior ocular surgery or laser treatment, glaucoma or ocular hypertension, uveitis or other intraocular inflammation, retinal or optic nerve disease, corneal or lens opacity sufficient to degrade image quality, diabetes mellitus, hypertension or any other systemic disease known to affect retinal microvasculature, or use of systemic vasoactive or antiangiogenic medications. Patients who had previously received isotretinoin and those from whom imaging of adequate quality could not be obtained at baseline were also excluded. Enrollment was consecutive among patients meeting these criteria during the study period.

Isotretinoin Exposure

The dermatology department prescribed and monitored isotretinoin according to the standard institutional regimen; dosing was not modified for this study. Treatment began at 0.5 mg/kg/day and was raised, after several weeks and according to tolerance, to between 0.5 and 1.0 mg/kg/day, with a target cumulative dose of 120 to 150 mg/kg and a usual course length of 16 to 24 weeks. Individual daily and cumulative doses were not recorded in the analytic dataset. Exposure is therefore described at the level of the prescribing regimen rather than as a measured per-patient variable; consequently, no dose-response analysis was possible, which is acknowledged as a limitation. Because the usual course is shorter than the observation period, some patients are likely to have completed or to be completing treatment by the month-6 visit, so that the visit reflects the end of the treatment course or shortly afterwards rather than mid-treatment.

Ethical Approval

The study was approved by the Clinical Research Ethics Committee of Erzincan Binali Yıldırım University (meeting date: 26.04.2021, meeting number: 06, decision number: 06/05, official document number: E-21142744-804.99-77544, 04.05.2021). The approved protocol title concerned retinal microvascular changes in patients receiving oral isotretinoin. The study was planned prospectively and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants before enrollment. All authors worked at this university's hospital during the study.

Ophthalmic Imaging Protocol

All imaging was performed by a single experienced operator on the Nidek RS-3000 Advance platform (Nidek Co., Ltd., Gamagori, Japan) using NAVIS-EX software version 1.4.0.1. Pupils were dilated with topical tropicamide 1% before each

MAIN POINTS

- Whether oral isotretinoin affects the retina and its microvasculature is still unclear, and longitudinal optical coherence tomography angiography (OCTA) data collected during treatment remain scarce.
- Over 6 months of treatment in 34 patients (68 eyes), no structural or microvascular endpoint on optical coherence tomography or OCTA showed a significant change after correction for multiple testing.
- Patient-clustered generalized estimating equations that account for both eyes and repeated visits, combined with false discovery rate and Holm correction, guard against inflated significance that can arise when many correlated imaging metrics are tested simultaneously.
- The few nominal fluctuations observed are best interpreted as exploratory signals rather than evidence of isotretinoin-related retinal microvascular toxicity.

session, and scanning began after adequate mydriasis. To limit diurnal variation, each patient was imaged at approximately the same time of day at every visit. Structural OCT used the device's macular map protocol centred on the fovea, from which Early Treatment Diabetic Retinopathy Study (ETDRS) sectoral retinal thickness, central macular thickness, and ganglion cell measurements were exported. OCTA was performed using a 6×6 mm macular scan centred on the fovea. Perfusion and foveal avascular zone (FAZ) metrics were derived from the superficial retinal capillary plexus, which was segmented by the device as the layer between the internal limiting membrane and the posterior boundary of the inner plexiform layer; the manufacturer's default projection-artifact removal was applied. Automatic segmentation was reviewed for every scan, and scans with segmentation errors were excluded rather than being manually corrected. All values used in this report are device-derived outputs; no third-party or custom software was used for quantification.

Image-Quality Control

Each acquisition was reviewed for signal quality, motion artifacts, segmentation reliability, and other clinically relevant artifacts. Scans were repeated until the image-quality index was at least 0.70. Images with a quality index below 0.70, those that could not reach this threshold despite repeated acquisition, or those with clinically relevant artifacts or unreliable segmentation were excluded.

Outcomes

The outcomes were the device-derived OCT and OCTA parameters, defined as follows: Superior and inferior ganglion cell measurements are the mean ganglion cell complex thicknesses in the corresponding hemifields, expressed in micrometers. FAZ area (mm^2) and FAZ perimeter (mm) denote the area and boundary length of the device-delineated avascular zone in the superficial capillary plexus. The FAZ circularity index is the dimensionless ratio $4\pi A/P^2$, which equals 1 for a perfect circle and decreases as the boundary becomes more irregular. Central macular thickness and the ETDRS sectoral retinal thickness values are full-thickness retinal measurements within the standard ETDRS subfields, expressed in micrometers. ETDRS9 sectoral perfusion is the device-reported vessel area density within each ETDRS subfield of the superficial capillary plexus, expressed as the percentage of the subfield area occupied by the flow signal. Twenty-seven such endpoints were analysed. Because no single primary OCTA endpoint was prespecified before analysis, the OCT/OCTA variables were treated as an exploratory outcome family and were interpreted primarily using multiplicity-adjusted results.

Statistical Analysis

Continuous variables are reported as mean $\pm$ standard deviation with median and interquartile range (IQR), and categorical variables as counts and percentages. The main longitudinal analysis used Gaussian generalized estimating equation (GEE) with patient-level clustering to account for both eyes of each patient and repeated visits.¹³ An exchangeable working correlation structure was specified, and robust sandwich

standard errors were used. Time was entered as a categorical factor with baseline as the reference, and models were adjusted for eye laterality, age, and sex. These three covariates were prespecified on structural rather than empirical grounds: laterality, because the unit of analysis is the eye and both eyes of each patient contribute, so a systematic right-left offset would otherwise enter the residual variance; and age and sex, because both are established determinants of macular thickness, ganglion cell thickness, and OCTA perfusion density in healthy eyes, and adjusting for them reduces residual variance and thereby sharpens the time contrasts. None of the three is on the causal path between isotretinoin exposure and retinal outcome; therefore, their inclusion cannot bias the time effect. The systemic disease variable was not included because it was constant across the analytic cohort. Overall time effects were tested using Wald tests with 3 degrees of freedom. Endpoint-level multiplicity across the 27 endpoints was controlled with the Benjamini–Hochberg FDR.¹⁴ Pairwise time contrasts, 6 per endpoint and 162 in total, were corrected within each endpoint with the Holm¹⁵ method and across all endpoint-contrast combinations with a global FDR. Sensitivity analyses included patient-averaged GEE models, patient-averaged Friedman tests, and GEE models with time-by-eye interaction terms. Analyses were performed in Python using the statsmodels and SciPy libraries; an equivalent IBM SPSS Statistics GENLIN/GEE syntax was prepared for local verification of the reported models.

RESULTS

Study Population and Data Completeness

Of the 54 patients initially enrolled and assessed at baseline, 20 did not complete all four scheduled visits and were excluded from the complete-case analysis (Figure 1). All 20 were lost to follow-up because they did not attend one or more

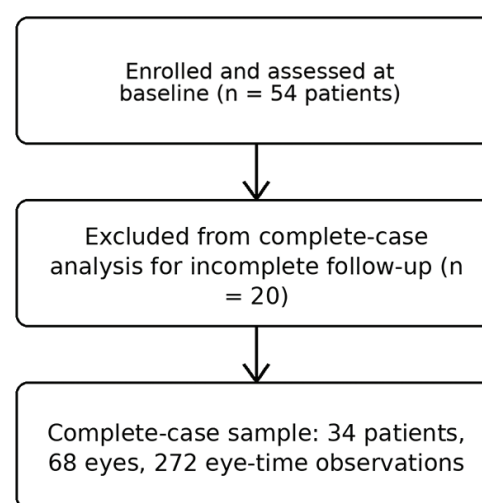


Figure 1. Flow of participants through the study. Of 54 patients enrolled and assessed at baseline, 20 were excluded from the complete-case analysis because they did not attend all four scheduled visits. The final analytic sample included 34 patients, 68 eyes, and 272 eye-time observations.

scheduled visits; no further reason was documented. None were withdrawn by the investigators, and none discontinued because of an ocular adverse event or any other ocular finding. The final dataset included 34 patients who completed the baseline, month 1, month 3, and month 6 visits, corresponding to 68 eyes and 272 eye-time observations, with no missing visits. Mean age was 20.71 ± 3.33 years, with a median of 20.5 years (IQR, 18.0–23.8). Of the patients, 22 (64.7%) were female and 12 (35.3%) were male. No additional systemic disease was recorded in the analytic dataset, and baseline laterality was balanced, with 34 right and 34 left eyes included (Table 1).

Descriptive OCT/OCTA Trends

Descriptive values changed little across the four visits. Selected structural, FAZ, and perfusion parameters are summarized in Table 2 and should be interpreted in conjunction with the clustered longitudinal models.

Primary Longitudinal GEE Analysis

In the primary patient-clustered GEE analysis, no endpoint remained significant after FDR correction; i.e., no significant change over time was detected for any endpoint. The smallest omnibus time-effect P value was observed for inner-nasal ETDRS9 perfusion (Wald $\chi^2 = 9.786$, $df = 3$, $P = 0.0205$), which did not survive FDR adjustment ($q = 0.5284$) (Table 3).

Exploratory Pairwise Time Contrasts

A small number of pairwise comparisons crossed the nominal 0.05 threshold, but none met confirmatory criteria after Holm correction within the endpoint or after global FDR correction across endpoints. The strongest nominal contrast was inner-temporal ETDRS9 perfusion between months 1 and 6 (difference = 1.338, $SE = 0.532$, $P = 0.0119$; Holm $P = 0.0715$; global FDR $q = 0.5934$) (Table 4).

Table 1. Study Characteristics and Data Structure

Characteristic	Value
Study design	Prospective longitudinal bilateral-eye study
Study site	Erzincan Binali Yıldırım University Mengücek Gazi Training and Research Hospital
Study period	01 June 2021 to 01 June 2022
Patients enrolled, n	54
Patients analyzed (completed all visits), n	34
Eyes, n	68
Eye-time observations, n	272
Scheduled visits	Baseline, month 1, month 3, month 6
Age, mean $\pm$ SD	20.71 ± 3.33 years
Age, median (IQR)	20.5 (18.0–23.8) years
Female/male, n (%)	22 (64.7%) / 12 (35.3%)
Additional systemic disease	None recorded in the analytic dataset
Baseline laterality	34 OD and 34 OS eyes
Image quality rule	OCT/OCTA images with quality index <0.70 were excluded

SD, standard deviation; IQR, interquartile range; OD, oculus dexter; OS, oculus sinister; OCT, optical coherence tomography; OCTA, optical coherence tomography angiography.

Table 2. Selected OCT/OCTA Parameters Across Follow-up (Eye-Level Mean $\pm$ SD)

Endpoint	Baseline	Month 1	Month 3	Month 6
Superior ganglion cell thickness (μm)	109.16 ± 5.63	109.50 ± 5.40	109.01 ± 6.24	109.10 ± 6.32
Inferior ganglion cell thickness (μm)	110.24 ± 5.79	108.96 ± 5.40	110.01 ± 5.67	110.07 ± 6.35
FAZ area (mm^2)	0.30 ± 0.05	0.29 ± 0.05	0.30 ± 0.05	0.29 ± 0.05
FAZ perimeter (mm)	2.97 ± 0.36	2.95 ± 0.32	3.03 ± 0.30	2.98 ± 0.33
FAZ circularity index (dimensionless, $4\pi A/P^2$)	0.47 ± 0.05	0.47 ± 0.06	0.47 ± 0.06	0.45 ± 0.05
Central macular thickness, CMT (μm)	260.26 ± 10.68	258.19 ± 13.25	258.43 ± 13.74	259.62 ± 12.29
Outer ETDRS mean perfusion (% vessel area density)	53.43 ± 2.05	53.27 ± 1.61	53.06 ± 1.67	53.24 ± 1.87
Inner ETDRS mean perfusion (% vessel area density)	40.18 ± 2.22	40.28 ± 2.04	40.24 ± 2.36	40.22 ± 2.34
Outer ETDRS mean retinal thickness (μm)	332.53 ± 6.57	333.09 ± 7.43	333.05 ± 7.03	333.11 ± 6.03
Inner ETDRS mean retinal thickness (μm)	342.08 ± 6.83	342.57 ± 6.71	342.20 ± 6.24	342.87 ± 6.03

Perfusion values are vessel area density in the superficial capillary plexus, expressed as the percentage of subfield area occupied by flow signal; thickness values are in micrometers; FAZ area is in mm^2 and FAZ perimeter in mm; the FAZ circularity index is dimensionless. FAZ, foveal avascular zone; ETDRS, Early Treatment Diabetic Retinopathy Study; OCTA, optical coherence tomography angiography.

Sensitivity Analyses

Sensitivity analyses were concordant with the primary analysis. Neither patient-averaged GEE models nor patient-averaged Friedman tests detected an FDR-confirmed time effect at the endpoint level; time-by-eye interaction models did not suggest a laterality-specific pattern (Table 5).

Effect Estimates and Confidence Intervals

To complement hypothesis testing, the ten largest nominal pairwise contrasts were expressed as model-based differences with Wald 95% confidence intervals. Differences were small

in native units: approximately 1 to 3 percentage points for sectoral perfusion, a few micrometers for retinal thickness, and approximately 0.02 for the dimensionless FAZ circularity index. Contrasts whose confidence intervals excluded zero were nominally significant, but none remained significant after the Holm or global FDR correction. When interpreted as estimates rather than tests, the intervals place an upper bound on what may have been missed: across all endpoints and contrasts, the data exclude sectoral perfusion changes larger than about 2.9 percentage points, retinal thickness changes larger than about 5.8 μm , and FAZ circularity changes larger than about 0.04 (Table 6).

Table 3. Primary GEE Overall Time-Effect Results with the Smallest Nominal *P* values

Endpoint	Wald χ^2	df	Raw <i>P</i>	FDR <i>q</i>	FDR-significant
ETDRS9 perfusion, inner nasal	9.786	3	0.0205	0.5284	No
ETDRS9 perfusion, outer nasal	7.655	3	0.0537	0.5284	No
ETDRS9 perfusion, inner temporal	7.455	3	0.0587	0.5284	No
ETDRS retinal thickness, outer inferior	6.701	3	0.0821	0.5540	No
FAZ circularity index	5.834	3	0.120	0.6028	No
ETDRS9 perfusion, inner superior	5.580	3	0.134	0.6028	No
ETDRS9 perfusion, outer superior	4.967	3	0.174	0.6721	No
ETDRS retinal thickness, inner superior	3.055	3	0.383	0.9913	No

Models used Gaussian GEE with patient-level clustering, exchangeable working correlation, robust sandwich standard errors, and adjustment for eye laterality, age, and sex. FDR significance was defined as $q < 0.05$.

ETDRS, Early Treatment Diabetic Retinopathy Study; FAZ, foveal avascular zone; GEE, generalized estimating equation; FDR, false-discovery rate.

Table 4. Exploratory Pairwise Contrasts With the Smallest Nominal *P* values

Endpoint	Contrast	Estimate	SE	Raw <i>P</i>	Holm <i>P</i>	Global FDR <i>q</i>
ETDRS9 perfusion, inner temporal	Month 6–Month 1	1.338	0.532	0.0119	0.0715	0.5934
ETDRS retinal thickness, outer inferior	Month 6–Baseline	3.221	1.305	0.0136	0.0817	0.5934
ETDRS9 perfusion, inner nasal	Month 1–Baseline	1.603	0.657	0.0146	0.0878	0.5934
ETDRS9 perfusion, outer nasal	Month 3–Baseline	−1.412	0.584	0.0157	0.0942	0.5934
FAZ circularity index	Month 6–Month 1	−0.020	0.009	0.0290	0.1738	0.5934
ETDRS9 perfusion, inner superior	Month 3–Month 1	−1.279	0.588	0.0294	0.1767	0.5934
ETDRS9 perfusion, outer superior	Month 3–Month 1	0.956	0.439	0.0296	0.1776	0.5934
ETDRS9 perfusion, inner temporal	Month 1–Baseline	−1.221	0.565	0.0309	0.1544	0.5934
ETDRS9 perfusion, outer nasal	Month 6–Month 3	1.279	0.600	0.0330	0.1648	0.5934
ETDRS9 perfusion, inner superior	Month 3–Baseline	−1.324	0.648	0.0411	0.2056	0.6660

Pairwise estimates are model-based differences between the two visits shown. Holm *P* values are within-endpoint adjusted; global FDR *q* values are calculated across all endpoint-contrast combinations.

ETDRS9, Early Treatment Diabetic Retinopathy Study 9; FAZ, foveal avascular zone; FDR, false-discovery rate.

Table 5. Summary of Sensitivity Analyses

Analysis family	Endpoint with smallest raw <i>P</i>	Smallest raw <i>P</i>	Smallest FDR <i>q</i>	No. $q < 0.05$
Patient-averaged GEE overall time	ETDRS9 perfusion, inner nasal	0.0205	0.5284	0
Patient-averaged Friedman test	ETDRS9 perfusion, inner superior	0.118	0.9920	0
Time $\times$ eye interaction GEE	Superior ganglion cell thickness	0.1457	0.9118	0

ETDRS9, Early Treatment Diabetic Retinopathy Study 9; GEE, generalized estimating equation; FDR, false-discovery rate.

Table 6. Effect Estimates and 95% Confidence Intervals for the Largest Nominal Pairwise Contrasts

Endpoint	Contrast	Estimated difference	95% CI
ETDRS9 perfusion, inner temporal	Month 6-Month 1	1.338	0.30 to 2.38
ETDRS retinal thickness, outer inferior	Month 6-Baseline	3.221	0.66 to 5.78
ETDRS9 perfusion, inner nasal	Month 1-Baseline	1.603	0.32 to 2.89
ETDRS9 perfusion, outer nasal	Month 3-Baseline	-1.412	-2.56 to -0.27
FAZ circularity index	Month 6-Month 1	-0.020	-0.038 to -0.002
ETDRS9 perfusion, inner superior	Month 3-Month 1	-1.279	-2.43 to -0.13
ETDRS9 perfusion, outer superior	Month 3-Month 1	0.956	0.10 to 1.82
ETDRS9 perfusion, inner temporal	Month 1-Baseline	-1.221	-2.33 to -0.11
ETDRS9 perfusion, outer nasal	Month 6-Month 3	1.279	0.10 to 2.46
ETDRS9 perfusion, inner superior	Month 3-Baseline	-1.324	-2.59 to -0.05

Differences are model-based GEE contrasts expressed in native units: percentage points for ETDRS9 perfusion, micrometers for ETDRS retinal thickness, and dimensionless units for FAZ circularity index. The 95% confidence interval is the Wald interval (estimate $\pm$ 1.96 $\times$ SE). None of these contrasts remained significant after within-endpoint Holm or global FDR correction.

ETDRS, Early Treatment Diabetic Retinopathy Study; GEE, generalized estimating equation; FDR, false-discovery rate; FAZ, foveal avascular zone; CI, confidence interval; SE, standard error.

DISCUSSION

In this prospective, longitudinal bilateral-eye study, no retinal structural or OCTA microvascular changes that survived correction for multiple testing were detected over 6 months of oral isotretinoin therapy. A few endpoint-level and pairwise comparisons were nominally significant, but these findings were not robust after FDR or Holm adjustments. The most cautious interpretation is that the observed sectoral changes represent small longitudinal fluctuations rather than reproducible evidence of treatment-related retinal microvascular toxicity. We emphasise that this is a statement about what was detected, not a demonstration that no effect exists; the distinction matters in a study of this size and is developed below.

These findings are broadly aligned with OCT studies that reported no clinically meaningful short-term change in central macular thickness, choroidal thickness, or retinal nerve fiber layer parameters during isotretinoin treatment.^{7,9} They do not exclude the possibility of subtle changes in selected patients or specific vascular compartments, because choroidal vascular shifts and OCTA-derived signals have been reported in other cohorts.^{10,11} Differences in imaging platform, segmentation algorithm, scan-quality threshold, cumulative dose, treatment duration, and patient selection may explain some of the heterogeneity across studies.

The patient-clustered GEE approach is important in this setting. Treating two eyes from the same patient as independent can underestimate standard errors and overstate statistical significance. The models used in this study account for bilateral-eye and repeated-visit correlations while adjusting for age, sex, and eye laterality. Multiplicity control is also central because OCT/OCTA devices generate multiple, correlated structural and perfusion endpoints; interpretation based solely on nominal *P* values would overemphasize isolated sectoral findings.

Statistical significance and clinical relevance are separate questions, and it is worth stating what the observed magnitudes would mean if they were real. The largest nominal

perfusion contrast was 1.34 percentage points in vessel area density, observed against a sectoral baseline of roughly 40 to 53 percentage points, which corresponds to a relative change of approximately 3%. The largest retinal thickness contrast was 3.2 μ m for a sectoral baseline above 330 μ m; this value is close to 1% and is below the test-retest repeatability generally reported for spectral-domain OCT thickness measurements. The FAZ circularity change of 0.02 falls within the visit-to-visit variability of that index. None of these approaches the magnitude at which retinal perfusion loss becomes clinically visible or alters management, and none was accompanied by a change in visual acuity or the structural parameters that would ordinarily accompany true ischemic remodelling. The upper confidence bounds reported above are the more informative quantities: they indicate that any change escaping detection in this cohort would itself have been small.

The image-quality rule is clinically relevant. Motion, segmentation error, projection artifact, and low signal can distort OCTA-derived parameters.^{5,6} Requiring an image-quality index of at least 0.70 reduces the likelihood that apparent temporal changes reflect acquisition noise. Nonetheless, device-derived perfusion measures should be interpreted in relation to the scan protocol, segmentation reliability, and the absence of an untreated control group.

Study Limitations

The study has several limitations; two merit particular consideration. First, there was no parallel control group of untreated acne patients. Every participant received isotretinoin; therefore, the design cannot separate a treatment effect from seasonal, diurnal, developmental, or device-related drift over the same 6 months. In a young cohort followed for a calendar year, such drift is non-negligible, and its absence from our estimates reflects an assumption rather than an observation. A concurrent untreated acne group measured on the same schedule would have made the comparison internal and would have been the single change most likely to strengthen a future study. Second, axial length and refractive error were

not recorded. OCTA measurements are scaled by ocular magnification: longer eyes yield systematically larger apparent FAZ dimensions and lower apparent vessel density unless a magnification correction is applied. Because our analysis is longitudinal and within-eye, magnification is constant within each patient and cannot generate a spurious time effect; therefore, the primary comparison is protected. What is lost is the ability to interpret absolute values, to compare them with cohorts of different refractive composition, or to test whether myopic eyes respond differently. Additionally, the sample was small and predominantly composed of young participants. No a priori power calculation was available; therefore, the absence of an FDR-confirmed change should be interpreted as indicating that no change was detected rather than as proof that none occurred. The complete-case design may introduce selection bias because 20 of the 54 enrolled patients did not complete all visits and therefore were excluded. Individual cumulative isotretinoin dose, acne severity, and diurnal variation in perfusion measurements were not available in the analytic dataset; therefore, a dose-response relationship could not be examined. Because no single primary OCTA endpoint was prespecified, the study should be considered exploratory.

CONCLUSION

Using a longitudinal, patient-clustered analytical framework, this prospective study detected no FDR-confirmed retinal structural or OCTA microvascular changes over 6 months of oral isotretinoin therapy. The sectoral fluctuations observed were small and did not withstand correction for multiple comparisons. Within the limits of sample size, follow-up, and imaging protocol, the study provides no reproducible OCT/OCTA evidence that oral isotretinoin alters retinal microvascular parameters during treatment, but remains unable to exclude effects smaller than the bounds reported above.

Ethics

Ethics Committee Approval: This study was approved by the Clinical Research Ethics Committee of Erzincan Binali Yıldırım University (meeting date: 26.04.2021, meeting number: 06, decision number: 06/05, official document number: E-21142744-804.99-77544, 04.05.2021).

Informed Consent: Written informed consent was obtained from all participants before enrollment.

Footnotes

Author Contributions:

Concept Design – A.M.S., E.İ.; Data Collection or Processing – A.M.S., M.G.K.; Analysis or Interpretation – E.İ., M.G.K.; Writing, Reviewing and Editing – A.M.S., E.İ., M.G.K.

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Factors Associated with Pelvic Fractures in Polytrauma Patients with Orthopaedic Injuries

Can Çamoğlu¹, Soner Sarı², Alperen Yiğit², Batuhan Gencer³, Özgür Doğan²

¹Clinic of Orthopaedics and Traumatology, Şırnak State Hospital, Şırnak, Türkiye

²Clinic of Orthopaedics and Traumatology, University of Health Sciences Türkiye, Ankara Bilkent City Hospital, Ankara, Türkiye

³Clinic of Orthopaedics and Traumatology, Marmara University Pendik Training and Research Hospital, İstanbul, Türkiye

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ORCID IDs of the authors: C.Ç. 0000-0002-9623-4439, S.S. 0000-0001-8863-9332, A.Y. 0000 0003 3714 9004, B.G. 0000 0003 0041 7378, Ö.D. 0000 0002 5913 0411.

ABSTRACT

Objective: To calculate the proportion of pelvic fractures in patients admitted to the emergency department due to polytrauma and to reveal the associated risk factors.

Methods: A total of 200 polytrauma patients with orthopaedic injuries were retrospectively evaluated. Demographic data such as age, gender, mechanism of injury, fracture locations, the Injury Severity Score (ISS), and the operation status of all patients were recorded. Fractures were categorized according to their anatomical locations: lower extremity fractures, upper extremity fractures, and pelvic fractures.

Results: Among the patients, 79 (39.5%) had lower extremity fractures, 112 (56%) had upper extremity fractures, and 57 (28.5%) had pelvic fractures. Statistical analysis revealed significant correlations between the presence of pelvic fractures and female gender ($P = 0.005$), higher ISS scores ($P = 0.005$), the absence of requirement for surgery or fracture fixation ($P < 0.001$ for each), and the absence of upper and lower extremity fractures ($P < 0.001$ and $P = 0.002$, respectively). Detailed risk analysis was conducted after 2 x 2 cross tabulations were constructed, and it revealed that female gender, no requirement for surgery or fixation, and absence of upper or lower extremity fractures are associated with an increased likelihood of pelvic fractures among victims of polytrauma.

Conclusion: The estimated proportion of pelvic fractures in patients with polytrauma and associated orthopaedic injuries is 28.5%. In this selected cohort of polytrauma patients with concomitant orthopaedic injuries, the absence of upper- and lower extremity fractures requiring surgical fixation was associated with pelvic fractures. However, this finding should be interpreted cautiously in light of potential patient selection bias and the influence of post-injury surgical decision-making. Further studies incorporating multivariable analyses are needed to clarify these associations.

Keywords: Polytrauma, pelvis, trauma

INTRODUCTION

Traumas are among the most common causes of morbidity and mortality worldwide.¹⁻³ The most common causes of polytrauma include high-energy injuries, such as traffic accidents, falls from height, and gunshot wounds. However, it may also be observed after low-energy injuries in elderly individuals or in individuals with osteoporotic bone structure.^{4,5} Polytrauma is defined as simultaneous, severe injury to at least two body regions or

organs.²⁻⁶ Pelvic fractures are of particular significance in such cases, given their association with hemodynamic instability and concomitant organ injuries. Indeed, pelvic fractures are the most common cause of death following brain injury in patients with polytrauma.⁷

Pelvic fractures, defined as fractures within the pelvic region, typically arise from high-energy injuries. These fractures are challenging to monitor and treat due to their complex



Corresponding author: Soner Sarı, E-mail: sarisnr97@gmail.com

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anatomy. They may manifest as isolated injuries or as part of a multifaceted trauma pattern. Expeditious detection and prompt intervention are paramount to the patient's prognosis and to the management of the injury. Displaced or dissociated pelvic fractures have been shown to cause severe hemodynamic instabilities^{2,4,5} while non-displaced or minimally displaced pelvic fractures attract less attention. Although fragmented, comminuted, or unstable pelvic fractures can be readily identified by physical examination or direct anteroposterior radiography, fissures of the iliac wing or fractures of the pubic rami may initially be overlooked, particularly in cases of polytrauma. However, these relatively minor injuries may result in secondary comorbidities due to delayed mobilization and the consequent need for anticoagulant therapy arising from immobilization. The challenge in managing these non-displaced fractures arises from the tendency of healthcare professionals to prioritize the treatment of more visible and urgent injuries, potentially overlooking these less conspicuous yet significant cases. This underscores the paramount importance of identifying pelvic fractures in patients with polytrauma. However, studies that evaluate the association between pelvic fractures and other orthopaedic injuries, as well as the predictability of such fractures, are scarce. The objective of the present study was to calculate the proportion of pelvic fractures among patients admitted to the emergency department for polytrauma and to identify fracture relationships associated with increased risk of pelvic fracture.

MATERIALS AND METHODS

Study Population and Data Collection

Following the approval of the University of Health Sciences Türkiye, Ankara Bilkent City Hospital Clinical Research Ethics Committee (decision no: TABED 1-25-1337, date: 04.06.2025), all patients who presented to the emergency department of the study center between 2019 and 2020 with a diagnosis of polytrauma, including orthopaedic injuries, and who were referred to the orthopaedics and traumatology clinic were investigated, regardless of the treatment method applied. Due to the retrospective nature of this study, the requirement for informed consent was waived. The inclusion criteria for the study were polytrauma, defined as injuries in more than one system,

and orthopaedic fractures. Patients who were referred to the orthopaedic clinic for other reasons (e.g., forensic necessity) and patients with severe soft tissue injuries without underlying fractures were excluded from the study. Pelvic fractures were evaluated and confirmed using computed tomography. Only pelvic ring fractures were included in the pelvic fracture group; acetabular fractures were excluded. Within the specified date range, 200 patients were retrospectively evaluated according to the inclusion and exclusion criteria.

Evaluation of the Patients

Demographic data of all patients, such as age, gender, mechanism of injury, fracture localization, the Injury Severity Score (ISS), and operation status, were recorded. The injury mechanisms were categorized under two subheadings: low-energy injuries (e.g., fall after sprain with accompanying head trauma, fall from pavement, fall from stairs with less than three steps, assault) and high-energy injuries (e.g., fall from height, traffic accidents, motorcycle accidents, firearm injuries). Fractures in all patients were categorized according to anatomical location: lower extremity fractures, upper extremity fractures, and pelvic fractures.

ISS is a system used to determine the severity of polytrauma (Table 1).^{2,3,8,9} It is one of the most commonly used anatomical scoring systems in this field. First, the severity of the injuries to the six body regions was determined using the Abbreviated Injury Scale (AIS). The ISS is calculated as the sum of the squares of the AIS scores of the three most seriously injured anatomical regions (i.e., the three anatomical regions with the highest AIS scores) (Table 1). Although the ISS can theoretically range from 1 to 75, any anatomical region with an AIS score of 6 automatically results in an ISS of 75. An AIS score of 6 indicates maximal injury severity (currently untreatable). After initial hospitalization, all patients were also evaluated for the need for surgery performed for any reason by a clinic other than orthopaedics and traumatology. Procedures that could be performed in the emergency department, such as chest tube insertion, were not categorized as surgical interventions. Requirements for fracture fixation were also recorded. To ascertain the need for operation and fixation, only surgeries performed at the time of initial hospital admission were considered; surgical requirements during subsequent follow-up were disregarded.

MAIN POINTS

- The proportion of pelvic fractures among polytrauma patients with concomitant orthopaedic injuries was 28.5 percent.
- Pelvic ring fractures were significantly associated with female sex and elevated Injury Severity Scores.
- The absence of concomitant upper or lower extremity fractures and the lack of need for emergency surgical fixation were paradoxically identified as significant clinical indicators of pelvic injury.
- Clinicians should maintain a high index of suspicion for severe pelvic trauma in polytrauma patients, particularly those lacking overt extremity fractures.

Table 1. Injury Severity Score and Abbreviated Injury Scale

Anatomical regions	Abbreviated Injury Scale (AIS)
Head-neck	Minor
Skin-soft tissue	Moderate
Face	Serious but not life-threatening
Thorax	Serious and life-threatening
Abdomen	Critical, survival doubtful,
Extremity-pelvis	Maximal (currently untreatable)
Injury Severity Score, sum of the squares of the 3 highest AIS scores.	

Statistical Analysis

Statistical analyses were conducted using IBM SPSS Statistics (version 25.0, IBM Corp., Armonk, NY, USA). The investigation of the data revealed that both the "age" and "ISS" parameters were not normally distributed. Consequently, median, interquartile range (IQR), and minimum and maximum values were used to describe these parameters, while categorical data were summarized as frequencies (percentages). The analysis of categorical parameters was conducted using the chi-square test, while Fisher's exact test was employed when the chi-square assumption was not met. For risk analysis, 2 x 2 cross-tabulations were constructed and odds ratios (ORs) were computed with 95% confidence intervals. A *P* value less than 0.05 was considered statistically significant.

RESULTS

The median age of the patients included in the study was 41 years (IQR: 38; range: 2-95 years), and the median ISS score was 16.5 (IQR: 16; range: 4-75). Of the patients, 79 (39.5%) had lower extremity fractures, 112 (56%) had upper extremity fractures, and 57 (28.5%) had pelvic fractures. A total of 76 (38%) patients underwent surgical intervention for fracture care. Detailed patient data are shown in Table 2.

Statistical analysis revealed significant correlations: the presence of pelvic fractures was significantly associated with female gender (*P* = 0.005), higher ISS scores (*P* = 0.005), absence of requirement for surgery or fracture fixation (*P* < 0.001 for each), and absence of upper and lower extremity fractures (*P* < 0.001 and *P* = 0.002, respectively) (Table 3). Detailed risk analysis was conducted after 2 x 2 cross tabulations were constructed, which revealed that female gender, absence of requirement for surgery or fixation, and absence of upper or lower extremity

fractures were associated with an increased likelihood of pelvic fractures in victims of polytrauma (OR: 1.87, 95% CI: 1.22-2.87; OR: 2.3, 95% CI: 1.33-4; OR: 2.88, 95% CI: 1.55-5.36; OR: 3, 95% CI: 1.83-4.91; and OR: 2.21, 95% CI: 1.28-3.83) (Table 4).

DISCUSSION

Determining the relationship between pelvic fractures and demographic characteristics, as well as other fracture types in polytrauma, will facilitate early diagnosis and rapid intervention. While fractures of the pelvis that are fragmented, comminuted, or unstable can be readily identified through physical examination or direct anteroposterior radiography, fissures of the iliac wing or fractures of the pubic rami may initially be overlooked, particularly in cases of polytrauma. However, these relatively minor injuries can potentially result in secondary comorbidities due to the delay in mobilization and the necessity of blood thinners, which arises from the immobilization. Therefore, it is important to determine which fractures or patient characteristics are more likely to be associated with these minor injuries that may be overlooked in polytrauma patients. In this way, high-risk patients can be identified and necessary evaluations can be conducted. This is the most important aspect of our study. Our most important finding was that female gender, higher ISS score, the absence of a requirement for surgery or fixation, and the absence of upper or lower extremity fractures were associated with an increased likelihood of pelvic fractures in victims of polytrauma. The prevalence of pelvic fractures ranges from 2% to 8% of all fractures, with literature on polytrauma cases indicating increases of up to 25%.^{10,11} In this study, the proportion of pelvic fractures in patients with orthopaedic injuries was 28.5%, indicating that approximately one in every four patients with orthopaedic injuries had a pelvic fracture. The median ISS in the present cohort was 16.5 (IQR:

Table 2. Demographic characteristics and fracture distribution of polytrauma patients

	Number of patients (n)	Percentage (%)
Age (years)*	41 (38) (2-95)	
Gender	Female	65
	Male	135
Injury Severity Score*	16.5 (16) (4-75)	
Mechanism of injury	Low-energy	48
	High-energy	152
Requirement for surgery	Yes	81
	No	119
Requirement for fracture fixation	Yes	76
	No	124
Lower extremity fracture	Yes	79
	No	121
Upper extremity fracture	Yes	112
	No	88
Pelvis fracture	Yes	57
	No	143

*median (interquartile range) (minimum-maximum range was used as descriptive statistics. n, number of patients.

Table 3. Factors Associated with Pelvic Fractures Among Polytrauma Patients with Orthopaedic Injuries

		With pelvic fractures (n=57)	Without pelvic fractures (n=143)	P
Gender	Female	27 (41.5%)	38 (58.5%)	0.005
	Male	30 (22.2%)	105 (77.8%)	
Age (years)		50 (36) (4-94)	40 (38) (2-95)	0.116
Injury Severity Score		18 (18) (8-75)	13 (14) (4-75)	0.005
Mechanism of injury	Low-	9 (18.8%)	39 (81.3%)	0.086
	High-	48 (31.6%)	104 (68.4%)	
Requirement for surgery	No	44 (37%)	75 (63%)	0.001
	Yes	13 (16%)	68 (84%)	
Requirement for fracture fixation	No	47 (37.9%)	77 (62.1%)	<0.001
	Yes	10 (13.2%)	66 (86.8%)	
Lower extremity fracture	No	44 (36.4%)	77 (63.6%)	0.002
	Yes	13 (16.5%)	66 (83.5%)	
Upper extremity fracture	No	40 (45.5%)	48 (54.5%)	<0.001
	Yes	17 (15.2%)	95 (84.8%)	

n, number of patients; p, statistical significance value.

Table 4. Odds Ratio Analysis of Factors Associated with Pelvic Fractures in Polytrauma Patients

		With pelvic fractures (n=57)	Without pelvic fractures (n=143)	P	OR (95% CI)
Gender	Female	27 (41.5%)	38 (58.8%)	0.005	1.87
	Male	30 (22.2%)	105 (77.8%)		(1.22-2.87)
Requirement for surgery	No	44 (37%)	75 (63%)	0.001	2.3
	Yes	13 (16%)	68 (84%)		(1.33-4)
Requirement for fracture fixation	No	47 (37.9%)	77 (62.1%)	<0.001	2.88
	Yes	10 (13.2%)	66 (86.8%)		(1.55-5.36)
Lower extremity fracture	No	44 (36.4%)	77 (63.6%)	0.002	2.21
	Yes	13 (16.5%)	66 (83.5%)		(1.28-3.83)
Upper extremity fracture	No	40 (45.5%)	48 (54.5%)	<0.001	3
	Yes	17 (15.2%)	95 (84.8%)		(1.83-4.91)

n, number of patients; p, statistical significance value; OR, odds ratio; CI, confidence interval.

16; range: 4-75). According to the extant literature, an ISS score greater than 15 is typically indicative of severe or major trauma, with a concomitant significant increase in mortality risk within this cohort.¹² Our median ISS score suggests that major trauma was more prevalent in our cohort. The association between pelvic fractures and higher ISS should be interpreted cautiously, as pelvic injuries themselves contribute directly to the ISS calculation. Therefore, this association may partly reflect the structure of the scoring system rather than an independent predictive relationship.^{2,3,8,9}

In the present study, female gender was significantly associated with pelvic fractures ($P = 0.005$, OR: 1.87, 95% CI: 1.22-2.87). This association can be attributed to the anatomical and biomechanical differences inherent in the female pelvis. The existing literature supports the notion that the prevalence of pelvic fractures is significantly higher in women than in men and that this may be related to decreased bone mineral

density. A higher prevalence of osteoporosis in women has been suggested as a possible cause of increased susceptibility to pelvic fractures.¹³ In a study by Demetriades et al.,¹⁴ reported that pelvic fractures showed a higher prevalence in women and this could be explained by biomechanical and hormonal factors. However, there are also studies in the literature indicating that pelvic fractures resulting from high-energy trauma are more common in male patients.^{15,16} This contradiction may be attributable to differences in study populations and sample sizes.

In the present study, the absence of concomitant fractures of the upper and lower extremities among patients with pelvic fractures was statistically significant. This finding is hypothesized to be related to the mechanism of injury. One possible explanation is that the initial point of impact may influence the distribution of injury energy and the resulting skeletal damage. It can be hypothesized that patients who

sustain the initial impact to an extremity may have a lower probability of concomitant pelvic fractures, whereas patients who experience a direct blow to the pelvis may have a lower probability of concomitant extremity fractures. However, this proposed mechanism remains hypothetical and cannot be confirmed by the present retrospective data. The extant literature on the subject is largely consistent, and pelvic fractures are found to be associated with abdominal, thoracic and genitourinary injuries, rather than upper and lower extremity fractures.^{2,17-19} Martínez et al.¹⁷ reported that pelvic fractures frequently occurred in conjunction with costal fractures and spinal injuries as a result of high-energy traumas. Figler et al.¹⁸ demonstrated that the bladder and urethra were the most frequently injured organs due to their position in the pelvis. In a study by Giannoudis et al.,¹⁹ 21.2% of patients with pelvic ring injuries had chest trauma, 16.9% had head injuries, and 7.8% had two or more long bone fractures. The most common injury accompanying pelvic fractures was not analyzed in this study. This is one of the study's limitations.

The correlation identified in the present study between the absence of fracture fixation and the occurrence of pelvic fractures can be interpreted as a consequence of the association between pelvic and extremity fractures. Musculoskeletal injuries represent the most prevalent indication for surgical intervention in polytrauma patients, with Balogh²⁰ documenting that 70% of patients with polytrauma necessitated orthopaedic surgical procedures. The literature has demonstrated that the number of surgeries increases in the presence of extremity injuries in polytrauma patients, and lower extremity fractures usually require surgical intervention.²¹ Because the absence of upper and lower extremity fractures increases the frequency of pelvic fractures in polytrauma, an association between the absence of a requirement for surgery or fixation and pelvic fractures is expected. Considering the relationship between pelvic fractures and genitourinary, abdominal, and thoracic injuries, pelvic fractures are expected to be directly related to the need for surgery. However, in our study, "the absence of requirement for surgery" increased the proportion of pelvic fractures by 2.3 times. We believe that this situation is not related to displaced or unstable fractures of the pelvis, but rather to minor injuries such as pubic ramus fractures or iliac crest fissures. In other words, we believe that the increased pelvic fracture rate in the absence of a need for surgery is due to minor pelvic injuries. It is important to note that the pelvic fractures were not classified in our study and that we examined these minor and major/displaced/unstable fractures under the same heading, which constitutes a limitation of the study.

Study Limitations

The retrospective design of the study and the limited sample size are the most significant limitations. The inability to perform subgroup analyses by pelvic fracture type is a significant limitation. The analysis could not be performed because of the limited number of patients, reliance on retrospectively collected screening data, and missing data in patient files. Furthermore, the inclusion of only polytrauma patients with orthopaedic fractures may have introduced selection bias, while

the absence of multivariable analysis and the influence of post-injury surgical decision-making limit the interpretation of the observed associations as independent risk factors or predictors. A further significant limitation of the present study is that it did not examine subsequent outcomes of pelvic fractures, nor did it include a mortality analysis, despite the acknowledged high correlation between pelvic fractures and mortality. However, given that the primary focus of the study was on the presence of pelvic fractures, these analyses were not conducted.

CONCLUSION

The estimated proportion of pelvic fractures among patients with polytrauma and associated orthopaedic injuries is 28.5%. In this selected cohort of polytrauma patients with concomitant orthopaedic injuries, the absence of extremity fractures requiring surgical fixation was associated with the presence of pelvic fractures. However, this association should be interpreted cautiously, considering the potential influence of patient selection and subsequent surgical decision-making. Further prospective, multicenter studies with larger cohorts are needed to better understand these associations and assess their independent significance.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained from the University of Health Sciences Türkiye, Ankara Bilkent City Hospital Clinical Research Ethics Committee (decision no: TABED 1-25-1337, date: 04.06.2025).

Informed Consent: Informed consent was waived due to the retrospective nature of the study. Permission to use patient data was obtained from the hospital management.

Footnotes

Author Contributions

Concept Design – C.Ç., S.S., A.Y., B.G., Ö.D.; Data Collection or Processing – C.Ç., S.S., A.Y.; Analysis or Interpretation – C.Ç., S.S., A.Y., B.G., Ö.D.; Literature Review – C.Ç., S.S., A.Y.; Writing, Reviewing and Editing – C.Ç., S.S., A.Y., B.G., Ö.D.

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The Inverse Morphological Relationship Between Posterior Tibial Slope and Medial Proximal Tibial Angle: A 3D Computed Tomography Study

Oğuzhan Tanoğlu¹, Hakan Cici¹, İlayda İncebiyık², Adem Osmanoglu², Habib Budak²

¹Department of Orthopedics and Traumatology, İzmir Demokrasi University Faculty of Medicine, İzmir, Türkiye

²Clinic of Orthopedics and Traumatology, Buca Seyfi Demirsoy Research and Training Hospital, İzmir, Türkiye

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ORCID IDs of the authors: O.T. 0000-0001-8984-9008, H.C. 0000-0002-9511-4865, İ.İ. 0000-0002-3289-7102, A.O. 0009-0006-2380-744X, H.B. 0009-0005-1882-5697.

ABSTRACT

Objective: The anatomical relationship between the posterior tibial slope (PTS) in the sagittal plane and the medial proximal tibial angle (MPTA) in the coronal plane is of critical importance for preoperative planning. The objective of this study is to investigate the effect of increased tibial slope on MPTA using three-dimensional anatomical models reconstructed from computed tomography.

Methods: A retrospective analysis was conducted on a cohort of 50 healthy adults. The dataset includes parameters such as gender, age, MPTA, and PTS for each adult. The subjects were divided into two cohorts based on their PTS values: those with a slope of 10° or higher were assigned to Group 2 (n=25), while those with 10° or lower were assigned to Group 1 (n=25).

Results: According to the data, MPTA angles in Group 1, which presented lower slope values ranged between 84.8° and 96.2°. In Group 2, which is characterized by higher PTS angles, the PTS angles ranged between 83.4° and 90.1°.

Conclusion: A tendency toward decreased MPTA values is observed when PTS reaches or exceeds 10°. This indicates that higher sagittal slope angles may be morphologically associated with varus alignment in the coronal plane.

Keywords: Deformity, three-dimensional imaging, anatomical models, osteotomy

INTRODUCTION

The successful reconstruction of the lower extremity depends on a comprehensive understanding of the three-dimensional (3D) morphology of the distal femur and proximal tibia.^{1,2} Measured in the coronal plane, the medial proximal tibial angle (MPTA) serves as the primary determinant of varus or valgus alignment and reflects the load distribution.³ In the sagittal plane, the posterior tibial slope (PTS) directly affects the anteroposterior stability and kinematic biomechanics of the knee.⁴ Prior studies have determined the relationship between the increased PTS angles and anterior cruciate ligament (ACL) ruptures.⁵ Increased PTS angles are mentioned as a risk factor for the failure of ACL reconstructions.⁶ Therefore, the authors

suggest slope reduction osteotomies for the failed cases of ACL reconstruction.⁷ MPTA is a widely used anatomical parameter for determining the deformity of the proximal tibia in the coronal plane. Although the measurement method is frequently in two-dimensional (2D), the morphology is virtually 3D, that has components in both coronal and sagittal plane.⁸ Besides, there is an interaction between other anatomical parameters and MPTA, which affects each other.⁹ Recent literature highlights that the PTS exhibits significant inter-individual variability and that an increased PTS can generate adverse shear forces on knee biomechanics.¹⁰ Although classical approaches often consider coronal and sagittal alignment parameters to be independent, it has been established that these two



Corresponding author: Oğuzhan Tanoğlu, E-mail: drtanoglu@gmail.com

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planes display simultaneous variations in certain specific phenotypes.¹⁰ Based exclusively on the provided dataset, this study investigates alterations in the MPTA, the primary coronal alignment parameter, when the PTS exceeds the 10° threshold.

MATERIALS AND METHODS

The Institutional Review Board approval for this research was obtained from the Ankara City Hospital Ethics Committee (decision no.: E1/1309/2020, date: 11.11.2020). At the time that ethical approval was obtained, the corresponding author was affiliated with Erzincan Binali Yıldırım University. As there was no active ethics committee for clinical research at the university during that period, the approval was secured from an external institution. Angiographic computed tomography (CT) images of adults older than 18 years of age were reviewed via the hospital registration system. Patients with a history of surgical procedures or fracture fixation of the right lower extremity, prior oncological interventions, any signs of osteoarthritis of the hip, knee, or ankle joints, deformity correction surgeries, or incomplete CT images were excluded from the study. CT images of 78 adults who met the study inclusion criteria were retrieved from radiological records. Given the retrospective design of this study and the use of pre-existing radiological records from the hospital database, obtaining informed patient consent was not required. CT images of adults were imported into Materialise Mimics Innovation Suite version 27 (Materialise, Leuven, Belgium). Only the right tibiae were segmented using the advanced segmentation option with thresholds between 250-2000 Hounsfield unit. The surfaces of the medial and lateral tibial plateaus were scanned (Figure 1), and an anatomical plane was created using the Create primitives option (Figure 2). The total length of the tibia was separated into three parts. The proximal and distal 1/3 borders were scanned, and two spheres were created. The center points of the spheres were merged, and the anatomical axis of the tibia was determined (Figure 3). The medial and lateral tibial plateaus were scanned, and two separate anatomical planes were created. The centers of the planes were merged to form a line used to measure MPTA in the coronal plane. The anatomical landmarks are transferred to Materialise 3-matic 19 (Materialise, Leuven, Belgium). MPTA in adults was measured between the tibial joint line and the tibial anatomical axis in the coronal plane (Figure 4). PTS angles were measured between the proximal tibial plane and the tibial anatomical axis in the

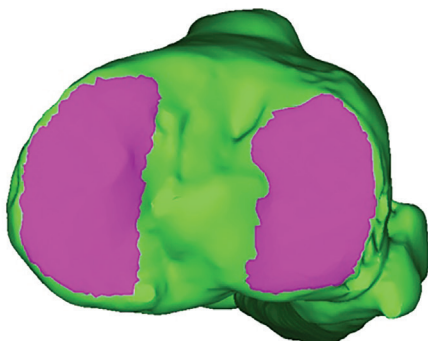


Figure 1. Scanning of medial and lateral plateau.

sagittal plane (Figure 5). The study group was divided into two main categories based on mean PTS values. Based on an a priori power analysis conducted using G*Power, a minimum sample size of 21 patients per group was determined. We randomly selected 25 adults with higher PTS angles and 25 adults with lower PTS angles from the cohort. The threshold of the PTS values as 10 degrees was defined according to the results of a 3D anatomical study by Tanoğlu et al.², which investigated the mean PTS angles of healthy adults in Turkish population. The demographic data and the measurement results were analyzed with JASP 95.4 (University of Amsterdam, Amsterdam, Netherlands). The distribution of the data was evaluated using the Shapiro-Wilk test, and the results indicated that the data were normally distributed. Therefore, the Student's t-test was used to compare the results between study groups.

RESULTS

The mean age of Group 1 was 49.8 ± 8.4 , and Group 2 was 47.1 ± 8.5 . We observed no statistically significant difference in mean age between the study groups ($P = 0.268$) (Table 1). Group 1

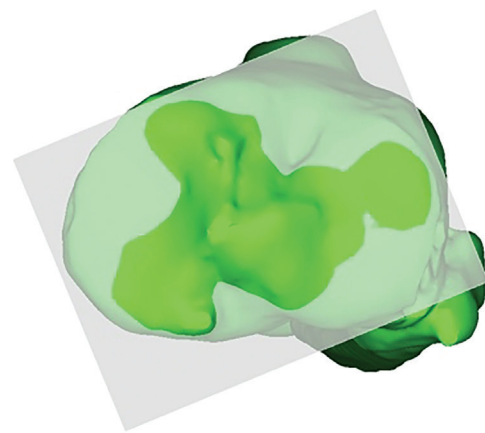


Figure 2. Anatomical plane created according to the scanned medial and lateral plateau.

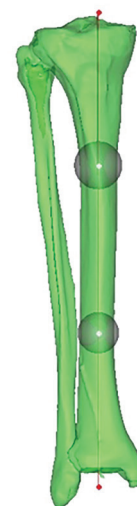


Figure 3. 3D anatomical axis of tibia.
3D, three-dimensional.

includes 25 adults with PTS values ranging from 4.1° to 9.3°. Group 2 includes 25 adults with PTS values ranging from 10° to 14.4°. The mean PTS angles were 7.2 ± 1.5 in Group 1 and 11.4 ± 1.4 in Group 2. The mean MPTA was 88.7 ± 3.3 in Group 1 and 86.6 ± 2 in Group 2. The study results revealed a statistically significant difference in mean MPTA values between study groups ($P = 0.011$).

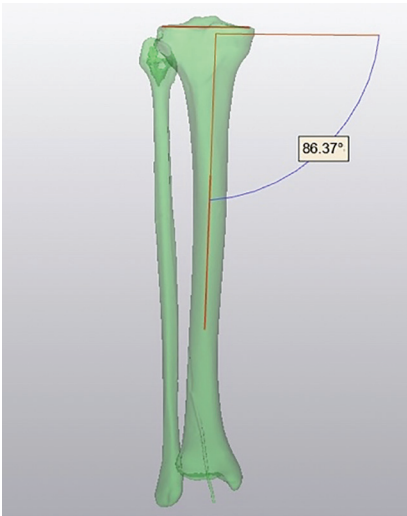


Figure 4. Medial Proximal Tibial Angle measurement in coronal plane.

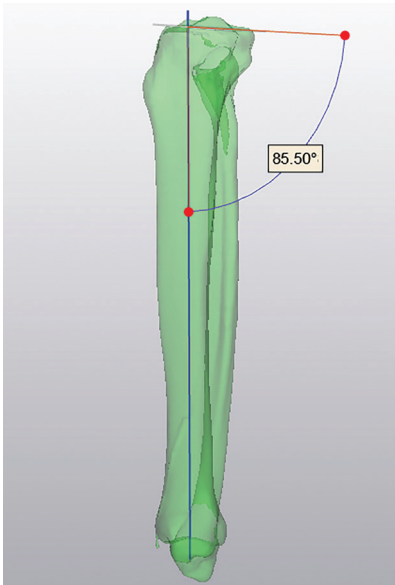


Figure 5. Posterior Tibial Slope measurement in sagittal plane.

DISCUSSION

The primary finding of the present study is an inverse morphological correlation between the PTS and the MPTA. Our data demonstrate that when the PTS reaches or exceeds 10° (Group 2), MPTA values tend to decrease significantly. Conversely, lower PTS values (Group 1) correspond to a higher MPTA. This morphological relationship suggests that an increase in sagittal slope is associated with an adaptation or accompanying deformity profile that tends toward varus alignment in the coronal plane. Biomechanically, the concurrent presence of an elevated PTS and coronal varus malalignment can profoundly disrupt knee kinematics, alter cruciate ligament tension, and exacerbate pathological loading on the medial compartment.^{4,5} Historically, lower extremity alignment studies have frequently evaluated the coronal and sagittal planes as isolated parameters. However, relying on standard 2D radiography often leads to measurement inaccuracies due to rotational variations.¹¹ Use of advanced 3D anatomical models reconstructed from CT scans ensures exceptional measurement accuracy compared to standard 2D radiographs. 3D reconstruction programs allow for precise, reproducible anatomical measurements of bone structures that 2D imaging often misrepresents due to rotational variances.¹¹

These biomechanical and morphological findings hold substantial clinical relevance, particularly in the surgical management of knee deformities. Genu varum, primarily driven by the proximal tibia, is the most prevalent deformity detected in severe knee arthrosis.¹² In younger adults, opening wedge high tibial osteotomy is frequently employed as a joint-preserving procedure to shift abnormal mechanical loads from the medial plateau to the lateral compartment.³ Furthermore, an increased PTS angle is a well-established risk factor for both primary ACL ruptures and subsequent graft failures.⁵⁻⁷ Slope-reducing osteotomies, which are frequently performed in this patient population, serve to protect the graft by decreasing the mechanical load applied to the ACL.^{6,7,13}

Our study results validate that preoperative planning for single-plane correction of the deformity is insufficient, and 3D biplanar planning is crucial to uncovering the intricate relationship between these anatomical parameters. Consequently, in the preoperative planning of corrective osteotomies, it is imperative to address not only varus malalignment but also excessive PTS. This correlation underscores that the existing MPTA should be considered in preoperative planning for slope-reducing surgery.

Table 1. Comparison of the Study Groups

	Group 1		Group 2		P value
	Mean	SD	Mean	SD	
Age	49.8	8.4	47.1	8.4	0.26
MPTA	88.7	3.3	86.6	2	0.011*
PTS	7.2	1.5	11.4	1.4	<0.001*

*Statistically significant. SD, standard deviation; MPTA, medial proximal tibial angle; PTS, proximal tibial slope.

Study Limitations

This study has some limitations. First, the retrospective design and the relatively small sample size (50 adults) may limit the generalizability of the findings. Furthermore, the cohort consisted entirely of healthy individuals. Consequently, the effect of degenerative morphological changes on this multiplanar relationship remains unclear. Future research should prioritize conducting a comparative 3D-CT study to evaluate these lower-limb anatomical parameters in healthy adult cohorts and in patients with advanced knee osteoarthritis, to determine how the PTS-MPTA relationship behaves in the presence of joint degeneration.

CONCLUSION

Our results validate that traditional single-plane preoperative planning is insufficient for managing complex proximal tibial deformities. The intricate morphological relationship between the sagittal and coronal planes mandates comprehensive 3D biplanar evaluation. When performing planned corrective osteotomies—whether addressing medial compartment osteoarthritis or offloading an ACL reconstruction—surgeons must avoid evaluating these parameters in isolation. It is imperative to meticulously account for both varus malalignment and excessive PTS to ensure optimal surgical outcomes and to restore physiological knee kinematics.

Ethics

Ethics Committee Approval: The Institutional Review Board approval for this research was obtained from the Ankara City Hospital Ethics Committee (decision no.: E1/1309/2020, date: 11.11.2020).

Informed Consent: Due to the retrospective design of this study, obtaining informed consent from the patients was not required; permission was obtained from the hospital administration to use the radiological records from the hospital database for the study.

Footnotes

Author Contributions

Concept Design – O.T.; Data Collection or Processing – İ.İ., A.O., H.B.; Analysis or Interpretation – O.T., H.C.; Literature Review – İ.İ., A.O., H.B.; Writing, Reviewing and Editing – O.T., H.C.

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Diagnostic Value of the Third-Trimester Fibrinogen-to-Albumin Ratio for Predicting PE and Small-for-Gestational-Age Neonates: A Retrospective Study

 Zulfiyya Gahramanova¹,  Betül Kalkan²,  Okay Alptekin¹

¹Clinic of Gynecology and Obstetric, Mengücek Gazi Training and Research Hospital, Erzincan, Türkiye

²Department of Gynecology and Obstetric, Erzincan Binali Yıldırım University Faculty of Medicine, Erzincan, Türkiye

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ORCID IDs of the authors: Z.G. 0000-0003-3656-5495, B.K. 0000-0003-4742-5308, O.A. 0000-0001-6404-4158.

ABSTRACT

Objective: To evaluate whether the fibrinogen-to-albumin ratio (FAR), measured mainly in the third trimester, is associated with and can predict preeclampsia (PE) and small-for-gestational-age (SGA) neonates in pregnant women.

Methods: In this retrospective study involving 30 patients (PE group) and 330 controls, the FAR was calculated, and its role in predicting PE and SGA was investigated. Additionally, gestational age at delivery, birth weight, percentile values, hemogram parameters, and biochemical variables were compared between the groups.

Results: FAR did not demonstrate sufficient diagnostic power to predict PE [receiver operating characteristic, area under the curve (AUC)= 0.527]; however, decreased serum albumin level was significantly associated with increased risk of PE [odds ratio (OR)= 0.768]. Albumin was identified as an independent predictor of PE (multivariate OR= 0.768, 95% confidence interval: 0.662-0.891, $p < 0.001$). Furthermore, no significant association was found between FAR and birth weight, SGA, or birth weight percentile values.

Conclusion: FAR alone does not possess sufficient diagnostic power (AUC= 0.527). Albumin emerged as a consistent predictor across all analyses (univariate OR= 0.768; multivariate OR= 0.768). Urea and alanine aminotransferase were also identified as independent predictors in the multivariate model. No significant association was detected between FAR and birth weight or SGA.

Keywords: Fibrinogen, albumin, PE, fibrinogen-to-albumin ratio

INTRODUCTION

Preeclampsia (PE), affecting approximately 5-10% of pregnant women, is a pregnancy complication characterized by hypertension and proteinuria occurring after the 20th week of gestation.^{1,2} PE, a multifactorial disorder characterized by hypertension and organ dysfunction, is associated with maternal and neonatal mortality and morbidity worldwide.²

An increasing incidence of PE has been observed in the United States in recent years. This trend has been attributed to a higher prevalence of known risk factors such as obesity and advanced maternal age.³

Although the etiology of PE has not been fully elucidated, clinical and pathological studies indicate that systemic inflammatory response, immune dysfunction, abnormalities in the coagulation and fibrinolytic systems, and aberrant placental play central roles in the pathogenesis of the disease.² Moreover, a more pronounced hypercoagulable state has been reported in preeclamptic pregnancies compared with normotensive pregnancies, and alterations in the coagulation system are considered to contribute to the disease process.⁴

Early prediction and recognition of PE are among the primary objectives of obstetric care, as appropriate clinical surveillance and timely intervention can prevent serious maternal and



Corresponding author: Betül Kalkan, E-mail: betul.yilmaz@erzincan.edu.tr

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perinatal complications. To this end, various biomarkers and imaging parameters, including soluble fms-like tyrosine kinase-1, placental growth factor, and uterine artery Doppler indices, have been extensively investigated for their potential in predicting PE.²

In recent years, a growing number of studies have evaluated the clinical utility of the fibrinogen-to-albumin ratio (FAR), derived from routine laboratory parameters and reflecting systemic inflammation and coagulation activity, in predicting the presence and severity of PE. However, the diagnostic and prognostic value of FAR for PE has not yet been conclusively established.

Fibrinogen (Factor I) is one of the major proteins in plasma, present in high concentrations, and plays a central role in the coagulation cascade. As the principal structural component of clot formation, fibrinogen is an indispensable element of the coagulation process; effective clot formation cannot occur in the absence of fibrinogen, even when other coagulation factors are present at normal levels.⁵ Synthesized in hepatic parenchymal cells, fibrinogen is a hexameric glycoprotein with a molecular weight of approximately 340 kDa. Its normal plasma concentration ranges from 200 to 400 mg/dL, with a half-life of approximately 3 to 5 days.⁶

Albumin, another important plasma protein synthesized in the liver, possesses antioxidant, anti-inflammatory, and tissue-repair-supporting properties. Albumin, the most abundant protein in plasma, tends to decrease in conditions such as chronic inflammation, malnutrition, and liver disease.⁷⁻⁹ During pregnancy, serum albumin levels decline due to physiological plasma volume expansion and hemodilution; mild hypoalbuminemia is frequently regarded as a normal finding.⁹

The FAR, calculated from serum fibrinogen and albumin levels, has attracted attention as a novel composite biomarker that simultaneously reflects inflammatory and coagulation processes. FAR has been shown to be useful in predicting clinical outcomes in various cardiovascular diseases and malignancies.^{10,11} In obstetrics, its prognostic value has been

investigated in threatened miscarriage, recurrent pregnancy loss, and several obstetric complications, and promising results have been reported.¹²

During inflammatory processes, FAR increases as a consequence of rising fibrinogen levels and declining albumin levels. Consequently, FAR is regarded as a composite indicator of the degree of systemic inflammation. Recent studies have suggested that FAR may be an important marker for evaluating numerous inflammation-associated conditions and predicting clinical outcomes.^{10,11}

MATERIALS AND METHODS

This retrospective cohort study was conducted in the Department of Obstetrics and Gynecology at Erzincan Binali Yıldırım University Mengücek Gazi Training and Research Hospital. Pregnant women with a gestational age ≥ 20 weeks who were followed at our clinic between January 2023 and June 2024 and who had serum fibrinogen and albumin levels measured were included in the study.

Ethical approval was obtained from the Erzincan Binali Yıldırım University Non-Interventional Clinical Research Ethics Committee (decision no.: 485828, date: 29.09.2025). All procedures were conducted in accordance with the principles of the Declaration of Helsinki. The study was retrospective; therefore, informed consent was not required, as only anonymized data from routine clinical records and patient files were analyzed and no intervention was performed on the patients.

The study population comprised 360 pregnant women, classified into the PE group ($n=30$) and the control group ($n=330$). PE was diagnosed based on the clinical assessment recorded at admission, according to the diagnostic criteria of the Ministry of Health of the Republic of Türkiye, including admission blood pressure ($\geq 140/90$ mmHg). Because individual blood pressure values could not be retrieved retrospectively from the electronic records, severity subclassification could not be performed.

Exclusion criteria included platelet (PLT) count $<100,000/\text{mm}^3$ for other than PE (either pre-existing or occurring during pregnancy), nephropathy, elevated liver enzymes attributable to causes other than PE, active bleeding, and intrahepatic cholestasis of pregnancy.

The study included pregnant women aged 18 years or older who were hospitalized for the following indications: decreased fetal movement, threatened preterm labor, trauma, hypertension, membrane rupture, uterine contractions, or other obstetric reasons. Demographic characteristics, gestational age at delivery, mode of delivery, birth weight, and neonatal percentile values were obtained from patients' files.

Advanced maternal age was defined as ≥ 35 years. Small for gestational age (SGA) was defined as a birth weight below the 10th percentile for gestational age. The FAR was calculated by dividing serum fibrinogen (mg/dL) by serum albumin (g/L) and reported as a unitless ratio. Levels of fibrinogen, albumin, hemoglobin, PLT, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, urea, and calcium were

MAIN POINTS

- The fibrinogen-to-albumin ratio (FAR), measured mainly in the third trimester, did not show sufficient diagnostic power for predicting preeclampsia (PE) (area under the curve= 0.527), and combining it with other laboratory parameters did not significantly improve discrimination.
- Lower serum albumin was the strongest independent predictor of PE (multivariate odds ratio= 0.768, 95% confidence interval: 0.662-0.891; $p < 0.001$), followed by urea and alanine aminotransferase.
- FAR was not associated with birth weight, small-for-gestational-age status, birth weight percentile, or maternal age.
- Serum albumin, a low-cost routine test, deserves further evaluation as a PE biomarker in larger prospective multicenter studies.

obtained from routine laboratory assessments performed at the time of hospitalization or approximately 3–4 weeks before delivery.

The primary analysis compared FAR and other laboratory parameters between the PE and control groups. The secondary analysis evaluated the association between FAR and birth weight, gestational age at delivery, and neonatal percentile values across the entire study population.

Statistical Analysis

The normality of the data was assessed using the Shapiro-Wilk test. Normally distributed continuous variables are presented as mean ± standard deviation and compared between groups using the independent samples t-test. Non-normally distributed continuous variables were expressed as medians [interquartile range (Q1–Q3)] and analyzed using the Mann-Whitney U test. Categorical variables were presented as frequencies and percentages and were compared using Fisher’s Exact Test.

The diagnostic performance of FAR for predicting PE was evaluated using receiver operating characteristic (ROC) curve analysis. The 95% confidence intervals (CIs) for the area under the curve (AUC) were calculated using the bootstrap method with 2,000 iterations. The optimal cut-off point was determined based on the Youden Index. The DeLong test was used for comparisons between ROC curves. Associations between variables were assessed using Spearman’s correlation analysis. Multivariate logistic regression analysis was performed to identify independent predictors of PE. In the multivariate model, FAR was included a priori as the marker of interest, and albumin, urea, and ALT were included based on their univariate association with PE.

AST was not entered because of its strong correlation with ALT (Spearman $r = 0.609$) to avoid collinearity. Model fit was assessed using the Nagelkerke R-squared and the Hosmer-Lemeshow test. A two-sided P value of <0.05 was considered statistically significant. ROC analyses, bootstrap CIs (2,000 iterations), DeLong comparisons were performed using Python; figures were generated using Python; the remaining analyses were performed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Comparison of Clinical and Laboratory Characteristics

The clinical, demographic, and laboratory characteristics of all 360 pregnant women included in the study are presented in Table 1. The group-specific distributions of key laboratory parameters are illustrated in Figure 1 using box plots.

Serum albumin levels were significantly lower in the PE group compared with the control group (31.67 ± 4.19 g/L vs. 33.66 ± 2.27 g/L; $P < 0.001$). This difference is also reflected in Figure 1, where box plots demonstrate a marked separation between the two groups.

FAR did not differ significantly between the two groups (control: 10.98 ± 3.01 ; PE: 11.51 ± 3.54 ; $P = 0.365$; Table 1). The expected increase in fibrinogen was limited in this sample.

Urea was significantly elevated in the PE group [15.00 (12.00 – 18.00) vs. 17.50 (14.25 – 21.75) mg/dL; $P = 0.008$; Table 1), consistent with other studies in the literature.¹³ Gestational age at delivery was significantly shorter in the PE group [38.00 (37.00 – 39.00) vs. 37.00 (36.50 – 38.00) weeks; $P = 0.008$].

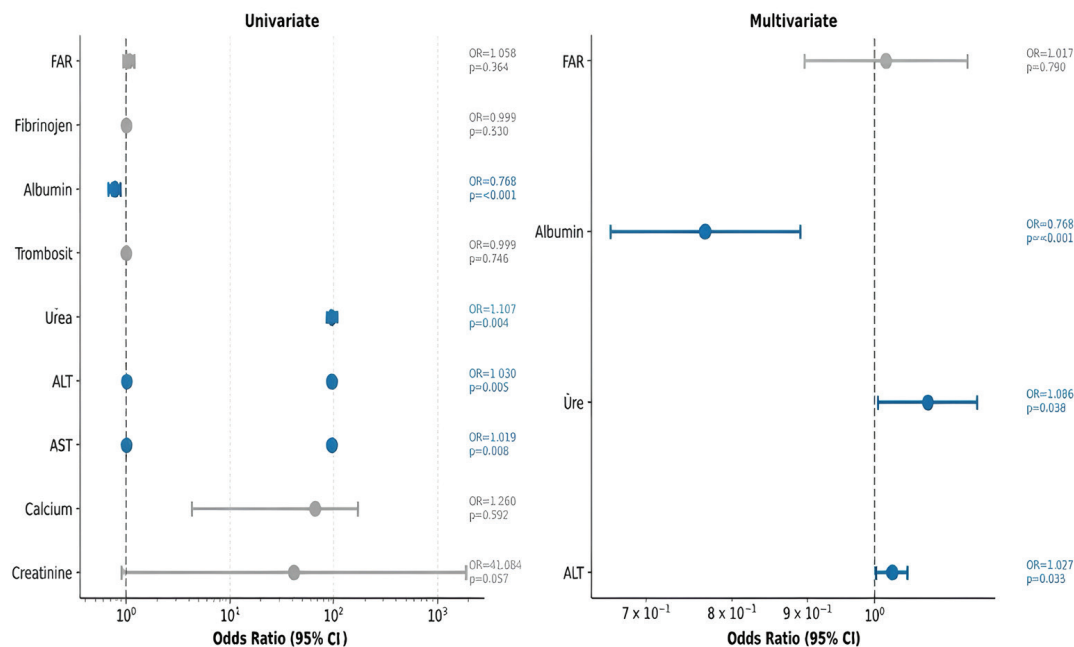


Figure 1. Box plots of FAR, albumin, fibrinogen, platelet count, urea and ALT according to the control (blue) and PE (gray) groups. Albumin ($P < 0.001$) and urea ($P = 0.008$) differed significantly between the groups. P values shown in gray indicate statistical significance.

FAR, fibrinogen-to-albumin ratio; ALT, alanine aminotransferase; PE, preeclampsia; CI, confidence interval; OR, odds ratio.

ALT, AST, PLT count, creatinine, calcium, and birth weight did not differ significantly between the groups (all $p > 0.05$; Table 1).

Correlation Analysis Between FAR and Clinical Parameters

Spearman correlation results between FAR and eight clinical parameters are presented in Table 2. A statistically significant negative correlation was identified between FAR and AST ($r = -0.170$; $P = 0.001$). No significant correlations were found with other parameters (PLT, urea, ALT, calcium, creatinine, birth weight, or percentile).

ROC Analysis of FAR and Combined Models

The standalone diagnostic accuracy of FAR and that of the combined models are presented in Table 3, and the corresponding ROC curves are shown in Figure 2.

The AUC value for FAR alone was 0.527 (95% CI: 0.420-0.633). That the CI encompasses the 0.500 reference value indicates that FAR does not significantly exceed the chance level (Table 3). The optimal threshold determined by the Youden index was 12.60, yielding a sensitivity of 43.3%, a specificity of 69.1%, and

an negative predictive value of 93.1%.

The ROC performance of models combining FAR with six parameters is also presented in Table 3. None of the FAR-combined models provided a statistically significant improvement over FAR alone.

Univariate and Multivariate Logistic Regression

Univariate and multivariate logistic regression results are presented in Table 4, and comparative forest plots are presented in Figure 3. In univariate analysis, albumin was the strongest predictor [odds ratio (OR)= 0.768; 95% CI: 0.670-0.880; $P < 0.001$; Table 4]. An OR below 1 indicates that each 1 g/L increase in albumin level is associated with a 23.2% reduction in the odds of PE. Urea (OR= 1.107; $P = 0.004$), ALT (OR= 1.030; $P = 0.005$), and AST (OR= 1.019; $P = 0.008$) also demonstrated significant associations. FAR did not yield a significant result (OR= 1.058; $P = 0.364$). In the multivariate model, albumin (OR= 0.768; $P < 0.001$), urea (OR= 1.086; $P = 0.038$), and ALT (OR= 1.027; $P = 0.035$) were identified as independent predictors (Table 4). FAR lost its independent predictive significance (OR= 1.017; $P = 0.790$). The model

Table 1. Comparison of Demographic and Clinical Parameters Between Control and PE Groups

Variable	n (C/PE)	Control (n=330)	PE (n=30)	P value	Test
Age (years)	330/30	28.35 ± 5.52	29.80 ± 6.41	0.176	t-test
FAR	330/30	10.98 ± 3.01	11.51 ± 3.54	0.365	t-test
Fibrinogen (mg/dL)	330/30	368.40 ± 99.39	356.58 ± 93.29	0.531	t-test
Albumin (g/L)	330/30	33.66 ± 2.27	31.67 ± 4.19	<0.001*	t-test
Hemoglobin (g/dL)	330/30	11.74 ± 1.39	11.77 ± 1.47	0.908	t-test
Platelet (x10 ³ /uL)	330/30	214.00 (183.00-252.00)	229.00 (199.75-244.75)	0.561	MWU
Creatinine (mg/dL)	330/30	0.60 (0.50-0.60)	0.60 (0.50-0.67)	0.113	MWU
Urea (mg/dL)	330/30	15.00 (12.00-18.00)	17.50 (14.25-21.75)	0.008*	MWU
Calcium (mg/dL)	330/30	8.77 ± 0.43	8.82 ± 0.60	0.593	t-test
ALT (U/L)	330/30	10.00 (8.00-13.00)	12.00 (9.00-16.75)	0.074	MWU
AST (U/L)	330/30	19.00 (15.25-22.00)	20.00 (15.25-24.00)	0.459	MWU
Gestational age (weeks)	318/19	38.00 (37.00-39.00)	37.00 (36.50-38.00)	0.008*	MWU
Birth weight (g)	308/18	3105.20 ± 516.30	2915.28 ± 513.52	0.130	t-test

Mean ± SD, normally distributed; median (Q1-Q3), non-normally distributed variables. * $P < 0.05$.

C/PE, control/PE patient count; MWU, Mann-Whitney U; SD, standard deviation; FAR, fibrinogen-to-albumin ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table 2. Spearman Correlation Analysis Between FAR and Clinical Parameters

Parameter	Spearman r	P value	n	Interpretation
Platelet	0.077	0.147	360	Not significant
Urea	-0.025	0.643	360	Not significant
ALT	-0.097	0.067	360	Not significant
AST	-0.170	0.001*	360	Weak negative
Calcium	-0.020	0.712	360	Not significant
Creatinine	0.078	0.141	360	Not significant
Birth weight	0.046	0.408	326	Not significant
Birth weight Percentile	-0.029	0.603	328	Not significant

r, spearman coefficient. * $P < 0.05$. Strength: $|r| > 0.70$ = Strong; 0.40-0.70 = Moderate; 0.20-0.40 = Weak.

ALT, alanine aminotransferase; AST, aspartate aminotransferase.

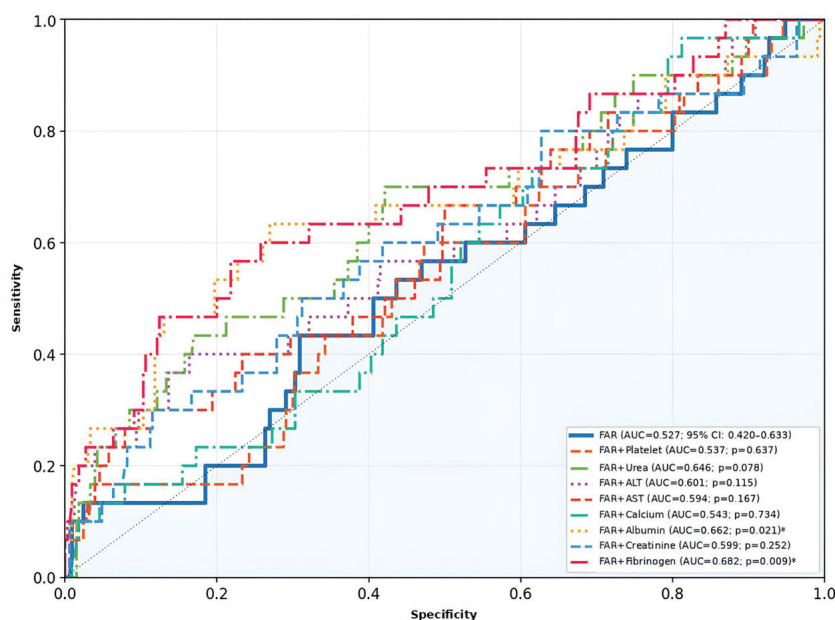


Figure 2. Comparison of ROC curves for FAR alone (thick blue line) and the combined models (coloured dashed lines). None of the combined models provided a statistically significant improvement over FAR alone on the DeLong test (Table 3). The dashed black line represents the chance-level reference line.

FAR, fibrinogen-to-albumin ratio; ROC, receiver operating characteristic; AUC, area under the curve; CI, confidence interval; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table 3. ROC Analysis and DeLong Comparison of FAR Alone and Combined Models

Model	AUC	95% CI	Sensitivity (%)	Specificity (%)	DeLong P
FAR (reference)	0.527	0.420-0.633	43.3	69.1	-
FAR+platelet	0.537	0.434-0.642	16.7	96.1	0.637
FAR+urea	0.646	0.538-0.753	70.0	57.9	0.078
FAR+ALT	0.601	0.482-0.716	40.0	83.6	0.115
FAR+AST	0.594	0.480-0.706	26.7	92.1	0.167
FAR+calcium	0.543	0.440-0.646	96.7	18.8	0.734
FAR+creatinine	0.599	0.487-0.710	50.0	68.8	0.252

FAR alone, negative predictive value 93.1%; Youden threshold 12.60. Combined AUCs derived from logistic regression probability scores. DeLong p: comparison against FAR alone.

AUC, area under the curve; CI, confidence interval (bootstrap, n = 2,000); FAR, fibrinogen-to-albumin ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Table 4. Univariate and Multivariate Logistic Regression Results

Variable	Univariate OR (95% CI)	Univariate P	Multivariate OR (95% CI)	Multivariate P
FAR	1.058 (0.937-1.194)	0.364	1.017 (0.896-1.155)	0.790
Albumin	0.768 (0.670-0.880)	<0.001	0.768 (0.662-0.891)	<0.001
Urea	1.107 (1.032-1.186)	0.004	1.086 (1.005-1.173)	0.038
ALT	1.030 (1.009-1.051)	0.005	1.027 (1.002-1.052)	0.035
AST	1.019 (1.005-1.033)	0.008	Not included	-
Fibrinogen	0.999 (0.995-1.003)	0.530	Not included	-
Platelet	0.999 (0.992-1.005)	0.746	Not included	-
Calcium	1.260 (0.541-2.933)	0.592	Not included	-
Creatinine	41.084 (0.897-1881.870)	0.057	Not included	-

The multivariate model included FAR, albumin, urea, and ALT. Model fit: Nagelkerke R-squared = 0.178; Hosmer-Lemeshow p = 0.085.

OR, odds ratio; CI, confidence interval; FAR, fibrinogen-to-albumin ratio; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

showed an acceptable fit (Nagelkerke R-squared =0.178; Hosmer-Lemeshow $P = 0.085$).

Association of FAR with Birth Weight and SGA

No significant correlation was found between FAR and birth weight among the 326 patients with available birth weight data (Spearman $r = 0.046$; $P = 0.408$; $n=326$). FAR did not differ significantly between 32 SGA and 296 non-SGA infants (median: 11.95 vs. 11.04; $P = 0.118$). The ROC AUC for FAR in predicting SGA was 0.584, which is close to chance level. The correlation between FAR and birth weight percentile was also non-significant ($r = -0.029$; $P = 0.603$). These results are summarized in Table 5.

Association Between Maternal Age, FAR and SGA

No statistically significant correlation was identified between age and FAR across all patients (Spearman $r = -0.007$; $P = 0.894$;

$n=360$; Table 6). This finding is supported by the scatter plot, in which the nearly horizontal regression line indicates that age has no deterministic effect on FAR.

No significant correlation was found in either the control group ($r = -0.023$; $P = 0.683$) or the PE group ($r = 0.102$; $P = 0.592$) when the groups were analyzed separately (Table 6). When the advanced maternal age (≥ 35 years, $n=63$ in the whole sample) and normal-age (<35 years, $n=297$) groups were compared, no significant difference in FAR values was observed [advanced-age median: 10.84 (9.64-12.83) vs. normal-age median: 11.05 (8.76-13.05); $P = 0.492$], indicating that FAR is independent of maternal age.

Regarding SGA, no statistically significant difference was found in maternal age when comparing mothers of 32 SGA infants and 296 non-SGA infants (26.0 vs. 28.0 years; $P = 0.149$; Table 6). Age

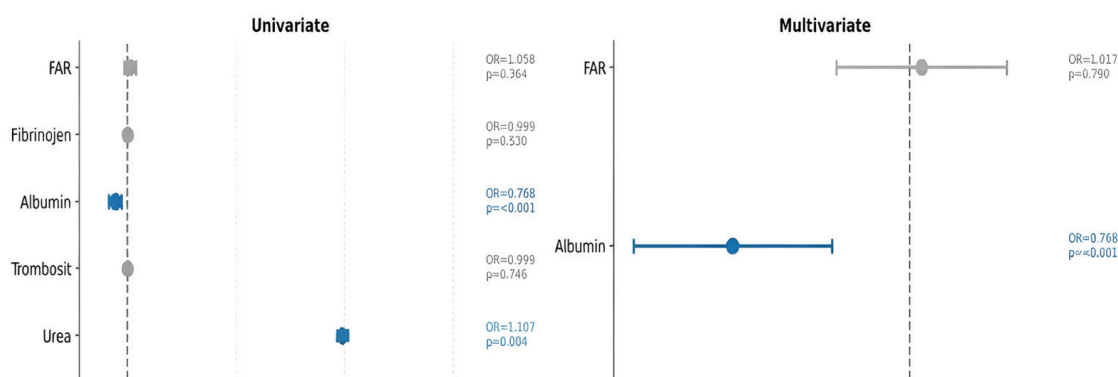


Figure 3. Univariate (left panel) and multivariate (right panel) logistic regression forest plot. Blue dots indicate variables with $P<0.05$, and gray dots indicate non-significant variables. Albumin stands out as the strongest predictor in both analyses.

Table 5. Association Between FAR and SGA

Analysis	P value	Interpretation
FAR, SGA ($n=32$) vs. non-SGA ($n=296$), median	11.95 vs. 11.04; $P = 0.118$ (MWU)	Not significant
FAR $\geq$ SGA (ROC AUC)	0.584	Close to chance level

FAR correlations with birth weight and birth-weight percentile are presented in Table 2.

MWU, Mann-Whitney U test; AUC, area under the curve; FAR, fibrinogen-to-albumin ratio; SGA, small for gestational age; ROC, receiver operating characteristic.

Table 6. Association of Maternal Age with FAR and SGA

Analysis	Value/statistic	P value	Interpretation
Age-FAR (all patients)	$r = -0.007$ ($n=360$)	0.894	Not significant
Age-FAR (control group)	$r = -0.023$ ($n=330$)	0.683	Not significant
Age-FAR (PE group)	$r = 0.102$ ($n=30$)	0.592	Not significant
Age-birth weight percentile	$r = 0.041$ ($n=328$)	0.457	Not significant
Age, SGA vs. non-SGA (median, MWU)	26.0 vs. 28.0 years	0.149	Not significant
Age $\geq$ SGA (logistic regression)	OR = 0.954 (0.891-1.023)	0.185	Not significant
Age $\geq$ SGA (ROC AUC)	0.423	-	Inverse association (AUC < 0.5)
Advanced age (≥ 35) vs. SGA (Fisher)	2/56 (3.6%) vs. 30/272 (11.0%)	0.134	Not significant

Correlations by Spearman; MWU, Mann-Whitney U; Fisher, Fisher's Exact test. Advanced-age counts in the SGA rows refer to the birth-weight-available subset ($n=328$); in the whole sample ($n=360$) the ≥ 35 -year group was $n=63$.

SGA, small for gestational age; FAR, fibrinogen-to-albumin ratio; PE, preeclampsia; ROC, receiver operating characteristic; AUC, area under the curve; OR, odds ratio.

was not an independent predictor of SGA (OR= 0.954; 95% CI: 0.891-1.023; $P = 0.185$). ROC analysis showed that age had no clinical utility in predicting SGA (AUC= 0.423; an AUC below 0.5 indicates an inverse association). No significant correlation was found between age and birth weight percentile ($r = 0.041$; $P = 0.457$), and SGA prevalence did not differ between the advanced age and normal age groups (3.6% vs. 11.0%; Fisher $P = 0.134$).

DISCUSSION

In this study, the diagnostic value of FAR for predicting PE was examined using rigorous statistical methods, and the effect of maternal age on FAR and SGA was evaluated in additional analyses.

FAR alone did not demonstrate sufficient diagnostic power (AUC= 0.527; Table 3). The limited performance of FAR is primarily attributable to the absence of a significant difference in fibrinogen levels between the two groups.

Albumin emerged as a consistent predictor across all analyses (univariate OR= 0.768, multivariate OR= 0.768; Table 4). Urea and ALT were also identified as independent predictors in the multivariate model. No significant association was found between FAR and birth weight or SGA (Table 2, Figure 3).

In a retrospective cohort study encompassing 3,249 pregnancies, patients were classified into control, mild, and severe PE groups. The investigators reported that FAR was independently associated with PE and could be a useful biomarker for clinical risk stratification. FAR could also contribute to the planning of delivery management and perinatal care, although the need for larger, prospective, multicenter studies to validate these findings was emphasized.³ One study reported that FAR demonstrated superior diagnostic performance for predicting PE severity compared with other routine laboratory indicators. Because fibrinogen and albumin levels are readily obtained as part of routine biochemical workup, FAR may serve as a practical and cost-effective biomarker in clinical practice.¹ Although FAR levels were slightly higher in the PE group in our study, the difference was not statistically significant ($P = 0.365$), and discriminatory power in ROC analysis limited. These discrepancies may be attributable to differences in patient populations, sample sizes, and study designs.

Studies evaluating FAR for the early prediction of PE, its disease severity, and its association with adverse perinatal outcomes have reported FAR as a promising biomarker. Given its low cost, ease of access, and strong predictive potential, it has been proposed that FAR could be integrated into first-trimester screening protocols alongside existing biomarkers.²

In contrast, our study assessed FAR during the second and third trimesters and investigated the association between FAR measured at more advanced gestational weeks and PE. This represents a different timing compared with previous studies, which predominantly focus on early pregnancy. While our findings suggest that FAR may be associated with PE even at advanced gestational ages, they demonstrate that it does not exhibit strong standalone diagnostic performance. This discrepancy may be explained by temporal variability in FAR throughout pregnancy and by variation in the pathophysiology

of PE across trimesters.

In a study evaluating the diagnostic performance of FAR for predicting PE, which comprised 131 PE cases and 131 control cases, the combination of second-trimester FAR and PLT distribution width (PDW) demonstrated better diagnostic performance than FAR alone. Nevertheless, the investigators emphasized the need for prospective studies to validate these findings.¹⁴ In our study, PDW was not included in the analysis and only FAR was evaluated. The possibility that adding PDW to the model might improve diagnostic performance is consistent with the literature, but this could not be confirmed with our current data and remains an area for investigation in future multivariate prospective studies.

Serum albumin level in cases of PE has been reported to reflect disease severity and to serve as a useful biomarker for determining the timing of delivery.¹⁵ Studies examining the association between serum albumin levels and PE have reported that hypoalbuminemia is associated with increased vascular permeability, systemic inflammation, and endothelial dysfunction; consequently, low albumin levels may correlate with the presence and severity of PE. It has been further emphasized that albumin, a negative acute-phase reactant, decreases during the inflammatory response and may have clinical implications, particularly in hypertensive disorders of pregnancy.^{16,17} In our study, serum albumin levels were significantly lower in the PE group compared with the control group, and albumin was identified as the strongest independent predictor of PE in multivariate analysis (OR= 0.768; $P < 0.001$). These results are consistent with findings reported in the literature and support the notion that albumin is not merely a nutritional indicator but also a potent biomarker reflecting the systemic inflammatory burden and endothelial damage in PE.

In a study evaluating the role of first-trimester Hemoglobin, Albumin, Lymphocyte, PLT (HALP) score and FAR in predicting PE, FAR levels were significantly higher in both severe and non-severe PE cases than in healthy pregnant women.¹⁷ In our study, FAR values were also numerically higher in the PE group than in the control group, although the difference was not statistically significant. However, FAR alone did not demonstrate sufficient discriminatory power in ROC analysis, suggesting that while FAR may be associated with PE, it may not serve as a robust standalone diagnostic marker.

Significantly elevated serum urea levels have been reported in preeclamptic pregnancies compared with normotensive pregnancies.¹⁸ Similarly, in our study, serum urea levels were significantly higher in the PE group than the control group. This finding is consistent with impaired renal function and reduced glomerular filtration rate in PE, which lead to accumulation of nitrogenous waste products; this interpretation is supported by the literature.

In a study evaluating 62 fetal growth restriction (FGR) cases and 62 control cases, first-trimester C-reactive protein, fibrinogen, and FAR levels were significantly higher in the FGR group.¹² In our study, however, no statistically significant association was found between SGA and FAR. This discrepancy may be related to the small number of SGA cases ($n=32$) and the study's overall

limited sample size. Larger multicenter prospective studies are needed to elucidate more clearly the relationship between FAR and SGA.

FAR has been reported as a prognostic marker in hepatocellular carcinoma, where its prognostic value varied according to AST level.¹⁰ Because both fibrinogen and albumin are synthesized in the liver, hepatic dysfunction can affect both proteins and, thereby, the FAR. In our study, the FAR-AST correlation was weak ($r = -0.170$). Although statistically significant, it was of limited clinical relevance and was not in the same direction as the strong positive correlation reported in hepatocellular carcinoma.

Because the number of PE cases in our study was limited and the majority were classified as non-severe, patients were evaluated as an overall PE group. In this context, FAR levels in non-severe PE cases were similar to those in the control group.

Furthermore, in our study, FAR and other laboratory parameters were evaluated primarily during the third trimester, approximately one month prior to delivery, whereas many studies have measured these parameters during the first and second trimesters. This difference in timing may be an important factor explaining the heterogeneity observed across study results.

Study Limitations

The imbalance between the PE group ($n=30$) and the control group ($n=330$) limited the statistical power for FAR analyses. The retrospective study design and the absence of data on the precise timing of measurements represent major limitations. The SGA group ($n=32$) should be interpreted with caution, given the small sample size. Severity classification for PE could not be directly applied because blood pressure data were unavailable; most patients in the PE group had non-severe disease. Because of the small number of events (30 events; approximately 7.5 events per variable), the multivariate model is at risk of overfitting, and the AUCs for the combined model were derived in-sample and are therefore optimistic. The creatinine OR, with its very wide CI, and the ALT discrepancy between the Mann-Whitney and regression analyses reflect the narrow measurement range, possible outliers, and the small number of events, and both should be interpreted with caution. Severe PE cases below 34 weeks of gestation were referred for delivery to a tertiary university hospital with a neonatal intensive care unit, according to the regional referral chain; therefore, gestational age at delivery was unavailable for 11 and birth weight for 12 of the 30 patients in the PE group, resulting in missing delivery data. In addition, the control group included women hospitalized for various obstetric indications (e.g., preterm premature rupture of membranes, trauma, threatened preterm labor), which may themselves affect fibrinogen and albumin levels and thus FAR.

CONCLUSION

The FAR demonstrated limited diagnostic performance as a standalone marker for predicting PE, with an AUC of 0.527 on ROC analysis (Table 3). In contrast, albumin was identified as the strongest independent predictor of PE in all analyses (multivariate OR= 0.768; $P < 0.001$; Table 4), a finding that is consistent with the existing literature.¹⁵

No statistically significant association was found between FAR and birth weight, SGA, maternal age, or birth weight percentile (Tables 2, 5 and 6). Moreover, advanced maternal age (≥ 35 years) had no significant effect on either FAR or SGA (Table 6).

FAR measurements in our study were predominantly obtained during the third trimester or within 3-4 weeks before delivery. This constitutes an important difference from studies in the literature that rely on first- and second-trimester FAR measurements. In light of these findings, it is necessary to compare the clinical value of FAR in predicting PE across pregnancy trimesters. Prospective, multicenter studies comparing second-trimester FAR measurements with peripartum data are particularly recommended.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the Erzincan Binali Yıldırım University Non-Interventional Clinical Research Ethics Committee (decision no.: 485828, date: 29.09.2025).

Informed Consent: Informed consent was waived due to the retrospective nature of the study. Permission to use patient data was obtained from the hospital management.

Footnotes

Author Contributions

Concept Design – Z.G., B.K.; Data Collection or Processing – Z.G.; Analysis or Interpretation – O.A.; Literature Review – Z.G., B.K., O.A.; Writing, Reviewing and Editing – Z.G., B.K.

Declaration of Interests: The authors declare no conflict of interest.

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The Effect of Cinnarizine Pretreatment on Gastric Ischemia-Reperfusion-Induced Oxidative Damage in Rats

Seval Bulut¹, Zeynep Süleyman², Taha Abdulkadir Çoban³, Halis Süleyman¹

¹Department of Medical Pharmacology, Erzincan Binali Yıldırım University Faculty of Medicine, Erzincan, Türkiye

²Department of Internal Medicine Nursing, Erzincan Binali Yıldırım University Faculty of Health Sciences, Erzincan, Türkiye

³Department of Medical Biochemistry, Erzincan Binali Yıldırım University Faculty of Medicine, Erzincan, Türkiye

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ORCID IDs of the authors: S.B. 0000-0003-4992-1241, Z.S. 0000-0003-0128-7990, T.A.Ç. 0000-0003-1711-5499, H.S. 0000-0002-9239-4099.

ABSTRACT

Objective: Gastric ischemia-reperfusion (I/R) injury is a significant clinical condition occurring in settings such as peptic ulcer bleeding, hemorrhagic shock, and surgery. Its pathogenesis involves oxidative imbalance and increased intracellular calcium. Cinnarizine, a calcium channel blocker with antioxidant properties, may exert protective effects. This study investigated the effect of cinnarizine pretreatment on gastric I/R injury.

Methods: Twenty-four male Wistar albino rats were randomly allocated to four groups (n=6): healthy group (HG), sham-operated (SOG), gastric I/R (GIR), and cinnarizine + I/R (C+GIR). The C+GIR group received cinnarizine orally at 10 mg/kg. One hour later, the SOG, GIR, and C+GIR groups were anesthetized with thiopental sodium (25 mg/kg, intraperitoneally). Access to the stomach was achieved via a ~2-2.5 cm midline laparotomy. In the GIR and C+GIR groups, the celiac artery was temporarily occluded to induce 1 hour of ischemia, whereas the SOG underwent no clamping. Reperfusion lasted 3 hours. Euthanasia was performed using thiopental sodium (50 mg/kg, intraperitoneally) and gastric tissues were subsequently excised. These tissues were assessed for malondialdehyde (MDA), total glutathione (tGSH), superoxide dismutase (SOD), and catalase (CAT) levels.

Results: The GIR group demonstrated significantly elevated MDA levels and decreased levels of tGSH, SOD, and CAT compared with the HG group (p<0.001). Conversely, pretreatment with cinnarizine in the C+GIR group markedly lowered MDA levels while maintaining tGSH levels and SOD and CAT activities (p<0.001).

Conclusion: Cinnarizine pretreatment may protect gastric tissue against I/R-induced oxidative damage by preserving redox balance.

Keywords: Cinnarizine, ischemia-reperfusion, oxidative stress, antioxidant

INTRODUCTION

Gastric ischemia-reperfusion (I/R)-induced injury is a clinically significant condition encountered in various pathological and surgical contexts, including peptic ulcer disease, severe blood loss, operative procedures.¹ I/R injury has been described as a stepwise process that begins with hypoxic damage and is exacerbated by the overproduction of reactive oxygen species

(ROS).² Reduced or interrupted blood flow causes cells to shift toward anaerobic metabolism to produce adenosine triphosphate (ATP), resulting in intracellular acidosis and energy depletion.^{2,3} In addition, impairment of ATPase-dependent ion transport disrupts intracellular calcium (Ca²⁺) homeostasis, leading to Ca²⁺ overload.³ Elevated intracellular Ca²⁺ levels are closely associated with the activation of



Corresponding author: Seval Bulut, E-mail: sevalbulut2010@hotmail.com

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apoptotic signaling pathways, Ca^{2+} -dependent proteolytic enzymes, and ultimately cellular injury.^{2,4,5} Reperfusion, although essential for restoring oxygen supply to ischemic tissues, paradoxically exacerbates tissue damage.⁶ This phenomenon is largely attributed to the accumulation of hypoxanthine and xanthine oxidase during the ischemic phase.² Upon reperfusion, tissue reoxygenation activates xanthine oxidase-mediated metabolism of hypoxanthine, resulting in excessive ROS production.^{3,7} When ROS generation exceeds the capacity of endogenous antioxidant defense systems, oxidative stress develops.⁶ Consequently, excessive ROS targets various biomolecules, including membrane lipids, proteins, and deoxyribonucleic acid, leading to cellular damage.⁶ Experimental studies have demonstrated increased levels of the oxidant marker malondialdehyde (MDA) and a decline in endogenous antioxidant defenses, such as glutathione (GSH) and superoxide dismutase (SOD), in gastric tissues exposed to I/R injury in rats.^{1,8} Previous research has reported that gastric I/R injury is associated with elevated intracellular Ca^{2+} levels, which contribute to the development of acute mucosal lesions.⁹ Given the underlying mechanisms of I/R injury, current evidence suggests that pharmacological agents possessing antioxidant and Ca^{2+} channel-blocking activities may confer protection against gastric damage induced by I/R. Cinnarizine, a piperazine-based compound examined in this study for gastric I/R-related effects, possesses antihistaminic, antioxidant, antiserotonergic, antidopaminergic, and Ca^{2+} channel-blocking properties.¹⁰ It is used primarily to treat nausea, vomiting, and vertigo associated with vestibular disorders.^{10,11} Experimental trials have indicated that cinnarizine exerts protective effects against oxidative stress by limiting MDA elevation and preserving GSH levels in various tissues.¹² Accumulating evidence indicates that lipophilic Ca^{2+} channel blockers, such as cinnarizine, may inhibit lipid peroxidation in cellular membranes.^{11,13} In addition, cinnarizine has been reported to prevent oxidative cell death by inhibiting intracellular Ca^{2+} influx.¹³ Moreover, it has been shown to reduce indomethacin-induced gastric lesions by improving mucosal blood flow.¹⁴ These findings suggest that cinnarizine may have therapeutic or preventive effects against I/R-induced gastric injury. Despite the growing interest in pharmacological modulation of ischemia-reperfusion injury, no direct evidence is available regarding the effects of cinnarizine in gastric I/R models. For this reason, we aimed to examine the effect of cinnarizine pretreatment on the extent of gastric damage induced by I/R.

MAIN POINTS

- Gastric ischemia-reperfusion (I/R) increased malondialdehyde and decreased total glutathione, superoxide dismutase and catalase levels.
- Cinnarizine pretreatment attenuated oxidative stress in gastric I/R.
- Cinnarizine preserved the endogenous antioxidant defense system.
- Cinnarizine may have therapeutic potential against gastric I/R injury.

MATERIALS AND METHODS

Animals

A total of twenty-four male Wistar albino rats (250-270 g) were procured from the Experimental Animals Application and Research Center of Erzincan Binali Yıldırım University, Türkiye. Before experimental procedures, all animals were maintained under standardized laboratory conditions and subjected to a 7-day acclimatization period to minimize environmental stress. After adaptation, rats were randomly assigned to four groups. Throughout the study, animals were housed under controlled environmental conditions, including a temperature of $22 \pm 2^\circ\text{C}$, relative humidity of 45-65%, and a 12-hour light/dark cycle. Standard laboratory chow and tap water were supplied ad libitum. All experimental procedures were carried out in the same institutional animal research facility. All experimental procedures were carried out following approval from the Erzincan Binali Yıldırım University Local Ethics Committee for Animal Experiments (date: 25.12.2025, decision no.: 66).

Experimental Groups

An equal number of rats ($n = 6$) were randomly assigned to each of the four experimental groups: healthy group (HG), sham-operated group (SOG), I/R group (GIR), and cinnarizine-pretreated GIR (C+GIR).

Chemicals

Cinnarizine (Nobel Pharmaceuticals, Düzce, Türkiye) and thiopental sodium (I.E. Ulagay, İstanbul, Türkiye) were used in this study.

Experimental Design

Prior to the experimental procedures, all rats were fasted for 12 hours with free access to water. After completion of the fasting period, animals in the C+IRG group received cinnarizine (10 mg/kg) by oral gavage. The dose of cinnarizine was determined based on previously published studies in rats demonstrating its gastroprotective effects.¹⁴ The HG, SOG, and GIR groups each received equivalent volumes of physiological saline via the same route. One hour after administration of cinnarizine or physiological saline, rats in the SOG, GIR, and C+GIR groups were anesthetized with thiopental sodium (25 mg/kg, intraperitoneally). Under anesthesia, a 2-2.5 cm midline abdominal incision was made to access the stomach. Meanwhile, in the SOG group, no manipulation of the celiac artery was performed, and the surgical incision was closed with sutures. In the GIR and C+GIR groups, following the 1-hour ischemic period, the vascular clips were removed, and a 3-hour reperfusion phase was initiated in the gastric tissue.¹ Upon completion of the protocol, all animals were euthanized by intraperitoneal injection of a lethal dose of thiopental sodium (50 mg/kg). Levels of MDA, tGSH, SOD, and catalase (CAT) were determined in the harvested tissues.

Biochemical Analyses

Sample Preparation

Gastric samples were rapidly frozen in liquid nitrogen and pulverized to obtain a fine powder. The powdered tissues were homogenized on ice in 50 mM phosphate buffer (pH 7.4) using a 1:10 (w/v) dilution. Subsequently, centrifugation was carried out at $10,000 \times g$ for 10 minutes under refrigerated conditions (4°C). Following centrifugation, the supernatants were carefully collected and stored for biochemical analyses. Prior to analysis, all samples were stored at -80°C .

Analysis of MDA, tGSH, SOD, and CAT in Gastric Tissues

Gastric tissue levels of MDA (Cat. No. 10009055), GSH (Cat. No. 703002), and SOD (Cat. No. 706002) were measured using rat-specific enzyme-linked immunosorbent assay kits obtained from Cayman Chemical Company (Ann Arbor, MI, USA). All experimental procedures were performed in strict accordance with the manufacturer's guidelines. CAT activity in tissue samples was determined spectrophotometrically following the method described by Góth.¹⁵ The Bradford¹⁶ technique was employed to quantify total protein levels in the tissues for normalization purposes.

Statistical Analysis

Data analysis was carried out using IBM SPSS Statistics (version 27, IBM Corp., Armonk, NY, USA), while graphical outputs were prepared with GraphPad Prism (version 9, GraphPad Software, San Diego, CA, USA). Results are expressed as mean $\pm$ standard deviation. Prior to statistical comparisons, the data distribution was evaluated using the Shapiro-Wilk test to verify normality. Since all variables met the assumption of normality, intergroup differences were examined using One-Way analysis of variance. The assumption of homogeneity of variance was checked with Levene's test, and multiple comparisons were subsequently performed using Tukey's post-hoc test. Statistical significance was defined at a threshold of $P < 0.05$.

RESULTS

Analysis of MDA Levels in Gastric Tissues

HG and SOG exhibited comparable MDA levels, with no statistically significant difference (Figure 1, Table 1; $P = 0.944$). In contrast, MDA levels were significantly elevated in the GIR

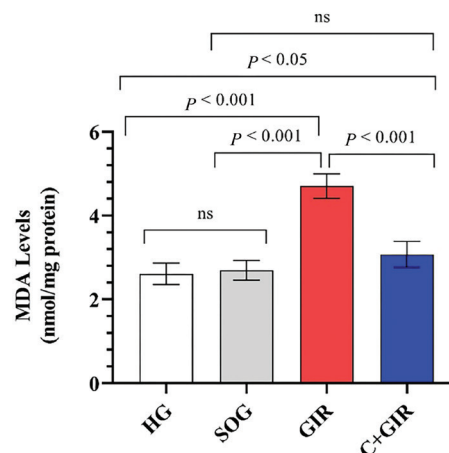


Figure 1. Effects of cinnarizine on MDA levels in gastric I/R injury. Bars represent mean $\pm$ standard deviation, $n = 6$ /each group. Statistical analysis was performed using One-Way ANOVA followed by Tukey's HSD post-hoc test. A P value < 0.05 was considered statistically significant.

Ns, non-significant; MDA, malondialdehyde; HG, healthy control; SOG, sham-operated group; GIR, ischemia-reperfusion group; C+GIR, cinnarizine pretreated ischemia-reperfusion group; I/R, ischemia-reperfusion.

compared with those in both the HG and SOG ($P < 0.001$). On the other hand, cinnarizine pretreatment in the C+GIR significantly attenuated the I/R-induced increase in MDA levels ($P < 0.001$). Following cinnarizine pretreatment, MDA levels in the C+GIR approached those observed in the HG ($P = 0.042$) and the SOG ($P = 0.125$).

Analysis of tGSH Levels in Gastric Tissues

As presented in Figure 2 and Table 1, no significant difference in tGSH levels was observed between the HG and SOG ($P = 0.445$). A marked reduction in tGSH content was observed in the GIR compared with both control groups (HG and SOG) ($P < 0.001$). Cinnarizine pretreatment effectively counteracted this ischemia-reperfusion-induced depletion of tGSH ($P < 0.001$). In the C+GIR group, tGSH values were restored toward those of the HG ($P = 0.092$) and SOG ($P = 0.768$).

Table 1. Effects of Cinnarizine on Oxidative Stress Parameters in Gastric Ischemia-Reperfusion Injury

Biochemical Variables		Experimental groups (n=6 /each group)			
		HG	SOG	GIR	C + GIR
		Mean ± SD			
Gastric tissues	MDA (nmol/mg protein)	2.61 ± 0.26 ^a	2.70 ± 0.24 ^{ac}	4.70 ± 0.29 ^b	3.07 ± 0.31 ^c
	tGSH (nmol/mg protein)	5.61 ± 0.42 ^a	5.34 ± 0.22 ^a	2.53 ± 0.21 ^b	5.17 ± 0.33 ^a
	SOD (U/mg protein)	6.80 ± 0.30 ^a	6.55 ± 0.37 ^a	2.89 ± 0.29 ^b	5.44 ± 0.30 ^c
	CAT (U/mg protein)	5.96 ± 0.27 ^a	5.84 ± 0.20 ^a	3.62 ± 0.26 ^b	5.32 ± 0.22 ^c

^{a, b, c}: Within the same row, groups sharing the same letter are not significantly different ($P > 0.05$), whereas groups with different letters differ significantly ($P < 0.05$). Statistical analysis was performed using One-Way ANOVA followed by Tukey's HSD post-hoc test. A P value < 0.05 was considered statistically significant. SD, standard deviation; MDA, malondialdehyde; tGSH, total glutathione; SOD, superoxide dismutase; CAT, catalase; HG, healthy control; SOG, sham-operated group; GIR, ischemia-reperfusion group; C+GIR, cinnarizine pretreated ischemia-reperfusion group.

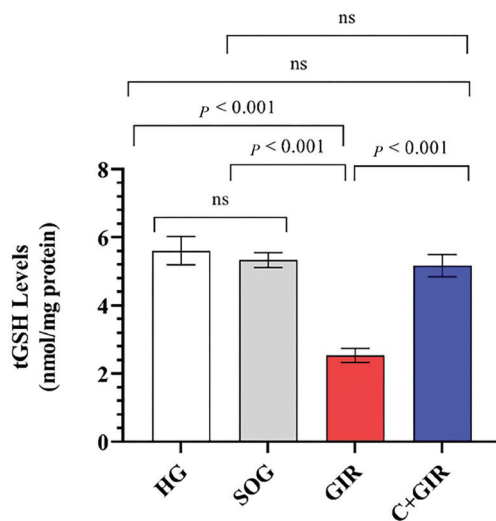


Figure 2. Effects of cinnarizine on tGSH levels in gastric I/R Injury. Bars represent mean $\pm$ standard deviation, $n = 6$ /each group. Statistical analysis was performed using One-Way ANOVA followed by Tukey's HSD post-hoc test. A P value < 0.05 was considered statistically significant.

ns, non-significant; tGSH, total glutathione; HG, healthy control; SOG, sham-operated group; GIR, ischemia-reperfusion group; C +GIR, cinnarizine pretreated ischemia-reperfusion group; I/R, ischemia-reperfusion.

Analysis of SOD and CAT Activities in Gastric Tissues

As shown in Figure 3A-B and Table 1, no statistically significant differences in SOD ($P = 0.542$) or CAT ($P = 0.845$) activities were observed between the HG and SOG. In contrast, both SOD and CAT were reduced in the GIR compared with the HG and SOG

($P < 0.001$). Cinnarizine pretreatment markedly attenuated this I/R-induced decline, leading to a significant improvement in antioxidant enzyme activities ($P < 0.001$). However, SOD and CAT in the C+GIR still differed significantly from those observed in the HG and SOG ($P < 0.05$).

DISCUSSION

In rats subjected to gastric I/R injury, the effect of cinnarizine on tissue protection was examined. Exposure to I/R alone resulted in higher MDA levels in gastric tissue, whereas tGSH content and activities of SOD and CAT were markedly reduced. These alterations indicate enhanced lipid peroxidation and suppression of the endogenous antioxidant defense system. In the cinnarizine + I/R group, oxidative stress-related alterations were substantially mitigated. Although both groups underwent the same ischemic insult, lower levels of oxidative damage markers were observed in the cinnarizine-treated group, suggesting a potential protective effect against I/R-induced tissue injury.

The findings of the current trial indicate that oxidative stress plays a central role in the development of gastric I/R injury. In tissues exposed to I/R, excessive ROS production occurs during both ischemia and, particularly, during reperfusion. This leads to the activation of lipid peroxidation, ultimately resulting in the disruption of cellular membrane integrity.¹⁷ MDA, a key end product of lipid peroxidation, is closely associated with cellular damage and cell death. Previous studies have demonstrated increased MDA levels in gastric tissues affected by I/R injury.^{8,18} I/R-induced augmentation of MDA levels in gastric tissues was confirmed, whereas cinnarizine treatment significantly suppressed this increase. To the best of our knowledge, The impact of cinnarizine on gastric MDA levels has not been directly addressed in the literature. However, cinnarizine has

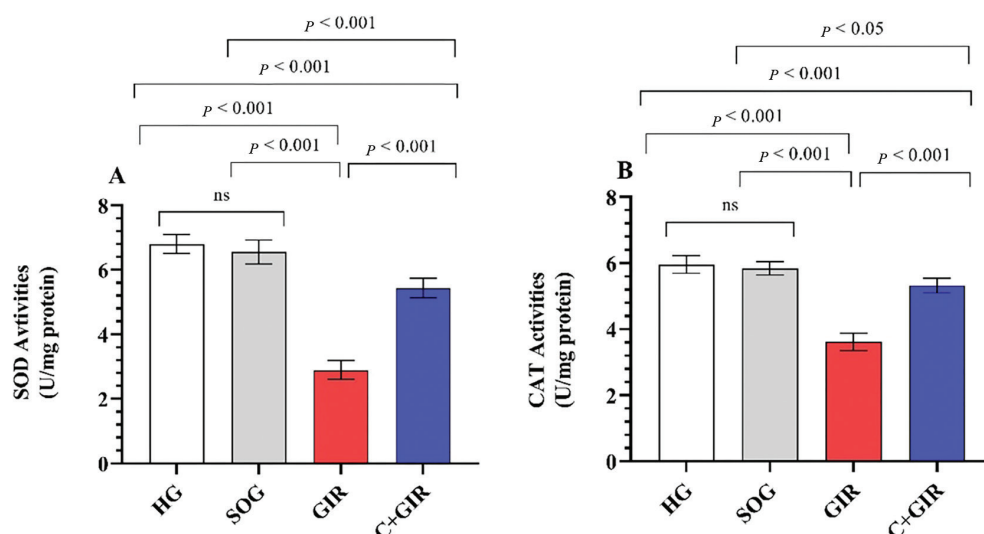


Figure 3A, B. Effects of cinnarizine on SOD and CAT activities in gastric I/R Injury. Bars represent mean $\pm$ standard deviation, $n = 6$ /each group. Statistical analysis was performed using one-way ANOVA followed by Tukey's HSD post-hoc test. A P value < 0.05 was considered statistically significant.

ns, non-significant; SOD, superoxide dismutase; CAT, catalase; HG, healthy control; SOG, sham-operated group; GIR, ischemia-reperfusion group; C +GIR, cinnarizine pretreated ischemia-reperfusion group; I/R, ischemia-reperfusion.

been reported to significantly reduce naphthalene-induced increases in MDA levels in liver and lung tissues as well as in plasma.¹⁹ The extent of tissue injury and the maintenance of cellular viability during I/R exposure largely depend on the capacity of endogenous antioxidant defense systems. When the excessive production of ROS cannot be adequately neutralized by antioxidant mechanisms, oxidative stress develops, leading to subsequent cellular damage.²⁰ Therefore, in the present study, gastric tissues were also evaluated for endogenous antioxidants, including tGSH, SOD, and CAT.

GSH is a major endogenous antioxidant synthesized in mammalian cells.⁸ It contributes to the maintenance of cellular redox homeostasis by directly reacting with ROS and serving as a cofactor in antioxidant enzyme systems.²¹ Previous studies have reported that I/R injury induces a marked reduction in GSH levels in gastric tissues as a consequence of increased oxidative stress and excessive ROS production.^{7,8} In the present study, gastric tGSH levels were significantly decreased following I/R, whereas cinnarizine treatment effectively prevented this decline. Despite the lack of direct evidence regarding the effects of cinnarizine on gastric GSH levels, cinnarizine has been reported to reverse GSH depletion in lung tissue in an experimental model of asthma, supporting its modulatory role in antioxidant defense systems.²²

Gastric tissues were further evaluated for SOD and CAT activities. SOD and CAT are sequential and complementary antioxidant enzymes. SOD acts on superoxide radicals by converting them into hydrogen peroxide and oxygen. This intermediate is subsequently broken down by CAT into water and oxygen, thereby preventing the generation of highly reactive hydroxyl radicals.²³ Experimental studies have shown that gastric I/R injury significantly reduces SOD and CAT activities, thereby weakening the antioxidant defense capacity of the tissue against oxidative stress.^{1,24} Consistent with the literature, the present findings demonstrated a decrease in SOD and CAT following I/R in gastric tissues. However, cinnarizine pretreatment appeared to be effective in preserving SOD and CAT activities despite I/R exposure. Existing literature indicates that no studies have evaluated the effects of cinnarizine on gastric SOD and CAT activities. Nevertheless, cinnarizine has been reported to prevent the reduction in SOD activity in lung tissue in an experimental model of asthma.²² In addition, flunarizine, a Ca²⁺ channel antagonist, has been shown to significantly attenuate I/R-induced reductions in gastric SOD activity. Its gastroprotective effect has been associated with its antioxidant properties and inhibition of cytoplasmic Ca²⁺ accumulation.²⁵

Study Limitations

This study has several limitations. Inflammatory cytokines, apoptotic markers, mitochondrial function parameters, and molecular Ca²⁺-signaling pathways were not evaluated. The absence of histopathological assessment is another limitation of this study. Furthermore, the use of a single dose and a single reperfusion time point limits the ability to determine dose-response relationships and time-dependent effects. Future studies employing different doses and varying reperfusion

durations may provide further insight into the optimal protective dose range of cinnarizine and the temporal profile of its effects.

CONCLUSION

A rat model of gastric I/R injury was used to investigate the protective effects of cinnarizine. The findings demonstrated that exposure to I/R resulted in a significant increase in the oxidant marker MDA in gastric tissues, accompanied by a marked reduction in key components of the endogenous antioxidant defense system, namely tGSH, SOD, and CAT. In contrast, cinnarizine-treated rats exhibited a significant attenuation of the I/R-induced increase in MDA levels and substantially prevented decreases in tGSH, SOD, and CAT activities. These results indicate that cinnarizine is effective in reducing oxidative stress and supporting the endogenous antioxidant defense system in gastric I/R injury.

Ethics

Ethics Committee Approval: All experimental procedures were carried out following approval by the Erzincan Binali Yıldırım University Local Ethics Committee for Animal Experiments (decision no.: 66, date: 25.12.2025).

Informed Consent: Since this is an animal experiment study, patient consent is not required.

Footnotes

Author Contributions: Concept Design – S.B., H.S.; Data Collection or Processing – S.B., T.A.Ç.; Analysis or Interpretation – S.B., T.A.Ç.; Literature Review – S.B., Z.S.; Writing, Reviewing and Editing – S.B., Z.S., H.S.

Declaration of Interests: The authors declare no conflict of interest.

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Big Evidence with Small Samples: A Methodological Perspective on N-of-1 (Single Case Experimental Designs) Based on Medical Studies

 Mehmet Tahir Huyut¹,  Orhan Aydın²

¹Department of Biostatistics and Medical Informatics, Erzincan Binali Yıldırım University Faculty of Medicine, Erzincan, Türkiye

²Department of Special Education, Division of Autism Spectrum Disorder, Ankara University Faculty of Educational Sciences, Ankara, Türkiye

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ORCID IDs of the authors: M.T.H. 0000-0002-2564-991X, O.A. 0000-0002-9695-2414.

ABSTRACT

N-of-1 or single-case experimental designs (SCEDs) are gaining renewed importance as robust methodological approaches for assessing individualized treatment response in modern medicine. This overview aims to present the methodological foundation of SCEDs, its applications in medical research, and its position within evidence-based medicine from a disciplinary perspective. This study evaluates the advantages, limitations, and emerging synthetic approaches to the method through a review of the current literature and an examination of selected clinical N-of-1 applications. Findings indicate that SCEDs provide high internal validity, particularly when treatment response varies among individuals, and allow for strong statistical inferences even with small samples. However, methodological limitations, including limited external validity, measurement burden, and carry-over effects, underscore the importance of combining multiple N-of-1 trials using hierarchical models. In recent years, the literature has emphasized that the “large evidence with small samples” approach is preferable from both ethical and methodological perspectives, forming the basis for personalized treatment decisions. This review provides a comprehensive framework for applying SCEDs in medical research and suggests that multiple N-of-1 syntheses will be the primary driver of population-level inference. Consequently, SCEDs can be considered a powerful component of modern medicine because they prioritize individual response over the average patient in clinical decision-making.

Keywords: N-of-1 trials, single-case experimental designs, evidence-based medicine, personalized medicine, small-sample Inference, individual treatment response

INTRODUCTION

1. Epistemological Foundations: The Crisis of the “Average Patient” and the Need for Personalized Evidence

The gold standard of *modern medicine*, evidence-based medicine (EBM), primarily relies on randomized controlled trials (RCTs) that utilize a nomothetic approach, aiming to discover general laws applicable to populations.^{1,2} However, this “average patient” paradigm creates an epistemological gap when applied to the individual in clinical practice.³ While RCTs provide high external validity for populations, they often

mask inter-individual variability, leading to the “tyranny of the average”.^{4,5} In medical research, the tension between the nomothetic (population-based) and idiographic (individual-based) approaches has sparked significant criticism regarding the clinical utility of aggregate data.^{6,7}

Traditional EBM often struggles to address heterogeneous treatment effects, in which a medication that benefits the majority may be ineffective or even harmful to an individual.⁸⁻¹⁰ This limitation necessitates a shift toward a more nuanced understanding of evidence, one that prioritizes the unique



Corresponding author: Mehmet Tahir Huyut, **E-mail:** mehmettahirhuyut@gmail.com

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biological and clinical context of the single patient.¹¹ The search for individual-specific evidence is not a rejection of EBM, but rather an evolution toward a more precise and person-centered methodology.¹²

2. The Bridging Role of SCEDs: Harmonizing Clinical Practice and Scientific Rigor

Single-case experimental designs (SCEDs), or N-of-1 trials, emerge as a critical bridge between the rigor of experimental research and the reality of clinical practice.^{13,14} Unlike anecdotal case reports, SCEDs utilize systematic manipulation, repeated measurements, and baseline comparisons to establish causal relationships within a single unit.^{15,16} This methodology allows the clinician to act as a researcher, transforming routine care into a disciplined scientific inquiry.¹⁷

By integrating the idiographic focus on the individual with the nomothetic requirement for rigorous control, SCEDs resolve the historical tension in medical knowledge acquisition.^{18,19} They provide a structured framework for assessing treatment efficacy in conditions in which traditional RCTs are impractical, such as rare diseases or highly personalized interventions.²⁰ Consequently, SCEDs function as a methodological cornerstone, ensuring that the evidence used in clinical decision-making is both scientifically robust and directly relevant to the patient being treated.²¹

MAIN POINTS

- N-of-1 (single-case experimental) designs provide a rigorous methodological framework for evaluating individualized treatment response. These designs offer high internal validity in situations where inter-individual heterogeneity limits the applicability of group-based evidence.
- Single-case experimental designs (SCEDs) act as a methodological bridge between nomothetic (population level) and idiographic (individual level) evidence. These approaches allow for causal inference at the patient level while remaining compatible with aggregate and hierarchical synthesis.
- Through repeated measurements and systematic manipulation of experimental phases, SCEDs enable the detection of clinically meaningful change at the individual level. This structure allows robust effect estimation even in small samples, supporting the concept of “big evidence from small samples.”
- Methodological challenges such as carry-over effects, autocorrelation, and limited external validity represent key analytical concerns in N-of-1 studies. These challenges can be effectively addressed through randomization, washout periods, time-series modeling, and Bayesian or meta-analytic aggregation of multiple single-case trials.
- SCEDs offer a practical and ethically sound approach for implementing personalized medicine in clinical research and practice. This is particularly relevant for chronic, heterogeneous, or treatment-resistant conditions, where individual response patterns are more informative than population averages.

3. The Role of SCEDs Research Today

SCEDs research has gained renewed importance in recent years, particularly in fields such as personalized medicine, pharmacogenetics, rehabilitation, behavioral medicine, and neuropsychology. The current research paradigm has moved beyond the classical RCT, which emphasizes average group effects, to understanding the variability of individual responses.^{4,22} In this context, SCEDs are considered a “self-randomized experiment”.²¹

In personalized medicine and pharmacogenetics, treatment response varies depending on genetic, biochemical, and environmental factors.²² This variability makes traditional RCT results based on group averages insufficient for clinical decision-making. The SCEDs approach allows direct testing of treatment efficacy in a single individual, thereby contributing to personalized treatment optimization. For example, the use of N-of-1 protocols is recommended to optimize drug responses in diseases with heterogeneous courses, such as chronic pain, hypertension, and major depression.²⁰⁻²² Such studies allow for the systematic testing of variables such as patient-specific doses, drug combinations, or treatment duration. Recently, N-of-1 studies have begun to be addressed not only at the individual level but also at the aggregate level. “Meta-analytic N-of-1 syntheses,” which combine studies with multiple SCEDs, have made it possible to produce generalizable findings at the group level by combining individual evidence.^{7,9,14,15,23} This approach is considered a methodological revolution, with the potential to simultaneously examine both individual efficacy and within-population variation. Particularly with the development of Bayesian hierarchical approaches in statistical modeling, aggregating studies with SCEDs has become both more powerful and more reliable.²⁴

Furthermore, the development of digital health technologies and artificial intelligence-based data analysis methods has made it possible to collect and analyze data at the individual level. Owing to mobile health applications, wearable sensors, and remote monitoring systems, the applicability of N-of-1 protocols in daily clinical practice has significantly increased.²⁵ Artificial intelligence algorithms can be used to automatically detect trends, predict efficacy, and generate personalized treatment recommendations from individual behavioral and physiological data streams.²⁶ These developments enable SCEDs to function not only as a research methodology but also as a clinical decision support system.

As a result, SCEDs have become a crucial component of the contemporary scientific paradigm, which is centered on individual heterogeneity. As a complement to classical group studies, SCEDs offer a new epistemological framework based on the principle of “evidence from the individual, not the population.” This approach is shaping the future of micro-level evidence-based practices in both clinical science and behavioral research.

4. Experimental Control Methods with SCEDs

The primary goal of SCEDs is to demonstrate the causal effect of an independent variable (treatment, medication, behavioral intervention, etc.) on a specific individual. To this end, the

participant serves as an experimental control, thus minimizing confounding effects arising from interindividual variation.¹⁶

Experimental control is achieved through systematically organized phases, ensuring a prediction-verification-replication pattern. The following section addresses design types in SCEDs. The following section addresses the use of randomization techniques in SCEDs, the control of carry-over bias and autocorrelation, and procedures for ensuring measurement reliability.

4.1. Types of SCEDs

The most commonly used methods for providing experimental control in SCEDs are interrupted time-series designs, which are structured around the presence or absence of an independent variable across specific phases. The main designs are as follows:

AB Design

It is the simplest form, comprising a baseline phase (A) and an intervention phase (B). However, it is limited in its ability to establish causality because it cannot be used to test effects with or without the intervention.^{15,16} Figure 1 shows the basic AB design, which is a quasi-experimental design. It provides only estimated information about the effect of the intervention.

Graphics features:

- X-axis: Sessions/Time/Measurement
- Y-axis: Dependent variable (e.g., pain score)
- Vertical line: Phase change/transitions (A→B)
- *Comment:* The intervention performed in Phase B resulted in a decrease in the patient's pain score.

However, this provides only a predictive *inference* regarding the intervention's effectiveness. To empirically support a strong hypothesis of the intervention's effectiveness, the intervention must be examined through the dimensions of "verification" and "replication."

- *Note:* Data lines and the forecast display line are used here for ease of understanding. They are not typically displayed in studies.

ABAB (Reversal/Withdrawal) Design

The sequence A (baseline), B (intervention), A (withdrawal of the intervention), B (re-intervention) is used to test the intervention effect. If the behavior or outcome variable

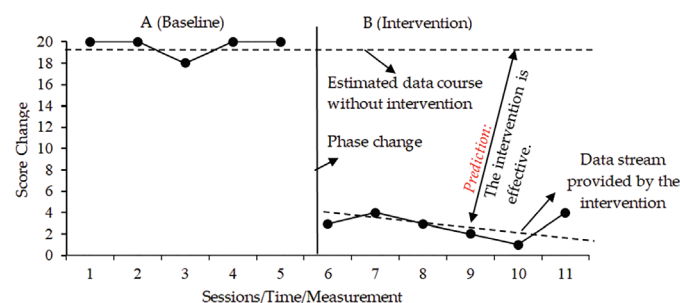


Figure 1. Basic AB design.

increases during the intervention period, returns to its previous level during the withdrawal period, and changes again during the re-intervention, this provides strong evidence for a causal relationship.^{16,27-29} Figure 2 illustrates the ABAB design and provides information on its use as an experimental design for reversible measures (e.g., blood sugar and blood pressure levels). It provides information about the intervention's effect through the dimensions of prediction, verification, and replication, and offers functional evidence of that effect.

Comment: Measured values systematically during the B phases. Since the values worsen in the A phases, where no intervention occurs and only measurements are taken, demonstrating the effectiveness of the intervention through the prediction-confirmation-replication triad establishes a strong causal relationship

Multiple Baseline/Probe Design

The intervention is initiated gradually across different behaviors, settings, or individuals. This provides a powerful alternative experimental control in situations where withdrawal is unethical (e.g., in medical or educational settings).¹⁹ Figure 3 illustrates the multiple-baseline design, which provides an experimental framework applicable to both reversible and non-reversible clinical outcomes. Reversible measures include physiological parameters (e.g., arterial blood pressure, blood glucose, heart rate), biochemical and hormonal indicators (e.g., thyroid hormone levels, inflammatory biomarkers, electrolytes), symptom-based assessments (e.g., pain intensity, anxiety or stress scores, fatigue levels), performance or functional measures (e.g., gait speed, cognitive reaction time, attentional performance), and daily-life indicators (e.g., sleep duration and quality, physical activity levels). The design is also suitable for non-reversible outcomes, such as axonal loss in multiple sclerosis, tumor size progression, manifestations of neurodegenerative diseases, and clinical worsening of congestive heart failure. In this design, the effect of the intervention is evaluated, compared with conditions where no intervention has been implemented, using the dimensions of prediction, confirmation, and replication to seek functional evidence. The key difference between the multiple probe and the multiple baseline designs is that, in the multiple probe design, measurements taken in Phase A are spaced. For example, if measurements are not taken in sessions 6-7 for the second and third graphs in Figure 2, and in session 9 for the third graph, this would constitute a variation of the multiple probe design.

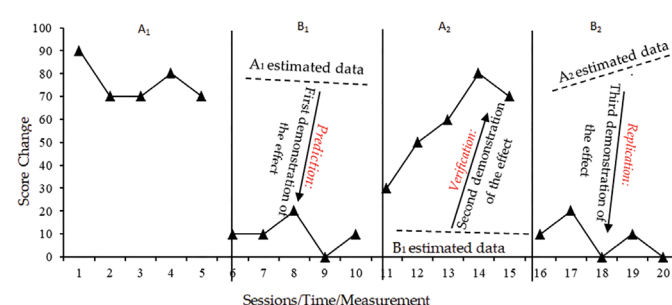


Figure 2. ABAB design.

Comment: In Figure 3, three participants are depicted in a stacked multiple-baseline design in which the intervention is introduced at different time points. In the upper panel, an improvement in the outcome is observed immediately following the onset of the intervention phase, whereas the remaining two participants continue to exhibit low, stable values, as reflected by ongoing A-phase measurements. This pattern provides preliminary prediction-level evidence regarding the potential effectiveness of the intervention. When the intervention is subsequently introduced to the participant in the middle panel, a similar improvement emerges, whereas the participant in the lower panel—who has not yet received the intervention—continues to display unfavorable trends. This provides confirmatory evidence supporting the effect of the intervention. Finally, when the intervention is implemented for the third participant in the lower panel, a consistent improvement is again observed, demonstrating replication of the effect. Collectively, this sequential pattern of prediction, confirmation, and replication provides strong experimental evidence for the intervention's impact on the dependent variable.

ABCBC or Multiple Intervention Reversal Designs

Systematic application of different variants of an intervention (e.g., doses or intensity levels) can be used for dose-response analysis.³⁰ Figure 4 illustrates the ABCBC design and provides information on the experimental design for reversible measurements. B and C are compared as separate interventions. While these interventions may have completely different treatment protocols, B and C may represent two variations in the delivery methods of an intervention. This provides information about which intervention is more ethical through prediction, validation, and replication, and supplies empirical evidence that one intervention is functionally more successful than the other.

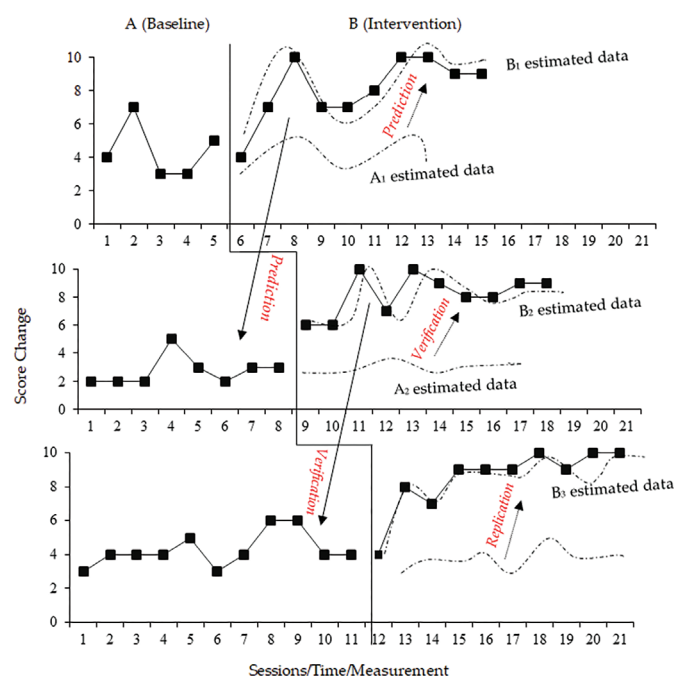


Figure 3. Multiple starting level design.

Alternating Treatment Design (ATD) or Multielement Design

A single-case experimental approach compares two or more interventions (e.g., Drug A and Drug B) through rapid, successive alternations. Randomized sequencing of intervention conditions minimizes carryover effects and allows for a direct comparison of treatment efficacy.⁹ Figure 5 illustrates the structure of the alternating treatment design. In an alternating treatment design, experimental control is established through the systematic divergence of data patterns across conditions as the interventions are presented rapidly in alternating order. At the outset, the researcher predicts that if the interventions truly differ in their effects, the data will begin to separate by condition at the initial measurement points. When such divergence emerges during data collection, the prediction is confirmed. The repeated alternation of conditions (B-C-B-C-...) provides replication by demonstrating the effect of each intervention multiple times across the measurement series. Together, these processes of prediction, confirmation, and replication yield strong experimental control, supporting the conclusion that observed changes in the dependent variable are attributable to the specific intervention delivered.^{16,28,29}

Comment: In Figure 5, interventions B and C are implemented to reduce the desired value. Although baseline values were high, the measured values after interventions B and C were lower than baseline. However, in the initial dataset, intervention C consistently produced lower values than intervention B, indicating greater effectiveness based on predictions in that dataset and confirmed by subsequent validation and replication.

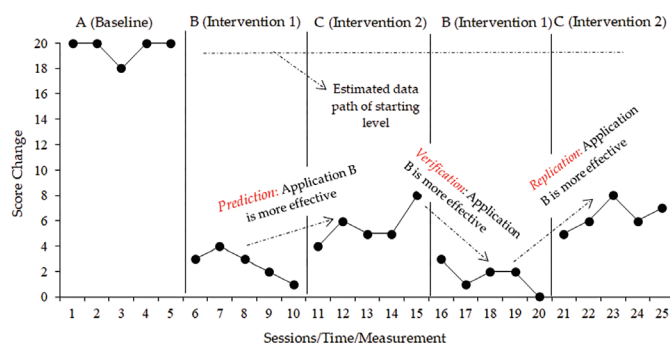


Figure 4. ABCBC design.

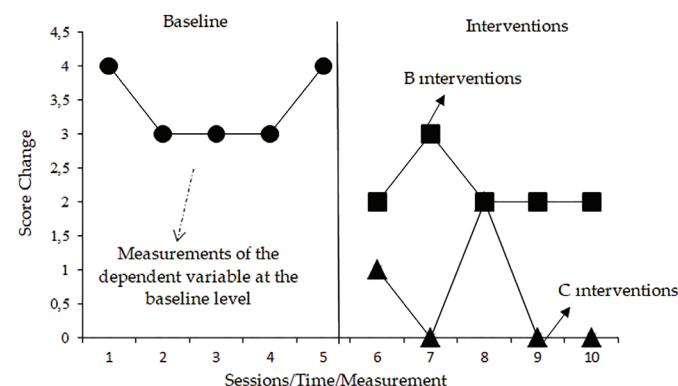


Figure 5. Design of rotational interventions.

4.2. Randomization, Washout, and Carry Over Effects

In modern N-of-1 protocols, randomization and washout periods play a critical role in improving internal validity. Randomizing the order of interventions minimizes expectancy and order effects.²² The washout period between interventions is the expected interval for the physiological or psychological effects of the previous treatment to subside. This is particularly important in pharmacological studies, to control for carry-over effects (the effect of the previous period carrying over into the next period).³¹

4.3. Trend and Autocorrelation Control

SCEDs data are discrete-time series, so issues such as trend (the natural direction of change) and autocorrelation (the dependency between successive observations) are common.^{32,33} This can lead to false-positive results or to an overestimation of the intervention effect.

Therefore, in the analysis:

- Autoregressive integrated moving average (ARIMA) models are used to account for trend and autocorrelation by modeling temporal dependence.³⁴

Alternatively, to determine whether the observed changes are random or not and to assess the statistical significance of the results, randomization tests³⁴ or Bayesian time series models³⁵ are preferred.

4.4. Measurement Reliability and Standardization

A strength of SCEDs is that repeated measurements on the same individual minimize measurement error. However, the following practices are recommended for reliable results:

- *Stable Conditions*: The measurement environment, time period and observer conditions are kept constant.
- *Standard Protocols*: Data collection and application procedures are carried out according to predetermined protocols.

- *Independent observers*: For reliability in observational data, the measurement of the dependent variable is made by at least two independent evaluators.^{29,30}

Application Examples of SCEDs Methods in Medical Research

The ten clinical studies presented in Table 1 were selected using purposive sampling rather than through systematic review. The primary inclusion criteria were: (1) the rigorous application of core SCED design principles (e.g., ABAB/reversal, multiple baseline, or alternating treatments), (2) representation across diverse medical specialties, including oncology, neurology, psychiatry, and chronic disease management and (3) the inclusion of both pharmacological and non-pharmacological interventions. Accordingly, ten illustrative examples of N-of-1 medical studies are summarized in Table 1, with each study described in terms of its objective, participant profile, selected design type, measurement framework (dependent and independent variables), applied intervention, and key outcomes.

These examples were chosen to demonstrate how different SCED architectures can generate high internal validity and support clinical decision-making across a wide range of medical contexts. Collectively, the studies illustrate the versatility of N-of-1 designs, showing their applicability to diverse conditions—from chronic pain and psychiatric disorders to rehabilitation and oncology—and their utility for purposes ranging from optimizing pharmacotherapy to evaluating individualized behavioral interventions. Many of these studies incorporate strategies to strengthen internal validity, such as randomization, washout periods, appropriate SCED selection, and time-series or autocorrelation-adjustment methods (e.g., ARIMA models, randomization tests, or Bayesian approaches).^{2,7} For clinical readers, the primary purpose of presenting these examples is to demonstrate how efficacy evidence can be generated at the individual patient level and how such evidence may inform personalized treatment decisions in real-world practice (Table1).

Table 1. The following tables contain brief, standardized summaries of each of the 10 sample clinical trials (purpose, design, measurements, intervention, outcome, and role of SCEDs in the study)

Example study	Study characteristics	Explanation
Effectiveness of transcranial direct current stimulation in chronic daily headaches: an experimental single case report with a novel protocol Rahimi et al., 2022 ³⁵	Objective	To evaluate the efficacy of tDCS for chronic daily headache at the individual level
	Participants	Adult female patient diagnosed with chronic headache
	Design	ABAB design
	Measures/dependent variables	Daily pain scores (VAS), pain intensity and diary records, frequency of analgesic use
	Intervention/independent variables	tDCS application (10-session protocol; separated by washout periods, 2 mA, 20 min, targeting dorsolateral prefrontal cortex)
	Outcomes	Pain intensity significantly decreased during tDCS periods, with reduced analgesic use
	Role of SCEDs	Allowed direct observation of neuromodulation effects at the individual level, guiding personalized treatment

Table 1. Continued

Example study	Study characteristics	Explanation
N-of-1 trials in cancer drug development. Cancer Discovery	Objective	To examine the effects of combined adaptive immunotherapy and chemotherapy on cancer progression at the individual level
	Participants	Adult male patient with advanced solid tumor
	Design	AB design
	Measures/dependent variables	Tumor size, immune cell profile (CD8 ⁺ , Treg), quality of life, clinical response, immune activation markers
	Intervention/independent variables	Immunotherapy + chemotherapy combination (adaptive dosing and cyclic chemotherapy regimen)
	Outcomes	Tumor regression, increased CD8 ⁺ proportion, and well-tolerated treatment course
	Role of SCEDs	Enabled individual response monitoring and dynamic dose optimization, illustrating a personalized oncology approach
N-of-1 trials comparing a non-steroidal anti-inflammatory drug with paracetamol in osteoarthritis	Objective	To compare the individual efficacy of NSAIDs versus paracetamol for osteoarthritis pain
	Participants	51 patients with osteoarthritis (each conducted as a separate N-of-1 trial)
	Design	Multiple crossover N-of-1 design (similar to alternating treatment design)
	Measures/dependent variables	Daily pain (VAS), morning stiffness, medication preference, pain scores, patient satisfaction
	Intervention/independent variables	NSAID versus paracetamol periods
	Outcomes	No significant difference for most patients, though some showed clear individual preference
	Role of SCEDs	Supported individualized treatment decisions without relying on group averages, providing a robust model for personalized medication selection in clinical practice
Efficacy and safety of amantadine as a treatment for apathy after brain injury: two single-case experimental design studies	Objective	To evaluate the individual effects of amantadine on post-brain injury apathy
	Participants	Two adults with traumatic brain injury
	Design	Multiple baseline across participants design
	Measures/dependent variables	Apathy Evaluation Scale (AES), neuropsychological performance tests
	Intervention/independent variables	Amantadine treatment (100-200 mg/day)
	Outcomes	Both cases showed reduced apathy scores and increased cognitive alertness
	Role of SCEDs	Enabled testing of drug efficacy at the individual level in rare and heterogeneous neurological cases
Personalized (N-of-1) trials for depression: a systematic review	Objective	To compare two different antidepressants in patients with major depressive disorder to identify the most suitable treatment at the individual level
	Participants	20 adults diagnosed with major depression, each followed as a separate N-of-1 trial
	Design	Multiple crossover N-of-1 design (similar to alternating treatments; e.g., Drug A → Washout → Drug B → Washout, repeated as needed)
	Measures/dependent variables	Depression severity (PHQ-9, HAM-D), side effect profile, daily mood tracking, functional assessments
	Intervention/independent variables	Type of antidepressant used
	Outcomes	Significant individual differences observed in 40% of patients; no difference at the group level
	Role of SCEDs	Guided personalized drug selection and informed clinical application of pharmacogenetic approaches

Table 1. Continued

Example study	Study characteristics	Explanation
Treating hypertension in children with N-of-1 trials	Objective	To personalize antihypertensive treatment by evaluating individual responses to three medications using repeated ABPM
	Participants	42 children with primary hypertension; 32 were included in the final analysis
	Design	N-of-1 randomized crossover design in which three antihypertensive medications—lisinopril, amlodipine, and hydrochlorothiazide—were prescribed in randomized order and individual treatment responses were compared
	Measures/dependent variables	Repeated 24-hour ambulatory blood pressure measurements (ABPM) and treatment-related adverse effects
	Intervention/independent variables	Three antihypertensive medications: lisinopril, amlodipine, and hydrochlorothiazide, administered in sequential treatment periods according to the N-of-1 protocol
	Outcomes	Individual treatment preferences varied: 16 preferred lisinopril, 8 amlodipine, and 4 hydrochlorothiazide; 4 remained uncontrolled
	Role of SCEDs	Enabled individualized comparison of treatment responses and identification of the preferred antihypertensive for each patient
Genetically-guided medical nutrition therapy in type 2 diabetes mellitus and pre-diabetes: a series of N-of-1 superiority trials	Objective	To examine how various diets or treatments affect individual glucose responses and develop personalized glycemic control strategies
	Participants	12 adults with type 2 diabetes, monitored using continuous glucose monitoring (CGM)
	Design	Alternating treatments design (e.g., low-carbohydrate diet vs. standard diet; periods randomized)
	Measures/dependent variables	Continuous glucose measurements (CGM), postprandial glucose response, daily mean glucose, glycemic variability, HbA1c (secondary)
	Intervention/independent variables	Two different diet/treatment protocols (e.g., low-carbohydrate vs. standard diet) or individualized lifestyle interventions
	Outcomes	Glucose responses varied widely among individuals; the “optimal” protocol differed for each patient
	Role of SCEDs	Enabled identification of personalized glycemic management strategies and showed that N-of-1 designs can generate individual glucose response profiles without requiring large sample sizes
Status of N-of-1 trials in chronic pain management: a narrative review	Objective	To evaluate the individual effects of mindfulness therapy on chronic pain
	Participants	Adults with chronic low back pain
	Design	ABAB design
	Measures/dependent variables	Pain intensity (VAS/NRS), attention to pain and emotion regulation scales, sleep quality, stress level
	Intervention/independent variables	8-week mindfulness program (mindfulness-based practices: breath awareness, body scan, emotion regulation)
	Outcomes	Pain intensity and stress decreased markedly during mindfulness periods, with functional improvements; effects returned during baseline periods.
	Role of SCEDs	Enabled precise individual assessment of intervention effects, guiding personalized treatment decisions and showing patient-specific impacts of non-pharmacological approaches.
An N-of-1 trial service in clinical practice: testing the effectiveness of stimulants for attention-deficit/hyperactivity disorder	Objective	To evaluate individual response and optimal dosage of stimulant medications in pediatric Attention-Deficit/Hyperactivity Disorder (ADHD)
	Participants	45 children (aged 6–12 years) clinically diagnosed with ADHD
	Design	Multiple crossover randomized double-blind placebo-controlled N-of-1 design (Repeated paired blocks: Drug A vs. Drug B vs. Placebo)
	Measures/dependent variables	Conners' Rating Scales (teacher and parent daily behavior scores), ADHD symptom severity scores, and side effect logs
	Intervention/independent variables	Methylphenidate vs. dexamphetamine vs. placebo administered in randomized, double-blind treatment cycles
	Outcomes	71% of participants identified a superior medication and dose, optimizing long-term individual treatment
	Role of SCEDs	Enabled precise, patient-specific drug selection in pediatric psychiatry without relying on group averages

Table 1. Continued

Example study	Study characteristics	Explanation
Melatonin for chronic insomnia in Angelman syndrome: a randomized placebo-controlled N-of-1 trial series Braam et al., 2008 ⁴⁴	Objective	To evaluate the individual effects of melatonin on sleep duration and quality
	Participants	Adult with chronic insomnia
	Design	ABAB design (Melatonin → Placebo → Melatonin → Placebo)
	Measures/dependent variables	Sleep onset latency, total sleep time, sleep efficiency, subjective sleep quality (sleep diary, digital monitoring)
	Intervention/independent variables	3 mg melatonin vs. placebo (taken at bedtime)
	Outcomes	Melatonin periods shortened sleep onset latency and improved efficiency, while placebo periods showed clear deterioration
	Role of SCEDs	Effective for identifying individual responses in sleep disorders
SCEDs: Single-case experimental designs; tDCS: Transcranial direct current stimulation; NSAIDs: Non-steroidal anti-inflammatory drugs; Treg: Regulatory T cells; RCTs: Randomized controlled trials; HbA1c: HemoglobinA1c; ACE inhibitor: Angiotensin-converting enzyme inhibitor		

DISCUSSION

Overall Review—SCEDs: The Individualized Dimension of Evidence-Based Medicine

SCEDs (N-of-1) represent the individualized dimension of EBM. While the classic application of EBM provides decision support based on population averages, SCEDs personalize clinical choices by providing control at the individual level.^{2,9} Therefore, SCEDs stand out as a paradigm that pushes the boundaries of the “average patient” idea, producing first-order evidence of individual responses and preferences in medical decision-making.

Advantages of SCEDs

- *Superiority in Internal Validity:* SCEDs allow each participant to serve as their own control, enabling within-subject comparisons that minimize the influence of confounding variables and strengthen causal inference.^{14,16,28,29}
- *Clinical Applicability:* Single-case designs provide direct answers to the patient-level question of “which treatment is effective?”, thereby contributing to individualized clinical decision-making. This approach offers substantial value for treatment selection, dose optimization, and the assessment of treatment tolerability.⁴⁵
- *Ethical Advantages:* Particularly when alternative interventions are being evaluated, single-case designs help ensure that patients are not exposed to an ineffective treatment for extended periods. In most SCEDs, all participants receive one or more forms of the intervention over time, which enhances ethical acceptability.¹⁵
- *Resource Efficiency (Big Evidence from Small Samples):* Single-case designs have the potential to reduce the financial and time-related burdens associated with large RCTs. With proper planning and sufficient repetitions, even small samples can yield strong internal validity.

Limitations of SCEDs

External Validity Limitation: A result valid for a single case cannot automatically be generalized to the population;

therefore, hierarchical/aggregate analyses of multiple N-of-1 studies are required to transfer information from SCEDs to the population level.^{7,45}

Repeated Measurement Burden: Daily or more frequent measurements can increase patient burden, reduce patient compliance, and compromise data quality. The balance between measurement frequency and utility should be carefully established during the design phase.

Carry-over Effects: In pharmacological interventions, the possibility that effects from the previous period will spill over into the next period poses a threat. Washout times and randomized period orders should be controlled, and, if necessary, carryover effects should be addressed using appropriate statistical models.

Measurement Reliability and Objectivity: When subjective measures (e.g., VAS) are used, measurement error and placebo/expectancy effects can confound the results. Independent observers and objective measurements (biomarkers, actigraphy, CGM, etc.) should be included whenever possible.

Analytical Complexity: Constructs such as time-series dependence, autocorrelation, trend, and seasonality should be included in the analysis; otherwise, *P* values may be misleading.

Evidential Value and Hierarchy: The Position of the N-of-1

The placement of SCEDs within the evidence hierarchy is purpose-dependent. RCTs are essential for population-level generalizability, whereas SCEDs offer the strongest evidence for individualized treatment decisions.^{9,46} An optimal contemporary approach is to combine RCTs with multiple N-of-1 studies. Utilizing SCEDs for patient-specific decisions and integrating RCT and SCED evidence in meta-analyses will guide future evidence-based research, policy development, and clinical guidelines.

Statistical Methods and Analysis Recommendations (Guide for Researchers)

Below are principles that researchers can practically apply in the design-analysis process:

Design Phase

- *Design Selection*: Based on the research question, select the appropriate ABAB, multiple baseline/probe design, or alternating intervention designs.³⁴
- *Randomization*: Randomize the order of phase or intervention delivery to reduce the risk of a carryover effect.
- *Wash-Out Duration*: Based on the pharmacodynamics of the intervention; inadequate wash-out increases the risk of a carryover effect.
- *Measurement Plan*: Plan for both short-term (daily) and longer-term (weekly/monthly) measurements; minimize measurement burden.
- *Preregistration*: Registration reduces reporting bias and stabilizes the analysis plan.

Analysis Phase

- *Dependency Control*: Use AR(1), ARIMA, or mixed-effects models to assess autocorrelation between measurements. For example, ARIMA effectively models trends and autocorrelation.³³
- *Randomized Testing*: When stage orders are randomized, permutation-based tests offer robust and distribution-independent options.^{35,47}
- *Bayesian Approaches*: Suitable for expressing uncertainty in SCEDs, using prior knowledge, and combining multiple N-of-1 studies with a hierarchical model.²⁴
- *Effect Size and Clinical Significance*: Don't just look at the *P* value; Effect sizes (Performance-criteria based effect size/PCES³², Tau-U⁴⁸, % change, mean stage change⁴⁸, and patient-specific thresholds should be reported.
- *Meta-Analysis*: Hierarchical (mixed/Bayesian) models are preferred when combining multiple N-of-1 studies; this allows the individual response distribution and the population mean to be estimated together.^{7,33}

Ethical, Experimental Guidance and Clinical Decision Tool: A Practical Guide for Practitioners (Step-by-Step Guide)

The following steps provide a practical roadmap for researchers/clinical teams designing a study with SCEDs:

- *Clarify The Research Question*: Treatment assignment, dose optimization, or intervention comparison?
- Suitable candidates include patients with variable drug response or uncertain treatment preferences.
- *Choose and Justify The Design*: ABAB, multiple baseline, alternating interventions, or ABCBC; specify the ethical and practical pros and cons of each.
- *Create The Measurement Set*: Determine the primary and secondary dependent variables, measurement frequency, and objective measures (if possible).

- *Plan Wash-Out and Duration*: Base the wash-out period on pharmacokinetic properties, and ensure a sufficient number of replicates.
- *Randomization and Blinding*: Implement double-blind or at least blinded assessment if applicable.
- *Data Management and Quality Control*: Verify daily records, independent observer assessments, missing data strategies.
- *Predefine The Analysis Plan*: Check for autocorrelation, effect size, randomization test, or Bayesian analysis methods.
- *Confirmed Consent and Ethical Review*: The design, burden, and potential benefits/harms should be clearly explained to the patient.
- *Reporting*: Consolidated Standards of Reporting Trials (CONSORT) guidelines should be followed; transparency regarding design type, randomization, washout, number of measurements, missing data, and analysis decisions should be reported.^{14,36}

To facilitate the translation of SCEDs methodology into routine clinical practice, the following checklist provides a quick reference for clinicians to determine the suitability of an N-of-1 trial for a specific patient or clinical scenario.

When to Consider an N-of-1 (SCEDs) Trial?

- *High Inter-individual Variability*: When standard population-based treatments yield inconsistent results across different patients.
- *Chronic and Stable Conditions*: When the disease course is long-term and symptoms are relatively stable (e.g., hypertension, chronic pain, insomnia, or stable neurodegenerative symptoms).
- *Treatment Optimization*: When seeking the optimal dosage or comparing the efficacy/safety of two similar therapeutic options for a specific individual.
- *Rare Diseases*: When the rarity of the condition precludes the recruitment of a sufficient sample size for a traditional RCT.
- *Assessing Idiosyncratic Responses*: When a patient experiences unusual side effects or benefits that are not documented in large-scale trials.

When are SCEDs Inappropriate (Contraindications)?

- *Acute or Rapidly Progressing Conditions*: If the clinical status changes too rapidly to allow for stable baseline measurements (e.g., acute infections, life-threatening emergencies).
- *Curative Treatments*: If the intervention is intended to provide a permanent cure, as this prevents the "return to baseline" required for withdrawal designs (e.g., surgical procedures, definitive antibiotic courses).
- *Prolonged Washout Periods*: If a drug's pharmacological effects persist for an extended period, making crossover transitions impractical due to carry-over effects.

• *High Measurement/Burden of Care*: If the frequency of data collection or the complexity of the monitoring protocol significantly compromises the patient's quality of life or clinical safety.

Reporting and Publication Standards

Transparent reporting is crucial in single-subject studies. Reporting should include full disclosure of design type, period lengths, washout details, measurement frequency, missing data, and statistical methods. Supplementary materials (raw data, time-series graphs) prepared according to single-subject research standards (Kratochwill et al.³⁴) facilitate peer review.

Future Directions: Holistic Data Analysis, Digital Integration, and Systematic Evidence

While this overview provides a comprehensive methodological framework, it is not a systematic review. Future research should conduct systematic reviews and meta-analyses of existing N-of-1 trials within specific disease categories. Such efforts would be instrumental in synthesizing individual-level evidence into broader clinical guidelines, thereby enhancing the external validity of SCEDs' findings at the population level.

In this context, the future of SCEDs encompasses two parallel directions: (1) translating multiple N-of-1 studies into population-level inferences through hierarchical, Bayesian, or meta-analytic approaches; and (2) integrating real-time data collection and analysis via digital health and artificial intelligence applications. Together, these developments make the goal of "big evidence from small samples" achievable both methodologically and in practice.^{4,26,32}

CONCLUSION

A Clinical and Methodological Call

SCEDs are one of the most practical ways to implement the individualized dimension of EBM. The proposition that "the future of medical science lies in understanding the individual response, not the average patient" is increasingly being proven true not only rhetorically but also in practice.⁴⁷ However, this potential must be used responsibly: Without rigorous design, reliable measurements, appropriate statistical adjustments, and strategies for examining the data holistically, SCEDs can lead to misinterpretations or conclusions with low generalizability. We recommend that researchers design integrated studies that utilize the strong internal validity of single-subject studies while addressing the need for external validity through multiple N-of-1 syntheses and modern statistical approaches.

Footnotes

Author Contributions

Concept Design – M.T.H., Data Collection or Processing – M.T.H., Analysis or Interpretation – M.T.H., O.A., Literature Review – M.T.H., O.A., Writing, Reviewing and Editing – M.T.H.

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Erratum



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On page 169 of the manuscript, Reference 6 required revision. Accordingly, the necessary correction has been indicated in bold within the text below.

The uncorrected version is as follows:

Celik P, Celik S, Hastaoglu F. Acil servise basvuran geriatric hasta profilinin belirlenmesi. J Acad Emerg Med. 2015;14(3):117-121. [CrossRef]

The corrected version is as follows:

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