The Medical Bulletin of Haseki

E-ISSN: 2147-2688

2025
Volume 63
Issue
November 5

www.hasekidergisi.com

The Medical Bulletin of Haseki

Editorial Board

galenos yayınevi

Publisher Contact
Address: Molla Gurani Mah. Kacamak Sk. No: 21/1 34093 Istanbul, Türkiye
Phone: +90 (530) 177 30 97 / +90 (539) 307 32 03
E-mail: info@galenos.com.tr/yayin@galenos.com.tr
Web: www.galenos.com.tr
Publisher Certificate Number: 14521

Online Publishing Date: December 2025
E-ISSN: 2147-2688
International scientific journal published quarterly.

The Medical Bulletin of Haseki

Scientific Advisory Board

The Medical Bulletin of Haseki

Scientific Advisory Board

The Medical Bulletin of Haseki

Scientific Advisory Board

The Medical Bulletin of Haseki

Please refer to the journal's webpage **(https://www.hasekidergisi.com/**) for "About Us", "Instructions to Authors" and "Peer Review & Ethic".

The editorial and publication process of the Medical Bulletin of Haseki are shaped in accordance with the guidelines of ICMJE, WAME, CSE, COPE, EASE, and NISO. The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing.

The Medical Bulletin of Haseki is indexed in **Emerging Sources Citation Index (ESCI), EBSCO Database, Gale, Turkish Medline-National Citation Index, Excerpta Medica/EMBASE, SCOPUS, TÜBİTAK/ULAKBİM, CINAHL, DOAJ, Hinari, GOALI, ARDI, OARE, AGORA, ProQuest, J-Gate, IdealOnline** and **Türkiye Citation Index**.

Owner: Mehmet Mesut Sonmez on University of Health Sciences Türkiye, İstanbul Haseki Training and Research Hospital

Responsible Manager: Akif ERBİN

T.C. SAĞLIK BAKANLIĞI HASEKİ EĞİTİM VE ARAŞTIRMA HASTANESİ

The Medical Bulletin of Haseki

Contents

Original Articles

Case Report

Index

Original Article

DOI: 10.4274/haseki.galenos.2025.44153
Med Bull Haseki 2025;63(5):236-244

Diagnostic Value of Hematological and Biochemical Markers in Pediatric Appendicitis: A ROC-Based Retrospective Evaluation

Sevgi Buyukbese Sarsu

University of Health Sciences Türkiye, Gülhane Faculty of Medicine, Gaziantep City Hospital, Department of Pediatric Surgery, Gaziantep, Türkiye

Abstract

Aim: Acute appendicitis is the most common surgical emergency in children; however, its diagnosis often remains challenging due to non-specific clinical features and overlapping laboratory results with other abdominal conditions. We aimed to evaluate the diagnostic accuracy of routine hematological and biochemical markers as accessible and reliable tools to support early and precise diagnosis of pediatric appendicitis.

Methods: This retrospective study included pediatric patients aged 0-18 years who were evaluated in the pediatric surgery department between April and June 2025. Patients were divided into three groups based on clinical, radiological, and histopathological findings: histopathologically confirmed acute appendicitis (n=63), right lower quadrant pain with appendicitis excluded (n=71), and healthy controls (n=74). Laboratory parameters analyzed at admission included white blood cell, neutrophils, lymphocytes, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio, mean corpuscular volume, red cell distribution width, mean platelet volume (MPV), platelet count, C-reactive protein (CRP), albumin, total bilirubin, direct bilirubin, sodium, and phosphorus. Diagnostic performance was evaluated using receiver operating characteristic curve analysis and multivariable logistic regression.

Results: Multivariable logistic regression identified seven independent predictors of acute appendicitis: direct bilirubin, albumin, CRP, MPV, sodium, NLR, and total bilirubin. Among these, direct bilirubin [odds ratio (OR)=8.34, p=0.0006) and albumin (OR=0.42, p=0.0021) were the strongest independent predictors, while CRP, MPV, and sodium also remained significant (p<0.05). Although NLR and total bilirubin were included in the model, their associations did not reach statistical significance. The final seven-parameter PediACS model demonstrated excellent discriminative performance (area under the curve=0.903; 95% confidence interval: 0.86-0.94). Using the optimal cut-off value (0.214) determined by the Youden Index, the model achieved 85.7% sensitivity, 89.7% specificity, and an overall diagnostic accuracy of 87.9%.

Conclusion: The PediACS model, derived from routine laboratory parameters, reliably distinguishes acute appendicitis from non-specific abdominal pain in children. It represents a practical, reproducible, and cost-effective diagnostic tool, particularly beneficial when imaging resources are limited or radiological results are inconclusive.

Keywords: Appendicitis, child, biomarkers, serum albumins, bilirubin, C-reactive protein, logistic models, ROC curve, sensitivity and specificity, nomograms

Introduction

Acute appendicitis is one of the most frequent indications for emergency surgical intervention in children. Delayed or missed diagnoses can lead to serious complications, such as perforation, abscess formation, and peritonitis, resulting in increased morbidity and prolonged hospital stay (1,2). In pediatric patients, diagnostic accuracy is often hindered by atypical clinical manifestations, limited communication abilities, and non-specific physical findings (3,4). Therefore, prompt and accurate diagnosis is crucial to reduce complications and avoid unnecessary surgical procedures (5).

Corresponding Author: Asst.prof., Sevgi Buyukbese Sarsu, University of Health Sciences Türkiye, Gülhane Faculty of Medicine, Gaziantep City Hospital, Department of Pediatric Surgery, Gaziantep, Türkiye
E-mail: sarsubuyukbese@gmail.com **ORCID:** orcid.org/0009-0004-5561-0014
Received: 08.08.2025 **Accepted:** 28.10.2025 **Publication Date:** 28.11.2025

Cite this article as: Buyukbese Sarsu S. Diagnostic value of hematological and biochemical markers in pediatric appendicitis: a ROC-based retrospective evaluation. Med Bull Haseki. 2025;63(5):236-244

Recent studies have explored the diagnostic value of routinely available laboratory biomarkers in pediatric appendicitis. Conventional inflammatory indicators such as white blood cell (WBC) count, C-reactive protein (CRP), and neutrophil-to-lymphocyte ratio (NLR) are widely used because they are simple, inexpensive, and accessible (6,7). Moreover, biochemical parameters including mean platelet volume (MPV), platelet-to-lymphocyte ratio (PLR), albumin, bilirubin fractions, and electrolytes such as sodium and phosphorus have been proposed as complementary diagnostic tools (8-10). These easily obtainable and cost-effective markers are especially useful in clinical settings where imaging resources are limited or radiological findings remain inconclusive.

We hypothesized that combining hematological and biochemical parameters would improve diagnostic accuracy for pediatric acute appendicitis compared with the use of individual markers. Accordingly, this study aimed to evaluate the diagnostic performance of routine laboratory parameters, determine optimal cut-off values through receiver operating characteristic (ROC) analysis, and construct a multivariable predictive model—the PediACS model—based on seven readily available indicators: direct bilirubin, albumin, CRP, MPV, sodium, total bilirubin, and NLR. By integrating these parameters into a single scoring system, the PediACS model is expected to serve as a practical, reliable, and cost-effective decision-support tool that enhances diagnostic precision and contributes to improved clinical management, particularly in resource-limited settings.

Materials and Methods

Compliance with Ethical Standards

This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the University of Health Sciences Türkiye, Gaziantep City Hospital Non-Interventional Clinical Research Ethics Committee (approval no.: 243/2025, date: 16.07.2025). Given the retrospective nature of the study, the requirement for informed consent was waived by the committee. All patient data were anonymized prior to analysis.

Study Design

This retrospective cross-sectional study included pediatric patients aged 0-18 years admitted to the tertiary pediatric surgery clinic between April and June 2025. Based on clinical, radiological, and histopathological findings, the patients were categorized into three groups: (1) The appendicitis group (n=63), consisting of children with acute appendicitis confirmed by surgical and histopathological examination; (2) the abdominal pain group (n=71), including children presenting with right lower quadrant pain in whom appendicitis was clinically and radiologically excluded; and (3) the healthy control group (n=74), comprising children with normal clinical and laboratory findings who presented for routine health evaluations.

The inclusion criteria included pediatric patients aged 0-18 years who presented with acute abdominal pain. Eligible participants included those with a preliminary diagnosis of acute appendicitis who underwent appendectomy and had histopathological confirmation. Patients with abdominal pain due to other conditions, such as mesenteric lymphadenitis or gastroenteritis, managed conservatively during the same period, were also included. Healthy individuals in the same age range with complete blood count results and basic biochemical test results served as the control group. Only cases with complete medical records, laboratory data, and pathology reports were included in the final analysis.

Patients older than 18 years; those with incomplete laboratory data for any of the analyzed hematological or biochemical parameters (n=6); or those without histopathological confirmation of acute appendicitis (n=3) were excluded from the study. In addition, individuals who underwent surgical procedures other than appendectomy and patients with chronic systemic diseases that could affect biomarker levels (such as malignancy, immunodeficiency, chronic liver or kidney disease, or systemic infection; n=7) were excluded. After all exclusions were applied, 208 patients with complete medical and laboratory data were included in the final analysis. Laboratory inconsistencies arising from preanalytical or postanalytical errors (n=2) were also excluded. For patients with repeated admissions for the same diagnosis, only the first admission was analyzed.

Demographic characteristics (age, sex) and laboratory results were obtained from the hospital's electronic medical database. The analyzed hematological and biochemical parameters included WBC count, neutrophils, lymphocytes, NLR, PLR, mean corpuscular volume (MCV), red cell distribution width (RDW-CV), MPV, platelet count, CRP, albumin, total bilirubin, direct bilirubin, sodium, and phosphorus. Among these parameters, NLR, CRP, albumin, MPV, sodium, and total and direct bilirubin demonstrated the strongest diagnostic potential and were incorporated into the final multivariable diagnostic model, termed PediACS.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA) and MedCalc version 20.0 (MedCalc Software Ltd., Ostend, Belgium). Data distribution was assessed using the Kolmogorov-Smirnov test. Continuous variables were

expressed as medians (interquartile ranges) and compared among groups using the Kruskal-Wallis test, whereas categorical variables were analyzed using the chi-square or Fisher's exact test, as appropriate. Receiver operating characteristic curve analysis was performed to evaluate the diagnostic performance of each parameter. The area under the curve (AUC) was calculated, and sensitivity, specificity, and optimal cut-off values were determined using the Youden Index. Variables showing statistical significance in ROC analysis were subsequently included in a multivariable logistic regression model to identify independent predictors of acute appendicitis. Model performance was assessed using odds ratios (ORs) with 95% confidence intervals (CIs), the Hosmer–Lemeshow goodness-of-fit test for calibration, and the AUC for discrimination.

A diagnostic scoring system, the PediACS model, was constructed from seven parameters that remained significant in the multivariable regression analysis (direct bilirubin, albumin, CRP, MPV, sodium, total bilirubin, and NLR). Each variable was assigned a weighted score according to its regression coefficient, and a nomogram was developed to facilitate individualized risk estimation. The clinical utility of the model was further evaluated using decision curve analysis (DCA). A two-tailed p-value <0.05 was considered statistically significant.

Results

Comparison of baseline laboratory parameters among the study groups revealed statistically significant differences across multiple hematological and biochemical variables (Table 1). NLR, CRP, and albumin showed the strongest statistical differences (p<0.001), whereas MPV, phosphorus, and MCV also differed significantly (p<0.05).

Receiver operating characteristic analysis identified NLR as the most accurate biomarker (AUC=0.845, sensitivity=83%, specificity=75%), followed by CRP (AUC=0.815) and albumin (AUC=0.810) (Table 2).

Multivariable logistic regression identified seven independent predictors of acute appendicitis: direct bilirubin, albumin, CRP, MPV, sodium, NLR, and total bilirubin (Table 3). Among these, direct bilirubin (OR=8.34, p=0.0006) and albumin (OR=0.42, p=0.0021) were the strongest predictors, while CRP, MPV, and sodium also remained significant (p<0.05). The PediACS model demonstrated excellent discrimination (AUC, 0.903; 95% CI, 0.86-0.94; sensitivity, 85.7%; specificity, 89.7%) (Figure 1). No significant multicollinearity was detected among the predictors (all variance inflation factors <2.5; Table 4).

A nomogram was generated to visualize the scoring structure of the PediACS model (Figure 2). Each variable contributed proportionally to the total diagnostic score according to its β coefficient, and the relative contribution of each variable is illustrated in Figure 3, where direct bilirubin and albumin accounted for the largest diagnostic weights.

Table 1. Baseline laboratory parameters showing significant differences (Kruskal–Wallis test)

Parameter	H Statistic	p-value
NLR	102.73	p<0.000001
NEU	72.55	p<0.000001
LYM	63.59	p<0.000001
PLR	59.78	p<0.000001
CRP	49.53	p<0.000001
RDW-CV	25.45	≈ 0.000003
WBC	25.09	≈ 0.000004
Phosphorus	10.16	≈ 0.0062
MCV	9.27	≈ 0.0097
MPV	8.17	0.017
Albumin	72.55	p<0.0001
PLT	6.65	0.036

Statistical tests: Kruskal-Wallis test for continuous variables, chi-square or Fisher's Exact test for categorical variables
NLR: Neutrophil-to-lymphocyte ratio, NEU: Neutrophil, LYM: Lymphocyte, PLR: Platelet-to-lymphocyte ratio, CRP: C-reactive protein, RDW-CV: Red cell distribution width, WBC: White blood cell count, MCV: Mean corpuscular volume, MPV: Mean platelet volume, PLT: Platelet count

Table 2. Diagnostic performance of single biomarkers (ROC analysis)

Parameter	AUC	Cut-off	Sensitivity	Specificity
NLR	0.845	2.87	0.83	0.75
CRP	0.815	5.50	0.71	0.81
NEU	0.808	7.00	0.79	0.79
PLR	0.754	134.35	0.81	0.66
Albumin	0.810	4.15	0.77	0.72
MPV	0.709	7.85	0.68	0.71
Total bilirubin	0.701	0.80	0.65	0.76

Abbreviations and tests: Table 1 plus ROC analysis for diagnostic accuracy
NLR: Neutrophil-to-lymphocyte ratio, CRP: C-reactive protein, NEU: Neutrophil, PLR: Platelet-to-lymphocyte ratio, MPV: Mean platelet volume, ROC: Receiver operating characteristic, AUC: Area under the curve

Table 3. Optimal cut-off and overall model performance (PediACS model)

Metric	Value
AUC	0.903
Optimal cut-off (Youden Index)	0.214
Sensitivity	85.7%
Specificity	89.7%
Accuracy	87.9%
F1 score	0.87

Abbreviations and tests: Same as Table 1 plus ROC and Youden Index analysis
ROC: Receiver operating characteristic, AUC: Area under the curve

Calibration analysis demonstrated strong agreement between predicted and observed probabilities (Table 5). The Hosmer-Lemeshow goodness-of-fit test (p>0.05) and the calibration curve (Figure 4) confirmed accurate probability estimation across the entire prediction range.

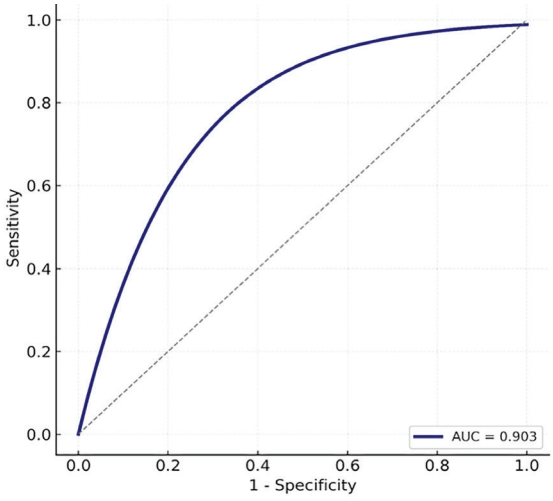

Figure 1. Receiver Operating Characteristic (ROC) Curve for the PediACS Model

The ROC curve illustrates excellent discrimination between acute appendicitis and control groups (AUC=0.903, 95% CI 0.86-0.94). The optimal cut-off determined by the Youden Index (0.214) yielded 85.7% sensitivity and 89.7% specificity. The curve confirms strong diagnostic performance of the model across all evaluated biomarkers

ROC: Receiver operating characteristic, AUC: Area under the curve, CI: Confidence interval

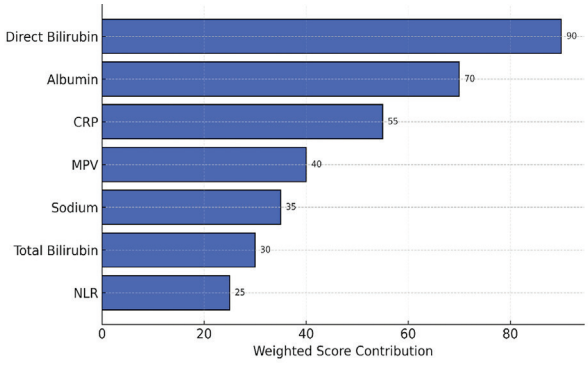

Figure 2. Nomogram-based scoring algorithm for the PediACS model (7-parameter model)

Nomogram-based scoring algorithm for the PediACS model showing the relative contribution of seven laboratory parameters to the total diagnostic score. Direct bilirubin and albumin had the highest predictive weights, followed by CRP, MPV, and sodium. The weighted points correspond to each parameter's β coefficient derived from the multivariable logistic regression model

CRP: C-reactive protein, MPV: Mean platelet volume, NLR: Neutrophil-to-lymphocyte ratio

The optimal cut-off value determined by the Youden Index (0.214) yielded a sensitivity of 85.7%, a specificity of 89.7%, an overall diagnostic accuracy of 87.9%, and an F1 score of 0.87. A comparative ROC analysis indicated that the PediACS model achieved a significantly higher AUC than that of the best-performing single biomarker, NLR (ΔAUC=+0.058, p=0.031; Table 6). Decision curve analysis (Figure 5) demonstrated greater net clinical benefit across a broad range of threshold probabilities, confirming the model's clinical utility in accurately distinguishing pediatric appendicitis.

Discussion

The present study assessed the diagnostic performance and clinical applicability of the PediACS model, a seven-parameter framework that combines biochemical and inflammatory markers to improve the diagnostic accuracy for pediatric acute appendicitis. The model demonstrated excellent discrimination (AUC=0.903, 95% CI: 0.86-0.94), good calibration (Hosmer-Lemeshow p>0.05), and a clear net clinical benefit across probability thresholds from 0.2 to 0.7 in the DCA. These results indicate that the model not only performs well statistically but also has significant potential as a practical tool for supporting individualized clinical decision-making in pediatric emergency settings.

Each biomarker incorporated into the PediACS model reflects a distinct physiological mechanism underlying appendiceal inflammation. Among these, direct (conjugated) bilirubin was identified as the strongest independent predictor (OR=8.34). Its increase is most likely attributable to cytokine-mediated hepatocellular cholestasis induced by interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). These pro-inflammatory cytokines impair both bile excretion and conjugation during the early stages of inflammation, thereby interfering with hepatocellular bile transport processes (11,12). In line with these observations, several previous studies have reported significantly elevated serum bilirubin levels in patients with appendicitis (13-16). Taken together, these findings support the hypothesis that hepatic metabolic alterations are closely associated with the systemic inflammatory response.

Conversely, hypoalbuminemia represents a classic negative acute-phase response, primarily resulting from cytokine-mediated suppression of hepatic protein synthesis and an increase in vascular permeability (17). In our study, reduced serum albumin levels were significantly associated with appendicitis and remained an independent inverse predictor in the multivariable analysis (OR=0.42; p=0.0021), consistent with its progressive decline under elevated inflammatory stress.

C-reactive protein is rapidly synthesized by hepatocytes in response to IL-6 and IL-1β signaling (18,19). Its plasma

Table 4. Multivariable logistic regression analysis (PediACS 7-parameter model)					
Parameter	**β**	**SE**	**p-value**	**OR (Exp)**	**95% CI (Lower-upper)**
Direct bilirubin	2.121	0.613	0.0006 ★	8.34	2.51-27.75
Albumin	-0.868	0.273	0.0021 ★	0.42	0.25-0.73
MPV	0.554	0.257	0.031 ★	1.74	1.05-2.87
CRP	0.030	0.012	0.0049 ★	1.03	1.01-1.06
Sodium	0.174	0.081	0.028 ★	1.19	1.02-1.40
NLR	0.174	0.133	0.181	1.19	0.92-1.55
Total bilirubin	0.385	0.348	0.269	1.47	0.74-2.89

Abbreviations and tests: As above; multivariable logistic regression used for independent predictors; Hosmer-Lemeshow test for model calibration
SE: Standard error, OR: Odds ratio, CI: Confidence interval, MPV: Mean platelet volume, CRP: C-reactive protein, NLR: Neutrophil-to-lymphocyte ratio
★ Statistically significant at the 0.05 level (p<0.05)

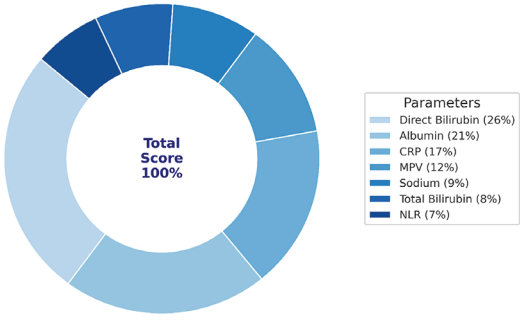

Figure 3. PediACS nomogram: score contribution distribution (7-parameter model)

Distribution of the relative contribution of seven laboratory parameters to the PediACS diagnostic model, expressed as percentage of total score. Direct bilirubin and albumin accounted for the highest proportions, followed by CRP, MPV, and sodium. The model composition demonstrates balanced weighting across inflammatory and biochemical markers

CRP: C-reactive protein, MPV: Mean platelet volume, NLR: Neutrophil-to-lymphocyte ratio

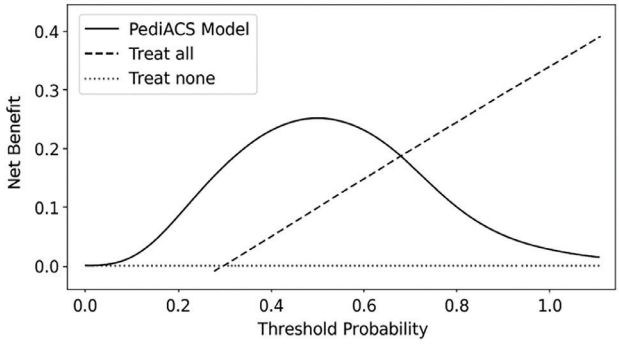

Figure 4. Calibration curve for the PediACS model

Calibration curve for the PediACS model showing strong agreement between predicted and observed probabilities of acute appendicitis. The blue line represents the model's performance, while the dashed gray line indicates perfect calibration. The model demonstrates excellent calibration with Hosmer-Lemeshow goodness-of-fit test (p>0.05)

concentration rises markedly during inflammatory conditions, thereby making CRP both a key component of the innate immune response and a clinically valuable marker for assessing the severity of systemic inflammation (20-24). The plasma CRP level shows a positive correlation with the intensity of inflammation, which explains its widespread clinical use as a non-specific but highly sensitive biomarker in various infectious and inflammatory diseases (25,26). Accordingly, the CRP-to-albumin ratio (CAR) serves as a sensitive indicator of inflammatory intensity and disease progression. As CRP levels rise and albumin concentrations decrease during inflammation, an elevated CAR value reflects a greater systemic inflammatory burden and has been associated with poorer clinical outcomes (27).

In appendicitis, hyponatremia is commonly attributed to the non-osmotic release of antidiuretic hormone (ADH, vasopressin) triggered by systemic inflammation. Circulating pro-inflammatory cytokines, including IL-6 and IL-1β, can cross the blood–brain barrier and activate the hypothalamic supraoptic and paraventricular nuclei. This cytokine-mediated stimulation leads to ADH hypersecretion, excessive renal free-water reabsorption, and ultimately dilutional or inflammation-related hyponatremia (28). In our cohort, lower sodium levels demonstrated a modest but statistically significant association with appendicitis (OR=1.19), supporting this proposed pathophysiological mechanism. Consistent with previous evidence, hyponatremia has also been suggested as a potential indicator of complicated appendicitis, and recent reports further reinforce this association (29).

Inflammatory laboratory markers—including CRP, NLR, and MPV—serve as important indicators of the systemic inflammatory response (6,7). C-reactive protein remains the primary acute-phase reactant in humans and reliably reflects the degree of inflammation.

The NLR, derived easily and inexpensively from routine differential counts, provides additional insight into subclinical inflammation. By integrating stress-related neutrophilia with relative lymphopenia, the NLR

Table 5. Model calibration and multicollinearity assessment		
Variable	**VIF**	**Tolerance**
CRP	1.82	0.55
Albumin	1.49	0.67
Direct bilirubin	1.36	0.73
Sodium	1.24	0.80
MPV	1.21	0.82
NLR	2.11	0.47
Total bilirubin	1.40	0.71
Hosmer–Lemeshow	p>0.05	Good fit

VIF: Variance inflation factor, CRP: C-reactive protein, MPV: Mean platelet volume, NLR: Neutrophil-to-lymphocyte ratio

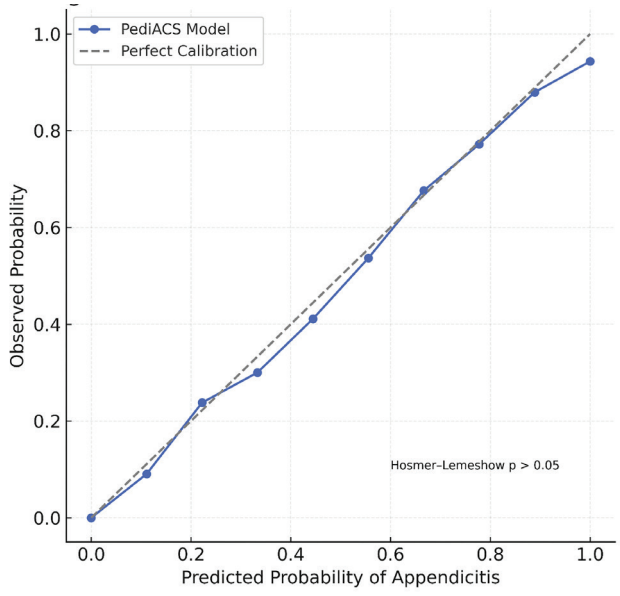

Figure 5. Decision Curve Analysis (DCA) for the PediACS Model (7-Parameter Version)

Decision curve analysis demonstrates that the PediACS model (solid line) provides a higher net benefit across a wide range of threshold probabilities compared with the "treat-all" (dashed line) and "treat-none" (dotted line) strategies. The model achieves the greatest clinical benefit between threshold probabilities of approximately 0.2 and 0.7, supporting its utility for individualized surgical decision-making in pediatric appendicitis

quantitatively reflects the imbalance between innate and adaptive immune activity. Through this mechanism, NLR may contribute meaningful supplementary information regarding both the onset and severity of appendicitis, thereby supporting clinical decision-making (30).

Mean platelet volume, an indicator of platelet size and activation, has frequently been investigated as a biomarker of systemic inflammation. Previous studies have shown that MPV correlates with inflammatory burden, and that elevated MPV levels in complicated appendicitis (e.g., perforated or gangrenous cases) may help identify high-risk patients who require urgent surgical intervention (31).

Table 6. Comparative ROC analysis (best single marker vs PediACS model)			
Model	**AUC**	**ΔAUC vs best single marker**	**p-value**
Best single marker (NLR)	0.845	–	–
PediACS model	0.903	+0.058	0.031

Abbreviations and tests: As in Table 1 plus ROC comparison between curves (DeLong test). NLR: Neutrophil-to-lymphocyte ratio

The elevation of MPV in acute appendicitis can be explained by the systemic inflammatory response and the accompanying increase in platelet activation (32). Under inflammatory conditions, thrombopoiesis accelerates, resulting in the release of larger, immature platelets into the circulation. These platelets are metabolically more active and exhibit greater proinflammatory potential, which accounts for the higher MPV values observed in acute inflammation, including appendicitis (33,34). In complicated forms such as perforated or gangrenous appendicitis, the inflammatory response is particularly intense, leading to a more pronounced increase in MPV. Ishizuka et al. (34) have shown that cytokines—especially IL-6—play a pivotal role in modulating megakaryocyte proliferation and platelet activation during severe inflammation. This cytokine-mediated stimulation promotes the release of larger platelets, reinforcing the value of MPV as a marker of inflammatory severity, particularly in complicated appendicitis.

When considered collectively, these hematologic and biochemical markers reflect the multidimensional inflammatory profile underlying appendiceal disease. Reactive oxygen species play a central role in inflammation-related tissue injury; free radicals released from polymorphonuclear leukocytes promote lipid peroxidation, increase microvascular permeability, and contribute to cellular damage. Evidence from the literature indicates that oxidative stress markers—such as malondialdehyde, thiobarbituric acid–reactive substances, and superoxide dismutase—are significantly elevated in advanced or gangrenous appendicitis (35). Overall, these observations suggest that systemic inflammation and oxidative stress jointly contribute to appendiceal tissue damage and metabolic dysregulation, which may further explain the biochemical alterations observed in bilirubin and albumin levels within the PediACS model.

The diagnostic potential of classical inflammatory biomarkers such as WBC count, neutrophil count, and CRP has been extensively investigated (6). Although these parameters often demonstrate moderate to high diagnostic accuracy when assessed individually, they typically lose independent predictive strength in multivariable analyses.

This finding underscores the rationale for using multivariate modeling in the PediACS framework, which integrates biomarkers with complementary biological relevance. The early stage of infection is typically characterized by neutrophilia and relative lymphopenia (36); consequently, the NLR has recently been proposed as a useful biomarker for detecting bacterial infections (37). Previous studies have shown that leukocyte count and NLR are markedly higher in complicated appendicitis compared to uncomplicated cases, with particularly elevated NLR values observed in perforated appendicitis (38).

Although individual biomarkers such as the (NLR, AUC=0.845), (CRP, AUC=0.815), and albumin (AUC=0.810) demonstrated strong univariate diagnostic accuracy, their predictive power diminished when analyzed together, suggesting overlapping biological variance. This pattern aligns with previous reports in the literature. Buyukbese and Sarac (6) reported similarly high diagnostic accuracy for CRP (AUC=0.887) and WBC count (AUC=0.845), whereas Birben et al. (39) observed moderate diagnostic performance for bilirubin-based indices (AUC ≈ 0.77-0.79). Similarly, diagnostic models based on the NLR have demonstrated high discriminative ability in pediatric populations, emphasizing the clinical relevance of inflammatory biomarkers in appendicitis. In addition, several investigations have suggested that NLR and bilirubin levels may aid in establishing the diagnosis or assessing disease severity. However, reported diagnostic performance varies widely across studies, largely due to methodological differences and heterogeneity in patient populations.

While Zhang et al. (8) also identified CRP, NLR, and direct bilirubin as significant biomarkers associated with complicated appendicitis, our findings further highlight the pivotal role of direct bilirubin as the most specific and independently predictive parameter within the multivariate diagnostic framework.

Beyond classical inflammatory markers such as CRP, NLR, and MPV, the PediACS model also incorporates biochemical indicators including direct and total bilirubin, sodium, and albumin, thereby reflecting the multifactorial nature of appendiceal inflammation. Although CRP and sodium individually exhibited limited diagnostic discrimination, their inclusion enhanced the model's overall diagnostic accuracy through synergistic interactions between inflammatory and biochemical pathways. This integrative structure outperformed the best single biomarker (ΔAUC=0.058, p=0.031). In contrast to previous studies that evaluated isolated parameters such as NLR in small or heterogeneous pediatric cohorts-which consequently reported variable diagnostic performance and limited generalizability (40). The present analysis demonstrates that combining inflammatory and biochemical markers yields superior diagnostic performance with robust internal calibration. Similarly, earlier studies focusing on a limited set of biochemical markers, particularly bilirubin and CRP, in selective cohorts with normal WBC counts have shown inconsistent diagnostic accuracy.

The nomogram visualization further supports these findings, illustrating how biochemical and inflammatory markers are integrated into a clinically applicable framework for individualized risk estimation. This multivariable synergy reflects the dynamic interaction between biochemical and inflammatory processes, as hepatic, hematologic, and electrolyte alterations co-evolve throughout the course of appendiceal inflammation. In the nomogram analysis, direct bilirubin showed the greatest contribution to the overall risk score, whereas low albumin levels acted as a negative predictor, consistent with previous biochemical evidence on the suppressive effects of systemic inflammation on hepatic protein synthesis (17). Collectively, these findings confirm that the nomogram effectively captures the integrated diagnostic value of biochemical and inflammatory parameters. Among the available inflammatory indicators, MPV is one of the markers that has shown the greatest variability across studies. While some investigations have reported lower MPV values in acute appendicitis, others have described higher values, resulting in inconsistent diagnostic performance. These discrepancies limit the utility of MPV as a reliable standalone biomarker. Nevertheless, when combined with other hematological and biochemical parameters that reflect different components of the inflammatory response, MPV can contribute meaningfully within a multivariable diagnostic framework.

This pattern is consistent with the findings of our study: although MPV demonstrated limited independent diagnostic power, it enhanced the overall performance of the multivariate PediACS model by complementing other markers. Similarly, parameters such as total bilirubin and the NLR may show strong performance in ROC analyses but lose statistical significance once overlapping biological pathways are accounted for in multivariable modeling. These observations highlight the importance of integrating multiple indicators rather than relying on any single biomarker in the diagnostic assessment of pediatric appendicitis.

At the Youden-optimized cut-off value of 0.214, the PediACS score achieved 85.7% sensitivity and 89.7% specificity, yielding an overall diagnostic accuracy of 87.9%. Decision-curve analysis (Figure 5) demonstrated a consistent net clinical benefit across threshold probabilities between 0.2 and 0.7, outperforming both the "treat-all" and "treat-none" strategies.

In practical terms, when ultrasonography provides inconclusive results, a high PediACS score may warrant early surgical consultation or short-interval reassessment, whereas a low score may support conservative management and reduce unnecessary imaging.

Because the model is based solely on routinely available laboratory parameters, it can be implemented across diverse clinical environments without additional cost or specialized training.

Most pediatric studies evaluating bilirubin fractions, CRP, or albumin individually have reported AUC values between 0.70 and 0.85, often without calibration or decision-curve validation. By achieving an AUC of 0.903, the PediACS model demonstrates superior diagnostic performance and clinical applicability.

Moreover, its nomogram-based visualization provides a user-friendly framework for estimating patient-specific risk, thereby bridging the gap between statistical modeling and bedside decision-making.

Future external validations should explore age-specific physiological adaptations, taking into account developmental differences in bilirubin metabolism, albumin synthesis, and sodium regulation.

In addition, large-scale multicenter prospective studies are warranted to assess the model's ability to reduce unnecessary imaging and negative appendectomy rates, particularly when integrated with ultrasonography or existing clinical scoring systems.

Study Limitations

This study has several limitations. First, its retrospective and single-center design may limit the generalizability of the findings to broader pediatric populations. Second, although the sample size was statistically adequate, the study period was relatively short; therefore, future research involving larger, multicenter cohorts is warranted for external validation. Third, imaging interpretations and surgical decision-making were based on clinical judgment, potentially introducing interobserver variability. Finally, only routinely available laboratory parameters were analyzed, and the potential diagnostic contribution of emerging biomarkers or advanced imaging modalities was not assessed.

Despite these limitations, the study has notable strengths. It is among the few pediatric appendicitis investigations to integrate both inflammatory and biochemical parameters into a unified multivariate diagnostic model. By relying exclusively on routine, low-cost laboratory data, the PediACS model enhances clinical feasibility and accessibility, particularly in resource-limited settings. Moreover, its excellent discriminatory capacity (AUC=0.903) and strong calibration (Hosmer–Lemeshow $p>0.05$) confirm its reliability as a practical, objective, and cost-effective decision-support tool for the early diagnosis of acute appendicitis in children.

Conclusion

The PediACS model, which integrates seven routinely available biochemical and inflammatory parameters, demonstrated excellent diagnostic accuracy (AUC=0.903) for detecting acute appendicitis in children. Its multivariable design provides a practical, accessible, and low-cost clinical decision-support tool, particularly valuable when ultrasonographic findings are inconclusive. The model's strong calibration and high overall performance underscore its potential for routine implementation in pediatric emergency settings. Nonetheless, prospective multicenter studies involving diverse pediatric populations are needed to confirm and externally validate its diagnostic reliability and generalizability.

Ethics

Ethics Committee Approval: This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the University of Health Sciences Türkiye, Gaziantep City Hospital Non-Interventional Clinical Research Ethics Committee (approval no.: 243/2025, date: 16.07.2025).

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

Footnotes

Financial Disclosure: This study received no financial support.

References

1. Shannon LC, Smith J, Brown A, et al. Postoperative antibiotics, outcomes, and resource use in children with gangrenous appendicitis. JAMA Surg. 2024;159:1-7.
2. Cameron DB, Anandalwar SP, Graham DA, et al. Development and implications of an evidence-based and public health-relevant definition of complicated appendicitis in children. Ann Surg. 2020;271:962-8.
3. Echevarria S, Rauf F, Hussain N, et al. Typical and atypical presentations of appendicitis and their implications for diagnosis and treatment: a literature review. Cureus. 2023;15:e37024.
4. Li D, Zhang Z, Wan J, et al. Clinical features and perforation predictors of appendicitis in infants and toddlers under 3: a retrospective two-center study. Sci Rep. 2025;15:14338.
5. Almaramhy HH. Acute appendicitis in young children less than 5 years: review article. Ital J Pediatr. 2017;43:15.
6. Buyukbese Sarsu S, Sarac F. Diagnostic value of white blood cell and C-reactive protein in pediatric appendicitis. Biomed Res Int. 2016;2016:6508619.
7. Hajibandeh S, Hajibandeh S, Hobbs N, Mansour M. Neutrophil-to-lymphocyte ratio predicts acute appendicitis and distinguishes between complicated and uncomplicated appendicitis: a systematic review and meta-analysis. Am J Surg. 2020;219:154-63.
8. Zhang Q, Zhao H, Wang F, Li W, Zhang P. Diagnostic value of laboratory parameters for complicated appendicitis: a two center study. Biomed Rep. 2024;20:77.

9. Assiri AM, Alrowabah NA, Alobaidi AA. Using neutrophil-lymphocyte ratio, mean platelet volume, and hyponatremia to diagnose appendicitis in children. IJMDC. 2023;7:867-73.
10. Feng W, Yang Q, Zhao X, Li M, Cui H. C-reactive protein and albumin ratio for the diagnosis of complicated appendicitis in children. Res Square. 2020.
11. Ikeda S, Mitaka T, Harada K, Sato F, Mochizuki Y, Hirata K. Tumor necrosis factor-alpha and interleukin-6 reduce bile canalicular contractions of rat hepatocytes. Surgery. 2003;133:101-9.
12. Kosters A, Karpen SJ. The role of inflammation in cholestasis: clinical and basic aspects. Semin Liver Dis. 2010;30:186-94.
13. Brienza N, Dalfino L, Cinnella G, Diele C, Bruno F, Fiore T. Jaundice in critical illness: promoting factors of a concealed reality. Intensive Care Med. 2006;32:267-74.
14. Emmanuel A, Murchan P, Wilson I, Balfe P. The value of hyperbilirubinaemia in the diagnosis of acute appendicitis. Ann R Coll Surg Engl. 2011;93:213-7.
15. Hong YR, Chung CW, Kim JW, et al. Hyperbilirubinemia is a significant indicator for the severity of acute appendicitis. J Korean Soc Coloproctol. 2012;28:247-52.
16. Pogorelić Z, Lukšić AM, Mihanović J, Đikić D, Balta V. Hyperbilirubinemia as an indicator of perforated acute appendicitis in pediatric population: a prospective study. Surg Infect (Larchmt). 2021;22:1064-71.
17. Li Y, Chen L, Yang X, et al. Dynamic association of serum albumin changes with inflammation, nutritional status and clinical outcomes: a secondary analysis of a large prospective observational cohort study. Eur J Med Res. 2025;30:679.
18. Wang MY, Zhang CM, Zhou HH, et al. Identification of a distal enhancer that determines the expression pattern of acute phase marker C-reactive protein. J Biol Chem. 2022;298:102160.
19. Singh SK, Prislovsky A, Ngwa DN, et al. C-reactive protein lowers the serum level of IL-17, but not TNF-α, and decreases the incidence of collagen-induced arthritis in mice. Front Immunol. 2024;15:1385085.
20. Rajab IM, Majerczyk D, Olson ME, et al. C-reactive protein in gallbladder diseases: diagnostic and therapeutic insights. Biophys Rep. 2020;6:49-67.
21. Wang Q, Shi Y. Photoacoustic viscoelasticity imaging for the detection of acute hepatitis: a feasibility study. Biophys Rep. 2020;6:1-8.
22. Jimenez RV, Wright TT, Jones NR, Wu J, Gibson AW, Szalai AJ. C-reactive protein impairs dendritic cell development, maturation, and function: implications for peripheral tolerance. Front Immunol. 2018;9:372.
23. Cottin V, Valenzuela C. C-reactive protein as a candidate biomarker in fibrotic interstitial lung disease. Respirology. 2024;29:195-8.
24. Zhang SC, Wang MY, Feng JR, Chang Y, Ji SR, Wu Y. Reversible promoter methylation determines fluctuating expression of acute phase proteins. Elife. 2020;9:e51317.
25. Plebani M. Why C-reactive protein is one of the most requested tests in clinical laboratories? Clin Chem Lab Med. 2023;61:1540-5.
26. Zhou HH, Tang YL, Xu TH, Cheng B. C-reactive protein: structure, function, regulation, and role in clinical diseases. Front Immunol. 2024;15:1425168.
27. Symeonidis NG, Pavlidis ET, Psarras KK, et al. Preoperative hyponatremia indicates complicated acute appendicitis. Surg Res Pract. 2022;2022:1836754.
28. Shuaib A, Alhamdan N, Arian H, Sallam MA, Shuaib A. Hyperbilirubinemia and hyponatremia as predictors of complicated appendicitis. Med Sci (Basel). 2022;10:36.
29. Anand S, Krishnan N, Birley JR, Tintor G, Bajpai M, Pogorelić Z. Hyponatremia-a new diagnostic marker for complicated acute appendicitis in children: a systematic review and meta-analysis. Children (Basel). 2022;9:1070.
30. Sidharth RG, Gunasekaran G, Kishore A, Jeffrey RS, Paramsivam S. Predictive value of neutrophil-to-lymphocyte ratio in differentiating perforated from non-perforated appendicitis: a cross-sectional study in a tertiary care hospital, Tamil Nadu. Cureus. 2024;16:e62030.
31. C A S, Vincent A. Mean platelet volume as an emerging biomarker for diagnosing acute appendicitis: a retrospective study. Cureus. 2024;16:e75247.
32. Aydogan A, Akkucuk S, Arica S, et al. The analysis of mean platelet volume and platelet distribution width levels in appendicitis. Indian J Surg. 2013;77:495-500.
33. Tullavardhana T, Sanguanlosit S, Chartkitchareon A. Role of platelet indices as a biomarker for the diagnosis of acute appendicitis and as a predictor of complicated appendicitis: a meta-analysis. Ann Med Surg (Lond). 2021;66:102448.
34. Ishizuka M, Shimizu T, Kubota K. Neutrophil-to-lymphocyte ratio has a close association with gangrenous appendicitis in patients undergoing appendectomy. Int Surg. 2012;97:299-304.
35. Koltuksuz U, Uz E, Ozen S, Aydinç M, Karaman A, Akyol O. Plasma superoxide dismutase activity and malondialdehyde level correlate with the extent of acute appendicitis. Pediatr Surg Int. 2000;16:559-61.
36. Takahashi J, Shono Y, Hirabayashi H, et al. Usefulness of white blood cell differential for early diagnosis of surgical wound infection following spinal instrumentation surgery. Spine (Phila Pa 1976). 2006;31:1020-5.
37. Naess A, Nilssen SS, Mo R, Eide GE, Sjursen H. Role of neutrophil to lymphocyte and monocyte to lymphocyte ratios in the diagnosis of bacterial infection in patients with fever. Infection. 2017;45:299-307.
38. Ribeiro AM, Romero I, Pereira CC, et al. Inflammatory parameters as predictive factors for complicated appendicitis: a retrospective cohort study. Ann Med Surg (Lond). 2022;74:103266.
39. Birben B, Akkurt G, Akın T, Surel AA, Tez M. Efficacy of bilirubin values in diagnosing acute appendicitis in patients with normal white blood cell count and predicting complicated appendicitis. Ulus Travma Acil Cerrahi Derg. 2021;27:50-4.
40. Marjanović V, Zečević M, Budić I, et al. Percentage of neutrophils and neutrophil-to-lymphocyte ratio in distinguishing late from early prehospital presentation of perforated appendicitis in children. Niger J Clin Pract. 2024;27:1435-40.

Original Article

DOI: 10.4274/haseki.galenos.2025.09797
Med Bull Haseki 2025;63(5):245-251

Comparison of Neutrophil-to-Albumin Ratio and Neutrophil-to-Lymphocyte Ratio in Acute Phase Positive and Negative Ankylosing Spondylitis Patients

Beliz Karatas

Erzincan Binali Yıldırım University Mengücek Gazi Training and Research Hospital, Department of Internal Medicine, Division of Rheumatology, Erzincan, Türkiye

Abstract

Aim: Acute phase reactants may not always be positive in ankylosing spondylitis (AS). We herein aimed to investigate whether the neutrophil-to-albumin ratio (NAR) is useful in monitoring AS activity.

Methods: One hundred nineteen patients who were followed up in the internal medicine rheumatology clinic between August 2024 and September 2024 and diagnosed with AS according to the axial spondyloarthropathy classification criteria of the Association for the Assessment of SpondyloArthritis international Society and the 1984 modified New York criteria were included in our study. The C-reactive protein (CRP), neutrophil-to-lymphocyte ratio (NLR), NAR and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) status of the patients were recorded at two visits, before and 6 months following treatment.

Results: A significant correlation was found between the initial NLR and the NLR values after 6 months, and between the initial and 6-month BASDAI values in both the CRP positive and CRP negative groups (p=0.000 and p=0.017). It was observed that the changes in individual NAR and NLR values and BASDAI values for each case were parallel (p=0.012).

Conclusion: C-reactive protein values in AS are not always consistent with disease activity, but the NLR may be helpful in such cases. As for the NA ratio, we found that each patient should be evaluated individually.

Keywords: Ankylosing spondylitis, neutrophil-to-albumin ratio, neutrophil-to-lymphocyte ratio

Introduction

Ankylosing spondylitis (AS) is a chronic, systemic inflammatory disease that primarily affects the sacroiliac joint and spine. It represents the archetype of spondyloarthropathies (SpA) (1). Inflammatory low back pain is the most important symptom: Morning stiffness in the hip in the second half of the night is quite specific. Decreased vertebral movements are observed in the advanced stages of the disease. Ankylosing spondylitis can also present as peripheral arthritis, which is often oligoarticular and asymmetric, frequently affecting the lower extremities and limited to the vertebrae. The most common extra-articular involvement is anterior uveitis. Other types of involvement include inflammatory bowel disease, psoriasis, apical pulmonary fibrosis, and aortic valve insufficiency (2). The disease typically begins in the third decade of life, and the male/female ratio for radiographic axial spondyloarthritis is two to one, while for non-radiographic axial spondyloarthritis it is one to one. The most common genetic association is with human leukocyte antigen-B27 (HLA-B27) (3). The incidence estimates of AS range from 0.05% to 1.4%, while the prevalence estimates range from 0.1% to 1.4% per

Corresponding Author: Beliz Karatas, MD, Erzincan Binali Yıldırım University Mengücek Gazi Training and Research Hospital, Department of Internal Medicine, Division of Rheumatology, Erzincan, Türkiye
E-mail: drblz1989@gmail.com **ORCID:** orcid.org/0000-0003-1894-8062
Received: 13.10.2024 **Accepted:** 28.09.2025 **Publication Date:** 28.11.2025

Cite this article as: Karatas B. Comparison of neutrophil-to-albumin and neutrophil-to-lymphocyte ratio in acute phase positive and negative ankylosing spondylitis patients. Med Bull Haseki. 2025;63(5):245-251

10,000 person-years (4). In Türkiye, the overall prevalence of SpA, including AS, is 1.05% (5).

When treatment is ineffective, this disease can lead to permanent disability. Scores are calculated to monitor activity. Among the components of these scores, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) are frequently used (6,7). However, ESR and other acute phase reactants are not always associated with disease activity, and changes in ESR are observed in less than 50% of patients. Interleukin 6 and tumor necrosis factor can be considered markers of inflammation in AS, but centers that can routinely test for them are in the minority (1). For this reason, a search for other acute phase reactants has been initiated while easily calculable and cost-effective indicators related to systemic inflammation have been investigated. Based on the knowledge that neutrophil and platelet levels increase with inflammation and lymphocyte levels decrease in autoimmune diseases, neutrophil, monocyte, lymphocyte, and platelet counts and indices derived from these, such as the neutrophil-to-lymphocyte ratio (NLR), monocyte-lymphocyte ratio, and platelet-lymphocyte ratio, have thus gained prominence (1). However, rapid changes in these values, especially during infections, may lead to misleading results independent of AS activity, as they develop in parallel with their half-lives (8). Therefore, we aimed to utilize albumin levels, which have a long half-life and serve as a negative acute phase reactant associated with chronic inflammation (9) and to examine changes in neutrophil-to-albumin ratios (NAR) in AS. In doing so, we used the method of comparison with the NLR emphasized in the previous studies. Our study is the first to evaluate the NAR in AS. In this study, we hypothesized that the NAR may be useful in monitoring the activity of AS.

Materials and Methods

Compliance with Ethical Standards

The ethics committee application was received from Erzincan Binali Yildirim University Non-Interventional Clinical Research Ethics Committee (approval no.: 2024-09/02, date: 11.07.2024). Our work is in accordance with the provisions of the Helsinki Declaration (revised in Brazil in 2013). Informed consent has been obtained in writing from the patients.

Study Design

One hundred nineteen patients who were followed up in our hospital's internal medicine rheumatology clinic between August 2024 and September 2024 and diagnosed with AS according to the axial spondyloarthropathy classification criteria of the Association for the International Assessment of Spondyloarthritis and the 1984 modified New York criteria (7) were included in our study.

The patients selected during these months had applied to our clinic for the first time 6 months ago and were newly diagnosed at that time. The criteria for inclusion in the study also included being at least 18 years old, not being pregnant, and not breastfeeding. The patients' gender, age (years), medications, comorbidities, disease duration (months), and HLA-B27 status were evaluated. C-reactive protein status, NLR, NAR, and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) were recorded at two visits, before and 6 months following treatment. In this study, it was important that the variations in the BASDAI score and acute phase reactants varied in parallel with the BASDAI status, rather than the effectiveness of the treatment or which treatment the patient received.

Total blood count values were measured by flow cytometry, albumin values by spectrophotometric methods, and CRP values by nephelometric methods. NLR and NAR were calculated with mathematical ratios. For CRP, values of 5 mg/L and above were considered positive, while values below this were considered negative. The BASDAI score is obtained by calculating patients' general fatigue, neck, back, and hip pain, peripheral arthritis, tenderness, and morning stiffness on a scale of 0 to 10. The BASDAI score of 4 and above was considered positive, while values below this were evaluated as negative. The BASDAI score positivity, CRP value positivity, and possible increases in the NLR and the NAR were associated with inflammation and disease activity.

Statistical Analysis

Patients were grouped as positive and negative based on their initial CRP values. The initial BASDAI status of these groups was statistically compared with the initial median values of NAR and NLR, as well as the BASDAI status after 6 months with the median values of NAR and NLR from that same period. Additionally, the initial median values of NAR and NLR for each case were compared with the median values 6 months following treatment. Chi-square, paired t-test, Wilcoxon two-related-samples t-test, and Kruskal-Wallis test were used with SPSS version 22. A p-value of 0.05 or below was considered significant. The study was conducted retrospectively.

Results

Demographic Data

The total number of patients was 119. Sixty-four patients were male (54%), and 55 patients were female (46%). The average age of the patients was 41.9±10.3. The distribution of patients according to the medications they used is as follows: ten patients using methotrexate (8.5%), 18 patients using duloxetine (15%), 3 patients using hydroxychloroquine (2.5%), 84 patients using sulfasalazine (70.6%), 10 patients using colchicine (8.5%),

Table 1. Demographic characteristics of patients	
Age (year), mean ± *SD, (min-max)	41.9±10.3 (19-69)
Female, (n%)	55 (46)
Male, (n%)	64 (54)
Methotrexate using, (n%)	10 (8.5)
Duloxetine using, (n%)	18 (15)
Hydroxychloroquine using, (n%)	3 (2.5)
Sulfasalazine using, (n%)	84 (70.6)
Colchicine using, (n%)	10 (8.5)
**NSAID using, (n%)	85 (71.4)
Adalimumab using, (n%)	33 (28)
İnfliximab using, (n%)	15 (12.5)
Certolizumab using, (n%)	7 (6)
Golimumab using, (n%)	13 (11)
Etanercept using, (n%)	11 (9)
Secukinumab using, (n%)	8 (6.5)
‡FMF, (n%)	7 (6)
Behçet's disease, (n%)	4 (3.5)
Uveitis, (n%)	13 (11)
Psoriasis, (n%)	1 (0.8)
¶UC, (n%)	4 (3.2)
Crohn's disease, (n%)	2 (1.6)
†Type 2 DM, (n%)	2 (1.6)
‡‡HT, (n%)	2 (1.6)
Fibromyalgia, (n%)	39 (33)
Peripheral arthritis, (n%)	81 (68)
§§NLR (beginning), median (min-max)	1.970 (0.55-12.44)
§§NLR (after 6 months), median (min-max)	1.762 (0.62-6.86)
¶¶NAR (beginning), median (min-max)	0.111 (0.06-2.41)
¶¶NAR (after 6 months), median (min-max)	0.101 (0.01-1.70)
Duration of disease (month), mean ± *SD (min-max)	56.2±55.8 (0-228)
††BASDAI positive cases, (n%)	107 (90)
††BASDAI positive cases (after 6 months), (n%)	46 (31)
***CRP positive cases, (n%)	59 (49)
***CRP positive cases (after 6 months), (n%)	29 (25)
‡‡‡HLA-B27 positive cases, (n%)	60 (51)

Explore and frequency test were used
*SD: Standard deviation, **NSAIDs: Non-steroidal anti-inflammatory drugs, ‡FMF: Familial mediterranean fever, ¶UC: Ulcerative colitis, †Type 2 DM: Type 2 diabetes mellitus, ‡‡HT: Hypertension, §§NLR: Neutrophil-to-lymphocyte ratio, ¶¶NAR: Neutrophil-to-albumin ratio, ††BASDAI: Bath Ankylosing Spondylitis Disease Activity Index, ***CRP: C-reactive protein, ‡‡‡HLA-B27: Human leukocyte antigen-B27

85 patients using non-steroidal anti-inflammatory drugs (71.4%), 33 patients using adalimumab (28%), 15 patients using infliximab (12.5%), 7 patients using certolizumab (6%), 13 patients using golimumab (11%), 11 patients using etanercept (9%), and 8 patients using secukinumab (6.5%). Seven patients had familial Mediterranean fever (6%); 4 patients had Behçet's disease (3.5%); 13 patients had uveitis (11%); 1 patient had psoriasis (0.8%); 4 patients had ulcerative colitis (3.2%); 2 patients had Crohn's disease (1.6%); 2 patients had type 2 diabetes mellitus (1.6%); 2 patients had hypertension (1.6%); 39 patients had fibromyalgia (33%); and 81 patients had peripheral arthritis (68%) as comorbidities. The initial NLR median (min-max) was 1.970 (0.55-12.44); the NLR median after 6 months was 1.762 (0.62-6.86); the initial NAR median (min-max) was 0.111 (0.06-2.41); and the NAR median after 6 months was 0.101 (0.01-1.70). The disease duration was 56.2±55.8 months. At the beginning, 107 patients had positive BASDAI scores (90%), while 6 months later, 46 patients had positive BASDAI scores (31%). There were 59 patients with positive CRP values at the beginning (49%), and 6 months later, there were 29 patients with positive CRP values (25%). There were 60 patients (51%) who were HLA-B27 positive (Table 1).

Relation Between Acute Phase Reactants and BASDAI Scores

Initial CRP positivity was correlated with a high initial BASDAI score, and CRP positivity after 6 months correlated with changes in BASDAI score at that time (Table 2).

The relation between the initial NLR values and initial BASDAI scores and the NLR values and BASDAI scores six months later was also compared between cases with initial CRP positive and negative statuses. Both the CRP positive and CRP negative groups showed that the initial NLR values changed in correlation with the initial BASDAI scores, and the NLR values after 6 months changed in correlation with the BASDAI scores after 6 months. However, when we analyzed the NAR values, this situation was not valid for either group (Table 3). In Table 4, the change in acute phase reactants was examined based on the first and 6-month BASDAI values of each case in groups with initial CRP values that were positive or negative. Accordingly, the NLR and NAR values changed in parallel with the case-specific BASDAI scores. However, there was no significant increase in NAR values in cases where the initial CRP

Table 2. Relationship between BASDAI scores at baseline and 6-month follow-up and CRP				
Acute phase reactant	**Relationship with ¶BASDAI (p-value)**	**¶BASDAI is high (n%)**	**¶BASDAI (after 6 months) (p-value)**	**n (%)**
****CRP positivity**	0.048*	56 (47)	-	-
****CRP positivity (after 6 months)**	-	-	0.001*	19 (16)

Chi-square test was used
*: p-value is significant (≤0.05), **CRP: C-reactive protein, ¶BASDAI: Bath Ankylosing Spondylitis Disease Activity Index

Table 3. Relationship between BASDAI scores at baseline and 6 month follow-up and acute phase reactants

Acute phase reactant	In ‡‡CRP positive group relationship with ¶BASDAI (p-value)	Relationship with ¶BASDAI in ‡‡CRP positive group (after 6 months) (p-value)	Median values of beginning (min-max)	Median values after 6 months (min-max)	In ‡‡CRP negative group relationship with ¶BASDAI (p-value)	Relationship with ¶BASDAI in ‡‡CRP negative group (after 6 months) (p-value)	Median values of beginning (min-max)	Median values after 6 months (min-max)
****NLR (beginning)**	0.032*	-	1.97 (0.55-6.65)		0.002*	-	1.95 (0.9-12.44)	-
****NLR (after 6 months)**	-	0.024*	-	1.69 (0.65-4.74)	-	0.05*		1.79 (0.62-6.86)
‡NAR (beginning)	0.083	-	0.122 (0.06-2.41)		0.159	-	0.103 (0.06-1.63)	-
‡NAR (after 6 months)	-	0.3	-	0.107 (0.05-1.61)	-	0.13	-	0.099 (0.01-1.7)

Paired t-test, Wilcoxon two related samples test, and Kruskal-Wallis test were used
*: p-value is significant (≤ 0.05), **NLR: Neutrophil-to-lymphocyte ratio, ‡NAR: Neutrophil-to-albumin ratio, ‡‡CRP: C-reactive protein, ¶BASDAI: Bath Ankylosing Spondylitis Disease Activity Index

Table 4. Changes in NLR and NAR values according to BASDAI changes in patients with positive and negative initial CRP values

	‡‡CRP positive cases			‡‡CRP negative cases		
Acute phase reactant	**Cases with high initial ¶BASDAI**	**Cases with low ¶BASDAI after 6 months**	***p-value**	**Cases with high initial ¶BASDAI**	**Cases with low ¶BASDAI after 6 months**	***p-value**
****NLR (median, min-max)**	2.17 (0.86-6.65)	1.60 (0.65-4.74)	0.000*	2.19 (1.05-12.44)	1.75 (0.62-3.74)	0.014*
‡NAR (median, min-max)	0.12 (0.06-2.41)	0.092 (0.05-1.61)	0.000*	0.118 (0.06-1.55)	0.086 (0.05-1.24)	0.000*
	Cases with low initial ¶BASDAI	**Cases with high ¶BASDAI after 6 months**	***p-value**	**Cases with low initial ¶BASDAI**	**Cases with high ¶BASDAI after 6 months**	***p-value**
****NLR (median, min-max)**	1.13 (0.86-1.56)	1.61 (1.59-2.10)	0.017*	1.31 (1.05-2.41)	1.99 (1.38-6.86)	0.149
‡NAR (median, min-max)	0.089 (0.07-0.11)	0.12 (0.09-1.18)	0.109	0.094 (0.07-1.63)	0.12 (0.07-1.7)	0.012*

Paired t-test, Wilcoxon two related samples test, and Kruskal-Wallis test were used
*: p-value is significant (≤0.05), **NLR: Neutrophil-to-lymphocyte ratio, ‡NAR: Neutrophil-to-albumin ratio, ‡‡CRP: C-reactive protein, ¶BASDAI: Bath Ankylosing Spondylitis Disease Activity Index

value was positive and the first BASDAI score was low, even if the BASDAI score was high after 6 months. Similarly, in cases where the initial CRP value was negative and the first BASDAI score was low, despite an increase in the BASDAI score after 6 months, no significant increase in the NLR value was observed.

Finally, when we investigated acute phase reactants that could help predict disease activity, we concluded that the initial CRP and initial NLR values are predictors of the BASDAI score (Table 5).

Discussion

In this study, in which we investigated the ability of NLR and NAR values to detect inflammation in acute phase positive and negative AS cases, we only recorded the patients' CRP values. The reason for this is that CRP is more specific to inflammation, compared to ESR, and fewer factors affect its levels. While CRP stands out in the follow-up of inflammatory arthritis, ESR is more valuable in connective tissue diseases (10).

When we grouped a total of 119 patients according to acute phase reactants, 59 cases (49%) had a positive initial CRP value, while 60 cases (51%) were negative. In the literature, ESR and CRP have been found to be positive in about 40% of AS patients (11). In this regard, our study is consistent with the literature.

In our study, among the patient group with a positive initial CRP value, CRP and NLR stood out as acute phase reactants that showed a change associated with the BASDAI scores at baseline and 6 months later. In this group, the initial CRP and NLR values of patients having a high baseline BASDAI score

Table 5. Predictors of BASDAI score			
Acute phase reactant	**p-value**	**Exp (B)**	**95% confidence interval**
Baseline **CRP	0.044*	8.8	1.06-73.09
Baseline ‡NLR	0.006*	79.1	3.47-1802.13
Baseline §NAR	0.545	51.5	0.056-3.65

Paired t-test, Wilcoxon two related samples test, and Kruskal-Wallis test were used
*: p-value is significant (≤0.05), BASDAI: Bath Ankylosing Spondylitis Disease Activity Index, **CRP: C-reactive protein, ‡NLR: Neutrophil-to-lymphocyte ratio, §NAR: Neutrophil-to-albumin ratio

were significantly higher than those of patients having a low baseline BASDAI score, and this was also valid for the visits at 6-month follow-up. The study conducted by Karoli et al. (12) on 200 patients diagnosed with AS in India found that cases with a high BASDAI score (>4) also had elevated CRP and NLR values. In this regard, our study is consistent with the literature. In line with this information, a positive CRP value at the time of diagnosis in an AS patient may predict the presence of inflammation with a high BASDAI score and indicate that CRP can be used as an activity marker. Additionally, studies indicating that NLR values parallel CRP, indicates that the NLR can also be used for this purpose just like CRP.

However, in the group with a negative initial CRP value, we identified the NLR as the only acute phase reactant that showed a significant difference between patients with high and low initial BASDAI scores, and it followed a parallel trend with the BASDAI score. According to the meta-analyses by Xu et al. (13), which encompass a total of 10 studies involving 765 AS patients and 701 healthy individuals, it has been noted that the NLR values in patients are related to BASDAI and CRP levels, and that NLR values may indicate disease activity. In addition, a study conducted by Kucuk et al. (14) with 102 AS patients in Türkiye found that the NLR values in cases with severe disease activity were significantly higher than those in cases with mild disease activity, and there was a positive correlation with the BASDAI score. In this regard, it can be concluded that the NL ratio may serve as an alternative acute phase reactant in the follow-up of AS cases where CRP values are negative and CRP is insufficient in determining disease activity. The group with a negative initial CRP value and a high initial BASDAI score showed a decrease in BASDAI score and a decrease in the NL ratio after 6 months of treatment compared to initial values. However, in the group with a negative initial CRP value and a low initial BASDAI score, no increase was observed in the NL rate compared to the initial values, despite a high BASDAI score after 6 months of treatment. It was a surprising finding in our research. When we investigated the reason for this, we discovered that non-inflammatory pathologies could lead to an increase in the BASDAI score 6 months after treatment in selected cases. Fibromyalgia and mechanical joint diseases are the main causes that can lead to a misleading increase in the BASDAI score. According to the study conducted by Gao (15) with 121 patients diagnosed with AS in China, the BASDAI score in patients accompanied by fibromyalgia was higher compared to those without. The lack of an increase in the NL ratio has allowed us to determine that the condition is not inflammatory, thus making it a reliable parameter. However, there are also studies in the literature indicating that the NL ratio does not show a linear relationship with the BASDAI score and disease activity (16,17). Therefore, more studies are needed to determine whether the NLR can be used in the context of negative acute phase reactant cases in AS.

On the other hand, this situation, observed for the NLR and CRP, was not valid for the NAR, which is another variable we investigated. There was no significant difference in the NA ratios between patients with high and low BASDAI scores in both the acute phase positive and negative groups at the first visit. This was also the case after 6 months. However, a significant difference appeared between the NA ratios measured at the beginning of the study and after 6 months, in parallel with the variation in the BASDAI score calculated at the beginning and after 6 months for each case. If a patient's high initial BASDAI score decreased after 6 months, it was also observed that this patient's NAR was also lower after 6 months. The reason for this situation may be the effects of individual factors such as liver functions, gender differences, and nutritional status on albumin, along with the relatively long half-life of albumin, which is about 3 weeks (18). Therefore, the result may be necessary to compare the result with the case itself. In our study, we did not have any patients with liver failure. Since the albumin value was not evaluated independently, differences between genders were not examined, and nutritional status was not assessed. Another finding in our study was that in cases where the BASDAI score was low in the initial CRP positive group and the BASDAI score was high after 6 months, there was no increase in the NAR compared to the initial score. When we investigated the reason for this, we found that the number of cases was insufficient and that statistical analysis could not be conducted.

When we researched the literature on this topic, we could not find any studies evaluating the NA ratio in AS. The lack of studies on the variation of NAR between AS patients and healthy individuals makes it impossible to establish a cut-off value for it or to comment on the existence of a significant difference. However, in the study conducted by Feng et al. (19) on lung cancer cases, it was concluded that the NAR was closely related to inflammation and exhibited an increase. In addition, a study conducted by Karasu et al. (20) in Türkiye has shown that NAR was high in cases of acute myocardial infarction. In line with these studies, it suggests that the level of the NAR increases in inflammatory conditions; thus, it may be used to monitor inflammatory processes. This also supports the idea that NAR can be used in the follow-up of rheumatological diseases, and it indicates that more research is needed.

Finally in our study, acute phase reactants that could predict disease activity were identified as CRP and NLR. That is, in cases where the initial CRP value is positive, the CRP indicates that the BASDAI score will be higher and the disease will progress more aggressively. In cases with negative CRP, NLR served this purpose. In the study conducted by Chen et al. (21) with 156 patients, elevated CRP levels were associated with the disease's poor prognosis, and our research was consistent with the literature in this regard.

Study Limitations

Our limitations include the small number of cases and the lack of cut-off values for CRP, NAR, and NLR. Despite these limitations, this study stands out as the first to comparatively evaluate the NAR alongside established inflammatory markers such as NLR and CRP in relation to BASDAI scores among AS patients. Moreover, the inclusion of both acute-phase–positive and –negative subgroups provides a comprehensive insight into the potential clinical utility of NAR as an alternative activity marker in CRP-negative cases.

Conclusion

We have shown that CRP levels in AS do not always correlate with disease activity, and that the NLR may be helpful in such cases. As for the NAR, further studies are needed to explore whether each patient should be evaluated individually, suggesting that there may be an alternative acute phase reactant in AS cases.

Ethics

Ethics Committee Approval: The ethics committee application was received from the Erzincan Binali Yildirim University Non-interventional Clinical Research Ethics Committee (approval no.: 2024-09/02, date: 11.07.2024).

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

Acknowledgments

We would like to thank Dr. Assistant Professor Ziynet Çınar for their contributions to our work in the field of biostatistics.

Footnotes

Financial Disclosure: This study received no financial support.

References

1. Wei Y, Zhang S, Shao F, Sun Y. Ankylosing spondylitis: from pathogenesis to therapy. Int Immunopharmacol. 2025;145:113709.
2. Ibrahim AM, El-Nahal AS, Ahmed HA, Youssif ZA, Abdelsalam MA. Hematological indices: potential markers of disease activity in ankylosing spondylitis patients treated with biological drugs. Egypt J Intern Med. 2024;36:74.
3. Hammad AI, Mahmoud FA. The correlation of both neutrophil/lymphocyte and platelet/lymphocyte ratios with disease activity in ankylosing spondylitis patients. Egypt Rheumatol Rehabil. 2025;52:38.
4. Crossfield SSR, Marzo-Ortega H, Kingsbury SR, Pujades-Rodriguez M, Conaghan PG. Changes in ankylosing spondylitis incidence, prevalence and time to diagnosis over two decades. RMD Open. 2021;7:e001888.
5. Onen F, Akar S, Birlik M, et al. Prevalence of ankylosing spondylitis and related spondyloarthritides in an urban area of Izmir, Turkey. J Rheumatol. 2008;35:305-9.
6. Li Y, Ma D, Yang L. Experiences and perceptions of patients with ankylosing spondylitis: a systematic review and meta-synthesis of qualitative studies. PLoS One. 2024;19:e0311798.
7. Schneeberger EE, Citera G, de Leon DP, et al. Simplified ankylosing spondylitis disease activity score (SASDAS) versus ASDAS: a post hoc analysis of a randomized controlled trial. J Rheumatol. 2022;49:1100-8.
8. Buonacera A, Stancanelli B, Colaci M, Malatino L. Neutrophil to lymphocyte ratio: an emerging marker of the relationships between the immune system and diseases. Int J Mol Sci. 2022;23:3636.
9. Gulhar R, Ashraf MA, Jialal I. Physiology, acute phase reactants. 2023 Apr 24. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan–.
10. Tezcan ME. Laboratory tests in rheumatology. Editors: Kısacık B, Bes C, Koca SS, Kasapcopur O. Clinical Rheumatology. Updated 3rd edition. İstanbul: EMA Medical Bookstore Publishing; 2023. p. 250-252.
11. Sarı I. Clinical findings, diagnosis, laboratory, monitoring and prognosis in axial spondyloarthritis. Editors: Kısacık B, Bes C, Koca SS, Kasapcopur O. Clinical Rheumatology. Updated 3rd edition. İstanbul: EMA Medical Bookstore Publishing; 2023. p.674.

12. Karoli Y, Avasthi S, Mahapatra S, Karoli R. Clinical profile of ankylosing spondylitis at a teaching hospital. Ann Afr Med. 2022;21:204-7.
13. Xu S, Ma Y, Wu M, et al. Neutrophil lymphocyte ratio in patients with ankylosing spondylitis: a systematic review and meta-analysis. Mod Rheumatol. 2020;30:141-8.
14. Kucuk A, Uslu AU, Ugan Y, et al. Neutrophil-to-lymphocyte ratio is involved in the severity of ankylosing spondylitis. Bratisl Lek Listy. 2015;116:722-5.
15. Gao R. Effects of fibromyalgia on the disease activity and treatment of patients with axial spondyloarthritis. J Musculoskelet Neuronal Interact. 2021;21:440-6.
16. Seng JJB, Kwan YH, Low LL, Thumboo J, Fong WSW. Role of neutrophil to lymphocyte ratio (NLR), platelet to lymphocyte ratio (PLR) and mean platelet volume (MPV) in assessing disease control in Asian patients with axial spondyloarthritis. Biomarkers. 2018;23:335-8.
17. Sariyildiz A, Benlidayi IC, Turk I, Acemoglu SSZ, Unal I. Evaluation of the relationship between blood cell markers and inflammation, disease activity, and general health status in ankylosing spondylitis. Rev Assoc Med Bras (1992). 2023;69:e20230722.
18. Busher JT. Serum albumin and globulin. In: Walker HK, Hall WD, Hurst JW, editors. Clinical methods: the history, physical, and laboratory examinations. 3rd ed. Boston: Butterworths; 1990.
19. Feng C, Yu H, Lei H, Cao H, Chen M, Liu S. A prognostic model using the neutrophil-albumin ratio and PG-SGA to predict overall survival in advanced palliative lung cancer. BMC Palliat Care. 2022 May;21:81.
20. Karasu M, Karaca Y, Yıldırım E, Kobat MA, Er F. Neutrophil-to-albumin ratio: a promising tool for CAD assessment in non-ST elevation AMI. Eur Rev Med Pharmacol Sci. 2023;27:11832-9.
21. Chen CH, Chen HA, Liao HT, Liu CH, Tsai CY, Chou CT. The clinical usefulness of ESR, CRP, and disease duration in ankylosing spondylitis: the product of these acute-phase reactants and disease duration is associated with patient's poor physical mobility. Rheumatol Int. 2015;35:1263-7.

Original Article

DOI: 10.4274/haseki.galenos.2025.29484
Med Bull Haseki 2025;63(5):252-257

Impact of the Severity of Steatosis due to Non-alcoholic Fatty Liver Disease on Bone Mineral Density in Patients with Osteoporosis

Aylin Ayyildiz*, Selda Ciftci Inceoglu**, Canan Alkim***, Banu Kuran**

*University of Health Sciences Türkiye, Basaksehir Cam ve Sakura City Hospital, Clinic of Physical Medicine and Rehabilitation, Istanbul, Türkiye

**University of Health Sciences Türkiye, Sisli Hamidiye Etfal Training and Research Hospital, Clinic of Physical Medicine and Rehabilitation, Istanbul, Türkiye

***University of Health Sciences Türkiye, Sisli Hamidiye Etfal Training and Research Hospital, Clinic of Gastroenterology, Istanbul, Türkiye

Abstract

Aim: Osteoporosis can occur in individuals with liver disease, and its development is thought to be influenced by the activity of the underlying hepatic disorder, as noted in previous studies. This study aimed to investigate the influence of the degree of liver steatosis, an important indicator of non-alcoholic fatty liver disease (NAFLD) severity, on bone mineral density (BMD).

Methods: Between August 2022 and July 2023, this cross-sectional study enrolled patients aged 50 to 90 years who were followed at an osteoporosis clinic and had a diagnosis of NAFLD confirmed by ultrasonography (US). Based on abdominal ultrasound findings, patients were classified into four groups reflecting the degree of liver steatosis. The groups were compared with respect to T-scores and BMD at the total spine, femur, and femoral neck, measured by dual-energy X-ray absorptiometry.

Results: Of the 792 patients assessed for eligibility, 248 met the inclusion criteria. The participants had a mean age of 65.1±11.2 years, and 97.6% (n=242) were female. The mean body mass index was 27.31±4.58 kg/m^2. Significant between-group differences were detected for lumbar total T-scores. A significant difference was identified in the lumbar spine T-score evaluation between the grade 3 and grade 0 groups in abdominal US imaging.

Conclusion: Because NAFLD and osteoporosis share several risk factors and involve chronic inflammation, NAFLD is considered a potential risk factor for bone loss. The findings of our study demonstrate that the risk of low BMD in the lumbar spine is elevated, particularly in advanced stages of liver steatosis.

Keywords: Bone mineral density, liver steatosis, non-alcoholic fatty liver disease, osteoporosis

Introduction

Non-alcoholic fatty liver disease (NAFLD) represents a continuum of chronic liver disorders encompassing simple steatosis, non-alcoholic steatohepatitis, fibrosis, cirrhosis, and ultimately hepatocellular carcinoma (HCC) (1). Non-alcoholic fatty liver disease, a metabolic disorder of the liver, is increasingly prevalent and is estimated to affect approximately 25% of the global population. It is estimated that 3.5 million new cases are diagnosed annually (2). Non-alcoholic fatty liver disease is the most common chronic liver condition globally and imposes a significant health and economic burden (3). Recently, the terminology has been updated to metabolic dysfunction-associated fatty liver disease and subsequently to metabolic dysfunction-

Corresponding Author: Aylin Ayyildiz, MD, University of Health Sciences Türkiye, Basaksehir Cam ve Sakura City Hospital, Clinic of Physical Medicine and Rehabilitation, Istanbul, Türkiye
E-mail: aylin.mrt93@gmail.com **ORCID:** orcid.org/0000-0002-7163-8234
Received: 21.04.2025 **Accepted:** 28.10.2025 **Publication Date:** 28.11.2025

Cite this article as: Ayyildiz A, Ciftci Inceoglu S, Alkim C, Kuran B. Impact of the severity of steatosis due to non-alcoholic fatty liver disease on bone mineral density in patients with osteoporosis. Med Bull Haseki. 2025;63(5):252-257

associated steatotic liver disease (MASLD), following the Delphi consensus statement. This revision reflects recognition that metabolic disorders—including abdominal obesity, type 2 diabetes, dyslipidemia, insulin resistance, and cardiovascular disease—are commonly associated with NAFLD (4).

Osteoporosis is a common systemic condition characterized by decreased bone mass and deterioration of bone microarchitecture. According to the World Health Organization, it affects approximately 6.3% of men aged 50 years or older and 21.2% of women aged 50 years or older worldwide. Additionally, it is estimated that approximately 37 million fragility fractures occur annually among individuals older than 55 years, corresponding to approximately 70 fractures per minute globally (5). A substantial body of research has highlighted the causal relationship between NAFLD and osteoporosis (1). These two diseases share similar risk factors, including older age, obesity, type 2 diabetes mellitus, and a sedentary lifestyle (6). The prevalence of osteoporosis is higher in patients with chronic liver disease than in people without liver disease, with figures ranging from 10% to 40% (7). Studies have shown that the prevalence of osteoporotic fractures is 2.5 times as high in individuals with NAFLD as in those without the condition (8). Substantial evidence from the literature substantiates the presence of osteoporosis in individuals with chronic liver disease. Nevertheless, no study has yet evaluated the impact of disease severity on osteoporosis.

In our study, we hypothesized that bone mineral density (BMD) would be lower in patients with a high degree of hepatic steatosis. The purpose of this study was to evaluate how different levels of liver steatosis affect BMD in patients with NAFLD. Consequently, this study will facilitate the incorporation of liver stenosis as a risk factor into osteoporosis clinical practice.

Materials and Methods

Compliance with Ethical Standards

The study received approval from the Ethics Committee of the University of Health Sciences Türkiye, Sisli Hamidiye Etfal Training and Research Hospital, (approval number: 4061, date: 29.08.2023). Informed consent was obtained from all participants during their initial outpatient visit. The research was conducted in accordance with the Declaration of Helsinki and adhered to the Strengthening the Reporting of Observational Studies in Epidemiolog guidelines for observational studies.

Study Design

Patients followed at the osteoporosis outpatient clinic of our hospital between August 2022 and July 2023 were screened in this cross-sectional study. Throughout the study period, 792 patients followed at our osteoporosis clinic were assessed for eligibility. Inclusion criteria required that participants be aged 50-90 years, be either men or postmenopausal women, and have undergone abdominal ultrasonography (US) within six months of their dual-energy X-ray absorptiometry (DXA) assessments. Furthermore, they were required to have US-defined NAFLD and a diagnosis of NAFLD by a gastroenterologist at our hospital. Individuals with any disease that would impair liver function (e.g., hemochromatosis, HCC, cirrhosis), a history of malignancy, or a history of surgery and biopsy of the liver, bile ducts, or intestines were excluded from this study.

Patients meeting the inclusion criteria underwent abdominal US to assess the degree of hepatic steatosis, on the basis of which they were assigned to one of four groups. The grading of steatosis was as follows: Grade 0 (absent)—normal liver echotexture; Grade 1 (mild)—slight, diffuse increase in echogenicity with clear visualization of the diaphragm and portal vein walls; Grade 2 (moderate)—moderate echogenicity elevation with partial blurring of the diaphragm and portal vein walls; and Grade 3 (severe)—pronounced echogenicity increase, with poor or absent visualization of the diaphragm, portal vein wall, and posterior right hepatic lobe (9). Demographic and clinical data, including age, sex, height, weight, body mass index (BMI), and comorbid conditions, were retrieved from the hospital records. Laboratory assessments included albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase, alkaline phosphatase, phosphorus, calcium, 25-hydroxyvitamin D, and international normalized ratio. Bone mineral density and T-scores for the lumbar spine (L1-L4 and L2-L4), femoral neck, and total femur were determined using DXA.

Statistical Analysis

Descriptive statistics included the mean, standard deviation, median, minimum, maximum, frequencies, and proportions. The Kolmogorov–Smirnov test was employed to assess the distribution of variables. The homogeneity of variances was evaluated using the Bartlett's test; since the variances were homogeneous, the Analysis of Variance F-test p-value was used. Subsequent between-group comparisons were conducted using the Bonferroni correction for post-hoc analyses. Statistical analyses were conducted using Jamovi (The Jamovi Project, 2024; Version 2.5) (Computer software), retrieved from https://www.jamovi.org, Sydney, Australia.

Results

Of 792 patients screened according to the study criteria, 248 were included in the study (Figure 1). Participants had a mean age of 65.1±11.2 years, and 242 (97.6%) were female. Their mean BMI was 27.31±4.58 kg/m². The remaining demographic and clinical features are provided in Table 1.

Abdominal US scores were used to divide patients into four groups, which were then compared with total lumbar spine, femoral neck, and total femur T-scores. A Statistically significant difference was identified between the groups for lumbar total T-scores (p=0.027). Nevertheless, no significant differences were observed between the groups for femoral neck and total femur T-scores (p=0.073 and p=0.088, respectively) (Table 2). To ascertain the stages at which this statistically significant difference occurred, a post-hoc comparison was conducted using the Bonferroni correction. Consequently, it was determined that the notable difference was between Grade 0 and Grade 3 (p=0.46). There was no significant difference among the remaining groups (p=0.995, p=0.436) (Table 3).

All patients were evaluated, without grouping, for potential correlations between AST, ALT, and CRP levels and T-scores of the total lumbar spine, femoral neck, and total femur, as well as BMD values. Nevertheless, no statistically significant correlation was identified (p>0.05).

Discussion

The findings of our investigation demonstrated that the presence of advanced liver steatosis is associated with a reduction in BMD in the lumbar spine in patients diagnosed with NAFLD. Chen et al. (10) reported that individuals with moderate-to-severe NAFLD had an increased risk of osteoporosis, whereas no significant association was observed in patients with mild NAFLD. In a cohort study by Shen et al. (11), patients with NAFLD had an increased risk of both osteoporosis and osteopenia. Additionally, a correlation was identified between the increase in fibrosis markers and the decrease in BMD. In a cross-sectional study of postmenopausal women, Lee et al. (12) found that NAFLD patients had reduced BMD, particularly at the lumbar spine and femoral neck, whereas no significant decrease was observed in the total femur. According to Moon et al. (13), postmenopausal women diagnosed with NAFLD exhibited reduced BMD at the lower lumbar spine, whereas this association was not observed among premenopausal women. In the current study, both male and postmenopausal female participants were assessed. The analysis revealed that lumbar spine BMD varied with the degree of hepatic steatosis. In contrast, BMD values at the total femur and femoral neck showed no statistically significant differences. Xie et al. (14) demonstrated a positive association between BMD and the presence of advanced fibrosis or cirrhosis. Furthermore, an independent negative correlation between NAFLD and BMD was observed in individuals aged 20-59 years.

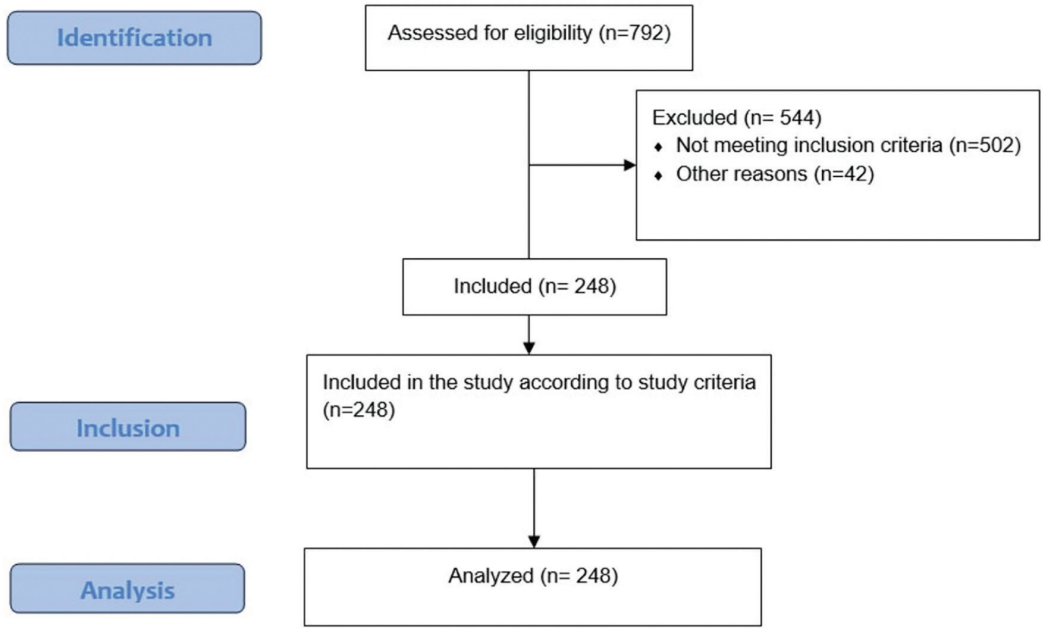

Figure 1. Flow chart of the study

Table 1. Demographic and clinical values of patients		
	Mean/ number	Standard deviation %
Age	65.1	11.2
Sex (number)		
Female	242	97.6
Male	6	2.4
Height (cm)	157.218	7.028
Weight (Kg)	67.375	11.300
Body mass index (kg/m²)	27.313	4.589
L1-L4 BMD	0.911	0.129
L2-L4 BMD	0.927	0.138
L1-L4 total T-score	-2.279	1.050
L2-L4 total T-score	-2.341	1.069
Femur neck BMD	0.745	0.106
Femur total BMD	0.795	0.113
Femur neck T-score	-2.102	0.810
Femur total T-score	-1.655	0.936
Albumin (g/dL)	4.737	3.673
ALP (IU/L)	69.395	25.250
ALT (IU/L)	17.354	12.532
AST (IU/L)	20.493	9.584
Phosphorus (mg/dL)	3.543	0.572
GGT (IU/L)	20.069	16.532
Calcium (mg/dL)	9.54	0.485
25-hidroxy vitamin D (ng/mL)	30.558	12.544
INR	1.050	0.264
APTT	28.031	11.801
PT	13.285	3.678

BMD: Bone mineral density, ALP: Alkaline phosphatase, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, GGT: Gamma-glutamyl transpeptidase, INR: International normalized ratio, APTT: Activated partial thromboplastin time, PT: Prothrombin time

Table 2. Comparison of lumbar total, femur neck and femur total T-scores according to abdominal USG grading				
Abdominal USG	**N**	**Mean**	**SD**	**p**
L1-L4 T Score				**0.027***
Grade 0	71	-2.55	0.901	
Grade 1	63	-2.10	0.997	
Grade 2	62	-2.35	1.099	
Grade 3	52	-2.05	1.171	
FN T-score				0.073
Grade 0	71	-2.26	0.724	
Grade 1	63	-1.93	0.891	
Grade 2	62	-2.18	0.764	
Grade 3	52	-2.00	0.840	
FT T-score				0.088
Grade 0	71	-1.88	0.955	
Grade 1	63	-1.53	0.869	
Grade 2	62	-1.65	0.932	
Grade 3	52	-1.51	0.960	

*: P≤0.05 means statistically significant, One-Way ANOVA (Fisher's)
USG: Ultrasound, SD: Standard deviation, FN: Femur neck, FT: Femur total

Table 3. Intergroup comparison of lumbar total (L1-L4) T-scores				
Abdominal USG	**Grade 0**	**Grade 1**	**Grade 2**	**Grade 3**
Grade 0				
Mean difference	—	-0.446	-0.201	**-0.4946**
p-value	—	0.064	0.679	**0.046***
Grade 1				
Mean difference		—	0.245	-0.0481
p-value		—	0.549	0.995
Grade 2				
Mean difference			—	-0.2932
p-value			—	0.436
Grade 3				
Mean difference				—
p-value				—

*: P≤0.05 means statistically significant, Tukey Post-hoc test
USG: Ultrasound

Despite numerous supporting studies, the risk of osteoporosis in individuals with NAFLD remains a subject of debate within the scientific community. In their retrospective cohort analysis, Sung et al. (15) found that the presence of NAFLD was not significantly associated with the occurrence of osteoporosis. It is plausible that the observed discrepancy arises from the larger representation of mild NAFLD cases and the comparatively brief observation period of two years. In a meta-analysis conducted by Vachliotis et al. (6), numerous studies reported no association between NAFLD and osteoporosis. Nevertheless, it is imperative to acknowledge that these studies were conducted in heterogeneous groups. Consequently, it is not possible to reach a definitive conclusion. Furthermore, although some studies have not identified a correlation between NAFLD and BMD, elevated serum ALT levels have been linked to reduced BMD (16-18). It has been demonstrated that increases in ALT levels serve as indicators of liver damage in advanced chronic liver disease. This finding provides further evidence to support the hypothesis that a reduction in BMD may occur in the context of advanced steatosis. However, the present study did not identify a correlation between ALT levels and BMD.

The pathophysiology of osteoporosis in chronic liver diseases is complex. The mechanism underlying the

development of NAFLD involves increased osteoclastic activity, modulated via the receptor activator of nuclear factor κB ligand (RANKL)/RANK–osteoprotegerin (OPG) pathway. Receptor activator of nuclear factor κB ligand, via its receptor, RANK, stimulates osteoclastogenesis; in contrast, OPG, a second RANKL receptor that acts as a decoy, restrains osteoclastogenesis and inhibits bone loss by binding RANKL, thereby preventing the RANK-RANKL cascade. In chronic liver diseases, the concentration of soluble RANKL in the extracellular matrix increases, thereby accelerating bone turnover. Despite the increase in OPG production to promote hemostasis and elevate the OPG/RANKL ratio, this increase is not sufficient. Furthermore, chronic liver diseases are characterized by sustained inflammatory processes. Proinflammatory cytokines, including interleukin-1, interleukin-6, and tumor necrosis factor-α (TNF-α) when produced, cause bone resorption in two ways: directly by increasing osteoclast activity and indirectly by increasing RANKL production. Furthermore, TNF-α has been demonstrated to enhance osteoclastogenesis via colony-stimulating factor-1 receptor gene expression, a mechanism that operates independently of the RANKL pathway (19). Furthermore, the synthesis of select hepatokines, including fibroblast growth factor-21 and insulin-like growth factor-binding protein-1, is elevated during the pathogenesis of NAFLD. These can cause bone loss via two distinct mechanisms: first, through the action of RANKL, and second, through binding to integrin-beta. Fibroblast growth factor-21 suppresses the maturation of bone marrow–derived mesenchymal stem cells into osteoblasts through interaction with the peroxisome proliferator-activated receptor-γ (6).

The term "NAFLD" has been modified to reflect its association with metabolic disorders, including obesity. This enabled the establishment of an association between NAFLD and osteoporosis, the most severe metabolic bone disease. Previously, it was postulated that elevated BMI was associated with increased BMD. It was argued that osteocytes are active in response to mechanical loading, particularly in relation to weight, and that bone formation is enhanced as a result (20). Nevertheless, a considerable body of recent research has demonstrated that fracture risk is elevated in individuals with obesity even when BMD is within the normal or high range (21). In particular, low muscle mass in obesity reduces the mechanical load applied to bone, thereby increasing the risk of falls in sarcopenic obesity. Metabolically unhealthy obesity, often accompanied by reduced muscle mass, is an important determinant of osteoporosis and fracture susceptibility. In a comprehensive review, Khanmohammadi and Kuchay (22) reported that individuals with MASLD have an increased risk of low BMD and fractures. Nevertheless, it was underscored that additional research is imperative, particularly for this recently designated disease with evolving diagnostic criteria.

Study Limitations

The main limitation of this research is the relatively small sample size. In the present study, NAFLD diagnosis and grading were performed using US, whereas neither elastography nor liver biopsy was used. The study population included only postmenopausal patients and male patients; premenopausal patients were excluded. Despite these limitations, our study can be considered the first to reveal the effect of liver steatosis on osteoporosis. Further research is required to assess this issue in greater depth.

Conclusion

This study demonstrated that patients with NAFLD showing advanced grades of hepatic steatosis tend to have reduced BMD, particularly at the lumbar spine. These observations imply that increasing steatosis severity may play a role in bone deterioration, underscoring the need to recognize NAFLD as a possible contributing factor in osteoporosis management.

Ethics

Ethics Committee Approval: The study received approval from the Ethics Committee of the University of Health Sciences Türkiye, Sisli Hamidiye Etfal Training and Research Hospital, (approval number: 4061, date: 29.08.2023).

Informed Consent: Informed consent was obtained from all participants during their initial outpatient visit.

Footnotes

Authorship Contributions

Concept: A.A., S.C.I., B.K., Design: A.A., S.C.I., C.A., Data Collection or Processing: S.C.I., B.K., Analysis or Interpretation: S.C.I., C.A., Literature Search: A.A., S.C.I., Writing: A.A., B.K.

Conflict of Interest: No conflicts of interest or competing interests have been reported by the authors or any individuals with control over the content of this article.

Financial Disclosure: No financial support was received for this paper or the research that resulted in this paper.

References

1. Zhou Y, Ni Y, Wang Z, Prud'homme GJ, Wang Q. Causal effects of non-alcoholic fatty liver disease on osteoporosis: a Mendelian randomization study. Front Endocrinol (Lausanne). 2023;14:1283739
2. Stefan N, Cusi K. A global view of the interplay between non-alcoholic fatty liver disease and diabetes. Lancet Diabetes Endocrinol. 2022;10:284-96.
3. Younossi Z, Anstee QM, Marietti M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol. 2018;15:11-20.
4. Rinella ME, Lazarus JV, Ratziu V, et al. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. J Hepatol. 2023;79:1542-56.
5. Tao J, Li H, Wang H, Tan J, Yang X. Metabolic dysfunction-associated fatty liver disease and osteoporosis: the mechanisms and roles of adiposity. Osteoporos Int. 2024;35:2087-98.
6. Vachliotis ID, Anastasilakis AD, Goulas A, Goulis DG, Polyzos SA. Nonalcoholic fatty liver disease and osteoporosis: a potential association with therapeutic implications. Diabetes Obes Metab. 2022;24:1702-20.
7. Guañabens N, Parés A. Osteoporosis in chronic liver disease. Liver Int. 2018;38:776-85.
8. Li M, Xu Y, Xu M, et al. Association between nonalcoholic fatty liver disease (NAFLD) and osteoporotic fracture in middle-aged and elderly Chinese. J Clin Endocrinol Metab. 2012;97:2033-8.
9. Ferraioli G, Soares Monteiro LB. Ultrasound-based techniques for the diagnosis of liver steatosis. World J Gastroenterol. 2019;25:6053-62.
10. Chen DZ, Xu QM, Wu XX, et al. The combined effect of nonalcoholic fatty liver disease and metabolic syndrome on osteoporosis in postmenopausal females in Eastern China. Int J Endocrinol. 2018;2018:2314769.
11. Shen Z, Cen L, Chen X, et al. Increased risk of low bone mineral density in patients with non-alcoholic fatty liver disease: a cohort study. Eur J Endocrinol. 2020;182:157-64.
12. Lee DY, Park JK, Hur KY, Um SH. Association between nonalcoholic fatty liver disease and bone mineral density in postmenopausal women. Climacteric. 2018;21:498-501.
13. Moon SS, Lee YS, Kim SW. Association of nonalcoholic fatty liver disease with low bone mass in postmenopausal women. Endocrine. 2012;42:423-9.
14. Xie R, Liu M. Relationship between non-alcoholic fatty liver disease and degree of hepatic steatosis and bone mineral density. Front Endocrinol (Lausanne). 2022;13:857110.
15. Sung J, Ryu S, Song YM, Cheong HK. Relationship between non-alcoholic fatty liver disease and decreased bone mineral density: a retrospective cohort study in Korea. J Prev Med Public Health. 2020;53:342-52.
16. Umehara T. Nonalcoholic fatty liver disease with elevated alanine aminotransferase levels is negatively associated with bone mineral density: cross-sectional study in U.S. adults. PLoS One. 2018;13:e0197900.
17. Xia MF, Lin HD, Yan HM, et al. The association of liver fat content and serum alanine aminotransferase with bone mineral density in middle-aged and elderly Chinese men and postmenopausal women. J Transl Med. 2016;14:11.
18. Purnak T, Beyazit Y, Ozaslan E, Efe C, Hayretci M. The evaluation of bone mineral density in patients with nonalcoholic fatty liver disease. Wien Klin Wochenschr. 2012;124:526-31.
19. Yang YJ, Kim DJ. An overview of the molecular mechanisms contributing to musculoskeletal disorders in chronic liver disease: osteoporosis, sarcopenia, and osteoporotic sarcopenia. Int J Mol Sci. 2021;22:2604.
20. Walsh JS, Vilaca T. Obesity, type 2 diabetes and bone in adults. Calcif Tissue Int. 201;100:528-35.
21. Sadeghi O, Saneei P, Nasiri M, Larijani B, Esmaillzadeh A. abdominal obesity and risk of hip fracture: a systematic review and meta-analysis of prospective studies. Adv Nutr. 2017;8:728-38.
22. Khanmohammadi S, Kuchay MS. Effects of metabolic dysfunction-associated steatotic liver disease on bone density and fragility fractures: associations and mechanisms. J Obes Metab Syndr. 2024;33:108-20.

Original Article

DOI: 10.4274/haseki.galenos.2025.46855
Med Bull Haseki 2025;63(5):258-266

Outcomes After Initial Rehydration with Isotonic Saline or Ringer's Lactate in Pediatric Acute Gastroenteritis (1-59 Months): A Single-center Retrospective Cross-sectional Study

Ceren Yilmaz Donmez*, Berker Okay**, Halil Ugur Hatipoglu***, Kamil Sahin***

*Ardahan State Hospital, Clinic of Pediatrics, Ardahan, Türkiye
**Istanbul Medipol University Faculty of Medicine, Department of Pediatrics, Istanbul, Türkiye
***University of Health Sciences Türkiye, Istanbul Haseki Training and Research Hospital, Clinic of Pediatrics, Istanbul, Türkiye

Abstract

Aim: The optimal choice of initial intravenous crystalloid solution in pediatric acute gastroenteritis (AGE) remains a matter of clinical debate due to differing effects on acid–base status and overall recovery. In this context, we aimed to compare clinical recovery and laboratory changes in children with AGE treated with isotonic saline (IS) versus Ringer's lactate (RL) as initial replacement therapy.

Methods: This single-center retrospective cross-sectional study included patients aged 1-59 months who presented with dehydration due to AGE and received initial intravenous rehydration with either IS or RL between January 2022 and February 2023. The primary outcome was the change in a standardized dehydration score from presentation to post-treatment. Secondary outcomes were pre- and post-treatment blood gas and electrolyte values. The comparisons used appropriate statistical tests.

Results: Both groups showed significant clinical improvement after fluid therapy. Dehydration scores decreased, and lactate levels fell significantly from baseline in each group ($p<0.001$ for both). Post-treatment electrolyte and acid-base parameters improved in both arms, with no serious adverse events. Between-group differences favored RL for overall clinical recovery and metabolic profile, although most laboratory changes were similar across groups.

Conclusion: In pediatric AGE, initial isotonic crystalloid replacement rapidly improves clinical and laboratory parameters. Due to its balanced composition and demonstrated benefits in overall recovery, RL may be favored as the primary replacement solution, whereas IS continues to serve as a viable alternative. Prospective randomized trials are warranted to confirm these findings.

Keywords: Child, dehydration, gastroenteritis, fluid therapy, sodium chloride, Ringer's lactate

Introduction

Acute gastroenteritis (AGE) is a significant cause of mortality and morbidity in childhood. Diarrhea, the most prominent symptom of AGE, is the second most common cause of death among children under five, following respiratory tract infections. Each year, 1.7 billion children suffer from AGE, and 525,000 of them lose their lives (1). Dehydration severity can be predicted in patients with significant fluid loss due to acute diarrhea using the clinical dehydration scale (CDS). The severity of dehydration can be determined by physical examination (2).

Corresponding Author: Berker Okay, MD, Istanbul Medipol University Faculty of Medicine, Department of Pediatrics, Istanbul, Türkiye
E-mail: drberkerokay@gmail.com **ORCID:** orcid.org/0000-0002-1506-9110
Received: 22.11.2024 **Accepted:** 16.09.2025 **Publication Date:** 28.11.2025

Cite this article as: Yilmaz Donmez C, Okay B, Hatipoglu HU, Sahin K. Outcomes after initial rehydration with isotonic saline or ringer's lactate in pediatric acute gastroenteritis (1-59 months): a single-center retrospective cross-sectional study. Med Bull Haseki. 2025;63(5):258-266

Blood gas measurements in children with acute diarrhea can quickly detect acid-base imbalances, including blood potential hydrogen (pH), actual and standard bicarbonate values (HCO_3 act, HCO_3 st), base excess [BE(B), BE(ecf)] in blood and extracellular fluid, and electrolyte levels (3). Metabolic acidosis detected in blood gas can be classified as normal anion gap or increased anion gap metabolic acidosis. Hyperchloremic metabolic acidosis is a typical example of normal anion gap metabolic acidosis. Abnormal gastrointestinal bicarbonate loss in acute diarrhea can lead to hyperchloremic acidosis when large amounts of high-chloride solutions, such as isotonic saline (IS), are given (4). In addition, every 1-unit change in serum pH is associated with an average change in ionized calcium (iCa) by 0.36 and potassium by 0.6 mmol/L (5,6). Isotonic saline is an isotonic sodium chloride solution containing 154 meq of sodium and 154 meq of chloride per liter (7). Ringer's lactate (RL) contains 130 meq of sodium, 109 meq of chloride, 4 meq of potassium, 3 meq of calcium, and 28 meq of lactate per liter, with lactate being metabolized in the liver to become HCO_3 (8).

The aim of our study is to compare changes in laboratory values and clinical conditions of children presenting to the emergency department with acute diarrhea when IS or RL is preferred as the initial rehydration solution. We assessed post-treatment clinical and laboratory changes after initial rehydration with IS vs. RL and determine which solution offers superior overall recovery. We hypothesized that RL, compared with IS, would yield greater clinical recovery, reflected by a larger reduction in a standardized dehydration score and a more favorable metabolic profile (higher pH and lower lactate/chloride) without increasing adverse events.

Materials and Methods

Compliance with Ethical Standards

The study protocol was conducted in accordance with the Helsinki Declaration. Ethical approval to conduct this study was obtained from the University of Health Sciences Türkiye, Istanbul Haseki Training and Research Hospital Clinical Research Ethics Committee (approval no.: 74-2023, date: 12.04.2023). Written consent was obtained from the parents of enrolled children.

Study Design and Patient Selection

We conducted a retrospective cross-sectional study. The study included patients aged 1 month to 5 years who presented to pediatric emergency departments with acute diarrhea and significant dehydration and received initial rehydration treatment between January 01, 2022, and February 28, 2023. The study was conducted in a single academic hospital located in the city center.

Patients receiving RL as replacement therapy (Group 1) and those receiving IS as replacement therapy (Group 2) were categorized accordingly. Patients with venous blood gas results and dehydration scores in their anamnesis, before and after replacement therapy, were included in the study. Patients with chronic causes of diarrhea (short bowel syndrome, malabsorption disease, celiac disease, etc.); those with mild dehydration; those with additional diseases that could cause electrolyte imbalance (renal tubular acidosis, adrenal diseases, cystic fibrosis, diabetic ketoacidosis, etc.); those with less than 30 minutes or more than 90 minutes between pre- and post-replacement blood gas analysis; those who had capillary or arterial blood gases; and those receiving replacement fluids other than IS or RL were excluded. Malnourished [<-2 standard deviation score (SDS) weight] and obese (>+2 SDS weight) patients were also excluded. Patients with life-threatening conditions, patients with elevated acute phase reactants suggestive of bacterial diarrhea, and patients with inflammatory bowel disease were excluded. Patients were selected based on inclusion and exclusion criteria, forming the sample group. The sample size was determined using the G*power Version 3.1.6 program with a standard effect size of 0.63, resulting in a total of 100 cases (50 for each group) for a study power of 90% and a 5% error rate (9).

During system screening, 149 patients were identified for Group 1. Of these, 47 patients were excluded due to deficiencies in blood gas analysis, other diagnostic tests, and/or incomplete medical histories. The remaining 102 patients were further reduced to 55 through random sampling, and these 55 patients were then included in the study. For Group 2, 428 patients were identified through system scans. Of these, 278 patients were excluded from the study due to the absence of pre- and post-replacement treatment blood gases in the system (e.g., delayed blood gas after fluid administration, clotted blood gas results due to treatment refusal, patients leaving without undergoing follow-up tests, etc.). Additionally, 41 patients were excluded from the study in Group 2 due to deficiencies in other blood tests or medical history information, such as dehydration scores and weight. To ensure equality between the groups, 55 patients were selected from the remaining 109 patients using the method of random sampling and included in the study (Figure 1).

Definitions, Scoring, Treatment, and Equality of Tests

Acute diarrhea was defined as lasting no more than seven days with more than three watery stools per day (1). The CDS from the European Society for Paediatric Gastroenterology, Hepatology, and Nutrition 2014 guidelines was used to determine dehydration scores.

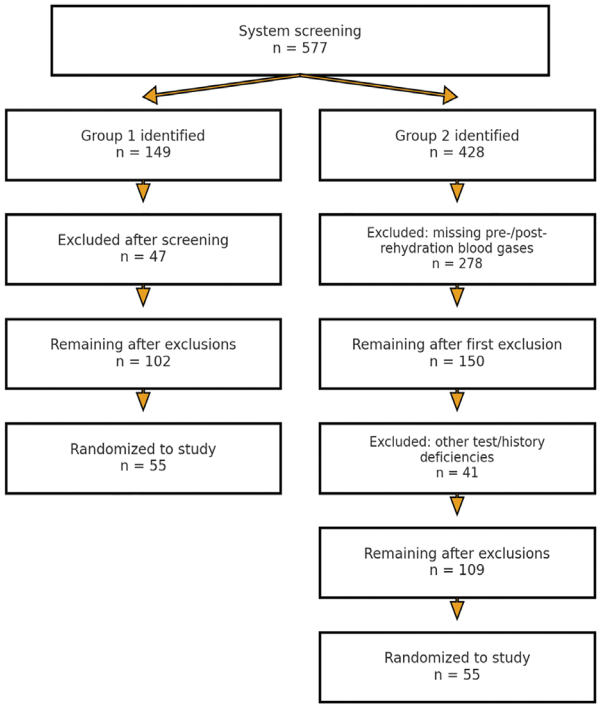

Figure 1. Flowchart of study

The scale evaluates four clinical signs—general condition, eyes, mucous membranes (tongue), and the presence of tears—with a scoring range of 0-2 points for each. A score of 0 indicates no or mild dehydration, 1-4 points indicate moderate dehydration, and 5-8 points indicate severe dehydration (2). Dehydration scores before and after treatment were calculated only for patients assessed by pediatric doctors. The anion gap was calculated as sodium - (bicarbonate + chloride) (10). According to the National Institute for Health and Care Excellence guidelines, glucose-free fluids containing 131-154 mEq/L of sodium are recommended for children with diarrhea and vomiting; hence the comparison between RL and IS (11). Patients who received 20 mL/kg of replacement fluid within 1 hour to ensure treatment protocol uniformity were included in the study. As a hospital policy, we administer initial fluid therapy to all children with moderate to severe dehydration at a dose of 20 mL/kg in 1 hour. All tests and scoring were performed immediately after the end of bolus treatment.

Statistical Analysis

Statistical analysis was conducted using SPSS 28.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The Shapiro-Wilk test was used to assess normal distribution. Descriptive statistics were presented, including numbers and percentages for categorical variables and mean, standard deviation, minimum, maximum, and median values for numerical variables. Categorical variables were compared using the chi-square test. Student's t-test and the Mann-Whitney U test were used to compare mean or median values between the two groups, depending on the sample distribution. The Wilcoxon test was used to determine whether there was a difference between the measurements at two different times or conditions. A paired samples t-test was used to test the significance of the difference between the arithmetic means of two related groups, provided that the condition of normality of distribution was met. The statistical significance level was set at $p<0.05$.

Results

Of the 110 patients included in the study, 51 (46.4%) were male, and 59 (53.6%) were female. Among patients receiving RL (Group 1) as replacement therapy, 26 (47.3%) were male, and 29 (52.7%) were female, with a median age of 22 months (min.: 1 months, max.: 58 months). Patients receiving IS (Group 2) as replacement therapy included 25 (45.5%) males and 30 (54.5%) females, with a median age of 26 months (min: 1 month, max: 59 months). No statistically significant differences were observed between the groups regarding gender and age ($p=1$ and $p=0.974$, respectively).

29% of the patients were severely dehydrated, while 71% had moderate dehydration. The dehydration score at admission in Group 1 was statistically significantly higher than in Group 2 ($p=0.008$). There was no statistically significant difference in the dehydration scores during the first hour between the groups after treatment ($p=0.260$). The change in the dehydration score with treatment in Group 1 was statistically significant and higher ($p<0.001$) (Figure 2).

Comparisons of pre- and post-replacement therapy venous blood gases and blood gas changes between the groups are shown in Table 1. Changes in laboratory values with replacement therapy for patients are presented in Table 2.

Discussion

The most significant outcome of our study is that in moderately to severely dehydrated patients diagnosed with AGE, RL solution provided more effective improvement in clinical and laboratory findings compared to IS as initial replacement therapy. Another important finding is that in children with metabolic acidosis due to diarrhea, replacement therapy with RL corrected acidosis more robustly compared to IS while also lessening the potential electrolyte disturbances associated with pH elevation. Despite the retrospective nature of our study, it facilitated the comparison of two distinct fluid therapies in children by evaluating blood tests and dehydration scores before and after fluid administration. This approach may serve

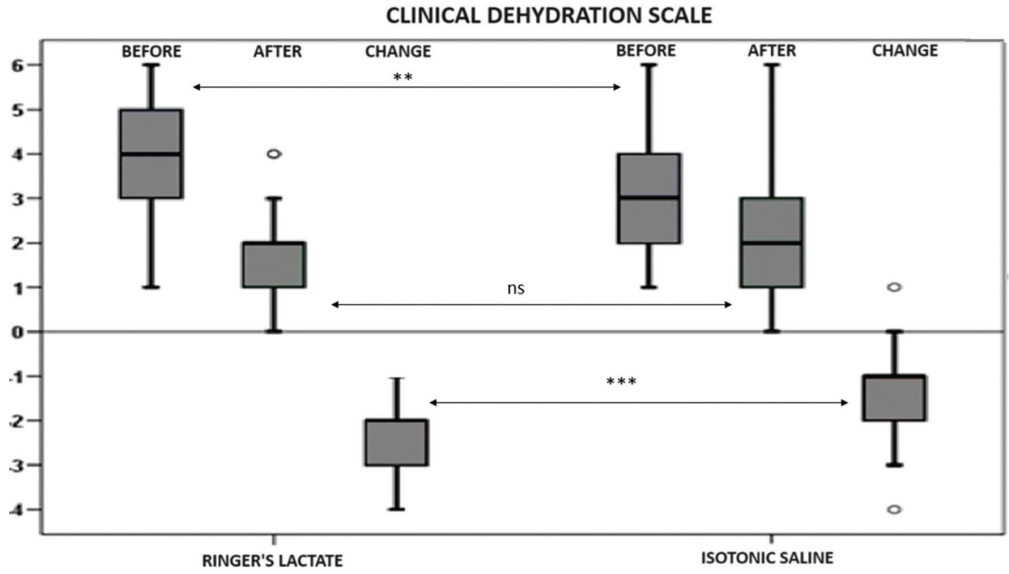

Figure 2. Change in dehydration scores before and after treatment by fluid type (IS vs RL). Error bars show 95% CIs
IS: Isotonic saline, RL: Ringer's lactate, CIs: Confidence intervals

as a guide for future prospective studies. In our cohort, both solutions produced rapid improvement. However, the magnitude of recovery—reflected by the larger reduction in the dehydration score and lactate—was more pronounced with RL after initial rehydration.

In studies, the dehydration rate among hospitalized patients with AGE requiring intravenous replacement therapy is reported to be between 26% and 55% (12,13). Recent studies in age groups similar to our study have shown that the rate of severe dehydration in children with diarrhea varies between 3.3 and 12.5% (14,15). In our study, 29% of the cases were severe, and 71% were moderately dehydrated. Our cases, according to the CDS, included patients with moderate to severe dehydration requiring intravenous replacement therapy. Since mildly dehydrated patients were not included in our study, our rate of severe dehydration may appear higher compared to other studies. Clinical dehydration scale scoring indicates the severity of a patient's dehydration at the time of admission and can also assist physicians in determining the patient's clinical status and the rate of recovery following treatment. In our emergency department cohort, RL produced a greater reduction in both the dehydration score and lactate after initial rehydration, suggesting a more favorable acid-base shift. Across most electrolytes, between-arm differences were small, indicating that both crystalloids effectively restored intravascular volume during the index visit.

In a study by Kartha et al. (9), comparing RL and IS treatments in children with AGE during acute dehydration, no significant difference was observed in the resolution of dehydration symptoms between the groups. In our study, the dehydration score at admission in Group 1 was higher compared to Group 2, and the decrease in dehydration scores with treatment in Group 1 was significantly greater. This difference may be attributed to the fact that our study applied a rapid 20 mL/kg fluid replacement within 1 hour, compared to the 6-hour slow fluid protocol used by Kartha et al. (9). The preference for RL as a replacement solution for AGE patients may contribute to a greater improvement in clinical and dehydration parameters. In both groups of our study, irrespective of the type of replacement solution, a decrease in dehydration scores was observed with treatment. The majority of dehydration was noted to be hypovolemic, and improvement could be achieved with intravenous fluid therapy (16).

In a study of patients hospitalized with a diagnosis of AGE, 84.8% were found to have acidosis in blood gas analysis (17). In our study, this rate was observed to be 49%, and the difference might be attributed to the absence of patients requiring hospitalization on the ward. Another study conducted in India showed no significant difference in changes in blood pH levels between RL and IS treatments in patients diagnosed with AGE; however, there was a tendency for an increase in both groups (18). In a similar study on the same patient group, but with different treatment protocols, where 100 cc/kg of fluid was administered over 3 or 6 hours with simultaneous oral rehydration solution support, the results may differ due to these various factors. In a study by Kartha et al. (9), comparing RL and IS during a 6-hour treatment period, both groups exhibited an increasing trend in pH levels, with no significant difference between the groups.

Table 1. Comparison of pre- and post-replacement therapy blood gas results and their changes in the groups

		Group 1	Group 2	p-value
pH	Before replacement	7.33±0.05	7.36±0.05	0.020#
	After replacement	7.38±0.04	7.35±0.05	<0.001#
	Change	0.045 (-0.02; 0.16)	-0.01 (-0.10; 0.07)	<0.001#
	p	**<0.001**ø	**0.013**ø	
pCO_2 (mmHg)	Before replacement	31.05±5.18	30.66±4.95	0.685#
	After replacement	30.40±4.44	31.27±5.39	0.360#
	Change	-0.5 (-10.1; 7.4)	0.5 (-10.1; 18.6)	0.151#
	p	0.266ø	0.356ø	
HCO_3 st (mmol/L)	Before replacement	17.45±2.53	18.29±2.61	0.091#
	After replacement	19.37±2.34	17.92±2.54	0.002#
	Change	1.7 (-1.6; 6.3)	-0.3 (-3.7; -2)	<0.001#
	p	**<0.001**ø	**0.032**ø	
HCO_3 act (mmol/L)	Before replacement	16.29±3.24	16.97±3.27	0.273#
	After replacement	17.96±2.934	16.69±3.23	**0.034**#
	Change	1.2 (-2.4; 5.8)	0 (-5.1; 3.6)	**<0.001**#
	p	**<0.001**ø	0.266ø	
BE (B) (mmol/L)	Before replacement	-8.1 (-16.3; -0.4)	-6.9 (-14.1; 1)	0.140#
	After replacement	-6.3 (-12.4; 0)	-7.5 (-16.4; -0.6)	**0.005**#
	Change	1.8 (-1.9; 7.2)	-0.5 (-5.1; 2.4)	**<0.001**#
	P	**<0.001**ø	**0.020**ø	
BE (ecf) (mmol/L)	Before replacement	-9.2 (-19; -0.13)	-8.1 (-16; 0.5)	0.319#
	After replacement	-7.3 (-14.3; -0.1)	-8.6 (-18.2; -1.2)	**0.012**#
	Change	1.9 (-5.67; 7.5)	-0.3 (-5.1; 4.4)	**<0.001**#
	p	**<0.001**ø	0.153ø	

The values are presented as median (minimum; maximum) and mean ± standard deviation
ø: Paired t-test, #: Student t-test, HCO_3 act: Actual bicarbonate, HCO_3 st: Standard bicarbonate (not affected by respiratory acid-base imbalance), BE(B): Base excess in blood, BE(ecf): Extracellular fluid base excess, pH: Potential of hydrogen, pCO_2: Partial pressure of carbon dioxide

Similarly, in another study in India comparing fluid treatments in 72 children with acute diarrhea, similar to our study, RL solution was found to cause a greater increase in serum pH compared to IS (19). A retrospective analysis of blood gas results in children under 5 years undergoing craniofacial surgery in Uruguay revealed more acidosis in the IS group compared to the RL group (20).

In our study, the group receiving RL treatment showed an increase in pH levels, indicating the correction of acidosis. In contrast, the group receiving IS treatment exhibited a statistically significant decrease in pH levels, suggesting a deepening of acidosis. We believe that the exacerbation of acidosis with IS treatment may be attributed to the increase in chloride ions, a negatively charged anion at high concentrations in the solution, leading to hyperchloremic metabolic acidosis by reducing bicarbonate (21,22). Additionally, we posit that the lactate in the content of RL solution, when metabolized in the liver and converted to bicarbonate, may contribute to an improvement in pH values (23). This signal persisted after the two-step screening and the random selection of cases was balanced to equalize groups, with no signal of harm compared with IS in this dataset. Taken together, these study-specific observations support the pragmatic use of RL when metabolic acidosis predominates at presentation, while acknowledging IS as a reasonable alternative when RL is unavailable.

We observed a significant increase in serum HCO_3 levels in Group 1 while noting a decrease in Group 2 following fluid therapies. Previous studies have demonstrated that balanced solutions result in a greater increase in serum bicarbonate levels compared to IS (18,19,24). In a study involving adult patients, the effects of RL and IS treatments on blood gas were investigated, and similar to our findings, a decrease in bicarbonate levels after treatment was observed in the group receiving IS (25).

Table 2. The change in laboratory values with replacement therapy in patients				
		Group 1	**Group 2**	**p-value**
Sodium (mmol/L)	Before replacement	133.92±2.43	133.95±3.51	0.490*
	After replacement	133.51±1.94	134.18±4.90	0.260*
	Change	-0.6 (-4.5; 4.4)	0.4 (-21.4; 6)	**0.005***
	p¥	0.043	0.061	
Potassium (mmol/L)	Before replacement	3.90±0.48	3.98±0.39	0.354#
	After replacement	3.74±0.43	3.82±0.51	0.352#
	Change	-0.09 (-1.28; 0.42)	-0.26 (-1.17; 0.77)	0.921#
	p∅	0.001	**0.008**	
Chloride (mmol/L)	Before replacement	106.04±3.89	106.22±5.41	0.661*
	After replacement	107.04±3.14	107.85±5.57	0.351*
	Change	1 (-10; 6)	2 (-24; 10)	**0.026***
	p¥	<0.001	<0.001	
Ionized calcium (mmol/L)	Before replacement	1.20±0.07	1.20±0.07	0.671*
	After replacement	1.19±0.05	1.17±0.09	0.320#
	Change	-0.02 (-0.14; 0.19)	-0.03 (-0.29; 0.13)	0.304#
	p∅	0.205	0.017	
Glucose (mg/dL)	Before replacement	74.31±18.70	70.85±19.34	0.267*
	After replacement	75.62±17.81	72.60±22.09	0.163*
	Change	1 (-30; 82)	0 (-28; 95)	0.912*
	P¥	0.877	0.866	
Lactat (mmol/L)	Before replacement	2.03±0.75	1.76±0.60	0.083*
	After replacement	1.55±0.42	1.46±0.49	0.283#
	Change	-0.37 (-2.81; 0.54)	-0.27 (-1.43; 0.83)	0.131#
	p∅	<**0.001**	<**0.001**	
Anion GAP	Before replacement	10.43±3.60	9.45±4.48	0.322*
	After replacement	7 (0.1-15.9)	8.1 (-11.9-17.5)	0.090#
	Change	-3.2 (-9.9; 3.2)	-1.4 (-22.7; 23.6)	**0.001***
	p¥	<**0.001**	0.011	

*: p<0.05, **: p<0.01, ***: p<0.001, All values are presented as median (minimum-maximum) and mean ± standard deviation
#: Student t test, *: Mann Whitney U test, ∅: Paired t-test, ¥: Wilcoxon test

Reviewing our study alongside the aforementioned literature, we find that the increase in bicarbonate levels associated with RL use is due to the lactate content in the solution, while the decrease in bicarbonate levels with IS is attributed to the chloride content (26). Therefore, considering our study and the findings of other related studies, we suggest that in dehydrated patients presenting with diarrhea to pediatric emergency clinics, choosing RL solution may lead to a more robust improvement with an effective increase in bicarbonate levels and subsequent improvement in blood pH compared to IS.

Studies have shown that intravenous fluid therapy with balanced solutions such as RL does not lead to a significant change in serum sodium concentration (9,19,27). According to the results of our study, the decrease in serum sodium values in the group receiving RL treatment may be associated with the lower sodium content of the RL solution (≈130 mEq/L), which is slightly lower than typical plasma sodium levels. This association is particularly relevant in pediatric patients with a diagnosis of AGE, dehydration, and hypernatremia. The use of balanced solutions, like RL, in this patient group may contribute to the easier maintenance of serum electrolyte balance. Additionally, hyponatremia is not a contraindication for RL.

The increase in chloride levels was observed to be higher in the group receiving IS solution in our study compared to the RL group. Despite being referred to as physiological serum, studies have shown that the excessive

use of IS solution, which is not truly a physiological fluid, can lead to elevated chloride levels (28,29). Our study's findings are consistent with these results, indicating that the use of IS solutions can result in higher chloride levels. In a study by Bampoe et al. (30) the use of RL solution in patients undergoing elective surgery was found to reduce the incidence of hyperchloremia compared to IS. The results obtained in our study seem to be consistent with these findings, suggesting that the lower chloride levels in the content of RL solution compared to IS may have a mitigating effect. This implies that choosing RL over IS may reduce the likelihood of a severe course of the disease, especially in patients at high risk of hyperchloremia and related metabolic acidosis. In cases where acidosis is already present, RL may be the preferred choice over IS in patients diagnosed with AGE.

In our study, we observed a decrease in iCa levels in the group receiving IS treatment, while no significant change was observed in the group receiving RL treatment. Ionized calcium levels respond sensitively to changes in serum pH. An increase of 1 unit in serum pH decreases iCa by an average of 0.36 mmol/L and potassium by 0.6 mmol/L (5,6). Similarly, we observed that potassium levels decreased less in patients receiving RL treatment compared to those receiving IS treatment. In our patients who were dehydrated due to diarrhea and whose existing acidosis was corrected with RL treatment, we noticed that, despite the increase in pH levels, iCa levels remained stable, and potassium levels decreased slightly. However, in patients receiving IS treatment, we observed a decrease in iCa and potassium levels despite no increase in pH. This suggests that the content of calcium and potassium in RL solution prevents electrolyte imbalances that can occur due to pH elevation. In terms of preventing potential complications, the use of RL may be considered safer than IS.

In our study, we observed that in the group treated with RL, post-treatment BE levels approached positive values, indicating an increase. In contrast, in the group receiving IS, we found that BE levels decreased after treatment. A study addressing this topic reported a significant decrease in BE levels with both fluid treatments (20). We observed concurrent changes in BE and pH values in both groups. This could be interpreted as indicating that acidosis may have a primarily metabolic origin. In our study, we identified a significant decrease in lactate levels in both treatment groups. Intravenous hydration with both types of fluid therapy may be associated with reduced lactate levels, facilitating the transport of more oxygen to the tissues. Despite the presence of lactate in RL solution, the stable serum lactate levels can be explained by the metabolism of lactate into bicarbonate, which results in decreased serum lactate levels as acid-base balance is achieved. This signal persisted after the two-step screening and balanced random selection of cases to equalize groups, with no signal of harm compared with IS in this dataset.

Study Limitations

Due to the retrospective design of our study, limitations in data availability prevented the comparison of changes in urea and creatinine levels between the groups. Additionally, information regarding the duration of patients' symptoms, the number of diarrheal episodes, the length of hospital stays, and rates of rehospitalization was not accessible. Although the groups were composed of similar patients without comorbidities, additional treatments or foods (e.g., zinc supplementation, hydration) administered by families during the treatment period without the physician's knowledge may have influenced the results, given the retrospective nature of the study. We believe that prospective studies could contribute new insights and knowledge to the medical field by offering more comprehensive data collection and analysis capabilities.

Conclusion

Our study indicates that for patients diagnosed with AGE, RL fluid therapy provides a more effective improvement in clinical and laboratory findings compared to IS fluid therapy. The high chloride levels in IS may delay the correction of acidosis, and we consider RL solution more advantageous in terms of potential complications such as hypokalemia and hypocalcemia during pH correction. Therefore, in the choice of replacement solution in cases of acute diarrhea, RL might be the preferred option if there are no contraindications.

Ethics

Ethics Committee Approval: Ethical approval to conduct this study was obtained from the University of Health Sciences Türkiye, Istanbul Haseki Training and Research Hospital Clinical Research Ethics Committee (approval no.: 74-2023, date: 12.04.2023).

Informed Consent: Written consent was obtained from the parents of enrolled children.

Footnotes

Authorship Contributions

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

Conflict of Interest: No conflicts of interest were declared by the authors.

Financial Disclosure: This study received no financial support.

References

1. World Health Organization. Diarrhoeal disease [Internet]. Geneva: World Health Organization; 2024 Mar 7 [cited 2025 Sep 11]. Available from: https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease
2. UNICEF. Diarrhoea [Internet]. New York: UNICEF Data; 2024 Nov 1 [cited 2025 Sep 11]. Available from: https://data.unicef.org/topic/child-health/diarrhoeal-disease/
3. Türk Biyokimya Derneği. Kan gazı, pH ve ilişkili diğer ölçümlerde preanalitik evre kılavuzu [Internet]. Ankara: Türk Biyokimya Derneği; 2023 Nov 30 [cited 2024 Jun 22]. Erişim adresi: https://www.turkbiyokimyadernegi.org.tr/upload/48/Dosyalar/tmp/Kan%20Gaz%C4%B1.pdf
4. Prough DS, Bidani A. Hyperchloremic metabolic acidosis is a predictable consequence of intraoperative infusion of 0.9% saline. Anesthesiology. 1999;90:1247-9.
5. Wang S, McDonnell EH, Sedor FA, Toffaletti JG. pH effects on measurements of ionized calcium and ionized magnesium in blood. Arch Pathol Lab Med. 2002;126:947-50.
6. Palmer BF, Clegg DJ. Physiology and pathophysiology of potassium homeostasis. Adv Physiol Educ. 2016;40:480-90.
7. Children's Mercy Kansas City. Acute gastroenteritis: clinical pathway synopsis [Internet]. 2024 Jan 17 [cited 2025 Sep 12]. Available from: https://www.childrensmercy.org/siteassets/media-documents-for-depts-section/documents-for-health-care-providers/block-clinical-practice-guidelines/mobileview/acute-gastroenteritis-synopsis.pdf
8. Türkiye İlaç ve Tıbbi Cihaz Kurumu. Polifleks Laktatlı Ringer kullanma talimatı [Internet]. 2023 Dec 1 [cited 2024 Jul 02]. Available from: https://titck.gov.tr/storage/Archive/2020/kubKtAttachments/T%C4%B0TCK%20POL%C4%B0FLEKS%20LAKTATLI%20R%C4%B0NGER%20UYGUN%20KT.pdf_3570a3ba-228e-4265-bf70-abb632416637.pdf
9. Kartha GB, Rameshkumar R, Mahadevan S. Randomized double-blind trial of Ringer lactate versus normal saline in pediatric acute severe diarrheal dehydration. J Pediatr Gastroenterol Nutr. 2017;65:621-6.
10. Kliegman RM, St. Geme JW 3rd, editors. Nelson Textbook of Pediatrics. 22nd ed. Philadelphia: Elsevier; 2024.
11. Kimberlin DW, Banerjee R, Barnett ED, Lynfield R, Sawyer MH, editors. Red Book: 2024–2027 Report of the Committee on Infectious Diseases. 33rd ed. Itasca (IL): American Academy of Pediatrics; 2024.
12. Üstebay S, Ülker Üstebay D, Ertekin Ö. The Frequency of adenovirus and rotavirus for children with acute gastroenteritis. Kafkas J Med Sci. 2019;9:6-10.
13. Olesen B, Neimann J, Böttiger B, et al. Etiology of diarrhea in young children in Denmark: a case-control study. J Clin Microbiol. 2005;43:3636-41.
14. Khales P, Razizadeh MH, Ghorbani S, et al. Human adenoviruses in children with gastroenteritis: a systematic review and meta-analysis. BMC Infect Dis. 2024;24:478.
15. Aydın A, Arslan N, Bim G, et al. The evaluation of admission signs and treatment response of patients with acute gastroenteritis. Dokuz Eylül Üniversitesi Tıp Fakültesi Dergisi. 2006;20:1-5.
16. Aydın A, Adal E. Evaluation of clinical and laboratory findings of dehydration in children. Türk Pediatri Arşivi. 1996;31-3.
17. Wildi-Runge S, Allemann S, Schaad UB, Heininger U. A 4-year study on clinical characteristics of children hospitalized with rotavirus gastroenteritis. Eur J Pediatr. 2009;168:1343-8.
18. Mahajan V, Sajan SS, Sharma A, Kaur J. Ringers lactate vs normal saline for children with acute diarrhea and severe dehydration- a double blind randomized controlled trial. Indian Pediatr. 2012;49:963-8.
19. Naseem M, Dubey AP, Mishra TK, Singh R. Effect of rehydration with normal saline versus Ringer lactate on serum sodium level of children with acute diarrhea and severe dehydration: a randomized controlled trial. Indian Pediatr. 2020;57:519-22.
20. Zunini GS, Rando KA, Cox RG. Fluid replacement in craniofacial pediatric surgery: normal saline or ringer's lactate? J Craniofac Surg. 2011;22:1370-4.
21. Plancarte C, Stopczynski T, Hamdan L, et al. Evaluating acute viral gastroenteritis severity: modified Vesikari and Clark scoring systems. Hosp Pediatr. 2024;14:430-7.
22. Jucá CA, Rey LC, Martins CV. Comparison between normal saline and a polyelectrolyte solution for fluid resuscitation in severely dehydrated infants with acute diarrhoea. Ann Trop Paediatr. 2005;25:253-60.
23. O'Connor MF, Roizen MF. Lactate versus chloride: which is better? Anesth Analg. 2001;93:809-10.
24. Rasheed S, Rafique S, Hussain AW, et al. Comparison of outcome between Ringer's lactate and normal saline fluid replacement in pediatric patients with acute watery diarrhoea. Pak Armed Forces Med J. 2020;101-5.
25. Scheingraber S, Rehm M, Sehmisch C, Finsterer U. Rapid saline infusion produces hyperchloremic acidosis in patients undergoing gynecologic surgery. Anesthesiology. 1999;90:1265-70.
26. Ho AM, Karmakar MK, Contardi LH, Ng SS, Hewson JR. Excessive use of normal saline in managing traumatized patients in shock: a preventable contributor to acidosis. J Trauma. 2001;51:173-7.
27. Kutacun Z. Hızlı ve uzun süreli izotonik sodyum klorür infüzyonunun asit-baz dengesine etkilerinin Ringer laktat ile karşılaştırılması [Internet]. 2005 [cited 2024 Jun 12]. Available from: https://acikbilim.yok.gov.tr/bitstream/handle/20.500.12812/28817/yokAcikBilim_10020180.pdf

28. Neyra JA, Canepa-Escaro F, Li X, et al. Association of hyperchloremia with hospital mortality in critically ill septic patients. Crit Care Med. 2015;43:1938-44.

29. Barker ME. 0.9% saline induced hyperchloremic acidosis. J Trauma Nurs. 2015;22:111-6.

30. Bampoe S, Odor PM, Dushianthan A, et al. Perioperative administration of buffered versus non-buffered crystalloid intravenous fluid to improve outcomes following adult surgical procedures. Cochrane Database Syst Rev. 2017;9:CD004089.

Original Article

DOI: 10.4274/haseki.galenos.2025.43534
Med Bull Haseki 2025;63(5):267-273

Comparison of Visual Evoked Potential in Patients with Attention-deficit/Hyperactivity Disorder in Medication and Non-medication Group

Betul Kırsavoglu*, Zeynep Unluturk**

*University of Health Sciences Türkiye, Kocaeli Derince Training and Research Hospital, Clinic of Psychiatry, Kocaeli, Türkiye

**University of Health Sciences Türkiye, Kocaeli City Hospital, Clinic of Neurology, Kocaeli, Türkiye

Abstract

Aim: Attention-deficit/hyperactivity disorder (AD/HD) is a common neuropsychiatric disorder resulting from disruptions in circuits that regulate attention and action. Many studies have revealed different parameters of AD/HD through neurophysiological measurements. The purpose of this study is to compare visual evoked potentials (VEPs) latency and amplitude in AD/HD patients. This study aims to discuss the effects of disease severity and psychostimulants use on the central nervous system based on VEP measurements.

Methods: The study was designed using a retrospective-descriptive methodology, including a total of 30 patients diagnosed with AD/HD, 15 of whom were taking psychostimulant medication and 15 of whom were not, who presented to the psychiatry outpatient clinic between January 2024 and June 2024. P-100 amplitude and latency were recorded for both eyes. In AD/HD patients, these values were compared based on the severity of the disease, between those who receive treatment and those who do not.

Results: There was no statistically significant difference between latency and amplitude values according to disease severity. The difference was statistically significant between P-100 latency according to psychostimulant use (p=0.046, p=0.016). With regard to the use of psychostimulants, there was no significant difference in the amplitude values.

Conclusion: VEP studies with increased sample size AD/HD, with additional confounding variables, will provide insight into information processing and shed light on the pathophysiology of AD/HD.

Keywords: Attention-deficit/hyperactivity disorder, visually evoked potentials, P-100

Introduction

Attention-deficit/hyperactivity disorder (AD/HD) is a chronic, heritable neurobehavioral condition marked by impulsivity, hyperactivity, and inattention, and affects 1.4-3% of the population (1). Approximately 60% to 80% of AD/HD symptoms last throughout adulthood. Therefore, AD/HD is not exclusively a childhood condition that subsides on its own after adolescence, and 4.4% of adults on average experience it (2). Although there are similarities in adult and childhood symptomatology, there are also important differences. Adults frequently experience emotional dysregulation, inattention compensated through depressive and anxious mechanisms, executive function (EF) -related symptoms, substance use disorders, and sleep disorders (3). Adults and children with AD/HD have varying levels of impairment in EFs, according to studies. The ability to mentally engage with ideas, wait before acting, meet unique, unexpected problems, resist temptations, and maintain attention is an example of EFs (4). Basic cognitive processes like attention management, cognitive inhibition, inhibitory control, working memory, and cognitive flexibility are examples of EFs, which are a fundamental to cognition (5). Working memory, also known as short-term memory, is affected in AD/HD,

Corresponding Author: Betul Kırsavoglu, MD, University of Health Sciences Türkiye, Kocaeli Derince Training and Research Hospital, Clinic of Psychiatry, Kocaeli, Türkiye
E-mail: betulkirsavoglu@hotmail.com **ORCID:** orcid.org/0000-0002-3703-9035
Received: 11.11.2024 **Accepted:** 20.10.2025 **Publication Date:** 28.11.2025

Cite this article as: Kırsavoglu B, Unluturk Z. Comparison of visual evoked potential in patients with attention-deficit/hyperactivity disorder in medication and non-medication group. Med Bull Haseki. 2025;63(5):267-273

where cognitive control, self-direction, self-regulation, and stimulus-driven behavioral responses are impaired, and is encoded with visual and auditory stimuli (6).

Researchers have used neurophysiological examinations to elucidate and correlate the complex neural pathways and etiology of neurodevelopmental mental diseases, such as AD/HD. Few studies have investigated visual evoked potential (VEP) patterns as a helpful tool for understanding visual processing (7). VEPs are electrical potentials triggered by brief visual stimuli, and recorded from the scalp the overlying the visual cortex. Signal averaging is used to extract VEP waveforms with an electroencephalogram (EEG) (8). Electrical changes in the central nervous system (CNS) caused by external stimuli are frequently recorded using evoked potentials. Clinically, short-latency brainstem auditory evoked response, somatosensory evoked potential, and VEPs are used. These evoked potentials represent the neuronal response to the given stimulus. Their amplitudes and latency are determined by the stimulus's physical properties. These waves have been researched in a variety of neurological and psychiatric syndromes, particularly schizophrenia, some types of anxiety disorders, and epilepsy. It is a non-invasive procedure that provides information about neural activity connected to sensory and cognitive information processing (9).

The goal of this study was to compare VEP latency and amplitude in one treated and one untreated group of 30 AD/HD patients, 15 of whom are on psychostimulant medication, and 15 of whom are not. We hypothesize that the cognitive impacts of disease severity and medication use in individuals with AD/HD can be demonstrated by VEP measurements.

Materials and Methods

Written informed consent was obtained from the patients in line with the ethical rules stated by the Declaration of Helsinki. Ethics approval was received on 09.03.2023 with approval number 2023-21, from the Scientific Research Ethics Committee of University of Health Sciences Türkiye, Kocaeli Derince Training and Research Hospital where the study was carried out.

Patients and Data

The sample size was calculated with power analysis by G*Power version 3.1.9.4. The minimum sample size was determined as 22 patients, with 11 patients in each group, based on the reference study and normal standard deviation at a 95% confidence level (1.81), as cited in the reference study (2).

Thirty patients aged between 18 and 65 years who applied to University of Health Sciences Türkiye, Kocaeli Derince Training and Research Hospital Psychiatry Outpatient Clinic with a diagnosis of AD/HD were included in the study. From this patient group, 15 patients who used psychostimulant drugs, and 15 who did not use them, were admitted to the psychiatry outpatient clinic between January 2024 and June 2024. The adult AD/HD Diagnostic Screening and Rating Scale, which has been validated for Turkish populations, was applied to all participants (10). The scale is a five-point Likert-type rating scale and consists of three subsections: Section 1, Attention Deficit: This section contains nine items based on symptoms of AD according to Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV). Section 2: Hyperactivity/ Impulsivity Section: This section also contains nine items based on symptoms of hyperactivity according to DSM-IV. Section 3: Characteristics and Problems Related to ADHD (Problem): This section, based on clinical experience and observations, contains a total of 30 items. The patients with total scores below 20 were considered mild, between 20-59 moderate, and above 59 severe AD/HD. Visual evoked potential measurements were performed on all patients. The obtained VEP measurements were compared according to the patients' disease severity and treatment status.

Participants had no eye disease, anisocoria, or pupillary abnormality. Visual acuity was normal, and no myotic or mydriatic drops were used.

Study Design

Pattern visual evoked potential were recorded with a Viasys Medelec Synergy device. Pattern visual evoked potential tests were performed in a dark, sound-isolated room. Surface electrodes were used, ensuring that the scalp was cleaned and the electrodes were entirely placed. The active electrode was placed 2 centimeters above the external occipital protuberance; the reference electrode was placed on the vertex; and the ground electrode was placed on the scalp border on the forehead.

The recordings were made in a 1-degree pattern size according to the International Society for Clinical Electrophysiology of Vision protocol. While the patients were looking at the fixation point the middle of the moving chessboard pattern on the screen 1 meter in front of them, the electrical potentials at the occipital cortex were recorded. The black-white chessboard pattern reversed at 2 reversals per second.

An average of 100 waves was recorded. The latency was measured in milliseconds (ms) and the amplitude was measured in microvolts (mV). The contrast was measured to be 99% according to the Michelson constant. When the cover or environmental artifacts exceeded 5%, the study was repeated.

Two negative waveforms (N75 and N135 peaks) and one positive waveform (P-100 peak) were recorded

for each eye (Figure 1). All recordings were made with every participant's eyes closed. Participants were closely followed by an experienced electrophysiology technician who ensured that they looked at the fixation point. Records were repeated for both eyes, of all patients, to ensure accuracy. P-100 latency and amplitude for both eyes of all patients were compared.

Statistical Analysis

Statistical Package for Social Science (IBM SPSS Statistics 25 software, Armonk, NY: IBM Corp.) was used for data analysis. Descriptive statistical methods were used. Continuous variables were expressed as mean ± standard deviation, and categorical variables were expressed as numbers. Metric data with a normal distribution were compared using the independent samples t-test. The Mann-Whitney U test was used to analyze the presence of significant differences when data were non-normally distributed. A p-value under 0.05 was considered statistically significant.

Results

Thirty patients were included in the study; 19 were male and 11 were female. Five women and 10 men were using psychostimulant drugs. The mean age of all participants was 25.53±8.1, while the mean age of women was 24.91±7.1 and that of men was 25.89±8.9. There was no statistically significant difference in the age or the gender distribution of the patients.

Adult AD/HD Diagnostic Screening and Rating Scale was applied to all patients. Eight (26.7%) of the patients were moderate and the other 22 (73.3%) were diagnosed with severe AD/HD. Five (62.5%) of the patients with moderate AD/HD were on medication, while 10 (45.5%) of the patients with severe AD/HD were on medication (Table 1).

The pattern VEP right P-100 latency, right P-100 amplitude, left P-100 latency, left P-100 amplitude, and mean P-100 latency values of all patients were compared. Mean left P-100 latency was 103.86±5.0 ms. Right P-100 latency was 105.73±5.6 ms. Left P-100 amplitude was 11.1±4.0 mV. Right P-100 amplitude was 10.6±4.4 mV. Four VEP values of each patient were compared according to medication use and AD/HD severity (Table 2).

The mean right P-100 latency of AD/HD patients who were taking medication was 107.6±4.7 ms, and of those who were not taking medication was 101.1±4.8 ms. The left P-100 latency of AD/HD patients who were taking medication was 108.4±4.8 ms, and of those who were not taking medication was 103.0±5.2 ms. The difference was statistically significant between P-100 latency according to pshycostimulant use (p=0.046, p=0.016). When the mean of right and left P-100 latency values were compared in the two groups, the difference was also statistically significant (p=0.008).

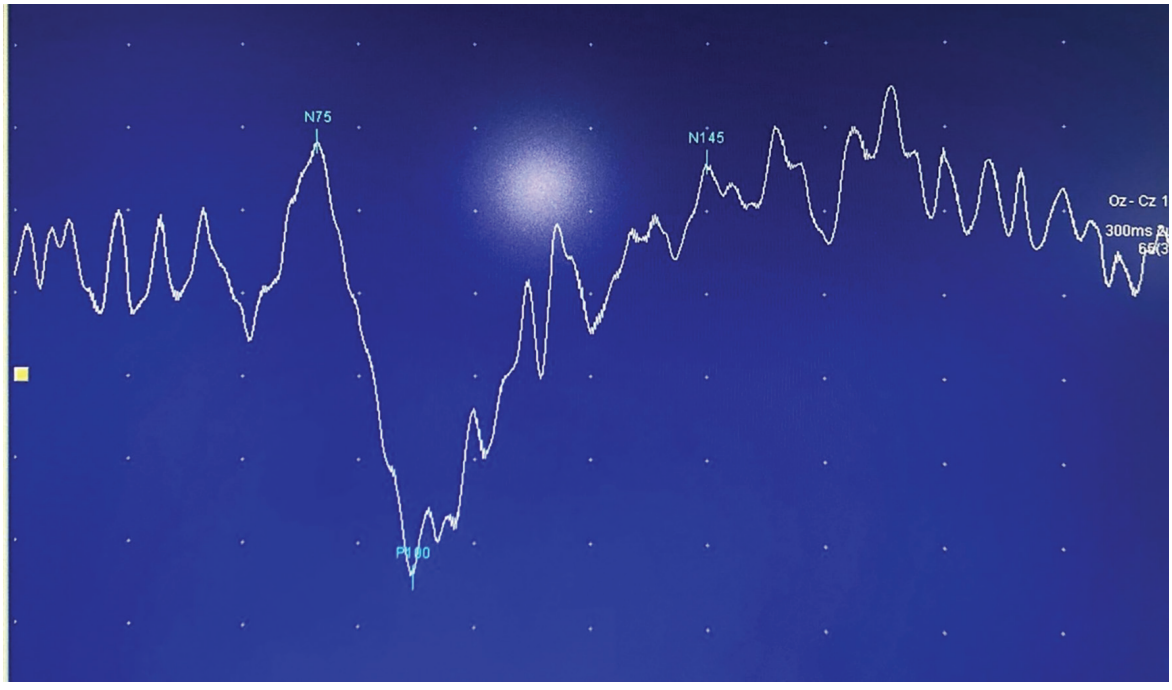

Figure 1. VEP-N75, P100, N145 waves
VEP: Visual evoked potential

Table 1. Demographics and clinical characteristics of the groups				
Characteristics	**Medication (n=15)**	**Non-medication (n=15)**	**Total (n=30)**	**p-value**
Age (years), mean ± SD	25.89±8.9	24.91±7.1	25.53±8.1	0.72
Gender (M/F)	10/5	9/6	19/11	0.71
AD/HD score, mean ± SD	58.4±10.2	56.7±9.8	57.6±9.9	0.65

Data are presented as mean ± SD or n. An independent samples t-test was used for normally distributed continuous variables. The Mann-Whitney U test was used for non-parametric data. P<0.05 was considered statistically significant
AD/HD: Attention-deficit/hyperactivity disorder, SD: Standard deviation, M: Male, F: Female

Table 2. P-100 values according to psychostimulant use and adhd severity						
	Psychostimulant medication		**p-value**	**AD/HD severity**		**p-value**
	Under-medication	**Non-medication**		**Moderate**	**Severe**	
Left P-100 latency (ms)	108.4±4.8	103.0±5.2	0.046*	106.4±5.2	103±4.7	0.098
Right P-100 latency (ms)	107.6±4.7	101.1±4.8	0.016*	105.8±4.4	105.6±6.1	0.777
Mean P-100 latency (ms)	107±3.9	102.6±4.6	0.008*	106.1±4.4	104.3±4.9	0.368
Left P-100 amplitude (mV)	10.4±3.9	11.9±4.2	0.321	9.4±2.6	11.8±4.4	0.163
Right P-100 amplitude (mV)	10.6±4.7	10.7±4.3	0.974	8.7±2.8	11.4±4.7	0.150
Mean P-100 amplitude (mV)	10.5±4.2	11.3±4.1	0.614	9.1±2.6	11.6±4.4	0.143

Data are presented as mean ± SD or n. Independent samples t-test was used for normally distributed continuous variables. Mann-Whitney U test was used for non-parametric data. A p-value <0.05 was considered statistically significant
P-100 values according to psychostimulant use and AD/HD severity
AD/HD: Attention-deficit/hyperactivity disorder, ms: Millisecond, mV: Microvolt

Discussion

In our study, left P-100 latency and right P-100 Latency values differed significantly between groups that used psychostimulants and those that did not. There was no noticeable difference when the latency values were compared according to the severity of AD/HD. Attention-deficit/hyperactivity disorder is a disorder that affects both simple and complicated cognitive processing, even though it involves behavioural problems. Distractibility, slow processing speed, and rising response time variability are examples of basic cognitive processes (11,12). Distractibility is the characteristic that leads individuals to become distracted from the intended stimuli. The failure to store the appropriate stimulus in memory or recording incorrect components is caused by distraction or task diversion. Distractibility in AD/HD is caused by an inability to reject unnecessary data or an excessive amount of attention to task-irrelevant stimuli (13-15). It is still unclear which stage of information processing is affected by attention problems in AD/HD and which regions of the CNS play a role in this problem. According to some theories, the CNS's three neural networks play a significant role in how effectively sustained attention processes are carried out. The alerting network ensures that this state of alertness is maintained and that the reaction is prepared. The excitatory network includes the right parietal lobe, the locus coeruleus and the right frontal lobe, particularly in some higher regions of the 6th Brodmann area. It seems that noradrenaline has a special role in the excitatory network's operation (16,17). Sensory stimuli are oriented via the orientation network. This network has been the subject of research, particularly with regard to visual stimuli. The parietal lobes, the oculomotor system, and the visual regions, particularly the fusiform gyrus, make-up the majority of the extrinsic network for processing visual stimuli. Acetylcholine appears to play a key role in the functioning of the orientation network (16,18). The executive-control network is focused on regulating intentional behavior, target identification, error detection, problem solving, and restraining automatic responses. This network consists of the basal ganglia and anterior cingulate gyrus. The executive-controller network appears to be particularly dependent on dopamine for proper function (16,19). Adults with AD/HD show impairments in both basic cognitive abilities such as slower processing speeds and increased distractibility and more advanced abilities such as problems with cognitive flexibility, selective attention, planning, verbal fluency, working memory, and memory functions (10,20). From the perspectives of neuroanatomy and chemistry, AD/HD is viewed as a very heterogeneous condition.

Measurement of evoked potentials has been used in several studies in an attempt to explain this complex relationship. Visual evoked potentials are recordings of electrical brain responses that occur in the occipital cortex in response to visual stimuli received by photoreceptors in response to visual stimuli. It measures the duration for

neuronal activity to transit from the retina to the occipital cortex and is used clinically to assess the pathway's integrity and function. The multiple stimulus-dependent waveforms are averaged by standard VEP. However, the positive wave on the midline occipital EEG electrode, marked P-100, which typically occurs about 100 ms after stimulation, is thought to be significant. For many disorders, their amplitude and latency are revealing (21,22). However, research has been conducted to determine whether measuring VEP-based wave qualities can be used to differentiate between various neurodevelopmental, neurodegenerative and mental diseases (23-25). Results from a study comparing VEP values of 12 healthy individuals, 12 patients with AD/HD, and 12 patients with bipolar mood disorder show that there is a significant difference in the neural activity of the visual systems in response to periodic optical stimuli among individuals with AD/HD, bipolar mood disorder, and healthy controls (2).

In a systematic review of EEG findings in adult AD/HD, Adamou et al. (26) revealed that EEG measurements differed for this disorder. Reasearch into the relationship between elevated theta (4-8 Hz) levels, alpha waves (8-10 Hz), beta waves (12-25 Hz), delta activity, and gamma band activity in EEG measurements and AD/HD has revealed inconsistent findings. Low gamma band activity has been observed in adults with AD/HD, which is consistent with research conducted in children and adolescents. The dysregulation of brain networks related to attention function was assumed to be the explanation of this (27-30). Studies focusing on event-related potential (ERP) measurements in AD/HD, on the other hand, have discovered these changes can be used as an informative tool. Event-related potentials studies are very beneficial in investigating a specific neural response triggered by cognition. A reaction to cognitive processing, such as viewing stimuli during assessment with scales, causes ERPs, which is a brief segment of the ongoing EEG recording (26,31). In adult AD/HD, it is visible that there is increased variability for both auditory and visual stimuli, as well as slowed cortical activity, which is consistent with the commonly predicted research findings (27,32). According to a study conducted by Leroy et al. (33), findings support the idea that earlier cortical levels of visual processing are impaired in the disorder, resulting in the formation of various ERP generators and EEG patterns in adult AD/HD. Hasler et al. (34) found that the functional networks responsible for bottom-up and top-down attention were less active, which suggests that people with AD/HD have less cortical capacity for activities involving these processes. There have been few studies that have investigated the severity of AD/HD symptoms, psychostimulant use, and ERP characteristics. Transcranial Magnetic Stimulation evoked potentials and ERPs were analyzed in a study by Hadas et al. (35); and right prefrontal cortex excitability was closely linked with AD/HD severity and behavioral impulsivity. According to studies assessing the severity of symptoms using ERP components such as mismatch negativity, it appears to predict the severity of AD/HD symptoms in children and adolescents. These results support the use of ERPs in assessing AD/HD symptoms in patients (36,37).

Methylphenidate (MPH) is the most commonly prescribed AD/HD medication as it helps increase and maintain alertness, tackle fatigue, and enhance focus. improvements in cognition, such as working and episodic memory (38,39). By comparing the ERPs of participants with AD/HD, following treatment with MPH, to participants who received a placebo, some EEG research in this field has attempted to answer this question. Unfortunately, findings for the N1, N2, P2, and P3 components' amplitudes and latencies varied between analyses (40,41).

Study Limitations

Including a larger number of volunteers in the study is important for the consistency of the results. In this respect, it is important that future studies in this field be conducted with a larger number of patients. Additionally, no comparison was made in the study regarding the medication types, doses, and the periods of intake by the patients due to difficulty in classification. Another limitation of the study is the lack of healthy control group and the exclusion of EEG findings. The strength of the study is that it is the first study to examine confounding factors that may affect information processing speed, such as disease severity and treatment intake, using neurophysiological measures of visual processing speed.

Conclusion

This study shows that the P-100 latency, which indicates the optic nerve to occipital cortex conduction, is longer in AD/HD patients using psychostimulant medication. This would suggest an additional process in the conditioning phase of a stimulus rather than the recording phase. As expected, if the distractibility and disease severity caused a difference between the treated/untreated and moderate to severe groups, longer latencies and smaller amplitudes would be expected in untreated and severe patients. Larger sample size VEP studies in AD/HD with additional confounding factors will provide an understanding of information processing and elucidate the pathophysiology of this disorder.

Ethics

Ethics Committee Approval: Ethics approval was received on 09.03.2023 with approval number 2023-21, from the Scientific Research Ethics Committee of University of Health Sciences Türkiye, Kocaeli Derince Training and Research Hospital which the study was carried out.

Informed Consent: Written informed consent was obtained from the patients in line with the ethical rules stated by the Declaration of Helsinki.

Acknowledgments

We would like to express our special gratitude and thanks to our colleague MD. Ezgi Yilmaz for imparting her knowledge and providing the necessary information regarding this research study.

Footnotes

Authorship Contributions

Concept: B.K., Design: Z.U., Data Collection or Processing: B.K., Z.U., Analysis or Interpretation: B.K., Z.U., Literature Search: B.K., Z.U., Writing: B.K., Z.U.

Conflict of interests: No conflict of interest were declared by the authors.

Financial Disclosure: This study received no financial support.

References

1. Thapar A, Cooper M. Attention deficit hyperactivity disorder. Lancet. 2016;387:1240-50.
2. Nazhvani AD, Boostani R, Afrasiabi S, Sadatnezhad K. Classification of ADHD and BMD patients using visual evoked potential. Clin Neurol Neurosurg. 2013;115:2329-35.
3. Weibel S, Menard O, Ionita A, et al. Practical considerations for the evaluation and management of Attention Deficit Hyperactivity Disorder (ADHD) in adults. Encephale. 2020;46:30-40.
4. Cristofori I, Cohen-Zimerman S, Grafman J. Executive functions. Handb Clin Neurol. 2019;163:197-219.
5. Chan RC, Shum D, Toulopoulou T, Chen EY. Assessment of executive functions: review of instruments and identification of critical issues. Arch Clin Neuropsychol. 2008;23:201-16.
6. Clark L, Blackwell AD, Aron AR, et al. Association between response inhibition and working memory in adult ADHD: a link to right frontal cortex pathology? Biol Psychiatry. 2007; 61:1395-401.
7. Hermens DF, Kohn MR, Clarke SD, Gordon E, Williams LM. Sex differences in adolescent ADHD: findings from concurrent EEG and EDA. Clin Neurophysiol. 2005; 116:1455-63.
8. Creel DJ. Visually evoked potentials. In: Kolb H, Fernandez E, Jones B, Nelson R, editors. Webvision: The Organization of the Retina and Visual System [Internet]. Salt Lake City (UT): University of Utah Health Sciences Center; 1995–. 2012 Mar 1 [updated 2015 Jul 14; cited 2025 Oct 22]. Available from: https://webvision.med.utah.edu/ PMID: 23035319.
9. Wu X, Tao M, Qiu Y. The diagnostic effect of integrated visual and auditory continuous performance and event-related potentials in ADHD. Am J Transl Res. 2024;16:5248-67.
10. Günay Ş, Savran C, Aksoy UM. Erişkin dikkat eksikliği hiperaktivite ölçeğinin (Adult ADD/AD/HD DSM IV-Based Diagnostic Screening and Rating Scale) dilsel eşdeğerlilik, geçerlik güvenirlik ve norm çalişmasi. Marmara Üniversitesi Atatürk Eğitim Fakültesi Eğitim Bilimleri Dergisi 21.21 (2005): 133-150.
11. Adams ZW, Roberts WM, Milich R, Fillmore MT. Does response variability predict distractibility among adults with attention-deficit/hyperactivity disorder? Psychol Assess. 2011;23:427-36.
12. Goth-Owens TL, Martinez-Torteya C, Martel MM, Nigg JT. Processing speed weakness in children and adolescents with non-hyperactive but inattentive ADHD (ADD). Child Neuropsychol. 2010;16:577-91.
13. Thorsen AL, Meza J, Hinshaw S, Lundervold AJ. Processing speed mediates the longitudinal association between ADHD symptoms and preadolescent peer problems. Front Psychol. 2018;8:2154.
14. Barkley RA. Behavioral inhibition, sustained attention, and executive functions: constructing a unifying theory of ADHD. Psychol Bull. 1997;121:65-94.
15. Gumenyuk V, Korzyukov O, Escera C, et al. Electrophysiological evidence of enhanced distractibility in ADHD children. Neurosci Lett. 2005;374:212-7.
16. Fan J, Wu Y, Fossella JA, Posner MI. Assessing the heritability of attentional networks. BMC Neurosci. 2001;2:14.
17. Witte EA, Marrocco RT. Alteration of brain noradrenergic activity in rhesus monkeys affects the alerting component of covert orienting. Psychopharmacology (Berl). 1997;132:315-23.
18. Davidson MC, Marrocco RT. Local infusion of scopolamine into intraparietal cortex slows covert orienting in rhesus monkeys. J Neurophysiol. 2000;83:1536-49. Erratum in: J Neurophysiol. 2003;89:3354
19. Simon H, Scatton B, Moal ML. Dopaminergic A10 neurones are involved in cognitive functions. Nature. 1980;286:150-1.
20. Butzbach M, Fuermaier ABM, Aschenbrenner S, Weisbrod M, Tucha L, Tucha O. Basic processes as foundations of cognitive impairment in adult ADHD. J Neural Transm (Vienna). 2019;126:1347-62.
21. Ghatol D, Widrich J. Intraoperative neurophysiological monitoring. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jul 24. Available from: https://www.ncbi.nlm.nih.gov/books/NBK563203/
22. Gronseth GS, Ashman EJ. Practice parameter: the usefulness of evoked potentials in identifying clinically silent lesions in patients with suspected multiple sclerosis (an evidence-based review): Report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology. 2000;54:1720-5

23. Šuštar Habjan M, Bach M, van Genderen MM, et al. ISCEV standard for clinical visual evoked potentials (2025 update). Doc Ophthalmol. 2025;151:97-112.

24. The temporal correlations of the VEP of the visual and motor cortices during the perception and mental reproduction of an image in normal children and in those with intellectual disorders]. Zh Vyssh Nerv Deiat Im I P Pavlova. 1997, 47:49-57.

25. Kim J, Sung IY, Ko EJ, Jung M. Visual evoked potential in children with developmental disorders: correlation with neurodevelopmental outcomes. Ann Rehabil Med. 2018;42:305-12.

26. Adamou M, Fullen T, Jones SL. EEG for diagnosis of adult ADHD: a systematic review with narrative analysis. Front Psychiatry. 2020;11:871.

27. Gonen-Yaacovi G, Arazi A, Shahar N, et al. Increased ongoing neural variability in ADHD. Cortex. 2016;81:50-63.

28. Hale TS, Smalley SL, Walshaw PD, et al. Atypical EEG beta asymmetry in adults with ADHD. Neuropsychologia. 2010;48:3532-9.

29. Woltering S, Jung J, Liu Z, Tannock R. Resting state EEG oscillatory power differences in ADHD college students and their peers. Behav Brain Funct. 2012;8:60.

30. Mercado-Aguirre I, Gutiérrez-Ruiz K, Contreras-Ortiz SH. Classification of auditory ERPs for ADHD detection in children. J Med Eng Technol. 2025; 49:69-78.

31. Kropotov JD, Pronina MV, Ponomarev VA, Poliakov YI, Plotnikova IV, Mueller A. Latent ERP components of cognitive dysfunctions in ADHD and schizophrenia. Clin Neurophysiol. 2019;130:445-53.

32. Herrmann MJ, Mader K, Schreppel T, et al. Neural correlates of performance monitoring in adult patients with attention deficit hyperactivity disorder (ADHD). World J Biol Psychiatry. 2010;11:457-64.

33. Leroy A, Petit G, Zarka D, et al. EEG dynamics and neural generators in implicit navigational image processing in adults with ADHD. Neuroscience. 2018; 373:92-105.

34. Hasler R, Perroud N, Meziane HB, et al. Attention-related EEG markers in adult ADHD. Neuropsychologia. 2016;87:120-33.

35. Hadas I, Hadar A, Lazarovits A, Daskalakis ZJ, Zangen A. Right prefrontal activation predicts ADHD and its severity: a TMS-EEG study in young adults. Prog Neuropsychopharmacol Biol Psychiatry. 2021;111:110340.

36. Yamamuro K, Ota T, Iida J, Nakanishi Y, Kishimoto N, Kishimoto T. Associations between the mismatch-negativity component and symptom severity in children and adolescents with attention deficit/hyperactivity disorder. Neuropsychiatr Dis Treat. 2016;12:3183-90.

37. Lee YJ, Jeong MY, Kim JH, Kim JS. Associations between the mismatch-negativity potential and symptom severity in medication-naïve children and adolescents with symptoms of attention deficit/hyperactivity disorder. Clin Psychopharmacol Neurosci. 2020;18:249-60.

38. Rösler M, Casas M, Konofal E, Buitelaar J. Attention deficit hyperactivity disorder in adults. World J Biol Psychiatry. 2010;11:684-98.

39. Parlatini V, Bellato A, Murphy D, Cortese S. From neurons to brain networks, pharmacodynamics of stimulant medication for ADHD. Neurosci Biobehav Rev. 2024;164:105841.

40. Berchio C, Kumar SS, Narzisi A, Fabbri-Destro M. EEG microstates in the study of attention-deficit hyperactivity disorder: a review of preliminary evidence. Psychophysiology. 2025;62:e14762.

41. García-Ponsoda S, Maté A, Trujillo J. Refining ADHD diagnosis with EEG: the impact of preprocessing and temporal segmentation on classification accuracy. Comput Biol Med. 2024;183:109305.

Original Article

DOI: 10.4274/haseki.galenos.2025.24008
Med Bull Haseki 2025;63(5):274-280

Evaluation of Eating Behaviors in Breath-holding Spells in Pediatrics: A Case-control Study

Orhan Coskun*, Sueda Is**, Ugur Topcu***, Enes Efe Is****

*University of Health Sciences Türkiye, Gaziosmanpasa Training and Research Hospital, Department of Pediatric Neurology, Istanbul, Türkiye

**Memorial Sisli Hospital, Clinic of Pediatrics, Istanbul, Türkiye

***University of Health Sciences Türkiye, Gaziosmanpasa Training and Research Hospital, Department of Pediatrics, Istanbul, Türkiye

****University of Health Sciences Türkiye, Sisli Hamidiye Etfal Training and Research Hospital, Department of Physical Medicine and Rehabilitation, Istanbul, Türkiye

Abstract

Aim: Breath-holding spells (BHS) are associated with various etiologies, including potential behavioral disorders. While factors like iron deficiency have been studied, the role of other micronutrients and specific eating behaviors remains under-investigated. Therefore, this study aimed to investigate vitamin deficiencies (folate and B12) and evaluate the eating behaviors of children with BHS.

Methods: This prospective, single-center, case-control study was conducted between June and September 2024. The study included 35 patients with BHS and 35 age- and sex-matched healthy controls. Blood and vitamin levels were compared, and the Children's Eating Behaviour Questionnaire (CEBQ) was administered to assess and compare eating patterns between the groups.

Results: Logistic regression analysis was performed to identify significant predictors for being in the patient group. Each unit increase in folate was associated with a 33% reduction in odds ratio (OR=0.67, p<0.001), and each unit increase in hemoglobin was associated with a 69% reduction in OR (OR=0.31, p=0.007). Regarding eating behaviors, only the "slow eating" subscale was a significant predictor; each point increase on this scale decreased the odds of being a patient by 13% (OR=0.87, p=0.025), indicating that faster eating is a risk factor. Ferritin, vitamin B12, and other CEBQ subscales, including "emotional overeating" and "enjoyment of food", were not significant predictors in the final model.

Conclusion: This study identifies folate deficiency and anemia as significant risk factors for BHS. This study is the first to report that children with BHS exhibit specific eating behaviors, such as rapid eating. These findings suggest that the clinical management of children with BHS should include screening for folate deficiency and providing guidance on observed eating behaviors.

Keywords: Breath-holding spells, feeding behavior, anemia, folic acid deficiency, infant

Introduction

Breath-holding spells (BHS) are common, generally benign paroxysmal events in early childhood that are often distressing to caregivers. The onset usually happens before the child is 18 months old, and up to 33% of cases have a family history of the condition (1). The pathophysiology is primarily linked to a dysregulation of the autonomic nervous system, with cyanotic spells precipitated by emotional triggers and pallid spells caused by vagally mediated cardiac inhibition (1-3). Supporting this neurophysiological basis, one study found significantly prolonged interpeak latencies in brainstem auditory evoked potentials among children with BHS, suggesting that delayed myelination of the brainstem may be a contributing factor (4,5).

Beyond autonomic dysfunction, both nutritional and psychosocial factors have been implicated in BHS. The link between iron deficiency and increased irritability is well-

Corresponding Author: Orhan Coskun, MD, University of Health Sciences Türkiye, Gaziosmanpasa Training and Research Hospital, Department of Pediatric Neurology, Istanbul, Türkiye
E-mail: dr.orhancoskun@hotmail.com **ORCID:** orcid.org/0000-0001-9229-404X
Received: 07.01.2025 **Accepted:** 20.10.2025 **Publication Date:** 28.11.2025

Cite this article as: Coskun O, Is S, Topcu U, Is EE. Evaluation of eating behaviors in breath-holding spells in pediatrics: a case-control study. Med Bull Haseki. 2025;63(5):274-280

established, and specific temperamental traits like low frustration tolerance have also been identified in these children (6,7). However, the roles of other micronutrients and specific eating behaviors, which could be linked to both temperament and nutritional status, remain under-investigated. We hypothesized that children with BHS would exhibit distinct eating behaviors (such as emotional or rapid eating) and have lower levels of specific micronutrients, particularly folate, compared to healthy controls.

While the roles of iron deficiency and temperament have been explored, the potential contribution of other micronutrient deficiencies and specific eating behaviors to BHS remains under-investigated. We hypothesized that children with BHS would exhibit distinct eating behaviors and have lower levels of key hematological parameters, particularly folate, compared to healthy controls. Therefore, the aim of this study was to test this hypothesis by evaluating eating behaviors and investigating the association between BHS and serum levels of folic acid and other hematological parameters. By identifying these nutritional and behavioral patterns, this study could contribute to a more comprehensive clinical assessment and provide a basis for targeted therapeutic approaches.

Materials and Methods

Compliance with Ethical Standards

This study was conducted in accordance with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Gaziosmanpasa Training and Research Hospital (approval no.: 20, date: 05.06.2024). Written informed consent was obtained from the parents or legal guardians of all participating children prior to their inclusion in the study.

Study Design and Participants

This prospective, single-center, case-control study was conducted at the Gaziosmanpasa Training and Research Hospital in Istanbul, Türkiye, between June 2024 and September 2024. The study included a patient group and a control group, with participants aged between 2 and 6 years.

The patient group consisted of 35 children with a diagnosis of BHS, recruited from the pediatric neurology outpatient clinic. Inclusion criteria were an established diagnosis of BHS confirmed by clinical evaluation and review of family-recorded videos. Exclusion criteria included the presence of epilepsy, cardiac rhythm disorders, or other chronic neuropsychiatric conditions (e.g., autism spectrum disorder), as well as regular medication use. All patients had normal cranial magnetic resonance imaging, sleep electroencephalogram (EEG), and electrocardiogram (ECG) findings. The Denver Developmental Screening Test was administered to all participants to ensure age-appropriate development.

The control group consisted of 35 healthy children matched for age, sex, and socio-economic status, recruited from the general pediatrics clinic. These children had no known acute or chronic diseases and were not on regular medication. For the control group, blood test results were obtained from the hospital's information system for age- and sex-matched healthy children who underwent routine blood tests during the same study period. The participant selection process is summarized in a flow diagram (Figure 1).

Procedures and Data Collection

For all participants, data on age, gender, height, weight, breastfeeding history, and number of siblings were collected. Body mass index (BMI) was calculated, and all participants were confirmed to be within the 5th and 95th percentiles for their age.

Eating behaviors were assessed using the Turkish validated version of the Children's Eating Behaviour Questionnaire (CEBQ), a 35-item parent-report tool that scores eight subscales of eating behavior (8,9).

Step 1. Total Number of Patients Screened: Potentially eligible patients followed in the Pediatric Neurology Clinic (n=42)
Step 2: Exclusion: Excluded Patients (n=7) **Refused to participate in the study (n=5)** **Incomplete test results (n=2) (EEG, MRI, ECG, Blood Results)**
Step 3: Case Group - Number of Patients with Breath-Holding Seizures Included in the Analysis as the Final Case Group (n=35)
Step 4: Control Group **Number of Healthy Children Matched by Age, Gender, and Socioeconomic Status Who Met the Inclusion Criteria and Included in the Analysis as the Control Group (n=35)**

Figure 1. Study flow diagram

The questionnaire is used in various patient groups and healthy populations and is an internationally validated tool (10-12).

For the patient group, blood samples were collected to measure serum levels of ferritin, vitamin B12, folate, and hemoglobin (Hb).

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 23.0. Descriptive statistics were presented as mean ± standard deviation for normally distributed variables and frequency (percentage) for categorical variables. Group comparisons were conducted using the independent samples t-test for normally distributed data and the Mann-Whitney U test for non-normally distributed data. Categorical variables were compared using the chi-squared test. Correlations were assessed using Pearson or Spearman's rho tests where appropriate. A p-value of <0.05 was considered statistically significant.

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 23.0. The normality of the distribution for continuous variables was assessed using the Kolmogorov-Smirnov test. Descriptive statistics were presented as mean ± standard deviation for normally distributed variables and as median (interquartile range) for non-normally distributed variables, while categorical variables were presented as frequency (percentage). For group comparisons, the independent samples t-test was used for normally distributed data, and the Mann-Whitney U test was used for non-normally distributed data. Categorical variables were compared using the chi-squared test. Correlations were assessed using Pearson or Spearman's rho tests where appropriate. A p-value of <0.05 was considered statistically significant.

Post-hoc power analysis was performed using the G*Power 3.1.9.7 program for an achievable sample size. In the post-hoc power analysis, the effect size was associated with power (1-β)=0.895 and power (1-β)=0.882 for the independent sample t-test when the significance criterion was d=0.7 and α=0.05, with the number of samples for the first group being 35, and the number of samples for the second group being 35. The statistical power (1-β) was found to be 0.917 for the chi-squared test, with an effect size of d=0.4, a significance level of α=0.05, and a total sample number of 70. For the correlation analysis, the effect size was calculated as power (1-β)=0.967, when the significance criterion was α=0.05 and the number of samples was 70.

Results

Sample Characteristics

The study included a total of 70 children, comprising 35 patients with BHS and 35 healthy controls. The two groups were well-matched, showing no statistically significant differences in gender, age, BMI, number of siblings, or history of breastfeeding (Tables 1, 2).

Comparison of Blood Parameters

The comparison of blood parameters revealed key differences between the groups (Table 3). Folate and Hb values were significantly lower in the patient group (p<0.001 for both). No significant differences were found for ferritin, vitamin B12, or other hematological parameters.

Although the mean folate level in the patient group was statistically lower, the rate of clinical folate deficiency (defined as a serum level <5.3 ng/mL) was found in 22.8% of patients (n=35) compared to 11.42% of controls (n=35).

Comparison of Eating Behaviors (CEBQ)

While the total CEBQ score did not differ between the groups, analysis of the subscales revealed significant behavioral differences (Table 4). The patient group scored

Table 1. Demographic findings

Total number (n, %)	**70 (100%)**
Female	40 (57.1%)
Male	30 (42.9%)
Groups (n, %)	**70 (100%)**
Patient	35 (50%)
Control	35 (50%)
Patient group (n, %)	**35 (100%)**
Female	20 (57.1%)
Male	15 (42.9%)
Control group (n, %)	**35 (100%)**
Female	20 (57.1%)
Male	15 (42.9%)
Age (year) (mean ± SD)	**3.31±1.22**
Patient	3.1±1.42
Control	3.52±0.95
Body mass index (kg/m²) (mean ± SD)	**19.51±4.15**
Patient	18.83±4.22
Control	20.2±4.02
Number of siblings (mean ± SD)	**1.47±1.2**
Patient	1.57±1.38
Control	1.37±1
History of breastfeeding (month) (mean ± SD)	**15.84±9.75**
Patient	13.9±9.29
Control	17.77±9.95
Type of breath-holding spells for the patient group (n, %)	**35 (100%)**
Cyanotic	16 (%45.7)
Pallid	19 (%54.3)

SD: Standard deviation

significantly higher on "emotional overeating" (p=0.021) and "enjoyment of food" (p=0.002). Conversely, the patient group scored significantly lower on the "slow eating" subscale (p=0.03), indicating a tendency to eat faster.

Predictors of Breath-Holding Spells: A Logistic Regression Analysis

In order to predict whether individuals were in the patient or control group, binary logistic regression analysis was performed using six different blood values (ferritin, B12, folic acid, Hb, white blood cells, and platelets). The logistic regression model was found to be statistically significant, χ^2 (6, n=70, n=42.24, p<0.001). The model explained 60% of the variance in health status (Nagelkerke R2) and correctly classified 77.1% of the cases. As a result of the analysis, the coefficients of folic acid and Hb levels were significant (p<0.05). When other variables were held constant, it was found that each unit increase in folic acid levels (e.g., 1 ng/mL) reduced the odds of being in the disease group by 33% [(OR=0.67, 95% confidence interval (CI): (0.54, 0.84), p<0.001)]. In other words, individuals with low folic acid levels were more likely to be ill. Similarly, each unit increase in Hb levels (e.g., 1 g/dL) reduced the odds of being in the disease group by 69% [OR=0.31, 95% CI: (0.14, 0.73), p=0.007]. The coefficients for other blood values (ferritin, B12, white blood cells, and platelets) were not statistically significant.

In the binary logistic regression analysis applied to predict whether individuals were in the control or patient group according to the scores obtained from the nutrition questionnaire, only the coefficient of the "slow eating" subscale was found to be significant; p<0.05. Each unit (e.g., 1 point) increase recorded in the "slow eating" score decreased the probability of being in the patient group by 13% [OR=0.87, 95% CI: (0.77, 0.98), p<0.025]. This result revealed that individuals who tend to eat faster were more likely to be part of the patient group. The coefficients of the total score obtained from the nutrition questionnaire and the other seven subscales (food craving, emotional overeating, food aversion, drinking craving, satiety craving, emotional undereating, and food pickiness) were not significant in the binary logistic regression model; p>0.05.

Discussion

In this study, we tested the hypothesis that children with BHS would exhibit distinct hematological and behavioral profiles compared to healthy controls. Our findings provide significant support for this hypothesis on two main fronts. First, supporting the hematological component, we found that children with BHS had significantly lower levels of Hb and folate. Second, supporting the behavioral component, we identified for the first time that children with BHS display specific eating patterns, namely a tendency for higher emotional overeating, greater enjoyment of food,

Table 2. Comparison of the patient group and the control group in terms of BMI, age, number of siblings and history of breastfeeding

	Patient group	Control group	
	[mean ± SD (median)]	[mean ± SD (median)]	p
Age	3.10±1.42 (2.87)	3.52±0.95 (3.79)	[a]p=0.156
Body mass index (kg/m2)	18.83±4.22 (18.12)	20.20±4.02 (19.83)	[a]p=0.169
Number of siblings	1.57±1.38 (1)	1.37±1 (1)	[b]p=0.774
Type of breath-holding spells for the patient group (month)	13.9±9.29 (17)	17.77±9.95 (24)	[b]p=0.075

Data are presented as mean ± standard deviation (median). Group comparisons were performed using the [a]Independent Samples t-test or the [b]Mann-Whitney U test SD: Standard deviation

Table 3. Comparison of blood values between the patient group and the control group

	Patient group	Control group	
	[mean ± SD (median)]	[mean ± SD (median)]	p
Ferritin (ng/ml)	53.25±142.7 (23.6)	30.55±29.72 (20.3)	[a]p=0.953
Vitamin B12 (pg/mL)	473.51±317.21 (398)	418.91±121.09 (388)	[a]p=0.972
Folic acid (ng/mL)	10.11±5.71 (8)	16.1±2.64 (15.6)	[a]p=0.000
Hemoglobin (g/dL)	11.39±1.32 (11.5)	12.32±0.7 (12.3)	[a]p=0.000
White blood cell count (/µL)	9968.29±2390.97 (10000)	8965.43±2622.35 (8780)	[b]p=0.099
Platelet count (/µL)	340942.86±105688.76 (357000)	316542.86±86290.67 (315000)	[a]p=0.106

Data are presented as mean ± standard deviation (median). Group comparisons were performed using the [a]Mann-Whitney U test or the [b]Independent samples t-test SD: Standard deviation

and faster eating. These parallel findings suggest a more complex interplay between nutritional status and behavior in the etiology of BHS.

This study should be considered a preliminary investigation into the complex relationship between BHS and hematological status. While the literature has predominantly focused on iron deficiency as a trigger for BHS, our results suggest that anemia itself, potentially influenced by the observed low folate levels, rather than iron store depletion (as indicated by normal ferritin levels), may be a key factor (13-15). This aligns with recent research demonstrating a relationship between folate/B12 deficiencies and spells and suggests that the clinical approach to anemic children with BHS should include a comprehensive assessment of all vitamins implicated in hematopoiesis, not just iron (16).

The most novel contribution of our study is the identification of specific eating behaviors in children with BHS. While initial group comparisons showed that children with BHS had higher scores for "emotional overeating" and "enjoyment of food", our logistic regression model identified that only the "slow eating" subscale was a significant independent predictor of group membership (OR=0.87, p=0.025). The odds ratio of less than one indicates that lower scores on this scale—reflecting a tendency for faster eating—were significantly associated with being in the patient group. This suggests that while emotional and hedonic aspects of eating may differ between the groups, the most robust and defining behavioral characteristic of children with BHS in our study is a tendency to eat more quickly. This finding of faster eating may be consistent with the known temperamental traits of children with BHS, such as higher impulsivity or lower frustration tolerance (7).

Table 4. Comparison of nutrition questionnaire scores between the patient group and the control group

	Patient group	Control group	
	[mean ± SD (median)]	[mean ± SD (median)]	p
Total score	92.34±16.72 (94)	86.63±11.33;(86)	[a]p=0.099
Subscale scores			
Food enthusiast	11±5.88 (9)	8.14±3.58 (7)	[b]p=0.078
Emotional overeating	6.69±2.19 (6)	5.69±2.29 (5)	[b]p=0.021
Enjoyment of food	16.69±5.73 (16)	12.57±4.6 (11)	[b]p=0.002
Drink enthusiast	8.29±3.49 (8)	7.37±2.4 (7)	[b]p=0.428
Satiety enthusiast	20.43±5.76 (19)	22.09±5.89 (22)	[a]p=0.238
Slow eating	10.06±3.66 (10)	12.46±4.72 (12)	[b]p=0.03
Emotional undereating	11.2±3.63 (11)	11.4±3.34 (11)	[b]p=0.559
Food selectivity	8±3.4 (8)	6.91±2.54 (7)	[a]p=0.134

Data are presented as mean ± standard deviation (median). Group comparisons were performed using the [a]Independent samples t-test or the [b]Mann-Whitney U test
SD: Standard deviation

These two primary findings—hematological and behavioral—may be interconnected. It is plausible that micronutrient deficiencies, such as folate deficiency observed in our patient group, could exacerbate disordered eating behaviors or the underlying temperamental traits. Conversely, specific eating patterns could contribute to inadequate micronutrient intake, creating a cyclical relationship that warrants further investigation. This study represents a pioneering effort to examine this intersection between feeding behaviors and BHS.

While our study focuses on factors present in early childhood, the long-term implications of these findings warrant consideration. A recent follow-up study by Polskaya and Aleksenko (17) examined the later outcomes of children with BHS, and future research could explore whether the nutritional and behavioral patterns we identified persist or resolve over time.

Study Limitations

Our study has several limitations that should be acknowledged. The primary limitation is the method of obtaining blood results for the control group, which was selected from the hospital's information system rather than being prospectively collected in the same manner as the patient group. This method introduces a potential for selection bias. Additionally, the single-center design and the relatively small sample size, although shown to be sufficient by power analysis, may limit the generalizability of our findings. A limitation, as noted by the reviewer, is that while the mean folate and Hb levels were statistically lower in the patient group, the mean values for both groups were within the normal clinical range. This issue raises important questions about the clinical significance of this statistical difference. However, the presence of a higher rate of folate deficiency in the patient group suggests that even subclinical variations may play a role. Our study was not designed to assess changes in eating behaviors after nutritional supplementation, and the reliance on a parental questionnaire carries an inherent risk of response bias. Another limitation of our study is the reliance on a single questionnaire to assess the complex nature of eating behaviors. Future studies could incorporate qualitative methods, such as clinical interviews or direct observation of feeding, to provide a richer understanding. Therefore, our findings on eating behaviors should be considered preliminary.

Despite these limitations, the study possesses significant strengths. Its prospective case-control design is a key strength. A key strength of our study is the rigorous diagnostic process used to ensure the integrity of our BHS cohort, which included EEG and ECG to exclude cardiac or epileptic etiologies. While a recent study by Hellström Schmidt et al. (18) has highlighted the potential for overuse of these tests in typical BHS cases, their application in our research context was crucial for diagnostic certainty and strengthening the validity of our findings. The use of the Denver Developmental Tests also ensured that developmental delays were not a confounding factor, thereby increasing the internal validity of our findings.

Conclusion

This study provides new insights into the pathophysiology of BHS by identifying two key findings. First, children with BHS have significantly lower Hb and folate levels compared to healthy controls. Furthermore, our findings suggest that impaired folate status may be a risk factor, a hypothesis that requires confirmation in larger studies. Second, this is the first study to demonstrate that children with BHS exhibit specific eating behaviors, namely, a tendency to eat rapidly, which may be linked to their underlying temperamental traits.

These findings carry notable clinical implications. Establishing standardized management approaches may benefit from incorporating routine screening for anemia and folate deficiency in children with BHS. In addition, providing families with guidance on managing the observed eating-related behavioral patterns may constitute an important component of a comprehensive therapeutic strategy.

Ethics

Ethics Committee Approval: This study was conducted in accordance with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University of Health Sciences Türkiye, Gaziosmanpasa Training and Research Hospital (approval no.: 20, date: 05.06.2024).

Informed Consent: Written informed consent was obtained from the parents or legal guardians of all participating children prior to their inclusion in the study.

Footnotes

Authorship Contributions

Design: O.C., S.I., E.E.I. Data Collection or Processing: O.C., E.E.I., Analysis or Interpretation: O.C., U.T., Literature Search: O.C., S.I., Writing: O.C., U.T.

Conflict of interests: No conflicts of interest were declared by the authors.

Financial Disclosure: This study received no financial support.

References

1. Flodine TE, Shah M, Mendez MD. Breath–holding spells. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Aug 17. Available from: https://scholarlyworks.lvhn.org/anesthesiology/464
2. Bjerring B, Debes NM. [Breath-holding spells in children]. Ugeskr Laeger. 2020;182:V07200504.
3. Leung AKC, Leung AAM, Wong AHC, Hon KL. Breath-holding spells in pediatrics: a narrative review of the current evidence. Curr Pediatr Rev. 2019;15:22-9.
4. Ozcora GDK, Kumandas S, Sagiroglu A, et al. Evaluating the brainstem in children with breath-holding spells. North Clin Istanb. 2022;9:610-5.
5. Goldman RD. Breath-holding spells in infants. Can Fam Physician. 2015;61:149-50.
6. Hothan KA, Al Judaibi RS, Alshabi MS, et al. Knowledge and awareness of breath-holding spells in infants and toddlers among parents in Saudi Arabia. Cureus. 2025;17:e77703.
7. Subbarayan A, Ganesan B, Anbumani, Jayanthini. Temperamental traits of breath holding children: a case control study. Indian J Psychiatry. 2008;50:192-6.
8. Wardle J, Guthrie CA, Sanderson S, Rapoport L. Development of the children's eating behaviour questionnaire. J Child Psychol Psychiatry. 2001;42:963-70.
9. Yılmaz R, Esmeray H, Erkorkmaz Ü. Çocuklarda yeme davranışı anketinin Türkçe uyarlama çalışması. Anatolian Journal of Psychiatry/Anadolu Psikiyatri Dergisi. 2011;12:287-94
10. Rahill S, Kennedy A, Walton J, McNulty BA, Kearney J. The factors associated with food fussiness in Irish school-aged children. Public Health Nutr. 2019;22:164-74.
11. Kozak A, Czepczor-Bernat K, Modrzejewska J, Modrzejewska A, Matusik E, Matusik P. Avoidant/restrictive food disorder (ARFID), food neophobia, other eating-related behaviours and feeding practices among children with autism spectrum disorder and in non-clinical sample: a preliminary study. Int J Environ Res Public Health. 2023;20:5822
12. Coskun O, Kipoglu O, Karacabey BN, et al. Evaluation of eating behaviors in childhood epilepsy with centrotemporal spikes: case-control study. Epilepsy Behav. 2021;120:108029.
13. Azab SF, Siam AG, Saleh SH, et al. Novel findings in breath-holding spells: a cross-sectional study. Medicine (Baltimore). 2015;94:e1150.
14. Hellström Schmidt S, Tedgård U, Pronk CJ. Breath-holding spells occur disproportionately more often in children with transient erythroblastopenia. Acta Paediatr. 2016;105:1088-93.

15. Ramachandran S, Alessandri A, Price J, Balasubramaniam S. Breath-holding spell and macrocytic anaemia in a toddler. Br J Haematol. 2014;166:156.

16. Aydın H, Bucak İ, Erdoğan S. Vitamin B12 and folic acid levels in patients with breath holding spells. Turkish J Pediatr Dis. 2023;17:113-7.

17. Polskaya AV, Aleksenko OV. The follow-up examination of children with breath-holding spells. Zh Nevrol Psikhiatr Im S S Korsakova. 2024;124:76-80.

18. Hellström Schmidt S, Smedenmark J, Jeremiasen I, Sigurdsson B, Eklund EA, Pronk CJ. Overuse of EEG and ECG in children with breath-holding spells and its implication for the management of the spells. Acta Paediatr. 2024;113:317-26.

19. Hellström Schmidt S, A Eklund E, Pronk CJ. Guidelines for uniform management of breath-holding spells. Lakartidningen. 2024;121:23209.

Original Article

DOI: 10.4274/haseki.galenos.2025.80299
Med Bull Haseki 2025;63(5):281-287

Intestinal Stoma Types, Indications and Complications in Pediatric Patients: A Three-year Single-center Experience with Necrotizing Enterocolitis as the Leading Indication

Fatma Sarac

University of Health Sciences Türkiye, Basaksehir Cam and Sakura City Hospital, Clinic of Pediatric Surgery, Istanbul, Türkiye

Abstract

Aim: This study aimed to analyze the distribution, clinical indications, and complication rates of pediatric intestinal stomas in our institution, with a particular focus on identifying the predominant underlying pathologies and their correlation with stoma type selection.

Methods: This retrospective observational cohort study included 78 pediatric patients who underwent intestinal stoma surgery between June 2020 and June 2023. All patients aged 0-18 years who underwent stoma-creation surgery were identified through our hospital's data system and clinical surgery register and evaluated for age, sex, primary disease, timing of stoma-creation, stoma type and location, and stoma-related complications. Patients who underwent esophagotomies or gastrostomies were excluded from the study; only those with intestinal stomas were included.

Results: Eighty-one stomas were performed on 78 patients: 55 ileostomies (68%), 22 colostomies (27%), and 4 jejunostomies (5%). Divergent (44.5%) and loop (42%) stomas were the most common. Necrotizing enterocolitis (NEC) was the leading indication (30.7%, n=24), followed by Hirschsprung disease (23.1%, n=18), meconium ileus (11.5%, n=9), and anorectal malformations (ARM) (10.3%, n=8). The overall complication rate was 78.2%, with skin excoriation being most frequent (64.1%, n=50). Neonates constituted 48.7% of patients (n=38), and 72% of patients were under one year of age.

Conclusion: Unlike most pediatric series, in which ARM predominate, NEC was the leading stoma indication in our cohort, reflecting institutional characteristics, including the absence of a dedicated neonatal surgery unit. Ileostomy was the most frequently performed procedure. Stoma indications and types vary significantly according to regional factors and the institutional infrastructure.

Keywords: Ostomy, ileostomy, colostomy, enterostomy, child, infant, newborn

Introduction

Intestinal stomas, surgical openings connecting the gastrointestinal tract to the abdominal wall, represent critical life-saving interventions in pediatric surgery. The word "stoma" comes from the Greek word for "mouth" and means moving intestinal contents through an opening in the abdomen, either temporarily or permanently. These procedures serve multiple purposes in both emergency and elective surgical scenarios, including fecal diversion, bowel decompression, and protection of distal anastomoses. While adult stoma indications primarily include colorectal malignancies, inflammatory bowel disease, diverticulitis, and trauma, pediatric populations present distinct indication patterns dominated by congenital anomalies and neonatal emergencies (1-3). Contemporary pediatric surgical practice demonstrates evolving trends in stoma use, with institutional variations reflecting differences in patient populations, availability of neonatal intensive care resources, and surgical expertise (4,5).

Corresponding Author: Assoc. Prof., Fatma Sarac, University of Health Sciences Türkiye, Basaksehir Cam and Sakura City Hospital, Clinic of Pediatric Surgery, Istanbul, Türkiye
E-mail: fsarac75@gmail.com **ORCID:** orcid.org/0000-0003-0894-7787
Received: 02.07.2025 **Accepted:** 16.11.2025 **Publication Date:** 28.11.2025

Cite this article as: Sarac F. Intestinal stoma types, indications and complications in pediatric patients: a three-year single-center experience with necrotizing enterocolitis as the leading indication. Med Bull Haseki. 2025;63(5):281-287

The predominant indications for pediatric intestinal stomas traditionally include anorectal malformations (ARM) and Hirschsprung disease (HD), accounting for the majority of cases in published series (6-8). However, acquired conditions, such as necrotizing enterocolitis (NEC), are increasingly significant indications, particularly in centers with high-volume neonatal intensive care units (9,10). Recent multicenter studies have highlighted substantial geographic and institutional variability in stoma patterns, with some centers reporting NEC as the leading indication, while others report ARM predominance (11-13). The selection of stoma type—whether loop, divided (divergent), or end configuration—and anatomical location (jejunum, ileum, or colon) depends on multiple factors, including underlying pathology, intestinal viability, surgeon preference, and institutional protocols. Contemporary evidence suggests that stoma-related complications remain common, ranging from 21% to 70% across series, with significant morbidity including prolapse, skin excoriation, stenosis, and fluid-electrolyte imbalances, which particularly affect younger patients (14-16).

We hypothesized that our institutional stoma patterns would differ from traditional literature reports due to the absence of a dedicated neonatal surgery unit and limited neonatal intensive care capacity, potentially resulting in a distinct distribution of underlying diagnoses and stoma types. Therefore, this study aimed to comprehensively analyze the types, indications, and complications of intestinal stomas performed at our pediatric surgery department over a three-year period. By characterizing our institutional experience, we seek to contribute to the understanding of how healthcare infrastructure and regional factors influence pediatric stoma surgery patterns, thereby providing valuable insights for resource allocation and surgical planning in similar clinical settings.

Materials and Methods

Compliance with Ethical Standards

This retrospective study was approved by the University of Health Sciences Türkiye, Basaksehir Cam and Sakura City Hospital Clinical Research Ethics Committee (approval number: 438, date: 27.09.2023). All procedures adhered to the ethical standards outlined in the 1964 Declaration of Helsinki and its subsequent amendments. The ethics committee waived the requirement for informed consent because the study was retrospective.

Study Design

This retrospective observational cohort study included all pediatric patients aged 0-18 years who underwent intestinal stoma-creation at our pediatric surgery department between June 2020 and June 2023. Patients were identified through systematic review of the hospital's electronic medical records system and the departmental surgical registry. Inclusion criteria were patients aged 0-18 years who underwent surgical creation of an intestinal stoma (jejunostomy, ileostomy, or colostomy) and had complete medical records available. Exclusion criteria were gastrostomies or esophagostomies, incomplete medical records, and stoma surgery performed at another institution (Figure 1).

Data Collection

Medical records were systematically reviewed to extract the following variables: patient demographics (age, sex), primary diagnosis, timing of stoma-creation, stoma characteristics (type, location, configuration), surgical technique, and postoperative complications. Stomas were classified by type (jejunostomy, ileostomy, or colostomy), configuration [divergent (completely divided), loop (continuity maintained), or end stoma], and location (based on anatomical segment). Complications were documented and categorized as skin excoriation, prolapse, retraction, stenosis, bleeding, parastomal hernia, ischemia or circulation impairment, and systemic complications (fluid-electrolyte imbalance).

Statistical Analysis

Data were analyzed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics are presented as mean ± standard deviation and median (interquartile range) for continuous variables and as frequencies and percentages

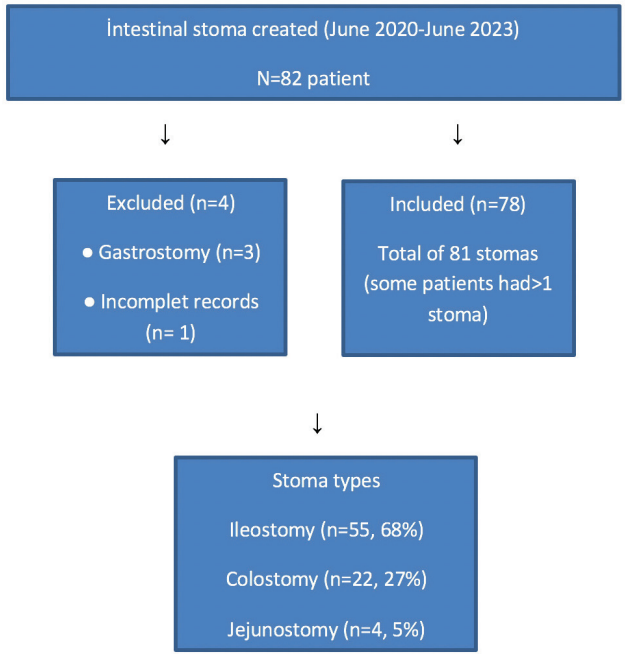

Figure 1. Study flow diagram

for categorical variables. For inferential statistics, the Independent samples t-test was used to compare normally distributed continuous variables between two groups, while the Mann-Whitney U test was applied to non-normally distributed continuous variables. Categorical variables were analyzed using Pearson's chi-square test or Fisher's exact test (when expected cell frequencies were <5). Multiple group comparisons for categorical variables were performed using the chi-square test for independence. Chi-square tests were used to compare complication rates between stoma types. If the sample size was fewer than five, as in jejunostomy, Fisher's exact test was used to assess configuration preferences by indication. A two-tailed p-value <0.05 was considered statistically significant.

Results

Patient Demographics and Stoma Characteristics

During the three-year study period, 81 stomas were performed on 78 patients. Three patients underwent multiple simultaneous stomas: ileostomy and colostomy (n=1), jejunostomy and ileostomy (n=1), and jejunostomy and colostomy (n=1). The gender distribution showed 40 males (51.3%) and 38 females (48.7%), with no significant difference by gender (p=0.832). Age distribution was as follows: neonates (0-28 days), 38 patients (48.7%); infants (1-12 months), 18 patients (23%); children (1-18 years), 22 patients (28.2%). Infants and neonates together represented 71.8% of the cohort (n=56).

Stoma Types and Configurations

Table 1 presents the distribution of stoma types and configurations. A chi-square test was used to analyze stoma types. Ileostomy was the most common (68%, n=55), followed by colostomy (27%, n=22), and jejunostomy (5%, n=4). The distribution of stoma configurations was divergent stomas in 36 cases (44.5%), loop stomas in 34 cases (42%), and end stomas in 11 cases (13.5%). No statistically significant difference was observed between the frequencies of divergent and loop stomas (p=0.742). All stomas were temporary.

Among ileostomies, the divergent configuration was most frequent (49%, n=27), followed by loop (40%, n=22) and end (11%, n=6) configurations. For colostomies, loop configuration predominated (50%, n=11), followed by divergent (36.3%, n=8) and end (13.6%, n=3).

Indications for Stoma-creation

Table 2 details the indications stratified by stoma type. Comparisons of indications were made using the chi-square test. If the sample size was fewer than 5, as

Table 1. Stoma types and configurations

Ostomy types	Jejunostomy (%)	Ileostomy (%)	Colostomy (%)	Total (%)
Divergent	1	27 (49)	8 (36.3)	36 (44.5)
Loop	1	22 (40)	11 (50)	34 (42)
End	2	6 (11)	3 (13.6)	11(13.5)
Total	4 (5)	55 (68)	22 (27)	81 (100)

Chi-square test was used to analyze stoma types. No statistically significant difference was observed between the frequencies of divergent and loop stomas (p=0.742)

Table 2. Indications for stoma creation

Stoma indications (number)	Ileostomy	Colostomy	Jejunostomy	Total
NEC (24)	21	3	1	25
HD (18)	11	7		18
Meconium ileus (9)	9			9
ARM (8)		8		8
Intestinal atresia (5)	4		2	6
Crohn's disease (3)	3	1		4
Trauma (2)		2		2
Volvulus (1)	1			1
Mechanical ileus (6)	5		1	6
FAP (1)	1			1
Latrogenic colon perforation (1)		1		1
Total (78 patient)	55	22	4	81

Chi-square test was used to compare stoma indications. There is a statistically significant difference between the NEC, HD and ARM groups (p<0.05)
NEC: Necrotizing enterocolitis, HD: Hirschsprung disease, ARM: Anorectal malformations, FAP: Familial adenomatous polyposis

in the jejunostomy group, Fisher's exact test was used. Necrotizing enterocolitis was the leading indication (30.7%, n=24), followed by HD (23.1%, n=18), meconium ileus (11.5%, n=9), ARM (10.3%, n=8), intestinal atresia (6.4%, n=5; 4 ileal, 1 jejunal), mechanical ileus (7.7%, n=6), Crohn's disease (3.8%, n=3), trauma (2.6%, n=2), volvulus (1.3%, n=1), familial adenomatous polyposis (1.3%, n=1), and iatrogenic colonic perforation during colonoscopy (1.3%, n=1).

Among NEC patients (n=24), 21 (87.5%) underwent ileostomy (predominantly diverting ileostomies), 3 underwent colostomy, and 1 underwent jejunostomy. Among HD patients (n=18), ileostomy was performed in 11 cases (61%) and colostomy in 7 cases (39%), with loop ileostomy and loop transverse colostomy preferred. All ARM patients (n=8) underwent colostomy: 7 received divergent sigmoid colostomies and 1 received a divergent transverse colostomy.

Complications

Table 3 summarizes stoma-related complications. The overall complication rate was 78.2% (61/78 patients). Skin excoriation was the most common complication (64.1%, n=50), followed by prolapse (6.4%, n=5: 4 in transverse loop colostomy, 1 in loop ileostomy); circulatory impairment (2.6%, n=2: both in severe NEC cases); bleeding (2.6%, n=2: both post-ileostomy for NEC); retraction (1.3%, n=1); and stenosis (1.3%, n=1).

Chi-square tests were used to compare complication rates between stoma types. Complications were more frequent in patients with NEC than in those with other indications (p=0.031). Skin excoriation affected 61.7% of NEC patients. One patient with stoma retraction required surgical revision. Patients with prolapse underwent early stoma closure. Other complications were managed conservatively with medical therapy. No significant differences in complication rates were found between ileostomy and colostomy (p=0.284) or between divergent and loop configurations (p=0.591).

Table 3. Stoma-related complications

Complication	n=patient number
Stenosis	1
Retraction	1
Prolapsus	5
Bleeding	2
Circulation impairment	2
Skin excoriation	50
Total	**61**

Chi-square test was used to compare complication rates between stoma types. While the most common complication was seen in NEC patients (p=0.031, p<0.05), no difference was observed between ileostomy and colostomy (p=0.591)
NEC: Necrotizing enterocolitis

Discussion

This three-year retrospective analysis of 78 pediatric patients undergoing 81 intestinal stomas revealed NEC as the predominant indication (30.7%), contrasting with most published series where ARM typically predominates. This finding validates our hypothesis that institutional infrastructure—specifically, the absence of a dedicated neonatal surgery unit—significantly influences stoma-indication patterns. Our results contribute to understanding how healthcare delivery models shape pediatric surgical practice patterns in different clinical settings.

The predominance of NEC in our series differs from that reported in traditional pediatric stoma literature, where ARM and HD typically constitute the leading indications (6-8,17). A recent multicenter European study by Schaart et al. (18) similarly reported institutional variation in stoma indications, with tertiary neonatal centers demonstrating higher NEC proportions. Our findings align with reports from institutions with high-volume neonatal intensive care units but limited neonatal surgical subspecialization. Conversely, specialized pediatric surgery centers with dedicated ARM programs report higher ARM stoma rates (19,20). Despite the presence of a sufficient number of specialized neonatal surgeons in our institution, adequate neonatal surgery services are lacking.

The relatively low proportion of ARM (10.3%) observed in our cohort likely reflects referral patterns and limited neonatal surgical capacity, as hypothesized. Many ARM patients may have been managed at institutions with specialized neonatal surgery units. This pattern has significant implications for surgical training and resource allocation in general pediatric surgery departments.

Hirschsprung disease was our second most common indication (23.1%), consistent with its established prevalence in pediatric stoma series (21,22). Recent guidelines from the European Society of Pediatric Gastroenterology, Hepatology, and Nutrition (2024) emphasize the continued role of temporary diversion in the management of HD, particularly in cases of total colonic aganglionosis and in critically ill presentations (23).

Meconium ileus (11.5%) constituted a significant proportion of our cases, reflecting the complex management of cystic fibrosis-associated intestinal complications. Contemporary approaches increasingly favor primary anastomosis when possible, reserving stomas for complicated cases with perforation, atresia, or volvulus (24,25).

The predominance of ileostomy (68%) in our series reflects the high proportion of NEC cases, in which ileal involvement is common. This finding is consistent with

recent data from neonatal surgical centers (26,27). The preference for a diverting ileostomy (49% of ileostomies) in NEC cases aligns with current practice patterns that prioritize adequate fecal diversion while minimizing distal limb complications (28).

Debate regarding loop versus divided (divergent) colostomy in ARM management persists in the contemporary literature (29,30). Although we used divergent sigmoid colostomy as our preferred approach for ARM (n=7), recent meta-analyses suggest that loop and divided techniques have comparable outcomes with respect to urinary tract infections and distal fecal impaction, while loop colostomy is associated with shorter operative times (31,32). However, proponents of divided colostomy cite lower prolapse rates and superior fecal diversion (33). Our institutional preference for divided sigmoid colostomy in ARM reflects traditional teaching and surgeons' experience, though prospective comparative studies are needed.

In HD patients, we preferentially performed loop ileostomy and loop transverse colostomy, with stoma location determined by identification of the transition zone. This approach is supported by recent literature emphasizing individualized stoma placement based on intraoperative findings and disease extent (34,35).

Our overall complication rate of 78.2% appears higher than in some reported series but falls within the wide range (21-70%) described in the pediatric stoma literature (1,14-16). The predominance of skin excoriation (64.1%) likely reflects multiple factors, including the young age of patients, liquid stool consistency from proximal stomas, and challenging ostomy care in neonates with fragile skin.

The strong association between NEC and complications ($p=0.031$) has been documented previously, with studies reporting increased rates of retraction, ischemia, and skin problems in this population (3,36).

Prolapse occurred in 6.4% of cases, predominantly in loop colostomies (n=4), consistent with the literature, which reports higher prolapse rates in loop versus divided configurations (37,38). Early stoma closure was our management approach for these cases, avoiding the morbidity associated with surgical revision.

Our low rates of stenosis (1.3%) and retraction (1.3%) compare favorably with published series, potentially reflecting adequate initial stoma construction and meticulous surgical technique (39). The patient requiring revision for retraction underwent a successful reoperation without subsequent complications.

Our findings demonstrate that stoma indication patterns are substantially influenced by institutional characteristics and healthcare delivery models. Centers without dedicated neonatal surgery units may experience different case mixes, emphasizing the importance of maintaining broad pediatric surgical expertise. The high complication rate, particularly skin excoriation, highlights the need for enhanced ostomy care protocols, potentially including early involvement of enterostomal therapy nurses and parental education programs.

Future research should focus on prospective comparison of stoma techniques, development of standardized complication reporting systems, and investigation of quality-of-life outcomes in pediatric ostomy patients. Multicenter collaborative studies would be valuable in establishing best practices adaptable to various institutional settings.

Study Limitations

Several limitations merit consideration in interpreting our findings. First, the retrospective design introduces potential selection and information biases and may involve incomplete documentation of some variables. Second, the absence of standardized protocols for stoma type selection reflects the involvement of multiple surgeons trained in different schools, introducing variability in surgical decision-making. This heterogeneity, while limiting protocol uniformity, may enhance external validity by reflecting real-world practice patterns. Third, our lack of a dedicated neonatal surgery unit limits the generalizability of our findings to specialized centers and likely influenced the distribution of indications. Fourth, the relatively small sample size (n=78) and single-center design limit statistical power for subgroup analyses and reduce broader applicability. Fifth, we lacked long-term follow-up data on the timing of stoma closure, definitive surgical procedures, and ultimate functional outcomes. Finally, we did not systematically assess quality-of-life measures or detailed cost analyses.

Despite these limitations, our study provides valuable real-world data reflecting pediatric stoma practice in a general pediatric surgical setting lacking neonatal subspecialization. Strengths include comprehensive inclusion over three years, detailed documentation of complications, and representation of diverse underlying pathologies. Our findings contribute important insights into how institutional infrastructure shapes pediatric surgical practice patterns; this information is valuable for healthcare planning and surgical training in similar settings. The demonstration of acceptable outcomes despite infrastructure limitations supports the feasibility of managing complex pediatric stoma cases in general pediatric surgery departments with appropriate expertise and resources.

Conclusion

This three-year institutional experience demonstrates that NEC and HD, rather than ARM, predominated as indications for stoma in our setting, reflecting the significant impact of institutional infrastructure, particularly the absence of a dedicated neonatal surgery unit, on pediatric surgical case distribution. Ileostomy was the most frequently performed procedure; divergent and loop configurations were used with similar frequency. While the overall complication rate was high (78.2%), most complications were managed conservatively, and skin excoriation was the most common complication, requiring enhanced ostomy-care protocols.

These findings underscore the importance of recognizing institutional and regional variations in pediatric stoma patterns when planning resources, developing clinical protocols, and designing training programs. Healthcare systems should consider these variations in surgical workforce planning and infrastructure development. Future multicenter prospective studies are needed to establish evidence-based guidelines adaptable to diverse clinical settings and to optimize outcomes for pediatric patients requiring intestinal diversion.

Ethics

Ethics Committee Approval: This retrospective study was approved by the University of Health Sciences Türkiye, Basaksehir Cam and Sakura City Hospital Clinical Research Ethics Committee (approval number: 438, date: 27.09.2023).

Informed Consent: The ethics committee waived the requirement for informed consent because the study was retrospective.

Footnotes

Financial Disclosure: This study received no financial support.

References

1. Vriesman MH, Noor N, Koppen IJ, Di Lorenzo C, de Jong JR, Benninga MA. Outcomes after enterostomies in children with and without motility disorders: a description and comparison of postoperative complications. J Pediatr Surg. 2020;55:2413-8.
2. Massenga A, Chibwae A, Nuri AA, et al. Indications for and complications of intestinal stomas in the children and adults at a tertiary care hospital in a resource-limited setting: a Tanzanian experience. BMC Gastroenterol. 2019;19:157.
3. Wolf L, Gfroerer S, Fiegel H, Rolle U. Complications of newborn enterostomies. World J Clin Cases. 2018;6:1101-10.
4. Pandiaraja J, Chakkarapani R, Arumugam S. A study on patterns, indications, and complications of an enteric stoma. J Family Med Prim Care. 2021;10:3277-82.
5. Adamou H, Habou O, Amadou Magagi I, et al. Pattern of lower intestinal ostomies in a low-income country: case of Southeast of Niger Republic. World J Surg. 2018;42:1581-9.
6. Almosallam OI, Aseeri A, Shanafey SA. Outcome of loop versus divided colostomy in the management of anorectal malformations. Ann Saudi Med. 2016;36:352-5.
7. van den Hondel D, Sloots C, Meeussen C, Wijnen R. To split or not to split: colostomy complications for anorectal malformations or hirschsprung disease: a single center experience and a systematic review of the literature. Eur J Pediatr Surg. 2014;24:61-9.
8. Youssef F, Arbash G, Puligandla PS, Baird RJ. Loop versus divided colostomy for the management of anorectal malformations: a systematic review and meta-analysis. J Pediatr Surg. 2017;52:783-90.
9. Oda O, Davies D, Colapinto K, Gerstle JT. Loop versus divided colostomy for the management of anorectal malformations. J Pediatr Surg. 2014;49:87-90.
10. Zani A, Eaton S, Puri P, et al. International survey on the management of necrotizing enterocolitis. Eur J Pediatr Surg. 2015;25:27-33.
11. Thyoka M, de Coppi P, Eaton S, et al. Advanced necrotizing enterocolitis part 1: mortality. Eur J Pediatr Surg. 2012; 22:8-12.
12. Rees CM, Eaton S, Kiely EM, Wade AM, McHugh K, Pierro A. Peritoneal drainage or laparotomy for neonatal bowel perforation? A randomized controlled trial. Ann Surg. 2008;248:44-51.
13. Allin BSR, Long AM, Gupta A, Lakhoo K, Knight M; British Association of Paediatric Surgeons Congenital Anomalies Surveillance System Necrotising Enterocolitis Collaboration. One-year outcomes following surgery for necrotising enterocolitis: a UK-wide cohort study. Arch Dis Child Fetal Neonatal Ed. 2018;103:F461-6.
14. Duchesne JC, Wang YZ, Weintraub SL, Boyle M, Hunt JP. Stoma complications: a multivariate analysis. Am Surg. 2002;68:961-6.
15. Chandramouli B, Srinivasan K, Jagdish S, Ananthakrishnan N. Morbidity and mortality of colostomy and its closure in children. J Pediatr Surg. 2004;39:596-9.
16. Aworanti O, Akani B, Nandi C, et al. Neonatal stoma complications: what predisposes to it? Afr J Paediatr Surg. 2021;18:149-54.
17. Levitt MA, Peña A. Anorectal malformations. Orphanet J Rare Dis. 2007;2:33.
18. Schaart MW, de Bruijn C, Tibboel D, et al. Intestinal stomas in neonates: high morbidity and mortality. Arch Dis Child Fetal Neonatal Ed. 2024;109:145-50.
19. Bischoff A, Levitt MA, Peña A. Bowel management for the treatment of pediatric fecal incontinence. Pediatr Surg Int. 2009;25:1027-42.

20. Wood RJ, Levitt MA. Anorectal malformations. Clin Colon Rectal Surg. 2018;31:61-70.

21. Langer JC, Rollins MD, Levitt M, et al. Guidelines for the management of postoperative obstructive symptoms in children with Hirschsprung disease. Pediatr Surg Int. 2017;33:523-6.

22. Nakamura H, Lim T, Puri P. Inflammatory bowel disease in patients with Hirschsprung's disease: a systematic review and meta-analysis. Pediatr Surg Int. 2018;34:149-54.

23. de Lorijn F, Kremer LC, Reitsma JB, Benninga MA. Diagnostic tests in Hirschsprung disease: a systematic review. J Pediatr Gastroenterol Nutr. 2006;42:496-505.

24. Del Pin CA, Czyrko C, Ziegler MM, Scanlin TF, Bishop HC. Management and survival of meconium ileus. A 30-year review. Ann Surg. 1992;215:179-85.

25. Burge DM, Drewett M. Meconium ileus and meconium peritonitis. In: Puri P, Höllwarth ME, editors. Pediatric surgery. 3rd ed. Springer; 2020. p. 421-31.

26. Hull MA, Fisher JG, Gutierrez IM, et al. Mortality and management of surgical necrotizing enterocolitis in very low birth weight neonates: a prospective cohort study. J Am Coll Surg. 2014;218:1148-55.

27. Rees CM, Pierro A, Eaton S. Neurodevelopmental outcomes of neonates with medically and surgically treated necrotizing enterocolitis. Arch Dis Child Fetal Neonatal Ed. 2007;92: F193-8.

28. Patel BN, Pasricha S, Forbes G, et al. Comparison of outcomes following primary anastomosis and stoma creation in gastrointestinal perforation in neonates. Pediatr Surg Int. 2020;36:591-7.

29. Kyrklund K, Koivusalo A, Rintala RJ, Pakarinen MP. Evaluation of bowel function and fecal continence in 594 Finnish patients with anorectal malformations. Dis Colon Rectum. 2017;60:293-8.

30. Mullassery D, Iacona R, Cross K, et al. Loop colostomies are safe in anorectal malformations. J Pediatr Surg. 2018;53: 2170-3.

31. Lee KH, Park JW, Choi YH, et al. Comparing loop and divided colostomy for anorectal malformation: a systematic review and meta-analysis. J Pediatr Surg. 2024;59:2468-78.

32. Ramirez-Amoros C, Carvalho C, San Basilio M, Martinez L, Encinas JL, Vilanova-Sanchez A. Classic divided sigmoidostomy vs loop sigmoidostomy in anorectal malformations: time for change? Pediatr Surg Int. 2024;40:252.

33. Liechty ST, Barnhart DC, Huber JT, Zobell S, Rollins MD. The morbidity of a divided stoma compared to a loop colostomy in patients with anorectal malformation. J Pediatr Surg. 2016;51:107-10.

34. Smith C, Ambartsumyan L, Kapur RP. Surgery, surgical pathology, and postoperative management of patients with hirschsprung disease. Pediatr Dev Pathol. 2020;23:23-39.

35. Kapur RP, Kennedy AJ. Transitional zone pull through: surgical pathology considerations. Semin Pediatr Surg. 2012;21:291-301.

36. Moss RL, Dimmitt RA, Barnhart DC, et al. Laparotomy versus peritoneal drainage for necrotizing enterocolitis and perforation. N Engl J Med. 2006;354:2225-34.

37. Ein SH, Shandling B, Wesson D, Filler RM. A 13-year experience with peritoneal drainage under local anesthesia for necrotizing enterocolitis perforation. J Pediatr Surg. 1990;25:1034-6; discussion 1036-7.

38. Chandler JC, Hebra A. Necrotizing enterocolitis in infants with very low birth weight. Semin Pediatr Surg. 2000;9:63-72.

39. Arumugam PJ, Bevan L, Macdonald L, Watkins AJ, Morgan AR, Beynon J, Carr ND. A prospective audit of stomas–analysis of risk factors and complications and their management. Colorectal Dis. 2003;5:49-52.

Original Article

DOI: 10.4274/haseki.galenos.2025.57060
Med Bull Haseki 2025;63(5):288-296

Evaluation of Intercondylar Notch and Tibial Slope Angles in Relation to Anterior Cruciate Ligament Injury: A Retrospective MRI Study in a Turkish Population

Ergul Cindemir*, Turkan Ikizceli**, Ridvan Karahasanoglu**, Yildiray Savas**

*University of Health Sciences Türkiye, Gaziantep City Hospital, Clinic of Radiology, Gaziantep, Türkiye

**University of Health Sciences Türkiye, Istanbul Haseki Training and Research Hospital, Clinic of Radiology, Istanbul, Türkiye

Abstract

Aim: Intrinsic variations in knee anatomy, including the intercondylar notch and tibial slope morphology, are associated with anterior cruciate ligament (ACL) injury risk. This study aimed to evaluate the association between the intercondylar notch angle (INA), medial and lateral tibial slope angles MTSA and LTSA, and femoral notch width (FNW) and the occurrence of ACL rupture by comparing patients with ACL injury and healthy controls.

Methods: This retrospective study included 413 individuals aged 20-40 years who underwent knee magnetic resonance imaging (MRI) between January 2016 and November 2019. The study group comprised 232 patients with complete ACL rupture, and 181 healthy individuals without ACL tears served as controls. Morphometric parameters, including the INA, MTSA and LTSA, FNW, intercondylar width (ICW), and notch width ratio (NWR), were measured and compared between groups. The NWR was calculated as FNW/ICW and classified as ≤0.27 or >0.27. Knees were also categorized by notch shape (A, U, W), and associations with age and sex were analyzed.

Results: The mean INA and FNW were significantly lower, while the LTSA was significantly higher in the ACL rupture group than in controls ($p<0.01$). The NWR was also significantly lower in the ACL group ($p<0.001$). An NWR ≤0.27 was associated with a 2.24-fold increased risk of ACL rupture, with an area under the curve of 0.724, indicating moderate diagnostic accuracy. No significant difference in notch shape types (A, U, W) was observed between groups ($p=0.169$).

Conclusion: Reduced notch dimensions, a lower NW ratio, and a steeper lateral tibial slope are significant anatomical risk factors for ACL rupture. Incorporating these MRI-based morphometric parameters into prevention programs may aid early identification of high-risk individuals. Prospective biomechanical studies are warranted to validate these findings and guide individualized prevention strategies.

Keywords: Anterior cruciate ligament injuries, knee joint, magnetic resonance imaging, risk factors

Introduction

The knee is the body's largest synovial joint, formed by the femoral condyles and tibial plateau, with the menisci positioned between them. Stability of the knee depends on static structures such as the capsule, menisci, and ligaments, as well as dynamic support from muscles and tendons. The anterior cruciate ligament (ACL), one of the primary stabilizers, lies within the joint capsule but outside the synovial membrane and consists of two bundles: the anteromedial and posterolateral (1-3).

Injuries to the ACL represent one of the most prevalent forms of knee pathology, particularly among athletes

Corresponding Author: Ergul Cindemir, MD, University of Health Sciences Türkiye, Gaziantep City Hospital, Clinic of Radiology, Gaziantep, Türkiye
E-mail: ergulkahraman@yahoo.com **ORCID:** orcid.org/0000-0003-1698-2291
Received: 03.07.2025 **Accepted:** 20.10.2025 **Publication Date:** 28.11.2025
***This study is based on the first author's medical dissertation completed on October 1, 2020.**

Cite this article as: Cindemir E, Ikizceli T, Karahasanoglu R, Savas Y. Evaluation of intercondylar notch and tibial slope angles in relation to anterior cruciate ligament injury: a retrospective MRI study in a Turkish population. Med Bull Haseki. 2025;63(5):288-296

participating in sports that involve cutting, pivoting, or physical contact. The ACL is a primary stabilizer of the knee, preventing anterior displacement and excessive internal rotation of the tibia. Rupture typically occurs during sudden deceleration or rotational loading of the joint (4-6). Numerous factors have been proposed to influence susceptibility to ACL injury, encompassing hormonal, genetic, biomechanical, and anatomical components. Among these parameters, decreased intercondylar notch dimensions and a steeper posterior tibial slope are regarded as important contributors to ligament rupture risk (7-9).

Magnetic resonance imaging (MRI), particularly T2-weighted and proton density (PD)-weighted sequences, plays a crucial role in the evaluation of ACL integrity. The ligament should be examined in sagittal, coronal, and axial planes, with attention to both primary and secondary signs of injury (10,11). Recent studies have highlighted the importance of intrinsic anatomical variations, particularly notch geometry and tibial slope, as key determinants of ACL injury risk (12,13). These morphometric parameters can be quantitatively assessed on MRI, providing valuable insights into the anatomical predisposition to ligament rupture.

We hypothesized that distinct morphological features of the knee joint may contribute to an increased susceptibility to ACL injury. Therefore, the present study aimed to investigate the relationship between the intercondylar notch angle (INA), medial tibial slope angle (MTSA) and lateral tibial slope angle (LTSA), and femoral notch width (FNW), and the occurrence of ACL rupture. By comparing these morphometric parameters between patients with ACL injury and healthy controls, we sought to identify anatomical features that may serve as potential predictive risk factors and contribute to more targeted prevention and individualized screening strategies.

Materials and Methods

Patient Selection

This retrospective cross-sectional study was conducted by reviewing knee MRIs acquired between January 1, 2016, and November 15, 2019, following approval approved by the Ethics Committee of University of Health Sciences Türkiye, Istanbul Haseki Training and Research Hospital (approval no.: 2020-22, date: 12.02.2020). A total of 413 participants were included in the study, comprising 232 patients with complete ACL ruptures and 181 healthy controls. All patients in the study group had complete ACL ruptures. The control group consisted entirely of individuals with no evidence of partial or complete ACL tears on knee MRI, exhibiting healthy knee morphology with intact ligamentous and osseous structures. In both the study and control groups, male and female participants aged between 20 and 40 years were included. Individuals younger than 20 or older than 40 years were excluded from the study. Those within this age range who had any history of knee ligament injury, previous knee surgery, knee deformities (genu varum or valgum), fractures involving the femur or tibia, or radiological evidence of osteoarthritis were also excluded. According to these criteria, 72 participants were excluded from the initial cohort.

Data Acquisition

Knee MRI scans were obtained using a 1.5-T MRI system (Achieva, Philips Medical Systems, The Netherlands). During image acquisition, patients were positioned supine with the knee in approximately 15 ° flexion and 10 ° external rotation. The knee imaging protocol was as follows:

- Sagittal T1-weighted (T1W): echo time (TE)=20 ms, repetition time (TR)=468 ms, matrix=240 × 284, field of view (FOV)=160 mm, number of excitations (NEX)=1
- Axial plane PD: TE=30 ms, TR=2300 ms, matrix=120 × 152, FOV=150 mm, NEX=1
- Coronal PD: TE=30 ms, TR=2300 ms, matrix=120 × 152, FOV=170 mm, NEX=1
- Sagittal PD: TE=30 ms, TR=2300 ms, matrix=120 × 160, FOV=180 mm, NEX=1

The slice thickness was set to 3 mm with an interslice gap of 0.5 mm.

Image Analysis

The ACL was anatomically evaluated in axial, coronal, and sagittal MRI sections. For each patient, age, sex, and the side of the knee assessed were recorded.

On sagittal T1W images, the INA was measured according to the approach proposed by Huang et al. (8). The INA was defined as the angle between the line drawn parallel to the femoral axis and the Blumensaat line tangent to the roof of the intercondylar notch (Figure 1).

On sagittal T1W images, the MTSA was measured as the angle between a line drawn perpendicular to the tibial axis and a line connecting the anterior and posterior peaks of the medial tibial plateau in its central section. This section is identified as the one where the tibiofemoral joint space is narrowest (Figure 2). Similarly, the LTSA was measured in the central section of the lateral tibial plateau, following the method described by Stijak et al. (9) (Figure 3).

Each measurement was performed three times for the same subject, and the mean value was used for analysis. The relationships between ACL injury risk and INA, MTSA, LTSA, sex, and age were then evaluated.

On axial PD images at the level of the popliteal groove and parallel to the line joining the posterior borders of

the femoral condyles, the intercondylar width (ICW) and FNW were measured according to the method described by Bouras et al. (14) (Figure 4). The notch width (NW) ratio was calculated as FNW/ICW.

On axial PD fat-suppressed images, the femoral notch morphology was classified as Type A, Type U, or Type W, following the criteria defined by van Eck et al. (15) (Figure 5). Type A notches were characterized by a narrowing configuration from the midsection toward the base and apex, reflecting a stenotic morphology. Type U notches exhibited a broader outline without tapering in the midsection, whereas Type W notches represented a wider variant of Type U, characterized by two distinct apices instead of a flat roof.

Subsequent analyses investigated which notch type was more frequently associated with ACL rupture and whether a significantly increased risk was observed in patients with narrow FNW or an NW ratio ≤0.27. The relationships between these morphological parameters, age, and sex were also evaluated.

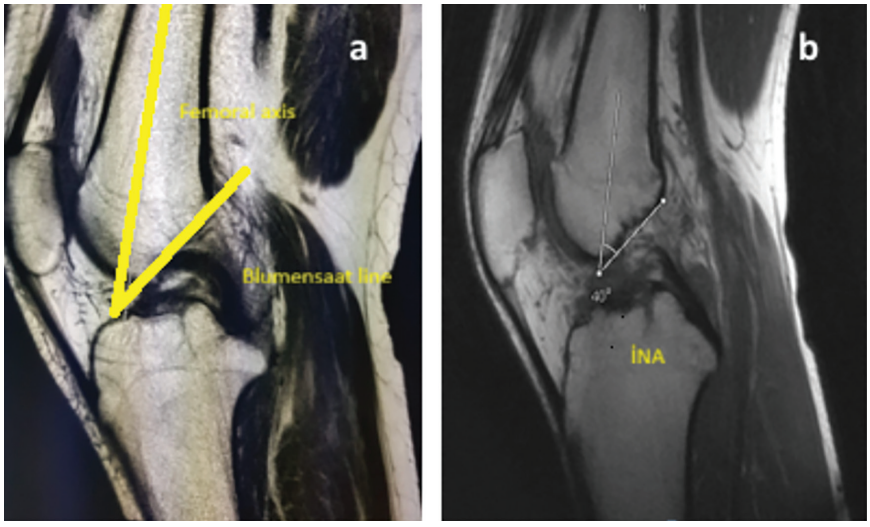

Figure 1. Measurement of the intercondylar notch angle. Sagittal T1-weighted MRI from two different patients show the angle between a line drawn parallel to the femoral axis and the Blumensaat line tangent to the roof of the intercondylar notch. The schematic illustration (a), and a sample patient measurement (b) are shown

MRI: Magnetic resonance imaging, INA: Intercondylar notch angle

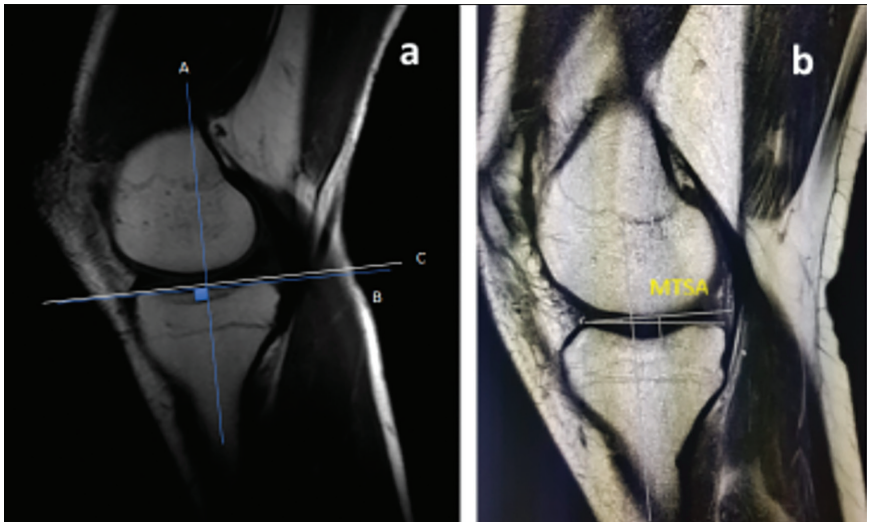

Figure 2. Measurement of the MTSA. On sagittal T1-weighted images, a line parallel to the tibial axis (A), a line perpendicular to the tibial axis (B), and a line connecting the anterior and posterior peaks of the medial tibial plateau in the central section (C) are drawn. The MTSA is defined as the angle between lines B and C. The schematic illustration (a), and a sample patient measurement (b) are shown

MTSA: Medial tibial slope angle

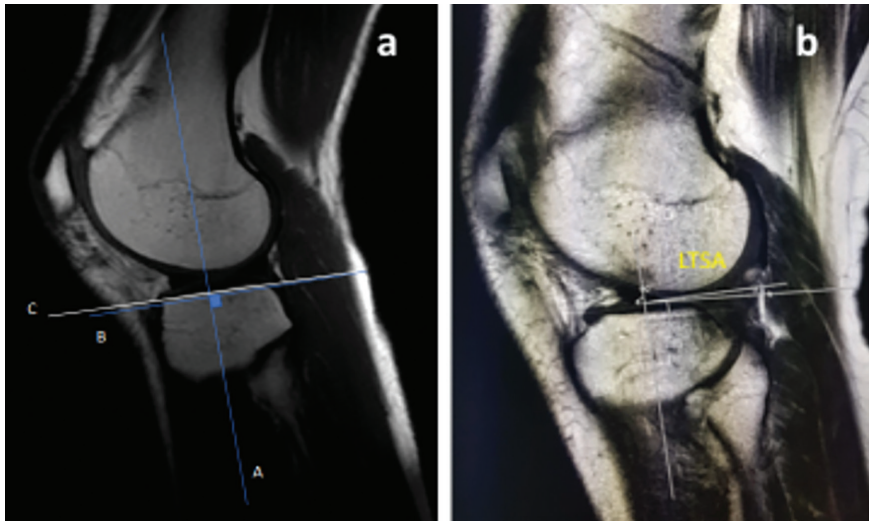

Figure 3. Measurement of the LTSA. On sagittal T1-weighted images, a line parallel to the tibial axis (A), a line perpendicular to the tibial axis (B), and a line connecting the anterior and posterior peaks of the lateral tibial plateau in the central section (C), are drawn. The LTSA is defined as the angle between lines B and C. The schematic illustration (a) and a sample patient measurement (b) are shown

LTSA: Lateral tibial slope angle

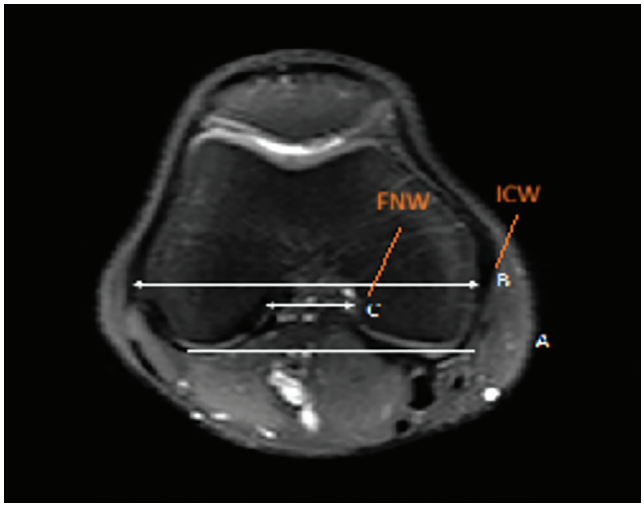

Figure 4. Measurement of femoral intercondylar notch width. A reference line is drawn parallel to the posterior borders of the femoral condyles (A) on an axial PD image passing through the popliteal groove. The intercondylar width (B) and the femoral notch width (C) are measured, and the notch width ratio is calculated as C/B

PD: Proton density, FNW: Femoral notch width, ICW: Intercondylar width

Statistical Analysis

All statistical analyses were performed using Number Cruncher Statistical System 2007 Statistical Software (Utah, USA). Descriptive statistics, including mean, standard deviation, median, and interquartile range, were calculated for all variables. The Shapiro-Wilk test was used to assess the normality of data distribution.

For comparisons between two independent groups, the independent samples t-test was applied to normally distributed variables, while the Mann-Whitney U test was used for non-normally distributed variables. The chi-square test was used to compare categorical data. Relationships between continuous variables were evaluated using the Pearson correlation test.

To determine the independent predictors of ACL injury, logistic regression analysis was performed, including variables that were significant in univariate analysis. The receiver operating characteristic (ROC) curve was used to determine the optimal cut-off value, and sensitivity, specificity, positive predictive value (PPV), negative predictive value, and positive likelihood ratio [LR (+)] were calculated.

All statistical results were evaluated at a significance level of $p<0.05$.

Results

A total of 413 individuals aged 20-40 years were evaluated, including 232 patients with complete ACL rupture (198 males, 34 females) and 181 healthy controls (144 males, 37 females). The mean age was 32.27±6.09 years in the study group and 31.30±6.15 years in the control group. The distribution of age, sex, and laterality (right/left knee) between groups was statistically homogeneous.

In the study group, 126 (54.3%) MRIs were of the right knee and 106 (45.7%) of the left; in the control group, 83 (45.9%) were of the right and 98 (54.1%) were of the left.

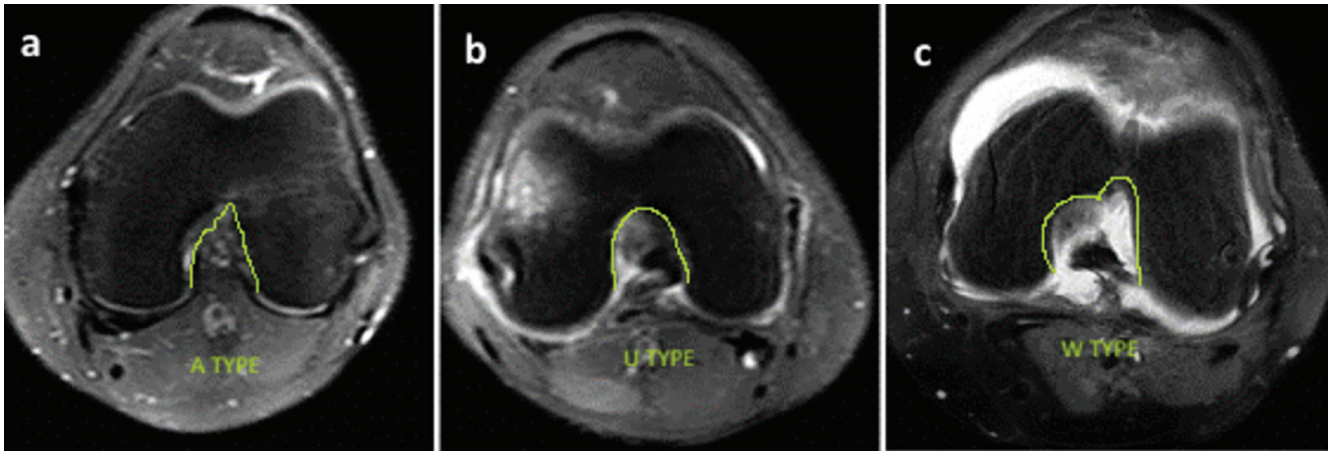

Figure 5. Classification of femoral notch shape on axial PD FS images. Knees were categorized into three types based on the notch configuration: type A, type U, and type W

PD: Proton Density, FS: Fat-Suppressed

The mean INA was significantly lower in the study group compared with controls (p=0.0001). LTSA values were significantly higher in the study group (p=0.002), while no significant difference was found in MTSA values (p=0.419) (Table 1).

The mean FNW was significantly lower (p=0.0001), and the mean ICW was significantly higher (p=0.036) in the study group compared with controls. The (NWR=FNW/ICW) was also significantly lower in the study group (p=0.0001). No significant difference was observed in knee notch shape types (A, U, W) between groups (p=0.169) (Table 1).

A significant negative correlation was detected between age and MTSA in the study group (r=-0.153, p=0.020) (Table 2).

Among the measured parameters, the NWR showed the highest diagnostic accuracy for ACL injury, with an area under the ROC curve (AUC) of 0.724 (0.678-0.767) (Table 3). For a cut-off of NWR ≤0.27, sensitivity was 69.4%, specificity 69.06%, PPV 74.2%, and LR (+) 2.24 (Table 4).

Overall, patients with low INA, high LTSA, narrow FNW, and NWR ≤0.27 had a significantly increased risk of ACL rupture (Table 5).

Table 1. Study and control group INA, MTSA, LTSA, FNW, ICW, NWR, and type comparison

		Control group n=181		Study group n=232		p
INA	**Mean ± SD**	39.2±3.81		36.93±4.56		**0.0001***
MTSA	**Mean ± SD**	3.32±2.46		3.38±2.31		0.419‡
	Median (IQR)	3 (1-5)		3 (2-5)		
LTSA	**Mean ± SD**	3.42±2.26		4.44±3.17		**0.002‡**
	Median (IQR)	3 (2-5)		4 (2-6)		
FNW	**Mean ± SD**	2.33±0.26		2.16±0.25		**0.0001***
ICW	**Mean ± SD**	7.98±0.55		8.09±0.55		**0.036***
NWR	**Mean ± SD**	0.29±0.03		0.26±0.03		**0.0001***
NWR	**>0.27 NWR**	125	69.06%	71	30.60%	**0.0001+**
	≤0.27 NWR	56	30.94%	161	69.40%	
Type	**A Type**	116	64.09%	130	56.03%	0.169+
	U Type	53	29.28%	77	33.19%	
	W Type	12	6.63%	25	10.78%	

*:Independent t-test ‡:Mann Whitney U test +:Chi-square test
INA: Intercondylar notch angle, MTSA: Medial tibial slope angle, LTSA: Lateral tibial slope angle, FNW: Femoral notch width, ICW: Intercondylar width, NWR: Notch width ratio, SD: Standard deviation, IQR: Interquartile range

Table 2. Pearson correlation test			
		Control group	Study group
		Age	Age
INA	**r**	0.112	0.045
	p	0.134	0.494
MTSA	**r**	0.124	**-0.153**
	p	0.096	**0.020**
LTSA	**r**	-0.086	-0.057
	p	0.251	0.388
FNW	**r**	**-0.229**	-0.075
	p	**0.002**	0.256
ICW	**r**	**-0.265**	-0.037
	p	**0.0001**	0.577
NWR	**r**	-0.084	-0.005
	p	0.260	0.934

p: Significance value, r: Correlation coefficient
INA: Intercondylar notch angle, MTSA: Medial tibial slope angle, LTSA: Lateral tibial slope angle, FNW: Femoral notch width, ICW: Intercondylar width, NWR: Notch width ratio

Table 3. Area under the ROC curve			
	AUC	**SE**	**95% CI**
NWR	0.724	0.025	0.678-0.767
FNW	0.678	0.027	0.630-0.723
INA	0.647	0.028	0.599-0.693
LTSA	0.588	0.028	0.539-0.636
ICW	0.558	0.028	0.509-0.607

ROC: Receiver operating characteristic, AUC: Area under the curve, SE: Standard error, CI: Confidence interval, NWR: Notch width ratio, FNW: Femoral notch width, INA: Intercondylar notch angle, LTSA: Lateral tibial slope angle, ICW: Intercondylar width

Discussion

Increasing evidence suggests that intrinsic knee morphology plays a significant role in determining the risk of ACL injury. Several morphometric parameters, including the INA, MTSA and LTSA, FNW, ICW, and the NW, have been extensively investigated as potential anatomical predictors of ACL rupture. Among these, the NWR, defined as the ratio of FNW to ICW, is regarded as one of the most reliable indicators, as it reflects the spatial capacity of the femoral notch to accommodate the ACL. A reduced NWR may limit the available space for the ligament, predisposing it to impingement and increased mechanical stress during knee motion.

In this study, we found that the INA and FNW were significantly lower, whereas the LTSA was significantly higher in patients with ACL rupture compared with healthy controls. No significant difference was observed in the MTSA between the groups. These findings support the hypothesis that morphological narrowing of the femoral notch and steeper tibial slopes increase ACL vulnerability by altering joint biomechanics. Our results are consistent with those reported by Huang et al. (8), who demonstrated significantly lower INA and higher LTSA values in patients with ACL injuries. Similarly, Dejour and Bonnin (16), as well as Khan et al. (17), emphasized that an increased lateral tibial slope promotes greater anterior tibial translation, thereby predisposing the ACL to excessive tensile stress and rupture.

These observations further emphasize the importance of notch geometry and tibial slope as key structural determinants of ACL integrity. In the present study, the NWR emerged as the strongest predictor of ACL injury,

Table 4. Cut-off points in the differential diagnosis of ACL injury by variables						
	Predictive value	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**LR (+)**
NWR	**≤0.27**	69.40	69.06	74.2	63.8	2.24
FNW	**≤2.21**	56.47	69.06	70.1	55.3	1.83
INA	**≤38**	64.22	60.77	67.7	57.0	1.64
LTSA	**>3**	55.17	62.43	65.3	52.1	1.47
ICW	**>7.83**	71.98	38.67	60.1	51.9	1.17

ACL: Anterior cruciate ligament, PPV: Positive predictive value, NPV: Negative predictive value, LR: Likelihood ratio, NWR: Notch width ratio, FNW: Femoral notch width, INA: Intercondylar notch angle, LTSA: Lateral tibial slope angle, ICW: Intercondylar width

Table 5. Logistic regression analysis to determine the factors affecting ACL injury					
	B	**P**	**OR**	**OR (95% CI)**	
				Lower bound	**Upper bound**
INA	-0.12	**0.0001**	0.89	0.84	0.94
LTSA	0.15	**0.0001**	1.16	1.07	1.25
FNW	-1.31	**0.021**	0.27	0.09	0.82
NWR (≤0.27)	-1.06	**0.0001**	0.35	0.20	0.61

ACL: Anterior cruciate ligament, B: Beta coefficient, P: Significance value, OR: Odds ratio, CI: Confidence interval, INA: Intercondylar notch angle, LTSA: Lateral tibial slope angle, FNW: Femoral notch width, NWR: Notch width ratio

with an area under the ROC curve of 0.724 and an odds ratio indicating a 2.24-fold increased risk among subjects with an NWR ≤0.27. These findings are consistent with the results of Souryal and Freeman (18) as well as the study by Anderson et al. (19), who identified an NWR below 0.27 as a critical threshold associated with greater injury risk. Similarly, Bouras et al. (14) reported that a narrow femoral notch and low NWR were significantly correlated with increased ACL rupture incidence, particularly in knees exhibiting type A notch morphology. Although the overall distribution of notch shapes (A, U, W) did not differ significantly between groups in our cohort, the proportion of type A knees was higher among patients with an NWR ≤0.27, further reinforcing the association between notch stenosis and ACL injury risk.

Our findings regarding the LTSA further corroborate previous evidence indicating that a steeper lateral tibial plateau increases anterior tibial translation under axial loading, thereby heightening stress on the ACL during pivoting or deceleration movements (20-22). Stijak et al. (9) similarly reported that a more vertical lateral tibial slope represents a key morphometric feature in patients with ACL rupture. Although no significant association was found between the MTSA and ACL injury, a significant negative correlation between age and MTSA was observed within the study group. This finding may reflect age-related adaptations in tibial geometry; however, its clinical relevance remains to be clarified.

Gender-based analyses in previous research have produced variable results. Hashemi et al. (23,24) reported that women with increased lateral tibial slope angles and decreased medial tibial depth are more susceptible to ACL injury, whereas men exhibit risk patterns associated with concomitant increases in both medial and LTSs. In the present study, no significant sex-based differences in ACL injury risk were identified, consistent with the findings of Vrooijink et al. (25) and van Diek et al. (26). However, both FNW and ICW values were lower in women than in men, suggesting that female knees may possess proportionally narrower intercondylar spaces despite similar NW ratios. Further investigations with larger female cohorts are warranted to elucidate potential gender-specific anatomical risk factors for ACL injury.

Several studies have explored the association between body mass index (BMI) and ACL injury risk, as increased body weight may influence tibial slope geometry and elevate knee joint loading (27,28). Although BMI data were not available in our retrospective cohort, this parameter may affect tibial plateau morphology and warrants further investigation. In addition, the absence of contralateral knee imaging limited our ability to evaluate potential bilateral or genetic predispositions, which have been proposed as contributing factors to ACL rupture (19,29).

In line with previous reports, our findings confirm that reduced intercondylar notch dimensions and an increased lateral tibial slope represent significant intrinsic risk factors for ACL rupture (30,31). Although non-contact ACL injuries, which account for the majority of cases and are multifactorial in origin, morphological narrowing of the intercondylar notch appears to play a consistent and reproducible role across different populations (32).

Study Limitations

There are certain limitations that should be considered when interpreting the findings of this study. Its retrospective design precludes causal inference, and the absence of data on BMI, activity level, and trauma mechanism reduces the generalizability of the findings. Furthermore, the lack of contralateral knee imaging prevented evaluation of potential bilateral or genetic predispositions, and minor variations in MRI acquisition parameters may have introduced measurement variability. Only patients with complete ACL ruptures were included, thereby excluding partial injuries that may exhibit different anatomical characteristics. Despite these limitations, the relatively large sample size and the use of a standardized measurement protocol enhance the reliability and robustness of our results.

Conclusion

In summary, our findings demonstrate that a smaller INA, reduced FNW, lower NWR, and steeper lateral tibial slope are significant morphological risk factors for ACL rupture. These findings suggest that intrinsic knee morphology plays a key role in injury susceptibility, independent of external factors such as sport type or trauma mechanism. Quantitative evaluation of these parameters on MRI may enhance injury risk prediction and support the development of targeted prevention strategies. Future prospective, multicenter, and biomechanical studies incorporating body composition and activity data are warranted to further elucidate the complex interplay between intrinsic anatomy and ACL injury susceptibility.

Ethics

Ethics Committee Approval: The study was approved by the Ethics Committee of University of Health Sciences Türkiye, Istanbul Haseki Training and Research Hospital (approval date:12.02.2020, approval number: 2020-22). All procedures performed in studies involving human participants were conducted in accordance with the ethical standards of the institutional research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Informed Consent: Written informed consent was obtained from all participants before undergoing magnetic resonance imaging.

Footnotes

Authorship Contributions

Surgical and Medical Practices: E.C., R.K., Concept: E.C., T.I., Design: E.C., T.I., Data Collection or Processing: E.C., R.K., Analysis or Interpretation: E.C., T.I., R.K., Y.S., Literature Search: E.C., Writing: E.C.

Conflict of interests: No conflict of interest were declared by the authors.

Financial Disclosure: This study received no financial support.

References

1. Amis AA, Dawkins GP. Functional anatomy of the anterior cruciate ligament. Fibre bundle actions related to ligament replacements and injuries. J Bone Joint Surg Br. 1991;73: 260-7.
2. Girgis FG, Marshall JL, Monajem A. The cruciate ligaments of the knee joint. Anatomical, functional and experimental analysis. Clin Orthop Relat Res. 1975:216-31.
3. Sakane M, Fox RJ, Woo SL, Livesay GA, Li G, Fu FH. In situ forces in the anterior cruciate ligament and its bundles in response to anterior tibial loads. J Orthop Res. 1997;15: 285-93.
4. Miller LS, Yu JS. Radiographic indicators of acute ligament injuries of the knee: a mechanistic approach. Emerg Radiol. 2010;17:435-44.
5. Shen L, Jin ZG, Dong QR, Li LB. Anatomical risk factors of anterior cruciate ligament injury. Chin Med J (Engl). 2018;131:2960-7.
6. Beynnon BD, Johnson RJ, Fleming BC, et al. The effect of functional knee bracing on the anterior cruciate ligament in the weightbearing and nonweightbearing knee. Am J Sports Med. 1997;25:353-9.
7. Lohmander LS, Englund PM, Dahl LL, Roos EM. The long-term consequence of anterior cruciate ligament and meniscus injuries: osteoarthritis. Am J Sports Med. 2007;35:1756-69.
8. Huang M, Li Y, Guo N, Liao C, Yu B. Relationship between intercondylar notch angle and anterior cruciate ligament injury: a magnetic resonance imaging analysis. J Int Med Res. 2019; 47:1602-9.
9. Stijak L, Herzog RF, Schai P. Is there an influence of the tibial slope of the lateral condyle on the ACL lesion? A case-control study. Knee Surg Sports Traumatol Arthrosc. 2008;16:112-7.
10. Ng WH, Griffith JF, Hung EH, Paunipagar B, Law BK, Yung PS. Imaging of the anterior cruciate ligament. World J Orthop. 2011;2:75-84.
11. Padron M, Lacalle ES, Oaso I, Schueller-Weidekamm C. MRI of the Cruciate ligaments. 2013;65-75. Available from internet: https://breitenseher.eu/books/978-3-9503296-9-8/leseprobe.pdf.
12. Zeng C, Borim FM, Lording T. Increased posterior tibial slope is a risk factor for anterior cruciate ligament injury and graft failure after reconstruction: a systematic review. J ISAKOS. 2025;12:100854.
13. Pradhan P, Kaushal SG, Kocher MS, Kiapour AM. Development of anatomic risk factors for ACL injuries: a comparison between ACL-injured knees and matched controls. Am J Sports Med. 2023;51:2267-74.
14. Bouras T, Fennema P, Burke S, Bosman H. Stenotic intercondylar notch type is correlated with anterior cruciate ligament injury in female patients using magnetic resonance imaging. Knee Surg Sports Traumatol Arthrosc. 2018;26:1252-7.
15. van Eck CF, Martins CA, Vyas SM, Celentano U, van Dijk CN, Fu FH. Femoral intercondylar notch shape and dimensions in ACL-injured patients. Knee Surg Sports Traumatol Arthrosc. 2010;18:1257-62.
16. Dejour H, Bonnin M. Tibial translation after anterior cruciate ligament rupture. Two radiological tests compared. J Bone Joint Surg Br. 1994;76:745-9.
17. Khan MS, Seon JK, Song EK. Risk factors for anterior cruciate ligament injury: assessment of tibial plateau anatomic variables on conventional MRI using a new combined method. Int Orthop. 2011;35:1251-6.
18. Souryal TO, Freeman TR. Intercondylar notch size and anterior cruciate ligament injuries in athletes. A prospective study. Am J Sports Med. 1993;21:535-9.
19. Anderson AF, Dome DC, Gautam S, Awh MH, Rennirt GW. Correlation of anthropometric measurements, strength, anterior cruciate ligament size, and intercondylar notch characteristics to sex differences in anterior cruciate ligament tear rates. Am J Sports Med. 2001;29:58-66.
20. Domzalski M, Grzelak P, Gabos P. Risk factors for Anterior Cruciate Ligament injury in skeletally immature patients: analysis of intercondylar notch width using magnetic resonance imaging. Int Orthop. 2010;34:703-7.
21. McLean SG, Oh YK, Palmer ML, et al. The relationship between anterior tibial acceleration, tibial slope, and ACL strain during a simulated jump landing task. J Bone Joint Surg Am. 2011;93:1310-7.
22. McLean SG, Lucey SM, Rohrer S, Brandon C. Knee joint anatomy predicts high-risk in vivo dynamic landing knee biomechanics. Clin Biomech (Bristol). 2010;25:781-8.
23. Hashemi J, Chandrashekar N, Gill B, et al. The geometry of the tibial plateau and its influence on the biomechanics of the tibiofemoral joint. J Bone Joint Surg Am. 2008;90:2724-34.

24. Hashemi J, Chandrashekar N, Mansouri H, et al. Shallow medial tibial plateau and steep medial and lateral tibial slopes: new risk factors for anterior cruciate ligament injuries. Am J Sports Med. 2010;38:54-62.

25. Vrooijink SH, Wolters F, Van Eck CF, Fu FH. Measurements of knee morphometrics using MRI and arthroscopy: a comparative study between ACL-injured and non-injured subjects. Knee Surg Sports Traumatol Arthrosc. 2011;19:12-6.

26. van Diek FM, Wolf MR, Murawski CD, van Eck CF, Fu FH. Knee morphology and risk factors for developing an anterior cruciate ligament rupture: an MRI comparison between ACL-ruptured and non-injured knees. Knee Surg Sports Traumatol Arthrosc. 2014;22:987-94.

27. Smith HC, Vacek P, Johnson RJ, et al. Risk factors for anterior cruciate ligament injury: a review of the literature - part 1: neuromuscular and anatomic risk. Sports Health. 2012;4: 69-78.

28. Kızılgöz V, Sivrioğlu AK, Aydın H, Ulusoy GR, Çetin T, Tuncer K. The combined effect of body mass index and tibial slope angles on anterior cruciate ligament injury risk in male knees: a case-control study. Clin Med Insights Arthritis Musculoskelet Disord. 2019;12:1179544119867922.

29. Dienst M, Schneider G, Altmeyer K, et al. Correlation of intercondylar notch cross sections to the ACL size: a high resolution MR tomographic in vivo analysis. Arch Orthop Trauma Surg. 2007;127:253-60.

30. Park JS, Nam DC, Kim DH, Kim HK, Hwang SC. Measurement of knee morphometrics using MRI: a comparative study between ACL-injured and non-injured knees. Knee Surg Relat Res. 2012;24:180-5.

31. Zeng C, Gao SG, Wei J, et al. The influence of the intercondylar notch dimensions on injury of the anterior cruciate ligament: a meta-analysis. Knee Surg Sports Traumatol Arthrosc. 2013;21:804-15.

32. Wordeman SC, Quatman CE, Kaeding CC, Hewett TE. In vivo evidence for tibial plateau slope as a risk factor for anterior cruciate ligament injury: a systematic review and meta-analysis. Am J Sports Med. 2012;40:1673-81.

Original Article

DOI: 10.4274/haseki.galenos.2025.07379
Med Bull Haseki 2025;63(5):297-303

The Clinical Effectiveness of Mesotherapy in Refractory Chronic Migraine

Elif Ozyigit*, Mert Zure*, Esra Topcu**, Mehmet Fatih Ucar***

*University of Health Sciences Türkiye, Kanuni Sultan Suleyman Training and Research Hospital, Clinic of Physical Medicine and Rehabilitation, Istanbul, Türkiye

**Amasya University, Sabuncuoglu Serefeddin Research and Training Hospital, Clinic of Physical Medicine and Rehabilitation, Amasya, Türkiye

***Istanbul Kultur University, Faculty of Science and Letters, Department of Maths and Computer Science, Istanbul, Türkiye

Abstract

Aim: This study aimed to evaluate the clinical effectiveness of mesotherapy in patients with refractory chronic migraine (CM). The goal was to determine its impact on pain frequency, duration, and intensity and on analgesic use, thereby informing alternative therapeutic strategies for this population.

Methods: This retrospective cohort study included 27 patients diagnosed with CM. These patients received mesotherapy injections containing 2 mL of 1% lidocaine, 40 mg of piroxicam in 2 mL, and 100 U of calcitonin in 1 mL at specific anatomical sites, including the glabella, supraorbital notch, infraorbital foramen, preauricular, postauricular, and temporal masseter, fronto-occipital, and trapezius muscle trigger points. Assessment parameters included the frequency of painful days per month (PDs), the number of analgesics per month (NoA), the duration of attacks per month (DoA), and the patients' visual analogue scale (VAS) scores. Evaluations were conducted before treatment and at 4, 8, and 12 weeks post-treatment.

Results: Significant improvements were observed in the NoA, DoA, PD, and VAS scores at 4 and 8 weeks post-treatment, compared with pre-treatment values. Although efficacy declined by week 12, scores remained higher than baseline. No adverse events were reported.

Conclusion: Mesotherapy appears to be an effective treatment for CM, with notable improvements in pain frequency, duration, and severity and a reduced need for analgesics over 12 weeks. To more thoroughly assess this efficacy, large-scale, prospective, randomized controlled studies are required.

Keywords: Pain, migraine disorders, mesotherapy, piroxicam, analgesics

Introduction

Headache is a common neurological symptom with a multifactorial etiology and represents one of the most frequent causes of outpatient visits worldwide. Common causes of headache include migraine, tension-type headache, and cervicogenic headache (1). Migraine is a condition characterized by recurrent headaches often accompanied by sensory, emotional, and cognitive symptoms, particularly affecting the productivity of the younger population aged 15-49 (1,2). The International Headache Society defines chronic migraine (CM) as a headache persisting for more than three months without excessive medication use, occurring on at least 15 days per month, with episodes lasting 4-72 hours, including at least eight days with migraine features. Accompanying symptoms may include nausea, vomiting, visual disturbances, and sensitivity to light, sound, and odors (1,3).

Corresponding Author: Elif Ozyigit, MD, University of Health Sciences Türkiye, Kanuni Sultan Suleyman Training and Research Hospital, Clinic of Physical Medicine and Rehabilitation, Istanbul, Türkiye
E-mail: dr.elifoz16@gmail.com **ORCID:** orcid.org/0000-0003-0997-7013
Received: 18.06.2025 **Accepted:** 29.10.2025 **Publication Date:** 28.11.2025

Cite this article as: Ozyigit E, Zure M, Topcu E, Ucar MF. The clinical effectiveness of mesotherapy in refractory chronic migraine. Med Bull Haseki. 2025;63(5):297-303

Chronic migraine is treated with a combination of pharmacological and non-pharmacological approaches to alleviate pain and reduce the frequency, duration, and intensity of attacks (1,4). Mesotherapy is an intradermal technique that involves injecting a specialized mixture into the superficial dermis using microneedles. Modern mesotherapy approaches advocate administering a solution in minimal quantities (5). Mesotherapy is thought to be effective in pain management by boosting endorphin levels, eliciting reflex responses to needle stimulation, and inducing local effects through the gradual diffusion of the medications (5,6). Mesotherapy can be deemed an effective treatment approach for headaches, lower back and neck pain, fibromyalgia, and musculoskeletal pain (5,7,8).

We hypothesized that mesotherapy, an intradermal injection technique using low-dose medication mixtures, could provide clinical benefit in refractory CM. There is a notable gap in the literature regarding studies demonstrating the effectiveness of mesotherapy in the treatment of CM. This study aims to assess the efficacy of mesotherapy in the management of CM.

Materials and Methods

Compliance with Ethical Standards

The principles of the Declaration of Helsinki were followed during this study. Ethical approval was obtained from the University of Health Sciences Türkiye, Kanuni Sultan Suleyman Training and Research Hospital Ethics Committee (approval no.: KAEK/2022.11.225, date: 21.11.2022). Informed consent for publication was obtained from the patients after they agreed that their anonymized case data could be summarized and analyzed. The signed documents are confidential.

Study Population

This retrospective interventional cohort study enrolled 27 patients diagnosed with CM who were referred to and treated at our integrative medicine and rehabilitation clinic between January 2019 and June 2022, and data extraction, analysis, and manuscript preparation were completed between January 2023 and December 2024. All participants met the criteria for refractory CM as outlined by the European Headache Federation (9). All patients had undergone at least one year of preventive therapy for CM prior to participating in the study. Signed informed consent was obtained from each participant.

Before mesotherapy, all patients underwent routine blood tests, including a complete blood count, thyroid function tests, liver function tests, kidney function tests, blood glucose measurement, and screening for markers of viral infections, including human immunodeficiency virus, Hepatitis B, and Hepatitis C. Patients were included in the study after the exclusion of infections, thyroid dysfunction, anemia, and systemic illnesses. Chronic illnesses and allergies were documented for each patient.

Patients were regularly monitored at 4-week intervals over a 12-week period to assess treatment effectiveness. The evaluation included the frequency of painful days per month (PD), the number of analgesics taken per month (NoA), the duration of attacks per month (DoA), and patients' visual analogue scale (VAS) scores, measured before treatment (baseline measurement) and at 4, 8, and 12 weeks post-treatment. Additionally, the use of analgesics (including ergot alkaloids, triptans, and others) was quantified as the number of doses per month.

None of the patients had contraindications to injections, including conditions such as malignant hypertension, medication overuse, intracranial pathologies such as open skull defects, known allergies to anesthetic agents or piroxicam, systemic or local infections, anticoagulant use, or a tendency toward vasovagal responses to injections.

The study flowchart is shown in Figure 1.

Mesotherapy Treatment Protocol

Mesotherapy employs various injection techniques to deliver drug mixtures precisely to the intended anatomical site. These injections can be administered via syringes, mechanical or electronic injection devices, or pneumatically powered portable injection guns. In this study, 10 mL syringes were used. Injections were performed at 21 predefined sites (glabella, supraorbital notch, infraorbital foramen, fronto-occipital, preauricular and postauricular zones, temporal trigger points, and masseter and trapezius muscles). Figures 2 and 3 show schematic illustrations of the anatomical sites for mesotherapy application and of the standardized injection, respectively.

Figure 2 legend: 1. glabella, 2. supraorbital notch, 3. infraorbital foramen, 4. fronto-occipital zone, 5. temporal trigger point, 6. preauricular zone, 7. posterior auricular, 8. masseter muscle, 9. trapezius muscle.

Two injection techniques were employed by an experienced physician in this field: profound intradermal injection at a depth of 2-4 mm and superficial intradermal injection at a depth of 1-2 mm, using sterile single-use needles measuring 0.3 mm × 4 mm and 0.3 mm × 13 mm, respectively. A total of 5 mL was administered per session: 2 mL of 1% lidocaine, 100 U of calcitonin in 1 mL, and 40 mg of piroxicam in 2 mL. All patients had three sessions of mesotherapy at 7 day intervals.

Statistical Analysis

Data were analyzed using IBM SPSS 25.0. Normality was tested using the Shapiro-Wilk test. The Wilcoxon signed-rank test was used for pairwise comparisons, the Friedman test for repeated measures, and the Mann-Whitney U test for gender differences. Effect sizes (r) and 95% confidence intervals were calculated. Significance

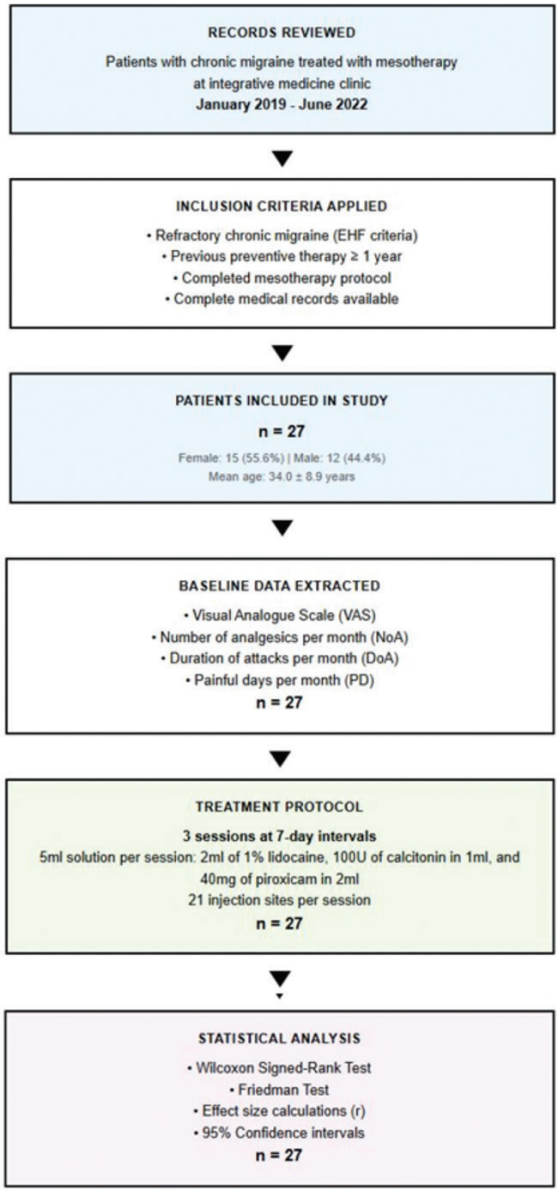

Figure 1. Study flowchart
EHF: European Headache Federation

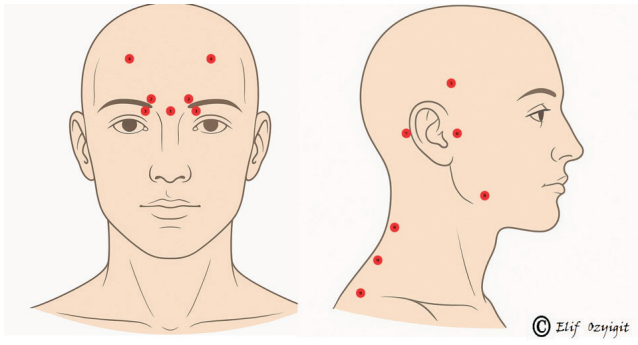

Figure 2. Anatomical sites for mesotherapy application

was set at $p<0.05$. The significance level for all statistical tests was set at $p<0.05$.

Results

The study comprised 27 participants, with 15 females and 12 males. Female patients had a mean age of 31.67±7 years, while male patients had a mean age of 36.9±10.5 years. The overall mean age across both genders was 34±8.936 years.

Mean values and standard deviations at baseline and weeks 4, 8, and 12 are presented in Table 1.

Baseline values were compared with those at weeks 4, 8, and 12 for each parameter (VAS, NoA, DoA, and PD). Pairwise comparisons and the resulting p-values are displayed in Table 2. Baseline means were higher than those at weeks 4, 8, and 12; hence, a statistically significant decrease in VAS, NoA, DoA, and PD values among patients receiving mesotherapy was observed. No adverse events were reported. Temporal changes in treatment outcomes are presented in Figure 4.

The analysis of variance results for dependent variables within the group is presented in Table 3. The Friedman test results indicated statistically significant differences in within-group variances among the groups. This suggests that the treatment effect varied significantly across the time points. The first assessment at 4 weeks post-application showed a notable improvement, and the last assessment at the 12th week showed a gradual reduction in effectiveness. However, the values after this decline did not exceed the initial values.

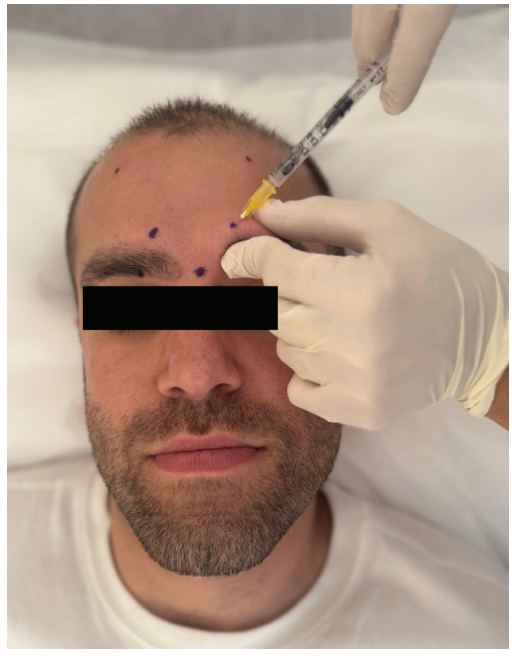

Figure 3. Model application for mesotherapy

Table 1. Group comparisons						
	(Mean ± SD)				Effect size (r)	Confidence interval (95%)
	Baseline	4.Week	8. Week	12. Week		
VAS	7.44±0.801	3.04±0.854	3.48±0.7	5.7±0.823	0.88	0.76-0.94
NoA	7.52±0.893	2.07±0.73	3.85±0.602	5.74±0.526	0.87	0.75-0.93
DoA	19.74±3.182	5.22±2.577	11.63±2.372	16.85±2.553	0.89	0.78-0.95
PD	17.93±3.012	5.59±1.947	9.04±1.48	10.89±1.717	0.89	0.78-0.95
Statistical test: Wilcoxon signed-rank test, effect size: r=Z/√N, where large effect r≥0.5 VAS: Visual analogue scales, NoA: Number of analgesic per month, DoA: Duration of attack per month, PD: Painful days per month, SD: Standard deviation						

Table 2. Comparison of baseline data and weekly data via Wilcoxon signed-rank test						
VAS baseline	VAS 4th week		VAS 8th week		VAS 12th week	
	Z-value	p-value	Z-value	p-value	Z-value	p-value
	-4.593[b]	0.000	-4.612[b]	0.000	-4.620[b]	0.000
NoA baseline	NoA 4th week		NoA 8th week		NoA 12th week	
	Z-value	p-value	Z-value	p-value	Z-value	p-value
	-4.585[b]	0.000	-4.642[b]	0.000	-4.517[b]	0.000
DoA baseline	DoA 4th week		DoA 8th week		DoA 12th week	
	Z-value	p-value	Z-value	p-value	Z-value	p-value
	-4.553[b]	0.000	-4.562[b]	0.000	-4.453[b]	0.000
PD baseline	PD 4th week		PD 8th week		PD 12th week	
	Z-value	p-value	Z-value	p-value	Z-value	p-value
	-4.550[b]	0.000	-4.549[b]	0.000	-4.556[b]	0.000
VAS: Visual analogue scales, NoA: Number of analgesic per month, DoA: Duration of attack per month, PD: Painful days per month						

Table 3. Comparison of related sample variances via friedman test	
Hypothesis	p-value
The distributions of VAS baseline, 4th week, 8th week and 12th week are the same	0.000
The distributions of NoA baseline, 4th week, 8th week and 12th week are the same	0.000
The distributions of DoA baseline, 4th week, 8th week and 12th week are the same	0.000
The distributions of PD baseline, 4th week, 8th week and 12th week are the same	0.000
VAS: Visual analogue scales, NoA: Number of analgesic per month, DoA: Duration of attack per month, PD: Painful days per month	

The analysis of whether the application of mesotherapy yielded different outcomes between male and female patients revealed that there were no significant gender-based differences in response to mesotherapy.

Discussion

The present study demonstrates substantial clinical improvements in CM management through mesotherapy, with effect sizes ranging from 0.86 to 0.90, indicating large clinical effects according to Cohen's criteria. The 59% reduction in VAS scores at 4 weeks (from 7.44 to 3.04) represents a clinically meaningful and substantial improvement in pain intensity.

Chronic migraine is a substantial neurological challenge, yet treatment options remain limited. Clinicians seek interventions that effectively alleviate pain while minimizing the occurrence of severe or intolerable side effects. Topiramate emerges as a pharmacological intervention capable of mitigating the progression to chronic headache in individuals experiencing episodic migraine (10). In addition, administering a greater occipital nerve (GON) block using a combination of lidocaine and methylprednisolone is a reliable alternative for managing refractory CM (11). In a recent randomized controlled trial, Chowdhury et al. (12) reported that combination therapy with topiramate and GON blocks reduced monthly headache days by approximately 6.9 days over a 12-week period in patients with. Our mesotherapy approach demonstrated greater efficacy with a smaller sample size, resulting in a reduction of 12.34 PD at 12

Figure 4. Mesotherapy treatment outcomes: temporal trends
VAS: Visual analogue scale

weeks, suggesting superior therapeutic benefit compared with this established combination therapy (12).

OnabotulinumtoxinA injection is another treatment option reported to show promise in reducing headache severity and frequency in individuals with chronic migraine (13,14). While direct comparison is limited by our retrospective, single-arm design, in contrast to the randomized, placebo-controlled Phase III Research Evaluating Migraine Prophylaxis Therapy trials, our mesotherapy protocol achieved a 69% reduction in painful days within 4 weeks, compared with botulinum toxin's approximately 50% reduction over 24 weeks, suggesting potential advantages in both the magnitude and onset of effects that warrant further investigation in controlled trials.

As an established therapeutic approach in integrative medicine, mesotherapy has been extensively studied in clinical trials for musculoskeletal pain and injuries (8,15-17). The findings of a comparative study by Akbas et al. (18), which assessed the effectiveness of mesotherapy using a mixture of thiocolchicoside, tenoxicam, and lidocaine, demonstrated that mesotherapy was more effective than intravenous dexketoprofen therapy in treating acute migraine attacks without aura. Building on this work on acute migraine attacks, our study is among the first to evaluate mesotherapy as a preventive treatment for CM, demonstrating substantial and sustained benefits over 12 weeks using a different medication combination.

The combination of lidocaine, piroxicam, and calcitonin in the mesotherapy solution potentially offers a multifaceted approach to managing CM. Several studies have investigated the use of lidocaine, particularly through injection or infusion, in various headache disorders (19). Lidocaine, a local anesthetic, can provide immediate pain relief by blocking voltage-gated sodium channels in neuronal membranes, thereby preventing the conduction of impulses along sensory nerves, particularly nociceptive C-fibers, a mechanism crucial for reducing the acute discomfort associated with migraine attacks (20). Piroxicam, a potent cyclooxygenase-2 inhibitor, helps reduce inflammation and pain by inhibiting endogenous prostaglandin production, thereby addressing the inflammatory component often linked to migraine pathophysiology (21). Calcitonin, a hormone known to influence calcium metabolism, has been suggested to have analgesic properties, possibly through its action on central pain pathways (22,23). The synergistic effect of these three components could have resulted in enhanced pain control, reduced frequency and intensity of migraine episodes, and an overall improvement in quality of life for patients, as reported in this study. Future studies should explore the specific mechanisms and long-term benefits of this combination therapy compared with traditional migraine treatments.

The significant findings from the Friedman test indicate meaningful differences in treatment effects across follow-up points, highlighting the temporal dynamics of mesotherapy's efficacy. This suggests that, while mesotherapy is beneficial, its peak effectiveness occurs earlier in the treatment period, necessitating potential consideration of ongoing or integrative treatment strategies to sustain benefits (24,25).

Study Limitations

Although this retrospective cohort study provides valuable insights into the efficacy of mesotherapy in the management of CM, it is essential to acknowledge its limitations, including the lack of a control group and potential biases inherent in retrospective analyses. The small sample size and relatively short follow-up period are additional limitations. Additionally, data on prior migraine treatments were unavailable for all patients, which represents a limitation in fully characterizing baseline clinical status. Despite these limitations, this study is among the few that explore the effectiveness of mesotherapy for treating CM, which may contribute to expanding therapeutic options in multimodal headache management. Additionally, the comprehensive outcome measures used and the large effect sizes reported support the scientific strength of the study.

To further validate these findings and to establish a more robust evidence base, randomized controlled trials are needed to enable direct comparisons of mesotherapy with standard treatments or with placebo, thereby minimizing confounding factors and providing higher-quality evidence regarding treatment efficacy and safety.

Conclusion

Mesotherapy appears to be an effective treatment for managing CM over a 12-week period, showing significant improvements in the duration, intensity, and frequency of pain, as well as a reduction in analgesic use. Large-scale, prospective, randomized controlled trials are needed to better evaluate the effectiveness of this.

Ethics

Ethics Committee Approval: Ethical approval was obtained from the University of Health Sciences Türkiye, Kanuni Sultan Suleyman Training and Research Hospital Ethics Committee (approval no.: KAEK/2022.11.225, date: 21.11.2022).

Informed Consent: Informed consent for publication was obtained from the patients after they agreed that their anonymized case data could be summarized and analyzed.

Footnotes

Authorship Contributions

Surgical and Medical Practices: E.O., E.T., Concept: E.O., M.Z., Design: E.O., M.Z., Data Collection or Processing: E.O., E.T., Analysis or Interpretation: E.O., M.Z., M.F.U., Literature Search: E.O., E.T., M.Z., M.F.U., Writing: E.O., E.T., M.Z., M.F.U.

Conflict of Interest: No conflicts of interest were declared by the authors.

Financial Disclosure: This study received no financial support

References

1. Kung D, Rodriguez G, Evans R. Chronic migraine: diagnosis and management. Neurol Clin. 2023;41:141-59.
2. Oliveira R, Gil-Gouveia R, Puledda F. CGRP-targeted medication in chronic migraine - systematic review. J Headache Pain. 2024;25:51.
3. Ray JC, Matharu M, Hutton E. Relationship of migraine and other forms of chronic pain. Handb Clin Neurol. 2024;199:517-24.
4. Zhao C, Li C, Yu X, Dai X, Zou W. Effectiveness and safety of pharmacological prophylaxis for chronic migraine: a systematic review and network meta-analysis. J Neurol. 2024;271:5762–77.
5. Topal İ, Yılmaz O. Effectiveness of mesotherapy in Post-COVID pain syndrome: retrospective cohort study of 96 patients. Clin Ther. 2024;46:e1-5.
6. Mammucari M, Paolucci T, Russo D, et al. A call to action by the Italian Mesotherapy Society on scientific research. Drug Des Devel Ther. 2021;15:3041-7.
7. Mammucari M, Maggiori E, Russo D, et al. Mesotherapy: from historical notes to scientific evidence and future prospects. ScientificWorldJournal. 2020;2020:3542848.
8. Koszela K, Mammucari M, Słupiński M, Woldańska-Okońska M. The role of mesotherapy in chronic back pain syndrome: what do we know? Rheumatol Int. 2025;45:206.
9. Martelletti P, Katsarava Z, Lampl C, et al. Refractory chronic migraine: a consensus statement on clinical definition from the European Headache Federation. J Headache Pain. 2014;15:47.
10. Zeinhom MG, Elsayed Khalil MF, Omar Youssif TY, et al. Efficacy and tolerability of valproate versus topiramate in migraine prevention, a randomized controlled multi-center trial. J Clin Neurosci. 2025;135:111156.
11. Chowdhury D, Tomar A, Deorari V, Duggal A, Krishnan A, Koul A. Greater occipital nerve blockade for the preventive treatment of chronic migraine: a randomized double-blind placebo-controlled study. Cephalalgia. 2023;43:3331024221143541.
12. Chowdhury D, Mundra A, Datta D, Duggal A, Krishnan A, Koul A. Efficacy and tolerability of combination treatment of topiramate and greater occipital nerve block versus topiramate monotherapy for the preventive treatment of chronic migraine: a randomized controlled trial. Cephalalgia. 2022;42:859-71.
13. Kępczyńska K, Domitrz I. Botulinum toxin-a current place in the treatment of chronic migraine and other primary headaches. Toxins (Basel). 2022;14:619.
14. Silberstein SD, Diener HC, Dodick DW, Sommer K, Lipton RB. Sustained benefits of onabotulinumtoxinA treatment in chronic migraine: an analysis of the pooled Phase 3 REsearch Evaluating Migraine Prophylaxis Therapy (PREEMPT) randomized controlled trials. Headache. 2024;64:838-48.
15. Paolucci T, Bellomo RG, Centra MA, Giannandrea N, Pezzi L, Saggini R. Mesotherapy in the treatment of musculoskeletal pain in rehabilitation: the state of the art. J Pain Res. 2019;12:2391-01.
16. Bifarini B, Gori F, Russo D, et al. Intradermal therapy (mesotherapy): the lower the better. Clin Ter. 2022;173:79-83.
17. Koszela K, Woldańska-Okońska M, Gasik R. Efficacy and safety of spinal collagen mesotherapy in patients with chronic low back pain in a three-month follow-up-retrospective study. J Clin Med. 2024;13:787.
18. Akbas I, Kocak MB, Kocak AO, et al. Intradermal mesotherapy versus intravenous dexketoprofen for the treatment of migraine headache without aura: a randomized controlled trial. Ann Saudi Med. 2021;41:127-34.
19. Berk T, Silberstein SD. The use and method of action of intravenous lidocaine and its metabolite in headache disorders. Headache. 201858:783-9.
20. Donath F, Mallefet P, Garreffa S, Furcha R. Efficacy of 8 mg lidocaine and 2 mg cetylpyridinium chloride (CPC) fixed-combination lozenges on sore throat pain intensity compared with 1 mg lidocaine and 2 mg CPC fixed-combination lozenges in subjects with sore throat due to upper respiratory tract

infection: a randomized double-blind parallel-group single-dose study. Trials. 2018;19:679.

21. Ravishankar K, Tayade H, Mandlik R. Sublingual piroxicam in migraine without aura. J Assoc Physicians India. 2011;59: 494-7.
22. Kumar G, Kirubahar R, Kanna M. Efficacy and safety of two different doses of intrathecal calcitonin, a randomized controlled study. Int J Res Med Sci. 2018;6:682-7.
23. Villar-Martinez MD, Goadsby PJ. Vestibular migraine: an update. Curr Opin Neurol. 2024;37:252-63.
24. Scaturro D, Vitagliani F, Signa G, et al. Neck pain in fibromyalgia: treatment with exercise and mesotherapy. Biomedicines. 2023;11:892.
25. Chen L, Li D, Zhong J, Qiu B, Wu X. Therapeutic effectiveness and safety of mesotherapy in patients with osteoarthritis of the knee. Evid Based Complement Alternat Med. 2018;2018:6513049.

Case Report

DOI: 10.4274/haseki.galenos.2025.84756
Med Bull Haseki 2025;63(5):304-307

Melanosis Coli Associated with SAPHO Syndrome: A Rare Coexistence Suggesting a Possible Gut–Bone Axis Link

Wei Yang*, Chendong He**

*Jiangsu Province Hospital of Chinese Medicine, Affiliated Hospital of Nanjing University of Chinese Medicine, Clinic of Radiology, Nanjing, China

**Nanjing Hospital of Chinese Medicine Affiliated to Nanjing University of Chinese Medicine, Clinic of Radiology, Nanjing, China

Abstract

We report the incidental detection of melanosis coli during a colonoscopy in a 72-year-old man with a history of laxative use. Subsequent imaging supported a diagnosis of SAPHO syndrome. The co-occurrence of these conditions is rare, and its clinical significance remains uncertain. This report aims to discuss potential underlying mechanisms, diagnostic challenges, and therapeutic considerations.

Keywords: melanosis coli, SAPHO syndrome, colononoscopy, constipation, positron-emission tomography, computed tomography

Introduction

Melanosis coli (MC) is a benign condition characterized by dark pigmentation of the colonic mucosa, most commonly associated with long-term laxative use (1,2). Synovitis, Acne, Pustulosis, Hyperostosis, and Osteitis (SAPHO) syndrome is a rare multisystem inflammatory disorder primarily affecting the skin, joints, and bones (3-5). The co-occurrence of MC and SAPHO syndrome is exceedingly rare, posing diagnostic and management challenges because both conditions are uncommon.

Case Report

A 72-year-old man was admitted with a two-week history of altered stool characteristics and a six-month history of diarrhea with melena. He denied vomiting, abdominal pain, or fever but reported a 5 kg weight loss. His history included chronic constipation managed with oral laxatives. Physical examination revealed normal skin and mucosal coloration, with no rash, edema, or joint abnormalities.

The laboratory tests showed that the high-sensitivity C-reactive protein level was high (69.37 mg/L) and the platelet count was high (485 × 10^9/L). The white blood cell count was normal (7.84 × 10^9/L), the red blood cell count was low (3.09 × 10^{12}/L), and the hemoglobin level was low (82 g/L). Serum biochemistry indicated elevated creatinine (110.7 µmol/L), hypokalemia (2.86 mmol/L), and hypocalcemia (1.94 mmol/L). Tumor markers, including alpha-fetoprotein, carcinoembryonic antigen, cancer antigen (CA) 125, CA 15-3, and CA 19-9, were within normal limits. Human leukocyte antigen B27 was negative, and levels of vitamins A, D, and E were normal. Stool analysis was positive for *Clostridium difficile*, and fecal calprotectin was elevated (64.84 µg/g).

Colonoscopy revealed mucosal edema in the ascending, transverse, and descending colon; multiple polyps; and diffuse pigmentation consistent with MC (Figure 1a-b). Histopathology revealed acute and chronic inflammatory cell infiltration, with pigment-laden macrophages, in the lamina propria, as well as ischemic changes. Positron emission tomography/computed tomography demonstrated multiple sclerotic lesions in the thorax, pelvis, and spine with adjacent soft-tissue swelling (Figure 2a-d). The sternoclavicular joints showed increased uptake (SUV_{max} of 4.5), and the ileocecal and colorectal regions showed marked uptake (SUV_{max} of 14.9;

Corresponding Author: Chendong He, MD, Nanjing Hospital of Chinese Medicine Affiliated to Nanjing University of Chinese Medicine, Clinic of Radiology, Nanjing, China
E-mail: hcd1222@163.com **ORCID:** orcid.org/0000-0002-2216-7245
Received: 13.08.2025 **Accepted:** 28.10.2025 **Publication Date:** 28.11.2025

Cite this article as: Yang W, He C. Melanosis coli associated with SAPHO syndrome: a rare coexistence suggesting a possible gut–bone axis link. Med Bull Haseki. 2025;63(5):304-307

Figure 2e), consistent with SAPHO syndrome. Symptomatic treatment with montmorillonite powder, cefotiam, and enteral nutrition led to improvement in diarrhea, and the patient was discharged at his request.

Discussion

Melanosis coli results from lipofuscin accumulation in macrophages within the colonic lamina propria. It is most frequently linked to chronic use of anthraquinone-containing laxatives (1,2) but can also occur in inflammatory bowel disease (IBD), ischemic colitis, or colorectal neoplasms (1,3). Anthraquinones disrupt the mucosal barrier and stimulate tumor necrosis factor-α (TNF-α) release, inducing epithelial apoptosis. The resulting apoptotic bodies are phagocytosed by macrophages, producing mucosal pigmentation (1,2). Other implicated factors include proton pump inhibitors, statins, and herbal medicines (2). Any factor that induces epithelial apoptosis,

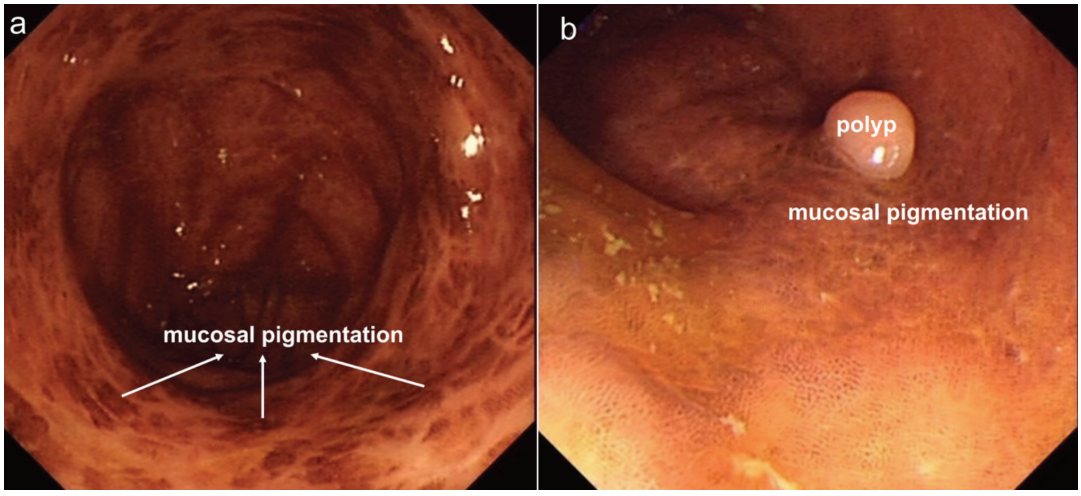

Figure 1. Colonoscopy showing markedly pigmented colonic mucosa and a polyp (a, b)

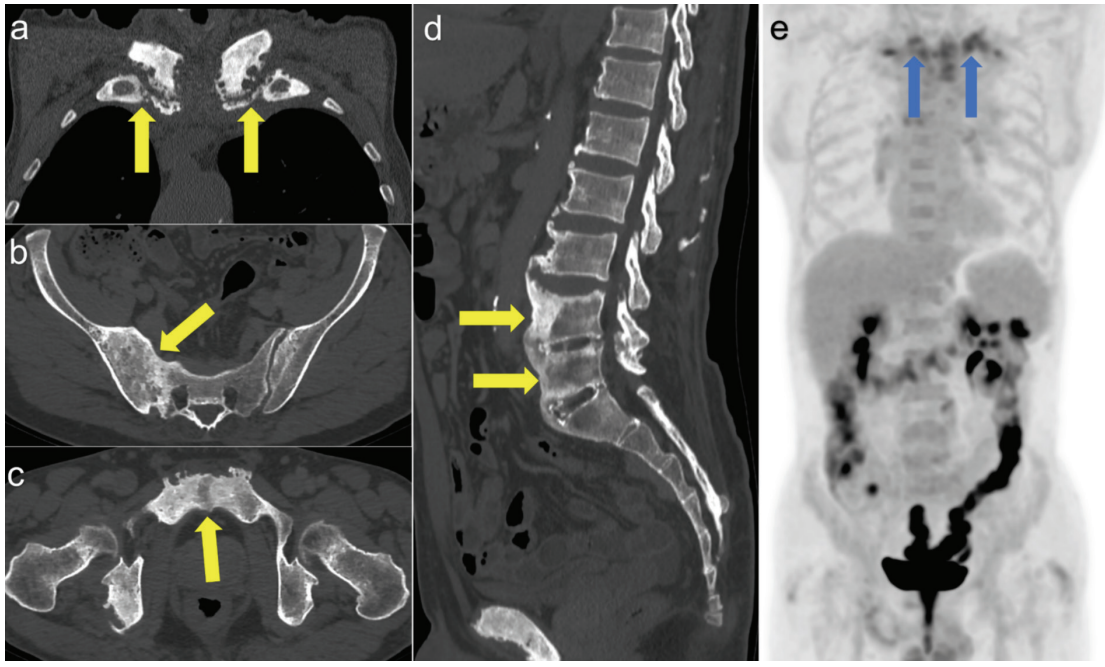

Figure 2. CT bone-window images demonstrating hypertrophic changes and osteosclerosis in the bilateral sternoclavicular joints (a, yellow arrow), right sacroiliac joint (b, yellow arrow), pubic symphysis (c, yellow arrow), and anterior vertebral segments (d, yellow arrow). Maximum-intensity projection PET/CT image showing increased radiotracer uptake in both sternoclavicular joints and the colorectal region (e, blue arrow)
PET/CT: Positron emission tomography/computed tomography

including non-steroidal anti-inflammatory drugs (NSAIDs) or IBD-related inflammation, may contribute to MC.

Synovitis, Acne, Pustulosis, Hyperostosis, and Osteitis syndrome is an uncommon autoinflammatory disorder within the spondyloarthropathy spectrum, typically characterized by chronic inflammation of the axial skeleton and the anterior chest wall (4,6). Although its exact pathogenesis remains unclear, autoimmune and infectious mechanisms have been implicated (6,7). Gastrointestinal involvement, including IBD and non-specific colitis, has been reported (3).

The simultaneous occurrence of MC and SAPHO syndrome raises questions about a potential underlying association. Gastrointestinal manifestations of SAPHO syndrome include chronic diarrhea, hematochezia, abdominal pain, and weight loss (4-5). Enteropathic SAPHO typically occurs in children and young adults, and its pathogenesis remains incompletely understood. It is thought to involve abnormal T-cell activation along the skin–gut–bone axis, resulting in dysregulation of inflammatory cytokines, including interleukin-1 beta (IL-1β), IL-6, IL-17, IL-18, and TNF-α (4).

Synovitis, Acne, Pustulosis, Hyperostosis, and Osteitis syndrome has been reported to present occasionally with rare intestinal manifestations, including Crohn's disease and ulcerative colitis (3). Both SAPHO syndrome and IBD are chronic inflammatory disorders with potential multisystem involvement. Available reports suggest that SAPHO syndrome associated with IBD occurs predominantly in females and is most commonly observed in patients with Crohn's disease. In a study by Hayem et al., 7.5% of patients with SAPHO syndrome had Crohn's disease (6). A few cases of ulcerative colitis co-occurring with SAPHO syndrome have also been documented (8). In the development of SAPHO syndrome, infection is considered a significant environmental factor (9). Tissue samples from bone and joint lesions in affected patients have revealed the presence of *Cutibacterium acnes (C. Acness)*, suggesting a potential role in disease pathogenesis. Cases of granulomatous colitis caused by *C. acnes* have been reported (3).

Synovitis, Acne, Pustulosis, Hyperostosis, and Osteitis syndrome is traditionally managed with therapies including NSAIDs, corticosteroids, and bisphosphonates. While NSAIDs are generally considered first-line agents for osteoarticular manifestations, they may exacerbate intestinal inflammation or precipitate disease flares (7,10). Corticosteroids can provide short-term relief of musculoskeletal and gastrointestinal symptoms, but long-term use may cause serious complications. Bisphosphonates inhibit osteoclast-mediated bone resorption, modulate local inflammatory responses, and show efficacy in refractory osteitis and hyperostosis; however, their use is limited in patients with concomitant enteropathy (8). Currently, no standard treatment exists for MC. The most important intervention is discontinuation of anthraquinone-containing laxatives. In patients with constipation, a healthy diet, prokinetic agents, and the maintenance of a stable intestinal microbiota are recommended.

Treatment of SAPHO syndrome in the context of MC presents significant clinical challenges. Modulation of the gut microbiota through microbe-based therapies has been proposed as a strategy. The gut–bone–skin axis underscores the immunological interdependence among the intestinal, skeletal, and cutaneous systems (10). Given the potential role of proinflammatory TNF- in both melanosis coli and SAPHO syndrome, TNF-α inhibitors such as infliximab and adalimumab may represent promising therapeutic options (3,5,10). Nevertheless, optimal treatment strategies should be determined through multidisciplinary collaboration involving gastroenterology, rheumatology, and dermatology specialists. A more profound understanding of SAPHO syndrome and its enteropathic variants is essential to improving the management of this potentially chronic and debilitating condition.

Conclusion

Although MC and SAPHO syndrome are distinct clinical entities, their co-occurrence raises questions regarding the potential interplay among chronic systemic inflammation, immune dysregulation, and colonic mucosal changes. Recognition of this rare association may enhance understanding of the extra-articular manifestations of SAPHO syndrome and their implications for gastrointestinal health.

Ethics

Informed Consent: Informed consent was obtained from the patient for the use and publication of anonymized clinical and imaging data.

Footnotes

Authorship Contributions

Concept: W.Y., C.H., Data Collection or Processing: W.Y., C.H., Writing: W.Y., C.H.

Conflict of interest: There are no conflicts of interest.

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

References

1. Yang N, Ruan M, Jin S. Melanosis coli: a comprehensive review. Gastroenterol Hepatol. 2020;43:266-72.
2. Zhang R, Huang C, Wu F, et al. Review on melanosis coli and anthraquinone-containing traditional Chinese herbs that cause melanosis coli. Front Pharmacol. 2023;14:1160480.

3. Naves JE, Cabré E, Mañosa M, Grados D, Olivé A, Domènech E. A systematic review of SAPHO syndrome and inflammatory bowel disease association. Dig Dis Sci. 2013;58:2138-47.
4. Yang Y, Chen Q, Zhong W. The role of cytokines in the pathogenesis of SAPHO syndrome. Front Immunol. 2024;15:1427784.
5. Wang Y, Gu M, Zheng Z, et al. Therapeutic approaches for SAPHO syndrome from the perspective of pathogenesis: a review of the literature. Front Immunol. 2025;16:1560398.
6. Przepiera-Będzak H, Brzosko M. SAPHO syndrome: pathogenesis, clinical presentation, imaging, comorbidities and treatment: a review. Postepy Dermatol Alergol. 2021;38:937-42.
7. Demirci Yildirim T, Sari İ. SAPHO syndrome: current clinical, diagnostic and treatment approaches. Rheumatol Int. 2024;44:2301-13.
8. Phillipps J, Mumtaz S, Valecha J, et al. Enteropathic SAPHO syndrome in ulcerative colitis responsive to bisphosphonates. Case Rep Rheumatol. 2024;2024:3558853.
9. Govoni M, Colina M, Massara A, Trotta F. "SAPHO syndrome and infections". Autoimmun Rev. 2009;8:256-9.
10. Almalaq AA Sr, Almasoud A, Almeghaiseeb E, et al. From the gut to the bone: a narrative review of the synovitis, acne, pustulosis, hyperostosis, and osteitis (SAPHO)-ınflammatory bowel disease (IBD) overlap syndrome. Cureus. 2025;17:e92230.

2025 Referee Index

Abdullah Agin
Akif Erbin
Ali Fuat Kaan Gök
Ali Kubilay Kubilay Korkut
Ali Yavuz Karahan
Alkan Çubuk
Arda Isik
Asli Okbay Güneş
Ayşe Çiğdem Tütüncü
Ayşe Nur Aksoy
Ayşe Serap Akgün
Aysenur Erbin
Baki Erdem
Bekir Turgut
Beliz Bilgili
Berna Caliskan
Betül Kirşavoğlu
Bilal Cetin
Burcu Ersöz Alan
Busra Yılmaz Tugan
Çiğdem Tura Bahadir
Cihan Comba
Dilan Colak
Duygu Gülmez Sevim
Duygu Yalınbaş Yeter
Egemen Cebeci
Elmas Biberci Keskin
Emine Küçükateş
Emine Tugba Alatas
Emre Balaban
Enes Efe İş
Ezgi Yilmaz
Fatma Saraç
Fatma YÜCEL BEYAZTAŞ
Feray Akbaş
Ferit Kerim Küçükler
Gamze Çıtlak
Hakan Yılmaz
Halil Lütfi Canat
Halit Özgül
Hasan Bulut
Hatice Köse
Ibrahim Cansaran Tanidir
Ilke Dolgun
İrfan Oğuz Şahin
Işil Bakirci
Kamil Sahin
Kamile Arıkan
Kemal Karaarslan
Levent Deniz
Mabdussamet Bozkurt
Mahir Topaloğlu
Mazhar Müslüm Tuna
Mehmet Akif Kilic
Mehmet Akif Ozturk
Mehmet Emirhan Işik
Meral Alagöz
Metin Savun
Muhammet İrfan Dönmez
Murat Uğurlucan
Nagehan Didem Sarı
Necla Gürbüz Sarıkaş
Numan Cim
Nursu Becet
Orkun Koban
Osman Kipoglu
Ozden Aksu
Ramazan Yilmaz
Recep Erçin Sönmez
Şebnem Çalık
Selin Betas Akin
Şemsi Nur Karabela
Şenay Güven Baysal
Senem Şaş
Şener Balas
Serhat Ege
Süleyman Ahbab
Tolgahan Atci
Tuba Selcuk Can
Turgut Anuk
Turkan Ikizceli
Veli Süha Öztürk
Yusuf Sahin
Zehra Zeynep Keklikkıran
Zerrin Özçelik