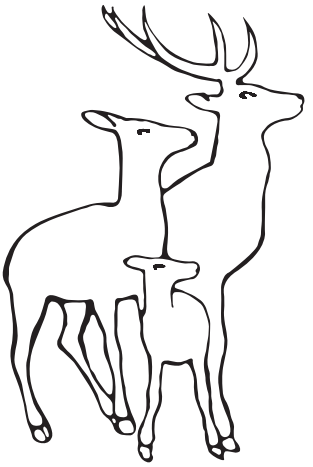
ISSN 0041-4301
Online ISSN 2791-6421
www.turkjpediatr.org

# THE TURKISH JOURNAL OF PEDIATRICS



Volume 67
Issue 6
November-December 2025



# THE TURKISH JOURNAL OF PEDIATRICS

www.turkjpediatr.org

**Volume 67 ▪ Issue 6**
**November-December 2025**

**ISSN: 0041-4301**
**Online ISSN: 2791-6421**

# THE TURKISH JOURNAL OF PEDIATRICS

**ISSN 0041-4301 Online ISSN 2791-6421**
www.turkjpediatr.org

Cilt: 67 Sayı: 6, Kasım-Aralık 2025



**YAYIN SAHİBİ**
**Yayımlayan Kurumlar Adına**
Elif Nursel ÖZMERT

**SORUMLU YAZI İŞLERİ MÜDÜRÜ**
Enver HASANOĞLU

**YAYIMLAYAN**
Türkiye Milli Pediatri Derneği
Hacettepe Üniversitesi Çocuk Sağlığı Enstitüsü
Uluslararası Çocuk Merkezi

**EDİTÖR ADRESİ**
The Turkish Journal of Pediatrics
P.K. 36, Samanpazarı 06240 Ankara, Türkiye
Faks: +90 (312) 305 22 64
E-posta: editorial@turkjpediatr.org

**YAYIN İDARE MERKEZİ**
The Turkish Journal of Pediatrics Editör Ofisi
Hacettepe Üniversitesi
İhsan Doğramacı Çocuk Hastanesi
06100 Ankara
Tel : +90 (312) 305 26 76
Faks: +90 (312) 305 22 64

**YAYININ TÜRÜ**
Uluslararası hakemli dergi

**YAYIN SIKLIĞI VE DİLİ**
İki aylık ▪ İngilizce

**BASIM YERİ**
Meteksan Matbaacılık ve Teknik Sanayi A.Ş.
Beytepe No: 3, 06530 Bilkent, Ankara, Türkiye
Tel: +90 (312) 266 44 10 (Pbx)

**BASIM TARİHİ:** 30.12.2025

**YAYINCILIK HİZMETLERİ**
Akdema Bilişim Yayıncılık ve Danışmanlık Tic. Ltd. Şti.
Kızılay Mah. Gazi Mustafa Kemal Bulvarı No: 23/8 06420
Çankaya/Ankara, Türkiye
Tel: +90 (533) 166 80 80 ▪ Web: www.akdema.com

**ISSN 0041-4301 Online ISSN 2791-6421**
www.turkjpediatr.org

Vol: 67 Issue: 6, November-December 2025



**PRODUCTION MANAGER**
**Owner on behalf of the Publishers**
Elif Nursel ÖZMERT

**ADMINISTRATOR**
Enver HASANOĞLU

**PUBLISHED BY**
Turkish National Pediatric Society
Hacettepe University Institute of Child Health
The International Children's Center

**EDITORIAL OFFICE**
The Turkish Journal of Pediatrics
P.K. 36, Samanpazarı 06240 Ankara, Türkiye
Fax: +90 (312) 305 22 64
E-mail: editorial@turkjpediatr.org

**SUBSCRIPTION ADDRESS**
The Turkish Journal of Pediatrics Editorial Office
Hacettepe University
İhsan Doğramacı Children's Hospital
06100 Ankara
Tel : +90 (312) 305 26 76
Fax: +90 (312) 305 22 64

**PUBLICATION TYPE**
International peer-reviewed journal

**PUBLICATION FREQUENCY AND LANGUAGE**
Bi-monthly ▪ English

**PRINTED BY**
Meteksan Matbaacılık ve Teknik Sanayi A.Ş.
Beytepe No: 3, 06530 Bilkent, Ankara, Türkiye
Tel: +90 (312) 266 44 10 (Pbx)

**PRINT DATE:** 30.12.2025

**PUBLISHING SERVICES**
Akdema Informatics, Publishing, and Consultancy Trade LLC
Kızılay Mah. Gazi Mustafa Kemal Bulvarı No: 23/8 06420
Çankaya/Ankara, Türkiye
Tel: +90 (533) 166 80 80 ▪ Web: www.akdema.com

# THE TURKISH JOURNAL OF PEDIATRICS



The Turkish Journal of Pediatrics is published by Hacettepe University Institute of Child Health, in partnership with the Turkish National Pediatric Society and the International Children's Center. The journal has been published since 1958 and has been available online since 2002. The Turkish Journal of Pediatrics is published 6 times a year. The journal does not have article processing charges or article submission charges.

The Turkish Journal of Pediatrics is a multidisciplinary, peer reviewed, open access journal that seeks to publish research to advance the field of Pediatrics. It publishes original articles, review articles, short communications, case reports, correspondence papers and letters to the editor. Articles published in this journal are evaluated in an independent and unbiased, double blinded peer-reviewed fashion by an advisory committee.

This publication is indexed in Web of Science - Science Citation Index Expanded (SCIE), PubMed/MEDLINE, Scopus, Embase, Directory of Open Access Journals (DOAJ), CABI Abstracts, ProQuest, EBSCOhost, BIOSIS Previews, Türkiye Citation Index and TR-Index.

# CONTENTS

VOLUME: 67 ISSUE: 6 NOVEMBER-DECEMBER 2025

## COMMENTARY



## ORIGINAL ARTICLES

# CONTENTS

VOLUME: 67 ISSUE: 6 NOVEMBER-DECEMBER 2025

## SHORT COMMUNICATION



## CASE REPORTS



## LETTERS TO THE EDITOR

The Turkish Journal of Pediatrics 2025; 67: 773-775
https://doi.org/10.24953/turkjpediatr.2025.7758

**Commentary**

# The rising epidemic of e-cigarette use among adolescents: an unpredictable threat

Demet Taş[1]

[1]*Division of Adolescent Medicine, Department of Pediatrics, Ankara Bilkent City Hospital, Faculty of Medicine, Ankara Yıldırım Beyazıt University, Ankara, Türkiye.*

E-cigarette use among adolescents is not just risky behaviour; it is a matter of concern for physicians and public health authorities because it can lead to nicotine addiction and encourage the use of conventional tobacco products, resulting in substantial and long-term health problems. In this context, the study entitled "Prevalence and predictors of e-cigarette use among adolescents in a tertiary pediatric clinic: insights from the new ECABA Scale," published in this issue of The Turkish Journal of Pediatrics by Can Oksay et al.[1], makes a substantial contribution to the international literature by evaluating e-cigarette awareness, prevalence of use, and related beliefs among adolescents.

Despite national access restrictions and legal regulations, the study demonstrated that 18.5% of adolescents had tried e-cigarettes at least once in their lifetime, while 9.2% were current users. Furthermore, adolescents who had experimented with or currently used conventional cigarettes exhibited significantly more favorable attitudes toward e-cigarettes.

According to data from the European School Survey Project on Alcohol and Other Drugs (ESPAD) 2024, the average prevalence of current e-cigarette use in the past 30 days among 15 to 16 year old students in Europe was reported to be 22%. Lifetime experience with e-cigarettes was approximately 44%.[2]

According to recent 2021 to 2022 data from the World Health Organization for the European Region, 32% of 15-year-old adolescents had tried at least one type of e-cigarette, and 20% reported use within the past 30 days, highlighting a rapid increase in use.[3]

Among adolescents, several factors contribute to the rapid increase in e-cigarette use, even in countries where sales to individuals under 18 years of age are legally restricted. These factors include widespread exposure to e-cigarette advertising on social media platforms across all age groups and the high accessibility of these products. Longitudinal studies conducted in adolescent populations have shown that adolescents who do not use any tobacco products but have experimented with e-cigarettes often transition to conventional cigarette use within a short period of time. This evidence indicates that e-cigarettes may function not as a harm reduction tool for adolescents, but rather as a gateway to nicotine dependence.[4]

Furthermore, concurrent use of e-cigarettes and conventional cigarettes, referred to as dual use, has been observed in many adolescents. In this pattern of use, adolescents are exposed not only to the toxic constituents of combustible tobacco and e-cigarettes, but also to very high levels of nicotine.[5]

E-cigarettes are devices that generate an aerosol by heating an e-liquid. These e-liquids typically

✉ Demet Taş ▪ demettas19691@hotmail.com

Received 18th December 2025, accepted 22nd December 2025.

contain nicotine, flavoring agents, propylene glycol, vegetable glycerin, and various chemical additives. Nicotine concentrations vary widely across products, and some have been reported to deliver nicotine amounts equivalent to those found in a full pack of 20 cigarettes, even with a single use. In addition, nicotine has been detected in certain e-liquids marketed as nicotine-free or labeled as containing zero percent nicotine. Substantial inconsistencies in nicotine content have also been observed even among e-cigarettes of the same brand and model, with many analyses reporting nicotine levels exceeding those declared by manufacturers.[6]

Nicotine has a very high addictive potential. In addition, due to its developmental characteristics, the adolescent brain rapidly increases dopaminergic activity in response to substances and certain behaviors, resulting in a stronger reward response. Consequently, the high nicotine content of e-cigarettes may lead to faster development of dependence in the more sensitive adolescent brain compared with adults. It is well established that adolescents remain at high risk of developing nicotine dependence even with intermittent use.[7]

Because of their developmental characteristics, adolescents tend to be more impulsive, and curiosity driven, making the flavored and colorful aromas and visual designs of e-cigarettes particularly attractive. The recent introduction of e-cigarette devices designed to resemble USB flash drives, highlighters, or other technological gadgets has further increased their popularity among adolescents and facilitated peer use. In the United Kingdom, following the market introduction in 2021 of disposable e-cigarette models that were more visually appealing and easier to use for adolescents, a striking 99% annual increase in e-cigarette use was observed, particularly among individuals aged 18 to 24 years. During the same period, the decline in conventional cigarette use remained limited.[8]

E-cigarettes have adverse health effects not only because they contain highly addictive nicotine, but also due to the presence of numerous harmful agents. These harmful components directly challenge marketing claims that e-cigarettes are harmless or clean products. Indeed, growing evidence increasingly associates e-cigarette use with serious health conditions, particularly severe clinical entities such as e-cigarette or vaping associated lung injury (EVALI).[9]

In conclusion, industry strategies targeting adolescents, together with the continued availability of e-cigarettes in our country despite national restrictions, threaten adolescent health. It would be beneficial to incorporate information on e-cigarette bans, industry tactics targeting adolescents, and the harm of e-cigarettes into school-based tobacco prevention programs tailored to adolescents' developmental level. Implementing evidence-based preventive and therapeutic interventions that challenge the belief that e-cigarettes are less harmful, within healthcare settings that have direct contact with adolescents, is of critical importance for protecting adolescent health.

## Author contribution

The author confirm contribution to the paper as follows: Conception and design: DT; literature review: DT; draft manuscript preparation and approval: DT. The author reviewed the results and approved the final version of the manuscript.

## Source of funding

The authors declare the study received no funding.

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES

1. Can Oksay S, Bilgin G, Gürler E, et al. Prevalence and predictors of e-cigarette use among adolescents in a tertiary pediatric clinic: insights from the new ECABA Scale. Turk J Pediatr 2025; 67: 776-788. https://doi.org/10.24953/turkjpediatr.2025.7284
2. European Union Drugs Agency. Key findings from the 2024 European School Survey Project on Alcohol and Other Drugs (ESPAD). 2025. Available at: https://www.euda.europa.eu/publications/data-factsheets/espad-2024-key-findings (Accessed on December 15, 2025).
3. Kiaer T, Hancock J. Alcohol, e-cigarettes, cannabis: concerning trends in adolescent substance use, shows new WHO/Europe report. 2024. Available at: https://www.who.int/europe/news/item/25-04-2024-alcohol--e-cigarettes--cannabis--concerning-trends-in-adolescent-substance-use--shows-new-who-europe-report (Accessed on December 15, 2025).
4. Chen G, Rahman S, Lutfy K. E-cigarettes may serve as a gateway to conventional cigarettes and other addictive drugs. Adv Drug Alcohol Res 2023; 3: 11345. https://doi.org/10.3389/adar.2023.11345
5. Jeon C, Jung KJ, Kimm H, et al. E-cigarettes, conventional cigarettes, and dual use in Korean adolescents and university students: prevalence and risk factors. Drug Alcohol Depend 2016; 168: 99-103. https://doi.org/10.1016/j.drugalcdep.2016.08.636
6. Raymond BH, Collette-Merrill K, Harrison RG, Jarvis S, Rasmussen RJ. The nicotine content of a sample of e-cigarette liquid manufactured in the United States. J Addict Med 2018; 12: 127-131. https://doi.org/10.1097/ADM.0000000000000376
7. Castro EM, Lotfipour S, Leslie FM. Nicotine on the developing brain. Pharmacol Res 2023; 190: 106716. https://doi.org/10.1016/j.phrs.2023.106716
8. Tattan-Birch H, Brown J, Shahab L, Beard E, Jackson SE. Trends in vaping and smoking following the rise of disposable e-cigarettes: a repeat cross-sectional study in England between 2016 and 2023. Lancet Reg Health Eur 2024; 42: 100924. https://doi.org/10.1016/j.lanepe.2024.100924
9. Amjad MA, Ocazionez Trujillo D, Estrada-Y-Martin RM, Cherian SV. E-Cigarette or vaping product use-associated lung injury: a comprehensive review. Int J Environ Res Public Health 2025; 22: 792. https://doi.org/10.3390/ijerph22050792

The Turkish Journal of Pediatrics 2025; 67: 776-788
https://doi.org/10.24953/turkjpediatr.2025.7284

**Original Article**

# Prevalence and predictors of e-cigarette use among adolescents in a tertiary pediatric clinic: insights from the new ECABA Scale

Sinem Can Oksay[1], Gülay Bilgin[2], Eda Gürler[1], Yadigar Öztürk[1], Deniz Mavi Tortop[3], Zeynep Reyhan Onay[1], Saniye Girit[1]

*[1]Division of Pediatric Pulmonology, Department of Pediatrics, Göztepe Prof. Dr. Süleyman Yalçın City Hospital, Faculty of Medicine, İstanbul Medeniyet University, İstanbul, Türkiye; [2]Division of Pediatric Pulmonology, Department of Pediatrics, Eskişehir City Hospital, Eskişehir, Türkiye; [3]Division of Pediatric Pulmonology, Department of Pediatrics, Şanlıurfa Training and Research Hospital, Şanlıurfa, Türkiye.*

## ABSTRACT

**Background.** Electronic cigarettes (e-cigarettes) are rapidly infiltrating youth culture under the guise of safety and social acceptance. Despite their prohibition in Türkiye, anecdotal reports suggest widespread access and experimentation among adolescents. This study aimed to determine the prevalence and predictors of e-cigarette use among adolescents attending a tertiary pediatric clinic, using the validated E-Cigarette Attitudes and Beliefs in Adolescents (ECABA) Scale to explore how beliefs shape behavior.

**Methods.** A cross-sectional design with consecutive sampling was employed. A total of 547 adolescents aged 14–18 years without psychological or organic illness participated. Data were collected using the 18-item ECABA Scale, along with demographic, cigarette, and e-cigarette use information. Logistic regression models were used to identify independent predictors of ever and current e-cigarette use.

**Results.** E-cigarette use was alarmingly common: 18.5% had ever tried, and 9.2% were current users. Males and those with peers who smoked or vaped were significantly more likely to use e-cigarettes ($p<0.001$). Higher ECABA total scores, reflecting more favorable attitudes, independently predicted both ever and current use (odds ratios 1.054 and 1.048 per unit increase, respectively). Subscales related to perceived physical harmlessness and social acceptance also emerged as significant predictors.

**Conclusions.** Adolescents who view e-cigarettes as harmless or socially acceptable are substantially more likely to use them, despite national restrictions. These findings expose a critical gap in prevention efforts and call for urgent school-based interventions to challenge the illusion of "safe vaping." The ECABA Scale may serve as an effective early warning tool to identify at-risk youth before nicotine dependence takes hold.

**Key words:** adolescents, attitudes; belief, e-cigarettes, predictors, public health.

Nicotine addiction has reinvented itself. Once confined to tobacco cigarette smoking, it now hides behind colorful flavors, sleek devices, and the illusion of safety. Worldwide, electronic cigarettes (e-cigarettes) have become the Trojan horse of the tobacco industry—marketing addiction as innovation, and vulnerability as freedom. Nowhere is this more alarming than among adolescents, a group deliberately targeted by advertising strategies that portray vaping as harmless, trendy, and socially acceptable.[1,2] As a result of these strategies, by

✉ Sinem Can Oksay ▪ drsinemcan@gmail.com

Received 21st Oct 2025, revised 19th Nov 2025, accepted 11th Dec 2025.

2024, in the United Kingdom, 18% of individuals aged 11–17 had tried e-cigarettes at least once, and 7.2% were identified as current users. In the United States, e-cigarette use among high school students increased from 1.5% in 2011 to 16.0% in 2015.[3] Although data are more limited in Asian countries, the reported prevalence of 'ever users' among adolescents ranges from 3.5% to 32%, reflecting regional and cultural variations.[4] In Türkiye, data on e-cigarette use among adolescents are limited. Although no nationally representative study assesses the prevalence of e-cigarette use in Türkiye, a local survey of high school students reported a prevalence of 15.4%.[5] The increasing prevalence of e-cigarette use among adolescents was highlighted in the 2023 report by the World Health Organization, which emphasized the urgency of taking immediate action in response to this concerning trend.[6]

A multinational study showed that adolescent experimentation greatly increases the risk of adult smoking.[7] Among adolescents, key factors contributing to the initiation of e-cigarette use include the desire for social acceptance, curiosity, peer influence, the appeal of flavored products, and the impact of persuasive marketing strategies delivered through social media.[8] However, due to cultural differences, patterns of e-cigarette use and the factors influencing such behavior may vary in our country. Against this backdrop, understanding how adolescents perceive e-cigarettes is not merely an academic exercise but a public health imperative. Beliefs about harm and social acceptance strongly influence experimentation and sustained use, yet culturally grounded data from Türkiye are lacking.

The primary aim of this study was to determine the prevalence and predictors of e-cigarette use among adolescents attending a tertiary pediatric outpatient clinic. The secondary aim was to explore the relationship between adolescents' beliefs and attitudes toward e-cigarettes—measured by the validated E-Cigarette Attitudes and Beliefs in Adolescents (ECABA) Scale[9], and their actual use behaviors.

## Materials and Methods

### *Study design and setting*

This cross-sectional, scale-based study was conducted between May and September 2025 at the pediatric outpatient clinics of a tertiary hospital in İstanbul, Türkiye. This study was conducted in accordance with the Declaration of Helsinki. Before starting the study, it was approved by the Istanbul Medipol University Non-Interventional Clinical Research Ethics Committee (approval number: 645, dated May 22, 2025). Written informed consent was obtained from parents or legal guardians, and age-appropriate assent was obtained from all participating adolescents.

### *Participants and data collection*

Adolescents aged 14–18 years who presented to the pediatric outpatient clinic for non-urgent visits and were not diagnosed with chronic physical or psychiatric disorders were invited to participate. Those who agreed to take part were first provided with an information sheet outlining the study and the questionnaires involved. In line with voluntary participation principles, individuals who did not provide consent or who submitted incomplete scales were excluded from the study. No financial or other incentives were offered to participants.

*Sociodemographic and Behavioral Variables:* Participants completed a structured questionnaire including demographic characteristics (age, gender, academic grade level, monthly household income, parental education level), self-reported knowledge about e-cigarettes, and smoking exposure variables (household smoking, close friends who smoke or use e-cigarettes, and frequency of use). Apart from age and gender, no personal identifying information was collected. The term 'smoking' refers exclusively to tobacco cigarette smoking, whereas the terms 'vaping' and 'e-cigarette use' are used to describe the use of electronic cigarettes. Participants were asked to self-rate their knowledge of e-cigarettes

as 'none,' 'low,' 'moderate,' or 'high.' These categories reflected participants' perceptions of their knowledge rather than objective testing; no examples, images, or definitions were provided to avoid inadvertently educating or encouraging experimentation. 'High knowledge' thus corresponds to participants' subjective assessment of being well-informed about e-cigarettes. To capture further how they learned about e-cigarettes, participants were also asked to report the source from which they first acquired this information; response options were coded as follows: friends (1), family (2), printed media (3), social media (4), online advertisements (5), and observing someone using an e-cigarette (6).

The terms 'ever smoked' and 'ever e-cigarette use' were used to describe individuals who had experimented with packaged cigarettes or e-cigarettes at any point in their lives. 'Current smoking' and 'current e-cigarette use' were defined as use within the past 30 days. In our study, academic grade level (9th, 10th, 11th, and 12th grades), monthly household income (<40,000 TRY, 40,000–60,000 TRY, >60,000 TRY; with the minimum wage in Türkiye being approximately 22,000 TRY), parental education level (illiterate, primary–middle school, high school–university), self-reported knowledge about e-cigarettes (none, low, moderate, high), and frequency of smoking or e-cigarette use (<1/week, 2–4/week, >4/week) were categorized and treated as ordinal variables.

*ECABA Scale*: As the data collection tool, the 18-item 'E-Cigarette Adolescent Beliefs and Attitudes (ECABA) Scale,' previously validated and shown to be reliable, was employed.[9] This scale is the first instrument developed explicitly in Türkiye for this age group. In the "E-cigarette Attitude and Belief Scale in Adolescents: A Validity and Reliability Study", Cronbach's alpha values were evaluated with a tiered approach: ≥ 0.90 excellent, ≥ 0.80 good, ≥0.70 acceptable, ≥ 0.60 questionable, ≥ 0.50 poor, and ≤ 0.50 unacceptable.[10] The internal consistency of the final version of the scale was assessed using Cronbach's alpha. A final exploratory factor analysis, conducted after removing semantically inconsistent items, yielded a five-factor structure comprising 18 items, all demonstrating satisfactory factor loadings (>0.50) and strong internal consistency (Cronbach's $\alpha$=0.88) (Supplementary Table S1).[9] The ECABA Scale is divided into five subscales: Physical Consequences of E-Cigarette, E-Cigarette versus Packaged Cigarettes (EC vs. PC), Establishing Identification, E-Cigarette Addiction, and Socialization.[9] Each item was rated on a 5-point Likert scale ranging from 1 ('strongly disagree') to 5 ('strongly agree'), with higher scores indicating more positive beliefs and attitudes toward e-cigarettes among adolescents. To provide readers with a better understanding of each subscale, representative items include: Physical Consequences of EC: 'E-cigarettes do not cause headache'; EC vs. PC: 'E-cigarettes do not contain nicotine, unlike classic cigarettes'; Establishing Identification: 'Seeing influencers use e-cigarettes makes me think more positively about them'; EC Addiction: 'E-cigarettes do not contain harmful or addictive substances'; and Socialization: 'There is no problem in using e-cigarettes to avoid being excluded from your circle of friends. The full 18-item ECABA Scale in English is provided as Supplementary Table S2.

### *Statistical analyses*

The minimum required sample size for the study was calculated as 384 participants using the G*Power software (version 3.1), based on a 95% confidence level and a 5% margin of error. Participants were recruited through a consecutive sampling method. To enhance reliability and account for potential exclusions, data were collected from a number of participants exceeding the calculated minimum requirement.

The demographic characteristics and descriptive attributes of the participants, as well as individual characteristic groups, were summarized using descriptive statistics such as frequency, percentage, and median with interquartile range. The normality of the

data was assessed using graphical methods and Kolmogorov–Smirnov normality tests. Depending on the data distribution, either parametric or non-parametric tests were applied. For comparisons of total and subscale scores of the ECABA Scale across different demographic and individual characteristic groups, the independent samples t-test or Mann–Whitney U test was used for two-group comparisons, and one-way ANOVA or Kruskal–Wallis test for comparisons involving more than two groups.

When a significant difference was detected in the ANOVA or Kruskal–Wallis tests, Bonferroni-corrected post hoc tests were performed to identify the source of the difference. Spearman's rank correlation coefficient (ϱ) was used to examine the relationships between ordinal variables and the total and subscale scores of the ECABA Scale. Our multivariable model was constructed by first including mandatory confounders identified a priori based on the literature and public health relevance (age, gender, and household income). In addition to these core variables, other potential covariates were evaluated using a change-in-estimate approach. Variables that altered the odds ratio (OR) of the primary independent variable by more than 10% upon inclusion—specifically, having close friends who smoke or use e-cigarettes, household smoking, and exposure to environments where e-cigarettes are used—were retained in the final multivariable model. Binary logistic regression analyses were conducted to evaluate whether ECABA scores could predict 'current' (currently using) and 'ever' (previously used) smoking and e-cigarette use. The first model (unadjusted) examined the bivariate relationship between each ECABA total and subscale score and cigarette or e-cigarette use. The analysis results were reported as OR with corresponding 95% confidence intervals (CI). All analyses were performed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). A p-value of less than 0.05 was considered statistically significant.

## Results

### *Study population*

A total of 662 adolescents were invited to participate in the study. Of these, 73 were excluded due to diagnosed chronic illnesses or lack of assent/parental consent. Among the remaining 589 adolescents, 42 were excluded due to incomplete responses on the scale. Consequently, the final study population consisted of 547 adolescents. A total of 547 adolescents with a median age of 16 (min-max: 14-18) years old participated in this scale. The female population comprised 51.4% (n = 281) of the participants. The participants' sociodemographic characteristics and environmental exposures are summarized in Table I. The most common sources where adolescents learned about e-cigarettes were, in order, friends, observing someone using them, and social media (respectively, 34.0%, 18.2%, 14.1%).

### *Prevalence of e-cigarette use and smoking*

Of all participants,18.5% of adolescents reported having ever used an e-cigarette, and 9.2% were current users within the past 30 days. For packaged cigarettes, the rates were slightly higher: 20.9% ever users and 14.4% current smokers. Table I presents the frequency of current e-cigarette use and smoking. Twelve point two percent of participants stated that they would not use e-cigarettes if they were not flavored.

### *Sociodemographic predictors of e-cigarette use*

Male adolescents were significantly more likely to have ever or currently used e-cigarettes compared with females ($p < 0.001$). E-cigarette use increased progressively with higher academic grade levels ($p = 0.023$). Adolescents reporting "high knowledge" about e-cigarettes paradoxically had higher odds of use ($p < 0.001$). Peer influence was one of the strongest predictors: those with friends who used e-cigarettes were almost twice as likely to report ever vaping ($p < 0.001$).

**Table I.** Characteristics, features, and demographic groups of the participants (N=547).

| | n (%) |
|---|---|
| Age (year), median (min-max) | 16 (14-18) |
| Female gender | 281 (51.4) |
| Academic grade | |
| 9th | 179 (32.7) |
| 10th | 85 (15.5) |
| 11th | 140 (25.6) |
| 12th | 143 (26.1) |
| Monthly income | |
| <40000 TRY | 317 (58.5) |
| 40-60000 TRY | 109 (20.1) |
| >60.000 TRY | 116 (21.4) |
| Mother educational grade | |
| Illiterate | 28 (5.1) |
| Primary-secondary school | 217 (39.9) |
| High school-university | 299 (55.0) |
| Father educational level | |
| Illiterate | 10 (1.8) |
| Primary-secondary school | 199 (36.6) |
| High school-university | 334 (61.5) |
| Peer and Household Exposures | |
| Close friend, EC using | 245 (44.7) |
| Close friend, smoking | 260 (47.5) |
| Ever exposure to EC use | 320 (58.7) |
| Household exposure to tobacco smoke | 370 (67.6) |
| EC self-rated knowledge level | |
| None | 90 (16.5) |
| Low | 232 (42.4) |
| Moderate | 174 (31.8) |
| High | 51 (9.3) |
| Number of smokers in the household | |
| Single smoker | 200 (54.1) |
| Multiple smokers | 170 (45.9) |
| E-cigarette use | |
| Ever used EC | 101 (18.5) |
| Current EC using (past 30 days) | 50 (9.2) |
| Frequency of EC use | |
| Once per week | 30 (60) |
| 2–4 times per week | 8 (16) |
| >4 times per week | 12 (24) |
| Tobacco cigarette use | |
| Ever smoked | 114 (20.9) |
| Current smoking (past 30 days) | 79 (14.4) |
| Frequency of smoking | |
| Once per week | 22 (27.8) |
| 2–4 times per week | 6 (7.6) |
| >4 times per week | 51 (64.6) |

EC: electronic cigarette, IQR: interquartile range, TRY: Turkish Liras

### *Association between ECABA Scale scores and e-cigarette use/smoking*

Table II presents a comparison of the ECABA Scale total and subscale scores according to gender, grade level, and smoking and e-cigarette use status. Male participants were more likely to believe that e-cigarettes positively contribute to socialization ($p< 0.001$). Participants whose close friends smoked cigarettes or used e-cigarettes demonstrated statistically significantly more positive beliefs and attitudes toward e-cigarettes across the total ECABA Scale score and all subscales (Table II). Individuals who had ever smoked or used e-cigarettes demonstrated statistically significantly more positive beliefs and attitudes toward e-cigarettes. Additionally, current e-cigarette users showed statistically significant positive responses specifically on the Physical Consequences of E-Cigarette subscale ($p=0.022$).

Table III presents correlations of ordinal variables with total and subscale scores of ECABA Scale. As academic grade level increased, participants demonstrated statistically significantly more positive responses to the Physical Consequences of E-Cigarette subscale items ($p=0.023$). As paternal education level increased, statistically significant increases in positive responses were observed in the scores (Table III). Additionally, we found that higher adolescent self-reported knowledge about e-cigarettes was associated with statistically significant more positive beliefs and attitudes across all scores (respectively, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p=0.029$,

p=0.003). As the frequency of e-cigarette use increased, statistically significant positive beliefs and attitudes were observed in the total score, three of the subscale scores (respectively, p=0.013, p=0.006, p=0.012, p=0.019) (Table III).

Crude and multivariable logistic regression analyses were performed to determine whether increases in the total and subscale scores constitute independent risk factors for e-cigarette and tobacco cigarette smoking. In

**Table II.** Comparison of total and subscale scores of ECABA Scale across characteristics, and demographic groups.

| | | Total score | Physical Consequences of EC | EC vs. PC | Establishing Identification | EC Addiction | Socialization |
|---|---|---|---|---|---|---|---|
| Gender | Male | 33 (18.8) | 10 (8) | 9.5 (9) | 3 (3) | 5 (4) | 2 (2) |
| | Female | 31 (17) | 8 (8) | 9 (8) | 3 (3) | 5 (4) | 2 (1) |
| | p value | 0.104 | 0.071 | 0.432 | 0.526 | 0.597 | <0.001 |
| Academic grade | 9th | 32 (18) | 7 (8) | 9 (9) | 4 (3) | 5 (4) | 32 (18) |
| | 10th | 30 (21) | 7 (8) | 9 (8) | 3 (3) | 4 (4) | 30 (21) |
| | 11th | 31 (15.3) | 9 (7) | 9 (8) | 3 (2.2) | 4.5 (4) | 31 (15.3) |
| | 12th | 34 (18) | 10 (8.5) | 11 (9.5) | 3 (3) | 5 (5) | 34 (18) |
| | p value | 0.260 | 0.098 | 0.195 | 0.765 | 0.051 | 0.260 |
| Household exposure to smoking | No | 32 (18) | 8 (8) | 10 (9) | 4 (3) | 5 (4) | 2 (2) |
| | Yes | 31.5 (17) | 9 (8) | 9 (8) | 3 (3) | 5 (4) | 2 (2) |
| | p value | 0.743 | 0.247 | 0.312 | 0.349 | 0.967 | 0.180 |
| Number of smokers in the household | Single | 32 (16) | 9 (8) | 9.5 (7) | 3 (3) | 5 (4) | 2 (2) |
| | Multiple | 31 (20.3) | 10 (8) | 9 (9) | 3 (3) | 5 (4) | 2 (1.2) |
| | p value | 0.611 | 0.902 | 0.385 | 0.402 | 0.518 | 0.077 |
| Close friend, smoking | No | 28 (17) | 7 (7) | 9 (8) | 3 (3) | 4 (4) | 2 (1) |
| | Yes | 34 (17.3) | 10 (8) | 11 (7) | 4 (3) | 5 (4.2) | 2 (2) |
| | p value | <0.001 | <0.001 | <0.001 | 0.022 | <0.001 | <0.001 |
| Close friend, EC using | No | 28 (17) | 7 (7) | 8 (8) | 3 (2) | 5 (4) | 2 (1) |
| | Yes | 36 (18) | 11 (8) | 11 (8) | 4 (3) | 5 (4) | 2 (2) |
| | p value | <0.001 | <0.001 | <0.001 | <0.002 | <0.009 | <0.001 |
| Ever used EC | No | 29 (16) | 8 (7) | 9 (8) | 3 (2) | 5 (4) | 2 (1) |
| | Yes | 38 (18) | 12 (8) | 12 (7) | 4 (4) | 6 (5) | 3 (3) |
| | p value | <0.001 | <0.001 | <0.001 | <0.003 | <0.001 | <0.001 |
| Current EC using (past 30 days) | No | 38 (17.5) | 11 (8.5) | 11 (8.5) | 5 (4) | 6 (4) | 3 (2.5) |
| | Yes | 42 (18.8) | 13.5 (6.7) | 13 (5.7) | 3 (3) | 7 (4.7) | 3 (4) |
| | p value | 0.104 | 0.022 | 0.082 | 0.142 | 0.163 | 0.507 |
| Ever smoking | No | 29.5 (17) | 7 (9) | 9 (9) | 3 (3) | 5 (4) | 2 (2) |
| | Yes | 38 (17) | 11.5 (8) | 11.5 (7) | 5 (4) | 6 (4) | 3 (2.7) |
| | p value | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |
| Current smoking (past 30 days) | No | 39 (13) | 12 (8) | 11 (6.5) | 5 (3.5) | 6 (4) | 3 (2.5) |
| | Yes | 37 (18) | 11 (8) | 12 (7) | 5 (5) | 6 (4) | 3 (2.5) |
| | p value | 0.770 | 0.628 | 0.746 | 0.935 | 0.764 | 0.868 |

Scores presented as median (interquartile range).
EC: electronic cigarette. ECABA: E-Cigarette Attitudes and Beliefs in Adolescents, PC: packaged cigarette.

**Table III.** Correlations of ordinal variables with total and subscale scores.

| | Total score | Physical Consequences of EC | EC vs. PC | Establishing Identification | EC Addiction | Socialization |
|---|---|---|---|---|---|---|
| Academic grade | 0.046 (p=0.282) | 0.097 (p=0.023) | 0.037 (p=0.389) | -0.037 (p=0.389) | 0.046 (p=0.278) | -0.014 (p=0.748) |
| Monthly income | 0.026 (p=0.545) | 0.003 (p=0.940) | 0.082 (p=0.057) | -0.019 (p=0.653) | -0.064 (p=0.139) | 0.051 (p=0.232) |
| Mother educational level | 0.032 (p=0.461) | 0.026 (p=0.544) | 0.050 (p=0.247) | -0.019 (p=0.666) | -0.014 (p=0.749) | 0.048 (p=0.259) |
| Father educational level | 0.119 (p=0.005) | 0.096 (p=0.026) | 0.150 (p=<0.001) | 0.043 (p=0.316) | 0.010 (p=0.811) | 0.088 (p=0.040) |
| EC knowledge level | 0.196 (p=<0.001) | 0.141 (p=<0.001) | 0.195 (p=<0.001) | 0.103 (p=<0.001) | 0.093 (p=0.029) | 0.129 (p=0.003) |
| Frequency of EC using | 0.349 (p=0.013) | 0.386 (p=0.006) | 0.351 (p=0.012) | 0.144 (p=0.318) | 0.126 (p=0.383) | 0.330 (p=0.019) |
| Frequency of smoking | -0.011 (p=0.923) | 0.086 (p=0.450) | 0.047 (p=0.680) | -0.160 (p=0.160) | -0.018 (p=0.876) | -0.043 (p=0.705) |

Values represent ϱ (Spearman's rank-order correlation coefficient)
EC: electronic cigarette. PC: packaged cigarette.

the crude model, each one-point increase in the ECABA Scale total score was associated with an approximately 6.6% increase in the likelihood of ever using an e-cigarette ($p < 0.001$) (OR = 1.066, 95% CI: 1.045-1.087) (Table IV). In the multivariate analysis, independent of demographic and social factors, the ECABA total score remained as a significant predictor, where each one-point increase was associated with a 4.6% higher likelihood of ever using e-cigarettes (OR = 1.046, $p = 0.003$), a 5.4% higher likelihood of ever smoking (OR = 1.054, $p < 0.001$), a 4.8% higher likelihood of being a current e-cigarette user (OR = 1.048, $p = 0.007$), and a 5.5% higher likelihood of being a current smoker (OR = 1.055, $p < 0.001$).

Among the subscales, the Physical Consequences subscale independently predicted both e-cigarette use and smoking (ever e-cigarette use: OR = 1.090, $p = 0.025$; current e-cigarette use: OR = 1.127, $p = 0.037$; ever smoking: OR = 1.113, $p = 0.002$; current smoking: OR = 1.130, $p = 0.002$). The Establishing Identification subscale was significantly associated with current e-cigarette use and smoking behavior (current e-cigarette use; OR = 1.363, $p = 0.035$, ever smoked: OR = 1.155, $p = 0.015$; current smoking: OR = 1.140, $p = 0.043$).

Fig. 1. demonstrates the logistic regression–based predicted probabilities of e-cigarette use and smoking across the range of ECABA total and subscale scores. As shown, higher ECABA Scale scores are significantly associated with both ever and current smoking. Total scores and the 'Physical Consequences of E-Cigarette' and 'Establishing Identification' subscales independently increase the likelihood of smoking, whereas other subscales show no significant associations.

## Discussion

In this study, we evaluated e-cigarette awareness, prevalence, and related beliefs among a large cohort of adolescents using the ECABA Scale, a validated national instrument explicitly designed for this population. Despite strict national regulations, we found that nearly one in five adolescents had experimented with e-cigarettes and almost one in ten were current users, underscoring the growing public health impact of vaping among youth in Türkiye. Beyond documenting prevalence, our findings

**Table IV.** Univariate and multivariable logistic regression analysis for using e-cigarette / tobacco cigarette smoking.

| | Ever Used EC | | | | Current EC Use | | | |
|---|---|---|---|---|---|---|---|---|
| ECABA Total or Subscale Scores | Univariate OR (95% CI) | p | Multivariable OR (95% CI) | p | Univariate OR (95% CI) | p | Multivariable OR (95% CI) | p |
| ECABA Total Score | 1.066 (1.045-1.087) | <0.001 | 1.046 (1.015-1.077) | 0.003 | 1.073 (1.047-1.100) | <0.001 | 1.048 (1.013-1.085) | 0.007 |
| Physical Consequences | 1.113 (1.046-1.185) | <0.001 | 1.090 (1.010-1.176) | 0.025 | 1.164 (1.070-1.267) | <0.001 | 1.127 (1.007-1.261) | 0.037 |
| EC vs. PC | 1.040 (0.978-1.106) | 0.206 | | | 1.040 (0.956-1.132) | 0.353 | | |
| Establishing Identification | 0.993 (0.891-1.107) | 0.903 | | | 0.845 (0.722-0.988) | 0.036 | 0.702 (0.568-0.866) | <0.001 |
| EC Addiction | 1.079 (0.980-1.188) | 0.118 | | | 1.097 (0.966-1.246) | 0.153 | | |
| Socialization | 1.089 (0.932-1.273) | 0.282 | | | 1.223 (1.001-1.495) | 0.049 | 1.363 (1.022-1.819) | 0.035 |
| | Ever Smoked | | | | Current Smoking | | | |
| ECABA Total or Subscale Scores | Univariate OR (95% CI) | p | Multivariable OR (95% CI) | p | Univariate OR (95% CI) | p | Multivariable OR (95% CI) | p |
| ECABA Total Score | 1.057 (1.038-1.077) | <0.001 | 1.054 (1.032-1.077) | <0.001 | 1.054 (1.033-1.075) | <0.001 | 1.055 (1.029-1.082) | <0.001 |
| Physical Consequences | 1.136 (1.068-1.207) | <0.001 | 1.113 (1.041-1.190) | 0.002 | 1.130 (1.060-1.210) | <0.001 | 0.130 | 0.002 |
| EC vs. PC | 0.975 (0.917-1.036) | 0.419 | | | | | | |
| Establishing Identification | 1.128 (1.022-1.246) | 0.017 | 1.155 (1.029-1.296) | 0.015 | 1.130 (1.010-1.260) | 0.029 | 0.140 | 0.043 |
| EC Addiction | 1.080 (0.984-1.187) | 0.104 | | | 1.054 (0.947-1.173) | | | |
| Socialization | 0.989 (0.848-1.153) | 0.892 | | | 1.022 (0.862-1.212) | | | |

CI: confidence interval, EC: electronic cigarette, ECABA: E-Cigarette Attitudes and Beliefs in Adolescents, OR: odds ratio, PC: packaged cigarette.

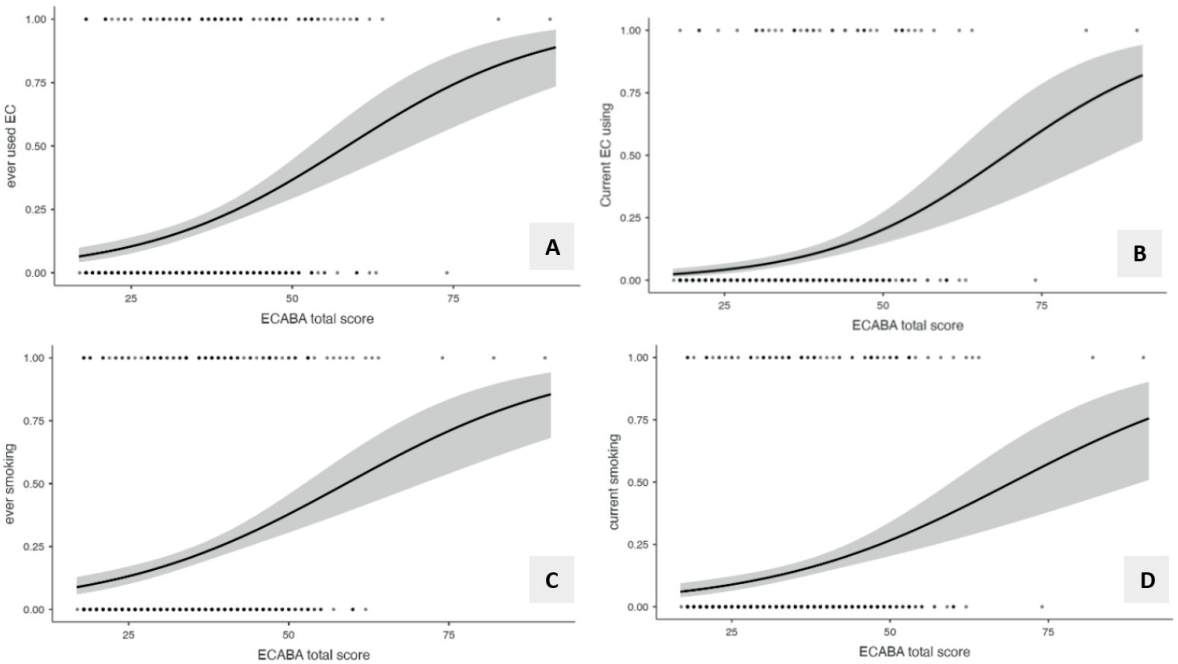



**Fig. 1.** Logistic regression predicted probability curves for packaged cigarette and e-cigarette use based on the ECABA total and subscale scores. (A) ECABA total score and ever e-cigarette use. (B) ECABA total score and current e-cigarette use. (C) ECABA subscales and ever packaged cigarette use. (D) ECABA subscales and current packaged cigarette use.

The Y-axis represents the probability of use (0 = no, 1 = yes), and the X-axis represents the ECABA total score. The black line indicates the model's predicted probability, and the shaded area represents the 95% confidence interval. Dots represent individual participant responses. ECABA: E-Cigarette Attitudes and Beliefs in Adolescents

highlight that adolescents' perceptions—particularly beliefs related to physical harmlessness and social acceptability—play a central role in shaping e-cigarette behavior. Moreover, higher ECABA Scale scores were identified as predictors of both ever trying e-cigarettes and current e-cigarette use, underscoring the scale's ability to capture attitudinal patterns that directly translate into behavioral outcomes.

When examining the sources through which participants learned about e-cigarettes, they primarily reported gaining information from their peer group, followed by observing others using e-cigarettes, and, thirdly, through social media. These findings highlight the significant influence of the social environment and exposure on adolescents' perceptions and attitudes toward e-cigarettes.[11] Adolescents who had been in settings where e-cigarettes were used, or who had close friends who used them, consistently demonstrated higher ECABA scores, reflecting more favorable beliefs and attitudes. This pattern aligns with previous research showing that peer use and social exposure are among the strongest predictors of adolescent e-cigarette initiation.[12,13] Dual users—those who used both cigarettes and e-cigarettes—also exhibited markedly more favorable beliefs, a finding consistent with studies such as Hanafin et al. in Ireland, where smokers perceived e-cigarettes as a harm-reducing alternative.[14] This suggests that product use may actively shape perceptions, reinforcing attitudes that portray e-cigarettes as less harmful. Similar findings from South Africa further indicate that adolescent e-cigarette users tend to view the product as harmless or 'safer' than packaged cigarettes.[15] Collectively, these results highlight the powerful role of peer modeling, observational learning,

and behavioral reinforcement in forming adolescents' perceptions of e-cigarettes.

Another notable pattern was the association between higher academic grade levels and more positive beliefs regarding the physical consequences of e-cigarettes. Several factors may explain this pattern. Previous studies report that with increasing age and grade level, students tend to perceive the potential harms of e-cigarettes as less significant and normalize their use within this group.[14-16] For instance, older high school students are more frequently exposed to online content and peer environments that portray e-cigarettes as less harmful. This exposure—coupled with increased autonomy, more unsupervised time with peers, and greater access to social media platforms where vaping-related promotional messages, influencer content, and harm-minimizing narratives are widespread—may gradually reduce perceived physical risks and contribute to higher subscale scores. Moreover, as adolescents grow older, the initial drivers of experimentation, such as curiosity or social pressure, may evolve into more personal motivations, including enjoyment, stress relief, and behavioral or physical dependence, further reinforcing positive beliefs and attitudes toward e-cigarettes. Taken together, these mechanisms are consistent with previous literature indicating that older adolescents are more likely to view e-cigarettes as less harmful and to normalize their use.

In our study, no significant relationship was found between maternal education level and positive beliefs or attitudes toward e-cigarettes. However, higher paternal education was associated with increased total ECABA Scale scores and subscale scores, particularly those reflecting beliefs related to physical health and comparisons with tobacco cigarette smoking. Previous findings on this topic are inconsistent: some studies report associations with maternal education,18 while others identify paternal education as the relevant factor.[16,17] These discrepancies suggest that sociocultural context may shape how parental education influences adolescents' perceptions of e-cigarettes.

The association between self-rated high knowledge and positive ECABA Scale scores warrants careful interpretation. We found that adolescents who reported having greater knowledge about e-cigarettes scored higher on the scale, indicating more positive beliefs and attitudes. It is important to note that the 'high knowledge' reported by adolescents likely reflects perceived knowledge, shaped by exposure to promotional or misleading online content, rather than an evidence-based understanding. Therefore, higher self-rated knowledge may actually indicate greater susceptibility to industry-driven narratives portraying e-cigarettes as harmless, which in turn contributes to more positive beliefs and attitudes. This likely reflects the widespread presence of misleading promotional content in industry-sponsored information.

In our study, increases in total ECABA Scale scores were identified as an independent predictor of a higher likelihood of ever trying e-cigarettes. The literature supports this finding; for example, one study reported that each one-unit increase in students' positive attitude scores toward e-cigarettes was associated with a 15% increase in the likelihood of lifetime e-cigarette use.[18]

E-cigarette aerosols contain harmful chemicals such as nicotine, flavoring agents, tobacco-specific nitrosamines, heavy metals (nickel, tin, lead), volatile organic compounds, and formaldehyde, which can cause both acute and chronic physical damage.[19] We found a significant positive association between participants' belief that e-cigarettes are not physically harmful and having ever used e-cigarettes, as measured by the 'Physical Consequences of E-Cigarette' subscale. The literature also indicates that young e-cigarette users are twice as likely as non-users to believe that e-cigarettes are harmless.[1,20,21] Such misperceptions may serve as cognitive justifications that facilitate initiation, enabling adolescents to minimize or dismiss potential

physical risks and align their beliefs with their behavior. These attitudes may also stem from widespread harm-minimizing messages in social media and marketing, which frame e-cigarettes as 'safer' alternatives and contribute to reduced risk perception among youth.

In our study, each one-unit increase in the 'Physical Consequences of E-Cigarette' subscale score was associated with an increased risk of current e-cigarette use. Consistent with prior research, this finding indicates that adolescents who minimize the physical harms of e-cigarettes are at greater risk of ongoing use.[20,22] We also observed that higher scores on the 'Socialization' subscale predicted current use, indicating that adolescents who view e-cigarettes as socially facilitating may be more inclined to use them. This aligns with those of Bernat et al., who reported that 15% of high school students aged 14–17 perceived e-cigarette users as having more friends and being cooler—a proportion that increased to 25% among current e-cigarette users.[23] These findings resonate with prior literature showing that risk minimization and social acceptance are key cognitive distortions among adolescent users. What is new here is that these associations were observed using a culturally adapted and validated scale, offering one of the first real-world applications of the ECABA Scale as a behavioral screening tool.

Our study demonstrated that each one-point increase in the total ECABA Scale score was associated with higher odds of both ever smoking and current smoking, suggesting that positive beliefs and attitudes toward e-cigarettes may generalize to combustible tobacco use behaviors. This finding aligns with previous research indicating that favorable perceptions of alternative nicotine products, such as e-cigarettes, can reduce risk perceptions related to tobacco cigarette smoking and potentially facilitate smoking initiation, particularly among adolescents.[24] Such cross-product normalization highlights that misconceptions about e-cigarettes may have broader implications beyond vaping itself, underscoring the need for prevention strategies that address risk perception across multiple forms of nicotine use.

In our study, scores on the 'Physical Consequences of E-Cigarette' and 'Establishing Identification' subscales were significantly and positively associated with the likelihood of having ever smoked and being a current smoker. This suggests that increased beliefs in the low physical harm of e-cigarettes and greater identification with e-cigarette use are linked to higher probabilities of ever smoking and current smoking. The literature indicates that positive attitudes toward e-cigarettes may contribute to decreased risk perception of nicotine products overall among adolescents, thereby facilitating transitions to other tobacco products such as cigarettes.[24] In this context, positive beliefs toward e-cigarette use may not be confined to vaping behavior alone. Still, they could also play a role in the transition to more harmful tobacco products such as cigarettes, potentially increasing the risk of dual use during this process.

This study has several limitations. First, the cross-sectional design prevents establishing causal relationships among beliefs, attitudes, and e-cigarette use. Second, data were self-reported, which may introduce recall bias or social desirability bias, potentially leading to under- or over-reporting of e-cigarette use and related behaviors. Third, the study was conducted in a single region and may not be fully generalizable to all adolescents in Türkiye. Nonetheless, the sample size was robust, and the findings provide essential baseline data for future population-based studies. These limitations should be considered when interpreting the magnitude and direction of the associations observed. The strength of the study is that this is the first study in Türkiye to evaluate adolescent e-cigarette use using a validated national-scale instrument. It has a large, well-characterized sample allowing meaningful subgroup comparisons. Unlike many prevalence studies, this research simultaneously examined behavioral prevalence and psychosocial

predictors, linking beliefs directly to actual use behaviors.

### *Conclusion*

The normalization of e-cigarette use among adolescents, even in a legally restricted setting, exposes a silent failure in tobacco control. Total ECABA Scale scores independently predicted current e-cigarette use, supporting its value in identifying at-risk youth. Beliefs in physical harmlessness and social acceptance are robust psychological gateways to nicotine initiation. The ECABA Scale proved useful in identifying these misperceptions and could serve as an early-warning tool within school or clinic settings. Future multicenter and population-based studies are urgently needed to expand these findings and guide national prevention policies. Unless preventive action keeps pace with the industry's adaptation, we risk losing a generation not to ignorance, but to illusion—the illusion that vaping is different.

## Supplementary materials

Supplementary materials for this article are available online at https://doi.org/10.24953/turkjpediatr.2025.7284

## Declaration of generative AI and AI-assisted technologies in the writing process

We acknowledge that we employed ChatGPT 4 to assist us in refining the clarity of our writing while developing the draft of this original article. We always maintained continuous human oversight (editing and revising) and verified the AI-generated output.

## Ethical approval

The study was approved by Istanbul Medipol University Non-Interventional Clinical Research Ethics Committee (date: May 22, 2025, number: 645).

## Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: SCO, GB, ZRO, DM; data collection: SCO, GB, DM, EG, YÖ; analysis and interpretation of results: SCO, EG, YÖ, SG; draft manuscript preparation: SCO, GB, ZRO, SG. All authors reviewed the results and approved the final version of the manuscript.

## Source of funding

The authors declare the study received no funding.

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES

1. Greenhill R, Dawkins L, Notley C, Finn MD, Turner JJD. Adolescent awareness and use of electronic cigarettes: a review of emerging trends and findings. J Adolesc Health 2016; 59: 612-619. https://doi.org/10.1016/j.jadohealth.2016.08.005
2. Doherty J, Davison J, McLaughlin M, et al. Prevalence, knowledge and factors associated with e-cigarette use among parents of secondary school children. Public Health Pract (Oxf) 2022; 4: 100334. https://doi.org/10.1016/j.puhip.2022.100334
3. U.S. Department of Health and Human Services. E-Cigarette use among youth and young adults - a report of the surgeon general. 2016. Available at: https://escholarship.org/content/qt9p15c3w3/qt9p15c3w3.pdf (Accessed on July 19, 2025).
4. Ko K, Ting Wai Chu J, Bullen C. A Scoping review of vaping among the Asian adolescent population. Asia Pac J Public Health 2024; 36: 664-675. https://doi.org/10.1177/10105395241275226
5. Kurtuluş Ş, Can R. Use of e-cigarettes and tobacco products among youth in Turkey. Eurasian J Med 2022;54:127-132. https://doi.org/10.5152/eurasianjmed.2022.20168

6. World Health Organization (WHO). Urgent action needed to protect children and prevent the uptake of e-cigarettes. 2023. Available at: https://www.who.int/news/item/14-12-2023-urgent-action-needed-to-protect-children-and-prevent-the-uptake-of-e-cigarettes

7. Chen X, Yu B, Wang Y. Initiation of electronic cigarette use by age among youth in the U.S. Am J Prev Med 2017; 53: 396-399. https://doi.org/10.1016/j.amepre.2017.02.011

8. Gentzke AS, Wang TW, Cornelius M, et al. Tobacco product use and associated factors among middle and high school students - national youth tobacco survey, United States, 2021. MMWR Surveill Summ 2022; 71: 1-29. https://doi.org/10.15585/mmwr.ss7105a1

9. Can Oksay S, Alpar G, Bilgin G, Tortop DM, Onay ZR, Gürler E. E-cigarette attitude and belief scale in adolescents: a validity and reliability study. Turk Thorac J 2025; 26: 290-297. https://doi.org/10.4274/ThoracResPract.2025.2025-5-5

10. Mallery P, George D. SPSS for Windows step by step. United States: Allyn & Bacon, Inc; 2000. Available at: https://dl.acm.org/doi/abs/10.5555/557542 (Accessed on December 18, 2024).

11. Ajzen I, Fishbein M. Understanding attitudes and predictiing social behavior. Englewood Cliffs, NJ: Prentice-Hall; 1980.

12. Valente TW, Piombo SE, Edwards KM, Waterman EA, Banyard VL. Social network influences on adolescent e-cigarette use. Subst Use Misuse 2023; 58: 780-786. https://doi.org/10.1080/10826084.2023.2188429

13. Dilektasli AG, Guclu OA, Ozpehlivan A, et al. Electronic cigarette use and consumption patterns in medical university students. Front Public Health 2024; 12: 1403737. https://doi.org/10.3389/fpubh.2024.1403737

14. Hanafin J, Sunday S, Clancy L. Sociodemographic, personal, peer, and familial predictors of e-cigarette ever use in ESPAD Ireland: a forward stepwise logistic regression model. Tob Induc Dis 2022; 20: 12. https://doi.org/10.18332/tid/144234

15. van Zyl-Smit RN, Filby S, Soin G, Hoare J, van den Bosch A, Kurten S. Electronic cigarette usage amongst high school students in South Africa: a mixed methods approach. EClinicalMedicine 2024; 78: 102970. https://doi.org/10.1016/j.eclinm.2024.102970

16. Kurdyś-Bykowska P, Kośmider L, Bykowski W, Konwant D, Stencel-Gabriel K. Epidemiology of traditional cigarette and e-cigarette use among adolescents in Poland: analysis of sociodemographic risk factors. Int J Environ Res Public Health 2024; 21: 1493. https://doi.org/10.3390/ijerph21111493

17. AlMuhaissen S, Mohammad H, Dabobash A, Nada MQ, Suleiman ZM. Prevalence, knowledge, and attitudes among health professions students toward the use of electronic cigarettes. Healthcare (Basel) 2022; 10: 2420. https://doi.org/10.3390/healthcare10122420

18. Vichayanrat T, Chidchuangchai W, Karawekpanyawong R, Phienudomkitlert K, Chongcharoenjai N, Fungkiat N. E-cigarette use, perceived risks, attitudes, opinions of e-cigarette policies, and associated factors among Thai university students. Tob Induc Dis 2024; 22: 10.18332/tid/186536. https://doi.org/10.18332/tid/186536

19. Bush A, Lintowska A, Mazur A, et al. E-cigarettes as a growing threat for children and adolescents: position statement from the European Academy of Paediatrics. Front Pediatr 2021; 9: 698613. https://doi.org/10.3389/fped.2021.698613

20. Aly AS, Mamikutty R, Marhazlinda J. Association between harmful and addictive perceptions of e-cigarettes and e-cigarette use among adolescents and youth-a systematic review and meta-analysis. Children (Basel) 2022; 9: 1678. https://doi.org/10.3390/children9111678

21. Cornelia M, Alexandra C, Ioana-Cristina P. It´s harmless! The reasons behind vaping behavior among youth: risks, benefits, and associations with psychological distress. Psihologija. Forthcoming 2025. https://doi.org/10.2298/PSI230824013M

22. Bluestein MA, Harrell MB, Hébert ET, et al. Associations between perceptions of e-cigarette harmfulness and addictiveness and the age of e-cigarette initiation among the Population Assessment of Tobacco and Health (PATH) youth. Tob Use Insights 2022; 15: 1179173X221133645. https://doi.org/10.1177/1179173X221133645

23. Bernat D, Gasquet N, Wilson KO, Porter L, Choi K. Electronic cigarette harm and benefit perceptions and use among youth. Am J Prev Med 2018; 55: 361-367. https://doi.org/10.1016/j.amepre.2018.04.043

24. Wills TA, Knight R, Sargent JD, Gibbons FX, Pagano I, Williams RJ. Longitudinal study of e-cigarette use and onset of cigarette smoking among high school students in Hawaii. Tob Control 2017; 26: 34-39. https://doi.org/10.1136/tobaccocontrol-2015-052705

The Turkish Journal of Pediatrics 2025; 67: 789-797
https://doi.org/10.24953/turkjpediatr.2025.6453

**Original Article**

# Plasma cotinine levels and sleep disturbances in children exposed to environmental tobacco smoke

Gizem Özcan[1], Fazılcan Zirek[1], Nisa Eda Çullas İlarslan[2], Fatih Günay[2], Filiz Bakar Ateş[3], Özge Yılmaz[4], Nazan Çobanoğlu[1]

*[1]Division of Pediatric Pulmonology, Department of Pediatrics, Faculty of Medicine, Ankara University, Ankara, Türkiye; [2]Department of Pediatrics, Faculty of Medicine, Ankara University, Ankara, Türkiye; [3]Department of Biochemistry, Faculty of Pharmacy, Ankara University, Ankara, Türkiye; [4]Division of Pediatric Pulmonology, Allergy and Immunology, Department of Pediatrics, Faculty of Medicine, Celal Bayar University, Manisa, Türkiye.*

## ABSTRACT

**Background.** The relationship between sleep disturbances and exposure to environmental tobacco smoke (ETS) in children is a growing health concern. This study aimed to evaluate the association between ETS exposure and sleep disorders in healthy children, and to determine whether there is a difference in this relationship between secondhand smoke (SHS) and thirdhand smoke (THS) exposure.

**Methods.** Healthy children aged 4–12 years who presented to the pediatric outpatient department were consecutively enrolled in this cross-sectional study. Plasma cotinine levels were measured to validate the exposure. The Children's Sleep Habits Questionnaire was used to assess sleep disorders in all children.

**Results.** Of the 203 children we evaluated, with a median (Q1-Q3) age of 8.3 (6–10) years, 99 (49.8%) were female. Children exposed to ETS had significantly more sleep disturbances than children who were not exposed to ETS (p = 0.042). However, there was no significant difference in the plasma cotinine levels (p = 0.239) or the prevalence of sleep disorders (p = 0.648) between children exposed to SHS and those exposed to THS.

**Conclusions.** Exposure to both SHS and THS is associated with an increase in plasma cotinine and a higher prevalence of sleep disorders in children. These findings highlight the importance of reducing children's exposure to all forms of ETS to promote healthy sleep and overall well-being.

**Key words:** children, cotinine, environmental smoke exposurei sleep, thirdhand smoking.

Environmental tobacco smoke (ETS) consists of the side stream and exhaled mainstream smoke from cigarettes and pipes. ETS exposure includes both secondhand and thirdhand smoke: secondhand smoke (SHS) refers to smoke passively inhaled by non-smokers, whereas thirdhand smoke (THS) refers to the residual contamination that persists after SHS has dispersed.[1] The World Health Organization estimates that 40% of children worldwide are exposed to ETS.[2] In a study conducted with adolescents in Hong Kong, 23.2% were exposed to SHS at home. When THS was also considered, this proportion rose to 63.3%. Exposure to SHS and THS within the home was linearly associated with respiratory symptoms.[3]

✉ Gizem Özcan ▪ gizemaltay87@hotmail.com

Received 18th Jun 2025, revised 31st Jul 2025, 8th Sep 2025, 3rd Oct 2025, 26th Nov 2025, accepted 11th Dec 2025.

Adequate sleep quality and duration are critical for children's health and development. Insufficient sleep can lead to poor academic performance, behavioral issues, and mental health problems.[4] Identifying modifiable factors that compromise sleep is therefore essential. Sleep problems associated with active smoking and the use of smokeless tobacco include reduced sleep duration, difficulty initiating and maintaining sleep, and disruptions in sleep architecture.[5] Polysomnography studies—evaluating acute nicotine patches in healthy, non-smoking adults via transdermal nicotine patches—provide strong evidence of nicotine's negative effects on sleep.[6,7]

Two main hypotheses have been proposed regarding the causal relationship between nicotine and sleep disturbances. The first suggests that nicotine disrupts the regulation of the sleep-wake cycle and that a decrease in blood nicotine levels during sleep may lead to nocturnal cravings and withdrawal symptoms, thereby disrupting sleep. In addition, irritation of the upper airway caused by tobacco smoke has been suggested to contribute to overall sleep disturbances.[8]

Studies conducted in adult populations[9,10] have demonstrated a positive association between SHS exposure and poor sleep. However, research on the relationship between ETS exposure and sleep problems in children remains limited. Although case-control studies have reported a statistical association between passive cigarette smoke exposure and sleep-disordered breathing in children, only a few have verified smoking exposure using a biomarker.[11,12] To the best of our knowledge, no study has directly compared the effects of SHS and THS on sleep disorders in children.

This study therefore aimed to evaluate the association between ETS exposure and sleep disorders in healthy children, to determine whether this relationship differs between SHS and THS exposure, and to objectively quantify exposure by measuring plasma cotinine levels.

## Materials and Methods

### *Ethics approval*

This study was approved by the local ethics committee (Ankara University Ethics Board, Approval No. 10-805-19), in addition to the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. Written informed consent was obtained from the parents, and children over eight years of age.

### *Patient selection*

Healthy children aged 4–12 years who were not taking any medications that affect sleep and who presented to the pediatric outpatient department of our hospital between June 2019 and March 2020 were consecutively enrolled in this cross-sectional study. Based on a previous report[13], the required sample size was calculated as 104 participants per group (exposed to ETS and not exposed to ETS), for a total of 208 participants, assuming a standard deviation of 12, a hypothesized effect size of 5, and a study power of 0.85.

Children with a diagnosis of allergic rhinitis, asthma, attention deficit hyperactivity disorder, autism spectrum disorders, cystic fibrosis, dysmorphic disorders, infections, non-cystic fibrosis bronchiectasis, obesity, adenotonsillary hypertrophy, sleep-disordered breathing diagnosed with polysomnography, type 1 diabetes mellitus, or other chronic diseases were excluded. Children who declared active smoking were also excluded from the study. Parents of all eligible children received detailed information about the study, and those who provided informed consent were included.

### *Questionnaires*

Two pediatricians conducted face-to-face questionnaire surveys. The first questionnaire collected information on parents' smoking habits, including the current smoking status

of the accompanying parent, the other parent, and any third parties (e.g., babysitters). Based on the parents' responses, the pediatricians classified children as having either "parent-reported ETS exposure" or "no parent-reported ETS exposure". Exposure to SHS and THS was differentiated according to whether smoking occurred in the presence of the child. Children living in households where smoking occurred indoors were classified as having SHS exposure, whereas those in households with no indoor smoking but with individuals who smoked outside and subsequently entered the home were classified as having THS exposure. In addition, parents were asked about their child's daily caffeine consumption with a yes/no question. For all participants, sex, current age, and body mass index (BMI), along with BMI z scores, were recorded.

The same evaluators administered an abbreviated, validated version of the Children's Sleep Habits Questionnaire (CSHQ) to assess sleep patterns and identify sleep problems.[13] CHSQ has been validated in our language and its validity and reliability has been tested.[14] The CSHQ includes 33 items relating to several key sleep domains that encompass major presenting clinical sleep complaints: bedtime behavior and sleep onset, sleep duration, anxiety around sleep, behavior occurring during sleep and night waking, sleep-disordered breathing, parasomnias, and morning waking/ daytime sleepiness. The CSHQ also inquires about the child's total daily sleep hours with an open-ended question. Parents were asked to recall their children's sleep behaviors over a typical week. Items were rated on a three-point scale as follows: "usually" if the sleep behavior occurred five to seven times per week, "sometimes" for two to four times per week, or "rarely" for none to once a week. The CSHQ yields a total sleep disturbance score (range, 33–99) and scores for the following eight scales: bedtime resistance (score range, 6–24), sleep onset delay (score range, 1–3), sleep duration (score range, 3–9), sleep anxiety (score range, 4–12), night waking (score range, 3–9), parasomnias (score range, 7–21), sleep-disordered breathing (score range, 3–9), and daytime sleepiness (score range, 8–24). A higher score is indicative of more sleep problems. As a previous receiver operating characteristic curve analysis suggested a cutoff score of 41 or higher yielded the best diagnostic confidence (i.e., correctly identified 80% of the clinical sample in the study), with children who obtained a score of ≥41 considered to "have sleep disturbances" and those who received a score <41 considered to "not have sleep disturbances." The following cutoff scores were observed for the eight scales: ≥7, "have bedtime resistance"; ≥2, "have sleep onset delay"; ≥4, "have sleep duration problem"; ≥5, "have sleep anxiety"; ≥4, "have night waking problems"; ≥8, "have parasomnias"; ≥4, "have sleep-disordered breathing"; and ≥10, "have daytime sleepiness."[13]

### *Measurement of plasma cotinine levels*

Cotinine, a metabolite of nicotine, is a reliable biomarker of exposure to tobacco smoke. Plasma levels provide a view of exposure over the previous 48–72 hours. However, owing to the stability of exposure patterns over time, a one-time cotinine measurement is considered representative of typical daily exposure.[15]

Plasma was collected from every child using a standard phlebotomy procedure and stored at -20 °C until analysis (maximum of 3 months). The direct barbituric acid assay was modified by Barlow et al.[16] and was used to measure cotinine levels. Briefly, 200 μL of plasma, 100 μL sodium acetate buffer (4 M, pH 4.7), 40 μL of KCN in $H_2O$ (1.5 M), 40 μl chloramine-T in $H_2O$ (0.4 M), and 200 μL barbituric acid in acetone: $H_2O$ (78 mM, 50% v/v) were sequentially added into 1 mL polypropylene tubes. The tubes were mixed and incubated at room temperature for 15 min. The reaction was stopped by the addition of 40 μL of sodium metabisulfite (1M in $H_2O$), and absorbance was measured at 490 nm using a spectrophotometer (Thermo, Germany), with $H_2O$ serving as the blank. All results were expressed as 'cotinine equivalents ng/mL' by comparing the absorbance at 490 nm of each

unknown with that of a 100 ng/mL cotinine solution $H_2O$ standard (BTLab, China). Results were expressed in ng/mL.

### *Statistical analysis*

The analysis used the Statistical Package for the Social Sciences (SPSS) version 22.0. Because the continuous variables did not show a normal distribution, data were presented as median (Q1-Q3). Categorical variables were expressed as frequencies and percentages. Comparisons between two independent groups were performed using the Mann–Whitney U test. Associations between categorical variables were analyzed using the chi-square test. The Spearman correlation test was employed to examine the relationship between two quantitative variables. Statistical significance was set at $p<0.05$.

## Results

### *Characteristics of patients*

Two hundred and eight consecutive participants were included in the study, but five participants had to be excluded due to missing data in their questionnaires. Of the 203 children we evaluated, with a median (Q1-Q3) age of 8.3 (6–10) years, 99 (49.8%) were female. Fifty-two (25.6%) children were in the preschool age group. The children's median z-score of BMI was 0 (-0.8 – 0.8). Parents reported that 192 (94.6%) children had daily caffeine intake. The median sleep time was 9.5 (9-10.5) hours. Of the 102 children exposed to ETS, 63 (61.8%) were exposed to SHS, while 39 (38.2%) were exposed to THS, according to parent reports. One hundred two children (50.2%) had parent-reported ETS exposure; 52 (25.6%) were exposed to paternal smoking, 14 (6.9%) to maternal smoking, 29 (14.3%) were exposed to both paternal and maternal smoking, and 7 (3.4%) were exposed to third-party smoking.

The median value of the total CSHQ score was 41 (38–46). One hundred eleven children (57.7%) were categorized in the "have sleep disturbances" group based on their total CSHQ score. According to their scores on the eight scales, 128 (63.1%) children had bedtime resistance, 43 (21.2%) had sleep onset delay, 68 (33.5%) had problems with sleep duration, 141 (69.5%) had sleep anxiety, 96 (47.3%) had night waking problems, 79 (38.9%) had parasomnias, 31 (15.3%) had sleep-disordered breathing, and 131 (64.5%) had daytime sleepiness. The median plasma cotinine level was 6.2 (4–10.6) (Table I).

**Table I.** Demographic data, questionnaire and laboratory results of the study group (N=203).

| | |
|---|---|
| Demographic Data | |
| Female sex, n(%) | 99 (49.8) |
| Age (years), median (IQR) | 8.3 (6-10) |
| Pre-school age group, n(%) | 52 (25.6) |
| BMI z score, median (IQR) | 0 (-0.8–0.8) |
| Daily caffeine intake, n (%) | 192 (94.6) |
| Sleep time (hours), median (IQR) | 9.5 (9–10.5) |
| Questionnaires | |
| Parent-reported ETS exposure, n (%) | 102 (50.2) |
| Parent-reported SHS exposure, n (%) | 63 (61.8) |
| Parent-reported THS exposure, n (%) | 39 (38.2) |
| ETS exposure by the mother, n (%) | 14 (6.9) |
| ETS exposure by the father, n (%) | 52 (25.6) |
| ETS exposure by the mother and father, n (%) | 29 (14.3) |
| ETS exposure by third party, n (%) | 7 (3.4) |
| Sleep disturbance according to CHSQ, n (%) | 111 (57.7) |
| Bedtime resistance, n (%) | 128 (63.1) |
| Sleep onset delay, n (%) | 43 (21.2) |
| Sleep duration problems, n(%) | 68 (33.5) |
| Sleep anxiety, n(%) | 141 (69.5) |
| Night waking problems, n (%) | 96 (47.3) |
| Parasomnias, n (%) | 79 (38.9) |
| Sleep-disordered breathing, n (%) | 31 (15.3) |
| Daytime sleepiness, n (%) | 131 (64.5) |
| Plasma cotinine levels (ng/mL), median (IQR) | 6.2 (4–10.6) |

BMI, body mass index; CHSQ, Children's Sleep Habits Questionnaire; ETS, environmental tobacco smoke; IQR, interquartile range; SHS, secondhand smoke; TSH, thirdhand smoke.

### *Comparison of groups with and without parent-reported ETS exposure*

Differences between children with "parent-reported ETS exposure" and those with "no parent-reported ETS exposure" in terms of plasma cotinine levels, sleep time, and sleep disturbances, according to the CSHQ and its subgroups, are presented in Table II. Plasma cotinine level was statistically significantly higher in the parent-reported ETS exposure group than no parent-reported ETS exposure group (Medians 10.6 [10.1–11.1] vs 4 [3.4–4.4] ng/mL, p <0.001). Sleep disturbances according to CHSQ were statistically more common in the parent-reported ETS group exposure than no parent-reported ETS exposure group (61.8% vs 47.5%, p=0.042).

### *Comparison of groups with SHS and THS exposure*

Differences between children "with SHS exposure" and "with THS exposure" in terms of plasma cotinine levels, sleep time, CSHQ total sleep disturbance scores, and scores on the eight scales are presented in Table III. Sleep disturbances were present in 23 (59%) of children exposed to THS and 40 (63.5%) of children exposed to SHS. No statistically significant difference was found between the two groups (p=0.648).

### *Correlations between plasma cotinine levels and CSHQ total sleep disturbance scores*

There were no significant correlations between plasma cotinine levels and CSHQ total sleep disturbance scores on the CSHQ in the overall study population (rho 0.070, p= 0.323).

## Discussion

In this study investigating the effects of ETS on the sleep of healthy children, we found that children exposed to ETS were more likely to experience sleep disorders. Plasma cotinine levels were significantly higher in children with parent-reported ETS exposure compared to those without such exposure. However, no significant difference was found between the cotinine levels and sleep disturbances of children exposed to SHS and those exposed THS.

Previous studies[17-20] have shown that SHS exposure increases the frequency of sleep-disordered breathing (SDB) in children and reduces sleep quality. To avoid this confounding

**Table II.** Comparison of groups with and without environmental tobacco smoke exposure.

| | No parent-reported ETS exposure (n: 101) | Parent-reported ETS (n: 102) | p value |
|---|---|---|---|
| Plasma cotinine levels (ng/mL), median (IQR) | 4 (3.4–4.4) | 10.6 (10.1–11.1) | <0.001** |
| Sleep time (hours), median (IQR) | 9.5 (8.6-10.5) | 9.5 (8.9-10.5) | 0.657 |
| Sleep disturbance according to CHSQ, n (%) | 48 (47.5) | 63 (61.8) | 0.042* |
| Bedtime resistance, n (%) | 58 (57.4) | 70 (68.6) | 0.254* |
| Sleep onset delay, n (%) | 22 (21.8) | 21 (20.6) | 0.313* |
| Sleep duration problems, n(%) | 36 (35.6) | 32 (31.4) | 0.217* |
| Sleep anxiety, n (%) | 67 (66.3) | 74 (72.5) | 0.377* |
| Night waking problems, n (%) | 41(40.6) | 55 (53.9) | 0.152* |
| Parasomnias, n (%) | 33 (32.7) | 46 (45.1) | 0.187* |
| Sleep-disordered breathing, n (%) | 17 (16.8) | 14 (13.1) | 0.617* |
| Daytime sleepiness, n (%) | 66 (65.3) | 65 (63.7) | 0.253* |

*Chi-square test, ** Mann-Whitney U test

BMI, body mass index; CHSQ, the Children's Sleep Habits Questionnaire; ETS, environmental tobacco smoke; IQR, inter quartile range; SHS, secondhand smoke; THS, thirdhand smoke.

**Table III.** Comparison of groups with second- and thirdhand smoke exposure.

| | Parent-reported SHS exposure (n: 63) | Parent-reported THS exposure (n: 39) | p value |
|---|---|---|---|
| Plasma cotinine levels (ng/mL), median (IQR) | 10.6 (10–11.1) | 10.6 (10.4–11.2) | 0.239* |
| Sleep time (hours), median (IQR) | 9.5 (8.5–10.3) | 9.5 (9–10.5) | 0.305* |
| CSHQ total sleep disturbance score, median, (IQR) | 43 (33-56) | 41 (33-62) | 0.133* |
| Sleep disturbance according to CHSQ, n (%) | 40 (63.5) | 23 (59) | 0.401** |
| Bedtime resistance, n (%) | 43 (68.3) | 27 (69.2) | 0.549** |
| Sleep onset delay, n (%) | 16 (25.4) | 5 (12.8) | 0.1** |
| Sleep duration problems, n (%) | 16 (25.4) | 16 (41) | 0.76** |
| Sleep anxiety, n (%) | 48 (76.2) | 26 (66.7) | 0.206** |
| Night waking problems, n (%) | 33 (52.4) | 22 (56.4) | 0.424** |
| Parasomnias, n (%) | 29 (46) | 17 (43.6) | 0.426** |
| Sleep-disordered breathing, n (%) | 10 (15.9) | 4 (10.3) | 0.312** |
| Daytime sleepiness, n (%) | 44 (69.8) | 21 (53.8) | 0.078** |

*Mann-Whitney U test, **Chi-square test
CHSQ, the Children's Sleep Habits Questionnaire; ETS, environmental tobacco smoke; IQR, inter quartile range; SD, standard deviation; SHS, secondhand smoke; THS, thirdhand smoke.

effect, we excluded children diagnosed with SDB with polysomnography. Consequently, the number of participants with problems in the SDB subgroup of the CSHQ was the lowest among all subgroups in our study.

Yolton et al.[8] evaluated 232 children with asthma who were exposed to SHS, and reported that 92.7% of the children had sleep disorders. In our study, this rate was considerably lower. The strong association between asthma and sleep disorders is well established[21,22], likely due to complex bidirectional interaction. Therefore, children with asthma were excluded from our sample; we believe this contributed to the lower prevalence of sleep disorders observed.

Although we found a statistically significant difference in cotinine levels between the ETS-exposed group and the non-ETS-exposed group based on parent reports, no correlation was observed between cotinine levels and CSHQ scores. Yolton et al.[8] reported significant associations between log-transformed serum cotinine levels and several sleep domains, including bedtime resistance, sleep anxiety, parasomnias, sleep-disordered breathing, daytime sleepiness, and total sleep disturbance, although they found no associations with sleep onset latency or sleep duration.

In our study, parents who smoked were asked whether they did so inside or outside their homes; Approximately 38% reported smoking outdoors. These children were classified as having THS exposure only. No significant difference in plasma cotinine levels was found between children THS and SHS-exposed. Consistent with previous studies[23,24], avoiding smoking in the child's presence reduces SHS exposure but does not eliminate ETS exposure. Many parents may underestimate THS risks, believing children are unaffected by ETS if smoking occurs in another location. Studies have shown that the proportion of parents who recognize THS is harmful ranges from 42.4% to 91%, yet this awareness is not consistently associated with implementing home or car smoking bans.[25]

The effects of THS, a relatively recent component in ETS research, remain poorly understood. Children are believed to be more vulnerable to THS than adults due to spending more time indoors and having developing respiratory systems.[26] Lidón-Moyano et al.[27] demonstrated

that salivary cotinine levels in adults exposed to THS at home were comparable to those exposed to SHS. Matt et al.[28] reported that children living in homes where adults smoked outside had ETS exposure levels 5–7 times higher than those in non-smoking homes, and children in homes with indoor smoking had levels 3-8 times higher than those with outdoor-only smoking. Similarly, Protano et al.[29] demonstrated that median urinary cotinine concentrations in children increased significantly with higher levels of household ETS exposure.

Nicotine uptake from SHS and THS can be assessed using several metabolites, with cotinine being the most commonly used. A study[30] of 4485 non-smokers aged 3–17 years participating in the 2013–2016 National Health and Nutrition Examination Survey used random forest models to identify the best combination of biomarkers and reported exposures to distinguish ETS-exposed children. The strongest predictors were the number of smokers in the home, serum cotinine, serum hydroxycotinine, and urine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol. Reliable serum cotinine measurement methods include gas chromatography, high-performance liquid chromatography, radioimmunoassay, and enzyme-linked immunosorbent assay.[16,31] However, these require expensive equipment, limiting routine use in low- and middle-income countries. In our study, plasma cotinine measurement was chosen for its simplicity, speed, and low cost, but the lack of a cutoff value for ETS exposure in plasma cotinine measurements remains a limitation.

Risk factors for pediatric sleep problems can be classified as biological (e.g., sex, age, body weight), environmental (e.g., ETS, heavy metals, air pollution), and social (e.g., family life, socioeconomic status, screen exposure).[32] Potential contributors, such as heavy metal and air pollution exposure, socioeconomic status, co-sleeping, and screen exposure, could be investigated through parent questionnaires. The absence of these variables in our analysis may have limited our ability to identify additional risk factors. Future research should measure SHS and THS exposure with greater precision to better clarify their impact on sleep disturbances. Plasma cotinine measurement may serve as a practical screening tool in low- and middle-income countries if validated in larger studies and accompanied by standardized cutoffs. There are studies on certain foods and medications thought to affect cotinine levels.[33] One of the limitations of our study was that we did not ask about these foods and medications. Combining objective sleep assessments, such as polysomnography or actigraphy, with parent-reported questionnaires could also improve the accuracy of findings.

In conclusion, ETS exposure increases the risk of sleep disturbances in children, and both SHS and THS contribute to elevated plasma cotinine levels. Efforts to reduce ETS exposure in children should address both SHS and THS exposure patterns to effectively protect pediatric sleep health.

## Ethical approval

The study was approved by Ankara University Ethics Board (number: 10-805-19).

## Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: GÖ, NÇ, ÖY; data collection: NEÇİ, FG, FBA; analysis and interpretation of results: GÖ, FZ ; draft manuscript preparation: GÖ, NÇ, ÖY. All authors reviewed the results and approved the final version of the manuscript.

## Source of funding

The authors declare the study received no funding.

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES


1. Wu JX, Lau ATY, Xu YM. Indoor secondary pollutants cannot be ignored: third-hand smoke. Toxics 2022; 10: 363. https://doi.org/10.3390/toxics10070363
2. World Health Organization (WHO). Second-hand smoke. Available at: http://www.who.int/gho/phe/secondhand_ smoke/en/ (Accessed on Aug 23, 2023).
3. Leung LT, Ho SY, Wang MP, Lam TH. Secondhand smoke from multiple sources, thirdhand smoke and respiratory symptoms in Hong Kong adolescents. Nicotine Tob Res 2018; 20: 192-198. https://doi.org/10.1093/ntr/ntw302
4. Paavonen EJ, Aronen ET, Moilanen I, et al. Sleep problems of school-aged children: a complementary view. Acta Paediatr 2000; 89: 223-228. https://doi.org/10.1080/080352500750028870
5. Sabanayagam C, Shankar A. The association between active smoking, smokeless tobacco, second-hand smoke exposure and insufficient sleep. Sleep Med 2011; 12: 7-11. https://doi.org/10.1016/j.sleep.2010.09.002
6. Choi JB, Lee YJG, Jeong DU. Transdermal nicotine patch effects on EEG power spectra and heart rate variability during sleep of healthy male adults. Psychiatry Investig 2017; 14: 499-505. https://doi.org/10.4306/pi.2017.14.4.499
7. Davila DG, Hurt RD, Offord KP, Harris CD, Shepard JW. Acute effects of transdermal nicotine on sleep architecture, snoring, and sleep-disordered breathing in nonsmokers. Am J Respir Crit Care Med 1994; 150: 469-474. https://doi.org/10.1164/ajrccm.150.2.8049831
8. Yolton K, Xu Y, Khoury J, et al. Associations between secondhand smoke exposure and sleep patterns in children. Pediatrics 2010; 125: e261-e268. https://doi.org/10.1542/peds.2009-0690
9. Nakata A, Takahashi M, Haratani T, et al. Association of active and passive smoking with sleep disturbances and short sleep duration among japanese working population. Int J Behav Med 2008; 15: 81-91. https://doi.org/10.1080/10705500801929577
10. Ohida T, Kaneita Y, Osaki Y, et al. Is passive smoking associated with sleep disturbance among pregnant women? Sleep 2007; 30: 1155-1161. https://doi.org/10.1093/sleep/30.9.1155
11. Jara SM, Benke JR, Lin SY, Ishman SL. The association between secondhand smoke and sleep-disordered breathing in children: a systematic review. Laryngoscope 2015; 125: 241-247. https://doi.org/10.1002/lary.24833
12. Safa F, Chaiton M, Mahmud I, Ahmed S, Chu A. The association between exposure to second-hand smoke and sleep disturbances: a systematic review and meta-analysis. Sleep Health 2020; 6: 702-714. https://doi.org/10.1016/j.sleh.2020.03.008
13. Owens JA, Spirito A, McGuinn M. The Children's Sleep Habits Questionnaire (CSHQ): psychometric properties of a survey instrument for school-aged children. Sleep 2000; 23: 1043-1051.
14. Akçay D, Akçay BD. Hekim Bozkurt Ö. Reliability and validity of Turkish Sleep Disturbance Scale for children. Anatolian Journal of Psychiatry 2020; 21(Suppl 1): 70-77. https://doi.org/10.5455/apd.65084
15. Avila-Tang E, Al-Delaimy WK, Ashley DL, et al. Assessing secondhand smoke using biological markers. Tob Control 2013; 22: 164-171. https://doi.org/10.1136/tobaccocontrol-2011-050298
16. Barlow RD, Stone RB, Wald NJ, Puhakainen EV. The direct barbituric acid assay for nicotine metabolites in urine: a simple colorimetric test for the routine assessment of smoking status and cigarette smoke intake. Clin Chim Acta 1987; 165: 45-52. https://doi.org/10.1016/0009-8981(87)90217-8
17. Forastiére F, Corbo GM, Michelozzi P, et al. Effects of environment and passive smoking on the respiratory health of children. Int J Epidemiol 1992; 21: 66-73. https://doi.org/10.1093/ije/21.1.66
18. Kuehni CE, Strippoli MPF, Chauliac ES, Silverman M. Snoring in preschool children: prevalence, severity and risk factors. Eur Respir J 2008; 31: 326-333. https://doi.org/10.1183/09031936.00088407
19. Sogut A, Yilmaz O, Dinc G, Yuksel H. Prevalence of habitual snoring and symptoms of sleep-disordered breathing in adolescents. Int J Pediatr Otorhinolaryngol 2009; 73: 1769-1773. https://doi.org/10.1016/j.ijporl.2009.09.026
20. Malakasioti G, Gourgoulianis K, Chrousos G, Kaditis A. Interactions of obstructive sleep-disordered breathing with recurrent wheezing or asthma and their effects on sleep quality. Pediatr Pulmonol 2011; 46: 1047-1054. https://doi.org/10.1002/ppul.21497
21. Kavanagh J, Jackson DJ, Kent BD. Sleep and asthma. Curr Opin Pulm Med 2018; 24: 569-573. https://doi.org/10.1097/MCP.0000000000000526
22. Reiter J, Ramagopal M, Gileles-Hillel A, Forno E. Sleep disorders in children with asthma. Pediatr Pulmonol 2022; 57: 1851-1859. https://doi.org/10.1002/ppul.25264
23. Winickoff JP, Friebely J, Tanski SE, et al. Beliefs about the health effects of "thirdhand" smoke and home smoking bans. Pediatrics 2009; 123: e74-e79. https://doi.org/10.1542/peds.2008-2184

24. Shehab K, Ziyab AH. Beliefs of parents in Kuwait about thirdhand smoke and its relation to home smoking rules: a cross-sectional study. Tob Induc Dis 2021; 19: 66. https://doi.org/10.18332/tid/140090

25. Vanzi V, Marti F, Cattaruzza MS. Thirdhand smoke knowledge, beliefs and behaviors among parents and families: a systematic review. Healthcare (Basel) 2023; 11: 2403. https://doi.org/10.3390/healthcare11172403

26. Ferrante G, Simoni M, Cibella F, et al. Third-hand smoke exposure and health hazards in children. Monaldi Arch Chest Dis 2013; 79: 38-43. https://doi.org/10.4081/monaldi.2013.108

27. Lidón-Moyano C, Fu M, Pérez-Ortuño R, et al. Third-hand exposure at homes: assessment using salivary cotinine. Environ Res 2021; 196: 110393. https://doi.org/10.1016/j.envres.2020.110393

28. Matt GE, Quintana PJE, Hovell MF, et al. Households contaminated by environmental tobacco smoke: sources of infant exposures. Tob Control 2004; 13: 29-37. https://doi.org/10.1136/tc.2003.003889

29. Protano C, Andreoli R, Manini P, Vitali M. How home-smoking habits affect children: a cross-sectional study using urinary cotinine measurement in Italy. Int J Public Health 2012; 57: 885-892. https://doi.org/10.1007/s00038-012-0354-0

30. Merianos AL, Mahabee-Gittens EM, Stone TM, et al. Distinguishing exposure to secondhand and thirdhand tobacco smoke among U.S. children using machine learning: NHANES 2013-2016. Environ Sci Technol 2023; 57: 2042-2053. https://doi.org/10.1021/acs.est.2c08121

31. Stanley SD, Gairola CG, Diana J, et al. Development and characterization of an ELISA for cotinine in biological fluids. Inhalation Toxicology 1993; 5: 403-413. https://doi.org/10.3109/08958379308998395

32. Liu J, Ji X, Rovit E, Pitt S, Lipman T. Childhood sleep: assessments, risk factors, and potential mechanisms. World J Pediatr 2024; 20: 105-121. https://doi.org/10.1007/s12519-022-00628-z

33. Tzoupis H, Papavasileiou KD, Papatzelos S, et al. Systematic review of naturally derived substances that act as inhibitors of the nicotine metabolizing enzyme cytochrome P450 2A6. Int J Mol Sci 2024; 25: 8031. https://doi.org/10.3390/ijms25158031

The Turkish Journal of Pediatrics 2025; 67: 798-807
https://doi.org/10.24953/turkjpediatr.2025.6725

Original Article

# Predictive value of serum hsa_circ_0061346, hsa_circ_0000095, and hsa_circ_0068606 expression levels on the severity of retinopathy of prematurity

Chenglv Liu[1], Lijie Dong[2], Qiaoling Yang[2], Lang Bai[1]

*[1]Department of Ophthalmology, Nanfang Hospital, Southern Medical University, Guangzhou, China; [2]Department of Ophthalmology, Huadu Maternal and Child Health Hospital, Guangzhou, China.*

## ABSTRACT

**Background.** To investigate the diagnostic and pathological staging value of serum hsa_circ_0061346, hsa_circ_0000095, and hsa_circ_0068606 levels in retinopathy of prematurity (ROP).

**Methods.** A total of 70 infants with ROP (140 eyes) treated at our hospital from October 2018 to October 2023 were enrolled as the ROP group, while 70 healthy preterm infants (140 eyes) of the same gestational age without ROP were selected as the control group. The relative expression levels of serum hsa_circ_0061346, hsa_circ_0000095, and hsa_circ_0068606 were detected by quantitative real-time PCR. Logistic regression analysis was used to identify factors influencing ROP occurrence. The diagnostic efficacy of the three circular RNAs (circRNAs) was evaluated using receiver operating characteristic (ROC) curve analysis.

**Results.** The relative expression levels of hsa_circ_0061346 in serum were significantly higher in the ROP group than in the control group (6.27 ± 3.60 vs. 0.72 ± 0.31, $P < 0.05$), whereas the levels of hsa_circ_0000095 (1.98 ± 1.38 vs. 3.90 ± 1.75) and hsa_circ_0068606 (1.18 ± 0.51 vs. 7.71 ± 4.45) were significantly lower (all $p < 0.05$). Multivariate logistic regression showed that abnormal expression of these circRNAs was an independent risk factor for ROP. Notably, the combined diagnostic performance of the three circRNAs yielded an area under the ROC curve of 0.983, with a sensitivity of 100% and a specificity of 97.14%.

**Conclusion.** The combined detection of serum hsa_circ_0061346, hsa_circ_0000095, and hsa_circ_0068606 may provide a novel approach for the early diagnosis and severity assessment of ROP.

**Key words:** circular RNA, retinopathy of prematurity, biomarker.

Retinopathy of prematurity (ROP) is a leading cause of blindness in preterm infants, characterized pathologically by abnormal retinal vascular proliferation and fibrosis. With advancements in neonatal intensive care, the survival rate of preterm infants has significantly increased, but the incidence of ROP has also risen, posing serious threats to visual health and imposing substantial burdens on affected families.[1-3] Currently, the diagnosis and staging of ROP primarily rely on fundus examination, which requires considerable operator expertise and cannot provide early warning signals at the molecular level. It is therefore imperative to identify biomarkers with greater sensitivity and specificity.

Recent studies have shown that circular RNAs (circRNAs) participate in the pathogenesis of various retinal diseases by regulating angiogenesis, inflammatory responses, and hypoxia-related stress.[4,5] However, the role

✉ Lang Bai ▪ bailangsfy@126.com

Received 29th Jul 2025, revised 10th Sep 2025, 30th Oct 2025, 22nd Nov 2025, accepted 11th Dec 2025.

of circRNAs in ROP has not been thoroughly investigated. circTFRC (hsa_circ_0068606) has emerged as a research focus in oncology, where its aberrant expression is closely associated with tumor initiation and progression.[6] Lin et al. demonstrated that hsa_circ_0068606 promotes tumor cell proliferation, migration, and invasion by regulating gene expression, acting as a miRNA sponge, and modulating signaling pathways (including ferroptosis-related pathways).[7] However, its expression profile in ROP requires further investigation. Additionally, hsa_circ_0000095 and hsa_circ_0068606, located on human chromosomes 1 and 3, respectively, warrant further exploration in the context of ROP. These findings suggest that circRNAs may serve as novel molecular biomarkers for ROP, yet their expression characteristics and clinical significance in this disease remain unclear.

To our knowledge, this study is the first to systematically measure the serum expression levels of hsa_circ_0061346, hsa_circ_0000095, and hsa_circ_0068606 in ROP patients, analyze their associations with disease onset and pathological staging, and evaluate the clinical utility of their combined detection. The aim of this study is to explore circRNA expression profiles in ROP and identify molecules that may contribute to earlier diagnosis and improved clinical management.

## Materials and Methods

### *Study population*

A total of 70 preterm infants diagnosed with ROP (the ROP group) and 70 healthy preterm infants serving as the normal control (NC) group were enrolled from a single center (the Department of Ophthalmology and Neonatology) between October 2018 to October 2023. This study was designed and conducted as a retrospective observational study. The purpose of sample collection during 2018–2023 was for routine diagnostic evaluation, biomarker preservation, and future research aimed at improving early detection of neonatal diseases, including but not limited to ROP. Parents or legal guardians provided written informed consent for the storage and future scientific use of the biological samples and clinical data at the time of collection, regardless of whether the infants later developed ROP. The same consent process was applied to the normal control group, as their samples were likewise collected during routine clinical care and retained in accordance with institutional biobanking policies.

The sample size was estimated using the formula for comparing two independent means, with a two-sided $\alpha$ of 0.05 and a power of 0.80. Preliminary data from our center indicated an expected mean difference of 0.8 in circRNA expression between the ROP and NC groups, with an estimated standard deviation of 1.5. Under these assumptions, the minimum required sample size was 63 infants per group. Considering a potential 10% attrition rate, 70 infants were ultimately included in each group to ensure adequate statistical power and study reliability.

Inclusion criteria were: 1) gestational age ≤ 36 weeks; 2) birth weight ≤ 2000 g; 3) absence of congenital ocular diseases; 4) ROP diagnosis consistent with the Guidelines for Retinopathy of Prematurity Screening[8]; 5) informed consent obtained and ethical approval granted. Exclusion criteria included: 1) severe systemic infections or hereditary metabolic diseases; 2) a history of ocular surgery or laser treatment.

All premature infants underwent ROP screening according to our unit protocol: infants with gestational age ≤ 36 weeks or birth weight ≤ 2000 g were included, and the first examination was conducted at 4 weeks postnatal age or 35 weeks postmenstrual age (whichever came later). Subsequent examinations were performed at 1–2 weeks intervals until the retina was fully vascularized or until ROP regression or progression was determined. Screening was terminated when complete retinal vascularization was observed or when

ROP had regressed without evidence of further progression.

ROP diagnosis and staging were performed via binocular indirect ophthalmoscopy after mydriasis, in accordance with the International Classification of Retinopathy of Prematurity (ICROP).[8] The severity of retinal lesions was classified into Stage I (n=4), Stage II (n=17), Stage III (n = 42), and aggressive posterior ROP (AP-ROP, n = 7). All examinations were performed by the same senior pediatric ophthalmologist to ensure diagnostic consistency.

This study followed the principles of the Declaration of Helsinki. Ethical approval for the retrospective use of previously collected samples and clinical data was obtained from the Institutional Ethics Committee (Approval No. 2025-033). The approval covered the use of previously collected clinical data and samples collected between October 2018 and October 2023.

### *Sample collection and processing*

Fasting venous blood (3 mL) was collected at 4 weeks postnatal age, allowed to stand at 4 °C for 30 minutes, and centrifuged at 3000 rpm (centrifugal radius: 8 cm) for 10 minutes. The supernatant serum was aliquoted into sterile microcentrifuge tubes and stored at -80 °C. Total RNA was extracted using TRIzol reagent (Invitrogen, USA), and RNA purity was assessed using a NanoDrop 2000 spectrophotometer (with an A260/A280 ratio of 1.8–2.0). The ROP stages reported in this study corresponded to the highest stage observed during follow-up, rather than the stage at the time of blood sampling.

### *CircRNA detection*

Linear RNA was digested with RNase R (20 U/μL, Beyotime, China) at 37 °C for 30 minutes, followed by reverse transcription using HiScript III RT SuperMix (Vazyme, Nanjing, China). The quantitative reverse transcription polymerase chain reaction (qRT-PCR) reaction system (20 μL) consisted of: 10 μL SYBR Green Master Mix, 2 μL cDNA template, 1 μL each of forward and reverse primers (10 μM), and $ddH_2O$ to a total volume of 20 μL. Primer sequences are given in Table I.

PCR conditions were: pre-denaturation at 95°C for 5 minutes; followed by 40 cycles of 95°C for 15 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method, normalized to β-actin as the internal reference.

### *Statistical analysis*

Data were analyzed using SPSS 25.0 software. Categorical data were expressed as frequencies or percentages and compared using the $\chi^2$ test. Normally distributed continuous data were presented as mean ± standard deviation ($x \pm s$) and compared using the t-test for two groups or one-way analysis of variance (ANOVA) for multiple groups, with pairwise comparisons performed using the SNK-q test. To identify risk factors for ROP, univariate logistic regression analyses were first conducted. Variables with a $p$ value <0.10 in the univariate analysis were

**Table I.** Primer sequences used in the study.

| | | |
|---|---|---|
| hsa_circ_0061346 | F | 5'-GAAGTGTGCCCCATTCTTTTAC-3' |
| | R | 5'- TTCGCAAACATCCATCCTCT-3' |
| hsa_circ_0000095 | F | 5'-GTATGCATACTACCTTGTACTGGTT-3' |
| | R | 5'- GACTATTGAAACCTGGAGAAACT-3' |
| hsa_circ_0068606 | F | 5'-CTGAACCAATACAGAGCAGACAT-3' |
| | R | 5'- GAACTGCCACACAGAAGAACTC-3' |
| β-actin | F | 5'-GTGACGTTGACATCCGTAAAGA-3' |
| | R | 5'- GCCGGACTCATCGTACTCC-3' |

subsequently included in the multivariate logistic regression model to calculate odds ratios (OR) and 95% confidence intervals (95% CI). The diagnostic performance was evaluated using receiver operating characteristic (ROC) curves, with the area under the curve (AUC) compared using the Z-test. For combined ROC curve analysis, the expression levels of the three circRNAs (hsa_circ_0061346, hsa_circ_0000095, and hsa_circ_0068606) were integrated into a logistic regression model to generate a predicted probability for each subject. These predicted probabilities were then used to construct a combined ROC curve, allowing assessment of the overall diagnostic performance of the three markers together. A *p*-value <0.05 was considered statistically significant.

## Results

### *Comparison of clinical characteristics between ROP and control groups*

A total of 70 preterm infants with retinopathy of prematurity (the ROP group) and 70 healthy preterm infants (NC group) were included. The mean gestational age of infants in the ROP group was 30.57±3.16 weeks, which was significantly lower than that of the NC group (34.97 ± 1.74 weeks, $p < 0.05$). Among the 70 ROP patients, 23 infants (32.9%) received treatment, while the remaining 47 infants (67.1%) experienced spontaneous regression of ROP (i.e., the disease resolved without intervention). No significant differences were observed between the two groups in terms of sex, singleton/twin status, gestational diabetes, maternal infection, gestational hypertension, bronchopulmonary dysplasia (BPD), or 1-minute Apgar scores ($p > 0.05$). However, the birth weight in the ROP group (1.56 ± 0.54 kg) was significantly lower than that in the NC group (2.38 ± 0.55 kg) ($p < 0.001$) (Table II, Fig. 1A). Additionally, the proportion of infants with neonatal respiratory distress syndrome (NRDS) was significantly higher in the ROP group compared to the NC group ($\chi^2 = 8.96$, $p = 0.0028$).

### *Comparison of serum circRNA expression levels between groups*

Serum expression of hsa_circ_0061346 was significantly higher in the ROP group (6.27 ± 3.60) compared to the NC group (0.72 ± 0.31). In contrast, expression levels of hsa_circ_0000095 (1.98 ± 1.38 *vs.* 3.90 ± 1.75) and hsa_circ_0068606 (1.18 ± 0.51 *vs.* 7.71 ± 4.45) were significantly lower in the ROP group compared to the NC group (Fig. 1B-D).

**Table II.** Comparison of clinical characteristics between ROP and control groups [n (%) or x±s]

| Clinical parameter | ROP (n=70) | Control (n=70) | t/χ2 | p |
|---|---|---|---|---|
| Gestational age (weeks) | 30.57 ± 3.16 | 34.97 ± 1.74 | 10.22 | <0.0001 |
| Birth weight (kg) | 1.56 ± 0.54 | 2.38 ± 0.55 | 8.84 | <0.0001 |
| Male sex | 36 (51%) | 45 (64%) | 2.37 | 0.12 |
| Twin birth | 14 (20%) | 17 (24%) | 0.37 | 0.54 |
| Gestational diabetes | 30 (43%) | 22 (31%) | 1.96 | 0.16 |
| Maternal infection | 36 (51%) | 25 (36%) | 3.52 | 0.06 |
| Gestational hypertension | 11 (16%) | 9 (13%) | 0.23 | 0.63 |
| BPD | 7 (10%) | 5 (7%) | 0.36 | 0.55 |
| NRDS | 28 (40%) | 12 (17%) | 8.96 | 0.0028 |
| Apgar score < 8 (1 min) | 7 (10 %) | 7 (10%) | 0.07 | 0.78 |

Percentages are column percentages.
BPD: Bronchopulmonary dysplasia, NRDS: Neonatal respiratory distress syndrome.

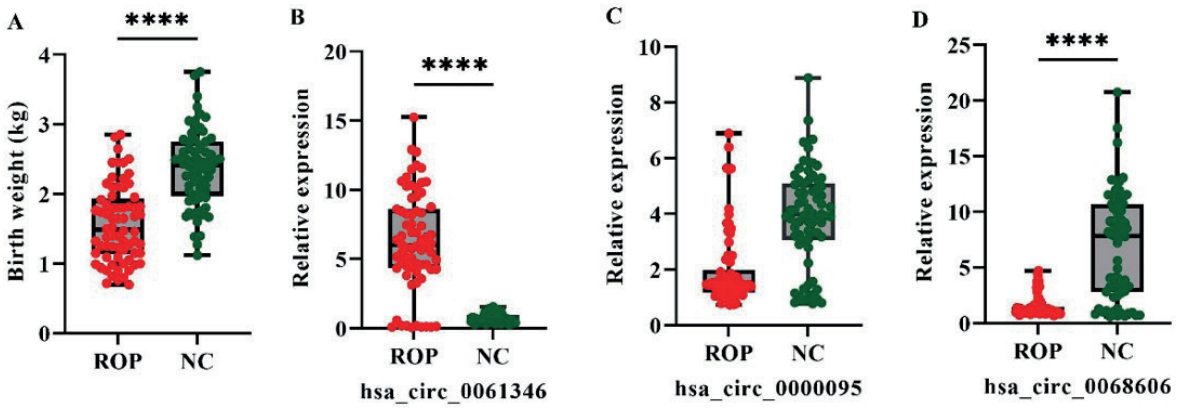



**Fig. 1.** Comparison of circRNA expression levels.

(A) Birth weight comparison between the ROP and control groups; Expression differences of (B) hsa_circ_0061346, (C) hsa_circ_0000095 and (D) hsa_circ_0068606 between the ROP and control groups. ****p < 0.0001.

ROP: retinopathy of prematurity, NC: normal control.

### *Changes in circRNA levels across different clinical characteristics in ROP*

The results in Table III indicate variations in the expression levels of the three circRNAs across different clinical characteristics. The expression of hsa_circ_0061346 was lower in the maternal infection group (5.47 ± 3.31) compared to the non-infection group (7.12 ± 3.74). The expression of hsa_circ_0000095 was significantly higher in the gestational diabetes group (1.79 ± 1.14) compared to the non-diabetes group (1.33 ± 0.77, $p = 0.046$). Notably, hsa_circ_0068606 expression was significantly higher in the BPD group (3.04 ± 2.17) compared to the non-BPD group (1.87 ± 1.24, $p = 0.03$) and in the 1-minute Apgar score < 8 group (2.97 ± 0.04) compared to the ≥ 8 group (1.88 ± 1.27, $p = 0.047$). No statistically significant differences were observed in the expression levels of the three circRNAs across sex, singleton/twin status, gestational hypertension, NRDS, or different pathological stages ($p > 0.05$).

### *Multivariate logistic regression analysis*

Univariate analysis (Table IV) revealed that gestational age (β = -0.641, OR = 0.527, $p < 0.0001$), birth weight (β = -2.493, OR = 0.083, $p < 0.0001$), and NRDS (β = 1.170, OR = 3.222, $p = 0.004$) were significantly associated with ROP occurrence. Lower gestational age and lower birth weight were associated with a higher risk of ROP, while NRDS was also associated with an increased risk. Notably, all three circRNA molecules (hsa_circ_0061346, hsa_circ_0000095, and hsa_circ_0068606) showed significant statistical associations ($p < 0.0001$ for all), with hsa_circ_0061346 expression associated with a higher risk of ROP (OR = 3.240), and higher expression of hsa_circ_0000095 and hsa_circ_0068606 associated with a lower risk (OR = 0.483 and 0.507, respectively). Other clinical factors, such as sex, singleton/twin status, and gestational complications, showed no significant associations.

Multivariate logistic regression analysis (Table V) indicated that, after adjusting for other variables, hsa_circ_0061346 (β = 1.195, OR = 3.303, 95% CI: 2.183–6.535, $p < 0.0001$) remained significantly associated with an increased risk of ROP, while hsa_circ_0000095 (β = -0.978, OR = 0.376, 95% CI: 0.188–0.574, $p = 0.0005$) and hsa_circ_0068606 (β = -1.291, OR = 0.275, 95% CI: 0.147–0.435, $p< 0.0001$) retained significant protective effects. Notably, gestational age ($p = 0.851$) and birth weight ($p = 0.089$) were no longer statistically significant in the multivariate model, and the effect of NRDS was attenuated ($p = 0.190$).

**Table III.** Comparison of three circRNAs with clinical characteristics in the ROP group

| Clinical parameter | hsa_circ_0061346 | hsa_circ_0000095 | hsa_circ_0068606 |
|---|---|---|---|
| Sex | *p*=0.201 | *p*=0.914 | *p*=0.925 |
| Male | 6.81±3.91 | 1.54±0.95 | 1.97±1.30 |
| Female | 5.70±3.19 | 1.52±1.00 | 2.00±1.49 |
| Singleton/twin | *p*=0.830 | *p*=0.930 | *p*=0.481 |
| Singleton | 6.32±3.79 | 1.52±0.92 | 1.93±1.29 |
| Twin | 6.08±2.79 | 1.55±1.16 | 2.22±1.75 |
| Gestational diabetes | *p*=0.502 | *p*=0.046 | *p*=0.987 |
| No | 6.53±3.61 | 1.33±0.77 | 1.98±1.25 |
| Yes | 5.94±3.61 | 1.79±1.14 | 1.99±1.56 |
| Maternal infection | *p*=0.054 | *p*=0.968 | *p*=0.964 |
| No | 7.12±3.74 | 1.53±1.09 | 1.99±1.31 |
| Yes | 5.47±3.31 | 1.53±0.86 | 1.98±1.47 |
| Gestational hypertension | *p*=0.559 | *p*=0.849 | *p*=0.111 |
| No | 6.16±3.65 | 1.52±0.92 | 1.87±1.26 |
| Yes | 6.86±3.42 | 1.58±1.24 | 2.60±1.87 |
| BPD | *p*=0.869 | *p*=0.149 | *p*=0.03 |
| No | 6.30±3.76 | 1.58±1.00 | 1.87±1.24 |
| Yes | 6.06±1.67 | 1.03±0.23 | 3.04±2.17 |
| NRDS | *p*=0.107 | *p*=0.472 | *p*=0.088 |
| No | 6.84±3.66 | 1.46±0.94 | 2.22±1.63 |
| Yes | 5.45±3.04 | 1.63±1.02 | 1.64±0.83 |
| 1 min Apgar score | *p*=0.239 | *p*=0.400 | *p*=0.047 |
| ≥8 | 6.44±3.59 | 1.50±0.91 | 1.88±1.27 |
| <8 | 4.75±3.61 | 1.82±1.46 | 2.97±0.04 |
| ROP stage | *p*=0.791 | *p*=0.111 | *p*=0.973 |
| I | 4.87±0.66 | 0.947±0.04 | 1.66±1.34 |
| II | 6.76±4.23 | 1.25±0.62 | 2.03±1.69 |
| III | 6.14±3.51 | 1.75±1.13 | 2.00±1.35 |
| AP-ROP | 6.70±3.80 | 1.20±0.37 | 1.98±0.97 |

Data are presented as mean ± standard deviation (SD).
BPD, Bronchopulmonary dysplasia; NRDS, Neonatal respiratory distress syndrome; ROP, retinopathy of prematurity; AP-ROP, aggressive posterior ROP.

### *ROC curve analysis*

The AUC for the individual diagnosis of ROP using serum hsa_circ_0061346, hsa_circ_0000095, and hsa_circ_0068606 was 0.862 (95% CI: 0.782–0.942) (Fig. 2A), 0.778 (95% CI: 0.693–0.862) (Fig. 2B), and 0.826 (95% CI: 0.748–0.903) (Fig. 2C), respectively. The combined diagnosis using all three circRNAs yielded an improved AUC of 0.983 (95% CI: 0.937–0.998) (Fig. 2D), with a sensitivity of 100% and a specificity of 97.14%. Z-test results indicated that the diagnostic performance of the combined approach was significantly superior to that of individual markers ($Z = -20.72$ to $-3.366$, all $p < 0.001$).

**Table IV.** Univariate logistic regression analysis of factors associated with ROP occurrence

| Factor | β | SE | Wald $\chi^2$ | p | OR | 95% CI |
|---|---|---|---|---|---|---|
| Sex | -0.531 | 0.346 | 2.356 | 0.125 | 0.588 | 0.297-1.154 |
| Gestational age | -0.641 | 0.103 | 38.726 | <0.0001 | 0.527 | 0.422-0.634 |
| Birth weight | -2.493 | 0.421 | 35.070 | <0.0001 | 0.083 | 0.034-0.177 |
| Singleton/twin | -0.249 | 0.409 | 0.371 | 0.779 | 0.542 | 0.346-1.734 |
| Gestational diabetes | 0.492 | 0.353 | 1.943 | 0.163 | 1.636 | 0.822-3.295 |
| Maternal infection | 0.645 | 0.346 | 3.474 | 0.062 | 1.906 | 0.973-3.783 |
| Gestational hypertension | 0.234 | 0.485 | 0.232 | 0.630 | 1.264 | 0.488-3.347 |
| BPD | 0.368 | 0.612 | 0.361 | 0.548 | 1.444 | 0.438-5.104 |
| NRDS | 1.170 | 0.400 | 8.556 | 0.004 | 3.222 | 1.499-7.267 |
| 1 min Apgar score | 0.164 | 0.218 | 0.566 | 0.454 | 1.178 | 0.771-1.834 |
| hsa_circ_0061346 | 1.176 | 0.230 | 26.143 | <0.0001 | 3.240 | 2.231-5.694 |
| hsa_circ_0000095 | -0.728 | 0.131 | 30.880 | <0.0001 | 0.483 | 0.367-0.615 |
| hsa_circ_0068606 | -0.679 | 0.134 | 25.653 | <0.0001 | 0.507 | 0.370-0.641 |

BPD, Bronchopulmonary dysplasia; NRDS, Neonatal respiratory distress syndrome; ROP, retinopathy of prematurity; SE, Standard error; OR, Odds Ratio.

**Table V.** Multivariate logistic regression analysis of factors associated with ROP occurrence

| Factor | β | SE | Wald $\chi^2$ | p | OR | 95% CI |
|---|---|---|---|---|---|---|
| Gestational age | -0.015 | 0.079 | 13.436 | 0.851 | 0.985 | 0.842-1.153 |
| Birth weight | -1.678 | 0.985 | 3.928 | 0.089 | 0.187 | 0.022-1.146 |
| NRDS | 0.952 | 0.726 | 0.924 | 0.190 | 2.592 | 0.619-11.14 |
| hsa_circ_0061346 | 1.195 | 0.262 | 15.914 | <0.0001 | 3.303 | 2.183-6.535 |
| hsa_circ_0000095 | -0.978 | 0.279 | 13.522 | 0.0005 | 0.376 | 0.188-0.574 |
| hsa_circ_0068606 | -1.291 | 0.271 | 41.196 | <0.0001 | 0.275 | 0.147-0.435 |

BPD, Bronchopulmonary dysplasia; NRDS, Neonatal respiratory distress syndrome; ROP, retinopathy of prematurity; SE, Standard error; OR, Odds Ratio.

## Discussion

ROP is a leading cause of childhood blindness and its pathogenesis has not yet been fully elucidated. CircRNAs, as a novel subtype of non-coding RNAs, have emerged as promising biomarkers and potential therapeutic targets in various ophthalmic diseases.[9,10] This study represents the first systematic evaluation of the expression profiles and clinical diagnostic utility of serum hsa_circ_0061346, hsa_circ_0000095, and hsa_circ_0068606 in ROP. Our findings demonstrate that these three circRNAs exhibit aberrant expression in ROP patients, with significant diagnostic and predictive value. Notably, their combined detection achieved an AUC of 0.983, with high sensitivity and specificity, highlighting their substantial potential as molecular biomarkers for early ROP detection. Specifically, serum hsa_circ_0061346 expression was significantly elevated in the ROP group and showed a significant association with an increased risk of ROP occurrence (OR=3.303, $p < 0.0001$). Recent studies have indicated that circRNAs play a critical regulatory role in retinal vascular diseases.[11,12] Li et al. reported the role of altered circRNA expression in peripheral blood mononuclear cells in retinal disorders[13], which supports the observations of this study.

In contrast, hsa_circ_0000095 and hsa_circ_0068606 exhibited significantly reduced

expression in the ROP group and were identified as protective factors, with OR values of 0.376 and 0.275, respectively. These findings suggest that these circRNAs may contribute to suppressing abnormal vascular proliferation associated with ROP or to promoting retinal vascular stability, potentially through competitive binding with miRNAs or the regulation of signaling pathways. hsa_circ_0068606 has been extensively studied in tumor biology, where it promotes cell proliferation and migration by regulating ferroptosis and MAPK-

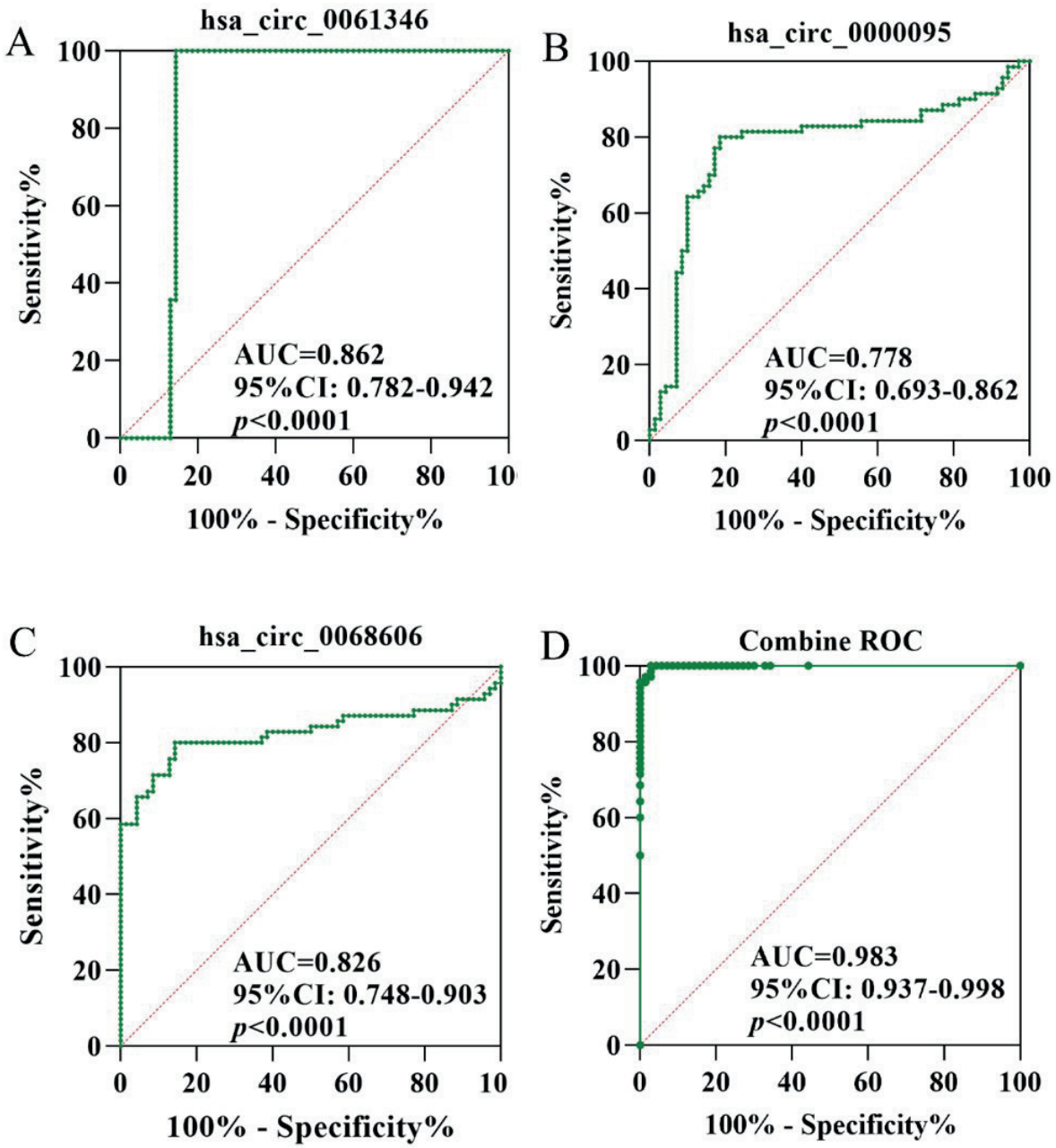



**Fig. 2.** ROC curves for serum hsa_circ_0061346, hsa_circ_0000095, and hsa_circ_0068606 in diagnosing ROP.

(A) ROC curve of hsa_circ_0061346; (B) ROC curve of hsa_circ_0000095; (C) ROC curve of hsa_circ_0068606; (D) Combined ROC curve of the three circular RNAs.

ROC: receiver operating characteristic, ROP: retinopathy of prematurity.

related signaling pathways.[7] Given that ROP pathogenesis involves hypoxia, iron metabolism dysregulation, and oxidative stress.[14-16] the reduced expression of hsa_circ_0068606 observed in this study may indicate a loss of its inhibitory effect on these aberrant signals, thereby promoting ROP progression.

Additionally, the associations between the three circRNAs and specific clinical characteristics suggest differential regulatory patterns under various pathological conditions. For instance, hsa_circ_0000095 expression was elevated in infants with gestational diabetes, while hsa_circ_0068606 levels were higher in those with BPD and lower 1-minute Apgar scores, potentially reflecting adaptive changes in circRNA expression across distinct pathological contexts, which warrants further investigation. Multivariate logistic regression analysis further supported the independent diagnostic value of circRNAs in ROP. After adjusting for perinatal risk factors, the significance of the three circRNAs persisted, whereas conventional indicators such as gestational age and birth weight were no longer statistically significant in the multivariate model. This finding underscores the central role of circRNAs in disease mechanisms and highlights the limitations of current clinical assessment systems, particularly in early disease stages, where molecular biomarkers may outperform traditional clinical characteristics.

However, this study has several limitations. First, the sample size was relatively small, and although intergroup matching was well-controlled, the generalizability and stability of the results needs further validation in multicenter studies with larger cohorts. Second, there was a significant difference in gestational age between the ROP and NC groups, which may have influenced the levels of the circRNAs examined and introduced a potential confounding factor in the interpretation of the findings. Third, the functional mechanisms of circRNAs were not thoroughly validated in *vitro* or in animal models. Future studies should incorporate functional experiments (e.g., miRNA binding and target gene regulation analyses) to elucidate their biological roles. Additionally, while qRT-PCR is a sensitive detection method, its routine clinical application faces challenges related to standardization and operational complexity.

In conclusion, this study demonstrates that hsa_circ_0061346, hsa_circ_0000095, and hsa_circ_0068606 exhibit significantly aberrant expression in the serum of ROP patients and are strongly associated with disease occurrence. Their combined detection substantially enhances diagnostic performance. As stable and specific molecular biomarkers, circRNAs hold promise as novel tools for early ROP screening and staging. Furthermore, these circRNAs may play a critical role in ROP pathogenesis, providing new insights for future mechanistic studies and the development of targeted interventions.

## Ethical approval

The study was approved by The Huadu District Maternal and Child Health Hospital (date: May 21, 2025, number: 2025-033).

## Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: CL, LB; data collection: CL, LD; analysis and interpretation of results: CL, QY; draft manuscript preparation: CL. All authors reviewed the results and approved the final version of the manuscript.

## Source of funding

The authors declare the study received no funding.

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES


1. Sabri K, Ells AL, Lee EY, Dutta S, Vinekar A. Retinopathy of prematurity: a global perspective and recent developments. Pediatrics 2022; 150: e2021053924. https://doi.org/10.1542/peds.2021-053924
2. Hong EH, Shin YU, Cho H. Retinopathy of prematurity: a review of epidemiology and current treatment strategies. Clin Exp Pediatr 2022; 65: 115-126. https://doi.org/10.3345/cep.2021.00773
3. Gebeşçe A, Uslu H, Keleş E, et al. Retinopathy of prematurity: incidence, risk factors, and evaluation of screening criteria. Turk J Med Sci 2016; 46: 315-320. https://doi.org/10.3906/sag-1407-127
4. Kim H, Kim J, Ryu J. Noncoding RNAs as a novel approach to target retinopathy of prematurity. Front Pharmacol 2022; 13: 1033341. https://doi.org/10.3389/fphar.2022.1033341
5. Zhou H, Song H, Wu Y, et al. Oxygen-induced circRNA profiles and coregulatory networks in a retinopathy of prematurity mouse model. Exp Ther Med 2019; 18: 2037-2050. https://doi.org/10.3892/etm.2019.7819
6. Yan Z, Duan C, Li X, et al. circ-TFRC downregulation suppresses ovarian cancer progression via miR-615-3p/IGF2 axis regulation. Cancer Cell Int 2024; 24: 152. https://doi.org/10.1186/s12935-024-03287-4
7. Lin Z, Zhong C, Shi M, et al. Circular RNA TFRC/SCD1 mRNA interaction regulates ferroptosis and metastasis in gastric cancer. Cell Death Dis 2025; 16: 436. https://doi.org/10.1038/s41419-025-07759-x
8. Wilkinson AR, Adams GGW, Fleck BW, Nieto-Hernandez R; Guideline Development Groups (GDG) of the Royal College of Paediatrics and Child Health (RCPCH) and the Royal College of Ophthalmologists (RCOphth). UK screening and treatment of retinopathy of prematurity updated 2022 guidelines. Early Hum Dev 2023; 177-178: 105715. https://doi.org/10.1016/j.earlhumdev.2023.105715
9. Hanineva A, Park KS, Wang JJ, DeAngelis MM, Farkas MH, Zhang SX. Emerging roles of circular RNAs in retinal diseases. Neural Regen Res 2022; 17: 1875-1880. https://doi.org/10.4103/1673-5374.335691
10. Zhang C, Hu J, Yu Y. CircRNA is a rising star in researches of ocular diseases. Front Cell Dev Biol 2020; 8: 850. https://doi.org/10.3389/fcell.2020.00850
11. Wu W, Zhang Y, Yang M. Emerging role of circular RNAs in the pathogenesis of retinoblastoma. Ophthalmic Res 2024; 67: 51-61. https://doi.org/10.1159/000535329
12. Sun LF, Chen XJ, Jin ZB. Emerging roles of non-coding RNAs in retinal diseases: a review. Clin Exp Ophthalmol 2020; 48: 1085-1101. https://doi.org/10.1111/ceo.13806
13. Li Y, Zhou H, Huang Q, et al. Potential biomarkers for retinopathy of prematurity identified by circular RNA profiling in peripheral blood mononuclear cells. Front Immunol 2022; 13: 953812. https://doi.org/10.3389/fimmu.2022.953812
14. Zhang L, Buonfiglio F, Fieß A, Pfeiffer N, Gericke A. Retinopathy of prematurity-targeting hypoxic and redox signaling pathways. Antioxidants (Basel) 2024; 13: 148. https://doi.org/10.3390/antiox13020148
15. Liu CQ, Liu XY, Ouyang PW, et al. Ferrostatin-1 attenuates pathological angiogenesis in oxygen-induced retinopathy via inhibition of ferroptosis. Exp Eye Res 2023; 226: 109347. https://doi.org/10.1016/j.exer.2022.109347
16. Graziosi A, Perrotta M, Russo D, et al. Oxidative stress markers and the retinopathy of prematurity. J Clin Med 2020; 9: 2711. https://doi.org/10.3390/jcm9092711

The Turkish Journal of Pediatrics 2025; 67: 808-817
https://doi.org/10.24953/turkjpediatr.2025.6685

**Original Article**

# Reflections of the 2021 update of the retinopathy of prematurity (ROP) guideline: a single-center retrospective comparative cohort analysis

Sibel Sevük Özumut[1], Ebru Yalın İmamoğlu[1], Serap Karaca[2], Berkay Kısakürek[2], Sertaç Arslanoğlu[3], Hüsnü Fahri Ovalı[3]

*[1]Department of Neonatology, Göztepe Prof. Dr. Süleyman Yalçın City Hospital, İstanbul, Türkiye; [2]Department of Ophthalmology, Göztepe Prof. Dr. Süleyman Yalçın City Hospital, İstanbul, Türkiye; [3]Department of Neonatology, Göztepe Prof. Dr. Süleyman Yalçın City Hospital, Faculty of Medicine, İstanbul Medeniyet University, İstanbul, Türkiye.*

## ABSTRACT

**Background.** We aimed to determine the risk factors for retinopathy of prematurity (ROP) and investigate the effects of the expanded screening criteria according to the 2021 update of the Turkish Neonatology Society guidelines on the clinical outcomes of premature infants and the incidence of severe ROP.

**Materials and Method.** Patient records of infants treated in the neonatal intensive care unit (NICU) between January-December 2020 and January-December 2023, who were identified as at-risk for ROP were retrospectively analyzed. Infants with severe ROP were compared with those without ROP or with mild ROP not requiring treatment in terms of risk factors.

**Results.** Among the cohort of 169 patients at risk of ROP, the median gestational age was 30.2 (interquartile range [IQR]: 27.4-32.1) weeks and the median birth weight was 1354 g (IQR: 920-1760). Severe ROP was detected in 2.9% (n=5) of the premature infants included in the study. When comparing the periods before and after the 2021 guideline update, the incidence of severe ROP was found to be 3.7% vs. 2.2%, respectively (p=0.085). After the 2021 update, the number of infants examined at ≥33 weeks increased approximately 2.5-fold, but no severe ROP was detected in this group. Small gestational age, low birth weight, multiple erythrocyte suspension transfusions, patent ductus arteriosus, prolonged oxygen duration, and prolonged invasive mechanical ventilation were found to be statistically significant risk factors for severe ROP ($p<0.05$).

**Conclusion.** ROP is a significant cause of disability in extremely premature infants. Early diagnosis and treatment with optimum screening criteria can reduce permanent visual damage. As the premature population at risk for ROP evolves, screening criteria must also adapt. The 2021 ROP guideline states that, due to variations in quality of care and patient populations, each center may define its own optimal screening criteria based on local data. However, it is essential to use the expanded 2021 criteria within national ROP screening programs.

**Key words:** prematurity, retinopathy of prematurity, permanent visual damage.

Retinopathy of prematurity (ROP) occurs due to abnormal development of retinal vessels in premature and low birth weight infants and is the leading cause of childhood blindness.[1,2] Technological developments in neonatal intensive care units and improvements in neonatal care have increased the survivorship of extremely premature infants, making it

✉ Sibel Sevük Özümüt ▪ sibel.ozumut@gmail.com

Received 22nd Jul 2025, revised 9th Oct 2025, 19th Nov 2025, accepted 20th Nov 2025.

possible to keep much smaller premature infants alive. As a result, the incidence of ROP is rising worldwide.[3-7] The most well-known risk factors for ROP are low birth weight and small for gestational age (SGA).[8] Many other factors including ethnicity and a country's level of development also influence the incidence of ROP. Therefore, different guidelines are used for the screening, diagnosis, and treatment of ROP around the world. Studies conducted in high-income countries have shown that infants born at ≥ 32 weeks are not at risk for ROP, and most infants born at >28 weeks who develop ROP have mild disease that spontaneously regresses without treatment.[9] On the contrary, countries with lower development indices tend to use broader screening criteria that more accurately reflect the population at risk of ROP.[10] Studies conducted in developing countries, including Türkiye, have reported cases of severe ROP requiring treatment in infants with birth weight (BW) between 1500-2500 grams.[11-14] Consequently, countries need to implement national ROP guidelines tailored to their own sociodemographic characteristics. In a multicenter study conducted by the Turkish Neonatology Society (TR-ROP study), the incidence of ROP in infants with a gestational age (GA) of 33-35 weeks was found to be 6.1% and the incidence of advanced stage ROP was 6 per thousand in this group.[15] The Turkish consensus guidelines on retinopathy of prematurity, created in 2016 by the Turkish Neonatal Society (TNS) in collaboration with the Turkish Ophthalmology Society, were updated in 2021 after evaluation of current literature as well as the TR-ROP study results, and the screening criteria were expanded.[16,17]

In this study, we aimed to investigate the effects of the expanded screening criteria according to the 2021 guideline update on the clinical outcomes of premature infants and the incidence of severe ROP.

## Materials and Methods

### *Study design and setting*

This was a single-center, cross-sectional, retrospective comparative study. The study was conducted in the neonatal intensive care unit (NICU) of a tertiary university hospital in İstanbul, Türkiye, with admits approximately 1000 patients per year. Patient records of infants treated in the NICU between January-December 2020 and January-December 2023, who were identified as at-risk for ROP and underwent ROP examination were analyzed. The study was approved by the Clinical Research Ethics Committee of Göztepe Prof. Dr. Süleyman Yalçın City Hospital (Registration no: 2023/0960).

### *Participants and definitions*

Searching of the data was performed using the hospital's software system for the following International Classification of Diseases 10th Revision (ICD-10) codes: H35.1 for ROP, P07.2 and P07.3 for prematurity.

Demographic and clinical characteristics, diagnostic information, and consultation notes of premature infants who were screened for ROP risk and whose records were fully accessible were retrospectively obtained from electronic patient files. Premature infants treated in the NICU in 2020 were treated and screened for ROP according to the 2016 TNS guidelines. According to this guideline, all infants with a gestational age of ≤ 32 weeks or a birth weight of ≤ 1500 grams as well as infants with BW > 1500 grams or GA > 32 weeks and an unstable clinical course (e.g. those who received cardiopulmonary support or were considered to be at risk by any means) were screened for retinopathy of prematurity.[16] Premature infants hospitalized in the NICU in 2023 were treated and followed for ROP according to the 2021 guideline update. According to this updated

guideline, all infants with a GA of < 34 weeks or a BW of ≤ 1700 grams were determined as at risk for retinopathy of prematurity. Preterm infants with a gestational age of ≥ 34 weeks or a BW of > 1700 grams who received cardiopulmonary support therapy or who were considered at risk for ROP by the clinician following them were also screened.[17]

Demographic characteristics of premature infants screened according to the 2016 and 2021 ROP guidelines as well as antenatal, natal and postnatal risk factors for ROP were reported. These risk factors were antenatal corticosteroid use, preeclampsia/eclampsia, infants of diabetic mothers, clinical chorioamnionitis, multiple pregnancy, mode of delivery, male gender, early gestational age, low birth weight, respiratory distress syndrome (RDS), prolonged duration of invasive mechanical ventilation, total duration of oxygen requirement, bronchopulmonary dysplasia (BPD) defined by oxygen requirement at a postmenstrual age of 36 gestational weeks (GW), intraventricular hemorrhage (IVH) > grade II according to Volpe staging[18], hemodynamically significant patent ductus arteriosus (PDA), necrotizing enterocolitis (NEC) ≥ stage II according to modified Bell's criteria [19] and the number of red blood cell (RBC) transfusions (20 mL/kg per transfusion).

Infants who died before the first ROP examination were excluded from the study. These infants were included only in the cohort mortality rate. After pupil dilation with 2.5% phenylephrine (Mydfirin, Alcon, USA) and 0.5% cyclopentolate (Sikloplejin, Abdi İbrahim, Turkey), anterior segment examination with a light source was performed, followed by fundus examination using an indirect ophthalmoscope and a 20-diopter lens. Infants with premature retinopathy were monitored at a frequency deemed appropriate by the ophthalmologist, while those without ROP findings were monitored every 1 to 2 weeks until retinal vascularization was complete. Findings were recorded in accordance with the International Classification of Premature Retinopathy.

### *Statistical analysis*

SPSS (Statistical Packages for Social Sciences: SPSS Inc, Chicago, IL, USA) software version 20.0 was used for all statistical calculations and analyses. Demographic data and diagnosis distributions were analyzed using descriptive analysis methods. In descriptive analyses, mean ± standard deviation was used for variables that conformed to normal distribution and median (interquartile range [IQR]) was used for variables that did not conform to normal distribution.

When comparing the incidence of severe ROP and clinical data before and after the 2021 ROP guideline update, an independent samples t-test was used for continuous variables with a normal distribution, while the Mann–Whitney U test was applied for variables without a normal distribution. Categorical variables were compared using the chi-square test, and Fisher's exact test was applied when appropriate. A p-value < 0.05 was considered statistically significant.

## Results

During the study period, 18.3% (n=195) of the 1061 newborns treated in the level 3 neonatal intensive care unit were identified as at risk for ROP. Of these infants 13.3% (n=26) died before their first ROP examination could be performed. Among those who died, 23% (n=6) had major congenital anomalies, and 65.3% (n=17) were extremely premature infants born between 22-24 weeks of gestation. The data of these infants were excluded from the analysis. The demographic and clinical characteristics of premature infants who were followed and treated due to the risk of ROP according to the criteria before and after the 2021 guideline update are compared in Table I. Severe ROP rates in infants evaluated according to screening criteria before and after the 2021 guideline update were 3.7% and 2.2%, respectively; this difference was not statistically significant. (p= 0.085) (Table II). When newborns at risk for ROP were grouped according to gestational

**Table I.** Comparison of demographic and clinical characteristics of newborns screened before and after the 2021 ROP guideline update

| Patients features | Before 2021 update, n=81 | After 2021 update, n=88 | p |
|---|---|---|---|
| Demographic features | | | |
| Gestational age,† weeks+days, median (IQR) | 29+5 (26+2-31+2) | 31+4 (28+5-33+1) | <0.001 |
| Birth weight,† g, median (IQR) | 1235 (833-1528) | 1497 (110-2022) | <0.001 |
| Male sex, n (%) | 46 (56.8%) | 37 (42%) | 0.306 |
| Vaginal delivery, n (%) | 14 (17.3%) | 8 (9%) | 0.262 |
| Multıple pregnancy n (%) | 11 (13.6%) | 8 (9%) | 0.82 |
| Twin | 9 (11.1%) | 7 (7.9%) | |
| Triplet | 2 (2.4%) | 1 (1.1%) | |
| Clinical features | | | |
| RDS, n (%) | 60 (74%) | 52 (59%) | 0.154 |
| PDA requiring treatment, n (%) | 19 (23.4%) | 18 (20.4%) | 0.808 |
| IVH > grade II, n (%) | 12 (14.8%) | 8 (9%) | 0.170 |
| NEC ≥ grade II, n (%) | 10 (12.3%) | 4 (4.5%) | 0.063 |
| BPD, n (%) | 29 (35.8%) | 24 (27.2%) | 0.249 |
| Mild | 10 (12.3%) | 14(15.9%) | |
| Moderate | 14 (17.2%) | 7 (7.9%) | |
| Severe | 5 (6.2%) | 3 (3.4%) | |
| Frequency of ET, n (%) | | | |
| Once | 19 (23.4%) | 14 (15.9%) | 0.185 |
| More than once | 16 (19.7%) | 11(12.5%) | |
| Severe ROP, n (%) | 3 (3.7%) | 2 (2.3%) | 0.837 |
| Duration IMV,† days, median (IQR) | 2 (0-11) | 1 (0-3) | 0.040 |
| Total days on oxygen,† median (IQR) | 13 (3-36) | 2 (0-15) | <0.001 |
| Duration of hospital stay,† days, median (IQR) | 40 (25-78) | 29 (12-55) | 0.019 |

BPD: Bronchopulmonary dysplasia; ET: Erythrocyte suspension transfusion; IMV: Invasive mechanical ventilation; IQR: Interquartile range (25th to 75th percentiles); IVH: Intraventricular hemorrhage; NEC: Necrotizing enterocolitis; PDA: Patent ductus arteriosus; RDS: Respiratory distress syndrome; ROP: Retinopathy of prematurity.
†Fisher's exact test p-value < 0.05.

age, severe ROP was observed only in babies born at 22-27 GW. Although the number of infants who underwent ROP examination at ≥ 33 gestational weeks increased approximately 2.5-fold after the 2021 update, no severe ROP cases were detected in this group (Table II).

Infants diagnosed with severe ROP requiring treatment were compared with those without ROP or with self-resolving ROP at any stage in terms of associated risk factors (Table III). Small gestational age, low birth weight, prolonged invasive mechanical ventilation, and duration of oxygen requirement significantly increased the risk of severe ROP (p< 0.001).

## Discussion

Improvements in neonatal care globally have increased the survival of extremely premature infants and the incidence of ROP is rising accordingly. In the light of rapidly evolving literature, the national ROP diagnosis and treatment guideline, originally created in 2016, was updated in 2021. In this study, we found that the changes in screening criteria with the

**Table II.** Severe ROP incidence by gestational age (before vs after 2021 update)

| Patient groups | | | Any ROP or no ROP n (%) | Severe ROP n (%) | P |
|---|---|---|---|---|---|
| Before 2021 update n=81 | GA groups | 22-27 wk | 20 (24.7%) | 3 (3.7%) | |
| | | 28-32 wk | 46 (56.7%) | 0 | |
| | | ≥ 33 wk | 12 (14.8%) | 0 | 0.085 |
| After 2021 update n=88 | GA groups | 22-27 wk | 13 (14.7%) | 2 (2.3%) | |
| | | 28-32 wk | 41 (46.5%) | 0 | |
| | | ≥ 33 wk | 32 (36.3%) | 0 | |

GA: gestational age; ROP: Retinopathy of prematurity.
Fisher's exact test p-value < 0.05.

**Table III.** Maternal and neonatal risk factors associated with severe retinopathy of prematurity

| | Any ROP or no ROP (n=164) | Severe ROP (n=5) | p |
|---|---|---|---|
| Antenatal features | | | |
| Antenatal steroid, two doses, n (%) | 79 (48.1%) | 3 (60%) | 0.68 |
| Preeclampsia, n (%) | 53 (21.3%) | 3 (60%) | 0.33 |
| Gestational diabetes, n (%) | 12 (7.3%) | 1 (20%) | 0.33 |
| Chorioamnionitis, n (%) | 10 (6%) | 0 (0) | 0.73 |
| Vaginal delivery, n (%) | 15 (9.1%) | 0 (0) | 0.61 |
| Clinical features | | | |
| Gestastional age,† weeks, median (IQR) | 31 (23-24) | 24 (24-25) | <0.001 |
| Male sex, n (%) | 91 (55.4%) | 0 (0%) | 0.018* |
| Birth weight,† g, , median (IQR) | 1440 (550-3590) | 560 (430-690) | <0.001 |
| IMV duration,† days, median (IQR) | 1 (0-85) | 70 (43-101) | <0.001 |
| Total days on oxygen,† median (IQR) | 6 (0-104) | 98 (70-156) | <0.001 |
| Hospital stay,† days, median (IQR) | 33 (3-180) | 125 (103-213) | <0.001 |
| IVH, n (%) | 19 (11.5%) | 1 (20%) | 0.48 |
| PDA, n (%) | 33 (20.1%) | 4 (80%) | 0.001* |
| NEC, n (%) | 11 (6.7%) | 3 (60%) | 0.004* |
| BPD, n ( %) | 48 (29.2%) | 5 (100%) | 0.003* |
| ET more than once, n (%) | 22 (13.4%) | 5 (100%) | <0.001* |

BPD: Bronchopulmonary dysplasia; ET: Erythrocyte suspension transfusion; IMV: Invasive mechanical ventilation; IQR: Interquartile range (25th to 75th percentiles); IVH: Intraventricular hemorrhage; NEC: Necrotizing enterocolitis; PDA: Patent ductus arteriosus; ROP: Retinopathy of prematurity.
†Fisher's exact test p-value < 0.05.

2021 update did not change the incidence of severe ROP or clinical outcomes in our cohort.

Severe ROP is a multifactorial disorder that can lead to blindness in premature infants. Its incidence is associated with well-known clinical risk factors such as younger gestational age and lower birth weight, the duration and concentration of oxygen therapy, hyperoxia/hypoxia, as well as sociodemographic risk factors related to countries and families. Due to the diversity of the cohort at risk for ROP, the incidence of severe ROP has been reported in a very wide range of 3-44% in the literature.[5-7,9,14,20] In Türkiye, two large multicenter studies conducted in 2015 and 2018 reported the rate of severe ROP as 5% and 6.7%, respectively.[11,15]In a 2012 study by the department of ophthalmology

of our hospital, examining premature infants at risk of ROP in our unit, the incidence of severe ROP was as high as 22.6%.[21]

In the current study, incidence of severe ROP was 2.9%, which is lower than both national multicenter studies and our unit's rate reported in 2012. The 85% reduction in severe ROP incidence in our unit in the last 13 years is likely associated with increased clinical experience of the healthcare team, improved access to advanced technical equipment, and strengthened multidisciplinary collaborations. As reported in the BIG-ROP study, the incidence of severe ROP is associated with the level of hospitals providing NICU services.[22] With the optimal quality of care of premature infants in NICU, risk factors that increase ROP such as NEC, BPD, IVH, and prolonged oxygen exposure and mechanical ventilation can be minimized.

Studies from developing countries including our own, have reported severe ROP cases requiring treatment in infants with a BW of 1500-2500 grams.[11,14,15,20] In line with these findings, the 2021 guideline update aims to detect ROP cases that may develop in more mature infants during screening and to enable their early treatment. An increase in the rate of severe ROP can be anticipated in programs conducted with expanded screening criteria. However, in a study by Kaya Guner and Inci Bozbıyık, the guideline was compared before and after the update and it was reported that although the rate of ROP at any stage increased after the update, the rate of severe ROP decreased.[23]

In the current study, the rates of severe ROP were similar between the cohorts before and after the 2021 guideline update. However, some important points should be taken into consideration when interpreting these results. In this comparative cross-sectional study, the number of premature babies between 22-27 GW- the group in which severe ROP was most frequently seen- was coincidentally higher in the pre-update group than in the post-update group. This may falsely create the impression that the rate of severe ROP decreased in the post-update period. As expected, median gestational age and birth weight were higher in this group because expanded screening criteria were used after the update. The duration of invasive mechanical ventilation, total oxygen use time, and hospital stay were longer in the pre-update group, which we believe is a reflection of this. In conclusion, the two retrospective cohorts compared in this study were not homogeneously distributed. Therefore, comparing the ROP rate before and after the update may be misleading. However, what is striking in our study is that there were no cases of severe ROP observed above 27 GW in either cohort. This result is parallel to the results of developed countries.[9,24]

Although the number of infants who underwent ROP examination at ≥33 GW increased by approximately 2.5 times following the expansion of screening criteria after the 2021 update, no severe ROP cases were detected. On the other hand, given that only a small number of patients require treatment and since ophthalmologic examination of these very premature infants may result in adverse conditions such as tachycardia, apnea and pain, some authors have proposed risk calculators such as DIGIROP (develop a prediction tool) calculator, in order to determine which infants most need ophthalmologic examination.[25] However, use of these calculators has not yet gained widespread acceptance.

Given the wide variation in healthcare systems, healthcare financing across countries and the socioeconomic status of families of premature infants, ROP screening should be tailored to local settings. The population of premature infants at risk for ROP may change as the quality of care in neonatal intensive care units improves and technical infrastructure becomes standardized. Therefore, the most appropriate screening criteria may also change in the future. Emerging technologies such as ROP video cameras and new biomarkers for the prevention, early diagnosis, and treatment of ROP will bring new opportunities for ROP screening.[10,26]

The 2021 ROP guideline also emphasizes that the quality of care in neonatal intensive care units may vary from unit to unit and that centers should determine the upper limits of birth weight and gestational age for ROP screening based on their own patient populations and epidemiological data. However, the National ROP Guideline updated in 2021 is based on the results of a large-scale study involving numerous centers across the country.[15] Considering the differences in quality of care among centers and the demographic characteristics of patients in our country, the use of the updated and expanded screening criteria is essential. This ensures that all infants at risk for severe ROP can be safely and effectively identified and monitored.

The most important risk factors for retinopathy of prematurity are small gestational age and low birth weight.[8] The Multicenter Trial of Cryotherapy for Retinopathy of Prematurity (CRYO-ROP), which demonstrated that these risk factors are inversely proportional to the risk of developing ROP, reported that each 100 g increase in BW reduced the probability of reaching threshold (severe) ROP by 27% and each weekly increase in GA reduced the probability of reaching the threshold disease by 19%.[27] There are many other studies showing similar results.[24,28-32] The results of the current study are consistent with the literature and confirm that small gestational age and low birth weight are important risk factors for severe ROP.

Mechanical ventilation treatment and oxygen delivery according to the infant's needs are vital for the survival of premature infants. However, it has also been reported that oxygen treatment is an important risk factor for ROP.[33] The increase in ROP due to high oxygen concentration can be explained by several mechanisms: An increase in free oxygen radicals and oxidant damage causes apoptosis and vasoobliteration in vascular endothelial cells.[34,35] BPD, which is associated with prolonged oxygen therapy, has been reported to contribute to the development of ROP. [28,36-38] In this study the increase in the duration of mechanical ventilation and oxygen treatment evidently increases the incidence of severe ROP. Great care should be taken when applying noninvasive ventilation methods in neonatal intensive care units, and it should not be forgotten that oxygen is a drug and that its dose must be adjusted precisely.

Studies have shown that repeated transfusions with adult blood increase the risk for ROP.[32,39,40] This is associated with the fact that 2.3 diphosphoglycerate in adult red blood cell suspensions causes lower oxygen binding to hemoglobin, thus increasing the amount of oxygen delivered to the tissues. Similar to the literature, we also observed that ≥ 2 RBC transfusions significantly increased the incidence of severe ROP. Strategies that reduce anemia in premature infants including late cord clamping after birth may reduce the incidence of ROP by enabling less frequent RBC transfusions.

Problems associated with advanced prematurity, including advanced NEC (stage ≥2), BPD, hemodynamically significant PDA, and IVH are known to increase the risk of ROP.[32,41-43] In this study due to the limited sample size, independent risk factors could not be analyzed using logistic regression. In the univariate analysis, hemodynamically significant PDA, advanced necrotizing enterocolitis, and bronchopulmonary dysplasia were found to significantly increase the incidence of severe ROP. These findings were consistent with the literature. However, advanced IVH, which is among the known risk factors, was not found to be associated with severe ROP in our study. This finding may be related to the low number of advanced IVH cases in our cohort. Awareness of the risk factors that contribute to ROP and implementing neonatal intensive care strategies that will reduce these factors may decrease the development of ROP.

### *Study strengths and limitations*

In this study, we reported the data before and after the 2021 TNS ROP guideline update with a comparative cohort analysis. This is one of the few studies investigating the results of the expanded screening criteria. Further guideline updates will likely be required as more data from similar studies accumulate and as the premature population at risk of ROP continues to evolve in the future.

This study has several limitations. First, the pre- and post-update cohorts compared retrospectively were not homogeneously distributed. Second, not all risk factors associated with ROP (such as genetic predisposition, postnatal growth restriction, and episodes of sepsis) were evaluated. The third limitation of the study is that, due to the small sample size, logistic regression analysis could not be performed to identify independent risk factors contributing to the development of ROP. Therefore, univariate analysis was used to evaluate the risk factors.

### *Conclusion*

Retinopathy of prematurity is an important cause of disability that is influenced by several risk factors and can result in permanent visual damage without screening and early treatment. Optimum ROP screening criteria and early treatment algorithms aim to ensure the survival of premature infants without sequelae. As the premature population at risk for ROP continues to change, it is likely that screening criteria will also need to adapt accordingly.

## Ethical approval

The study was approved by Istanbul Medeniyet University Göztepe Training and Research Hospital Clinical Research Ethics Committee (date: December 20, 2023, number: 2023/0960).

## Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: SSÖ, EYİ, FO; data collection: SSÖ, SK, BK; analysis and interpretation of results: SSÖ, FO, SA, EYİ, SK, BK; draft manuscript preparation: SSÖ, SA, FO, EYİ. All authors reviewed the results and approved the final version of the manuscript.

## Source of funding

The authors declare the study received no funding.

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES

1. Good WV, Hardy RJ, Dobson V, et al. The incidence and course of retinopathy of prematurity: findings from the early treatment for retinopathy of prematurity study. Pediatrics 2005; 116: 15-23. https://doi.org/10.1542/peds.2004-1413
2. Hartnett ME, Penn JS. Mechanisms and management of retinopathy of prematurity. N Engl J Med 2012; 367: 2515-2526. https://doi.org/10.1056/NEJMra1208129
3. Hussain N, Clive J, Bhandari V. Current incidence of retinopathy of prematurity, 1989-1997. Pediatrics 1999; 104: e26. https://doi.org/10.1542/peds.104.3.e26
4. Lad EM, Hernandez-Boussard T, Morton JM, Moshfeghi DM. Incidence of retinopathy of prematurity in the United States: 1997 through 2005. Am J Ophthalmol 2009; 148: 451-458. https://doi.org/10.1016/j.ajo.2009.04.018
5. Younge N, Goldstein RF, Bann CM, et al. Survival and neurodevelopmental outcomes among periviable infants. N Engl J Med 2017; 376: 617-628. https://doi.org/10.1056/NEJMoa1605566
6. Brumbaugh JE, Hansen NI, Bell EF, et al. Outcomes of extremely preterm infants with birth weight less than 400 g. JAMA Pediatr 2019; 173: 434-445. https://doi.org/10.1001/jamapediatrics.2019.0180

7. Ying GS, Quinn GE. Nationwide increase in the incidence of retinopathy of prematurity in the US-A growing problem? JAMA Ophthalmol 2023; 141: 486-487. https://doi.org/10.1001/jamaophthalmol.2023.0926

8. Avery GB, Glass P. Retinopathy of prematurity: progress report. Pediatr Ann 1988; 17: 528-533. https://doi.org/10.3928/0090-4481-19880801-10

9. Holmström G, Hellström A, Jakobsson P, Lundgren P, Tornqvist K, Wallin A. Evaluation of new guidelines for ROP screening in Sweden using SWEDROP - a national quality register. Acta Ophthalmol 2015; 93: 265-268. https://doi.org/10.1111/aos.12506

10. Gilbert CE. Screening for retinopathy of prematurity: does one size fit all? Arch Dis Child Fetal Neonatal Ed 2016; 101: F280-F281. https://doi.org/10.1136/archdischild-2015-310129

11. Bas AY, Koc E, Dilmen U, ROP Neonatal Study Group. Incidence and severity of retinopathy of prematurity in Turkey. Br J Ophthalmol 2015; 99: 1311-1314. https://doi.org/10.1136/bjophthalmol-2014-306286

12. Gilbert C. Retinopathy of prematurity: a global perspective of the epidemics, population of babies at risk and implications for control. Early Hum Dev 2008; 84: 77-82. https://doi.org/10.1016/j.earlhumdev.2007.11.009

13. Chaudhry TA, Hashmi FK, Salat MS, et al. Retinopathy of prematurity: an evaluation of existing screening criteria in Pakistan. Br J Ophthalmol 2014; 98: 298-301. https://doi.org/10.1136/bjophthalmol-2013-304018

14. Roohipoor R, Karkhaneh R, Farahani A, et al. Retinopathy of prematurity screening criteria in Iran: new screening guidelines. Arch Dis Child Fetal Neonatal Ed 2016; 101: F288-F293. https://doi.org/10.1136/archdischild-2015-309137

15. Bas AY, Demirel N, Koc E, et al. Incidence, risk factors and severity of retinopathy of prematurity in Turkey (TR-ROP study): a prospective, multicentre study in 69 neonatal intensive care units. Br J Ophthalmol 2018; 102: 1711-1716. https://doi.org/10.1136/bjophthalmol-2017-311789

16. Koç E, Baş YA, Özdek Ş, et al; TOD ROP Commission, TND ROP Working Group. Turkey retinopathy of prematurity guideline 2016. Turkish Neonatology Association and Turkish Ophthalmology Association; 2016: 4-53.

17. Koç E, Baş YA, Özdek Ş, et al; Turkish Neonatology Association, Turkish Ophthalmology Association. Turkish Retinopathy of Prematurity Guideline 2021. Turkish Neonatology Association and Turkish Ophthalmology Association; 2021: 3-84.

18. Inder TE, Perlman JM, Volpe JJ. Preterm intraventricular hemorrhage/posthemorrhagic hydrocephalus. In: Volpe JJ, editor. Volpe's Neurology of the Newborn. 6th. Philadelphia, PA: Elsevier; 2018: 637-698. https://doi.org/10.1016/B978-0-323-42876-7.00024-7

19. Walsh MC, Kliegman RM. Necrotizing enterocolitis: treatment based on staging criteria. Pediatr Clin North Am 1986; 33: 179-201. https://doi.org/10.1016/s0031-3955(16)34975-6

20. Gaber R, Sorour OA, Sharaf AF, Saad HA. Incidence and risk factors for retinopathy of prematurity (ROP) in biggest neonatal intensive care unit in Itay Elbaroud City, Behera Province, Egypt. Clin Ophthalmol 2021; 15: 3467-3471. https://doi.org/10.2147/OPTH.S324614

21. Akçakaya AA, Yaylali SA, Erbil HH, et al. Screening for retinopathy of prematurity in a tertiary hospital in Istanbul: incidence and risk factors. J Pediatr Ophthalmol Strabismus 2012; 49: 21-25. https://doi.org/10.3928/01913913-20110208-01

22. Özdek Ş, Ozdemir HB, Ozen Tunay Z, et al. Clinical and demographic characteristics of treatment requiring retinopathy of prematurity in big premature infants in Turkiye: Report No. 1 (BIG-ROP Study). Ophthalmologica 2024; 247: 293-303. https://doi.org/10.1159/000541053

23. Kaya Güner E, Inci Bozbiyik D. Evaluation of the update for screening for retinopathy of prematurity in a tertiary care center in Türkiye with retrospective cohorts. Turk J Med Sci 2024; 54: 1295-1301. https://doi.org/10.55730/1300-0144.5912

24. Lundgren P, Kistner A, Andersson EM, et al. Low birth weight is a risk factor for severe retinopathy of prematurity depending on gestational age. PLoS One 2014; 9: e109460. https://doi.org/10.1371/journal.pone.0109460

25. Pivodic A, Johansson H, Smith LEH, et al. Development and validation of a new clinical decision support tool to optimize screening for retinopathy of prematurity. Br J Ophthalmol 2022; 106: 1573-1580. https://doi.org/10.1136/bjophthalmol-2020-318719

26. Karunatilake M, Daspal S, Mugarab Samedi V, Rubab S. Screening for retinopathy of prematurity through utilization a pediatric retinal camera at Jim Pattison Children's Hospital: a vision for improved care. Glob Pediatr Health 2021; 8: 2333794X211039642. https://doi.org/10.1177/2333794X211039642

27. Schaffer DB, Palmer EA, Plotsky DF, et al. Prognostic factors in the natural course of retinopathy of prematurity. The Cryotherapy for Retinopathy of Prematurity Cooperative Group. Ophthalmology 1993; 100: 230-237. https://doi.org/10.1016/s0161-6420(93)31665-9

28. Kim SJ, Port AD, Swan R, Campbell JP, Chan RVP, Chiang MF. Retinopathy of prematurity: a review of risk factors and their clinical significance. Surv Ophthalmol 2018; 63: 618-637. https://doi.org/10.1016/j.survophthal.2018.04.002

29. Karna P, Muttineni J, Angell L, Karmaus W. Retinopathy of prematurity and risk factors: a prospective cohort study. BMC Pediatr 2005; 5: 18. https://doi.org/10.1186/1471-2431-5-18

30. Allvin K, Hellström A, Dahlgren J, Andersson Grönlund M. Birth weight is the most important predictor of abnormal retinal vascularisation in moderately preterm infants. Acta Paediatr 2014; 103: 594-600. https://doi.org/10.1111/apa.12599

31. Celebi AR, Petricli IS, Hekimoglu E, Demirel N, Bas AY. The incidence and risk factors of severe retinopathy of prematurity in extremely low birth weight infants in Turkey. Med Sci Monit 2014; 20: 1647-1653. https://doi.org/10.12659/MSM.892262

32. Thomas K, Shah PS, Canning R, Harrison A, Lee SK, Dow KE. Retinopathy of prematurity: risk factors and variability in Canadian neonatal intensive care units. J Neonatal Perinatal Med 2015; 8: 207-214. https://doi.org/10.3233/NPM-15814128

33. Patz A, Hoeck LE, DeLaCruz E. Studies on the effect of high oxygen administration in retrolental fibroplasia. Am J Ophthalmol 1952; 35: 1248-1253. https://doi.org/10.1016/0002-9394(52)91140-9

34. Sun Y, Hellström A, Smith LEH. Retinopathy of prematurity. In: Martin RJ, Fanaroff AA, Walsh MC, editors. Fanaroff and Martin's Neonatal-Perinatal Medicine. 12th ed. Philadelphia, PA: Elsevier; 2024: 2064-2072.

35. Hellström A, Smith LEH, Dammann O. Retinopathy of prematurity. Lancet 2013; 382: 1445-1457. https://doi.org/10.1016/S0140-6736(13)60178-6

36. Harrell SN, Brandon DH. Retinopathy of prematurity: the disease process, classifications, screening, treatment, and outcomes. Neonatal Netw 2007; 26: 371-378. https://doi.org/10.1891/0730-0832.26.6.371

37. Chen M, Citil A, McCabe F, et al. Infection, oxygen, and immaturity: interacting risk factors for retinopathy of prematurity. Neonatology 2011; 99: 125-132. https://doi.org/10.1159/000312821

38. Enomoto H, Miki A, Matsumiya W, Honda S. Evaluation of oxygen supplementation status as a risk factor associated with the development of severe retinopathy of prematurity. Ophthalmologica 2015; 234: 135-138. https://doi.org/10.1159/000433565

39. Wardle SP, Drury J, Garr R, Weindling AM. Effect of blood transfusion on lipid peroxidation in preterm infants. Arch Dis Child Fetal Neonatal Ed 2002; 86: F46-F48. https://doi.org/10.1136/fn.86.1.f46

40. Hesse L, Eberl W, Schlaud M, Poets CF. Blood transfusion. Iron load and retinopathy of prematurity. Eur J Pediatr 1997; 156: 465-470. https://doi.org/10.1007/s004310050641

41. Seiberth V, Linderkamp O. Risk factors in retinopathy of prematurity: a multivariate statistical analysis. Ophthalmologica 2000; 214: 131-135. https://doi.org/10.1159/000027482

42. Hagadorn JI, Richardson DK, Schmid CH, Cole CH. Cumulative illness severity and progression from moderate to severe retinopathy of prematurity. J Perinatol 2007; 27: 502-509. https://doi.org/10.1038/sj.jp.7211780

43. Fundora JB, Binenbaum G, Tomlinson L, et al. Association of surgical necrotizing enterocolitis and its timing with retinopathy of prematurity. Am J Perinatol 2023; 40: 1178-1184. https://doi.org/10.1055/s-0041-1733785

The Turkish Journal of Pediatrics 2025; 67: 818-828
https://doi.org/10.24953/turkjpediatr.2025.6251

**Original Article**

# Predictive value of dynamic plasma biomarkers for clinical outcomes in pediatric sepsis

Jiping Tian[1], Jing Song[1], Fudong Wang[1], Feng Liu[1], Lijun Jiang[1]

[1]*Department of Pediatrics, Affiliated Hospital of Yangzhou University, Yangzhou, Jiangsu Province, China.*

## ABSTRACT

**Background.** Pediatric sepsis is a heterogeneous syndrome; data on early biomarker kinetics and their link to severity are scarce.

**Methods.** We prospectively enrolled 80 children with sepsis (March 2022 – June 2024). C-reactive protein (CRP), procalcitonin (PCT), erythrocyte sedimentation rate (ESR), interleukin-6 (IL-6), serum amyloid-A (SAA), and D-dimer were measured at admission (T0), 72 hours (T1) later and on Day 7 (T2). Disease severity was assessed using the pediatric Sequential Organ Failure Assessment (pSOFA); length of stay (LOS) was recorded. Baseline values, Day 7 levels, and changes Δ(T2–T0) were correlated with pSOFA and LOS.

**Results.** Baseline inflammatory profiles differed by etiology: median CRP and PCT on admission were roughly doubled in bacterial versus viral disease, while IL-6 was highest in respiratory and abdominal infections. Nevertheless, all six markers decreased significantly over seven days ($p \leq 0.015$) and the proportional declines were uniform across pathogens or foci (interaction $p > 0.18$). Higher admission CRP, PCT, IL-6 and D-dimer modestly correlated with greater organ dysfunction ($r \leq 0.55$), whereas steeper week-long falls in the same markers tracked with larger pSOFA improvement ($r = –0.41$ to $–0.53$; all $p \leq 0.002$). SAA showed a weaker inverse association ($r = –0.32$, $p = 0.008$), whereas the decline in ESR was not significant. A pragmatic two-step algorithm (admission CRP ≥ 60 mg/L, PCT ≥ 3 ng/mL, IL-6 ≥ 200 pg/mL or D-dimer ≥ 1.5 mg/L; plus a ≥ 50% drop in IL-6 or PCT within 72 h) identified children who ultimately required intensive care unit (ICU) care or stayed ≥ 7 days with an area-under-the-curve of 0.91.

**Conclusions.** Both initial elevations and early declines in CRP, PCT, IL-6 and D-dimer mirror organ dysfunction and hospitalization duration in pediatric sepsis. Serial monitoring of these readily available markers may improve early risk stratification and guide therapy.

**Key words:** pediatric sepsis, dynamic plasma biomarkers, C-reactive protein, procalcitonin, interleukin-6.

Pediatric sepsis is an infection driven systemic inflammatory response that can quickly lead to organ failure and shock. The Surviving Sepsis Campaign criteria and the pediatric Sequential Organ Failure Assessment (pSOFA) score remain the main tools for grading severity.[1-3] Yet sepsis is still one of the top causes of childhood death worldwide.[1,4] Age related immune maturation shapes infection risk and disease course, making early diagnosis and precise risk stratification—and therefore timely treatment—difficult.[5-7].

Biomarkers underpin early diagnosis, therapy monitoring, and prognosis in sepsis, with serial measurements offering clearer views of response and disease course.[8,9] In children, key analytes include C-reactive protein (CRP), procalcitonin (PCT), erythrocyte sedimentation

✉ Lijun Jiang ▪ jianglijunjs@163.com

Received 2nd May 2025, revised 21st Jun 2025, accepted 25th Aug 2025.

rate (ESR), interleukin-6 (IL-6), serum amyloid-A (SAA), and D-dimer.[7,8] Although these tests sharpen diagnostic accuracy, they often require specialized assays and may encourage antibiotic overuse.[7] Composite panels can improve early screening but remain costly and unstandardized, highlighting the need for affordable, validated multimarker workflows across diverse pediatric populations.[7] Biomarker work in sepsis has focused mainly on adults, where markers such as vascular endothelial growth factor (VEGF) and soluble fms-like tyrosine kinase receptor-1 (sFlt 1) reliably flag poor outcomes.[10] Pediatric evidence is limited—only a handful of studies profile biomarkers in children.[11-13] Even fewer link serial declines to clinical recovery.[14] Comprehensive pediatric studies across illness severities are needed to sharpen risk stratification and tailor care.

The six biomarkers—CRP, SAA, PCT, IL-6, ESR, and D-dimer—represent distinct yet complementary pathways in the pathophysiology of sepsis, facilitating early diagnosis and management in pediatric patients. These biomarkers are routinely available within a 24-hour laboratory panel, with a turnaround time of less than two hours.[15] Competing candidates like presepsin and pro-adrenomedullin were excluded due to the lack of robust pediatric reference ranges or higher costs.[16] Studies indicate that IL-6 and PCT are particularly effective in distinguishing bacterial infections, while combinations of these biomarkers can yield high predictive values for sepsis severity.[8,17]

We aimed to track six biomarkers —CRP, PCT, IL-6, SAA, ESR, and D-dimer— over the first seven days of pediatric sepsis and see how their trends match pSOFA scores. We also tested whether these patterns predicted length of stay, intensive care unit (ICU) admission, and major complications. We expect high baseline levels and slow declines to signal worse illness and longer hospital stays, while faster falls should coincide with recovery.

## Materials and Methods

### *Study design and setting*

We carried out a single center, prospective observational study in the Department of Pediatrics, Affiliated Hospital of Yangzhou University, from March 2022 to June 2024. Consecutive children who met consensus diagnostic criteria for sepsis were enrolled and followed prospectively. Key inflammatory biomarkers were quantified at predefined time points from admission through Day 7, alongside detailed clinical assessments. All procedures adhered to institutional pediatric sepsis pathways and local standards of care.

The protocol was approved by the Affiliated Hospital of Yangzhou University Ethics Committee and conducted in accordance with the Declaration of Helsinki. Before enrolment, the child's parents or legal guardians received a comprehensive explanation of the study's objectives, procedures, potential risks, and benefits, and subsequently provided written informed consent. All participant data were anonymized to safeguard confidentiality.

Inclusion Criteria:

1. Children aged 1 month to 14 years were considered eligible if they presented with clinical signs of sepsis, according to the International Pediatric Sepsis Consensus Conference (IPSCC) criteria, i.e., suspected/proven infection plus ≥2 age-adjusted systemic inflammatory response syndrome (SIRS) criteria (abnormal temperature or leukocyte count mandatory).[18]
2. Children who had a documented need for inpatient care, and were able to undergo serial blood sampling at the designated time points
3. Patients were included irrespective of infection source or pathogen type, as long as the attending pediatrician deemed sepsis the most likely clinical diagnosis

4. Additional requirements included the ability of a parent or legal guardian to provide signed informed consent within 24 hours of admission
5. Willingness to comply with the study protocol.

Exclusion Criteria:

1. Children with known severe primary immunodeficiency
2. Children who were receiving or had recently received high-dose immunosuppressive therapy
3. Children with documented malignancies.
4. Patients with an anticipated survival of less than 3 months due to terminal non-infectious illnesses
5. Those whose parents or guardians declined serial blood sampling
6. Any child whose clinical team anticipated transfer to another facility
7. Those who were physically unable to participate.

### *Local pediatric sepsis pathway*

The hospital implements a nurse-triggered electronic Sepsis Early-Warning System (SEWS) in the emergency department. Key steps are: 1) Recognition and first hour (ED): SEWS ≥ 4 prompts immediate senior review. Blood cultures and complete blood count are drawn before antibiotics. Broad-spectrum therapy is delivered within 60 min (third-generation cephalosporin ± vancomycin for community-acquired, piperacillin-tazobactam ± amikacin for healthcare-associated). 2) Resuscitation bundle: Isotonic crystalloid 20–40 mL/kg is infused over the first 3 h. Point-of-care lactate, glucose and electrolytes are measured; vasoactive drugs are started if fluid-refractory shock occurs. 3) Admission criteria: Children with pSOFA ≥ 6, need for vasoactive or invasive ventilation proceed directly to the 15-bed pediatric ICU (PICU). Stable children (pSOFA < 6) are admitted to a 12-bed high-dependency observation unit (HDU) within the general pediatric ward (nurse:patient = 1:3, continuous monitoring). 4) Serial monitoring (ward or PICU): CRP, PCT, IL-6, SAA, ESR and D-dimer are repeated at 72 h and Day 7. pSOFA is recalculated at the same time-points. Daily fluid balance, vasoactive-inotropic score and respiratory support level are documented. 5) Pathogen-directed de-escalation: Antibiotics are narrowed to culture-confirmed monotherapy within 72 h whenever susceptibilities allow, following local antimicrobial-stewardship guidelines. 6) Step-down or discharge: Transfer from PICU/HDU to the general ward requires pSOFA ≤ 2, lactate < 2 mmol/L and no vasoactive support for ≥ 12 h. Discharge criteria include afebrile status for 24 hours, oral intake > 60% of requirement and caregiver education.

### *Data collection*

Upon admission (T0), demographics (age, sex, weight), clinical parameters (vital signs), and suspected infection sites (respiratory, abdominal, etc.) were recorded. Baseline blood samples were collected for CRP, PCT, IL-6, SAA, ESR, and D-dimer. Subsequent samples were drawn on Day 3 (T1) and Day 7 (T2), or at discharge if earlier. Information on interventions (antibiotics, vasopressors, mechanical ventilation), durations of hospital stay (LOS), and any complications was documented in standardized case report forms.

pSOFA was computed at admission (T0), 72 h (T1) and Day 7 (T2) using the validated pediatric adaptation of the SOFA score.[19] For each time-point we assigned 0–4 points to six organ systems: respiratory ($PaO_2/FiO_2$ ratio), coagulation (platelet count), liver (total bilirubin), cardiovascular (mean arterial pressure and/or vasoactive-inotropic score), central nervous system (Glasgow Coma Scale), and renal (serum creatinine). When a laboratory or clinical value was missing at a scheduled time point and there was no previous abnormal value for that organ system, a score of 0 was assigned. This conservative rule has been used

in prior pSOFA validation studies. The total pSOFA ranged from 0 to 24.

### *Biomarker sampling and laboratory methods*

Each blood sample (2–3 mL) was collected from a peripheral vein and immediately transported to the hospital laboratory. Levels of CRP and D-dimer were measured by immunoturbidimetric assay, PCT was determined by chemiluminescence, IL-6 and SAA were assayed by enzyme-linked immunosorbent assay (ELISA), and ESR was analyzed by an automated analyzer. All assays followed manufacturer-recommended protocols, and results were expressed in standard units, with calibration and internal quality control checks performed daily to ensure assay reliability.

### *Statistical analysis*

All data were entered into a secure electronic database and checked for completeness and accuracy. Descriptive statistics, including means ± standard deviations or medians (with interquartile ranges), were used to summarize continuous variables, while frequencies and percentages described categorical variables. Biomarker changes over time were analyzed by repeated-measures analysis of variance (ANOVA) or the Friedman test, with post hoc pairwise comparisons if significant. Spearman's correlation coefficients were calculated to assess relationships between biomarker levels (or their changes) and pSOFA scores. Where relevant, logistic regression techniques were employed to identify independent predictors of prolonged LOS or ICU admission. To enhance clinical utility, we generated ROC curves for CRP, PCT, IL-6 and D-dimer against (i) admission pSOFA ≥ 4 and (ii) hospital stay ≥ 7 days. Optimal cut-offs were selected by the Youden index and reported with area under the curve (AUC), sensitivity and specificity. Statistical significance was set at $p < 0.05$.

*Missing data*: Biomarker measurements were complete at admission; 5% were missing at 72 h and 6% at Day 7 due to hemolysis or insufficient volume. We used pair-wise deletion for non-modelled correlations and restricted maximum-likelihood estimation for mixed-effects models, both of which are unbiased under a missing-at-random assumption. Sensitivity analyses with last-observation-carried-forward produced identical direction and significance.

*Log-scale sensitivity analysis*: Because plasma biomarker elimination approximates first-order (exponential) decay, we repeated all correlation and regression analyses using natural-log–transformed concentrations. The change over 7 days was calculated as $\Delta\ln = \ln(T2) - \ln(T0) = \ln(T2/T0)$. Spearman correlations with ΔpSOFA and multivariable linear models (adjusted for age, time-to-antibiotic < 60 min and early de-escalation) were fitted. Predictive performance for favorable outcome (Day 7 pSOFA ≤ 2) was compared with DeLong's test.

## Results

### *Patient characteristics*

Eighty children fulfilled the enrolment criteria between March 2022 and June 2024 (Fig. 1). Their mean age was 6.2 ± 3.1 years (range 1–14); boys and girls were evenly represented. Only nine patients (11%) carried a significant underlying condition, most often congenital heart disease (5%). Respiratory (38%) and abdominal (25%) infections dominated the case-mix, and a microbiological pathogen was identified in 78% of episodes. Median time from symptom onset to hospital arrival was two days and the mean admission pSOFA score was 3.8 ± 1.5, indicating largely mild-to-moderate organ dysfunction (Table I).

Baseline biomarker concentrations varied meaningfully by both pathogen and infection focus. Children with proven bacterial sepsis entered the hospital with CRP and PCT levels roughly twice those seen in viral cases, while admission IL-6 was highest in respiratory and abdominal infections but lowest in urinary-tract disease (Supplementary Tables S1 and S2).

### *Management and global outcomes*

Timely adherence to the institutional sepsis pathway yielded favorable short-term outcomes. Only eight children (10%) required PICU admission and two (2.5%) underwent brief mechanical ventilation (median 2 days). The median hospital stay was nine days (interquartile range, Q1-Q3: 7–10), and no deaths occurred (Table II). These figures confirm that the cohort largely represents early-recognized, lower-severity sepsis.

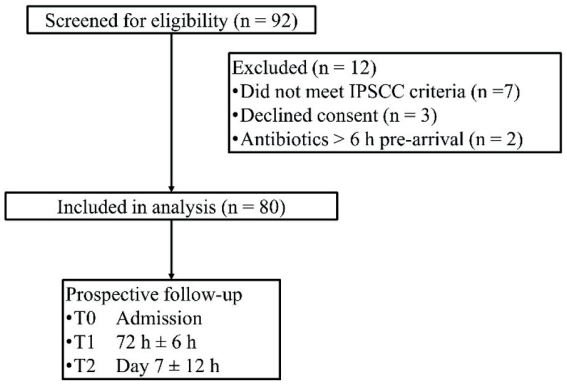



**Fig. 1.** Flowchart for participant enrollment.

**Table I.** Baseline characteristics.

| | |
|---|---|
| Age (years), mean±SD (range) | 6.2 ± 3.1 (1–14) |
| Sex, n (%) | |
| Male | 40 (50.0%) |
| Female | 40 (50.0%) |
| Weight (kg), median (IQR) | 15.0 (10.0–20.5) |
| Underlying conditions, n (%) | |
| Immunodeficiency | 3 (3.8%) |
| Congenital heart disease | 4 (5.0%) |
| Others (e.g., mild asthma) | 2 (2.5%) |
| Primary infection site, n (%) | |
| Respiratory | 30 (37.5%) |
| Abdominal / GI | 20 (25.0%) |
| Urinary tract | 10 (12.5%) |
| Others / Unknown | 20 (25.0%) |
| Pathogen identified, n (%) | |
| Bacterial | 35 (43.8%) |
| Viral | 25 (31.3%) |
| Fungal | 2 (2.5%) |
| Mixed / unknown | 18 (22.5%) |
| pSOFA score at admission | 3.8 ± 1.5 |
| Time from symptom onset to admission (days), median (IQR) | 2.0 (1.0–3.0) |

GI, gastrointestinal; IQR, interquartile range; pSOFA, Pediatric Sequential Organ Failure Assessment; SD, standard deviation.

### *Temporal kinetics of inflammatory biomarkers*

All six biomarkers decreased significantly over the first week ($p \leq 0.015$ for each). Median CRP fell by 69% (65 to 20 mg/L) and PCT by 71% (3.5 to 1.0 ng/mL). IL-6 showed the steepest drop —an 82% fall from 220 to 40 pg/mL— while ESR declined more modestly (32 to 24 mm/h). D-dimer reduction mirrored clinical improvement, halving from 1.8 to 0.8 mg/L (Table III).

Pathogen stratification revealed broadly similar kinetic patterns: regardless of whether infection was bacterial, viral or mixed, median CRP and IL-6 fell by at least 50% and 60% respectively over seven days, with no statistically significant interaction (Supplementary Table S3), demonstrating that serial rather than single measurements are needed to gauge treatment response, irrespective of microbiological etiology.

### *Relationship between biomarkers and organ dysfunction*

Higher CRP, PCT, IL-6 and D-dimer levels at admission correlated modestly with worse organ dysfunction (Spearman $r = 0.40 – 0.55$; all $p \leq 0.003$). Conversely, larger seven-day declines

**Table II.** Clinical management and outcomes.

| | |
|---|---|
| ICU admission, n (%) | 8 (10.0%) |
| Duration of ICU stay (days), median (IQR) | 5 (4–7) |
| Mechanical ventilation, n (%) | 2 (2.5%) |
| Duration of mechanical ventilation (days), median (IQR) | 2 (2–3) |
| Length of hospital stay (days), median (IQR) | 9 (7–10) |
| In-hospital mortality, n (%) | 0 (0.0%) |

IQR, interquartile range; ICU, intensive care unit

**Table III.** Biomarker levels at different time points.

| Biomarker | T0 (Admission) | T1 (72 hours) | T2 (Day 7) | Change Δ (T2–T0) | p-value |
|---|---|---|---|---|---|
| CRP (mg/L) | 65.2 (40.0–90.0) | 40.1 (25.0–60.0) | 20.0 (10.0–30.0) | –45.2 (–60.0 to –30.0) | < 0.001 |
| PCT (ng/mL) | 3.5 (1.0–8.0) | 2.0 (0.8–5.0) | 1.0 (0.5–2.0) | –2.5 (–5.0 to –1.0) | 0.002 |
| IL-6 (pg/mL) | 220 (150–320) | 120 (70–200) | 40 (20–70) | –180 (–250 to –80) | < 0.001 |
| SAA (mg/L) | 50.0 (35.0–80.0) | 35.0 (20.0–60.0) | 15.0 (10.0–25.0) | –35.0 (–55.0 to –20.0) | < 0.001 |
| ESR (mm/h) | 32 ± 9 | 28 ± 8 | 24 ± 7 | –8 ± 3 | 0.015 |
| D-dimer (mg/L) | 1.8 (1.0–3.0) | 1.2 (0.6–2.0) | 0.8 (0.4–1.5) | –1.0 (–2.2 to –0.5) | 0.010 |

Levels are presented as mean ± standard deviation, or median (interquartile range).
CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IL-6, interleukin-6; PCT, procalcitonin; SAA, serum amyloid A.

in the same four biomarkers tracked closely with greater pSOFA improvement (r = –0.41 to –0.53; p ≤ 0.002). SAA decline showed a weaker but still significant relationship (r = –0.32, p = 0.008), whereas the ESR decline was not statistically significant (r = –0.18, p = 0.11) (Table IV).

Importantly, neither pathogen category nor infection focus modified these biomarker-outcome relationships; all interaction p values exceeded 0.18 (Supplementary Table S4). Overall organ failure resolved quickly: mean pSOFA declined from 3.8 at admission to 2.9 at 72 h and 1.9 by Day 7 (Supplementary Table S5).

### *Biomarker profiles and length of hospital stay*

Children discharged within one week started with lower CRP, PCT, IL-6 and D-dimer values than peers remaining beyond Day 7, and their biomarkers fell more steeply—e.g. a median IL-6 drop of 200 pg/mL versus 100 pg/mL in the prolonged-stay group (Table V). These biochemical differences translated into a median hospital stay of five versus ten days, underscoring the prognostic value of early trajectories.

### *Diagnostic performance and sensitivity analyses*

Natural-log transformation confirmed that larger relative (percentage) falls in CRP, PCT, IL-6 and D-dimer remained robustly associated with ΔpSOFA after adjustment for age, time-to-antibiotic and early de-escalation (Supplementary Table S6).

Receiver-operating-characteristic analysis identified pragmatic admission cut-offs — CRP ≥ 60 mg/L, PCT ≥ 3 ng/mL, IL-6 ≥200 pg/mL and D-dimer ≥1.5 mg/L— that predicted either pSOFA ≥4 or hospital stay ≥7 days with AUCs between 0.75 and 0.86 (Supplementary Table S7). Combining the admission thresholds with a ≥50% fall in IL-6 or PCT by 72 h yielded a composite C-statistic of 0.91 for ICU admission or prolonged stay, suggesting a practical two-step algorithm for early risk stratification.

**Table IV.** Correlation of biomarker levels and changes with disease severity (pSOFA).

| Biomarker decline Δ(T2–T0) | Spearman r with ΔpSOFA | p-value |
|---|---|---|
| CRP | –0.44 | 0.001 |
| PCT | –0.47 | < 0.001 |
| IL-6 | –0.53 | < 0.001 |
| D-dimer | –0.41 | 0.002 |
| SAA | –0.32 | 0.008 |
| ESR | –0.18 | 0.11 |

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; IL-6, interleukin-6; PCT, procalcitonin; pSOFA, pediatric Sequential Organ Failure Assessment; SAA, serum amyloid A.

**Table V.** Association of admission biomarkers and their changes with length of hospital stay.

| Length of hospital stay | < 7 Days (n = 35) | ≥ 7 Days (n = 45) | p-value |
|---|---|---|---|
| CRP at T0 (mg/L) | 40.0 (25.0–60.0) | 75.0 (50.0–100.0) | 0.002 |
| ΔCRP (T2–T0) | –25.0 (–35.0 to –15.0) | –10.0 (–20.0 to 0.0) | 0.015 |
| PCT at T0 (ng/mL) | 2.0 (1.0–5.0) | 5.0 (2.0–10.0) | 0.005 |
| ΔPCT (T2–T0) | –2.0 (–4.0 to –1.0) | –1.0 (–2.0 to 0.5) | 0.020 |
| IL-6 at T0 (pg/mL) | 150 (80–220) | 280 (200–400) | < 0.001 |
| ΔIL-6 (T2–T0) | –200 (–260 to –120) | –100 (–150 to –50) | 0.008 |
| D-dimer at T0 (mg/L) | 1.0 (0.5–2.0) | 2.5 (1.5–3.5) | 0.010 |
| ΔD-dimer (T2–T0) | –1.5 (–2.0 to –0.8) | –0.5 (–1.0 to 0.0) | 0.025 |

Changes in levels are presented as median (interquartile range).
Δ, Change in; CRP, C-reactive protein; IL-6, interleukin-6; PCT, procalcitonin.

## Discussion

In our cohort of children with moderate sepsis, concentrations of all six biomarkers—CRP, PCT, IL 6, SAA, ESR, and D dimer—declined significantly during the first week of hospitalization. Higher baseline levels correlated with greater disease severity, while larger week long reductions were associated with shorter hospital stays and fewer ICU admissions.

Our data corroborate earlier pediatric studies: CRP, PCT, IL 6 and the remaining biomarkers fell sharply between admission and Day 7, and higher initial levels signaled greater disease severity.[20-22] Although these analytes are standard in adult sepsis, developmental differences alter their kinetics and diagnostic thresholds; adult cohorts therefore require different cut offs.[23,24] Direct comparisons confirm distinct temporal profiles of biomarker elevation in children, highlighting the need for age adjusted criteria.[25] Pediatric series also report higher ICU admission rates and more frequent acute kidney injury than adult counterparts.[26] The concordance of our findings with child focused investigations strengthens the evidence that PCT, in particular, is a sensitive marker for early diagnosis and prognosis in pediatric sepsis.[16,27,28] Beyond classical inflammatory markers, a 2025 study from our region showed that plasma thiol/disulfide imbalance—a surrogate of oxidative stress—distinguishes septic from non septic PICU patients and independently predicts organ failure.[29] Integrating such redox indices with the cytokine acute phase panel we report could further refine early risk assessment.

IL 6 peaked at admission and showed the strongest correlations with both pSOFA scores and length of stay. A prospective PICU study recently identified an IL 6 threshold of 178 pg/mL that predicted new onset multiple organ dysfunction syndrome (MODS) with 97% sensitivity.[30] Mechanistically, this early cytokine surge activates the canonical Janus kinase / signal transducer and activator of transcription 3 (JAK/STAT3) pathway, amplifying hepatic acute phase protein synthesis and promoting endothelial injury, thereby predisposing patients to coagulopathy.[31] PCT rose rapidly in our cohort as well but normalized sooner than CRP, a kinetic profile consistent with the BATCH randomized trial, where a PCT guided algorithm safely shortened intravenous antibiotic exposure compared with CRP based usual care.[32] The slower decline of CRP reflects its IL 6–dependent hepatic production and clearance dynamics.[33] SAA exhibited the highest admission spike yet maintained a prolonged plateau in children with extended hospitalization, mirroring evidence that high affinity binding to HDL stabilizes circulating SAA and delays catabolism.[34] Finally, persistently elevated D dimer through Day 3 independently predicted longer stays, echoing

a 2025 pediatric study that linked raised D dimer to micro coagulopathy and MODS.[35] Concurrently, an elevated lactate/albumin ratio (LAR) predicted a 28 day mortality in hospitalized children with nosocomial infection.[36] Because LAR reflects metabolic perfusion rather than inflammation, combining it with our inflammatory biomarkers could yield a more holistic bedside risk score.

The pronounced, time dependent declines in our biomarker panel illustrate the superiority of serial measurements over single snapshots: real time data enable earlier risk stratification and timelier therapeutic adjustments.[12,14] Children whose CRP or PCT levels remain elevated, or fall only slowly, may therefore require closer surveillance or intensified support, a strategy reinforced by threshold based algorithms that individualize care according to biomarker trajectories.[8,37,38] Embedding these trends into routine protocols allows clinicians to judge antibiotic response more accurately and to detect complications earlier, an approach now increasingly advocated for pediatric units, particularly those with constrained ICU capacity.[39-42] Collectively, our findings support dynamic biomarker monitoring as a cornerstone of personalized management, with the potential to improve outcomes in children with mild to moderate sepsis.

Pediatric mortality prediction still relies largely on physiology-based scores such as Pediatric Risk of Mortality (PRISM) III/IV, Pediatric Index of Mortality (PIM) 3 and Pediatric Logistic Organ Dysfunction (PELOD) 2, each calculated from the worst values in the first 24 h after PICU admission and each demanding a substantial laboratory and monitoring footprint.[43-46] In high resource settings PRISM III/IV discriminates mortality with an AUC around 0.84[47]; Chinese validation work reported a slightly lower AUC of 0.76 for PRISM IV but a comparable 0.80 for PELOD 2[44], underscoring real world variability. The newer pSOFA, which adapts SOFA thresholds for age, requires similar data volume yet achieved an AUC of 0.82 and out performed SIRS in multicenter Chinese cohorts.[48] Within our early recognition, ward based cohort the median admission pSOFA was 4 and fell to 2 by Day 7, mirroring the moderate severity case mix described in those national studies. Importantly, the two step kinetic rule we propose (a four marker admission panel plus IL 6 or PCT decline by 72 h) predicted ICU stay or prolonged LOS with a C statistic of 0.91, doing so before the 24 h data window that PRISM/PIM/PELOD require. Because the kinetic rule relies on assays already drawn for routine care, it offers a rapid, low cost triage adjunct that can flag low risk responders for early de escalation or identify children whose physiology has yet to deteriorate enough to trigger the traditional scores. Future multicenter work should test whether combining ΔpSOFA with this biomarker trajectory further boosts discrimination across the full spectrum of pediatric sepsis severity.

This was a single-center study conducted in a tertiary referral hospital; results may not apply to district hospitals or low-resource settings. Our cohort was drawn exclusively from a high-dependency observation unit that sits between the emergency department and the PICU. Although this model is increasingly common in tertiary centers, it is not universal. The resulting case-mix (moderate severity, zero mortality) may therefore under-represent the sickest pediatric sepsis phenotypes. Prospective validation in higher-acuity settings will be essential before our two-step biomarker algorithm can be generalized. The total sample size limited power for subgroup analyses, particularly the fungal subgroup. We enrolled patients under the 2005 IPSCC definition; although 94% also met the 2023 Phoenix criteria on admission, future studies should adopt Phoenix criteria prospectively. We adjusted for time-to-antibiotic and early de-escalation, but could not control for all potential confounders such as cytokine-directed therapies because none were used. Finally, advanced biomarkers like presepsin and pro-adrenomedullin were not measured; their additive value remains unknown.

In this prospective cohort of 80 children with early-recognized sepsis, four readily available admission biomarkers —CRP, PCT, IL-6, and D-dimer— identified the sickest quartile (pSOFA ≥4) with an AUC of 0.84. Fall in IL-6 or PCT within 72 h predicted rapid organ recovery with 85% sensitivity and 78% specificity. The combination of these admission cut-offs plus the 72-h reduction index achieved a C-statistic of 0.91 and a negative-predictive-value of 0.92 for ICU admission or length-of-stay ≥ 7 days. Taken together, this two-step algorithm can (i) support early de-escalation or shortening of antibiotic courses in low-risk responders, (ii) flag non-responders who may need escalation or adjunctive therapies, and (iii) provide an objective criterion for safe transfer from high-dependency care to the ward or for hospital discharge.

## Supplementary materials

Supplementary materials for this article are available online at https://doi.org/10.24953/turkjpediatr.2025.6251.

## Ethical approval

The study was approved by Ethics Committee of Affiliated Hospital of Yangzhou University (date: 08.06.2021, number: yz202134).

## Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: LJ; data collection: JT, JS, FW, FL; analysis and interpretation of results: JT, JS, FW, FL; draft manuscript preparation: JT, JS, FW, FL, LJ. All authors reviewed the results and approved the final version of the manuscript.

## Source of funding

The authors declare that the study is supported/funded by Maternal and Child Health Research Project of Jiangsu Province, grant number: F202071; Maternal and Child Health Outstanding Talent Project of Jiangsu Province, grant number: SWBFY 2021-9.

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES

1. Bracken A, Lenihan R, Khanijau A, Carrol E. The aetiology and global impact of paediatric sepsis. Curr Pediatr Rep 2023; 11: 204-213. https://doi.org/10.1007/s40124-023-00305-3
2. Vaughn LH. Sepsis. In: Pediatric Surgery. Cham: Springer; 2023: 85-95. https://doi.org/10.1007/978-3-030-81488-5_8
3. Plunkett A, Tong J. Sepsis in children. BMJ 2015; 350: h3017. https://doi.org/10.1136/bmj.h3017
4. Molloy EJ, Bearer CF. Paediatric and neonatal sepsis and inflammation. Pediatr Res 2022; 91: 267-269. https://doi.org/10.1038/s41390-021-01918-4
5. Wheeler DS. Introduction to pediatric sepsis. Open Inflamm J 2011; 4: 1-3. https://doi.org/10.2174/1875041901104010001
6. Wheeler DS, Wong HR, Zingarelli B. Pediatric Sepsis - Part I: "Children are not small adults!". Open Inflamm J 2011; 4: 4-15. https://doi.org/10.2174/1875041901104010004
7. Esposito S, Mucci B, Alfieri E, Tinella A, Principi N. Advances and challenges in pediatric sepsis diagnosis: integrating early warning scores and biomarkers for improved prognosis. Biomolecules 2025; 15: 123. https://doi.org/10.3390/biom15010123
8. Leonard S, Guertin H, Odoardi N, et al. Pediatric sepsis inflammatory blood biomarkers that correlate with clinical variables and severity of illness scores. J Inflamm (Lond) 2024; 21: 7. https://doi.org/10.1186/s12950-024-00379-w
9. Sumra B, Abdul QS. Pediatric sepsis: early detection, management, and outcomes - a systematic review. Int J Multidiscip Res 2020; 2: 1-15. https://doi.org/10.36948/ijfmr.2020.v02i06.12104
10. Whitney JE, Silverman M, Norton JS, Bachur RG, Melendez E. Vascular endothelial growth factor and soluble vascular endothelial growth factor receptor as novel biomarkers for poor outcomes in children with severe sepsis and septic shock. Pediatr Emerg Care 2020; 36: e715-e719. https://doi.org/10.1097/PEC.0000000000001638

11. Z Oikonomakou M, Gkentzi D, Gogos C, Akinosoglou K. Biomarkers in pediatric sepsis: a review of recent literature. Biomark Med 2020; 14: 895-917. https://doi.org/10.2217/bmm-2020-0016

12. Lanziotti VS, Póvoa P, Soares M, Silva JR, Barbosa AP, Salluh JI. Use of biomarkers in pediatric sepsis: literature review. Rev Bras Ter Intensiva 2016; 28: 472-482. https://doi.org/10.5935/0103-507X.20160080

13. Wang X, Li R, Qian S, Yu D. Multilevel omics for the discovery of biomarkers in pediatric sepsis. Pediatr Investig 2023; 7: 277-289. https://doi.org/10.1002/ped4.12405

14. Downes KJ, Fitzgerald JC, Weiss SL. Utility of Procalcitonin as a biomarker for sepsis in children. J Clin Microbiol 2020; 58: e01851-e01819. https://doi.org/10.1128/JCM.01851-19

15. Silman NJ. Rapid diagnosis of sepsis using biomarker signatures. Crit Care 2013; 17: 1020. https://doi.org/10.1186/cc13137

16. Zhu S, Zeng C, Zou Y, Hu Y, Tang C, Liu C. The Clinical Diagnostic Values of SAA, PCT, CRP, and IL-6 in Children with Bacterial, Viral, or Co-Infections. Int J Gen Med 2021; 14: 7107-7113. https://doi.org/10.2147/IJGM.S327958

17. Boenisch S, Fae P, Drexel H, Walli A, Fraunberger P. Are circulating levels of CRP compared to IL-6 and PCT still relevant in intensive care unit patients? Laboratoriumsmedizin 2013; 37. https://doi.org/10.1515/LABMED-2013-0029

18. Goldstein B, Giroir B, Randolph A; International Consensus Conference on Pediatric Sepsis. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. Pediatr Crit Care Med 2005; 6: 2-8. https://doi.org/10.1097/01.PCC.0000149131.72248.E6

19. Matics TJ, Pinto NP, Sanchez-Pinto LN. Association of organ dysfunction scores and functional outcomes following pediatric critical illness. Pediatr Crit Care Med 2019; 20: 722-727. https://doi.org/10.1097/PCC.0000000000001999

20. Tyagi N, Gawhale S, Patil MG, Tambolkar S, Salunkhe S, Mane SV. Comparative analysis of C-reactive protein and procalcitonin as biomarkers for prognostic assessment in pediatric sepsis. Cureus 2024; 16: e65427. https://doi.org/10.7759/cureus.65427

21. Kumar V, Neelannavar RV. Serum levels of CRP and procalcitonin as early markers of sepsis in children above the neonatal age group. Int J Contemp Pediatr 2019; 6: 411-415. https://doi.org/10.18203/2349-3291.IJCP20190037

22. Murthy GRR, Pradeep R, Sanjay KS, Kumar V, Sreenivas SK. Acute-phase reactants in pediatric sepsis with special reference to C-reactive protein and procalcitonin. Indian J Child Health 2015; 2: 118-121. https://doi.org/10.32677/IJCH.2015.v02.i03.005

23. Onyenekwu CP, Okwundu CI, Ochodo EA. Procalcitonin, C-reactive protein, and presepsin for the diagnosis of sepsis in adults and children. Cochrane Database Syst Rev 2017; 4: CD012627. https://doi.org/10.1002/14651858.CD012627

24. Aneja RK, Carcillo JA. Differences between adult and pediatric septic shock. Minerva Anestesiol 2011; 77: 986-992.

25. Marassi C, Socia D, Larie D, An G, Cockrell RC. Children are small adults (when properly normalized): transferrable/generalizable sepsis prediction. Surg Open Sci 2023; 16: 77-81. https://doi.org/10.1016/j.sopen.2023.09.013

26. Stanski NL, Gist KM, Hasson D, et al. Characteristics and outcomes of children and young adults with sepsis requiring continuous renal replacement therapy: a comparative analysis from the Worldwide Exploration of Renal Replacement Outcomes Collaborative in Kidney Disease (WE-ROCK). Crit Care Med 2024; 52: 1686-1699. https://doi.org/10.1097/CCM.0000000000006405

27. Baldua V, Castellino N, Kadam P, Avasthi B, Kabra N. Is procalcitonin a better option than CRP in diagnosing pediatric sepsis? RGUHS J Med Sci 2016; 6: 73-78. https://doi.org/10.26463/rjms.6_1_11

28. Plesko M, Suvada J, Makohusova M, et al. The role of CRP, PCT, IL-6 and presepsin in early diagnosis of bacterial infectious complications in paediatric haemato-oncological patients. Neoplasma 2016; 63: 752-760. https://doi.org/10.4149/neo_2016_512

29. Arı HF, Arı M, Ogut S. Oxidative stress and anti-oxidant status in children with sepsis. BMC Pharmacol Toxicol 2025; 26: 64. https://doi.org/10.1186/s40360-025-00895-2

30. Mohamed Said Abdelfattah WA, Hafez MZ, Ahmed ME, et al. Interleukin (6, 10): can be used as a prediction tool for multiple organ dysfunction syndrome in critically ill pediatric patients? Journal of Clinical Pediatrics and Mother Health 2024; 2: 019.

31. Hirano T. IL-6 in inflammation, autoimmunity and cancer. Int Immunol 2021; 33: 127-148. https://doi.org/10.1093/intimm/dxaa078

32. Waldron CA, Pallmann P, Schoenbuchner S, et al. Procalcitonin-guided duration of antibiotic treatment in children hospitalised with confirmed or suspected bacterial infection in the UK (BATCH): a pragmatic, multicentre, open-label, two-arm, individually randomised, controlled trial. Lancet Child Adolesc Health 2025; 9: 121-130. https://doi.org/10.1016/S2352-4642(24)00306-7

33. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J Clin Invest 2003; 111: 1805-1812. https://doi.org/10.1172/JCI18921

34. Sack GH. Serum amyloid A - a review. Mol Med 2018; 24: 46. https://doi.org/10.1186/s10020-018-0047-0

35. Shrikiran A, Palanichamy A, Kumar S, et al. D-dimer as a marker of clinical outcome in children with sepsis: a tertiary-care experience. Journal of Comprehensive Pediatrics 2025; 16: e152358. https://doi.org/10.5812/jcp-152358

36. Arı HF, Keskin A, Arı M, Aci R. Importance of lactate/albumin ratio in pediatric nosocomial infection and mortality at different times. Future Microbiol 2024; 19: 51-59. https://doi.org/10.2217/fmb-2023-0125

37. Alder MN, Lindsell CJ, Wong HR. The pediatric sepsis biomarker risk model: potential implications for sepsis therapy and biology. Expert Rev Anti Infect Ther 2014; 12: 809-816. https://doi.org/10.1586/14787210.2014.912131

38. Wong HR. Pediatric sepsis biomarkers for prognostic and predictive enrichment. Pediatr Res 2022; 91: 283-288. https://doi.org/10.1038/s41390-021-01620-5

39. He RR, Yue GL, Dong ML, Wang JQ, Cheng C. Sepsis biomarkers: advancements and clinical applications-a Narrative review. Int J Mol Sci 2024; 25: 9010. https://doi.org/10.3390/ijms25169010

40. Smith KA, Bigham MT. Biomarkers in pediatric sepsis. The Open Inflammation Journal 2011; 4(Suppl 1-M4): 24-30. https://doi.org/10.2174/1875041901104010024

41. Iragamreddy VR. Innovations in pediatric sepsis management: from biomarkers to bedside monitoring. IOSR J Dent Med Sci 2025; 24: 38-39. https://doi.org/10.9790/0853-2401013839

42. Schuetz P, Plebani M. Can biomarkers help us to better diagnose and manage sepsis? Diagnosis (Berl) 2015; 2: 81-87. https://doi.org/10.1515/dx-2014-0073

43. Arı HF, Reşitoğlu S, Tuncel MA, Şerbetçi MC. Comparison between mortality scoring systems in pediatric intensive care unit: reliability and effectiveness. Pamukkale Med J 2024; 17: 664-673. https://doi.org/10.31362/patd.1479595

44. Zhang Z, Huang X, Wang Y, et al. Performance of three mortality prediction scores and evaluation of important determinants in eight pediatric intensive care units in China. Front Pediatr 2020; 8: 522. https://doi.org/10.3389/fped.2020.00522

45. Angurana SK, Dhaliwal M, Choudhary A. Unified severity and organ dysfunction scoring system in pediatric intensive care unit: a pressing priority. Journal of Pediatric Critical Care 2023; 10: 181-183. https://doi.org/10.4103/jpcc.jpcc_50_23

46. Zhang L, Wu Y, Huang H, et al. Performance of PRISM III, PELOD-2, and P-MODS scores in two pediatric intensive care units in China. Front Pediatr 2021; 9: 626165. https://doi.org/10.3389/fped.2021.626165

47. Shen Y, Jiang J. Meta-analysis for the prediction of mortality rates in a pediatric intensive care unit using different scores: PRISM-III/IV, PIM-3, and PELOD-2. Front Pediatr 2021; 9: 712276. https://doi.org/10.3389/fped.2021.712276

48. Liu R, Yu ZC, Xiao CX, et al. Different methods in predicting mortality of pediatric intensive care units sepsis in Southwest China. Zhonghua Er Ke Za Zhi 2024; 62: 204-210. https://doi.org/10.3760/cma.j.cn112140-20231013-00282

The Turkish Journal of Pediatrics 2025; 67: 829-840
https://doi.org/10.24953/turkjpediatr.2025.6969

**Original Article**

# Clinical significance of human herpesvirus 6 detected in cerebrospinal fluid: a 10-year retrospective study in children

Jung Sook Yeom[1,2], Young-Soo Kim[2,3], Ji Sook Park[1,2], Eun Sil Park[1,2], Ji-Hyun Seo[1,2], Jae-Young Lim[1,2], Hyang-Ok Woo[1,2]

*[1]Department of Pediatrics, Gyeongsang National University Hospital, Jinju, Korea; [2]Institute of Medical Science, School of Medicine, Gyeongsang National University, Jinju, Korea; [3]Department of Neurology, Gyeongsang National University Hospital, Jinju, Korea.*

## ABSTRACT

**Background.** Human herpesvirus 6 (HHV-6) is occasionally detected in the cerebrospinal fluid (CSF) of young children, but its clinical significance remains uncertain. This study aimed to describe HHV-6–positive cases and to explore features that may help distinguish presumed infection from bystander detection.

**Methods.** We retrospectively reviewed pediatric patients with CSF HHV-6 detected by multiplex polymerase chain reaction or the FilmArray Meningitis/Encephalitis (FA-ME) panel between January 2015 and March 2025 at a single tertiary hospital. Cases were categorized as presumed HHV-6 infection or bystander detection based on clinical features and the presence of alternative pathogens or diagnoses. Clinical and laboratory findings were compared between the two groups.

**Results.** Among 1,865 children tested, HHV-6 was detected in 25 (1.3%; median age, 6 months), all of whom presented with fever. Seizures occurred in seven (28%) and ataxia in one (4%). Two patients developed encephalitis; one had abnormal imaging and later developed epilepsy. Seventeen patients were classified as presumed infection. In this group, rash was more prevalent (59% vs. 13%, $p = 0.04$), neutrophil and platelet counts were lower at admission and declined further at follow-up ($p < 0.05$), and aspartate aminotransferase (AST) levels were higher ($p < 0.01$) than those in the bystander infection group. CSF pleocytosis did not differ significantly between groups. Two patients received ganciclovir; both had HHV-6 detected early by the FA-ME panel, and one was subsequently diagnosed with bacterial sepsis.

**Conclusions.** HHV-6 encephalitis was uncommon. Rash, changes in neutrophil and platelet counts, along with elevated AST levels may help interpret CSF HHV-6 detection, but these findings require validation in larger studies incorporating virologic confirmation.

**Key words:** human herpesvirus 6, cerebrospinal fluid, exanthema, cytopenia.

Human herpesvirus 6 (HHV-6) infection is a common viral infection in early childhood.[1] Primary infection in this age group is usually symptomatic, and children often present with high fever or seizures, requiring medical evaluation.[2,3] Incidence peaks between 6 and 9 months, but approximately 20% of infections occur in infants aged < 6 months, in whom fever warrants particular clinical attention.[4] HHV-6 infection in most children resolves spontaneously; however, this virus may occasionally cause serious neurological

✉ Young-Soo Kim ▪ youngsookim0127@gmail.com

Received 5th Sep 2025, revised 27th Sep 2025, 2nd Nov 2025, accepted 4th Nov 2025.

Poster presented at the 58th KCNS Annual Symposium & 1st East Asian Pediatric Neurology Conference on May 22, 2025, in Gyeongju, Korea

complications such as encephalitis, even in immunocompetent children.[5] These observations suggest that HHV-6 infection poses a considerable clinical burden during early childhood.

The hallmark presentation of HHV-6 is exanthem subitum, characterized by several days of high fever followed by an abrupt rash.[6] When this characteristic rash is absent, diagnosis can be challenging, particularly in young febrile infants.[7] The recent adoption of the FilmArray Meningitis/Encephalitis (FA-ME) panel has enabled rapid detection within hours, which is significantly faster than traditional polymerase chain reaction (PCR) methods.[8] While this technological advancement improves diagnostic efficiency, careful clinical judgment is required because interpreting the significance of HHV-6 detection in cerebrospinal fluid (CSF) remains challenging.[8] Absence of CSF pleocytosis is frequently reported in HHV-6–associated neurological presentations, including encephalitis.[9] In addition, the presence of HHV-6 DNA does not always indicate a primary infection. It may instead reflect viral reactivation or chromosomal integration. HHV-6 is unique among human herpesviruses because it can be integrated into host chromosomes,[10] often through germline transmission. This process can lead to persistently high levels of viral DNA in the serum, whole blood, and occasionally CSF, even without active replication.[11] In a study of CSF samples from 200 children < 2 years old with suspected CNS infection, HHV-6 DNA was detected in 2.5% of cases with primary infection and in 2.0% with chromosomally integrated HHV-6.[11] In that study, HHV-6 DNA was not detected in the CSF of children aged > 2 years or adults as a result of primary infection.[11] These findings suggest that only about half of the HHV-6 DNA detected in the CSF of children aged < 2 years reflects true primary infection and that HHV-6 DNA detection may not indicate primary infection in older individuals.[11] These results highlight the need for caution when interpreting HHV-6 detection in CSF. Furthermore, chromosomally integrated HHV-6 (ciHHV-6) can be identified through persistently high viral loads or by detecting viral DNA in hair follicles;[1] however, these approaches may not be practical for clinical use. Therefore, clinical markers are required to help interpret HHV-6 detection in CSF; however, currently, such information is limited.

The aim of this study was to enhance clinical understanding by retrospectively reviewing pediatric cases of HHV-6 detected in CSF over the past decade. The primary objective was to describe the clinical features of these pediatric cases in detail. As a secondary objective, we analyzed the clinical differences between cases in which HHV-6 was presumed to be pathogenic and those in which it was considered unlikely to reflect active infection.

## Methods

### *Ethics statement*

This study was approved by the Institutional Review Board of the authors' institution, and informed consent was waived because of its retrospective design.

### *Study design*

This study was conducted at the Gyeongsang National University Hospital (a tertiary center with approximately 900 inpatient beds), serving Gyeongnam province in South Korea. We retrospectively analyzed the laboratory data of children with CSF specimens sampled by lumbar puncture and tested by multiplex PCR for six human herpesviruses (herpes simplex virus 1 and 2 [HSV-1 and -2], Epstein–Barr virus [EBV], cytomegalovirus [CMV], HHV-6, and varicella-zoster virus [VZV]) or the FA-ME panel as part of standard clinical care between January 2015 and March 2025. The FA-ME panel, which was adopted by our institution in 2021, detects 14 pathogens, including six bacteria (*Escherichia coli* K1, *Haemophilus influenzae*, *Listeria monocytogenes*, *Neisseria meningitidis*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae*), seven viruses (CMV, enterovirus,

HSV-1 and -2, HHV-6, human parechovirus, and VZV), and one fungus (*Cryptococcus neoformans/gattii*).

### *Data collection*

Clinical data of children with HHV-6 positive results were obtained through a medical chart review. Data regarding age, sex, clinical presentation or symptoms, fever duration, presence of rash, antimicrobial therapy, and radiographic findings were obtained. Results of additional infectious disease testing of CSF and other clinical specimens were also reviewed to determine alternative infectious etiologies. CSF parameters, including white blood cell (WBC) count, protein and glucose levels, were also collected. Other laboratory findings, including WBC count, platelet count, and C-reactive protein (CRP) levels, were recorded.

### *Investigation for HHV-6 positivity*

To interpret HHV-6 positivity in CSF samples, we conducted a detailed chart review. Patients were classified into presumed HHV-6 infection or presumed bystander detection. Presumed infection cases were defined as those in which the clinical presentation strongly suggested HHV-6 as the most plausible cause of illness, while recognizing that definitive virological proof (e.g., viral load quantification or serology) was not available. Presumed bystander cases were those in which HHV-6 detection was considered incidental or better explained by another pathogen or clinical condition.

A diagnosis of presumed HHV-6 infection was made when one of the following criteria was met: 1) a typical HHV-6 illness of 3–4 days of high fever followed by a roseola-like rash or 2) in the absence of other identifiable pathogens, clinical features compatible with HHV-6 infection, such as a roseola-like rash following fever shorter than the typical 3-4 days, febrile seizures in children aged < 2 years, meningitis or encephalitis with CSF pleocytosis, or unexplained fever in infants aged < 6 months. The bystander group included patients who did not meet the above criteria, or in whom alternative pathogens more plausibly accounted for the illness. All cases were independently reviewed by a pediatric neurologist and a pediatric infectious disease specialist who were directly involved in the clinical care of these patients, and discrepancies were resolved by consensus.

### *Analysis*

To identify distinguishing features of clinically significant cases, we compared the infection and bystander groups by sex, age, fever duration, presence of rash, seizure occurrence, CSF profile, and laboratory findings. Statistical analysis was performed using Fisher's exact test and the Mann–Whitney U test. For each variable, the relative difference (RD) was calculated with 95% confidence intervals (CIs) to estimate effect size. Statistical significance was set at $p < 0.05$.

## Results

### *Patient testing and prevalence of HHV-6*

Multiplex PCR testing for six human herpesviruses was performed in 1,665 patients. In addition, 232 patients were tested using the FA-ME panel, which has been implemented in clinical practice since August 2021. Among them, 32 patients underwent both tests, resulting in a total of 1,865 pediatric patients being tested. Of these, HHV-6 was detected in 25 patients (1.3%).

### *Characteristics of HHV-6-positive patients*

Table I shows the clinical characteristics of HHV-6-positive patients (n = 25). The median age was 6 months (interquartile range [IQR], 2–18 months), and the majority were male (56%, n = 15). All patients presented with fever, and seizures occurred in seven (28%). Of these, five had recurrent seizures, and two experienced only a single event. Ataxia was observed in one patient (4%). CSF pleocytosis (≥5 WBC/μL) was identified in 11 patients (44%). HHV-6 was detected by the FA-ME panel in 5 patients and

**Table I.** Summary of clinical findings for patients with HHV-6 detected in cerebrospinal fluid.

| No. of patient | Sex | Age (mo) | Symptoms | Fever duration (days) | Rash | CSF WBC (/μL) | CSF glucose (mg/dL) | CSF protein (mg/dL) | Blood HHV-6 | Other possible pathogens | Empirical / specific treatment | Brain imaging | Complication |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | F | 7 | Fever, seizures | 4 | + | 8 | 74 | 188 | + | None* | Acyclovir, IVIG, antibiotics / maintained | Focal hemorrhage, pons, midbrain and both lower thalami. | Epilepsy |
| 2 | M | 11 | Fever, seizures | 5 | + | 2 | 64 | 11 | + | None*† | None / none | Normal | None |
| 3 | F | 12 | Fever, seizures | 2 | - | 0 | 69 | 12 | + | None* | None / none | N/d | None |
| 4 | M | 18 | Fever, seizures | 4 | - | 0 | 72 | 21 | + | None*† | Antibiotics / none | Normal | None |
| 5 | M | 18 | Fever, seizures | 5 | + | 0 | 65 | 17 | + | Adenovirus* | Antibiotics / none | Normal | None |
| 6 | F | 11 | Fever, single seizure | 2 | - | 0 | 78 | 53 | N/d | None* | None / none | N/d | None |
| 7 | M | 6 | Fever, a single seizure | 3 | + | 5 | 68 | 28 | + | None* | Antibiotics / none | N/d | None |
| 8 | M | 2 | Fever, lethargy | 2 | - | 792 | 58 | 118 | + | None* | Antibiotics / none | Normal | None |
| 9‡ | M | 18 | Fever, ataxia | 4 | + | 1 | 57 | 26 | N/d | None* | Antibiotics / none | Normal | None |
| 10‡ | F | 36 | Fever, headache, lethargy | 5 | - | 23 | 54 | 24 | - | None*† | Antibiotics, IVIG / ganciclovir | Normal | None |
| 11 | M | 1 | Fever | 1 | + | 1 | 57 | 45 | - | None*† | Antibiotics / none | N/d | None |
| 12 | F | 2 | Fever | 3 | + | 6 | 64 | 27 | N/d | None* | Antibiotics / none | N/d | None |
| 13 | M | 3 | Fever | 1 | - | 0 | 62 | 24 | + | None* | Antibiotics / none | N/d | None |
| 14 | M | 5 | Fever | 4 | + | 2 | 70 | 68 | N/d | None* | Antibiotics | N/d | None |
| 15 | F | 26 | Fever | 7 | + | 0 | 53 | 31 | + | None*† | Antibiotics / none | N/d | None |

CMV, cytomegalovirus; CSF, cerebrospinal fluid; EBV, Epstein–Barr virus; HHV-6, human herpes virus-6; IVIG, intravenous immunoglobulin; N/d, not done; WBC, white blood cell; * Multiplex polymerase chain reaction (PCR) for respiratory viruses, including adenovirus, respiratory syncytial virus, influenza virus, parainfluenza virus, human bocavirus, human metapneumovirus, human coronavirus, rhinovirus, and enterovirus; † Multiplex PCR for acute gastroenteritis pathogens, including astrovirus, adenovirus, rotavirus, norovirus, *Campylobacter jejuni, Campylobacter coli, Clostridium perfringens, Vibrio cholerae, Vibrio vulnificus, Vibrio parahaemolyticus, Listeria monocytogenes, Salmonella* spp., *Bacillus cereus, Yersinia enterocolitica, Staphylococcus aureus*, and enterohemorrhagic *Escherichia coli;* ‡ Cerebrospinal fluid was tested using a FilmArray Meningitis/Encephalitis panel, which detects *Escherichia coli* K1, CMV, enterovirus, *Streptococcus pneumoniae*, HHV-6, herpes simplex virus types 1 and 2 (HSV-1/2), *Neisseria meningitidis, Streptococcus agalactiae, Cryptococcus neoformans/gattii, Haemophilus influenzae*, varicella zoster virus (VZV), *Listeria monocytogenes*, and human parechovirus; otherwise tested with multiplex PCR for herpesviruses including HSV-1/2, VZV, EBV, CMV, and HHV-6; § presumed bystander HHV-6

**Table I.** Continued.

| No. of patient | Sex | Age (mo) | Symptoms | Fever duration (days) | Rash | CSF WBC (/μL) | CSF glucose (mg/dL) | CSF protein (mg/dL) | Blood HHV-6 | Other possible pathogens | Empirical / specific treatment | Brain imaging | Complication |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 16 | F | 6 | Fever, lethargy | 1 | + | 1 | 65 | 23 | - | None* | Antibiotics / none | N/d | None |
| 17 | M | 1 | Fever | 1 | - | 2 | 47 | 75 | n/d | None* | Antibiotics / none | N/d | None |
| 18§ | F | 18 | Fever of unknown origin | 11 | - | 138 | 31 | 83 | + | CMV, EBV detected in CSF, none*† | Antibiotics / acyclovir added | Normal | Unknown (transfer) |
| 19§ | F | 0.7 | Fever, rash, diarrhea | 5 | + | 9 | 50 | 85 | n/d | None*†, compatible with cow milk allergy | Antibiotics / none | N/d | None |
| 20§ | F | 28 | Fever, headache, vomiting | 1 | - | 136 | 71 | 27 | n/d | Enterovirus in CSF | None / none | N/d | None |
| 21§ | M | 3 | Fever | 1 | - | 6 | 58 | 34 | n/d | Rhinovirus*, diagnosed with pyelonephritis | Antibiotics / none | N/d | None |
| 22§ | F | 4 | Fever, rhinorrhea | 2 | - | 5 | 57 | 78 | n/d | Rhinovirus* | Antibiotics / none | N/d | None |
| 23‡§ | M | 1 | Fever, lethargy | 2 | - | 1 | 58 | 73 | n/d | *Streptococcus agalactiae* sepsis | Antibiotics / ganciclovir | Normal | None |
| 24‡§ | M | 2 | Fever, rhinorrhea | 1 | - | 0 | 69 | 45 | n/d | Rhinovirus* | Antibiotics / none | N/d | None |
| 25‡§ | M | 44 | Fever, headache, vomiting | 5 | none | 1000 | 45 | 76 | n/d | Enterovirus in CSF | Antibiotics, acyclovir / stop acyclovir | N/d | None |

CMV, cytomegalovirus; CSF, cerebrospinal fluid; EBV, Epstein–Barr virus; HHV-6, human herpes virus-6; IVIG, intravenous immunoglobulin; N/d, not done; WBC, white blood cell; * Multiplex polymerase chain reaction (PCR) for respiratory viruses, including adenovirus, respiratory syncytial virus, influenza virus, parainfluenza virus, human bocavirus, human metapneumovirus, human coronavirus, rhinovirus, and enterovirus; † Multiplex PCR for acute gastroenteritis pathogens, including astrovirus, adenovirus, rotavirus, norovirus, *Campylobacter jejuni, Campylobacter coli, Clostridium perfringens, Vibrio cholerae, Vibrio vulnificus, Vibrio parahaemolyticus, Listeria monocytogenes, Salmonella* spp., *Bacillus cereus, Yersinia enterocolitica, Staphylococcus aureus*, and enterohemorrhagic *Escherichia coli;* ‡ Cerebrospinal fluid was tested using a FilmArray Meningitis/Encephalitis panel, which detects *Escherichia coli* K1, CMV, enterovirus, *Streptococcus pneumoniae*, HHV-6, herpes simplex virus types 1 and 2 (HSV-1/2), *Neisseria meningitidis, Streptococcus agalactiae, Cryptococcus neoformans/gattii, Haemophilus influenzae*, varicella zoster virus (VZV), *Listeria monocytogenes*, and human parechovirus; otherwise tested with multiplex PCR for herpesviruses including HSV-1/2, VZV, EBV, CMV, and HHV-6; § presumed bystander HHV-6

by multiplex PCR in 20. All participants were previously documented as healthy; however, no records of a systematic assessment of immune function were available. None had a history of receiving immunosuppressive therapy, including corticosteroids.

Acyclovir was administered to three patients (Patients 1, 18, 25; 16%). Only Patient 1 was classified as presumed HHV-6 infection, presenting with recurrent seizures and altered consciousness after 3 days of high fever. A rash developed on the fourth day. CSF pleocytosis was observed, and brain magnetic resonance imaging (MRI) revealed hyperintensity in the pons, midbrain, and bilateral lower thalami, accompanied by focal hemorrhage (Fig. 1.). Empirical treatment with acyclovir, antibiotics,

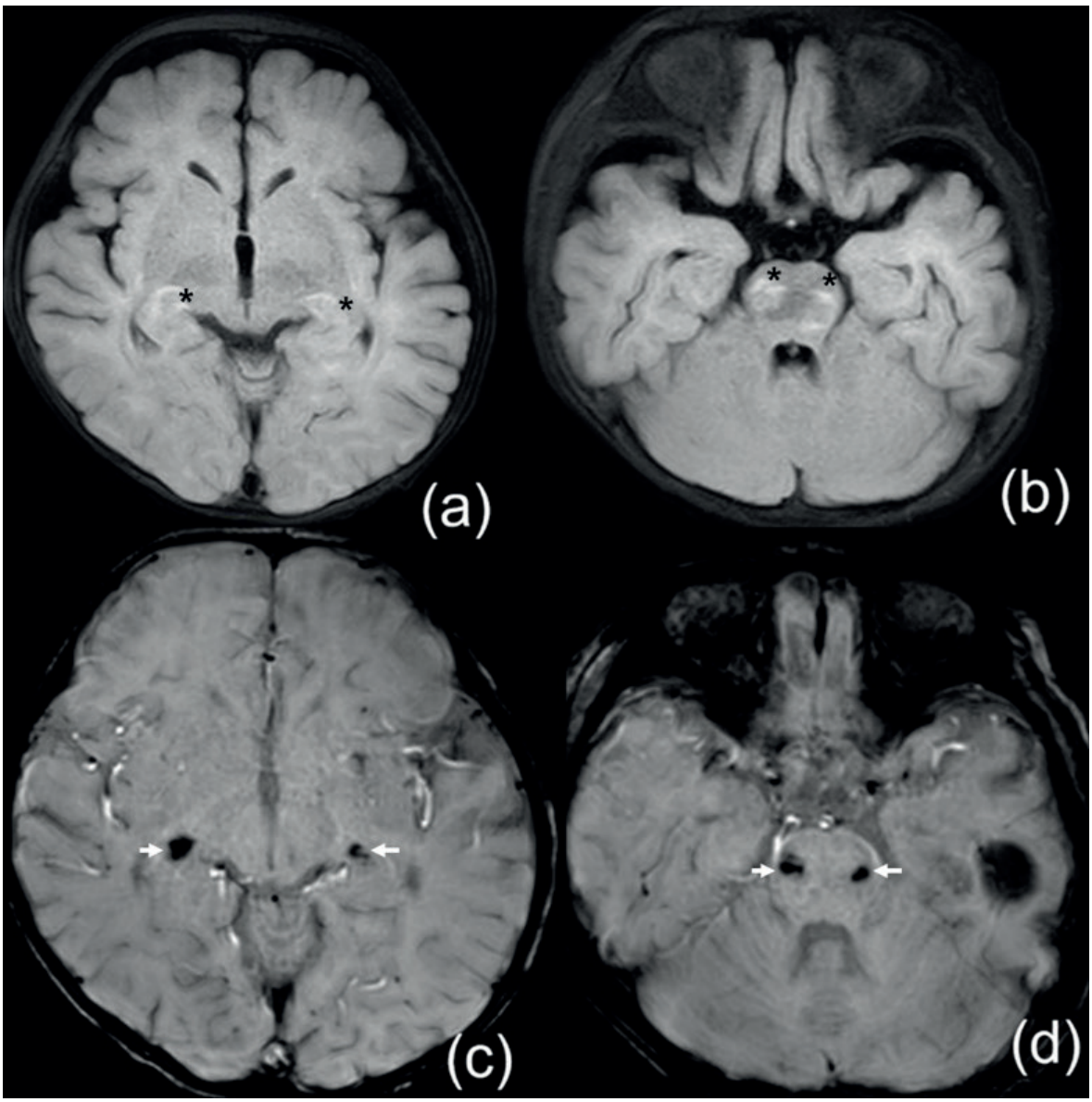



**Fig. 1.** Brain magnetic resonance imaging of patient no. 1, who presented with presumed HHV-6 encephalitis. Axial T2-weighted fluid-attenuated inversion recovery (FLAIR) images (a, b) demonstrate hyperintensity in the pons, midbrain, and bilateral lower thalami (asterisks). Susceptibility-weighted imaging (SWI) (c, d) reveals focal hemorrhages within these regions (arrows).

and intravenous immunoglobulin (IVIG) was initiated for suspected encephalitis. HHV-6 infection was confirmed on hospital day 5, when the patient was already improving; therefore, acyclovir was maintained without switching to ganciclovir. The patient recovered without acute sequelae but later developed epilepsy. Patient 25 presented with fever lasting 5 days, headache, lethargy, and CSF pleocytosis, prompting empirical treatment with acyclovir under the impression of encephalitis. Enterovirus and HHV-6 were detected, but treatment was stopped because several features indicated enterovirus infection, including the concurrent seasonal outbreak, marked CSF pleocytosis (>1,000/μL), and rapid clinical improvement within 1–2 days. Patient 18 was admitted with prolonged fever of unknown origin. EBV, CMV, and HHV-6 were detected on multiplex PCR, and acyclovir was started due to suspected EBV infection supported by serology. The patient was transferred before outcome assessment.

Ganciclovir was administered to two patients (4%), both of whom tested positive for HHV-6 using the FA-ME panel on the day of admission. They were treated empirically for lethargy and severe clinical presentation. Patient 10 had an encephalitic course with lethargy and CSF pleocytosis, and no alternative pathogen was identified; the case was therefore considered a presumed HHV-6 infection. The patient recovered fully while receiving ganciclovir and IVIG, and the brain MRI was normal. In contrast, Patient 23 was later diagnosed with group B streptococcal sepsis, and ganciclovir was discontinued.

**Table II.** Comparison between the presumed HHV-6 infection group and the presumed bystander group.

| | Presumed HHV-6 infection group (N=17) | Presumed bystander (N=8) | Relative Difference (RD) (95% CIs) | P |
|---|---|---|---|---|
| Age, months | 7 (2.5–18) | 3.5 (1.3–25.5) | -14.5% (-68.0%, 128.8%) | 0.67 |
| Male | 10 (59%) | 4 (50%) | 8.8% (-33.0%, 50.6%) | 1.0 |
| Fever duration, days | 3.5 (1.0–4.8) | 2.0 (1.0–5.0) | -10.9% (-57.5%, 86.8%) | 0.88 |
| Rash | 10 (59%) | 1 (13%) | 46.3% (13.6%, 79.1%) | 0.04* |
| Seizures | 7 (41%) | 0 (0%) | 41.2% (17.8%, 64.6%) | 0.06 |
| Hospital stay, days | 4.0 (3.5–6.0) | 4.5 (4.0–8.8) | -10.1% (-41.2%, 37.5%) | 0.71 |
| CSF pleocytosis, n | 5 (29%) | 6 (75%) | -45.6% (-82.6%, -8.6%) | 0.08 |
| CSF WBC, /μL | 1.0 (0–5.5) | 7.5 (2.0–137.5) | -69.4% (-97.1%, 222.0%) | 0.06 |
| CSF glucose, mg/dL | 64.0 (57.0–69.5) | 57.5 (46.5–66.3) | 15.4% (-3.1%, 37.5%) | 0.14 |
| CSF protein, mg/dL | 28.0 (22.0–60.5) | 74.5 (36.8–81.8) | -24.8% (-55.6%, 27.4%) | 0.03* |
| Initial ANC, /μL | 3,400 (1,780–5,900) | 4,980 (2,875-9,132) | -30.3% (-58.4%, 16.7%) | 0.21 |
| Follow-up ANC, /μL | 1,300.0 (530.0–2,080.0) | 2,965.0 (1,417.5–13,660.0) | -75.3% (-89.9%, -39.9%) | 0.02* |
| Initial platelets, $x10^3$/μL | 242.0 (186.0–316.5) | 302.0 (257.7–470.0) | -29.6% (-48.1%, -4.7%) | 0.04* |
| Follow-up platelet, $x10^3$/μL | 186.0 (144.0–243.5) | 322.0 (261.0–433.2) | -41.5% (-59.0%, -16.5%) | 0.01* |
| Follow-up interval, days | 2.0 (0.0–3.5) | 2.0 (0.25–4.5) | 6.5% (-53.6%, 144.5%) | 0.97 |
| C-reactive protein, mg/L | 1.7 (0.6-27.8) | 7.3 (2.0-28.9) | 65.5% (-57.4%, 543.7%) | 0.40 |
| AST, U/L | 48.0 (44.0-61.0) | 29.5 (21.7-42.0) | 74.8% (21.9%, 150.8%) | <0.01* |
| ALT, U/L | 20.0 (16.5-31.5) | 15.0 (13.2-19.0) | 58.6% (-12.5%, 187.3%) | 0.11 |
| Treatment with acyclovir | 1 (6%) | 2 (25%) | -19.1% (-51.1%, 12.9%) | 0.23 |
| Treatment with ganciclovir | 1 (6%) | 1 (8%) | -6.6% (-32.1%, 18.9%) | 1.00 |

Quantitative data are presented as median (interquartile range), qualitative data as number (percentage); ALT, alanine transaminase; ANC, absolute neutrophil count; AST, aspartate transaminase; CIs, confidence intervals; CSF, cerebrospinal fluid; HHV-6, human herpesvirus 6; *Statistical significance was set at $p < 0.05$, considered statistically significant.

IVIG was administered to both patients as part of empirical treatment for suspected encephalitis, and they were ultimately considered to have presumed HHV-6 infections (Patients 1 and 10).

### *Comparison between the infection and bystander groups*

Table II shows the comparison of patients classified as presumed HHV-6 infection (n = 17) and presumed bystander (n = 8). Among the patients with presumed infection, Patient 5 had adenovirus detected in respiratory specimens. However, the case was considered presumed HHV-6 infection by consensus, based on the presentation of typical exanthem subitum—several days of high fever followed by rash—and the absence of findings suggestive of adenovirus infection, such as conjunctivitis or respiratory symptoms. Among the patients with bystander detection, Patient 19 developed fever and rash almost simultaneously, accompanied by diarrhea and a positive cow's milk IgE. These findings were consistent with cow's milk protein allergy, and the case was therefore considered a bystander.

No significant differences were observed between the two groups in age, sex, fever duration, seizure presentation, length of hospital stay, CSF WBC count, CSF glucose level, absolute neutrophil count (ANC) at admission, CRP level, and antiviral use. In the presumed infection group, rash was more frequent (59% vs. 13%, RD = 46.3% [95% CI: 13.6 – 79.1%], p = 0.04) and CSF protein levels were significantly lower (median 28.0 vs. 74.5 mg/dL, RD = -24.8% [95% CI: -55.6 – 27.4%], p = 0.03). Follow-up studies showed significantly lower ANC (median 1,300 vs. 2,965/μL, RD = -75.3% [95% CI: -89.9% – -39.9%], p = 0.02) and platelet counts (median 186 vs. 322 × $10^3$/μL, RD = -41.5% [95% CI: -59.0% – -16.5%], p = 0.01) in the presumed infection group, whereas at admission the differences were smaller and only significant for platelet counts. Aspartate aminotransferase (AST) levels were higher in the presumed infection group (median 48.0 vs. 29.5 U/L, RD = 74.8% [95% CI: 21.9 – 150.8%], p < 0.01).

## Discussion

This study was retrospectively conducted to assess the clinical significance of HHV-6 detected in the CSF of pediatric patients over a 10-year period. Of the 25 HHV-6–positive-cases, 17 were classified as presumed HHV-6 infection, which is commonly associated with rash and cytopenia. The findings of this study may provide insights into markers of pathogenic HHV-6 infection and assist clinicians in interpreting CSF HHV-6 results in pediatric settings.

In our cohort, HHV-6 was detected in 1.3% (25/1,865) of children who were tested, with a median age of 6 months; nearly half of the patients were <6 months, slightly below the usual peak of 6–9 months.[4] This younger distribution likely reflects the frequent lumbar punctures for unexplained fever rather than clinical suspicion of exanthem subitum. A similar pattern was described by Pandey et al.,[12] who found HHV-6 in 2.5% of 1,005 children, with a median age of 0.55 years. Detection at such an early age may raise concerns about immune immaturity; nonetheless, antiviral therapy was rarely required. In our series, only one infant received ganciclovir before group B streptococcus sepsis was confirmed, while in the Pandey et al.[12] study, none were treated; nevertheless, all infants aged < 6 months recovered without complications. These findings suggest that, in otherwise healthy infants, HHV-6 detection alone should be interpreted with caution before initiating antiviral therapy. Pandey et al.[12] exclusively used the FA-ME panel, which is reported to have a lower sensitivity for HHV-6 than the conventional PCR used in most of our cases. Therefore, the higher detection rate in their study likely reflects population differences rather than assay performance. Studies focusing on specific clinical groups have shown even higher detection rates of approximately

6% in febrile seizure cases[13] and up to 33% in meningoencephalitis cases.[9]

All 25 HHV-6–positive patients in our cohort presented with fever; seizures occurred in seven (28%) and ataxia in one (4%). Antivirals were administered in five cases—three received acyclovir and two ganciclovir—with one patient in each group also given IVIG. These two (Patients 1 and 10) were ultimately classified as HHV-6 meningoencephalitis. While limbic involvement is typical in HHV-6 encephalitis, thalamic and brainstem lesions are also frequent in children and consistent with this case.[14] Focal hemorrhages, though rarely described, have been reported in fatal cases.[15] In contrast, our patient recovered without acute sequelae, though epilepsy later developed. The patient was treated with acyclovir and IVIG. However, because acyclovir has limited activity against HHV-6,[16] the favorable outcome was likely driven more by the addition of IVIG than by antiviral therapy itself. Patient 10, who presented with symptoms of encephalitis and a normal MRI result, also received ganciclovir plus IVIG soon after admission and recovered fully. Because both patients with encephalitis received combination therapy, the specific contribution of each component could not be determined. However, the favorable outcomes in our study contrast with those in a nationwide Japanese report, where nearly half of 60 pediatric patients developed long-term sequelae despite treatment.[17] In that cohort, 51.7% received antivirals, 55.0% steroids, and 31.7% immunoglobulins, but outcomes were not analyzed by treatment regimen.[17] By contrast, in a Korean series, better results were reported in patients treated with both antivirals and IVIG than in those who received antivirals alone,[18] consistent with our observations. Taken together, these findings suggest that early combination therapy, particularly including IVIG, may improve outcomes in HHV-6 encephalitis, although current evidence remains limited. Larger studies are needed to establish optimal treatment strategies. Notably, both patients in our study who received ganciclovir were diagnosed early using the FA-ME panel. While one case aligned with presumed HHV-6 encephalitis, the other was ultimately diagnosed as group B streptococcus sepsis. This contrast underscores the risk of overtreatment based on early detection alone and highlights the need to interpret HHV-6 results within the full clinical context.

Differentiating clinically significant HHV-6 infection from incidental detection remains a key challenge in interpreting CSF PCR results. In our cohort, 17 of 25 HHV-6–positive cases were classified as presumed infection, while 8 were considered bystander detections when another pathogen or a more plausible diagnosis was present. Among distinguishing features, rash was significantly more frequent in the presumed infection group (59% vs. 13%, $p = 0.04$)—an unexpected finding given that roseola-like rashes were part of the case definition. Ward et al.[11] similarly reported rash in 67% of confirmed primary infection cases but only 10% of ciHHV-6 cases, supporting its potential role as a clinical marker. The groups were also distinguished by the hematologic findings. At admission, ANC tended to be lower in the presumed infection group than in the bystander group; however, the difference was not statistically significant. In contrast, platelet counts were already significantly lower in the presumed infection group at admission. On follow-up, both ANC and platelet counts showed clearer differences in the presumed infection group. AST levels were also higher in this group. These results are consistent with those of prior studies suggesting HHV-6–associated bone marrow suppression.[19,20] Miura et al.[19] observed severe neutropenia in 30% of primary HHV-6 cases, particularly between days 5 and 10, often with thrombocytopenia, AST elevation, and chemokine increases, suggesting an inflammation-mediated contribution to myelosuppression. Consistent with this finding, our cohort showed progressive declines in ANC and platelet counts. AST levels were elevated at admission in the presumed infection group, but without follow-up data this finding

should be interpreted cautiously, as seizures—more frequent in this group—may also have contributed. In contrast, CSF pleocytosis did not differ between groups and was not useful for assessing clinical relevance, consistent with reports that pleocytosis is often absent even in HHV-6 CNS infections.[5,12] Finally, higher CSF protein in the presumed bystander group (74.5 vs 28.0 mg/dL; p = 0.03) may reflect age-related physiology (4 vs 7 months); hence we did not rely on this finding to distinguish the groups. This interpretation is supported by our effect size analysis, in which the CI for the RD included zero (RD = -24.8% [95% CI: -55.6% to 27.4%], indicating that the difference was not statistically robust.

This study has several limitations. First, as this was a retrospective study, some clinical details were incomplete. The immune status of patients is a key factor in interpreting the clinical relevance of HHV-6 detection. However, detailed data on immune function were not consistently available because of the retrospective design of the study. Second, the number of patients, especially those with HHV-6 encephalitis, was small, limiting generalizability and precluding robust statistical comparisons. Notably, the non-significant differences between the two groups were underpowered, with post-hoc power analysis showing values ranging from 0.03 to 0.49. Third, classification into presumed infection and bystander groups was based on clinical judgment rather than virological confirmation, and this approach may be debated. However, such clinical categorization is not unique to our study. Wang et al.[21] used a similar clinical classification and found strong concordance between clinical and virological definitions, noting that fever was the most reliable clinical feature distinguishing primary infection from bystander detection. Consistent with our findings, Pandey et al.[12] reported that approximately half of HHV-6–positive cases occurred in infants aged < 6 months, most of them presenting with fever. They also confirmed ciHHV-6 in only a minority of tested patients, all of whom were infected with another plausible pathogen.[12] These parallels suggest that, while not definitive, our classification appears clinically reasonable. Finally, we lacked confirmatory assays (viral load, serology, or ciHHV-6 testing) and long-term follow-up, limiting confirmation of active infection and assessment of late neurological outcomes. Prospective studies with standardized virological testing and longitudinal follow-up are needed to refine classification and clarify the clinical significance of HHV-6 detected in pediatric CSF.

In conclusion, HHV-6 detection in CSF may not always indicate active infection. Encephalitis was rare in our cohort, and although affected patients recovered without acute sequelae, the findings should be interpreted with caution because of the small sample size and limited follow-up. Clinical features, such as rash, together with laboratory changes like cytopenia and elevated AST, may help assess clinical relevance. Careful interpretation is especially important in young children, particularly when considering antiviral treatments.

## Ethical approval

This study was approved by the Institutional Review Board of Gyeongsang National University Hospital, with a waiver of informed consent due to its retrospective design (IRB No. GNUH-2025-05-20).

## Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: JSY, YSK; data collection: JSY; analysis and interpretation of results: JSY, JHS, YSK, JSP, ESP, JYL, HOW; draft manuscript preparation: JSY, YSK. All authors reviewed the results and approved the final version of the manuscript.

**Source of funding**

This work was supported by the New Faculty Research Support Grant from Gyeongsang National University in 2025 (grant no: GNU-NFRSG-0054).

**Conflict of interest**

The authors declare that there is no conflict of interest.

## REFERENCES

1. Dockrell DH. Human herpesvirus 6: molecular biology and clinical features. J Med Microbiol 2003; 52: 5-18. https://doi.org/10.1099/jmm.0.05074-0
2. Karimi A, Sakhavi M, Nahanmoghaddam N, et al. Evaluation of viral (HHV6, adenovirus, HSV1, enterovirus) and bacterial infection in children with febrile convulsion by serum PCR and blood culture mofid children's hospital 2016-2017. Arch of Pediatr Infect Dis 2018; 6: e63954. https://doi.org/10.5812/pedinfect.63954
3. Inoue J, Weber D, Fernandes JF, et al. HHV-6 infections in hospitalized young children of Gabon. Infection 2023; 51: 1759-1765. https://doi.org/10.1007/s15010-023-02077-w
4. Hall CB, Long CE, Schnabel KC, et al. Human herpesvirus-6 infection in children: a prospective study of complications and reactivation. N Engl J Med 1994; 331: 432-438. https://doi.org/10.1056/NEJM199408183310703
5. Nikolskiy MA, Lioznov DA, Gorelik EU, Vishnevskaya TV. HHV-6 in cerebrospinal fluid in immunocompetent children. BioMed 2023; 3: 420-430. https://doi.org/10.3390/biomed3030034
6. Okada K, Ueda K, Kusuhara K, et al. Exanthema subitum and human herpesvirus 6 infection: clinical observations in fifty-seven cases. Pediatr Infect Dis J 1993; 12: 204-208. https://doi.org/10.1097/00006454-199303000-00006
7. Yamamoto S, Takahashi S, Tanaka R, et al. Human herpesvirus-6 infection-associated acute encephalopathy without skin rash. Brain Dev 2015; 37: 829-832. https://doi.org/10.1016/j.braindev.2014.12.005
8. Mostyn A, Lenihan M, O'Sullivan D, et al. Assessment of the FilmArray® multiplex PCR system and associated meningitis/encephalitis panel in the diagnostic service of a tertiary hospital. Infect Prev Pract 2020; 2: 100042. https://doi.org/10.1016/j.infpip.2020.100042
9. Abdelrahim NA, Mohamed N, Evander M, Ahlm C, Fadl-Elmula IM. Human herpes virus type-6 is associated with central nervous system infections in children in Sudan. Afr J Lab Med 2022; 11: 1718. https://doi.org/10.4102/ajlm.v11i1.1718
10. Daibata M, Taguchi T, Nemoto Y, Taguchi H, Miyoshi I. Inheritance of chromosomally integrated human herpesvirus 6 DNA. Blood 1999; 94: 1545-1549.
11. Ward KN, Leong HN, Thiruchelvam AD, Atkinson CE, Clark DA. Human herpesvirus 6 DNA levels in cerebrospinal fluid due to primary infection differ from those due to chromosomal viral integration and have implications for diagnosis of encephalitis. J Clin Microbiol 2007; 45: 1298-1304. https://doi.org/10.1128/JCM.02115-06
12. Pandey U, Greninger AL, Levin GR, Jerome KR, Anand VC, Dien Bard J. Pathogen or bystander: clinical significance of detecting human herpesvirus 6 in pediatric cerebrospinal fluid. J Clin Microbiol 2020; 58: e00313-e00320. https://doi.org/10.1128/JCM.00313-20
13. Mamishi S, Kamrani L, Mohammadpour M, Yavarian J. Prevalence of HHV-6 in cerebrospinal fluid of children younger than 2 years of age with febrile convulsion. Iran J Microbiol 2014; 6: 87-90.
14. Crawford JR, Chang T, Lavenstein BL, Mariani B. Acute and chronic magnetic resonance imaging of human herpesvirus-6 associated encephalitis. J Pediatr Neurol 2009; 7: 367-373. https://doi.org/10.3233/JPN-2009-0331
15. Ahtiluoto S, Mannonen L, Paetau A, et al. In situ hybridization detection of human herpesvirus 6 in brain tissue from fatal encephalitis. Pediatrics 2000; 105: 431-433. https://doi.org/10.1542/peds.105.2.431
16. Amjad M, Gillespie MA, Carlson RM, Karim MR. Flow cytometric evaluation of antiviral agents against human herpesvirus 6. Microbiol Immunol 2001; 45: 233-240. https://doi.org/10.1111/j.1348-0421.2001.tb02612.x
17. Yoshikawa T, Ohashi M, Miyake F, et al. Exanthem subitum-associated encephalitis: nationwide survey in Japan. Pediatr Neurol 2009; 41: 353-358. https://doi.org/10.1016/j.pediatrneurol.2009.05.012

18. You SJ. Human Herpesvirus-6 may be neurologically injurious in some immunocompetent children. J Child Neurol 2020; 35: 132-136. https://doi.org/10.1177/0883073819879284

19. Miura H, Kawamura Y, Ozeki E, Ihira M, Ohashi M, Yoshikawa T. Pathogenesis of severe neutropenia in patients with primary human herpesvirus 6B infection. Pediatr Infect Dis J 2015; 34: 1003-1007. https://doi.org/10.1097/INF.0000000000000777

20. Hashimoto H, Maruyama H, Fujimoto K, Sakakura T, Seishu S, Okuda N. Hematologic findings associated with thrombocytopenia during the acute phase of exanthem subitum confirmed by primary human herpesvirus-6 infection. J Pediatr Hematol Oncol 2002; 24: 211-214. https://doi.org/10.1097/00043426-200203000-00010

21. Wang H, Tomatis-Souverbielle C, Everhart K, Oyeniran SJ, Leber AL. Detection of human herpesvirus 6 in pediatric CSF samples: causing disease or incidental distraction? Diagn Microbiol Infect Dis 2023; 107: 116029. https://doi.org/10.1016/j.diagmicrobio.2023.116029

The Turkish Journal of Pediatrics 2025; 67: 841-854
https://doi.org/10.24953/turkjpediatr.2025.5170

**Original Article**

# Assessment of factors affecting timing of discharge in pediatric cancer patients with febrile neutropenia

Ceren Üstün[1], Burça Aydın[2], Nilgün Kurucu[2], Bilgehan Yalçın[2], Ali Varan[2], Tezer Kutluk[2]

*[1]Department of Pediatrics, Faculty of Medicine, Hacettepe University, Ankara, Türkiye; [2]Division of Pediatric Oncology, Department of Pediatrics, Faculty of Medicine, Hacettepe University, Ankara, Türkiye.*

## ABSTRACT

**Background.** Febrile neutropenia is a common cause of hospital admissions among pediatric cancer patients. To optimize personalized approaches for hospitalization and antibiotic treatment, risk stratification has been proposed. This study aimed to explore the impact of clinical and laboratory parameters on risk stratification for patient discharge.

**Methods.** This prospective study included pediatric lymphoma and solid tumor patients who were hospitalized due to febrile neutropenia between June 2018 and June 2019. Patient characteristics, primary oncological diagnosis and disease status, comorbid conditions, time elapsed after the last course of chemotherapy, use of granulocyte-colony stimulating factor (G-CSF) prophylaxis, presence of port catheter, infection type, fever values/duration, physical examination findings, and duration of neutropenia were collected. Laboratory investigations including complete blood counts, acute phase reactants at the onset of the episode, culture results were also recorded.

**Results.** The study examined 142 febrile neutropenic episodes from 88 consecutive patients. The median age of the study group was 6.8 years, with 19.3% of cases being lymphoma and 80.7% having solid tumors. The median hospital stay was 7 days. Factors associated with longer hospitalization periods included a lymphoma diagnosis, presence of comorbid conditions, bone marrow involvement, and febrile neutropenic period during hospitalization. Patients presenting with fever ≥ 39 °C at admission, poor general appearance, hypotension, prolonged capillary filling time, and severe infection signs had longer hospital stays. In febrile neutropenic episodes, absolute monocyte count ≤ 100 cells/mm$^3$, platelet count ≤ 50,000/mm$^3$, and prolonged neutropenia delayed discharge time. Patients with microbiologically defined infections, especially those with positive catheter cultures, also had delayed discharge.

**Conclusion.** The diagnosis of lymphoma, poor general condition at admission, presence of microbiologically defined infection, thrombocytopenia, delayed recovery of absolute neutrophil counts, and prolonged fever duration were significant factors in determining the treatment duration and predicting discharge time.

**Key words:** febrile neutropenia, infections, pediatric oncology, quality of life, risk scoring, safe discharge.

Febrile neutropenia is a common complication in children receiving cancer chemotherapy. The immune system of these patients is compromised by both treatments and in some cases by the cancer itself. The type, duration, and intensity of cancer treatment are key risk factors for infections, often affecting various aspects of their immune defenses. Additional risk factors such as mucous membrane abnormalities, the presence of indwelling

✉ Ceren Üstün ▪ ceren.irmak@hacettepe.edu.tr

Received 31st Jul 2024, revised 30th Mar 2025, 20th Jul 2025, 10th Sep 2025, accepted 4th Oct 2025.

catheters, malnutrition, extensive antibiotic use, and frequent hospitalizations further elevate the risk of infection.[1]

The standard approach to febrile neutropenia management involves the initiation of empirical antibiotic therapy and close observation. However, challenges such as increasing antibiotic resistance, catheter-related complications and nosocomial infections reduce patients' quality of life and increase treatment costs, presenting significant problems. These issues become more pronounced with extended hospital stays.[2]

Therefore, it is crucial to identify patients who might be eligible for early discharge or outpatient treatment and to design their treatments accordingly. Several guidelines have been published based on the findings and outcomes of patient series[2-5], but most of these guidelines focus on adult patients. Studies involving children are scarce and often encompass smaller patient cohorts.[6,7] Consequently, there is a need for prospective studies that comprehensively cover all relevant variables in pediatric cases.

The aim of this study was to evaluate the impact of data obtained at admission and during follow-up on the discharge timing of pediatric cancer patients hospitalized with febrile neutropenia.

## Methods

This prospective study aimed to investigate the characteristics of febrile neutropenic episodes in patients under 18 years of age with lymphoma and solid tumors, treated at our Pediatric Oncology inpatient unit between June 1, 2018, and June 1, 2019. Leukemia cases were excluded from the study as their follow-up and treatment were managed by a different department within the institution. Patients who had undergone hematopoietic stem cell transplantation were also excluded from the study.

Demographic information of the patients during febrile neutropenic episodes, the location where fever began (outpatient or hospital), primary oncological diagnosis, primary disease status (remission, recurrent/resistant), comorbid conditions, time elapsed since last chemotherapy course, use of Granulocyte-colony stimulating factor (G-CSF) prophylaxis, presence of a port catheter, infection type (fever of unknown origin [FUO], clinically or microbiologically defined infection), fever value at the onset of the episode, physical examination findings (general condition, presence of hypotension/shock, capillary refilling time, mucositis, anal ulceration), highest recorded fever value, number of febrile days, and duration of neutropenia were recorded. Patients presenting with signs such as pallor, lethargy, poor perfusion, altered mental status, respiratory distress or dehydration were considered to be in poor general condition. This assessment was based on physicians' clinical observations during physical examinations. Laboratory investigations, including hemoglobin, leukocyte, absolute neutrophil and monocyte counts, platelet count, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) at the onset of the episode as well as blood, catheter, urine and respiratory tract culture results, were also recorded. In addition, the antibiotics used during the episodes, modification periods, and discharge times were documented. Throughout the study, the parameters in the patient follow-up form were recorded separately for each episode, even though multiple episodes could occur in the same patient.

Febrile neutropenia was defined as an absolute neutrophil count (ANC) below 500/mm$^3$ or between 500 and 1,000/mm$^3$ with an expected decrease to below 500/mm$^3$ within 48 hours, accompanied by an axillary body temperature of 38.3 °C or higher, or 38 °C sustained for more than one hour. The highest recorded fever value upon admission was documented as the body temperature measured at the time of diagnosing febrile neutropenia. In all patients presenting with a febrile neutropenic episode, at least one set of peripheral blood cultures and, if present, cultures from each lumen of the

central venous catheter were obtained promptly after the onset of fever. Following culture collection, broad-spectrum empirical antibiotic therapy (piperacillin-tazobactam, meropenem or cefepime) was initiated. In cases where fever persisted beyond 48–72 hours, or if there was clinical deterioration at the onset of the episode, suspicion of a resistant pathogen, or a possible catheter-related infection, the antibiotic regimen was modified accordingly. Cases defined solely by fever without demonstrable clinical and laboratory signs of infection were categorized FUO. A clinically defined infection referred to an infection clinically diagnosed when the microbiologically infectious agent could not be identified. Examples included pneumonia, mucositis, cellulitis, sinusitis, perianal infections, and neutropenic enterocolitis (typhlitis). A microbiologically defined infection was defined as either the presence of the infectious agent in the blood culture, without a defined clinical focus, or the identification of a microbiological agent at a clinical focus without corresponding growth in the blood culture. Serious infections included catheter infection, port pocket infection, pneumonia, meningitis, typhlitis, and sepsis.

Patients were categorized based on the chemotherapy regimens they received prior to the onset of febrile neutropenic episodes. The chemotherapy regimens included doxorubicin, alkylating agents, and platinum-based regimens. The onset of febrile neutropenia was recorded after administration of the final dose of chemotherapy. The duration between the last chemotherapy dose and the onset of the febrile neutropenia episode was documented.

Data on the duration of neutropenia for each febrile episode were also collected and defined as the number of consecutive days with ANC below 500/mm$^3$. Neutropenia duration was defined as the period from the onset of neutropenia (ANC < 500/mm$^3$) to the first recovery of ANC to > 500/mm$^3$. Prolonged neutropenia was defined as an ANC remaining below 500/mm$^3$ for more than 7 days, regardless of the underlying cause.

Discharge decisions were based on several criteria, including hemodynamic stability, being afebrile for at least 24–48 hours, control of the infectious focus, evidence of neutrophil recovery, and the availability of a safe and supportive home environment for continued monitoring. Early discharge was defined as the discontinuation of intravenous antibiotic therapy and hospital discharge in low-risk patients who had received at least 72 hours of intravenous antibiotics, remained afebrile for a minimum of 24 hours, and had no documented source of infection. This approach was considered appropriate irrespective of bone marrow recovery, provided that reliable outpatient monitoring could be ensured.

### *Statistical analysis*

Statistical analysis was performed using SPSS for Windows version 26.0 (SPSS Inc, USA). Numerical variables were expressed as mean ± standard deviation or median (range of distribution), while qualitative variables were presented as numbers and percentages. The assumption of normality for quantitative variables was assessed using the Shapiro-Wilk test. If a normal distribution was confirmed by the Shapiro-Wilk test, comparisons between two groups were conducted using the Student's t-test. If the assumption of homogeneity of variances was met, the Student's t-test was used; otherwise, the Welch test was applied. If a normal distribution was not present, the Mann-Whitney U test was used. Results were presented as mean ± standard deviation for the Welch test and Student's t-test, and as median (minimum–maximum) for the Mann-Whitney U test. Due to the violation of the homogeneity of variances assumption in the comparison of infection types, Welch's ANOVA was employed. Pairwise comparisons between groups for type of infection were conducted using the Tamhane T2 post hoc test. To evaluate type of infections, two dummy variables were generated. A multiple linear regression model was constructed using the variables that were found to be statistically significant in univariate

analyses. The backward elimination method was applied to identify the most relevant predictors influencing discharge time. To assess multicollinearity among independent variables, Variance Inflation Factors (VIF) were calculated. All VIF values were below 5, indicating that multicollinearity was not a concern. The coefficient of determination ($R^2$) was reported to evaluate the explanatory power of the final model. The significance level was set at $p<0.05$ for all analyses.

This study received ethical approval from Hacettepe University Non-Interventional Clinical Research Ethics Committee (No: GO 18/445). Written informed consent was obtained from parents/legal guardians for all participants.

## Results

During the 12-month follow-up period, a total of 142 febrile neutropenic episodes from 88 patients were included in the study. The median age of the patients was 6.8 years (range: 0.4-17.2 years), and the male-to-female ratio was 1.2:1. Solid tumors were more prevalent than lymphomas, accounting for 80.7% of cases compared to 19.3% for lymphomas. Among the lymphomas, non-Hodgkin lymphoma was the most frequent diagnosis, while neuroblastoma was the most common solid tumor type associated with febrile neutropenic episodes. Table I presents the characteristics of febrile neutropenic patients.

For patients experiencing febrile neutropenic episodes, the median white blood cell count at admission was 500/mm$^3$ (range: 0-6,000/mm$^3$), and the median ANC was 50/mm$^3$ (range: 0-900/mm$^3$), additional parameters are presented in Table II.

Regarding the febrile neutropenic episodes, the median duration of fever was 2 days (range: 1-16 days), and the median duration of neutropenia

**Table I.** Demographic characteristics of pediatric cancer patients with febrile neutropenia (N=88).

| | n (%) |
|---|---|
| Female / male gender | 40 (45.5) / 48 (54.5) |
| Diagnosis | |
| Lymphoma | 17 (19.3) |
| Non-Hodgkin lymphoma | 16 (18.1) |
| Hodgkin lymphoma | 1 (1.1) |
| Solid tumor | 71 (80.7) |
| Neuroblastoma | 17 (19.3) |
| Rhabdomyosarcoma | 14 (15.9) |
| Ewing sarcoma | 9 (10.2) |
| Central nervous system tumor | 8 (9) |
| Osteosarcoma | 7 (7.9) |
| Others* | 16 (18.1) |
| Comorbid condition | 18 (20.5) |
| Nephrotoxicity | 5 |
| Hepatotoxicity | 4 |
| Respiratory failure | 3 |
| Coagulation disorder | 3 |
| Immunodeficiency | 3 |
| Illness status | |
| Remission | 71 (80.7) |
| Recurrent / resistant disease | 17 (19.3) |
| Bone marrow involvement | 28 (20.5) |

*Wilms tumor, germ cell tumor, hepatoblastoma, retinoblastoma.

**Table II.** Laboratory parameters at the time of admission

| Parameter | Median (range) |
|---|---|
| Hb (gr/dL) | 8.8 (6.3-13.5) |
| WBC (/mm$^3$) | 500 (0-6,000) |
| ANC (/mm$^3$) | 50 (0-900) |
| AMC (/mm$^3$) | 0 (0-1,500) |
| Thrombocytes (/mm$^3$) | 69,500 (3,000-623,000) |
| CRP (mg/dL) | 4.05 (0.2-46.6) |
| ESR (mm/h) | 22 (2-96) |

AMC: absolute monocyte count, ANC: absolute neutrophil count, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, Hb: hemoglobin, WBC: white blood cell.

was 3 days (range: 1-12 days). Clinical features of the episodes are detailed in Table III. In 60 episodes (42.3%), the focus of fever could not be determined, while infection was identified in the remainder, as either clinical (40.1%) or microbiological (17.6%). In blood and catheter culture positive cases, the most common pathogen isolated was methicillin-resistant *Staphylococcus aureus* (MRSA). Other pathogens are detailed in Table IV. There was only one case (0.7%) in which mortality was attributed to *Candida*-related sepsis; no other infection-related deaths occurred.

The median duration from the last chemotherapy administration to the onset of febrile neutropenia was 7 days. A total of 44 patients (38%) had received regimens that included both doxorubicin and alkylating agents. During follow-up, the most frequently observed chemotherapy protocols preceding febrile neutropenic episodes were as follows: 22.5% of episodes were associated with regimens containing both platinum and alkylating agents, 10.4% with platinum-based regimens, 9.2% with alkylating agents alone, 6.3% with regimens including both doxorubicin and platinum, and 13.4% with other chemotherapeutic agents. A comparison of discharge times across the different chemotherapy regimen groups revealed no statistically significant difference ($p = 0.186$).

The median length of hospital stay for patients with febrile neutropenic was seven days (range: 3-25 days) with antibiotic treatment. Empirical antibiotic therapy was initiated with piperacillin-tazobactam in 87 episodes (61.3%) febrile neutropenic episodes, meropenem in 33 episodes (23.2%), and cefepime in 22 episodes (15.5%). In 67 episodes (47%), antibiotic modification was required due to persistent fever after 48–72 hours (n=26), hemodynamic instability (n=16), or positive culture results (n=25). Patients who required

**Table III.** Features of febrile neutropenic episodes (N=142).

| | n (%) |
|---|---|
| Number of episodes per patient, median (range) | 1 (1-5) |
| Status at the time of admission | |
| At hospital | 31 (21.8) |
| Outpatient | 111 (78.2) |
| Time since last chemotherapy, median (range) | 7 (0-22) |
| Prophylactic use of G-CSF | 60 (42) |
| Severity of neutropenia | |
| ANC < 100 /mm$^3$ | 102 (71.8) |
| ANC > 100 /mm$^3$ | 40 (28.2) |
| Monocyte level | |
| AMC < 100 /mm$^3$ | 100 (70.4) |
| AMC > 100 /mm$^3$ | 42 (29.6) |
| Thrombocytopenia (<50,000 /mm$^3$) | 61 (43) |
| Fever above 39 °C at admission | 45 (31.2) |
| Poor condition | 31 (21.8) |
| Hypotension | 16 (11.2) |
| Capillary refill time >2 sec | 16 (11.2) |
| Oral mucositis | 39 (27.4) |
| Anal ulceration | 11 (7) |
| Serious infection | 38 (26.7) |
| Catheter-related infection | 18 (12.6) |
| Pneumonia | 10 (7) |
| Sepsis | 5 (3.5) |
| Port pocket infection | 2 (1) |
| Typhlitis | 2 (1) |
| Meningitis | 1 (0.7) |
| Type of infection | |
| FUO | 60 (42.3) |
| Clinically defined | 57 (40.1) |
| Microbiologically defined | 25 (17.6) |
| Positive peripheral blood culture | 13 (9.1) |
| Positive central venous catheter culture | 17 (11.9) |

ANC: absolute neutrophil count, AMC: absolute monocyte count, FUO: fever unknown origin, G-CSF: granulocyte-colony stimulating factor.

**Table IV.** Microbiological characteristics of culture-positive rebrile neutropenia episodes

| | Peripheral blood (n) | Central venous catheter (n) |
|---|---|---|
| Methicillin-resistant *S. aureus* | 1 | 3 |
| Methicillin-resistant *S. epidermidis* | 4 | 6 |
| *Pseudomonas aeruginosa* | 2 | 1 |
| *Escherichia coli* | 1 | 0 |
| *Enterococcus faecalis* | 2 | 1 |
| *Enterocccus faecium* | 0 | 3 |
| *Acinetobacter pitti* | 1 | 0 |
| *Hesbaspirullum aquaticum* | 1 | 0 |
| *Klebsiella oxycota* | 1 | 1 |
| *Candida kefyr* | 0 | 2 |

antibiotic modification had a median discharge time of 10 days, compared to 5 days for those who did not require modification ($p < 0.001$, Table V). Although neutropenia (ANC < 500/mm$^3$) persisted in 8 episodes, patients were discharged early, after a median of 3 days once signs of bone marrow recovery were observed. Only one patient (0.7%) was re-hospitalized for recurrent fever within 48 hours after discharge and was successfully discharged again following treatment.

Lymphoma diagnosis, presence of comorbidities, presence of catheter, and antibiotic modification were all significantly associated with prolonged hospitalization (Table V). Although no statistically significant association was observed between the length of hospital stay and sex or disease status ($p = 0.652$ and $p = 0.095$, respectively), patients with relapsed or refractory disease tended to have longer hospitalizations compared to those in remission. Additionally, patients presenting with an absolute monocyte count (AMC) ≤ 100/mm$^3$ and a platelet count ≤ 50,000/mm$^3$ were more likely to experience prolonged hospital stays ($p < 0.001$ and $p = 0.04$, respectively). In contrast, no statistically significant association was found between hemoglobin levels ≤ 7 g/dL or severe neutropenia (ANC ≤ 100/mm$^3$) and time to discharge ($p = 0.12$ and $p = 0.81$, respectively). When the length of hospitalization was analyzed based on infection type during febrile neutropenic episodes, patients with microbiologically documented infections had significantly longer hospital stays compared to those with clinically or microbiologically documented infections ($p < 0.001$). Laboratory and microbiological parameters associated with prolonged hospitalization included prolonged neutropenia, prolonged fever and positive blood and/or catheter cultures ($p < 0.001$).

Multiple linear regression analysis identified several significant factors influencing discharge time (Table VI). Based on standardized regression coefficients (β), microbiologically defined infection was the strongest predictor of discharge timing, associated with a 2.8-day longer hospital stay compared with FUO ($p<0.001$, $\beta = 0.345$). Prolonged fever (>96 hours) and poor general condition were associated with 2.7- and 2.6-day longer stays, respectively ($p<0.001$, $\beta = 0.312$ and $\beta = 0.277$). Prolonged neutropenia (≥7 days) extended hospitalization by 2.9 days compared with neutropenia lasting <7 days ($p<0.001$, $\beta = 0.228$). Patients requiring antibiotic therapy modification stayed 1 day longer ($p=0.041$, $\beta = 0.123$), those with lymphoma had a 1.1-day longer stay than patients with solid tumors ($p=0.013$, $\beta = 0.117$), and thrombocytopenia added 0.9 days to hospitalization ($p=0.014$, $\beta = 0.113$). The model demonstrated strong explanatory power, with $R^2 = 0.737$ and adjusted $R^2 = 0.721$, indicating that approximately 72% of the variance in discharge timing was explained by these variables.

**Table V.** Simple linear regression analysis of variables affecting discharge duration.

| Variables | Days to discharge* | *p-value* |
|---|---|---|
| Gender | | |
| Female (n=70) | 7.8 ± 4.0 | 0.652[a] |
| Male (n=72) | 8.1 ± 4.2 | |
| Diagnosis | | |
| Lymphoma (n=30) | 9.5 ± 4.5 | 0.017[a] |
| Solid tumor (n=112) | 7.5 ± 0.3 | |
| Illness status | | |
| Recurrent/Resistant disease (n=32) | 9.3 ± 5.4 | 0.095[b] |
| Remission (n=110) | 7.5 ± 3.5 | |
| Bone marrow involvement | | |
| Lymphoma (n=30) | 9.7 ± 5.5 | 0.03[b] |
| Solid tumor (n=112) | 7.4 ± 3.4 | |
| Comorbid condition | | |
| Yes (n=30) | 9.6 ± 4.6 | 0.013[a] |
| No (n=112) | 7.5 ± 3.8 | |
| Central venous catheter | | |
| Yes (n=90) | 8.6 ± 4.5 | 0.005[b] |
| No (n=52) | 6.8 ± 2.9 | |
| Antibiotic therapy modifications | | |
| Yes (n=67) | 10.5 ± 4.3 | < 0.001[b] |
| No (n=75) | 5.6 ± 1.9 | |
| Fever at admission | | |
| ≥ 39 °C (n=45) | 9.6 ± 4.2 | < 0.001[a] |
| < 39 °C (n=97) | 7.1 ± 3.7 | |
| General condition | | |
| Poor (n=31) | 13.0 ± 4.2 | < 0.001[b] |
| Good (n=111) | 6.5 ± 2.6 | |
| Hypotension | | |
| Yes (n=16) | 13.0 ± 4.8 | < 0.001[a] |
| No (n=126) | 7.3 ± 3.5 | |
| Capillary refill time | | |
| >2 sec (n=16) | 13.0 ± 4.8 | < 0.001[a] |
| ≤2 sec (n=126) | 7.3 ± 3.5 | |
| Serious infection | | |
| Yes (n=38) | 12.4 ± 4.1 | < 0.001[b] |
| No (n=104) | 6.3 ± 2.5 | |
| Hemoglobin level | | |
| ≤ 7 g/dL (n=14) | 9 (4-25) | 0.12[c] |
| > 7 g/dL (n=128) | 7 (3-21) | |
| Seerity of neutropenia | | |
| ≤100 cells /mm$^3$ (n=102) | 8.0 ± 3.9 | 0.81[a] |
| >100 cells /mm$^3$ (n=40) | 7.8 ± 4.3 | |
| Absolute monocyte count | | |
| ≤100 cells /mm$^3$ (n=100) | 8.7 ± 4.2 | < 0.001[b] |
| >100 cells /mm$^3$ (n=42) | 6.1 ± 2.9 | |
| Thrombocyte count | | |
| ≤ 50,000 /mm$^3$ (n=61) | 8.7 ± 4.5 | 0.04[a] |
| > 50,000 /mm$^3$ (n=81) | 7.3 ± 3.5 | |
| Type of infection | | |
| Fever of unknown origin (n=60) | 5.6 ± 2.0[+] | < 0.001[d] |
| Clinically defined (n=57) | 8.5 ± 4.1[*] | |
| Microbiologically defined (n=25) | 12.2 ± 3.78[x] | |
| Peripheral blood culture | | < 0.001[a] |
| Positive (n=13) | 13.1 ± 4.0 | |
| Negative (n=129) | 7.4 ± 3.7 | |
| Central venous catheter culture | | < 0.001[a] |
| Positive (n=18) | 12.5 ± 3.2 | |
| Negative (n=69) | 7.5 ± 4.3 | |
| Prolonged neutropenia (≥ 7 days) | | < 0.001[a] |
| Yes (n=15) | 12.6 ± 5.1 | |
| No (n=123) | 7.3 ± 3.4 | |
| Prolonged fever (> 96 hours) | | < 0.001[b] |
| Yes (n=41) | 12.0 ± 4.3 | |
| No (n=101) | 6.3 ± 2.5 | |

[a,b,c,d] p-values are obtained from Student t-test, Welch test, Mann-Whitney U test, and Welch ANOVA respectively.
*Expressed as mean ± standard deviation, or median (range)
P < 0.05 printed bold.

**Table VI.** Multiple linear regression analysis of variables affecting discharge duration.

| Variables | B | 95% CI | β | p |
|---|---|---|---|---|
| Constant | 7.792 | 6.578-9.005 | - | 0.000 |
| Presence of lymphoma | 1.116 | 0.252-2.080 | 0.117 | 0.013 |
| Antibiotic therapy modification | 0.987 | 0.039-1.935 | 0.123 | 0.041 |
| Poor condition | 2.689 | 1.601-3.778 | 0.277 | <0.001 |
| Thrombocytopenia (< 50,000 /mm$^3$) | 0.911 | 0.184-1.639 | 0.113 | 0.014 |
| Prolonged neutropenia (≥ 7 days) | 2.931 | 1.747-4.114 | 0.228 | <0.001 |
| Prolonged fever (>96 hours) | 2.769 | 1.779-3.758 | 0.312 | <0.001 |
| FUO vs. microbiologically defined | -2.792 | -3.997- (-1.587) | 0.345 | <0.001 |
| Clinically vs. microbiologically defined | -1.425 | -2.517- (-0.334) | 0.175 | 0.011 |

B: unstandardized coefficients; β: standardized coefficients, CI: confidence interval, FUO: fever of unknown origin.
$R^2 = 0.737$, $R^2adj = 0.721$, $p<0.001$

## Discussion

The prompt initiation of broad-spectrum antibiotic therapy for all hospitalized children with febrile neutropenia has significantly reduced mortality rates.[8-11] The risk stratification is crucial to avoid toxicity, prevent the resistance, improve patient quality of life, predict prognosis, shorten antibiotic courses, hospital stays and reduce costs. Treatment algorithms for adult patients have been established based on high and low-risk febrile neutropenic categories.[4,12-14] Nonetheless, studies on risk classification and treatment algorithms for pediatric patients continue to progress.

Factors such as a diagnosis of leukemia or lymphoma[15-17], progressive disease[15], bone marrow involvement[15,16], and the presence of a central venous catheter[18] have been identified in literature as poor prognostic indicators in the treatment of febrile neutropenia among pediatric cancer patients. As our clinic mainly accepts patients with solid tumors and lymphomas, we observed that patients with lymphoma diagnosis had the longest hospital stay in our study. This may be attributed to their intensive chemotherapeutic regimens.[19-21] A prospective cohort study supports this observation, indicating that patients with hematological malignancies carry a higher risk of serious infections compared to those with solid tumors, likely attributed to the intensity of chemotherapy.[22] Despite these observations, we did not find a significant relationship between chemotherapy regimens and febrile neutropenia prognosis or discharge time in our study group. This lack of correlation may be influenced by the relatively small sample size of our study.

Ammann et al. reported that bone marrow involvement increases the risk of serious infection in febrile neutropenic pediatric cancer patients.[23] Similarly, we considered patients with bone marrow involvement to be at higher risk, and they indeed experienced prolonged hospitalization. Recurrent or resistant disease did not show a significant difference in terms of hospitalization duration, likely because the majority of patients in our study were newly diagnosed and in remission (80.7%). The prolonged hospitalization in febrile neutropenic episodes among patients with comorbid conditions supports findings from similar studies in the literature.[4,24]

Physical examination findings upon admission during febrile neutropenic episodes offer valuable insights into patient prognosis and treatment outcomes. In our study, patients presenting with fever of ≥ 39 °C, poor general condition, hypotension, prolonged capillary refill time, and severe infection had a more challenging and prolonged course of fever, resulting in a longer duration of antibiotic

administration. Evidence from the literature also supports the importance of general appearance and vital sign stability as indicators of the lack of serious infection, whereas a fever exceeding 38.5 °C, prolonged capillary refill time, and severe tachypnea are associated with an increased risk of infection. These physical examination findings and the overall general condition are essential markers of disease severity not only in febrile neutropenic patients but also across all patient groups.[20,25,26] Nevertheless, it is crucial to identify which examination findings carry greater predictive value than others. In our study, poor general condition on physical examination and prolonged fever demonstrated higher predictive value for hospitalization duration.

Determining the predictive value of laboratory tests is an essential aspect of numerous studies, including ours.[27,28] Notably, the neutrophil count stands out as a key parameter in diagnosing febrile neutropenia. In our study, a significant proportion (71%) of patients experienced severe neutropenia (ANC ≤ 100/mm$^3$). We found that the depth of neutropenia did not negatively impact the patients' days of antibiotic treatment or discharge time. Although our study did not establish a specific cutoff value for ANC, we observed a significantly longer hospital stay in febrile neutropenic patients with AMC ≤ 100/mm$^3$. Additionally, a platelet count of ≤ 50,000/mm$^3$ was associated with prolonged hospitalization. Our findings align with Amman et al.'s study, who reported that a platelet count less than 50,000/mm$^3$ and AMC less than 100/mm$^3$ were linked to an increased risk of serious infection in febrile neutropenic patients.[23] Similarly, Rackoff et al. reported that an AMC higher than 100/mm$^3$ was associated with a low risk of bacteremia.[29] In another study, an AMC lower than 100/mm$^3$ and prolonged neutropenia were associated with clinically or microbiologically defined infection.[30] Lima et al., also emphasized that a platelet count below 50,000/mm$^3$ at the onset of fever is particularly important as it indicates a higher likelihood of infection.[31] Alali et al. reported that the median length of stay for febrile neutropenia was three days shorter when patients were discharged when AMC >100/mm$^3$, which was associated with fewer unfavorable outcomes, resulting in reduced hospital days, shorter antibiotic courses, and lower costs.[32] Nevertheless, it is essential to classify patients presenting with an AMC ≤ 100/mm$^3$ and a platelet count of ≤ 50,000/mm$^3$ as high-risk. Such patients require careful management and should not to be considered for early discharge.

Regarding Hb and CRP values, our study did not identify specific threshold values. However, patients with low Hb levels and elevated CRP levels experienced later discharge. Notably, Rondinelli's study identified an Hb level less than 7 g/dL as a high-risk factor for serious infection complications.[18] Similarly, Ammann et al.'s study revealed that a CRP level higher than 90 mg/L increased the risk of serious infection.[23] In another study, febrile neutropenic children with a CRP higher than 90 mg/L, platelet count lower than 20,000/mm$^3$, and albumin levels lower than 2.5 g/dL were considered high-risk for complications and mortality.[33] Similar to the study conducted by Secmeer et al., CRP and procalcitonin levels were found to be higher in patients with neutropenic fever.[34] Although our study did not determine a specific CRP threshold, elevated CRP levels at admission may serve as a laboratory marker to predict the severity of infection and discharge duration. When examining the prognosis and discharge duration in febrile neutropenic patients, it becomes apparent that multiple laboratory parameters and clinical findings complicate the prediction of outcomes in individual patients. This likely explains why existing literature often assigns threshold values to specific data rather than determining comprehensive thresholds for all parameters.

The duration of neutropenia plays a crucial role in the decision to discharge for febrile neutropenic children with cancer. In our study group, patients with shorter neutropenia duration were discharged earlier, leading to shorter hospital stays. A retrospective study by Delebarre et al.

similarly emphasized prolonged neutropenia as the most critical parameter in predicting severe infection.[20] Although no standard neutrophil value is specified for discharge decisions in febrile neutropenic pediatric patients, current guidelines recommend discontinuing empirical antibacterial therapy after 48 hours if there is evidence of marrow recovery and blood cultures remain negative. This applies to both high-risk and low-risk febrile neutropenia patients who have been clinically well and afebrile for at least 24 hours, in accordance with the 2017 and 2023 guidelines.[7,35] In our study, eight febrile neutropenic cases were discharged despite an ANC < 500/mm$^3$ after at least three days of antibiotic treatment and a median of two days without fever during follow-up. Only one patient required re-hospitalization due to recurrent fever, and their subsequent infection was successfully treated.[36] Additionally, in another retrospective study evaluating 84 febrile neutropenic episodes in 56 patients as FUO, patients were discharged after at least 24 hours of afebrile and 72 hours of intravenous antibiotic therapy, irrespective of ANC levels. No re-hospitalization, deaths, or major complications were observed.[36] Likewise, in another study from the literature, 37 out of 83 low-risk febrile neutropenia episodes recovered after 48 hours of intravenous antibiotic treatment.[13] To facilitate early discharge planning, maintaining effective communication with parents and ensuring patients can promptly seek medical attention are essential prerequisites. However, in cases of low-risk febrile neutropenia where these conditions cannot be met, implementing early discharge remains challenging. Jackson et al. demonstrated that the Australian-UK-Swiss (AUS) rule and homecare criteria can safely identify children who can be discharged on oral antibiotics with parental monitoring, and that low-risk febrile neutropenia episodes in selected children require less than 24 hours of inpatient care.[37] Givone et al. established a consensus on criteria for initiating evidence-based step-down treatment in children with febrile neutropenia and low-risk infections, though no agreement was reached regarding the specific antimicrobial regimen for step-down therapy.[38] These findings highlight the need for further studies to better understand hospitalization duration and treatment decisions in febrile neutropenic children. Future research will play a crucial role in optimizing treatment protocols and ensuring safer discharge processes for these patients.

Febrile neutropenic patients experience infections resulting from reduced neutrophil function and compromised mucosal barriers, classified into different categories. In many cases, the source of fever cannot be identified. Clinically diagnosed infections are seen in 20-30% of febrile neutropenic episodes, however the etiological agent can only be identified microbiologically in only 10-30% of all cases.[4,39] Our microbiological infection rate was similar to that in the literature. We found a higher proportion of clinically diagnosed infections (40.1%), which may be attributed to the prospective design of our study, ensuring reliable data records and detailed physical examinations.

Our study did not analyze the hospital costs for febrile neutropenic patients. A study by Mueller et al. revealed that 40% of febrile neutropenic patients had a short hospital stay, and severe infections were rare.[40] Therefore, various studies have explored early discharge in low-risk febrile neutropenic patients to reduce the financial burden.[10,13] However, it should be emphasized that risk classification scores must be evaluated specifically for each pediatric oncology center.[6,13] In the study by Vargas et al., it was stated that outpatient management of low-risk patients had the potential to reduce the hospital treatment costs of febrile neutropenia events. This suggests that managing low-risk cases on an outpatient basis could alleviate the financial burden on the healthcare system compared to inpatient care.[41] Socioeconomic level of patients, hospital transportation options, available resources, and limitations of the social security system may vary significantly between regions, and all these factors influence early discharge decisions. A recent study demonstrated that

although ANC is commonly included in risk stratification strategies for the management of febrile neutropenia, socioeconomic factors that may influence readmission rates should also be taken into account.[42] According to our evaluation, these variables significantly affect the decision-making process. While our study suggests that early discharge without waiting for neutropenia resolution is possible for low-risk febrile neutropenic patients in accordance with pediatric febrile neutropenia guidelines, we observed that only 8 out of 142 febrile neutropenic episodes met this criterion. This was primarily due to the majority of patients (60.2%) coming from rural areas with lower socioeconomic levels.

Many studies conducted in children rely on retrospective data, limiting their findings to the routine evaluation parameters of a single clinic and not encompassing the broader literature. Due to the retrospective nature of such studies, certain clinical examination findings may not have been recorded, resulting in an emphasis on analyzing laboratory values alone. One of the strengths of our study is its prospective design. The higher rate of clinically diagnosed infections in our study might be attributed to this prospective approach and comprehensive evaluation. However, the limitations of our study include its single-center design and relatively limited sample size.

### *Conclusion*

We conclude that the diagnosis of lymphoma, presence of comorbid conditions, central catheter use, bone marrow involvement, and febrile neutropenic episodes occurring during hospitalization are indicative of high-risk group evaluation and delayed discharge. Patients with fever ≥ 39°C at the time of febrile neutropenic episodes, poor general condition, hypotension, prolonged capillary refill time, and severe infection findings also experienced prolonged hospital stays and antibiotic administration. Prolonged fever and neutropenia were further associated with extended hospitalization. These results suggest that it is possible to devise a treatment plan based on early risk group determination, allowing clinicians to predict appropriate discharge timing. Early discharge of low-risk patients not only reduces hospital costs but also significantly improves patient quality of life. Oral antibiotic therapy with close follow-up is yet to be standardized for low-risk febrile neutropenic patients across all centers. Our study reveals high-risk criteria in febrile neutropenic patients, thereby making it possible to identify low-risk patients. Discharging low-risk patients early or providing outpatient follow-up must be integrated in the care of the pediatric cancer patients.

### Acknowledgment

We would like to sincerely thank Osman Dağ, an expert biostatistician, for his invaluable consulting support. His guidance and assistance with the statistical analysis significantly contributed to the quality of this manuscript.

### Ethical approval

The study was approved by Hacettepe University Non-Interventional Clinical Research Ethics Committee (date: May 3, 2018, number: GO 18/445).

### Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: CÜ, BA, NK, BY, AV, TK; data collection: CÜ; analysis and interpretation of results: CÜ, BA, NK, BY, AV, TK; draft manuscript preparation: CÜ, BA. All authors reviewed the results and approved the final version of the manuscript.

### Source of funding

The authors declare the study received no funding.

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES


1. Nelson RC, Ivey JR, Eder AF. Delayed presentation of a septic transfusion reaction. Transfusion 2017; 57: 2309-2310. https://doi.org/10.1111/trf.13983
2. Sung L, Feldman BM, Schwamborn G, et al. Inpatient versus outpatient management of low-risk pediatric febrile neutropenia: measuring parents' and healthcare professionals' preferences. J Clin Oncol 2004; 22: 3922-3929. https://doi.org/10.1200/JCO.2004.01.077
3. de Naurois J, Novitzky-Basso I, Gill MJ, et al. Management of febrile neutropenia: ESMO Clinical Practice Guidelines. Ann Oncol 2010; 21(Suppl 5): v252-v256. https://doi.org/10.1093/annonc/mdq196
4. Freifeld AG, Bow EJ, Sepkowitz KA, et al. Clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the Infectious Diseases Society of America. Clin Infect Dis 2011; 52: e56-e93. https://doi.org/10.1093/cid/cir073
5. Phillips R, Hancock B, Graham J, Bromham N, Jin H, Berendse S. Prevention and management of neutropenic sepsis in patients with cancer: summary of NICE guidance. BMJ 2012; 345: e5368. https://doi.org/10.1136/bmj.e5368
6. Lehrnbecher T, Phillips R, Alexander S, et al. Guideline for the management of fever and neutropenia in children with cancer and/or undergoing hematopoietic stem-cell transplantation. J Clin Oncol 2012; 30: 4427-4438. https://doi.org/10.1200/JCO.2012.42.7161
7. Lehrnbecher T, Robinson P, Fisher B, et al. Guideline for the management of fever and neutropenia in children with cancer and hematopoietic stem-cell transplantation recipients: 2017 update. J Clin Oncol 2017; 35: 2082-2094. https://doi.org/10.1200/JCO.2016.71.7017
8. Cagol AR, Castro Junior CG, Martins MC, et al. Oral vs. intravenous empirical antimicrobial therapy in febrile neutropenic patients receiving childhood cancer chemotherapy. J Pediatr (Rio J) 2009; 85: 531-535. https://doi.org/10.2223/JPED.1956
9. Lehrnbecher T. Treatment of fever in neutropenia in pediatric oncology patients. Curr Opin Pediatr 2019; 31: 35-40. https://doi.org/10.1097/MOP.0000000000000708
10. Mueller EL, Walkovich KJ, Mody R, Gebremariam A, Davis MM. Hospital discharges for fever and neutropenia in pediatric cancer patients: United States, 2009. BMC Cancer 2015; 15: 388. https://doi.org/10.1186/s12885-015-1413-8
11. Kutluk T, Kurne O, Akyüz C, et al. Cefepime vs. Meropenem as empirical therapy for neutropenic fever in children with lymphoma and solid tumours. Pediatr Blood Cancer 2004; 42: 284-286. https://doi.org/10.1002/pbc.10442
12. Flowers CR, Seidenfeld J, Bow EJ, et al. Antimicrobial prophylaxis and outpatient management of fever and neutropenia in adults treated for malignancy: American Society of Clinical Oncology clinical practice guideline. J Clin Oncol 2013; 31: 794-810. https://doi.org/10.1200/JCO.2012.45.8661
13. Gil-Veloz M, Pacheco-Rosas DO, Solórzano-Santos F, Villasís-Keever MA, Betanzos-Cabrera Y, Miranda-Novales G. Early discharge of pediatric patients with cancer, fever, and neutropenia with low-risk of systemic infection. Bol Med Hosp Infant Mex 2018; 75: 352-357. https://doi.org/10.24875/BMHIM.18000015
14. Marti FM, Cullen MH, Roila F; ESMO Guidelines Working Group. Management of febrile neutropenia: ESMO clinical recommendations. Ann Oncol 2009; 20(Suppl 4): 166-169. https://doi.org/10.1093/annonc/mdp163
15. Alexander SW, Wade KC, Hibberd PL, Parsons SK. Evaluation of risk prediction criteria for episodes of febrile neutropenia in children with cancer. J Pediatr Hematol Oncol 2002; 24: 38-42. https://doi.org/10.1097/00043426-200201000-00011
16. Ammann RA, Bodmer N, Hirt A, et al. Predicting adverse events in children with fever and chemotherapy-induced neutropenia: the prospective multicenter SPOG 2003 FN study. J Clin Oncol 2010; 28: 2008-2014. https://doi.org/10.1200/JCO.2009.25.8988
17. Santolaya ME, Alvarez AM, Becker A, et al. Prospective, multicenter evaluation of risk factors associated with invasive bacterial infection in children with cancer, neutropenia, and fever. J Clin Oncol 2001; 19: 3415-3421. https://doi.org/10.1200/JCO.2001.19.14.3415
18. Rondinelli PI, Ribeiro Kde C, de Camargo B. A proposed score for predicting severe infection complications in children with chemotherapy-induced febrile neutropenia. J Pediatr Hematol Oncol 2006; 28: 665-670. https://doi.org/10.1097/01.mph.0000212996.94929.0b

19. Castagnola E, Fontana V, Caviglia I, et al. A prospective study on the epidemiology of febrile episodes during chemotherapy-induced neutropenia in children with cancer or after hemopoietic stem cell transplantation. Clin Infect Dis 2007; 45: 1296-1304. https://doi.org/10.1086/522533

20. Delebarre M, Garnier N, Macher E, et al. Which variables are useful for predicting severe infection in children with febrile neutropenia? J Pediatr Hematol Oncol 2015; 37: e468-e474. https://doi.org/10.1097/MPH.0000000000000440

21. Phillips RS, Sung L, Ammann RA, et al. Predicting microbiologically defined infection in febrile neutropenic episodes in children: global individual participant data multivariable meta-analysis. Br J Cancer 2016; 114: e17. https://doi.org/10.1038/bjc.2016.137

22. Delebarre M, Dessein R, Lagrée M, et al. Differential risk of severe infection in febrile neutropenia among children with blood cancer or solid tumor. J Infect 2019; 79: 95-100. https://doi.org/10.1016/j.jinf.2019.06.008

23. Ammann RA, Hirt A, Lüthy AR, Aebi C. Identification of children presenting with fever in chemotherapy-induced neutropenia at low risk for severe bacterial infection. Med Pediatr Oncol 2003; 41: 436-443. https://doi.org/10.1002/mpo.10320

24. Klastersky J, Paesmans M, Rubenstein EB, et al. The multinational association for supportive care in cancer risk index: a multinational scoring system for identifying low-risk febrile neutropenic cancer patients. J Clin Oncol 2000; 18: 3038-3051. https://doi.org/10.1200/JCO.2000.18.16.3038

25. Van den Bruel A, Thompson M, Buntinx F, Mant D. Clinicians' gut feeling about serious infections in children: observational study. BMJ 2012; 345: e6144. https://doi.org/10.1136/bmj.e6144

26. Kara SS, Tezer H, Polat M, et al. Risk factors for bacteremia in children with febrile neutropenia. Turk J Med Sci 2019; 49: 1198-1205. https://doi.org/10.3906/sag-1901-90

27. Klaassen RJ, Goodman TR, Pham B, Doyle JJ. "Low-risk" prediction rule for pediatric oncology patients presenting with fever and neutropenia. J Clin Oncol 2000; 18: 1012-1019. https://doi.org/10.1200/JCO.2000.18.5.1012

28. Orudjev E, Lange BJ. Evolving concepts of management of febrile neutropenia in children with cancer. Med Pediatr Oncol 2002; 39: 77-85. https://doi.org/10.1002/mpo.10073

29. Rackoff WR, Gonin R, Robinson C, Kreissman SG, Breitfeld PB. Predicting the risk of bacteremia in childen with fever and neutropenia. J Clin Oncol 1996; 14: 919-924. https://doi.org/10.1200/JCO.1996.14.3.919

30. Tezcan G, Kupesiz A, Ozturk F, et al. Episodes of fever and neutropenia in children with cancer in a tertiary care medical center in Turkey. Pediatr Hematol Oncol 2006; 23: 217-229. https://doi.org/10.1080/08880010500506719

31. Lima MAF, de Sá Rodrigues KE, Vanucci MF, et al. Bloodstream infection in pediatric patients with febrile neutropenia induced by chemotherapy. Hematol Transfus Cell Ther 2023; 45: 170-175. https://doi.org/10.1016/j.htct.2021.08.005

32. Alali M, Prather C, Danziger-Isakov LA, et al. Absolute monocyte count as early and safe marker for antibiotic cessation in febrile neutropenia without etiology in pediatric oncology patients. J Pediatr Hematol Oncol 2023; 45: e702-e709. https://doi.org/10.1097/MPH.0000000000002696

33. Das A, Trehan A, Bansal D. Risk factors for microbiologically-documented infections, mortality and prolonged hospital stay in children with febrile neutropenia. Indian Pediatr 2018; 55: 859-864.

34. Secmeer G, Devrim I, Kara A, et al. Role of procalcitonin and CRP in differentiating a stable from a deteriorating clinical course in pediatric febrile neutropenia. J Pediatr Hematol Oncol 2007; 29: 107-111. https://doi.org/10.1097/MPH.0b013e3180320b5b

35. Lehrnbecher T, Robinson PD, Ammann RA, et al. Guideline for the management of fever and neutropenia in pediatric patients with cancer and hematopoietic cell transplantation recipients: 2023 update. J Clin Oncol 2023; 41: 1774-1785. https://doi.org/10.1200/JCO.22.02224

36. Lehrnbecher T, Stanescu A, Kühl J. Short courses of intravenous empirical antibiotic treatment in selected febrile neutropenic children with cancer. Infection 2002; 30: 17-21. https://doi.org/10.1007/s15010-002-2094-1

37. Jackson TJ, Napper R, Haeusler GM, et al. Can I go home now? The safety and efficacy of a new UK paediatric febrile neutropenia protocol for risk-stratified early discharge on oral antibiotics. Arch Dis Child 2023; 108: 192-197. https://doi.org/10.1136/archdischild-2021-323254

38. Givone A, Duval-Destin J, Delebarre M, Abou-Chahla W, Lervat C, Dubos F. Consensus survey on the management of children with chemotherapy-induced febrile neutropenia and at low risk of severe infection. Pediatr Hematol Oncol 2024; 41: 172-178. https://doi.org/10.1080/08880018.2023.2218406

39. Agyeman P, Kontny U, Nadal D, et al. A prospective multicenter study of microbiologically defined infections in pediatric cancer patients with fever and neutropenia: Swiss Pediatric Oncology Group 2003 fever and neutropenia study. Pediatr Infect Dis J 2014; 33: e219-e225. https://doi.org/10.1097/INF.0000000000000326

40. Mueller EL, Croop J, Carroll AE. Fever and neutropenia hospital discharges in children with cancer: a 2012 update. Pediatr Hematol Oncol 2016; 33: 39-48. https://doi.org/10.3109/08880018.2015.1102998

41. Vargas C, Haeusler GM, Slavin MA, et al. An analysis of the resource use and costs of febrile neutropenia events in pediatric cancer patients in Australia. Pediatr Blood Cancer 2023; 70: e30633. https://doi.org/10.1002/pbc.30633

42. McCormick M, Richardson T, Rapkin L, Kalpatthi R. Risk factors for readmission following febrile neutropenia in pediatric oncology patients. J Pediatr Hematol Oncol 2023; 45: e496-e501. https://doi.org/10.1097/MPH.0000000000002585

The Turkish Journal of Pediatrics 2025; 67: 855-865
https://doi.org/10.24953/turkjpediatr.2025.5599

**Original Article**

# Prognostic value of serum vitamin D level for renal scarring in childhood acute pyelonephritis

Milad Mahzoon[1], Jamshid Yousefi[2], Anoush Azarfar[3], Mahmood Reza Khazaei[2]

*[1]Faculty of Medicine, MMS.C., Islamic Azad University, Mashhad, Iran; [2]Department of Pediatrics, MMS.C., Islamic Azad University, Mashhad, Iran; [3]Kidney Complications Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.*

## ABSTRACT

**Background.** Urinary tract infection (UTI) is a significant issue in childhood due to its high prevalence and potential long-term complications. Several studies have suggested that vitamin D deficiency is an influential factor in the progression of infection. This study aims to determine the value of serum vitamin D levels in predicting renal scarring among children with febrile UTIs.

**Methods.** This study was conducted through a census sampling method from September 2019 to November 2021, with a 6-month follow-up period to survey children with their first febrile UTI who were referred to the nephrology clinic of a tertiary academic hospital. Out of 193 referred children, 55 met the inclusion criteria. Patients were excluded if they had a previous history of UTI, recurrent or breakthrough infection, delay in treatment initiation, hospitalization, ultrasonographic abnormality, hypertension, neurogenic bladder, or renal failure. Five additional cases were excluded due to incomplete follow-up. The study was completed with 50 participants, aged between 3 and 98 months.

The main outcomes were measuring serum vitamin D levels during the acute phase of UTIs and conducting dimercaptosuccinic acid (DMSA) scans four to six months later. Logistic regression was used to determine the correlation between vitamin D levels and DMSA findings.

**Results.** Levels of 25-hydroxyvitamin D were significantly associated with renal scarring ($p = 0.0001$); mean serum concentrations were significantly lower in patients with renal scarring (20.7 ± 7.8 ng/mL) than in those without renal scarring (37.1 ± 11.4 ng/mL). A serum vitamin D concentration of less than 30 ng/mL was determined as the best predictor of post-UTI renal scarring (positive LR 4.25, Youden's j index 0.676).

**Conclusions.** The study showed a negative correlation between renal scarring and serum vitamin D levels. It has been found that serum vitamin D level is a good predictor of renal scarring.

**Key words:** dietary supplements, immune system, pyelonephritis.

Urinary tract infection (UTI) is a common problem in childhood that can lead to kidney damage and renal scarring. Hypertension, albuminuria, and chronic kidney disease are serious consequences of long-term kidney damage and renal scarring.[1,2] Several factors may affect UTI outcomes and lead to renal scarring. The severity and frequency of UTIs play a role, with more severe or recurrent infections increasing the likelihood of scar formation.[1,3] Recent studies have attempted to determine whether antioxidants, micronutrients, or vitamins have a preventive effect against UTI scars and complications.[4-8]

✉ Mahmood Reza Khazaei ▪ khazaeem@iaumshms.ac.ir

Received 6th Dec 2024, revised 8th Aug 2025, accepted 3rd Nov 2025.

Understanding the correlation between vitamin D levels and renal scarring in children with UTIs is an area of ongoing research. Vitamin D has well-known functions in bone and calcium metabolism, but newer findings show its immunomodulatory role and effect on defense mechanisms against infections.[9,10] Although high-dose vitamin D supplementation is generally well tolerated in children, vitamin D deficiency is common and has been associated with increased production of inflammatory cytokines, which might exacerbate tissue damage and renal scarring.[11,12] The ability of the immune system to kill pathogens may be compromised by vitamin D deficiency, leading to more severe and recurrent UTIs and ultimately resulting in renal scarring.[10,12,13] This prognostic study investigates whether serum vitamin D levels can predict the development of renal scars in children with febrile UTIs.

## Methods

### *Study design*

In this diagnostic / prognostic study, a complete enumeration survey method (census sampling) was used to evaluate 193 patients aged between one month and 11 years with urinary problems and the possibility of UTI referred to the nephrology clinic of our academic hospital from September 2019 until November 2021.

Participants' demographic information, such as age and gender, was collected and recorded from self-identified patients. Patient height and weight were recorded. Height-for-age and weight-for-age standard deviation scores (SDS) and malnutrition status were calculated using World Health Organization (WHO) growth standards. Malnutrition status was defined according to the WHO weight-for-age SDS criteria. For children from birth to 60 months, the WHO Child Growth Standards were applied using Anthro Survey Analyzer. AnthroPlus software was used for individuals from 61 months to 19 years to apply the WHO Reference 2007.[14] Clinical and laboratory findings were used to confirm acute uncomplicated pyelonephritis for enrollment in the study. Laboratory examinations consisted of urinalysis and urine culture, white blood cell count, blood urea nitrogen (BUN) and creatinine levels, serum C-reactive protein (CRP) level, erythrocyte sedimentation rate (ESR), alkaline phosphatase, phosphorus, calcium, and 25-hydroxyvitamin D levels which were obtained immediately after suspicion of clinical UTI. Urine samples for urinalysis and culture were obtained via mid-stream clean catch sampling in all continent and cooperative patients. Otherwise, catheterization with a catheter of the appropriate size was used in small children or incontinent individuals. All creatinine measurements were performed using the Jaffe method. Normal renal function for patients aged over 2 years was defined as an estimated glomerular filtration rate (eGFR) greater than 90 mL/min/1.73m², calculated using the Schwartz formula.[15] For children ≤ 24 months old, GFR within 1 standard deviation below the mean for age was considered normal.[16] The total serum level of 25-hydroxyvitamin D as an index test was measured in venous blood samples using a combination of enzyme immunoassay competition method with final fluorescent detection - enzyme linked fluorescent assay - (ELFA) by the VIDAS® 25 OH Vitamin D TOTAL kit, and was expressed as nanograms per milliliter (ng/mL).[17]

Ultrasonographic examinations were performed for all children during the first week after diagnosis. All enrolled patients received cefixime 8 mg/kg per day orally divided every 12 hours, followed by a week of oral antibiotics based on the antibiogram results. The efficacy of treatment was confirmed by a negative urine culture 3 days after discontinuation of antibiotic therapy. Monthly urine tests and urine cultures were conducted for four to six months in all patients to ensure no recurrence of infection. A Tc-99m 2,3-DMSA scan, the current gold standard for detecting renal scarring, was performed four to six months after the onset of pyelonephritis to assess kidney involvement.[18] Any DMSA scan report

indicating differential renal function (DFR) out of the range of 50%±5% and/or showing the existence of scarring in the kidney, regardless of the number, severity, unilateral or bilateral involvement, was considered a subject with scarring complications.

The study followed the Standards for Reporting Diagnostic Accuracy Studies (STARD) 2015 reporting guideline.[19]

### *Exclusion and inclusion criteria*

According to the clinical and laboratory criteria, the conditions for entering the study as pyelonephritis were as follows: The clinical criteria consisted of eight signs, including fever, hypothermia, shivering, abdominal or costovertebral pain, weakness, nausea, and vomiting. At least three out of eight clinical criteria, together with any of the following urinary symptoms such as irritability during urination, frequency, painful urination, malodor urine, the reappearance of bedwetting, cloudy or darker urine as well as frank hematuria were considered for the clinical diagnosis of pyelonephritis.[20] The laboratory criterion was a positive urine culture (a midstream urine sample with more than $10^5$ colony forming units (CFU)/mL, a catheterized urine culture of more than 50,000 cfu/mL or a suprapubic urine sample with more than 100 colonies of one type of organism) plus at least two of three of the following laboratory findings: first-hour ESR > 15 mm/hr, CRP > 5 mg/dL, and WBC > 10,000 per microliter. For children aged 2 to 24 months presenting with fever or hypothermia, a UTI was defined by a urinalysis indicative of infection (either pyuria of >10 white blood cells per high-power field or bacteriuria) in conjunction with a positive urine culture.[21]

Patients were excluded from the study if they did not have urinary symptoms, were hospitalized, or had a delay in initiation of treatment of more than 5 days from the onset of clinical signs or symptoms. Patients were also excluded if they experienced breathing problems, abnormal chest sounds, abnormal ear exam, fever with known alternative cause, had any positive history of UTIs, hypertension, known cases of obstructive urinary system disease, myelomeningocele, paraplegia, urinary stones, and immunodeficiency conditions. Patients who received immunosuppressive medications or vitamin D supplementation in the last month before UTI onset and those with abnormal eGFR were also excluded.

Children with ultrasonographic results such as trabeculated bladder wall, increased renal parenchymal echogenicity, irregular kidney borders, or with a solitary or ectopic kidney, as well as those with anatomical findings such as duplicated system, horseshoe kidney, renal cyst, mass or abscess; or with pelvicalyceal dilation (maximal anteroposterior mid pelvic diameter ≥4 mm) with or without ureteral dilatation, were excluded. Children were excluded based on a significant renal length discrepancy. For those under four years of age, a discrepancy was defined as a right kidney ≥6 mm longer than the left, or a left kidney ≥10 mm longer than the right. For older children, the threshold for exclusion was a discrepancy of ≥10 mm, regardless of which kidney was larger.[22]

### *Patient categorization and statistical analysis*

Differential renal function below 45% and/or a defined scar by DMSA scan was considered abnormal. Based on DMSA findings, groups with normal (no-scar) and abnormal (scar+) DMSA findings were assigned for statistical analysis. The Shapiro–Wilk test was used to assess the normality of the data distribution. Student's t-test was used for normal data and the Mann-Whitney test for non-normal data distribution. A receiver operating characteristic (ROC) curve was used to determine the serum vitamin D concentration that best predicted an abnormality by DMSA scan.[23] The positive likelihood ratio, Youden's J index, and decision threshold were used to compare various levels of serum vitamin D to determine the best cutoff for serum vitamin D concentrations for predicting the likelihood of post-pyelonephritis renal scar occurrence.[24] Serum Vitamin D levels

below the cut-off were considered positive index tests. Similarly, levels equal to/or above the cut-off were defined as negative index tests. By definition, the sensitivity was the proportion of those with abnormal DMSA scans who had positive index tests and the specificity was the proportion of those with normal DMSA scans who had negative index tests. The positive predictive value (PPV) was the proportion of those with positive index test results who had an abnormal DMSA scan. The negative predictive value (NPV) was the proportion of individuals with negative index test results who had a normal DMSA scan.[25] A p value of less than 5% was considered significant. Logistic regression was used in multivariate analysis. Prior to multivariate modeling, univariate screening was performed to reduce overfitting risk. Variables associated with the outcome at $p < 0.1$ were retained to enhance model stability. The SPSS statistical software v.21.3 (IBM, Chicago, IL, USA) and Analyse-it® 5.80.2 were used for statistical analysis.

### *Ethical considerations*

The study was conducted according to the guidelines of the Declaration of Helsinki, and was approved by the National Committee for Ethics in Biomedical Research.[26] Before implementing the plan, we obtained informed consent from the parents of all individuals included in the study. Notably, no additional costs were imposed on the patients.

## Results

From the initial 193 referred children, 143 patients were excluded for reasons mentioned in Fig. 1. Of these, 106 had one excluded factor,

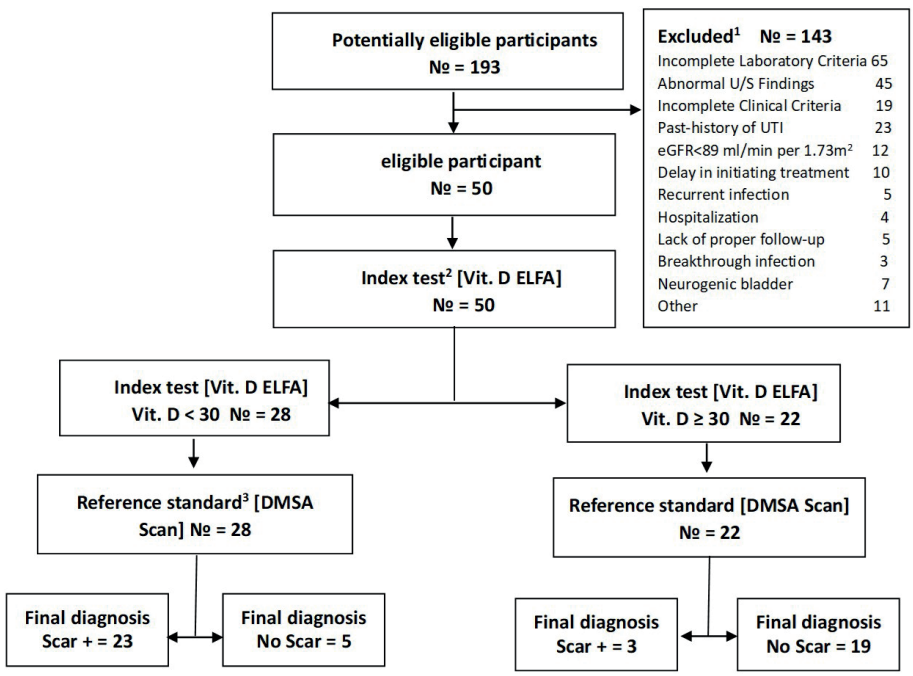



**Fig. 1.** Standard for Reporting Diagnostic Accuracy (STARD) diagram to report flow participants through the study [with vitamin D level < 30 ng/mL as index test defined cut-off by ROC curve analysis].

[1]37 patients had more than one involved exclusion factor.

[2]Index test: Serum vitamin D level measured by Enzyme-Linked Fluorescent Assay (ELFA) at the onset of UTI.

[3]Reference standard: Dimercaptosuccinic acid (DMSA) scan examination done 4 to 6 months later.

18 had two, eleven had three, six had four, and two had five involved factors. Fifty outpatients with a normal glomerular filtration rate, aged 3 to 98 months, with pyelonephritis were enrolled in the study; 28% (14 patients) were boys, and 72% were girls. The demographic and anthropometric characteristics of enrolled patients are presented in Table I. Kidney scars were revealed by DMSA scan in 26 people (52%), 17 girls (65%) and 9 boys (35%).

Urine culture results showed *Escherichia coli* in 36 patients (72%). Non-*E. coli* cultures consisted of *Enterobacter* (5), *Klebsiella* (4), *Pseudomonas* (2), *Citrobacter* (1), *Staphylococcus epidermidis* (1), and *Staphylococcus aureus* (1). Renal scars occurred in 55.5% and 42.8% of *E. coli* vs non-*E. coli* patients, respectively.

The mean vitamin D levels of patients were 28.58 ± 12.65 (7-60) ng/dL. There was no significant effect of gender regarding the average level of serum vitamin D; 30.1 ± 12.3ng/mL for girls and 24.8 ± 13.3 ng/mL for boys, respectively, t(48)= -1.33, p=0.19. Table I presents additional relevant information about ESR and CRP values, including mean ± standard deviation and median values in patients.

### *Correlation between the serum and urine parameters and renal scarring status*

The study compared the average vitamin D, ESR, and CRP values in scar and non-scar groups among girls, boys, and all patients.

The lowest and highest vitamin D levels were 7 and 39 ng/mL in the scar group, and 20 and 60 ng/mL in the non-scar group, respectively. There was a significant difference in vitamin D serum levels between the two groups ($p < 0.0001$). This difference was observed when the groups were subdivided by gender (Table II, Fig. 2). The comparison of average levels of CRP and ESR in both the scar and non-scar groups and their subdivided groups showed no significant differences, as presented in Table II.

The impact of various factors on renal scarring was examined via logistic regression analysis. The variables of age, sex, height-SDS, vitamin D concentration, ESR, CRP levels, and type

**Table I.** Demographic characteristics and laboratory data of the enrolled patients

| Characteristic | Total Patients (n=54) | Scar Group (n=26) | Non-Scar Group (n=24) | p -value* |
|---|---|---|---|---|
| Sex, n (%) | | | | 0.2782 |
| Male | 14(28) | 9 (34.6) | 5 (20.9) | |
| Female | 36 (72) | 17 (65.4) | 19 (79.1) | |
| Age (months), mean ± SD | 38.6 ± 26.2 | 46.2±25.2 | 30.41±25.3 | 0.0315 |
| Anthropometrics, mean ± SD | | | | |
| Height (cm) | 94.0 ± 18.1 | 98.7±15.6 | 88.5±19.7 | 0.0520 |
| Height-SDS | -0.066 ± 0.573 | -0.211±0.615 | 0.106±0.48 | 0.0465 |
| Weight (kg) | 14.55 ± 5.24 | 15.34±3.89 | 13.68±6.37 | 0.2679 |
| Weight-SDS | 0.188 ± 0.989 | -0.058±0.850 | 0.454±1.076 | 0.0671 |
| Malnutrition Status, n (%) | | | | |
| Overweight (SDS ≥ 2) | 0 (0) | 0 (0) | 0 (0) | |
| Normal (SDS ≥ -2 to 2) | 50 (100) | 26 (100) | 24 (100) | |
| Moderate underweight (SDS ≥ -2 to -3) | 0 (0) | 0 (0) | 0 (0) | |
| Severe underweight (SDS < -3) | 0 (0) | 0 (0) | 0 (0) | |

SDS: standard deviation score,
* Distribution of population is not normal at the 5% significance level

**Table II.** Serum levels of Vitamin D, CRP, and ESR with and without renal scarring in children with febrile urinary tract infections, categorized by sex

| Variable | No-scar group | | Scar group | | df | t statistic | p value |
|---|---|---|---|---|---|---|---|
| | mean ± SD | 95% CI | mean ± SD | 95% CI | | | |
| 25(OH)D (ng/mL) | | | | | | | |
| Females | 37.4±10.9 | 32.2 - 42.7 | 21.8±7.7 | 17.9 - 25.8 | 34 | -4.72 | <0.0001 |
| Males | 35.80±14.7 | 17.5 - 54.1 | 18.7±7.9 | 12.6 - 24.7 | 12 | -2.88 | 0.0138 |
| All | 37.08±11.42 | 32.3 - 41.9 | 20.73±7.77 | 17.6 - 23.9 | 48 | -5.96 | <0.0001 |
| ESR (mm/hr) | | | | | | | |
| Females | 47.0±22.2 | 36.3 - 57.7 | 47.9±26.9 | 34.0 - 61.7 | 34 | 0.11 | 0.914 |
| Males | 52.6±32.4 | 12.4 - 92.8 | 53.4±17.7 | 39.8 - 67.1 | 12 | 0.06 | 0.950 |
| All | 48.2±24.0 | 38 - 58.3 | 49.8±23.9 | 40.1 - 59.5 | 48 | 0.24 | 0.809 |
| CRP (mg/dL) | | | | | | | |
| Females | 23.5±12.7 | 17.1 - 29.8 | 32.6±14.6 | 25.9 - 39.4 | 1.93 | 375.50 | 0.0531 |
| Males | 28.6±13.8 | 11.4 - 45.8 | 33.6±22.9 | 159 - 51.2 | 12 | 0.44 | 0.67 |
| All | 24.5±12.8 | 18.2 - 30.9 | 33.0±17.5 | 26.9 - 39.0 | 48 | 1.93 | 0.595 |

25(OH)D: 25-hydroxyvitamin D, CI: confidence interval, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, SD: standard deviation.

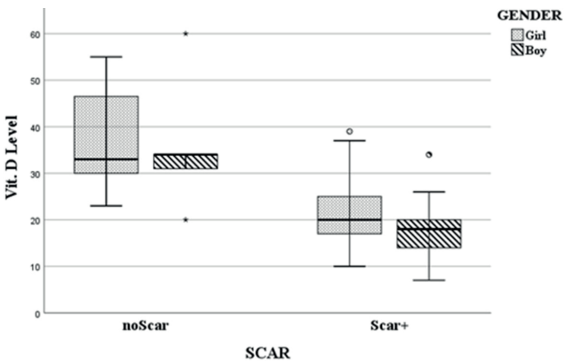



**Fig. 2.** Comparison of mean vitamin D levels with occurrence of scars in both sexes.

of urine culture (i.e., *E. coli* vs. non-*E. coli*) were considered. To mitigate overfitting and ensure model stability, univariate screening identified three variables ($p < 0.1$) for retention in the multivariate analysis: age, vitamin D concentration, and CRP. By controlling the effect of confounding variables, vitamin D level at the initial presentation of acute UTI has a significant relationship with renal scarring ($\beta = -.135$, $p < .003$), as detailed in Supplementary Table S1.

### *Receiver operating characteristic (ROC) curve analysis*

The area under the ROC curve (AUC) was 0.89 (95% confidence interval [CI]: 0.798 - 0.979; Fig. 3). Reporting on the increase in discrimination using the ROC curve and AUC ≥ 0.8 is relevant to obtaining insight into the incremental value of serum vitamin D levels and defining the prediction model.[23] Therefore, we can reject the null hypothesis that serum vitamin D level cannot predict the occurrence of renal scarring. However, defining a decision threshold or cut-off i.e. going from a prediction model to a prediction rule, is crucial to using the prediction test for decision-making in clinical practice.[27,28] The optimal threshold value or cutoff point for the serum vitamin D concentration was determined to be 30 ng/mL as shown in the decision threshold curve (Youden's J index = 0.676, t* = 30; Fig. 4, Supplementary Table S2). At this point, the estimated specificity and sensitivity are 0.792 and 0.885, respectively.

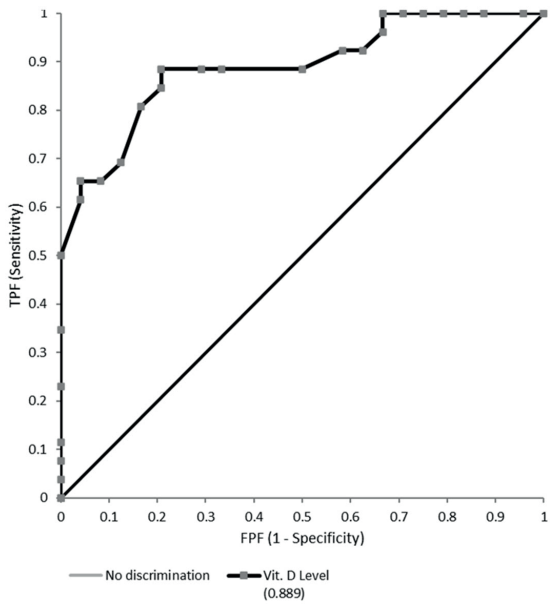



**Fig. 3.** Receiver operating characteristic (ROC) curve for different levels of serum vitamin D in patients with post-pyelonephritis kidney scars. The area under the curve (AUC) is equal to 0.889.

TPF: True Positive Fraction. FPF: False Positive Fraction.

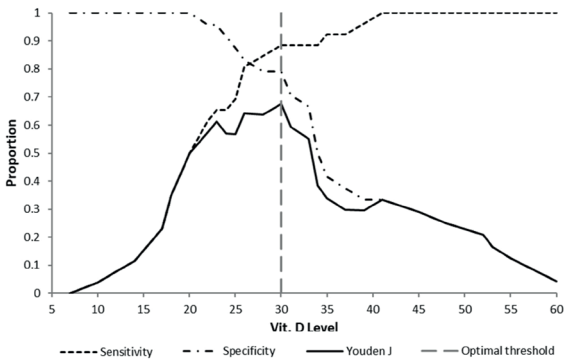



**Fig. 4.** Decision threshold curve for optimal threshold levels of serum vitamin D to predict post-pyelonephritis kidney scars. The performance metric curves from the clinical dataset study (50 test cases) show sensitivity, specificity, and the Youden J index as a function of the decision threshold to predict post-urinary tract infection renal scarring in children. The vertical line indicates the optimal threshold for vitamin D at 30 ng/dL serum level.

## Discussion

Childhood UTIs may lead to kidney scar formation and complications such as hypertension and renal failure.[1,2] Major risk factors for renal scarring include recurrent infections, delayed treatment, young age, and immune deficiency.[3] While vitamin D deficiency has been proposed as a risk factor, studies investigating the correlation between vitamin D intake and UTIs in children have yielded different outcomes.[12] Our research findings suggest that a higher serum vitamin D level at UTI onset is correlated with a reduced risk of renal scarring after the first pyelonephritic attack. Although we could not find any similar research, vitamin D deficiency was found to be an independent risk factor in patients with recurrent UTIs according to Sürmeli Döven's study.[12] Most studies have focused on the correlation between vitamin D levels and the occurrence of UTIs. Sherkatolabbasieh et al. reported no significant correlation between vitamin D levels and UTIs, whereas other researchers have shown a significant negative correlation, with lower vitamin D levels associated with a greater risk of childhood UTIs.[29-31]

Vitamin D, a fat-soluble vitamin, plays an important role in regulating bone metabolism and cell growth, and is critical for the optimal immune system.[32] Vitamin D is known as an immune response modulator and regulates innate immunity through macrophage and dendritic cell activity, as well as through an adaptive immune response via lymphocyte T cells.[10] Several observational studies have shown that vitamin D influences innate immunity through the upregulation of antimicrobial peptides, such as the CAMP/LL37 peptide, which has antibacterial properties.[9] Therefore, poor vitamin D status is associated with greater susceptibility to infections.

Strong laboratory and epidemiological evidence links vitamin D deficiency to increased rates of UTIs.[31,33-35] Adequate vitamin D levels can decrease pro-inflammatory cytokine levels released predominantly from innate immune cells.[36] Pro-inflammatory cytokine production is crucial for initiating the anti-infectious process, but their exacerbated production during severe inflammation may contribute to serious consequences. The capacity of interleukin (IL)-1 and tumor necrosis factor-alpha (TNF-a) to induce inflammatory mediator expression contributes to their pro-inflammatory properties.[37] IL-1 and TNF-a activate phospholipase, cyclooxygenase, and lipoxygenase leading to the release of prostaglandins, thromboxane, leukotrienes, and platelet-activating factor (PAF). Free radicals (superoxide [O2−], nitric oxide [NO]), and proteolytic enzymes are other mediators produced by target cells in response to IL-1 and TNF-a. Other cytokines, including chemokines such as IL-8 and some T-cell-derived cytokines, such as lymphotoxin-$\alpha$, are also involved in the cytokine cascade.[37] Insufficient vitamin D can induce an inflammatory cascade that leads to severe inflammation and additional organ damage.[12]

Vitamin D stimulates the synthesis of antimicrobial peptides, cathelicidins, and defensins, which exhibit broad-spectrum activity against viruses, bacteria, and fungal infections; reduce the concentration of proinflammatory cytokines; and increase the concentration of anti-inflammatory cytokines. Vitamin D is also involved in the differentiation, maturation, and proliferation of immune cells.[10] Mohanty et al found tight junction proteins such as occludin and claudin-14 induced by vitamin D during *E. coli* infection restores the bladder epithelial integrity and can prevent bacterial invasion through the epithelial barrier.[38]

The WHO recommends maintaining a serum vitamin D concentration above 20 ng/ml.[39] However, the National Institute of Health suggests that for certain at-risk groups, such as elderly individuals, pregnant women, and those with chronic conditions, a 25-hydroxyvitamin D level above 30 ng/mL is necessary.[40] This is supported by Vieth who argues that the current tolerable upper intake level of vitamin D may be too low and that higher serum levels may be more beneficial than harmful.[41]

In our study, the ROC curve analysis showed an AUC of 0.89, indicating that it is a reliable measurement to predict renal scarring on the DMSA scan.[23] The optimal prognostic threshold for serum vitamin D concentration was identified as 30 ng/mL, exceeding the WHO's sufficiency guideline of 20 ng/ml.[39]

These findings suggest that monitoring serum 25-hydroxyvitamin D levels may be important in assessing the risk of renal scarring in children with febrile UTIs. Vitamin D levels are influenced by ethnicity, genetic factors, and skin pigmentation, with higher melanin concentrations requiring greater sunlight exposure for adequate synthesis.[42-44] Therefore, future studies should include diverse geographic and racial/ethnic populations to enhance the generalizability of findings.

We need to acknowledge that our study has some limitations and points. First, radionuclide scans were intentionally deferred during the acute infection phase to minimize radiation exposure in pediatric patients. Instead, we relied solely on their medical history and ultrasound imaging to detect any evidence of renal scarring. Thus, previous non-obvious UTIs and related scarring or renal abnormalities from other issues could impact the selection bias in our study. Next, we attempted to eliminate all known confounding factors to assess the effect of vitamin D deficiency alone. Despite the exclusion of many patients, the net effect of vitamin D deficiency on renal scarring in pyelonephritis was ultimately analyzed. We suggest prospective studies could consider integrating early-phase imaging to better distinguish pre-existing damage from new-onset scarring.

### *Conclusion*

This study aimed to investigate the relationship between vitamin D levels and post-UTI renal scarring in children. A reverse correlation was found between post-pyelonephritis renal scarring and serum vitamin D levels. Moreover, the results indicated that only vitamin D levels were significantly related to renal scarring. The ROC curve analysis demonstrated that serum vitamin D levels exhibit an acceptable AUC, suggesting its potential utility as a prognostic marker for renal scarring. Serum vitamin D ≥ 30 ng/mL demonstrated prognostic value for renal scarring outcomes in pediatric pyelonephritis, warranting consideration as a risk-stratification biomarker in susceptible populations.

### Supplementary Materials

Supplementary materials for this article are available online at https://doi.org/10.24953/turkjpediatr.2025.5599

### Acknowledgments

Dr. Mojtaba Meshkat, Ph.D. in Biostatistics, contributed by critically reviewing statistical analysis. There was no financial compensation for this contribution.

### Ethical approval

The study was approved by Ethics Committee of the Faculty of Medicine, Mashhad Medical Sciences of Islamic Azad University (registered by The Ministry of Health and Medical Education of Iran; date: May 2019, number: IR.IAU.MSHD.REC.1398.033).

### Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: MM, AA, MRK, JY; data collection: MM; patient follow-up and control of inclusion/exclusion criteria: AA, JY, MRK. analysis and interpretation of results: MM, AA, MRK, JY; draft manuscript preparation: MM, MRK. All authors reviewed the results and approved the final version of the manuscript.

### Source of funding

The authors declare the study received no funding.

### Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES

1. Tullus K. Outcome of post-infectious renal scarring. Pediatr Nephrol 2015; 30: 1375-1377. https://doi.org/10.1007/s00467-015-3130-6
2. Baltu D, Salancı BV, Gülhan B, Özaltın F, Düzova A, Topaloğlu R. Albuminuria is associated with 24-hour and night-time diastolic blood pressure in urinary tract infection with renal scarring. Turk J Pediatr 2023; 65: 620-629. https://doi.org/10.24953/turkjped.2022.766
3. Shaikh N, Haralam MA, Kurs-Lasky M, Hoberman A. Association of renal scarring with number of febrile urinary tract infections in children. JAMA Pediatr 2019; 173: 949-952. https://doi.org/10.1001/jamapediatrics.2019.2504
4. Allameh Z, Salamzadeh J. Use of antioxidants in urinary tract infection. J Res Pharm Pract 2016; 5: 79-85. https://doi.org/10.4103/2279-042X.179567
5. Kahbazi M, Sharafkhah M, Yousefichaijan P, et al. Vitamin A supplementation is effective for improving the clinical symptoms of urinary tract infections and reducing renal scarring in girls with acute pyelonephritis: a randomized, double-blind placebo-controlled, clinical trial study. Complement Ther Med 2019; 42: 429-437. https://doi.org/10.1016/j.ctim.2018.12.007
6. Jorde R, Sollid ST, Svartberg J, Joakimsen RM, Grimnes G, Hutchinson MYS. Prevention of urinary tract infections with vitamin D supplementation 20,000 IU per week for five years. Results from an RCT including 511 subjects. Infect Dis (Lond) 2016; 48: 823-828. https://doi.org/10.1080/23744235.2016.1201853

7. Sedighi I, Taheri-Moghadam G, Emad-Momtaz H, et al. Protective effects of omega-3 fatty acids supplementation against renal parenchymal scarring in children with acute pyelonephritis: results of a pilot clinical trial. Curr Pediatr Rev 2022; 18: 72-81. https://doi.org/10.2174/1573396317666210909153643

8. Khan DSA, Naseem R, Salam RA, Lassi ZS, Das JK, Bhutta ZA. Interventions for high-burden infectious diseases in children and adolescents: a meta-analysis. Pediatrics 2022; 149: e2021053852C. https://doi.org/10.1542/peds.2021-053852C

9. Ismailova A, White JH. Vitamin D, infections and immunity. Rev Endocr Metab Disord 2022; 23: 265-277. https://doi.org/10.1007/s11154-021-09679-5

10. Baeke F, Takiishi T, Korf H, Gysemans C, Mathieu C. Vitamin D: modulator of the immune system. Curr Opin Pharmacol 2010; 10: 482-496. https://doi.org/10.1016/j.coph.2010.04.001

11. Brustad N, Yousef S, Stokholm J, Bønnelykke K, Bisgaard H, Chawes BL. Safety of high-dose vitamin D supplementation among children aged 0 to 6 years: a systematic review and meta-analysis. JAMA Netw Open 2022; 5: e227410. https://doi.org/10.1001/jamanetworkopen.2022.7410

12. Sürmeli Döven S, Erdoğan S. Vitamin D deficiency as a risk factor for renal scarring in recurrent urinary tract infections. Pediatr Int 2021; 63: 295-299. https://doi.org/10.1111/ped.14397

13. Flores ME, Rivera-Pasquel M, Valdez-Sánchez A, et al. Vitamin D status in Mexican children 1 to 11 years of age: an update from the Ensanut 2018-19. Salud Publica Mex 2021; 63: 382-393. https://doi.org/10.21149/12156

14. World Health Organization (WHO). Application tools: growth reference data for 5-19 years. Geneva: WHO; 2025. Available at: https://www.who.int/tools/growth-reference-data-for-5to19-years/application-tools (Accessed on Aug 10, 2025).

15. Schwartz GJ, Muñoz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. J Am Soc Nephrol 2009; 20: 629-637. https://doi.org/10.1681/ASN.2008030287

16. Chapter 1: definition and classification of CKD. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney International Supplements 2013; 3: 19-62. https://doi.org/10.1038/kisup.2012.64

17. Moreau E, Bächer S, Mery S, et al. Performance characteristics of the VIDAS® 25-OH Vitamin D Total assay - comparison with four immunoassays and two liquid chromatography-tandem mass spectrometry methods in a multicentric study. Clin Chem Lab Med 2016; 54: 45-53. https://doi.org/10.1515/cclm-2014-1249

18. Moorthy I, Wheat D, Gordon I. Ultrasonography in the evaluation of renal scarring using DMSA scan as the gold standard. Pediatr Nephrol 2004; 19: 153-156. https://doi.org/10.1007/s00467-003-1363-2

19. Bossuyt PM, Reitsma JB, Bruns DE, et al. STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies. Clin Chem 2015; 61: 1446-1452. https://doi.org/10.1373/clinchem.2015.246280

20. National Institute for Health and Care Excellence (NICE): Guidelines. Urinary tract infection in under 16s: diagnosis and management. London: NICE; 2020.

21. Subcommittee on Urinary Tract Infection, Steering Committee on Quality Improvement and Management; Roberts KB. Urinary tract infection: clinical practice guideline for the diagnosis and management of the initial UTI in febrile infants and children 2 to 24 months. Pediatrics 2011; 128: 595-610. https://doi.org/10.1542/peds.2011-1330

22. Khazaei MR, Mackie F, Rosenberg AR, Kainer G. Renal length discrepancy by ultrasound is a reliable predictor of an abnormal DMSA scan in children. Pediatr Nephrol 2008; 23: 99-105. https://doi.org/10.1007/s00467-007-0637-5

23. Nahm FS. Receiver operating characteristic curve: overview and practical use for clinicians. Korean J Anesthesiol 2022; 75: 25-36. https://doi.org/10.4097/kja.21209

24. Schisterman EF, Faraggi D, Reiser B, Hu J. Youden Index and the optimal threshold for markers with mass at zero. Stat Med 2008; 27: 297-315. https://doi.org/10.1002/sim.2993

25. Santini A, Man A, Voidăzan S. Accuracy of diagnostic tests. J Crit Care Med (Targu Mures) 2021; 7: 241-248. https://doi.org/10.2478/jccm-2021-0022

26. World Medical Association. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. Bull World Health Organ 2001; 79: 373-374.

27. Reilly BM, Evans AT. Translating clinical research into clinical practice: impact of using prediction rules to make decisions. Ann Intern Med 2006; 144: 201-209. https://doi.org/10.7326/0003-4819-144-3-200602070-00009

28. Steyerberg EW, Pencina MJ, Lingsma HF, Kattan MW, Vickers AJ, Van Calster B. Assessing the incremental value of diagnostic and prognostic markers: a review and illustration. Eur J Clin Invest 2012; 42: 216-228. https://doi.org/10.1111/j.1365-2362.2011.02562.x

29. Sherkatolabbasieh H, Firouzi M, Shafizadeh S, Nekohid M. Evaluation of the relationship between vitamin D levels and prevalence of urinary tract infections in children. New Microbes New Infect 2020; 37: 100728. https://doi.org/10.1016/j.nmni.2020.100728

30. Li X, Yu Q, Qin F, Zhang B, Lu Y. Serum vitamin D level and the risk of urinary tract infection in children: a systematic review and meta-analysis. Front Public Health 2021; 9: 637529. https://doi.org/10.3389/fpubh.2021.637529

31. Chidambaram S, Pasupathy U, Geminiganesan S, R D. The association between vitamin D and urinary tract infection in children: a case-control study. Cureus 2022; 14: e25291. https://doi.org/10.7759/cureus.25291

32. Hewison M. Vitamin D and the immune system: new perspectives on an old theme. Endocrinol Metab Clin North Am 2010; 39: 365-79, table of contents. https://doi.org/10.1016/j.ecl.2010.02.010

33. Shalaby SA, Handoka NM, Amin RE. Vitamin D deficiency is associated with urinary tract infection in children. Arch Med Sci 2018; 14: 115-121. https://doi.org/10.5114/aoms.2016.63262

34. Mahmoudzadeh H, Nikibakhsh AA, Pashapour S, Ghasemnejad-Berenji M. Relationship between low serum vitamin D status and urinary tract infection in children: a case-control study. Paediatr Int Child Health 2020; 40: 181-185. https://doi.org/10.1080/20469047.2020.1771244

35. Mahyar A, Ayazi P, Safari S, Dalirani R, Javadi A, Esmaeily S. Association between vitamin D and urinary tract infection in children. Korean J Pediatr 2018; 61: 90-94. https://doi.org/10.3345/kjp.2018.61.3.90

36. AlGhamdi SA, Enaibsi NN, Alsufiani HM, Alshaibi HF, Khoja SO, Carlberg C. A single oral vitamin D3 bolus reduces inflammatory markers in healthy saudi males. Int J Mol Sci 2022; 23: 11992. https://doi.org/10.3390/ijms231911992

37. Megha KB, Joseph X, Akhil V, Mohanan PV. Cascade of immune mechanism and consequences of inflammatory disorders. Phytomedicine 2021; 91: 153712. https://doi.org/10.1016/j.phymed.2021.153712

38. Mohanty S, Kamolvit W, Hertting O, Brauner A. Vitamin D strengthens the bladder epithelial barrier by inducing tight junction proteins during E. coli urinary tract infection. Cell Tissue Res 2020; 380: 669-673. https://doi.org/10.1007/s00441-019-03162-z

39. Rosen CJ. Clinical practice. Vitamin D insufficiency. N Engl J Med 2011; 364: 248-254. https://doi.org/10.1056/NEJMcp1009570

40. National Institutes of Health, U.S. Department of Health and Human Services. Vitamin D. 2018 [updated Sep18, 2023; Jan14, 2024]. Available at: https://ods.od.nih.gov/factsheets/VitaminD-HealthProfessional

41. Vieth R. Critique of the considerations for establishing the tolerable upper intake level for vitamin D: critical need for revision upwards. J Nutr 2006; 136: 1117-1122. https://doi.org/10.1093/jn/136.4.1117

42. Braegger C, Campoy C, Colomb V, et al. Vitamin D in the healthy European paediatric population. J Pediatr Gastroenterol Nutr 2013; 56: 692-701. https://doi.org/10.1097/MPG.0b013e31828f3c05

43. Martin CA, Gowda U, Renzaho AM. The prevalence of vitamin D deficiency among dark-skinned populations according to their stage of migration and region of birth: a meta-analysis. Nutrition 2016; 32: 21-32. https://doi.org/10.1016/j.nut.2015.07.007

44. Bahrami A, Sadeghnia HR, Tabatabaeizadeh SA, et al. Genetic and epigenetic factors influencing vitamin D status. J Cell Physiol 2018; 233: 4033-4043. https://doi.org/10.1002/jcp.26216

The Turkish Journal of Pediatrics 2025; 67: 866-874
https://doi.org/10.24953/turkjpediatr.2025.ID

**Original Article**

# Clinical value of serum and urine endocan in children with hemolytic uremic syndrome

Muhammet Akif Güler[1], Muhammet Çelik[2]

[1]*Department of Pediatric Nephrology, Erzurum City Hospital, Erzurum, Türkiye;* [2]*Department of Biochemistry, Faculty of Medicine, Atatürk University, Erzurum, Türkiye.*

## ABSTRACT

**Background.** Endocan (endothelial cell-specific molecule-1) is a soluble dermatan sulfate proteoglycan of the extracellular matrix released into the circulation by vascular endothelial cells and involved in vascular processes in which endothelial cell activation occurs. In this study, we aimed to evaluate serum and urinary endocan levels in children with hemolytic uremic syndrome (HUS) during the acute disease and follow-up period, compared with controls, and to evaluate associated clinical and laboratory parameters.

**Methods.** Children were evaluated in three groups: HUS patients in the active stage (Group 1, HUS-active stage, n=15), HUS patients followed until the resolution of active disease (Group 2, HUS-follow-up, n=10) and healthy controls (Group 3, n=15). Clinical parameters and renal outcomes were compared between the groups based on serum and urinary endocan levels.

**Results.** The pairwise group comparisons of the urinary endocan levels (median; Q1-Q3) revealed statistically significant differences between Group 1 (2148; 1592-3068 ng/gCr) and Group 2 (1274; 733-1565 ng/gCr), and between Group 1 and Group 3 (954; 517-1966 ng/gCr) ($P<0.01$), but not between Group 2 and Group 3 ($P>0.05$). The serum endocan level showed no statistically significant difference between the groups ($p>0.05$). When all groups were evaluated together, urinary endocan level showed positive correlations with white blood cell counts ($r= 0.63$, $P<0.001$), and with lactic dehydrogenase ($r= 0.51$, $P<0.001$), blood urea nitrogen ($r= 0.48$, $P<0.05$) and serum creatinine levels ($r= 0.50$, $P<0.001$). However, urinary endocan levels showed negative correlations with hemoglobin ($r= -0.57$, $P<0.001$) and platelet levels ($r= -0.37$, $P<0.05$), and estimated glomerular filtration rate ($r= -0.45$, $P<0.001$). The urinary endocan levels of patients with HUS decreased significantly during follow-up ($P<0.05$).

**Conclusions.** Our findings suggested that urinary endocan levels were significantly elevated in HUS patients in the active stage. In addition, several important laboratory parameters in the HUS clinic were associated with urine endocan levels.

**Key words:** hemolytic uremic syndrome; endocan; endothelial damage; children.

Hemolytic uremic syndrome (HUS) is a thrombotic microangiopathy, and it is characterized by hemolytic anemia, thrombocytopenia, and acute kidney injury (AKI).[1,2] It is more common in children under five years old, with an incidence of 5-6/100,000. In HUS, microthrombi formed due to vascular damage, causes platelet aggregation and ultimately leads to thrombocytopenia. At the same time, hemolytic anemia occurs with damage to erythrocytes as they pass through thrombosed vessels. These events result in

✉ Muhammet Akif Güler ▪ akif2532@gmail.com

Received 30th Mar 2024, revised 10th Jul 2024, 6th Feb 2025, 23rd Apr 2025, 11th Aug 2025, 17th Nov 2025, accepted 22nd Nov 2025.

ischemic organ damage, especially in the kidneys. HUS presents with general disease symptoms, hematological findings, signs of AKI and extrarenal findings such as seizures, colitis, cholecystitis, pancreatitis, elevated liver enzymes, and myocardial dysfunction.[2] In 2016, the International Consensus proposed a new classification system for HUS.[3]

Endocan (endothelial cell-specific molecule-1) is a 50-kDa soluble proteoglycan consisting of dermatan sulfate and a mature polypeptide of 165 amino acids.[4,5] It is expressed in vascular endothelial cells, pulmonary capillaries, kidneys (glomerular endothelial cells and tubular epithelial), cardiomyocytes, digestive system, liver, brain, thyroid gland, thymus, epididymis, skin, and lymph nodes.[6,7] Endocan is involved in various vascular processes that regulate endothelial activation, endothelial permeability, and cellular adhesion and proliferation.[8] Endocan may be an independent predictor or a new prognostic biomarker in immunoglobulin A (IgA) nephropathy[7] cardiovascular events due to chronic kidney disease (CKD)[9], chronic renal allograft injury[10], coronary artery diseases[11], cancers[12] and diabetic nephropathy.[13] Although endocan is present in extremely low concentrations in body fluids, it can be easily detected due to its stability in physiological conditions. Therefore, it can be a non-invasive diagnostic and prognostic marker in various systemic and renal diseases.[4]

Developing clinical prediction scores and rapid diagnostic tools is essential for early identification of patients with HUS and timely initiation of targeted therapies.[2] However, to date, no specific biomarker has been identified that can guide hospitalization decisions or reliably assess disease severity in children with HUS. Although endocan is associated with many diseases, no study has evaluated its relationship with HUS. Therefore, we aimed to evaluate serum and urinary endocan levels in children with HUS during the acute disease and follow-up period, compared to controls; and to evaluate associated clinical and laboratory parameters.

## Material and Methods

### *Patients and study design*

This study was prospectively conducted in the pediatric nephrology clinic of Atatürk University Faculty of Medicine in 2021 and 2022. Pediatric patients included in the study were evaluated in three groups: a) HUS patients in active stage (Group 1, HUS-active stage, n=15), b) HUS patients who could be followed until the resolution of active disease (Group 2, HUS-follow-up, n=10) and c) healthy controls (Group 3, n=15). The patients' age, sex, weight, and vital signs were recorded. Hematological and biochemical tests were performed. The estimated glomerular filtration rate (eGFR) was calculated using the Schwartz formula.[14] AKI was staged according to the KDIGO study.[15] The diagnosis of HUS was based on the presence of the triad of renal dysfunction showing an elevated serum creatinine level for age and height, hemolytic anemia (hemoglobin <8 g/dL or hematocrit <30%), and thrombocytopenia (platelet count <150x$10^9$/L).[2]

The clinical severity of HUS was evaluated on the following six items, with a score assigned to each item: 1) Prolonged anuria (longer than two weeks); 2) Kidney replacement therapy (KRT) requirement; 3) KRT lasting longer than four weeks; 4) Diagnosis of atypical HUS (aHUS), *Streptococcus pneumoniae*-associated hemolytic uremic syndrome (SP-HUS), cobalamin C-HUS, or atypical hemolytic uremic syndrome (aHUS) with diacylglycerol kinase epsilon (*DGKE*) gene variant; 5) Stage 3 AKI in the acute phase of the disease; and 6) Non-renal organ involvement (pancreatitis, elevated liver enzymes, colitis, cholecystitis, myocardial dysfunction, rhabdomyolysis, ulcerative-necrotic skin lesions, seizure, lethargy, and coma).[1,2] Urinary blood and protein measurements were performed semi-quantitatively using the photometric method in a fully automated H-800 Dirui urine analyzer (DIRUI, H-800, China). The patients were treated following the most recent guidelines and discharged from the hospital upon clinical improvement.[2,16,17]

### *Sample collection and storage*

Blood and urine samples were collected from all patients: Group 1 within the first 48 hours of admission (mean: 2.0±0.3 days), Group 2 during follow-up (mean: 2.2±0.7 months after the first admission), and Group 3 during the whole study period. Blood samples (3.5 mL) were collected into biochemistry tubes, and urine samples (5 mL) were collected into sterile urine tubes. The blood samples were kept at room temperature for 20 minutes, and then centrifuged at 4,000 rpm for 15 minutes. Serum samples obtained after centrifugation were transferred to Eppendorf tubes and aliquoted. The urine samples were centrifuged at 2,000 rpm for 15 minutes, and then the supernatant was transferred to microcentrifuge tubes and aliquoted. The aliquoted serum and urine samples were stored in an ultra-low temperature freezer at -80 °C until the study day.

### *Analyte assay techniques*

For the measurement of serum and urine endocan levels, the BT LAB enzyme-linked immunosorbent assay (ELISA) kit (Bioassay Technology Laboratory, Cat. No. E3160Hu, lot no. 202205005, China) was used. The experimental steps in the kit insert were applied to the automatic ELISA reader device, and the samples were analyzed with the ELISA method using the automatic Dynex ELISA reader device (Dynex Technologies Headquarters, Chantilly, USA). The results were expressed as ng/L. The sensitivity of the ELISA kit was 2.56 ng/L, and its detection range was 5-2,000 ng/L. The intra-assay and inter-assay coefficients of variation were below 4% and 10%, respectively. Urine creatinine levels were determined through the kinetic colorimetric measurement using the Jaffe method on the Roche Cobas c702 device (Roche Diagnostics GmbH, Mannheim, Germany). Urine creatinine was measured in the same urine specimens. The urine endocan level was expressed relative to the creatinine concentration: endocan/creatinine (ng/gCr).

### *Statistical analysis*

Data was analyzed using SPSS v. 25.0 for Windows software (IBM SPSS Inc., Chicago, IL, USA). The chi-square test was used to assess the sex distribution among the study groups. The Shapiro-Wilk test was used to determine the normality of the data. For nonparametric data, the Mann-Whitney U test was used to compare the means and medians of two groups. Analysis of variance (ANOVA) was used to compare group means. Pairwise comparisons were performed using the Duncan or Kruskal-Wallis tests (adjusted by the Bonferroni correction). The Spearman rho correlation test was applied to evaluate relationships among all continuous variables, both across the total sample and within groups. The results are shown as mean ± standard deviation or median (Q1-Q3), depending on data distribution. An alpha significance level of $P<0.05$ was considered statistically significant.

### *Ethics committee approval*

This study was conducted after receiving ethical approval from the Clinical Research Ethics Committee of Atatürk University Faculty of Medicine (2021/06-55). Written consent was obtained from the parents of the patients/healthy controls.

## Results

All Group 1 patients had clinical and laboratory findings consistent with HUS. Hemoglobin (Hb) and serum endocan levels were normally distributed ($P>0.05$), whereas the other analyzed characteristics were not ($P<0.01$). The demographic characteristics and the blood and urine results of the groups are given in Table I. The pairwise group comparisons of median urinary endocan levels revealed significant differences ($P<0.01$) between Group 1 and Group 2, and between Group 1 and Group 3, but not between Group 2 and Group 3 ($P>0.05$). Although the mean serum endocan

level was higher in Group 1, the difference between groups was not statistically significant. (P>0.05) (Table I, Fig. 1). There was also no significant correlation between the serum endocan levels and urinary endocan levels in all groups (r=0.29, P>0.05)

Most of the laboratory parameters in Group 1 differed significantly from those in the other groups (Table I). When all groups are evaluated together, the mean urinary endocan level positively correlated with most laboratory parameters (Table II). In Group 1, the serum

**Table I.** Demographic characteristics and laboratory findings of the study groups.

| | Group 1 (n=15) | Group 2 (n=10) | Group 3 (n=15) | P value |
|---|---|---|---|---|
| Demographic characteristics | | | | |
| Age (month) | 40.0 (20.0-56.0) | 37.5 (26.7-56.5) | 47.0 (33.0-73.0) | 0.576 |
| Body weight (kg) | 15.0 (10.0-21.0) | 14.3 (10.3-20.1) | 18.3 (14.0-24.0) | 0.491 |
| Female sex | 7 (46.6%) | 5 (50%) | 8 (54.4%) | 0.936 |
| Laboratory findings | | | | |
| eGFR (ml/min/1.73 $m^2$) | 16.9 (13.3-26.0)[b] | 133.5 (121.2-169.0)[a] | 173.0 (153.0-244.0)[a*] | <0.001 |
| WBC (N: 4.5–13.5 $\times 10^3 \mu L$) | 11.4 (9.8-20.3)[a] | 9.05 (7.61-10.38)[b] | 7.7 (6.7-9.6)[b] | 0.001 |
| Hemoglobin (N: 12–16 g/dL) | 8.43±1.56 [b] | 12.20±1.43[a] | 12.50±1.27[a] | 0.001 |
| Platelet (N: 150–450 $x10^3/\mu L$) | 41 (28-51)[a] | 301 (254-358)[b] | 334 (302-461)[b] | <0.001 |
| LDH (N: < 248 U/L) | 2400 (2047-2834)[a] | 289 (252-339)[b] | - | <0.001 |
| BUN (N: 7-17 mg/dL) | 64 (40-102)[a] | 12.7 (10.8-15.8)[b] | - | <0.001 |
| Cr (N: 0.4-1.2 mg/dL) | 3.0 (2.4-5.9)[a] | 0.39 (0.29-0.49)[b] | 0.33 (0.22-0.40)b | <0.001 |
| Serum endocan (ng/L) | 496.42±104.02[a] | 488.1±93.59[a] | 424.9±139.8[a] | 0.212 |
| Urinary endocan (ng/gCr) | 2148 (1592-3068)[a] | 1274 (733-1565)[b] | 954 (517-1966)[b] | 0.002 |

Data are presented as n (%), mean±SD or median (Q1-Q3).
[a,b]The difference between means/medians with the same letter is not significant, but the difference between means/medians with different letters is significant.
BUN, blood urea nitrogen; Cr, creatinine; eGFR, estimated glomerular filtration rate; LDH, lactate dehydrogenase; SD, standard deviation; Q1, 25th percentile; Q3, 75th percentile; WBC, white blood cell.

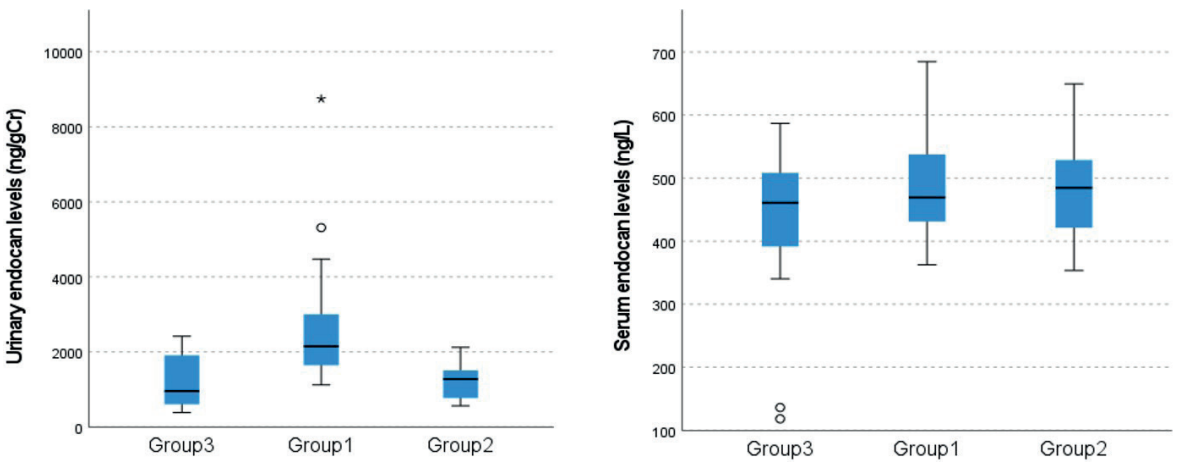



**Fig. 1.** Comparison of urinary and serum endocan levels of groups (Group 1: HUS-active stage, Group 2: HUS-follow-up and Group 3: healthy controls).
HUS, hemolytic uremic syndrome.

**Table II.** Correlation coefficients and significance levels in the parameters analyzed in the study groups.

| | Correlation coefficient (r) with- | |
|---|---|---|
| | Serum endocan level | Urinary endocan level (normalized to Cr) |
| General | | |
| White blood cell count | 0.17 | 0.63** |
| Hemoglobin level | -0.21 | -0.57** |
| Platelet count | 0.07 | -0.37* |
| Estimated glomerular filtration rate | -0.17 | -0.45** |
| Serum creatinine level | 0.16 | 0.50** |
| Blood urea nitrogen level | 0.13 | 0.48* |
| Lactate dehydrogenase level | 0.27 | 0.51** |
| Group 1 | | |
| Estimated glomerular filtration rate | -0.55* | -0.03 |
| Stage of AKI in the acute phase of the disease | 0.59* | 0.09 |
| Group 2 | | |
| The time taken until urine sample collection | -0.65* | - |
| Group 3 | | |
| White blood cell count | 0.60* | 0.60* |
| Serum endocan level | - | 0.53* |

*: P<0.05, **: P<0.01. AKI, acute kidney injury; Cr, creatinine.

and urinary endocan levels did not show a statistically significant correlation with the clinical severity score (r= 0.16, P=0.117; and r= 0.05, P=863), hypertension (r= -0.03, P=0.908; r= 0.36, P=0.187), urine protein (r= 0.32, P=0.250; and r= -0.19, P=0.497) and urine blood positivity (r= 0.40, P=0.143; and r= -0.23, P=0.408), erythrocyte sedimentation rate (r= 0.31, P=0.417; r= 0.05, P=0.898), C-reactive protein (CRP, r= -0.46, P=0.117; r= 0.05, P=0.972), respectively. Distribution of the clinical severity score and AKI stages of patients in Group 1 (n=15) is presented in Table III. In Group 1, patients with stage III AKI had a significantly higher mean serum endocan level (546.2±89.32 ng/L), when compared to Group 1 patients with stage I (363.3±1.20 ng/L) and stage II (419.3±28.38 (ng/L) AKI (P<0.05). No statistically significant difference was observed between urinary endocan levels according to AKI stages (P>0.05) (Table III). When Group 1 patients with (n=5) and without (n=10) hypertension were compared, there was no statistical difference in terms of serum (means±SD: 496.2±97.48 vs. 496.5±112.28 ng/L, P=0.953) and urine (medians [Q1-Q3]: 2238.2 [2005.8-4891.9] vs. 1767.4 [1399.8-2965.9] ng/gCr; P=0.206) endocan levels. There was no significant difference in serum (means±SD: 506.0±78.09 vs. 485.4±133.67 ng/L; P=0.463) and urinary (medians [Q1-Q3]; 1644.7 [1307.2-2837.8] vs. 2274.4 [1863.6-4471.9] ng/gCr; P=0.094) endocan levels in patients who needed KRT compared to those who did not need KRT.

## Discussion

HUS is a thrombotic microangiopathy characterized by thrombocytopenia, intravascular hemolysis and AKI.[18] Although HUS has various etiological causes, including environmental triggers or genetic mutations, all forms of HUS present with endothelial damage, in which there are microvascular lesions characterized by the formation of fibrin and platelet-rich thrombi.[2] Non-invasive diagnosis

**Table III.** Distribution of Group 1 (n=15) patients in terms of clinical severity score and AKI stage.

| | | Serum endocan level (mean±SD) | Urine endocan level (mean±SD) | n | % |
|---|---|---|---|---|---|
| Acute kidney injury | Stage I | 363.3±1.20[b] | 2384.8±773.8[a] | 2 | 13.3 |
| | Stage II | 419.3±28.38[b] | 2401.9±612.1[a] | 3 | 20.0 |
| | Stage III | 546.2±89.32[a*] | 3003.3±2460.8[a] | 10 | 66.7 |
| Parameters of clinical severity[&] | Stage III AKI in the acute phase of the disease | | | 10 | 66.7 |
| | KRT requirement | | | 8 | 53.2 |
| | Diagnosis of atypical HUS | | | 2 | 13.3 |
| | Anuria > 2 weeks | | | 2 | 13.3 |
| | Non-renal organ involvement | | | 2 | 13.3 |
| | KRT > 4 weeks | | | 1 | 6.7 |
| Clinical severity score | Score 0 | | | 3 | 20.0 |
| | Score 1 | | | 1 | 6.6 |
| | Score 2 | | | 5 | 33.3 |
| | Score 3 | | | 4 | 26.7 |
| | Score 4 | | | 0 | 0.0 |
| | Score 5 | | | 2 | 13.3 |

[a,b]While there is no significant difference between the means indicated with the same letter, there is a significant difference between the means indicated with different letters.
*P<0.05
[&]Patients received 1 point for each present parameter, and the clinical severity score was determined by summing all scores.
AKI, acute kidney injury; HUS, hemolytic uremic syndrome; KRT, kidney replacement therapy; SD, standard deviation.

of various kidney diseases remains a challenge in clinical practice. Blood urea nitrogen, serum creatinine levels and proteinuria are the commonly practiced diagnostic parameters to evaluate kidney pathologies; however, they are not specific.[4] The diagnostic methods for AKI due to many causes, including HUS, are mainly based on serum creatinine measurement. However, the decreased sensitivity and specificity of this marker, which does not always reflect the extent of renal parenchymal destruction, has led to the evaluation of alternative markers associated with inflammation and endothelial dysfunction, such as endocan.[19]

In the current study, although the mean serum endocan level was higher in Group 1, mean serum endocan levels showed no statistically significant difference between the groups (P>0.05). However, the mean urinary endocan level in Group 1 patients was significantly higher than in the other groups (P<0.05). Our findings suggest that urinary endocan levels may provide more valuable information than serum endocan levels in patients diagnosed with HUS. Also, higher urine endocan levels may indicate the active stage of the disease.

There is limited data on the metabolism and excretion of circulating endocan. Normally, endocan cannot pass through the negatively charged basement membrane in a healthy glomerulus, due to the presence of highly negatively charged dermatan sulfate, an essential component of endocan.[7] It is unclear whether the increase in serum endocan levels in renal diseases is due to increased production or decreased renal clearance. In addition, it remains unclear whether the increase in urinary endocan levels results from the release of this molecule from damaged renal tubular cells or from its leakage from plasma due to an impaired glomerular basement membrane.[4,9] In the literature, studies investigating serum endocan levels are more common than those investigating urinary endocan levels. In these

studies, serum endocan levels were found to be significantly higher in many diseases.[7,9-11,13,19-24] In our study, urinary endocan levels were significantly higher in patients with HUS. This finding suggests that endocan release into the urine may differ from endocan release into the circulation due to impaired glomerular permeability caused by endothelial damage in HUS.

Lee et al.[7] found that serum and urinary endocan levels were higher in adult patients with IGA nephropathy than in healthy controls. In the same study, plasma endocan levels did not differ significantly across CKD stages. However, patients with higher serum endocan levels had unfavorable renal outcomes. Urinary endocan levels were also higher in patients with poor renal function. In another study of adult patients, plasma endocan concentrations were significantly higher in CKD patients than in controls. They became progressively higher throughout the CKD stages. Plasma endocan concentrations were negatively correlated with eGFR and positively correlated with high-sensitivity CRP.[9] As we did not have any patient with CKD as a result of HUS, we could not make an evaluation to determine the relation of endocan and the presence of CKD in children with HUS.

Since inflammation and endothelial dysfunction are involved in the pathogenesis of AKI, it has been assumed that elevated serum endocan levels may reflect renal dysfunction in this patient group.[4] In our study, stage III AKI was detected in 10 of 15 patients in Group 1. The mean serum endocan level was significantly higher in 10 patients with AKI III than in the other five patients with AKI I and II ($P<0.05$). Furthermore, serum endocan levels were positively correlated with AKI stages ($r=0.59$, $P<0.05$). This finding suggests that serum endocan level may be used to determine patients with high AKI stage during the active stage of HUS.

Rahmania et al.[24] evaluated the predictive value of endocan in the requirement of KRT in a group of intensive care patients with AKI. They found higher serum endocan and creatinine levels in those who required RRT. In our study, KRT was needed in 8 of 15 patients in the active disease group (Group 1). There was no significant difference in serum and urinary endocan levels between the patients who needed KRT and those who did not. This difference may result from the sample differences since our sample did not include KRT patients requiring intensive care.

In Group 1, the serum and urinary endocan levels did not show a statistically significant correlation with the clinical severity score ($P>0.05$). These findings may be explained by the small number of patients included in the study and the low mean clinical severity score (2.20±1.56) in Group 1.

The clinical diagnosis of HUS is based on the triad of anemia (hemoglobin <8 g/dL), thrombocytopenia (platelets < $150 \times 10^9$/L), renal dysfunction, including hematuria, proteinuria, and an elevated serum Cr level.[25] Elevated white blood cell (WBC) count (greater than 20,000 per $mm^3$), and hematocrit (greater than 23%) are other risk factors for mortality and long-term complications from HUS.[26] In our study, when all groups were analyzed together, mean urinary endocan levels showed significant positive correlations with WBC count, and with lactate dehydrogenase, blood urea nitrogen, and creatinine levels, and significant negative correlations with hemoglobin level, platelet count, and eGFR. Taken together, these findings indicate that several key laboratory parameters relevant to the diagnosis and follow-up of HUS are closely associated with urinary endocan levels.

This study has certain limitations. One significant limitation is the small sample size, which was a result of the rarity of HUS cases. Therefore, larger-scale, multicenter clinical studies are needed to provide more meaningful insights into the applicability of endocan as a marker in the diagnosis, follow-up and prognosis of HUS patients.

In conclusion, urinary endocan levels were significantly higher in HUS patients in the active phase. The urinary endocan level may provide more valuable information than the serum endocan level in the clinical follow-up of patients with HUS. Also, serum endocan level may be used to evaluate AKI level during the active stage of HUS. We believe this study will guide future research investigating the long-term prognostic value of endocan in patients with HUS and exploring its potential as a target marker for the treatment and diagnosis of HUS.

## Ethical approval

The study was approved by Clinical Research Ethics Committee of Atatürk University Faculty of Medicine (date: September 30, 2021, number: 2021/06-55). Written consent was obtained from the parents of the patients and healthy controls.

## Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: MAG, MC; data collection: MAG; analysis and interpretation of results: MAG, MC; draft manuscript preparation: MAG, MC. All authors reviewed the results and approved the final version of the manuscript.

## Source of funding

The authors declare the study received no funding.

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES

1. Cody EM, Dixon BP. Hemolytic Uremic Syndrome. Pediatr Clin North Am 2019; 66: 235-246. https://doi.org/10.1016/j.pcl.2018.09.011
2. Manrique-Caballero CL, Peerapornratana S, Formeck C, Del Rio-Pertuz G, Gomez Danies H, Kellum JA. Typical and Atypical Hemolytic Uremic Syndrome in the Critically Ill. Crit Care Clin 2020; 36: 333-356. https://doi.org/10.1016/j.ccc.2019.11.004
3. Loirat C, Fakhouri F, Ariceta G, et al. An international consensus approach to the management of atypical hemolytic uremic syndrome in children. Pediatr Nephrol 2016; 31: 15-39. https://doi.org/10.1007/s00467-015-3076-8
4. Nalewajska M, Gurazda K, Marchelek-Myśliwiec M, Pawlik A, Dziedziejko V. The Role of Endocan in Selected Kidney Diseases. Int J Mol Sci 2020; 21: 6119. https://doi.org/10.3390/ijms21176119
5. Sarrazin S, Adam E, Lyon M, et al. Endocan or endothelial cell specific molecule-1 (ESM-1): a potential novel endothelial cell marker and a new target for cancer therapy. Biochim Biophys Acta 2006; 1765: 25-37. https://doi.org/10.1016/j.bbcan.2005.08.004
6. Zhang SM, Zuo L, Zhou Q, et al. Expression and distribution of endocan in human tissues. Biotech Histochem 2012; 87: 172-178. https://doi.org/10.3109/10520295.2011.577754
7. Lee YH, Kim JS, Kim SY, et al. Plasma endocan level and prognosis of immunoglobulin A nephropathy. Kidney Res Clin Pract 2016; 35: 152-159. https://doi.org/10.1016/j.krcp.2016.07.001
8. Leite AR, Borges-Canha M, Cardoso R, Neves JS, Castro-Ferreira R, Leite-Moreira A. Novel Biomarkers for Evaluation of Endothelial Dysfunction. Angiology 2020; 71: 397-410. https://doi.org/10.1177/0003319720903586
9. Yilmaz MI, Siriopol D, Saglam M, et al. Plasma endocan levels associate with inflammation, vascular abnormalities, cardiovascular events, and survival in chronic kidney disease. Kidney Int 2014; 86: 1213-1220. https://doi.org/10.1038/ki.2014.227
10. Su YH, Shu KH, Hu CP, et al. Serum Endocan correlated with stage of chronic kidney disease and deterioration in renal transplant recipients. Transplant Proc 2014; 46: 323-327. https://doi.org/10.1016/j.transproceed.2013.10.057
11. Çimen T, Efe TH, Akyel A, et al. Human Endothelial Cell-Specific Molecule-1 (Endocan) and Coronary Artery Disease and Microvascular Angina. Angiology 2016; 67: 846-853. https://doi.org/10.1177/0003319715625827
12. Huang X, Chen C, Wang X, et al. Prognostic value of endocan expression in cancers: evidence from meta-analysis. Onco Targets Ther 2016; 9: 6297-6304. https://doi.org/10.2147/OTT.S110295

13. Lv Y, Zhang Y, Shi W, et al. The Association Between Endocan Levels and Subclinical Atherosclerosis in Patients With Type 2 Diabetes Mellitus. Am J Med Sci 2017; 353: 433-438. https://doi.org/10.1016/j.amjms.2017.02.004

14. Schwartz GJ, Muñoz A, Schneider MF, et al. New equations to estimate GFR in children with CKD. J Am Soc Nephrol 2009; 20: 629-637. https://doi.org/10.1681/ASN.2008030287

15. Kellum JA, Lameire N, Aspelin P, et al. Kidney Disease: Improving Global Outcomes (KDIGO) acute kidney injury work group. KDIGO clinical practice guideline for acute kidney injury. Kidney Int Suppl 2012; 2: 1-138. https://doi.org/10.1038/kisup.2012.2

16. Igarashi T, Ito S, Sako M, et al. Guidelines for the management and investigation of hemolytic uremic syndrome. Clin Exp Nephrol 2014; 18: 525-557. https://doi.org/10.1007/s10157-014-0995-9

17. Sheerin NS, Glover E. Haemolytic uremic syndrome: diagnosis and management. F1000Res 2019; 8: F1000 Faculty Rev-F1000 Faculty1690. https://doi.org/10.12688/f1000research.19957.1

18. Jokiranta TS. HUS and atypical HUS. Blood 2017; 129: 2847-2856. https://doi.org/10.1182/blood-2016-11-709865

19. Samouilidou E, Athanasiadou V, Grapsa E. Prognostic and Diagnostic Value of Endocan in Kidney Diseases. Int J Nephrol 2022; 2022: 3861092. https://doi.org/10.1155/2022/3861092

20. Azimi A. Could "calprotectin" and "endocan" serve as "Troponin of Nephrologists"? Med Hypotheses 2017; 99: 29-34. https://doi.org/10.1016/j.mehy.2016.12.008

21. Raptis V, Bakogiannis C, Loutradis C, et al. Levels of Endocan, Angiopoietin-2, and Hypoxia-Inducible Factor-1a in Patients with Autosomal Dominant Polycystic Kidney Disease and Different Levels of Renal Function. Am J Nephrol 2018; 47: 231-238. https://doi.org/10.1159/000488115

22. Samouilidou E, Bountou E, Papandroulaki F, Papamanolis M, Papakostas D, Grapsa E. Serum Endocan Levels are Associated With Paraoxonase 1 Concentration in Patients With Chronic Kidney Disease. Ther Apher Dial 2018; 22: 325-331. https://doi.org/10.1111/1744-9987.12654

23. Oka S, Obata Y, Sato S, et al. Serum Endocan as a Predictive Marker for Decreased Urine Volume in Peritoneal Dialysis Patients. Med Sci Monit 2017; 23: 1464-1470. https://doi.org/10.12659/msm.900693

24. Rahmania L, Orbegozo Cortés D, Irazabal M, et al. Elevated endocan levels are associated with development of renal failure in ARDS patients. Intensive Care Med Exp 2015; 3(Suppl 1): A264. https://doi.org/10.1186/2197-425X-3-S1-A264

25. Liu Y, Thaker H, Wang C, Xu Z, Dong M. Diagnosis and Treatment for Shiga Toxin-Producing Escherichia coli Associated Hemolytic Uremic Syndrome. Toxins (Basel) 2022; 15: 10. https://doi.org/10.3390/toxins15010010

26. Boyer O, Niaudet P. Hemolytic-Uremic Syndrome in Children. Pediatr Clin North Am 2022; 69: 1181-1197. https://doi.org/10.1016/j.pcl.2022.07.006

The Turkish Journal of Pediatrics 2025; 67: 875-884
https://doi.org/10.24953/turkjpediatr.2025.6575

**Original Article**

# A prospective observational study on the underdiagnosis of pediatric abdominal migraine

Ayşe Büşra Paydaş[1], Aylin Yücel[2], Ahmet Sami Güven[3]

*[1]Department of Pediatric Cardiology, Faculty of Medicine, Necmettin Erbakan University, Konya, Türkiye; [2]Department of Pediatric Gastroenterology, Hepatology and Nutrition, Faculty of Medicine, Necmettin Erbakan University, Konya, Türkiye; [3]Department of Pediatric Neurology, Faculty of Medicine, Necmettin Erbakan University, Konya, Türkiye.*

## ABSTRACT

**Background.** Abdominal migraine is often considered a rare cause of chronic abdominal pain in children, but its true prevalence in specialized care and the specificity of current diagnostic criteria are not well understood. We aimed to determine the frequency of abdominal migraine in a tertiary pediatric gastroenterology clinic and to evaluate the diagnostic challenges posed by symptom overlap.

**Methods.** In this prospective study, 160 children (ages 5–18 years) with chronic recurrent abdominal pain were evaluated and followed for six months. Following comprehensive clinical, laboratory, and endoscopic assessments, patients were assigned to one of three final diagnostic groups: abdominal migraine, other disorders of gut-brain interaction (DGBI), or organic disease.

**Results.** The cohort of 160 patients was predominantly female (62.5%; mean age 11.6 ± 4.0 years). Abdominal migraine was the final diagnosis in 8.1% (n=13) of patients. Compared to the other groups, abdominal migraine was characterized by significantly longer pain duration ($p = 0.001$) and a higher prevalence of stress as a trigger. A key finding was the high rate of diagnostic overlap: 14.5% of patients with other DGBIs and 26.8% of patients with organic disease also fulfilled the Rome IV criteria for abdominal migraine. In these cases, a comprehensive evaluation identified a more appropriate primary diagnosis.

**Conclusions.** Abdominal migraine is a key diagnosis for unexplained pediatric abdominal pain, but its criteria lack specificity due to symptom overlap. A definitive diagnosis, therefore, requires a thorough clinical evaluation that extends beyond a symptom-based checklist to prevent misdiagnosis.

**Key words:** abdominal migraine, abdominal pain, children, functional gastrointestinal disorders, disorders of gut-brain interaction.

Chronic abdominal pain, defined as at least two episodes of pain severe enough to affect daily activities over two months, is a common pediatric complaint, affecting an estimated 9–15% of children and adolescents.[1,2] Given that up to 80% of cases lack a demonstrable organic cause, the diagnosis often falls under disorders of gut-brain interaction (DGBI).[3] DGBIs are understood to arise from complex mechanisms, including visceral hypersensitivity, motility disorders, and dysfunctions in the intestinal sensory and motor systems, with a prevalence ranging from 3–16% depending on the population studied.[4,5] The diagnostic framework for these conditions, the Rome criteria, was first established in the

✉ Ayşe Büşra Paydaş ▪ aysbsra93@gmail.com

Received 7th Jul 2025, revised 6th Aug 2025, 21st Oct 2025, accepted 2nd Nov 2025.

This study was presented as an oral presentation at the 2nd Meram Pediatrics International Congress in 2024.

1980s and most recently updated in 2016 as the Rome IV criteria.[6]

Abdominal migraine, a specific DGBI subtype, occurs in 0.2–4.1% of children.[7] It is defined by recurrent, paroxysmal episodes of severe, acute abdominal pain that is typically periumbilical, midline, or diffuse and lasts for at least one hour. These episodes are often accompanied by symptoms such as pallor, anorexia, nausea, vomiting, headache, and photophobia. According to the Rome IV criteria, the symptoms must occur on multiple occasions over at least six months, with a return to baseline health between attacks, and cannot be attributed to another medical condition after a thorough evaluation.[8]

The impact of DGBIs on children is significant, leading to a lower quality of life, increased school absenteeism, and more frequent hospitalizations, with symptoms persisting into adulthood for up to one-third of patients.[9,10] While early diagnosis can mitigate these effects, abdominal migraine remains frequently underdiagnosed.[11] Current management is often multimodal, involving psychological therapies, dietary changes, and neuromodulation, reflecting the limited pharmacological options available for children.[5]This study was therefore designed to determine the frequency of abdominal migraine in children presenting with chronic abdominal pain at a tertiary care clinic. We aimed to highlight its role as an essential differential diagnosis and investigate the factors contributing to its potential underdiagnosis.

## Materials and Methods

### *Study design and data collection*

This prospective, cross-sectional study was conducted at a pediatric gastroenterology outpatient clinic between November 2021 and November 2022. The study protocol adhered to the principles of the Declaration of Helsinki and received approval from the local institutional ethics committee. Written informed consent was obtained from all legal guardians and assent from participants where appropriate. We prospectively enrolled children aged 5–18 years who presented with chronic, recurrent abdominal pain. Patients were excluded if they had a pre-existing organic disease known to cause abdominal pain, such as inflammatory bowel disease, familial Mediterranean fever, or chronic hepatobiliary or renal conditions.

Over a six-month period, 602 patients were screened for abdominal pain. From this initial pool, 442 were excluded: 26 had inflammatory bowel disease, 328 were being managed for gastritis or gastroesophageal reflux, and 88 had experienced symptoms for less than the required two-month duration. The final study cohort comprised 160 patients with recurrent abdominal pain of at least two months' duration without a clear organic etiology at enrollment.

### *Data collection and follow-up*

At the initial visit, participants' guardians completed a structured questionnaire detailing demographic information, clinical symptoms, and family medical history. All patients were evaluated using the Rome IV criteria, and the presence of clinical alarm features (e.g., weight loss, nocturnal symptoms) was documented. Data on comorbid conditions, medication history, and factors reported to trigger or alleviate pain were also collected.

Participants were followed prospectively for six months. During this period, we recorded clinical outcomes, laboratory results, imaging findings, and, when clinically indicated, endoscopic and histopathological data. At the conclusion of the six-month follow-up, a final diagnosis was established for each patient. Based on this diagnosis, patients were stratified into three distinct groups for analysis:

- Group 1: Abdominal migraine
- Group 2: Other DGBIs (e.g., functional dyspepsia, irritable bowel syndrome)
- Group 3: Organic disease (diagnosed during the follow-up period)

A comparative analysis of demographic, clinical, and diagnostic findings was then performed across these three groups.

### *Statistical analysis*

The statistical analyses of the study were performed using SPSS 27.0 (IBM Inc., Armonk, NY, USA) software. The descriptive statistics were presented as mean ± SD or median (Q1-Q3) for numerical variables as necessary, and frequency (percentage) for categorical variables. The normality of the continuous variables was checked by the Kolmogorov-Smirnov test. The comparison between study groups (abdominal migraine, other DGBIs and organic disease) was performed using one-way ANOVA with Tukey HSD post-hoc test or Kruskal-Wallis test with K-W critical difference pairwise comparison for non-normally distributed variables. Chi-square test was employed to determine the relationships between categorical variables. In all analyses, $p < 0.05$ value was considered a statistically significant result for 5% type-I error.

## Results

### *Patient demographics and diagnostic stratification*

The study cohort comprised 160 pediatric patients with chronic recurrent abdominal pain. The majority were female (62.5%) with a mean age of 11.56 ± 3.98 years (range: 5–18 years) (Table I). After a six-month evaluation, patients were assigned to one of three diagnostic groups (Table II): Group 1 (abdominal migraine; 8.1%, n=13), Group 2 (other DGBIs; 47.5%, n=76), and Group 3 (organic disease; 44.4%, n=71). Patients in Group 3 were significantly older than those in Group 2 ($p < 0.001$), but no other significant demographic differences were found.

**Table I.** Demographic and clinical characteristics of the study cohort

| Characteristic | Value (*N* = 160) |
|---|---|
| Sex, n (%) | |
| Female | 100 (62.5) |
| Male | 60 (37.5) |
| Age (years), mean ± SD | 11.56 ± 3.98 |
| Anthropometric z-scores | |
| Weight, mean ± SD | -0.38 ± 0.09 |
| Height, mean ± SD | -0.21 ± 0.08 |
| Body mass index, median [Q1, Q3][a] | -0.29 [-1.28, 0.69] |
| Pain localization, n (%) | |
| Periumbilical | 69 (43.1) |
| Epigastric | 55 (34.4) |
| Widespread | 8 (5.0) |
| Other | 8 (5.0) |
| Not specified | 20 (12.5) |
| Pain duration | |
| Duration (minutes), median [Q1, Q3][a] | 60 [20, 180] |
| Distribution by duration, n (%) | |
| < 1 hour | 74 (46.3) |
| 1-72 hours | 85 (53.1) |
| > 72 hours | 1 (0.6) |
| Alarm features, n (%) | |
| Patients with alarm symptoms | 69 (43.1) |
| Specific features reported[b] | |
| Family history of IBD, celiac, or peptic ulcer | 33 (20.6) |
| Nocturnal diarrhea | 11 (6.9) |
| Arthritis symptoms | 9 (5.6) |
| Gastrointestinal bleeding | 8 (5.0) |
| Growth retardation | 6 (3.8) |
| Other (fever, vomiting, weight loss, etc.) | 15 (9.4) |

IBD = Inflammatory bowel disease; SD = Standard deviation; [a] Q1 = 1st quartile; Q3 = 3rd quartile; [b] Patients could present with multiple alarm features; therefore, percentages for specific features are calculated from the total cohort (*N* = 160) and do not sum to 43.1%.

**Table II.** Final diagnoses of patients stratified by category (*N* = 160)

| Diagnostic category and specific diagnosis | n | % |
|---|---|---|
| Organic diseases[a] | 71 | 44.4 |
| Chronic gastritis[b] | 43 | 26.9 |
| Gastroesophageal reflux disease | 17 | 10.6 |
| Celiac disease | 6 | 3.8 |
| Familial Mediterranean fever | 4 | 2.5 |
| Alkaline reflux gastritis | 3 | 1.9 |
| Duodenitis | 2 | 1.3 |
| Lactose intolerance | 1 | 0.6 |
| Disorders of gut-brain interaction (DGBI) | 89 | 55.6 |
| Functional abdominal pain–NOS | 27 | 16.9 |
| Functional dyspepsia | 22 | 13.8 |
| Irritable bowel syndrome | 15 | 9.4 |
| Abdominal migraine | 13 | 8.1 |
| Functional constipation | 12 | 7.5 |

[a] The sum of specific diagnoses may exceed the subtotal for the category as some patients had more than one organic disease; [b] *Helicobacter pylori*–positive and –negative gastritis were combined into a single category.
NOS: Not otherwise specified.

### *Pain triggers and alleviating factors*

Abdominal pain characteristics of the whole cohort are summarized in Table I. Specific triggers for abdominal pain were identified in 45.0% (n=72) of patients. Dietary factors were the most common trigger (30.6%), with spicy foods, acidic beverages, and fatty foods frequently implicated. Other reported triggers included hunger (4.4%), constipation (4.4%), and stress (3.8%). Factors providing pain relief were reported by 26.3% of patients, with defecation being the most frequent (13.8%), followed by the use of proton pump inhibitors (6.3%).

### *Medical history and comorbidities*

A personal history of migraine was rare (1.9%), but a family history was noted in 26.3% (n=42) of the cohort. Among patients diagnosed with abdominal migraine, none had a personal history of migraine, though 38.5% reported a positive family history. The majority of patients (85.6%) had no comorbid conditions. For the 23 patients with comorbidities, these included allergic/immunological disorders (3.1%), neurological conditions (2.5%), and renal disorders (2.5%). In patients with conditions like familial Mediterranean fever or nephrolithiasis, the chronic abdominal pain was determined to be unrelated to their primary disease.

### *Laboratory and endoscopic findings*

Upper endoscopy was performed in 33.8% (n=54) of patients, prompted by alarm symptoms (n=21) or severe dyspeptic complaints (n=33). Pathological findings were identified in 90.7% (n=49) of these procedures, with erythematous and nodular gastritis being the most common endoscopic abnormalities. Histopathological analysis confirmed chronic gastritis as the most frequent diagnosis. Serological screening for celiac disease via anti-endomysial and anti-tissue transglutaminase IgA antibodies yielded positive results in a small number of cases (2.5% and 2.3%, respectively).

### *Comparative analysis across diagnostic groups*

As detailed in Table III, the clinical features of abdominal pain varied significantly across the groups.

Duration and localization: Pain episodes in the abdominal migraine group (Group 1) were significantly longer than in Groups 2 and 3 ($p = 0.001$). Pain localization also differed ($p < 0.001$), with periumbilical pain being most common in the DGBI groups (Groups 1 and 2) and epigastric pain predominating in the organic disease group (Group 3).

Triggers and relief: Pain triggered by eating was significantly less frequent in Group 1, whereas stress-induced pain was more prevalent. Defecation provided relief most often in Group 2, while relief from proton pump inhibitors was a feature of Group 3 ($p = 0.001$).

**Table III.** Comparison of abdominal pain characteristics across diagnostic groups.

| Characteristic | Group 1: Abdominal Migraine (n = 13) | Group 2: Other DGBI (n = 76) | Group 3: Organic Disease (n = 71) | p |
|---|---|---|---|---|
| Localization, n (%) | | | | <0.001* |
| Periumbilical | 9 (69.2)[a] | 46 (60.5)[b] | 13 (18.3)[b] | |
| Epigastric | 0 (0.0)[a] | 14 (18.4)[b] | 42 (59.2)[c] | |
| Duration of pain (minutes), median [Q1, Q3] | 180 [180, 1185][a] | 60 [15, 120][b] | 60 [15, 180][b] | 0.001** |
| Duration, categorical, n (%) | | | | 0.005* |
| < 1 hour | 1 (7.7)[a] | 41 (53.9)[b] | 32 (45.1)[b] | |
| 1–72 hours | 12 (92.3)[a] | 34 (44.7)[b] | 39 (54.9)[b] | |
| Patients with alarm signs, n (%) | 6 (46.2) | 33 (43.4) | 30 (42.3) | 0.964* |
| Pain triggers, n (%) | | | | |
| Eating | 1 (7.7)[a] | 21 (27.6)[b] | 27 (38.0)[b] | 0.005* |
| Stress | 5 (38.5)[a] | 1 (1.3)[b] | 1 (1.4) )[b] | 0.001* |
| Pain relievers, n (%) | | | | |
| Defecation | 0 (0.0)[a] | 18 (23.7)[b] | 1 (1.4)[a] | 0.001* |
| PPI use | 0 (0.0)[a] | 3 (3.9)[b] | 7 (9.9) )[b] | 0.001* |

Within a row, values that do not share a common superscript letter (a, b, c) are significantly different from each other based on post-hoc pairwise comparisons ($p < 0.05$); *$p < 0.05$ based on the chi-square test; **$p < 0.05$ based on the Kruskal-Wallis test; DGBI: Disorders of gut-brain interaction; PPI: Proton pump inhibitor; Q1: 1st quartile; Q3: 3rd quartile.

Laboratory parameters: No significant differences were observed between groups regarding personal or family history of migraine. A comparison of laboratory parameters revealed statistically significant differences only in aspartate transaminase and alanine transaminase levels. However, as all values remained within the normal reference range for children, these findings were not considered clinically significant.

### *Overlap of diagnostic criteria*

A crucial finding was the high percentage of patients in other groups who met the formal criteria for abdominal migraine. Among patients ultimately diagnosed with another DGBI (Group 2) or an organic disease (Group 3), 14.5% and 26.8%, respectively, fulfilled all Rome IV criteria for abdominal migraine. In these patients, the diagnosis of abdominal migraine was ultimately excluded after a comprehensive clinical evaluation identified a more appropriate primary diagnosis.

## Discussion

This study offers new insights into the prevalence and diagnostic challenges of abdominal migraine in pediatric patients with chronic abdominal pain. Our findings demonstrate that abdominal migraine accounts for 8.12% of chronic abdominal pain cases in a tertiary care setting, which is notably higher than the 1-4.5% prevalence reported in population-based studies.[12] This discrepancy suggests that abdominal migraine may be underdiagnosed in general practice, supporting our hypothesis that this condition is "more than rare" in specialized gastroenterology clinics.

Most significantly, our study reveals critical limitations in the current diagnostic approach. While 15% (n=24) of our cohort initially met Rome IV criteria for abdominal migraine, only 8.12% (n=13) received this final diagnosis after comprehensive clinical evaluation. This finding highlights the need for careful clinical judgment beyond the algorithmic application

of symptom-based criteria and raises important questions about their specificity.

Chronic abdominal pain affects an estimated 9-15% of children and adolescents.[2] While some studies report higher prevalence in girls aged 9-10 years, others suggest equal sex distribution.[13,14,15] A recent Turkish study reported a mean age of 11.26 ± 3.80 years with 67% female predominance.[16] Our findings are consistent with these reports, demonstrating 62.5% female predominance and a mean age of 11.5 years.

Patients diagnosed with abdominal migraine in our cohort had a mean age of 10 years, with no significant differences in anthropometric parameters compared to other groups. Although abdominal migraine affects both sexes, literature reports a marked female predominance (1.6:1 ratio) with a prevalence among school-aged children of 1-4.5%.[2,12] Previous studies suggest abdominal migraine predominantly affects children aged 3-10 years, with a mean age of 7 years and bimodal peaks at ages 5 and 10.[7,17,18] The earlier peak may coincide with school entry as a potential stressor, though the underlying mechanism remains unclear.[7]

### *Pain characteristics and duration*

While reports of pain duration in abdominal migraine vary from 60 minutes to an average of 17 hours, our results offer important clarification.[17,19] We found that pain episodes in our abdominal migraine patients lasted approximately three hours on average significantly longer than in other diagnostic groups. This finding establishes pain duration as a useful distinguishing feature.

Our findings also revealed distinct trigger patterns that may aid in differential diagnosis. Stress was a significantly more prevalent trigger in the abdominal migraine group (30.8%) compared to eating-related pain (7.7%). This pattern suggests that stress sensitivity may be more characteristic of abdominal migraine than dietary sensitivity. While certain foods and stress are known triggers, our data suggest stress may be a more diagnostically significant factor.[14,20] Given that anxiety and depression are common in children with functional abdominal pain and that psychological distress increases the likelihood of symptoms persisting into adulthood, these findings emphasize the importance of assessing psychosocial factors.[8]

### *Comorbidity patterns and novel associations*

In our cohort, 85.6% of patients had no comorbidities. Among those who did, we identified a higher prevalence of renal disease in patients with abdominal migraine a novel finding. A 2022 study linked migraine headaches in children with chronic kidney disease to improved homeostasis post-transplantation.[21] Although no direct association with abdominal migraine has been reported, our finding suggests possible shared pathophysiological mechanisms that merit investigation in larger cohorts.

### *Diagnostic considerations and organic disease prevalence*

While organic causes are identified in approximately 5-10% of children with chronic abdominal pain, the majority are diagnosed with functional abdominal pain.[22] A Turkish study reported that 68.3% of such patients were diagnosed with functional pain, while 31.7% had an identifiable organic cause after full evaluation.[16] Our cohort demonstrated a higher frequency of organic disease (44.37%), which may be attributed to the referral pattern to our gastroenterology clinic, where patients predominantly present with alarm symptoms or suspected organic pathology. This higher organic disease rate in our population strengthens our findings, as it demonstrates that abdominal migraine diagnosis remained robust even in a population with high organic disease prevalence.

Population-based studies indicate that the abdominal migraine phenotype consists of poorly localized, midline, periumbilical (65-

80%), or diffuse (16%) pain.[12] Pain localization in our study most commonly involved the periumbilical area (69.2%), consistent with previous studies.[12,23] However, group-specific differences were noted: patients with abdominal migraine frequently reported diffuse pain, while epigastric pain was more common among those with organic disease. Such findings are consistent with diagnostic criteria for abdominal migraine, which describe pain as periumbilical, midline, or diffuse. The high frequency of gastritis in the organic disease group may explain the predominance of epigastric pain in this subset.

### *Diagnostic challenges and criteria limitations*

Our study has revealed significant diagnostic challenges: a substantial portion of patients with other functional disorders (14.5%) and organic disease (26.8%) met the full Rome IV criteria for abdominal migraine. This reveals significant limitations in the current diagnostic framework. Based on our findings, a definite diagnosis requires more than fulfilling symptom-based criteria. We propose that a definite diagnosis must include: (1) complete exclusion of organic pathology; (2) consideration of the overall clinical picture, not just isolated symptoms; (3) evaluation of response to migraine-specific therapy where appropriate; and (4) longitudinal follow-up to ensure diagnostic stability.

The Rome IV criterion stating that "symptoms cannot be explained by another medical condition after appropriate evaluation" proves crucial but requires careful interpretation. In our experience, this criterion necessitates a comprehensive assessment that goes beyond routine laboratory and imaging studies to include detailed evaluation of psychosocial factors, dietary patterns, and family history.

Our findings suggest that the current Rome IV criteria, while useful as screening tools, lack the specificity required for definitive diagnosis in complex clinical scenarios. This is particularly noteworthy given the established co-occurrence of multiple DGBI in individual patients.[24] In these cases, although patients met the abdominal migraine criteria, their primary complaints were attributed to other DGBI. The resulting high rate of false-positive diagnoses (46% of patients meeting criteria did not receive the final diagnosis) indicates that fulfilling diagnostic criteria does not equate to a confirmed diagnosis. We propose that future diagnostic criteria should incorporate stronger emphasis on characteristic pain patterns and duration, more specific requirements for associated symptoms, clearer guidelines for excluding overlapping functional disorders, and integration of family history weighting in diagnostic algorithms.

### *Impact on clinical management*

The diagnosis of abdominal migraine fundamentally alters the therapeutic approach compared to other functional gastrointestinal disorders. While traditional functional abdominal pain management focuses on dietary modifications, stress reduction, and symptomatic treatment, abdominal migraine diagnosis opens avenues for migraine-specific interventions including prophylactic medications, lifestyle modifications targeting migraine triggers, and specialized neurological follow-up.[10] In our cohort, patients with abdominal migraine demonstrated distinct clinical patterns: longer pain duration, greater stress sensitivity, and reduced food-related triggers compared to other functional disorders. These characteristics should guide clinicians toward more targeted interventions, including stress management techniques, sleep hygiene optimization, and consideration of migraine prophylaxis in severe cases. On top of that, the diagnosis carries important prognostic implications, as these patients require monitoring for potential evolution to classical migraine during adolescence, with evolution rates ranging from 25-70%.[12] This necessitates long-term follow-up strategies that differ significantly from typical functional abdominal pain management protocols.

### *Relationship with migraine headaches*

The relationship between abdominal migraine and classical migraine headaches represents one of the most intriguing aspects of this condition. In a prospective study of school-aged children (5-15 years), approximately 4.1% were diagnosed with abdominal migraine. In particular, children with migraine headaches were twice as likely to experience abdominal migraine, and vice versa.[3] Our findings provide additional context to this relationship: among patients with chronic abdominal pain, 1.9% had a personal history of migraine while 26.25% reported a family history of migraine. Family history of migraine was present in 38.46% (n=5) of patients diagnosed with abdominal migraine. This familial clustering is consistent with existing literature, which reports migraine family history in 65-90% of abdominal migraine cases compared to approximately 20% in controls.[12]

This disconnect between family history and personal migraine history in our abdominal migraine patients suggests several possibilities: (1) abdominal migraine may represent an earlier manifestation of migraine diathesis that precedes headache development, (2) genetic predisposition to migraine may manifest differently in pediatric populations, or (3) current diagnostic approaches may miss subtle migraine headache symptoms in children presenting primarily with abdominal complaints. Therefore, existing studies indicate a high prevalence of family history of migraine among children diagnosed with either abdominal migraine or migraine headaches.[25] Based on these data, we suggest that clinicians should inquire about personal and family history of migraines in patients with chronic abdominal pain, which may improve diagnostic accuracy and lead to more effective management.

The concept of abdominal migraine as a "migraine equivalent" or precursor syndrome has important clinical implications. Episodic syndromes associated with migraine—including abdominal migraine, cyclic vomiting syndrome, and infantile colic—are characterized by periodic symptoms in children with positive family histories of migraine and normal interictal neurological examinations.[26] These children face an increased risk of developing migraine during adolescence (25-70%, depending on the syndrome).[12] Up to 70% of patients later diagnosed with migraine retrospectively report a history of childhood "migraine equivalents," with abdominal migraine being the most common.[27]

While literature indicates that 58–70% of children with abdominal migraine also experience migraine headaches, none of our patients had a personal history of migraine, despite a high prevalence of family history. This pattern suggests these children may be in a pre-headache phase of migraine evolution, with important clinical implications: they require neurological monitoring and may benefit from early prevention strategies given their increased risk of developing migraine in adulthood.

### *Study limitations*

This study has several limitations. Its questionnaire-based design introduces potential recall bias. Stress, as a trigger of abdominal pain, was assessed subjectively based on patients' past experiences and was not evaluated using a standardized scale. The single-center, tertiary care setting may limit the generalizability of our findings. Furthermore, the small sample size of the abdominal migraine group restricts the statistical power of some observations, such as the association with renal disease, and requires cautious interpretation. Finally, treatment outcomes were not assessed, as the study's objective was prevalence and characterization.

### *Clinical implications*

This study demonstrates that abdominal migraine is a common and distinct clinical entity. Even when patients meet diagnostic criteria, a thorough evaluation to exclude organic pathology is essential. Clinicians should consider abdominal migraine as a

key differential diagnosis in children with persistent abdominal pain, particularly when there is a family history of migraine or a pattern of stress-related triggers. A comprehensive approach integrating clinical history, physical examination, and psychosocial assessment is crucial for accurate diagnosis and effective management.

## Ethical approval

The study was approved by Clinical Research Necmettin Erbakan University Ethics Committee (date: January 17, 2022, number: 2022/3837). Written informed consent was obtained from all participants and their legal guardians.

## Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: ASG, AY; data collection: ABP; analysis and interpretation of results: ABP, AY; draft manuscript preparation: ABP, AY, ASG. All authors reviewed the results and approved the final version of the manuscript.

## Source of funding

The authors declare the study received no funding.

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES

1. Gray L. Chronic abdominal pain in children. Aust Fam Physician 2008; 37: 398-400.
2. Niriella MA, Jayasena H, Nishad N, Wijesingha IP, Prabagar K. Abdominal migraine in adults: a narrative review. Cureus 2025; 17: e85958. https://doi.org/10.7759/cureus.85958
3. Di Lorenzo C, Colletti RB, Lehmann HP, et al. Chronic abdominal pain in children: a technical report of the American Academy of Pediatrics and the North American Society for pediatric gastroenterology, hepatology and nutrition. J Pediatr Gastroenterol Nutr 2005; 40: 249-261. https://doi.org/10.1097/01.mpg.0000154661.39488.ac
4. Faure C, Wieckowska A. Somatic referral of visceral sensations and rectal sensory threshold for pain in children with functional gastrointestinal disorders. J Pediatr 2007; 150: 66-71. https://doi.org/10.1016/j.jpeds.2006.08.072
5. Thapar N, Benninga MA, Crowell MD, et al. Paediatric functional abdominal pain disorders. Nat Rev Dis Primers 2020; 6: 89. https://doi.org/10.1038/s41572-020-00222-5
6. Drossman DA. Functional gastrointestinal disorders: history, pathophysiology, clinical features and Rome IV. Gastroenterology 2016; 150: 1262-1279. https://doi.org/10.1053/j.gastro.2016.02.032
7. Azmy DJ, Qualia CM. Review of abdominal migraine in children. Gastroenterol Hepatol (N Y) 2020; 16: 632-639.
8. Gajendran S, Sharma J, Sharma A. Functional abdominal pain disorders in children. Aust J Gen Pract 2025; 54: 363-366. https://doi.org/10.31128/AJGP-08-24-7369
9. de Jesus CD, de Assis Carvalho M, Machado NC. Impaired health-related quality of life in Brazilian children with chronic abdominal pain: a cross-sectional study. Pediatr Gastroenterol Hepatol Nutr 2022; 25: 500-509. https://doi.org/10.5223/pghn.2022.25.6.500
10. Sinopoulou V, Groen J, Gordon M, et al. Efficacy of interventions for the treatment of irritable bowel syndrome, functional abdominal pain-not otherwise specified, and abdominal migraine in children: a systematic review and network meta-analysis. Lancet Child Adolesc Health 2025; 9: 315-324. https://doi.org/10.1016/S2352-4642(25)00058-6
11. Uc A, Hyman PE, Walker LS. Functional gastrointestinal disorders in African American children in primary care. J Pediatr Gastroenterol Nutr 2006; 42: 270-274. https://doi.org/10.1097/01.mpg.0000189371.29911.68
12. Irwin S, Barmherzig R, Gelfand A. Recurrent gastrointestinal disturbance: abdominal migraine and cyclic vomiting syndrome. Curr Neurol Neurosci Rep 2017; 17: 21. https://doi.org/10.1007/s11910-017-0731-4

13. Malaty HM, Abudayyeh S, Fraley K, Graham DY, Gilger MA, Hollier DR. Recurrent abdominal pain in school children: effect of obesity and diet. Acta Paediatr 2007; 96: 572-576. https://doi.org/10.1111/j.1651-2227.2007.00230.x

14. Devanarayana NM, Mettananda S, Liyanarachchi C, et al. Abdominal pain-predominant functional gastrointestinal diseases in children and adolescents: prevalence, symptomatology, and association with emotional stress. J Pediatr Gastroenterol Nutr 2011; 53: 659-665. https://doi.org/10.1097/MPG.0b013e3182296033

15. Dhroove G, Saps M, Garcia-Bueno C, Leyva Jiménez A, Rodriguez-Reynosa LL, Velasco-Benítez CA. Prevalence of functional gastrointestinal disorders in Mexican schoolchildren. Rev Gastroenterol Mex 2017; 82: 13-18. https://doi.org/10.1016/j.rgmx.2016.05.003

16. Vatansever G, Tanca AK, Kırsaçlıoğlu CT, Demir AM, Kuloğlu Z. Evaluation of children with chronic abdominal pain and cost analysis. J Ankara Univ Fac Med 2021; 74: 324-331. https://doi.org/10.4274/atfm.galenos.2021.93585

17. Winner P. Abdominal migraine. Semin Pediatr Neurol 2016; 23: 11-13. https://doi.org/10.1016/j.spen.2015.09.001

18. Abu-Arafeh I, Russell G. Prevalence and clinical features of abdominal migraine compared with those of migraine headache. Arch Dis Child 1995; 72: 413-417. https://doi.org/10.1136/adc.72.5.413

19. Evans RW, Whyte C. Cyclic vomiting syndrome and abdominal migraine in adults and children. Headache 2013; 53: 984-993. https://doi.org/10.1111/head.12124

20. Escobar MA, Lustig D, Pflugeisen BM, et al. Fructose intolerance/malabsorption and recurrent abdominal pain in children. J Pediatr Gastroenterol Nutr 2014; 58: 498-501. https://doi.org/10.1097/MPG.0000000000000232

21. Elron E, Davidovits M, Eidlitz Markus T. Headache in pediatric and adolescent patients with chronic kidney disease and after kidney transplantation: a comparative study. J Child Neurol 2022; 37: 497-504. https://doi.org/10.1177/08830738221086432

22. Wright NJ, Hammond PJ, Curry JI. Chronic abdominal pain in children: help in spotting the organic diagnosis. Arch Dis Child Educ Pract Ed 2013; 98: 32-39. https://doi.org/10.1136/archdischild-2012-302273

23. Wilder-Smith CH, Schindler D, Lovblad K, Redmond SM, Nirkko A. Brain functional magnetic resonance imaging of rectal pain and activation of endogenous inhibitory mechanisms in irritable bowel syndrome patient subgroups and healthy controls. Gut 2004; 53: 1595-1601. https://doi.org/10.1136/gut.2003.028514

24. Van Tilburg MA, Walker LS, Palsson OS, et al. 820 prevalence of child/adolescent functional gastrointestinal disorders in a national US Community Sample. Gastroenterology 2014 ; 146: 143. https://doi.org/10.1016/S0016-5085(14)60508-4

25. Napthali K, Koloski N, Talley NJ. Abdominal migraine. Cephalalgia 2016; 36: 980-986. https://doi.org/10.1177/0333102415617748

26. Aurora SK, Shrewsbury SB, Ray S, Hindiyeh N, Nguyen L. A link between gastrointestinal disorders and migraine: Insights into the gut-brain connection. Headache 2021; 61: 576-589. https://doi.org/10.1111/head.14099

27. LenglarT L, Caula C, Moulding T, Lyles A, Wohrer D, Titomanlio L. Brain to belly: abdominal variants of migraine and functional abdominal pain disorders associated with migraine. J Neurogastroenterol Motil 2021; 27: 482-494. https://doi.org/10.5056/jnm20290

The Turkish Journal of Pediatrics 2025; 67: 885-891
https://doi.org/10.24953/turkjpediatr.2025.6132

**Short Communication**

# Oxytocin levels in children with childhood-onset fluency disorder

Erdoğan Özgür[1], Ercan Saruhan[2], Börte Gürbüz Özgür[3]

*[1]Department of Otorhinolaryngology, Faculty of Medicine, Dokuz Eylül University, İzmir, Türkiye; [2]Department of Biochemistry, Faculty of Medicine, Muğla Sıtkı Koçman University, Muğla, Türkiye; [3]Department of Child and Adolescent Psychiatry, Faculty of Medicine, İzmir Democracy University, Buca Seyfi Demirsoy Training and Research Hospital, İzmir, Türkiye.*

## ABSTRACT

**Background.** Evidence suggests a role for oxytocin in language development and cognitive functions in humans. However, there is a lack of research investigating the role of oxytocin in childhood-onset fluency disorder (stuttering). The aim of this study is to compare blood oxytocin levels between children diagnosed with stuttering and healthy controls.

**Methods.** Nineteen male children diagnosed with stuttering, aged between 6 and 11 years, and 27 typically fluent male children as a control group were included. All participants underwent psychiatric screening using the semi-structured interview The Kiddie Schedule for Affective Disorders and Schizophrenia School-Age Children-Present and Lifetime Version, and an ear, nose, throat examination. Serum oxytocin levels were determined using enzyme-linked immunosorbent assay.

**Results.** The median (Q1-Q3) blood oxytocin levels in the case group were 113.4 (90.19-136.3) pg/mL, while in the control group were 136.7 (105.4-203.7) pg/mL. A statistically significant lower level of oxytocin was observed in the case group compared to the control group (U=162, p=0.03).

**Conclusions.** We speculate a potential role of oxytocin in the etiology of developmental stuttering under the umbrella of neurodevelopmental disorders. The investigation of oxytocin, which plays a role in socialization and speech, in future studies on speech fluency disorders is intriguing in terms of its implications for clinical applications, including treatment.

**Key words:** stuttering, childhood, oxytocin, speech disorders, fluency disorder.

Childhood-onset fluency disorder, commonly referred to as stuttering, is a communication disorder marked by disruptions in the natural flow and timing of speech that are not typical for a person's age. These findings and symptoms are included among the diagnostic criteria for "childhood onset fluency disorder", which falls under the communication disorders classification within the category of neurodevelopmental disorders in the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5).[1] Approximately 80%-90% of all developmental stuttering cases begin before the age of 6.[1] Childhood stuttering is a multifactorial diagnosis involving genetic, psychological, neurological, and behavioral characteristics. In treatment, focus should be placed on each contributing factor.[2-5] Among

✉ Börte Gürbüz Özgür ▪ drborte@hotmail.com

Received 6th April 2025, revised 15th July 2025, revised 13th August 2025, accepted 3rd September 2025.

This study was presented as an oral presentation at the 44th Turkish National Congress of Otorhinolaryngology and Head and Neck Surgery, held in Antalya on November 15-19, 2023.

the neurogenic factors implicated in etiology are differences in the gray-white matter ratio of the brain, variations in neuronal network connections, atypical lateralization of hemisphere functions, and increased white matter connections in the right hemisphere.[6-8]

Oxytocin, a neurohypophyseal peptide, is secreted from the paraventricular and supraoptic nuclei and is primarily associated with uterine contractions during childbirth and milk ejection during lactation. In both men and women, oxytocin is believed to play a role in sexual and maternal behavior, social recognition, aggression, cognition, and neuromodulation through its central and peripheral effects.[9,10] It is believed that the activation of oxytocin receptors may play an important role in developmental processes and cell differentiation. The presence of oxytocin receptors in various brain regions supports this view. Some studies in both animals and humans have demonstrated the role of oxytocin in social communication, auditory processing, and speech behavior.[11,12] A recent study by Theofanopoulou suggests that oxytocin may play a role in language development and cognitive functions in humans.[13] Another piece of evidence supporting the effects of oxytocin on speech is the finding that oxytocin receptors are more abundant in the left hemisphere, where speech and auditory centers are located, compared to the right hemisphere.[14] All of these findings support the hypothesis that oxytocin plays a role in social relationships, auditory and speech behavior development in mammals.

Previous studies have reported the role of oxytocin in speech development. Considering studies suggesting the role of oxytocin in neurodegenerative and neurodevelopmental disorders[15], we decided to examine oxytocin levels in speech disorders, which are classified as neurodevelopmental disorders. A literature review did not reveal any study investigating the role of oxytocin in the etiology of speech fluency disorders in children. Our hypothesis posits that blood oxytocin levels in children with speech fluency disorders will be lower compared to healthy controls.

## Materials and Methods

### *Ethics and consent*

The study obtained ethical approval from the Muğla Sıtkı Koçman University Clinical Research Ethics Committee. The study was conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent forms were obtained from the children and their parents for participation in the study.

### *Participants*

Between January 2020 and July 2020, children presenting with complaints of impaired speech fluency, frequent repetitions, or prolongations of sounds or syllables at the Child and Adolescent Psychiatry and/or Ear, Nose, and Throat (ENT) outpatient clinics of Muğla Education and Research Hospital, and diagnosed with stuttering, were consecutively enrolled as the case group (n=28). To eliminate the potential influence of sex and age on oxytocin levels, only male participants in the prepubertal age group (6-11 years old) were included in the study. Age and sex matched typically fluent children without a psychiatric diagnosis upon psychiatric examination and with normal ENT examination, who presented to the ENT outpatient clinic for reasons other than communication problems (e.g. preoperative evaluation), were included as the control group.

### *Procedures*

Childhood-onset fluency disorder (stuttering) was diagnosed by using the DSM-5 criteria during psychiatric examination.[1] All participants were administered the Kiddie Schedule for Affective Disorders and Schizophrenia School-Age Children-Present and Lifetime Version-(K-SADS-PL) by a child and adolescent psychiatrist to exclude psychiatric diagnoses.

Children with non-fluency communication disorders, additional psychiatric diagnoses, neurological disorders, physical conditions such as cleft palate-lip that may contribute to speech disorder, and those clinically diagnosed with intellectual disability were excluded from the study. Additionally, all children presenting with stuttering underwent otolaryngological examination and hearing assessment by an ENT specialist. A socio-demographic data form prepared by the researchers was administered. This form included information such as the participants' and parents' age, education level, occupation, marital status, medical history, number of siblings, presence of motor movements accompanying stuttering, family history, onset time of stuttering, and whether the participant received treatment for stuttering.

### *Psychiatric assessment tools*

*The Kiddie Schedule for Affective Disorders and Schizophrenia School-Age Children-Present and Lifetime Version-Present And Life Time Version (K-SADS-PL) DSM-5:* K-SADS-PL, the semi-structured interview form used in this study, is designed to inquire about psychiatric diagnoses in children and adolescents aged 6-18 years in accordance with the DSM-5 diagnostic criteria.[16] A detailed psychiatric assessment is conducted with both parents and children face-to-face. The Turkish validity and reliability of this assessment have been established by Ünal et al.[17] In the case group, 9 cases were excluded due to comorbid psychiatric diagnoses.

### *Measurement of serum oxytocin concentrations*

Fasting venous blood samples were collected from participants into gel-separated blood tubes by venipuncture (between 8-10 a.m.). The blood tubes were centrifuged at 2000 g for 10 minutes and stored at -80 °C until analysis of oxytocin levels.

Serum oxytocin concentrations were measured by a human oxytocin enzyme-linked immunosorbent assay (ELISA) kit (Cat# E1046Hu, Bioassay Technology Laboratory, Shanghai, China) according to the instructions given in the package insert. The sensitivity of the oxytocin assay was 1.06 pg/mL. Inter-assay and intra-assay coefficients of variation of assay were < 10%. The measuring range for assay was 2 to 600 pg/mL.

### *Data analysis*

The data were analyzed using SPSS 29.0 (IBM Corp., USA). Descriptive statistics were reported using frequency, percentage, mean, standard deviation, and median (25th-75th percentile, Q1-Q3). The normal distribution of the data was assessed using the Kolmogorov-Smirnov test. Chi square test was used to compare categorical variables. Mann Whitney U test, one of the nonparametric tests, was used to compare two independent groups that were not normally distributed. A p-value less than 0.05 was considered statistically significant.

## Results

The study comprised 45 boys aged 6–11 years, including 19 diagnosed with stuttering (case group) and 27 typically fluent peers (control group). The median age of the case group was 9 (7-10) years, and 8 (7-10) years in the control group with no statistically significant difference in age between the groups (p=0.751). Among those diagnosed with stuttering, 8 (42.1%) had involuntary motor movements accompanying stuttering (e.g. jerking of the head, eye blinks, stomping the foot) and 12 (63.2%) had a family history of stuttering. The mean age of onset of stuttering symptoms was 5.05 ± 1.7 years. There was no statistically significant difference in sociodemographic characteristics such as grade level, age of parents, parental education level, employment status, and marital status between the case and control groups (Table I).

**Table I.** Sociodemographic characteristics of stuttering and control groups

| | Case (n=19) | Control (n=27) | p |
|---|---|---|---|
| Age, yr, median (Q1-Q3) | 9 (7-10) | 8 (7-10) | 0.751* |
| School, n (%) | | | 0.558** |
| Pre-school | 2 (10.5%) | 1 (3.7%) | |
| Primary school | 14 (73.7%) | 23 (85.2%) | |
| Secondary school | 3 (15.8%) | 3 (11.1%) | |
| Mother's age, yr, median (Q1-Q3) | 37 (34-40) | 38 (34-40) | 0.662* |
| Father's age, yr, median (Q1-Q3) | 40 (39-42) | 40.5 (38.75-43.25) | 0.772* |
| Maternal employment, n (%) | 9 (47.4%) | 12 (44.4%) | 0.453** |
| Paternal employment, n (%) | 18 (94.7%) | 26 (96.3%) | 0.422** |
| Mother's education level, n (%) | | | 0.749** |
| Less than high school | 12 (63.2%) | 15 (55.6%) | |
| High school graduate | 5 (26.3%) | 7 (25.9%) | |
| University graduate | 2 (10.5%) | 5 (18.5%) | |
| Father's education level, n (%) | | | 0.750** |
| Less than high school | 11 (57.9%) | 15 (55.6%) | |
| High school graduate | 4 (21.1%) | 8 (29.6%) | |
| University graduate | 4 (21.1%) | 4 (14.8%) | |
| Parental consanguinity, n (%) | 2 (10.5%) | 1 (3.7%) | 0.173** |
| Parents currently married, n (%) | 16 (84.2%) | 25 (92.6%) | 0.623** |
| Chronic disease in the child, n (%) | 0 (100%) | 2 (7.4%) | 0.504** |
| Family history of stuttering, n (%) | 12 (63.2%) | 1 (3.7%) | <0.001** |
| Speech language therapy, n (%) | 6 (31.6%) | N/A | - |
| Accompanying motor movement, n (%) | 8 (42.1%) | N/A | - |

N/A: not applicable
* Mann-Whitney U test, ** Chi square test.

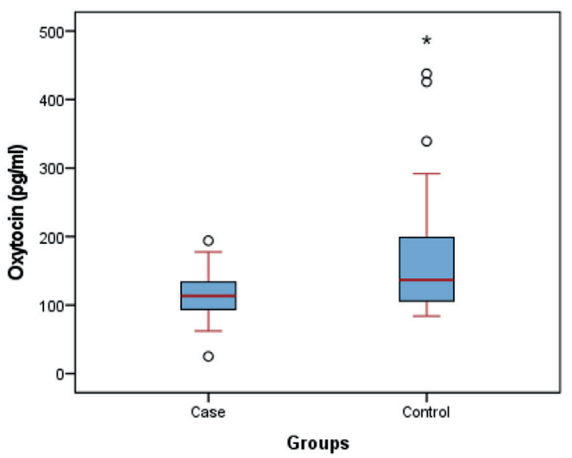



**Fig. 1.** Plasma oxytocin levels in stuttering and control groups (p=0.03).

The median blood oxytocin levels were 113.4 (90.19-136.3) pg/mL in the case group, and 136.7 (105.4-203.7) pg/mL in the control group (Fig. 1). Upon comparing the blood oxytocin levels between the groups, oxytocin levels in the case group were statistically significantly lower ($U$=162, p=0.03). There was no significant difference in blood oxytocin levels based on the presence or absence of involuntary motor movements accompanying stuttering in the case group ($U$=38, p=0.62).

## Discussion

There are limited studies on the role of oxytocin in speech disorders, but scientific evidence supports its effects on communication and speech. This study aims to investigate the relationship between oxytocin and stuttering, a speech disorder.

The results obtained from the study revealed that blood oxytocin levels in children with stuttering were significantly lower. This finding can be considered as an important clue suggesting the potential role of oxytocin neurohormone in the etiology of stuttering. Since there are no studies investigating blood oxytocin levels in individuals with stuttering in the literature, the results could not be compared. However, there are studies on certain hormones that may be associated with stuttering. Selcuk et al. reported that testosterone levels were higher in children with stuttering compared to the control group.[18] Researchers have suggested that high levels of sex hormones may increase stress in individuals, and it is proposed that high stress levels may trigger stuttering. In addition to serum testosterone levels, some studies have investigated the 2nd digit to 4th digit ratio (2D:4D) as an indicator of prenatal testosterone levels, and differences in prenatal testosterone levels have been reported to be associated with stuttering.[19,20] Mohammadi et al. reported that serum testosterone, dihydrotestosterone, and estradiol levels were significantly higher in the stuttering group.[21] The investigation of sex hormones in stuttering stems from the fact that stuttering is more prevalent in males.

In our study, we included patients of the same developmental period and male sex to exclude age and sex factors that could affect blood hormone levels. Although oxytocin is not a sex hormone, studies in adults have reported that blood oxytocin levels vary according to sex and age periods.[22,23] There is a need for studies investigating changes in oxytocin levels in children according to age periods.

The relationship between oxytocin and psychosocial behaviors, as well as its role in the etiology of certain psychiatric disorders, is intriguing. The discovery of oxytocin receptors being more densely populated in certain areas of the brain, particularly those associated with speech and hearing in the left hemisphere compared to the right hemisphere, as reported by Mitre et al., can be considered as a significant finding supporting the effects of oxytocin on speech.[14]

A recent study investigating the effects of intranasal oxytocin administration and the oxytocin receptor (rs53576) on speech using functional magnetic resonance imaging demonstrated that oxytocin increased brain activity in sensorimotor cortices, both dorsal and right ventral speech processing streams, as well as subcortical and cortical limbic and executive control regions. It was also shown that in some of these regions, the rs53576 *OXTR* polymorphism modulated brain activity associated with oxytocin administration.[24] Studies on the role of oxytocin in both normal speech and speech disorders will help elucidate its neuroimaging, neurophysiological, and biochemical effects.

In this study, anxiety disorders were also excluded from the study to eliminate the influence of additional psychopathologies on oxytocin levels. Oxytocin suppresses activity in amygdala neurons associated with other brain regions related to fear, such as the anterior cingulate cortex (ACC) and the medial prefrontal cortex (MPC).[25,26] It was discovered that oxytocin diminishes heightened activation of the ACC and MPC in individuals experiencing social anxiety disorder when exposed to sad faces.[26] The absence of a diagnosis of social anxiety disorder or other anxiety disorders in children of the age range studied in this research does not necessarily mean they won't develop them later in life. Additionally, which stuttering children will develop anxiety disorders remains a topic that requires further research. Whether current oxytocin levels serve as a biomarker for

anxiety disorders in stuttering children is also an intriguing topic for future studies.

The lack of examination into the relationship between stuttering severity and oxytocin and failure to compare oxytocin levels across different age groups (under 6 years old, over 11 years old) are limitations of this study. Another limitation is that the study was completed with a small sample size due to a decrease in the number of cases presenting to the clinic during the coronavirus disease 2019 pandemic. Restricting the age range to prepubertal and including only males can be considered the strengths of the study.

### *Conclusion*

This study is pioneering in its examination of oxytocin levels in stuttering. However, the role of oxytocin in the onset of speech disorders cannot be conclusively determined based on the observed differences in oxytocin levels alone.

In light of all this information, we believe that oxytocin may play a potential role in the etiology of developmental stuttering, which falls under the umbrella of neurodevelopmental disorders. Further studies examining oxytocin levels in speech disorders will help determine the reproducibility of our results. Additionally, research investigating the role of oxytocin in socialization and speech will shed light on its place in the etiology of speech fluency disorders, thus expanding treatment options in the literature.

## Ethical approval

The study was approved by Muğla Sıtkı Koçman University Clinical Research Ethics Committee (date: 16.01.2020, number: 01/VIII).

## Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: EÖ, ES, BGÖ; data collection: EÖ, ES, BGÖ; analysis and interpretation of results: EÖ, ES, BGÖ; draft manuscript preparation: EÖ, ES, BGÖ. All authors reviewed the results and approved the final version of the article.

## Source of funding

The authors declare the study received no funding.

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES

1. American Psychiatric Association, DSM-5 Task Force. Diagnostic and statistical manual of mental disorders: DSM-5. 5th ed. Washington, DC: American Psychiatric Association; 2013: xliv, 947. https://doi.org/10.1176/appi.books.9780890425596
2. Smith A, Weber C. How stuttering develops: the multifactorial dynamic pathways theory. J Speech Lang Hear Res 2017; 60: 2483-2505. https://doi.org/10.1044/2017_JSLHR-S-16-0343
3. Packman A. Theory and therapy in stuttering: a complex relationship. J Fluency Disord 2012; 37: 225-233. https://doi.org/10.1016/j.jfludis.2012.05.004
4. Starkweather CW, Givens-Ackerman J. Stuttering. Austin, TX: Pro-Ed; 1997.
5. Smith A, Kelly E. Stuttering: a dynamic, multifactorial model. In: Curlee RF, Siegel GM, editors. Nature and treatment of stuttering: new directions. 2nd ed. Boston, MA: Allyn & Bacon; 1997: 204-217.
6. Chang SE, Erickson KI, Ambrose NG, Hasegawa-Johnson MA, Ludlow CL. Brain anatomy differences in childhood stuttering. Neuroimage 2008; 39: 1333-1344. https://doi.org/10.1016/j.neuroimage.2007.09.067
7. Chang SE, Zhu DC. Neural network connectivity differences in children who stutter. Brain 2013; 136: 3709-3726. https://doi.org/10.1093/brain/awt275
8. Song LP, Peng DL, Jin Z, et al. Gray matter abnormalities in developmental stuttering determined with voxel-based morphometry. Zhonghua Yi Xue Za Zhi 2007; 87: 2884-2888.

9. Greenamyre JT, Betarbet R, Sherer TB. The rotenone model of Parkinson's disease: genes, environment and mitochondria. Parkinsonism Relat Disord 2003; 9(Suppl. 2): S59-S64. https://doi.org/10.1016/s1353-8020(03)00023-3

10. Gutkowska J, Jankowski M. Oxytocin: old hormone, new drug. Pharmaceuticals (Basel) 2009; 2: 168-183. https://doi.org/10.3390/ph203168

11. Boccia ML, Petrusz P, Suzuki K, Marson L, Pedersen CA. Immunohistochemical localization of oxytocin receptors in human brain. Neuroscience 2013; 253: 155-164. https://doi.org/10.1016/j.neuroscience.2013.08.048

12. Clark-Elford R, Nathan PJ, Auyeung B, et al. The effects of oxytocin on social reward learning in humans. Int J Neuropsychopharmacol 2014; 17: 199-209. https://doi.org/10.1017/S1461145713001120

13. Theofanopoulou C. Implications of oxytocin in human linguistic cognition: from genome to phenome. Front Neurosci 2016; 10: 271. https://doi.org/10.3389/fnins.2016.00271

14. Mitre M, Marlin BJ, Schiavo JK, et al. A distributed network for social cognition enriched for oxytocin receptors. J Neurosci 2016; 36: 2517-2535. https://doi.org/10.1523/JNEUROSCI.2409-15.2016

15. Ghazy AA, Soliman OA, Elbahnasi AI, Alawy AY, Mansour AM, Gowayed MA. Role of oxytocin in different neuropsychiatric, neurodegenerative, and neurodevelopmental disorders. Rev Physiol Biochem Pharmacol 2023; 186: 95-134. https://doi.org/10.1007/112_2022_72

16. Kaufman J, Birmaher B, Axelson D, Perepletchikova F, Brent D, Ryan N. Schedule for affective disorders and schizophrenia for school-aged children: present and life time version (K-SADS-PL) DSM-5 November 2016 working draft. New Haven: Yale University, Child and Adolescent Research and Education; 2016.

17. Ünal F, Öktem F, Çetin-Çuhadaroğlu F, et al. Reliability and validity of the schedule for affective disorders and schizophrenia for school-age children-present and lifetime version, DSM-5 November 2016-Turkish Adaptation (K-SADS-PL-DSM-5-T). Türk Psikiyatri Dergisi 2019; 30: 42-50. https://doi.org/10.5080/u23408

18. Selçuk EB, Erbay LG, Özcan ÖÖ, Kartalci Ş, Batcioğlu K. Testosterone levels of children with a diagnosis of developmental stuttering. Ther Clin Risk Manag 2015; 11: 793-798. https://doi.org/10.2147/TCRM.S83129

19. Montag C, Bleek B, Breuer S, et al. Prenatal testosterone and stuttering. Early Hum Dev 2015; 91: 43-46. https://doi.org/10.1016/j.earlhumdev.2014.11.003

20. Dönmez YE, Özcan Ö, Bilgiç A, Miniksar DY. The relationship between prenatal testosterone and developmental stuttering in boys. Turk J Pediatr 2019; 61: 193-199. https://doi.org/10.24953/turkjped.2019.02.007

21. Mohammadi H, Joghataei MT, Rahimi Z, et al. Sex steroid hormones and sex hormone binding globulin levels, CYP17 MSP AI (-34T:C) and CYP19 codon 39 (Trp:Arg) variants in children with developmental stuttering. Brain Lang 2017; 175: 47-56. https://doi.org/10.1016/j.bandl.2017.09.004

22. Marazziti D, Baroni S, Mucci F, et al. Sex-related differences in plasma oxytocin levels in humans. Clin Pract Epidemiol Ment Health 2019; 15: 58-63. https://doi.org/10.2174/1745017901915010058

23. Zak PJ, Curry B, Owen T, Barraza JA. Oxytocin release increases with age and is associated with life satisfaction and prosocial behaviors. Front Behav Neurosci 2022; 16: 846234. https://doi.org/10.3389/fnbeh.2022.846234

24. Vogt C, Floegel M, Kasper J, Gispert-Sánchez S, Kell CA. Oxytocinergic modulation of speech production-a double-blind placebo-controlled fMRI study. Soc Cogn Affect Neurosci 2023; 18: nsad035. https://doi.org/10.1093/scan/nsad035

25. Huber D, Veinante P, Stoop R. Vasopressin and oxytocin excite distinct neuronal populations in the central amygdala. Science 2005; 308: 245-248. https://doi.org/10.1126/science.1105636

26. Labuschagne I, Phan KL, Wood A, et al. Medial frontal hyperactivity to sad faces in generalized social anxiety disorder and modulation by oxytocin. Int J Neuropsychopharmacol 2012; 15: 883-896. https://doi.org/10.1017/S1461145711001489

The Turkish Journal of Pediatrics 2025; 67: 892-895
https://doi.org/10.24953/turkjpediatr.2025.6395

Case Report

# A case report: celiac disease and pediatric stuttering

Birce İzgi Akçay[1], Aysel Ünlüsoy[2], Necati Balamtekin[1]

[1]*Department of Pediatric Gastroenterology, Gülhane Training and Research Hospital, Ankara, Türkiye;* [2]*Department of Pediatric Gastroenterology, Bilkent City Hospital, Ankara, Türkiye*

## ABSTRACT

**Background.** Celiac disease is an immune-mediated disorder known to manifest not only with gastrointestinal symptoms but also with a wide range of extraintestinal features, including neuropsychiatric conditions.

**Case Presentation.** We describe the case of a 4-year-old girl who presented with isolated stuttering. Serologic tests revealed elevated anti-tissue transglutaminase antibodies, and a diagnosis of celiac disease was confirmed by duodenal biopsy. A strict gluten-free diet was initiated. The patient's speech disorder began to improve by the sixth month of treatment and resolved completely by the twelfth month of dietary adherence.

**Conclusion.** This case highlights the importance of considering celiac disease in the differential diagnosis of speech disorders in pediatric patients, especially when no other underlying cause is identified.

**Key words:** celiac disease, stuttering, pediatric, gluten-free diet, neurological manifestation, case report.

Celiac disease is an enteropathy that occurs in genetically susceptible individuals as a result of an abnormal immune response to gluten—a protein found in wheat, barley, and rye—leading to small intestinal mucosal damage. It is well known that the disease may present not only with gastrointestinal symptoms but also with various extraintestinal manifestations including, dermatologic, musculoskeletal, and neurological involvement.[1]

Stuttering is a speech disorder characterized by involuntary repetitions, prolongations, or pauses that interrupt the normal flow of speech. In children, it can be developmental or associated with various factors, including neurological disorders, medications, and food allergies. Wheat has been suggested as one of the food triggers potentially associated with stuttering.[2]

To the best of our knowledge, similar case reports describing the association between celiac disease and stuttering are rare in the literature. This article presents the case of a 4-year-old girl diagnosed with celiac disease during evaluation for stuttering. The resolution of symptoms following initiation of a gluten-free diet is discussed in light of current literature.

## Case Presentation

A 4-year-old girl was referred to the division of pediatric gastroenterology after serologic evaluation for suspected celiac disease revealed anti-tissue transglutaminase IgA and IgG levels above 200 IU/mL. The initial referral was made by a general pediatrician during assessment for persistent stuttering.

She was born at term via cesarean section, weighing 3300 grams, as the second child of

✉ Birce İzgi Akçay ▪ izgiakcay@gmail.com

Received 4th Jun 2025, revised 25th Jul 2025, accepted 10th Oct 2025.

a healthy 36-year-old mother and a healthy 37-year-old father. There was no prior history of growth retardation or health problems until the onset of stuttering around age 3. Psychosocial evaluation revealed a calm family environment, no exposure to preschool, and no identifiable stressors. No family history of stuttering or celiac disease was reported. Previous medical evaluations had found no organic pathology. Although brief psychotherapy was recommended, the family opted out after the diagnosis. There was no significant family history. Physical exam showed a weight of 14.2 kg (10th–25th percentile) and height of 102 cm (25th–50th percentile). Initial syllable repetitions were noted during speech. Other systems were normal. Laboratory findings: Hb 12.1 g/dL, WBC 6,000/mm$^3$, platelets 330,000/mm$^3$. Anti-tTG IgA and IgG >200 IU/mL. Vitamin A 385.6 μg/L (316-820), vitamin E 5.77 mg/L (6.6-14.3), vitamin C 2.38 mg/L (4-21), 25-hydroxy vitamin D 26.8 μg/L (30-100), folate 17.81 ng/mL (>5.38), ferritin 23.1 μg/L (13-232). Endoscopy showed atrophic changes in the duodenal and bulbar mucosa. Mucosal biopsies demonstrated Marsh type 3c lesions in the duodenal bulb and type 3a lesions in the second part of the duodenum.

She was diagnosed with celiac disease and started on a strict gluten-free diet. Speech began improving by month 6 and resolved completely by month 12.

Written informed consent was obtained from the patient's legal guardians for publication of this case.

## Discussion

Celiac disease is known for its gastrointestinal and extraintestinal spectrum. In atypical forms, symptoms may be limited to anemia, growth failure, or neurological issues.[3] In recent years, despite the paucity of data in peer-reviewed literature, stuttering has been proposed as a possible extraintestinal manifestation of celiac disease.[4] Language and speech skills begin to develop early in childhood and are acquired through the coordinated function of multiple systems, including the neuromotor and auditory pathways, as well as visual input. Although some publications suggest psychological mechanisms, current evidence generally supports that stuttering originates from altered brain morphology and/or function due to underlying organic abnormalities in the brain.[2,4]

Celiac disease is a malabsorptive condition, and nutrient and vitamin deficiencies resulting from malabsorption are among the key contributors to its clinical manifestations. Based on this pathophysiological mechanism, neurological disorders such as epilepsy and migraine are known to occur more frequently in patients with celiac disease.[5-7] In addition, early cognitive dysfunction has been reported in children diagnosed with celiac disease.[8] In our patient, laboratory tests at the time of diagnosis revealed deficiencies in vitamin C, vitamin E, and 25-hydroxyvitamin D levels. These findings support previous research suggesting that functional nutrient deficiencies may lead to diverse neurological symptoms in affected individuals.

Approximately 5% of children begin to stutter around the age of 3 during the period of speech acquisition. However, about 75% of these cases resolve spontaneously without intervention before reaching adolescence.[9] The first reported case suggesting a link between gluten and speech disturbance involved a child who developed aphasia after being hospitalized for severe diarrhea and was subsequently diagnosed with celiac disease. The child had lost speech ability entirely at the time of diagnosis, but following the initiation of a gluten-free diet, both speech and normal bowel function were reported to return. Around the same period, publications also emerged reporting an association between celiac disease and epilepsy.[10] In our patient, stuttering improved after the introduction of a gluten-free diet and resolved completely within a year. Notably, speech fluency began to improve by approximately six months after starting the gluten-free diet and reached

complete resolution by twelve months, without the need for any additional speech therapy. This gradual, time-dependent improvement in the absence of other interventions strengthens the plausibility of a gluten-related mechanism, although spontaneous developmental recovery cannot be fully excluded. While this could certainly be part of the child's natural developmental trajectory, the timing of both the onset and resolution of symptoms following dietary intervention may support a possible link between stuttering and celiac disease. Although peer-reviewed medical literature lacks robust evidence on the relationship between stuttering and celiac disease, there are various anecdotal reports on online platforms suggesting a connection between stuttering and food allergies—particularly gluten. Conversely, other reports dismiss any such association.[4] Undoubtedly, many similar anecdotes can be found on the internet, but these should be critically evaluated and validated through scientific research. Ultimately, current scientific studies clearly demonstrate the association between celiac disease and various neurological disorders.[5] Given that stuttering is known to be associated with neurological abnormalities, it may plausibly arise as a manifestation of gluten-related neurological dysfunction.

Although gluten ataxia usually occurs in middle-aged adults with classical findings such as gait ataxia, dysarthria, and nystagmus, early or subtle cerebellar involvement cannot be completely excluded in pediatric cases.[7] In our patient, the marked improvement in fluency after starting a strict gluten-free diet raises the possibility—albeit speculative—that the stuttering may have reflected an early or atypical form of gluten-related cerebellar dysfunction. This hypothesis cannot be confirmed without neuroimaging or serological testing, but it highlights an area that may warrant further investigation in future studies.

Importantly, brain MRI and MR spectroscopy were not performed at the time of diagnosis, as the family declined an extensive neurological work-up for stuttering. This represents a limitation of our case and prevents definitive exclusion of structural or functional cerebellar pathology. However, the temporal improvement in fluency following the gluten-free diet suggests that gluten-related cerebellar dysfunction or early gluten ataxia cannot be ruled out. This observation remains hypothesis-generating and highlights the need for further research on isolated speech disturbances as potential early manifestations of gluten-related neurological involvement in children.

We believe that in pediatric patients presenting with speech disorders, it would be a prudent clinical approach to consider celiac disease as part of the differential diagnosis—or at the very least, to exclude its presence.

## Ethical approval

Informed consent was obtained from the legal guardians.

## Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: BİA, NB; data collection: NB; analysis and interpretation of results: BİA, AÜ, NB; draft manuscript preparation: BİA, AÜ, NB. All authors reviewed the results and approved the final version of the manuscript.

## Source of funding

The authors declare the study received no funding.

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES

1. Catassi C, Verdu EF, Bai JC, Lionetti E. Coeliac disease. Lancet 2022; 399: 2413-2426. https://doi.org/10.1016/S0140-6736(22)00794-2

2. Laiho A, Elovaara H, Kaisamatti K, et al. Stuttering interventions for children, adolescents, and adults: a systematic review as a part of clinical guidelines. J Commun Disord 2022; 99: 106242. https://doi.org/10.1016/j.jcomdis.2022.106242

3. Balamtekin N, Uslu N, Baysoy G, et al. The presentation of celiac disease in 220 Turkish children. Turk J Pediatr 2010; 52: 239-244.

4. Hoggan R. Stutters, stammers, and gluten-is there a connection? J Gluten Sensitivity 2011; 10: 1-6.

5. Ford RP. The gluten syndrome: a neurological disease. Med Hypotheses 2009; 73: 438-440. https://doi.org/10.1016/j.mehy.2009.03.037

6. Hadjivassiliou M, Gibson A, Davies-Jones GA, Lobo AJ, Stephenson TJ, Milford-Ward A. Does cryptic gluten sensitivity play a part in neurological illness? Lancet 1996; 347: 369-371. https://doi.org/10.1016/s0140-6736(96)90540-1

7. Giuffrè M, Gazzin S, Zoratti C, et al. Celiac disease and neurological manifestations: from gluten to neuroinflammation. Int J Mol Sci 2022; 23: 15564. https://doi.org/10.3390/ijms232415564

8. Beas R, Godoy A, Norwood DA, et al. Cognitive impairment and insomnia in celiac disease: a systematic review and meta-analysis. Gut Liver 2024; 18: 1080-1084. https://doi.org/10.5009/gnl240063

9. Kell CA, Neumann K, von Kriegstein K, et al. How the brain repairs stuttering. Brain 2009; 132: 2747-2760. https://doi.org/10.1093/brain/awp185

10. Molteni N, Bardella MT, Baldassarri AR, Bianchi PA. Celiac disease associated with epilepsy and intracranial calcifications: report of two patients. Am J Gastroenterol 1988; 83: 992-994.

The Turkish Journal of Pediatrics 2025; 67: 896-903
https://doi.org/10.24953/turkjpediatr.2025.6009

Case Report

# Novel *FUCA1* variants in two families, including the first report of a contiguous gene deletion syndrome involving *FUCA1* and *HMGCL*

Mustafa Kılıç[1], Harun Yıldız[1], Firdevs Dinçsoy Bir[2]

[1]*Division of Metabolism, Department of Pediatrics, Ankara Etlik City Hospital, University of Health Sciences, Ankara, Türkiye;*
[2]*Department of Medical Genetics, Ankara Etlik City Hospital, Ankara, Türkiye.*

## ABSTRACT

**Background.** Fucosidosis is a rare, autosomal recessive lysosomal storage disorder caused by deficiency of an alpha-L-fucosidase due to pathogenic variants in the *FUCA1* gene, leading to the accumulation of fucoglycoconjugates in the lysosomes of the liver, brain, skin and other organs. Its main clinical features include progressive neurological deterioration, seizures, coarse facies, visceromegaly, angiokeratoma, growth retardation, recurrent sinopulmonary infections and dysostosis multiplex.

**Case Presentation.** Three patients with fucosidosis from two unrelated families with severe developmental delay, hearing loss, coarse facies but no hepatosplenomegaly and angiokeratoma are presented. A homozygous, novel nonsense mutation c.236G>A (p.Trp79*) in exon 1 of the *FUCA1* gene was identified in one family, and a homozygous novel 64.5 kb deletion, including *HMGCL* (exons 1-6), *FUCA1*, and *CNR2* (exon 2) genes in the other.

**Conclusions.** Fucosidosis should be considered in patients with delayed motor and cognitive development followed by progressive neurological deterioration, even in the absence of common features such as organomegaly and angiokeratoma. The pathogenic variants identified in both families were novel and consistent with fucosidosis type 1. To our knowledge, this is the first reported case of fucosidosis accompanied by 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) lyase deficiency resulting from a contiguous gene deletion involving the *HMGCL* gene at the 1p36.11 locus.

**Key words:** fucosidosis, alpha-L-fucosidase, HMG-CoA lyase deficiency, developmental delay, lysosomal storage disorder, 1p36.11 deletion.

Fucosidosis (OMIM #230000) is a rare, autosomal recessive neurodegenerative lysosomal storage disorder characterized by a deficiency of alpha-L-fucosidase due to pathogenic variants in the *FUCA1* gene (MIM *612280) located on chromosome 1p36.11. Alpha-L-fucosidase (EC 3.2.1.51) hydrolyzes fucose resides from glycolipids, glycoproteins and oligosaccharides. Its deficiency leads to the accumulation of oligosaccharides rich in alpha-L-fucose in many organs, particularly in the brain which are excessively excreted in urine.[1-8] Clinical features include neurological signs such as progressive psychomotor retardation, seizures, spasticity, and dystonia, along with coarse facies, visceromegaly, angiokeratomas, growth retardation, recurrent respiratory tract infections, and dysostosis multiplex.[1-3] Kyphoscoliosis, joint contractures, telangiectasia, hernia, hearing impairment and

✉ Mustafa Kılıç ▪ kilickorkmaz@yahoo.com.tr

Received 7th Mar 2025, revised 18th Jun 2025, accepted 23rd Sep 2025.

ophthalmological abnormalities (dilated and tortuous retinal veins and conjunctival vessels, corneal opacities, pigmentary retinopathy, diminished visual acuity) are also reported.[1-3] Previously, the disease was classified according two phenotypes based on age of onset and severity. Type 1 (severe form) follows a rapidly progressive neurodegenerative course with symptoms at the age before 1 to 2 years of age and death occurring in the first decade. Whereas type 2 (milder form) follows a slower neurologic deterioration with symptoms at the age of after 1 to 2 years and survive into the second or third decade of life.[3-5] It is now considered as a continuous clinical spectrum with variable severity of phenotype.[3-5] Its frequency is below 1 in 200,000 live births.[6] The highest incidence has been reported in Italians and the Mexican-Indian or Hispanic-American population.[3-8] The diagnosis is based on clinical suspicion, supported by appropriate clinical and radiological examinations followed by urinary examination for oligosaccharide excretion and then specific enzyme assay, usually on white blood cells and genetic analysis. Brain magnetic resonance imaging (MRI) findings include bilateral globus pallidus hyperintensities on T1-, marked hypointensity accompanying hyperintense curvilinear streak between their medial and lateral segments on T2-weighted images and diffuse symmetric white matter hyperintensities on T2-weighted images with normal appearance on T1-weighted images, indicative of hypomyelination.[9-11] Brain magnetic resonance imaging spectroscopy (MRS) findings include both the peak at the 3.8–3.9 ppm on being most prominent with short TE values (TE 30/35) that is broadening second creatinine peak, suggesting oligosaccharides and glycolipids and a doublet peak at the 1.2 ppm that inverts on TE 135, suggesting fucose peak. Although there is no specific treatment, patients have reported benefiting from bone marrow transplantation.[12] Earlier transplant has been reported to be more effective than transplant after full clinical manifestation. Here we present three fucosidosis type 1 patients from two unrelated families. This report includes the first case of fucosidosis accompanied by 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) lyase deficiency due to a contiguous gene deletion, along with two novel pathogenic variants in the *FUCA1* gene.

## Materials and Methods

In Patients 1 and 2, Sanger sequencing analysis of the *FUCA1* gene was performed using genomic DNA extracted from peripheral blood lymphocytes. The description of the variants utilized the human genome version 38 (hg38) and the transcript NM_000147.5 (RefSeq).

For Patient 3, DNA extracted from peripheral blood was used to perform a chromosomal microarray study using Infinium Global Screening Array Cyto (GSA-Cyto) chips on the Illumina iScan platform. Copy number variations (CNVs) were detected and visualized using the NxClinical (v.6.0) analysis software developed by Biodiscovery. The relevant positions were reported based on the Human Genome Build 37 (GRCh37/hg19) reference. The obtained data were evaluated using current databases (PubMed, OMIM, DGV, DECIPHER).

Detected SNVs and CNVs are classified based on the recommendations of ACMG-AMP 2015 and ACMG-ClinGen 2020 guidelines, respectively.[13,14]

## Case presentations

### *Patients 1 and 2*

A six-year-old Turkish male patient of Uzbek origin in Afghanistan was referred to our clinic due to an inability to sit and speak. His complaints began after the age of one; he had one seizure at the age of two, but was not started on medication as it did not recur. He was able to sit at the age of 12 months, but he was never able to stand, walk or speak. The parents were first-degree cousins. At presentation, he was spastic tetraplegic with growth retardation and could speak no meaningful words. He

had dystonia triggered by stimuli. His deep tendon reflexes were brisk. He had a depressed nasal bridge and coarse facial features. Skin examination was normal and there was no organomegaly. X-ray revealed a dysostosis multiplex including anterior beaking of vertebrae, lumbar hyperlordosis, widened and scalloped acetabular roof. Brain MRI showed marked hypointensity in both globi pallidi, diffuse symmetric white matter hyperintensities on T2-weighted images, cerebellar folia and cerebral atrophy with enlargement of the lateral ventricles. Electroencephalography showed epileptic activity. Ophthalmologic examination was normal, but the brainstem auditory evoked potentials test was bilaterally pathological. Tandem mass spectrometry, quantitative blood amino acid analysis, biotinidase activity, homocysteine, very long-chain fatty acids, transferrin isoelectric focusing and urine organic acid analysis were all in normal range. Urine glycosaminoglycan analysis was normal, but urine thin-layer chromatography showed increased oligosaccharides, particularly consistent with fucosidosis. The alpha-L-fucosidase enzyme level was measured at 1.9 nmol/hour/mg protein (N: >40), which was significantly low, diagnostic of fucosidosis. He is now 9.5 years old, responds to sound, has eye tracking, but is bedridden and fed with a gavage diet.

The patient's two-year-old male sibling with coarse facial features, developmental delay, hearing loss and dysostosis multiplex was also diagnosed with the same condition through enzymatic and molecular genetic analysis. His deep tendon reflexes were brisk. He had a depressed nasal bridge and coarse facial features. Skin examination was normal and there was no organomegaly. X-ray revealed a dysostosis multiplex including anterior beaking of vertebrae, lumbar hyperlordosis, widened and scalloped acetabular roof. He was able to sit at the age of 12 months and walk at the age of 1.5. He has eye tracking and can respond to sound. However bilateral mild hearing loss was detected and hearing aids were planned. Ophthalmologic examination and electroencephalography were normal. He is now 5.5 years old and has been hospitalized 3 times due to sinopulmonary infection. He can still walk but cannot run. He can only say 'mom' and 'dad'. He can feed himself but has started to lose weight (Table I).

Patients 1 and 2 were found to have the same homozygous variant in exon 1 of the *FUCA1* (NM_000147.5) gene, c.236G>A p.Trp79*, which

**Table I.** Comparison of clinical findings in our patients and literature

| Clinical Finding | Willems et al.[3] 1991 (%) | Patient 1[a] (Family 1) | Patient 2[a] (Family 1) | Patient 3 (Family 2) |
|---|---|---|---|---|
| Intellectual disability | 95% | + | + | + |
| Neurologic regression | 88% | + | + | + |
| Coarse face | 79% | + | + | + |
| Growth retardation | 78% | + | + | - |
| Recurrent infections | 78% | + | + | + |
| Dysostosis multiplex | 58% | + | + | + |
| Angiokeratoma | 52% | - | - | - |
| Seizure | 38% | + | - | - |
| Organomegaly | 30% | - | - | - |
| Hearing loss | 12% | + | + | + |
| Hernia | 9% | - | - | - |
| Loss of visual acuity | 6% | - | - | - |

[a]: Patients marked with the same superscript letter are siblings.

to our knowledge has not been previously documented in the literature. It is considered as a null variant in a gene where loss of function is recognized as a mechanism of the disease. Additionally, it has an extremely low frequency in gnomAD population databases, further supporting its potential clinical significance. It was classified as "pathogenic" based on recommendations of ACGM 2015 criteria with the evidence codes PVS1, PM2, PP4.[13] Due to the autosomal recessive inheritance of the disease, genetic counseling was provided to the family and parental segregation analysis is planned.

### *Patient 3*

A 3-year-old Turkish female patient previously followed up with a diagnosis of HMG-CoA lyase deficiency (OMIM #246450) at another hospital, was referred to us with complaints of macrocephaly, coarse face, hearing loss and severe intellectual disability. There were no specific features in the perinatal history. The parents were first-degree cousins and one of their previous children had died at age 2-year-and 6-month due to HMG-CoA lyase deficiency. The patient achieved head control at 4 months, sat with support at 8 months and was able to stand with support at 12 months, with no meaningful words, however she lost motor and cognitive skills following a febrile infection at 12 months. Physical examination revealed normal growth parameters, coarse facies, macrocephaly, pectus carinatum, absent head control and speech, spasticity and pes cavus in the lower extremities. She used a hearing aid. Ophthalmologic examination was normal (Table I). Tandem mass spectrometry revealed significantly elevated C6DC levels and urine organic acid analysis showed marked excretion of 3-methylglutaric acid, 3-methylglutaconic acid, and 3-hydroxy-3-methylglutaric acid, consistent with the diagnosis of HMG-CoA lyase deficiency. Brain MRI showed thinning of the corpus callosum, signal increase in bilateral frontoparietotemporal subcortical areas, all deep white matter, internal and external capsules on T2-weighted images. Although treatment was initiated early and the patient did not experience an acute attack, she developed severe developmental delay, hearing loss, coarse facial features, and macrocephaly which were unexpected findings. Because PCR amplification of exons 1-6 of the *HMGCL* gene was absent, chromosomal microarray analysis was performed to investigate the presence of a deletion. Chromosomal microarray analysis revealed the homozygous 64.5 kb deletion arr[GRCh37] 1p36.11 (24137225_24201779) x0 in the patient, which includes exons 1-6 of the *HMGCL* (NM_000191) gene, the whole *FUCA1* (NM_000147) gene, and exon 2 of the *CNR2* (NM_001841) gene (Fig. 1A). Loss-of-function mutations in both *HMGCL* and *FUCA1* are classified as pathogenic and are related to HMG-CoA lyase deficiency, and fucosidosis, respectively. Due to its complete overlap with established haploinsufficient / loss of function-sensitive genes that are exclusively associated with recessive conditions, it is classified as pathogenic according to the ACMG (2020) criteria. Segregation analysis was performed on the parents, and both were found to be heterozygous for the same deletion (Fig. 1B and Fig. 1C). Notably, to the best of our knowledge, the literature does not report any cases of simultaneous homozygous deletion of both the *HMGCL* and *FUCA1* genes. Unfortunately, due to the patient's passing from an infection urine oligosaccharide analysis and alpha-L-fucosidase enzyme level were not evaluated. In this patient, we considered the coexistence of HMG-CoA lyase deficiency and fucosidosis as the primary contributors to the clinical findings.

Written informed consents were obtained from the parents for the collection and publication of the patients' clinical information.

## Discussion

The first case of fucosidosis was described by Durand et al. in 1966.[1] To date, fewer than 150 cases and less than 50 different pathogenic variants in the *FUCA1* gene have been reported worldwide (the HGMD database professional

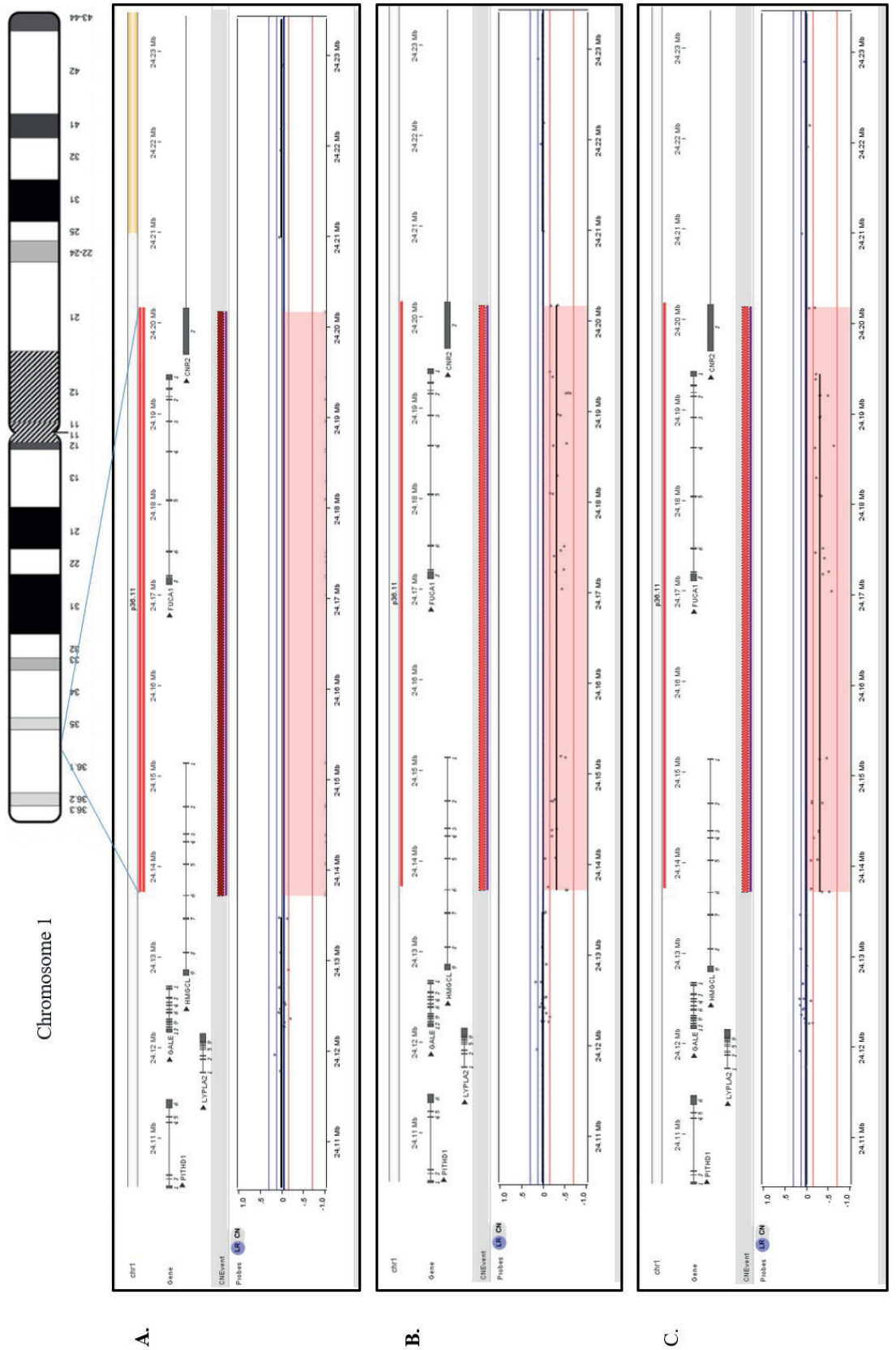



**Fig. 1.** Cytogenetic microarray results representing the 1p36.11 microdeletion of 64.5 kb arr[GRCh37]1p36.11(24137225_24201779)x0, including *HMGCL*, *FUCA1*, and *CNR2* genes in the patient (A). The same deletion in the heterozygous form in both the mother (B) and father (C).

2024.3, www.hgmd.cf.ac.uk; accessed 13 November 2024). Genotype-phenotype correlations are not well defined.[4-8] A wide range of severity can occur within the same family. However, as a rule, patients who are homozygous for null alleles such as the gross deletions or premature stop codon variants are expected to have loss of enzyme activity and a more severe phenotype. The majority of variants are intragenic small deletions/insertions (INDELs), nonsense and splice site variants, with fewer missense variants, all of which can be identified through sequence analysis. Previously, seven exonic deletions were reported in the *FUCA1* gene. However, loss of both of *FUCA1* and *HMGCL* genes has not been reported in the literature to date. The pathogenic variants detected in both families were novel and consistent with fucosidosis type 1 (severe form).

Importantly, the most striking feature of Patient 3 was the co-occurrence of two distinct inborn errors of metabolism—fucosidosis and HMG-CoA lyase deficiency—resulting from a contiguous gene deletion. While the co-existence of two Mendelian disorders in a single patient is not entirely unprecedented, what makes this case unique is the underlying genomic mechanism. Contiguous gene deletion syndromes in inherited metabolic disorders are exceedingly rare. Well-documented examples include glycerol kinase deficiency combined with Duchenne muscular dystrophy, and hypotonia-cystinuria syndrome, as well as various mitochondrial DNA deletions. To our knowledge, this is the first report of a contiguous gene deletion encompassing both *FUCA1* and *HMGCL*, leading to the simultaneous manifestation of two metabolic disorders. This finding underscores the importance of considering large-scale genomic rearrangements in patients with atypical or overlapping metabolic phenotypes, and it expands the spectrum of known contiguous gene deletion syndromes in the field of inborn errors of metabolism.

HMG-CoA lyase deficiency is an autosomal recessive disorder caused by biallelic pathogenic variants in *HMGCL*. HMG-CoA lyase deficiency typically presents with recurrent episodes of encephalopathy including hypoglycemia, metabolic acidosis, vomiting and a reduced level of consciousness. Most patients present in the first year of life. Neurological problems are common, particularly in neonatal-onset cases. A moderate protein or leucine restriction, low fat diet and carnitine supplements are usually preferred in the management. The genotypes poorly correlate with the clinical phenotype.[15] Metabolic investigations in the third patient were consistent with HMG-CoA lyase deficiency.

Fucosidosis should be considered in the differential diagnosis of patients with progressive neurologic deterioration. All three patients had severe developmental delay with neuromotor regression beginning at one year of age. Coarse facies, hearing loss and dysostosis multiplex were present in all of the patients. Hepatosplenomegaly and angiokeratoma were not found in any of the patients. Willems et al. reported visceromegaly and angiokeratoma in 30% and 52% of patients, respectively.[3]

In Patient 3, HMG-CoA lyase deficiency was diagnosed at the early neonatal period as the first diagnosis by selective screening due to an affected sibling history after which early treatment was started. Although she did not have metabolic attacks, her developmental milestones were significantly delayed. In addition, she had coarse facies, macrocephaly, hearing loss and dysostosis multiplex inconsistent with HMG-CoA lyase deficiency. Genetic studies were performed both to confirm HMG-CoA lyase deficiency and to investigate the possible second genetic disorder. Two inborn errors of metabolism including HMG-CoA lyase deficiency and fucosidosis were identified by a chromosomal microarray analysis. Therefore, comprehensive genetic and metabolic testing should be pursued in cases where clinical findings cannot be explained by a single disorder.

One of the key limitations of this study is the inability to perform enzymatic analysis and urinary oligosaccharide testing for Patient 3, as the patient was deceased. However, the clinical findings including macrocephaly, coarse facial features, and hearing loss strongly support the diagnosis of fucosidosis. Genetic analysis confirmed the diagnosis by identifying a homozygous deletion in the patient, with both parents being heterozygous carriers. The *CNR2* gene has not been associated with any Online Mendelian Inheritance in Man (OMIM) phenotype to date. The potential contribution of the *CNR2* deletion to a clinical entity with overlapping features cannot be entirely ruled out. However, this hypothesis requires further functional studies and additional experiments. Both families have received counseling regarding prenatal diagnostic options and the importance of genetic testing in future pregnancies.

In conclusion, angiokeratoma and hepatosplenomegaly may not be consistent findings in fucosidosis. When unexpected new findings emerge, the possibility of a second disease should be considered and additional diagnostic tests should be performed. To our knowledge, this is the first reported case in the literature of fucosidosis accompanied by HMG-CoA lyase deficiency resulting from a contiguous gene deletion, with merged phenotypes of both disorders.

## Ethical approval

A signed informed consent form was obtained from each participant's parents for the publication of this report.

## Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: MK, HY; data collection: MK, HY; analysis and interpretation of results: HY, FD, MK; draft manuscript preparation: MK, HY, FD. All authors reviewed the results and approved the final version of the manuscript.

## Source of funding

The authors declare the study received no funding.

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES

1. Durand P, Borrone C, Della Cella G. A new mucopolysaccharide lipid storage disease? Lancet 1966; 288: 1313-1314. https://doi.org/10.1016/S0140-6736(66)91718-1
2. Simon J, Frits AW. Glycosaminoglycans and oligosaccharides disorders: glycosaminoglycans synthesis defects, mucopolysaccharidoses, oligosaccharidoses and sialic acid disorders. In: Saudubray JM, Baumgartner MR, Ángeles García-Cazorla, Walter JH, editors. Inborn Metabolic Diseases Diagnosis and Treatment. 7th ed. Germany: Springer Nature; 2022: 765-783. https://doi.org/10.1007/978-3-662-63123-2_41
3. Willems PJ, Gatti R, Darby JK, et al. Fucosidosis revisited: a review of 77 patients. Am J Med Genet 1991; 38: 111-131. https://doi.org/10.1002/ajmg.1320380125
4. Stepien KM, Ciara E, Jezela-Stanek A. Fucosidosis-clinical manifestation, long-term outcomes, and genetic profile-review and case series. Genes (Basel) 2020; 11: 1383. https://doi.org/10.3390/genes11111383
5. Willems PJ, Seo HC, Coucke P, Tonlorenzi R, O'Brien JS. Spectrum of mutations in fucosidosis. Eur J Hum Genet 1999; 7: 60-67. https://doi.org/10.1038/sj.ejhg.5200272
6. Gowda VK, Srinivasan VM, Vegda H, Bhat M. Fucosidosis with pathogenic variant in FUCA1 gene. Indian J Pediatr 2020; 87: 867-868. https://doi.org/10.1007/s12098-020-03246-7
7. do Rosario MC, Purushothama G, Narayanan DL, Siddiqui S, Girisha KM, Shukla A. Extended analysis of exome sequencing data reveals a novel homozygous deletion of exons 3 and 4 in FUCA1 gene causing fucosidosis in an Indian family. Clin Dysmorphol 2023; 32: 112-115. https://doi.org/10.1097/MCD.0000000000000452
8. Tiberio G, Filocamo M, Gatti R, Durand P. Mutations in fucosidosis gene: a review. Acta Genet Med Gemellol (Roma) 1995; 44: 223-232. https://doi.org/10.1017/s0001566000001641

9. Galluzzi P, Rufa A, Balestri P, Cerase A, Federico A. MR brain imaging of fucosidosis type I. AJNR Am J Neuroradiol 2001; 22: 777-780.

10. Autti T, Joensuu R, Aberg L. Decreased T2 signal in the thalami may be a sign of lysosomal storage disease. Neuroradiology 2007; 49: 571-578. https://doi.org/10.1007/s00234-007-0220-6

11. Malatt C, Koning JL, Naheedy J. Skeletal and brain abnormalities in fucosidosis, a rare lysosomal storage disorder. J Radiol Case Rep 2015; 9: 30-38. https://doi.org/10.3941/jrcr.v9i5.2149

12. Miano M, Lanino E, Gatti R, et al. Four year follow-up of a case of fucosidosis treated with unrelated donor bone marrow transplantation. Bone Marrow Transplant 2001; 27: 747-751. https://doi.org/10.1038/sj.bmt.1702994

13. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015; 17: 405-424. https://doi.org/10.1038/gim.2015.30

14. Riggs ER, Andersen EF, Cherry AM, et al. Correction: Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). Genet Med 2021; 23: 2230. https://doi.org/10.1038/s41436-021-01150-9

15. Morris AAM. Disorders of ketogenesis and ketolysis. In: Saudubray JM, Baumgartner MR, Ángeles García-Cazorla, Walter JH, editors. Inborn Metabolic Diseases Diagnosis and Treatment. 7th ed. Germany: Springer Nature; 2022: 303-313. https://doi.org/10.1007/978-3-662-63123-2_13

The Turkish Journal of Pediatrics 2025; 67: 904-911
https://doi.org/10.24953/turkjpediatr.2025.6313

Case Report

# Hyperuricemia and elevated creatinine in a child with anemia

Emre Leventoğlu[1], Ayşe Şimşek[2], Hayriye Nermin Keçeci[3]

*[1]Department of Pediatric Nephrology, Konya City Hospital, Konya, Türkiye; [2]Department of Pediatric Hematology, Konya City Hospital, Konya, Türkiye; [3]Department of Pediatric Genetics, Konya City Hospital, Konya, Türkiye.*

## ABSTRACT

**Background.** Anemia in infancy is a frequent clinical challenge, often attributed to nutritional deficiencies. However, persistent or unexplained anemia warrants further investigation. In this case report, we describe an infant in whom anemia was first identified at six months of age; however, the underlying etiology became apparent only after the development of elevated serum creatinine and hyperuricemia.

**Case Presentation.** We present a 1.7-year-old boy with persistent normocytic anemia, hyperuricemia, and elevated creatinine, initially evaluated for hematologic causes without a clear diagnosis. Despite normal growth, laboratory findings revealed hypouricosuria, hyposthenuria, and mildly decreased kidney function. Imaging and immunologic work-up were unremarkable. Due to multisystem involvement, genetic testing was performed and identified a heterozygous variant in *REN* gene, suggesting a potential link to autosomal dominant tubulointerstitial kidney disease (ADTKD). Due to persistent anemia refractory to iron therapy, erythropoietin was initiated at a dose of 0.50 μg/kg/week, resulting in a 1.9 g/dL increase in hemoglobin after one month. The family was appropriately informed about the chronic nature of the kidney disease, and a lifelong follow-up strategy was established.

**Conclusion.** This case underscores the importance of considering ADTKD in pediatric patients presenting with unexplained anemia and mild kidney impairment, even in the absence of a family history. Early diagnosis can prevent unnecessary procedures such as kidney biopsy, allow the timely initiation of supportive treatments, and improve long-term outcomes. Pediatricians, pediatric hematologists and pediatric nephrologists should be aware of this diagnostic possibility, particularly when anemia is accompanied by hyperuricemia and elevated creatinine in infancy.

**Key words:** anemia, glomerular filtration rate, *REN* gene, renin-angiotensin system.

Anemia is a condition characterized by a deficiency of healthy red blood cells (RBCs) in the body and is among the most common hematological conditions in children, affecting more than 269 million children worldwide.[1] According to the World Health Organization, the prevalence of anemia in children under five years of age was approximately 40% in 2019.[2] The most common etiological cause in this age group is iron deficiency due to inadequate intake, which accounts for 25–50% of cases.[3] In addition, hemoglobinopathies, particularly sickle cell disease and thalassemia, constitute clinically significant causes, with a reported prevalence ranging from 6% to 9%.[4] Moreover, deficiencies of vitamins such as vitamin B12 and folate, infectious diseases including malaria and schistosomiasis, chronic blood loss, inflammatory conditions or chronic kidney diseases (CKD) represent important etiological

✉ Emre Leventoğlu ▪ dremrelevent@gmail.com

Received 16th May 2025, revised 8th Sep 2025, 3rd Nov 2025, accepted 4th Nov 2025.

factors for anemia in childhood.[4] During infancy, clinical signs suggestive of CKD include feeding difficulties, failure to thrive, developmental delay, polyuria, polydipsia, recurrent vomiting or episodes of dehydration, electrolyte disturbances and hypertension. Regarding anemia, it usually appears in children with CKD stage 3 and becomes more common and clinically significant in advanced stages.[5,6] Whatever the cause, it can lead to a reduced oxygen-carrying capacity of cells, causing clinical symptoms of anemia in infants such as fatigue, pallor, poor growth and failure to thrive.[7]

In rare types of anemia that do not fit previously described etiological categories, a group of patients remain undiagnosed despite comprehensive diagnostic evaluations. However, advances in targeted next-generation sequencing have facilitated the identification of the underlying genetic cause in more than 60% of such cases.[8] In this case report, we describe an infant in whom anemia was first identified at six months of age; however, the underlying etiology was identified through genetic analysis only after the development of elevated serum creatinine and hyperuricemia.

**Case Presentation**

A 1.7-year-old boy with anemia was referred to the department of pediatric nephrology for hyperuricemia and elevated creatinine. He was born full term from an uneventful pregnancy from unrelated parents. There was no family history of hematological or nephrological diseases. His birth weight was 3.4 kg at 39 weeks of gestation. There was no history of jaundice in the neonatal period. He was breastfed for the first 6 months of his life, received oral iron prophylaxis. There was no infection or symptoms of fever, rash, dysuria, hematuria, vomiting or bloody stools in his medical history, however, normocytic anemia was noticed when he presented with fatigue, pallor and poor growth at the age of 6 months.

At the age of 6 months, white blood cell (WBC) count was 9870/μL, hemoglobin (Hb) 7.1 g/dL, mean corpuscular volume (MCV) 90.2 fL, thrombocyte count 378000 /μL, and reticulocyte ratio 1.2%. In the peripheral smear, erythrocytes had normochromic normocytic appearance. There were no blasts; platelets were abundant and clustered. Serum iron level was slightly low (28 μg/dL), but total iron binding capacity, ferritin, folate and vitamin B12 levels were normal. Direct Coombs test was negative and haptoglobin was normal. Fecal occult blood was negative. In serum biochemistry, there was no elevated lactate dehydrogenase or hyperbilirubinemia, however, serum creatinine (0.71 mg/dL; normal range: 0.15-0.34 mg/dL)[9] and uric acid levels (6.9 mg/dL normal range: 2.7-4.5 mg/dL)[10]were high. Hemoglobin high performance liquid chromatography (HPLC), osmotic fragility test, glucose-6-phosphate dehydrogenase, pyruvate kinase, and 5′ nucleotidase levels were normal. Bone marrow aspiration was normocellular, normoblastic erythropoiesis was present, and no dysplasia was observed in normoblasts. The etiology of anemia could not be elucidated, and the patient was followed up with 2 mg/kg elemental iron daily.

At the time of referral to pediatric nephrology, physical examination showed normal growth with a body weight of 10 kg (10-25th percentiles) and a height of 79 cm (10-25th percentiles). His blood pressure was 80/50 mmHg (<90$^{th}$ percentile). Skin thickness and turgor were normal but markedly pale. Liver or spleen was not palpable. He had anemia (Hb 7.7 g/dL, MCV 85.5 fL, RBC 2.75 x10$^6$/μL, RDW 13.3%), but WBC and platelet count were normal. Kidney function tests showed elevated serum creatinine and reduced estimated glomerular filtration rate (eGFR) (0.83 mg/dL and 39 mL/min/1.73m$^2$, respectively). He had hyperuricemia (8.8 mg/dL). Serum electrolyte and albumin levels were normal. Blood gas analysis showed pH 7.33 and $HCO^3$ 19.2 mmol/L with negative base excess and normal anion gap. Urinalysis showed a pH of 6.5, specific gravity of 1002, and did not

show hematuria/proteinuria or glycosuria. Spot urine uric acid was 5.5 mg/dL (normal range 37-97 mg/dL) and fractional excretion of uric acid was as low as 4.59%.[11] Urine output was 4.4 mL/kg/hr. In the immunologic evaluation, serum complement levels were normal. Antinuclear antibodies, anti-neutrophil cytoplasmic antibodies and anti-double-stranded DNA were all negative (Table I). Ultrasonography showed normal-sized kidneys with normal parenchymal echogenicity. Renal Doppler ultrasound was normal. The genetic examination was performed due to persistent anemia, elevated creatinine, hypouricosuric hyperuricemia and hyposthenuria. Targeted next-generation sequencing revealed a heterozygous c.38T>C (p.Leu13Pro) variant of uncertain significance (VUS) in *REN* gene. The *REN* gene encodes the renin protein, and the variant was inherited in an autosomal dominant manner. Segregation analysis revealed that the variant was not present in either parent, indicating that the identified *REN* variant arose *de novo*. In analyses evaluating the clinical concordance of the identified variant, the patient's plasma renin activity was 1.1 ng/mL/h (normal range 2.9 to 24 ng/mL/h) with a low serum aldosterone level. Also, erythropoietin level was 2.9 mU/mL (normal range: 4-21 mU/mL). Therefore, the patient was diagnosed with autosomal dominant tubulointerstitial kidney disease (ADTKD).[12]

As the patient had not yet developed significant hypotension or hyperkalemia, a high-sodium diet or fludrocortisone therapy was not initiated at this stage. However, allopurinol and oral sodium bicarbonate therapies were initiated. Due to persistent anemia refractory to iron therapy, erythropoietin was initiated at a dose of 0.50 μg/kg/week, resulting in a 1.9 g/dL increase in hemoglobin after one month. The patient's clinical features and laboratory values at the time of initial referral to pediatric nephrology and at the end of the 9-month follow-up period are shown in Table I.

Written informed consent was obtained from parents for the use of clinical and laboratory data for this publication.

## Discussion

Autosomal dominant tubulointerstitial kidney disease is characterized by a bland urinary sediment and a gradual progression of CKD and eventually necessitates kidney replacement therapy. The most common causes of ADTKD include mutations in *UMOD*, *MUC1*, and *REN* genes.[12] In a study by Živná M et al., pathogenic variants were identified in the *UMOD* gene in 38% of cases, in the *MUC1* gene in 21%, and in the *REN* gene in only 3% of cases.[13] Pathogenic mutations in the *REN* gene lead to a reduction in the production and secretion of both wild-type prorenin and renin. Therefore, all affected patients exhibit low plasma renin and aldosterone levels.[14]

Fatigue, refusal to feed, and failure to thrive can be seen even in the infantile period in ADTKD caused by *REN* mutation. Early onset anemia, increased serum creatinine, decreased eGFR, hyperuricemia, mild hyperkalemia and acidosis are usually present.[15] Most patients develop kidney failure after the age of 35.[14] However, due to the rarity of the disease, the age of diagnosis may be delayed up to 57 years despite symptoms and signs at an early age.[16] Consistent with previously reported cases of ADTKD, our patient also exhibited hallmark features including anemia, hyperuricemia, and elevated serum creatinine. Although the diagnosis might appear somewhat delayed by a few months, it was established at the remarkably early age of 1.7 years, highlighting the potential for early detection when clinical and laboratory findings are carefully integrated and evaluated in the context of ADTKD. A non-specific kidney biopsy may also be performed in the diagnosis of ADTKD.[17] However, we believe that genetic analysis should be prioritized before considering interventional procedures such as kidney biopsy, particularly when there is a family history of CKD consistent with an

**Table I.** Clinical characteristics and laboratory results of the patient

| | At the time of referral to pediatric nephrology | At latest follow-up | Reference value |
|---|---|---|---|
| Age (year) | 1.7 | 2.4 | |
| Anthropometric measurements | | | |
| Height, cm (percentile) | 79 (10-25$^{th}$ p) | 87 (10-25$^{th}$ p) | |
| Weight, kg (percentile) | 10 (10-25$^{th}$ p) | 12.5 (25-50$^{th}$ p) | |
| Blood pressure | | | |
| SBP, mmHg (percentile) | 80 (<90$^{th}$ p) | 88 (<90$^{th}$ p) | |
| DBP, mmHg (percentile) | 50 (<90$^{th}$ p) | 49 (<90$^{th}$ p) | |
| Blood | | | |
| Hemoglobulin (g/dL) | 7.7 | 11.1 | 11-15.1 |
| MCV (fL) | 85.5 | 81.2 | 75-85 |
| RBC ($10^6$/μL) | 2.75 | 3.65 | 3.9-5.3 |
| RDW (%) | 13.3 | 12.8 | 11.5-14.5 |
| White blood cell (/μL) | 8540 | 9110 | 4000-10000 |
| Platelets (/μL) | 244000 | 315000 | 150000-400000 |
| Creatinine (mg/dL) | 0.83 | 0.68 | 0.15-0.34 |
| eGFR (mL/min/1.73m$^2$) | 39 | 53 | 90-120 |
| Uric acid (mg/dL) | 8.8 | 4.4 | 2.7-4.5 |
| Albumin (g/dL) | 4.2 | 4.2 | 3.5-5.2 |
| Calcium (mg/dL) | 8.6 | 8.8 | 8.4-10.2 |
| Phosphorus (mg/dL) | 4.2 | 4.3 | 2.3-4.5 |
| Magnesium (mg/dL) | 2.1 | 2.2 | 1.2-2.5 |
| Sodium (mmol/L) | 138 | 139 | 136-146 |
| Chloride (mmol/L) | 106 | 105 | 90-110 |
| Potassium (mmol/L) | 4.9 | 4.8 | 3.5-5.1 |
| pH | 7.33 | 7.39 | 7.35-7.45 |
| $HCO_3$ (mmol/L) | 19.2 | 24.1 | 22-28 |
| Base excess (mmol/L) | -5.8 | -0.4 | (-2) – (+2) |
| Anion gap (mEq/L) | 12.8 | 9.9 | 8-16 |
| C3 (mg/dL) | 112 | N/A | 79-152 |
| C4 (mg/dL) | 28.4 | N/A | 16-38 |
| ANA | Negative | N/A | Negative |
| ANCA | Negative | N/A | Negative |
| Anti-ds DNA | Negative | N/A | Negative |
| Plasma renin activity (ng/mL/h) | 1.1 | N/A | 2.9-24 |
| Aldosterone (ng/dL) | <4.0 | N/A | <36 |
| Erythropoietin (mU/mL) | 2.9 | N/A | 4-21 |
| Urine | | | |
| pH | 6.5 | 7.5 | 5.0-7.5 |
| Density | 1002 | 1020 | 1005-1030 |
| Protein/creatinine (mg/mg) | 0.19 | 0.11 | <0.2 |
| Leucocyte (/HPF) | 1 | 0 | <5 |
| Erythrocyte (/HPF) | 1 | 0 | <5 |
| Uric acid (mg/dL) | 5.5 | 10.1 | 37-97 |
| FeUA (%) | 4.59 | 6.77 | >10 |

ANA: anti-nuclear antibody, ANCA: anti-neutrophilic cytoplasmic antibody, Anti-dsDNA: anti-double stranded deoxyribonucleic acid, C3: Complement 3, C4: Complement 4, DBP: Diastolic blood pressure, eGFR: Estimated glomerular filtration rate, FeUA: Fractional excretion of uric acid, MCV: Mean corpuscular volume, N/A: not available, RBC: Red blood cell, RDW: Red cell distribution width, SBP: Systolic blood pressure.

autosomal dominant inheritance pattern. In our patient, the symptoms and findings were highly consistent with ADTKD, however there was no history of kidney disease in his family. This case highlights that when clinical and laboratory findings are highly consistent with our preliminary diagnosis, genetic testing should be performed appropriately, even in the absence of a family history.

The renin-angiotensin-aldosterone system plays a critical role in the diagnosis and pathophysiology of CKD. It regulates glomerular filtration pressure and fluid-electrolyte balance in the kidneys. Renin secreted by juxtaglomerular cells converts angiotensinogen to angiotensin I. Angiotensin II, a potent vasoconstrictor that is subsequently formed, stimulates aldosterone secretion. As a result, glomerular pressure increases, and sodium retention and potassium excretion occur in the distal tubules. However, renin levels do not always increase in CKD; they may increase in the early stages, decrease in the advanced stages, or be characteristically low in genetic disorders. Therefore, measuring renin-angiotensin-aldosterone activity or monitoring its effects is important in CKD for both diagnosis and prognosis.[18] In *REN* mutation, the decrease in eGFR may be due to insufficient renin production during embryonic development and less kidney mass than it should be. Decreased renin levels disrupt local renin-angiotensin-aldosterone activity and glomerular hemodynamic regulation. Insufficient angiotensin II cannot balance afferent and efferent arteriolar tone, leading to decreased filtration pressure, hypoperfusion, and eventual ischemia. Therefore, scar tissue forms, eGFR declines, and CKD progresses. The *REN* mutation also impairs sodium reabsorption in the distal tubule and collecting ducts, inhibiting potassium and hydrogen ion excretion, leading to hyperkalemia and non-anion gap metabolic acidosis in the long term. The stress and inflammation that develop due to intracellular ion imbalance increase interstitial fibrosis and structural damage. In addition, uric acid excretion from the kidney decreases, and serum uric acid levels rise. This accelerates oxidative stress and fibrosis, deepens tubulointerstitial damage, and causes CKD progression.[14] In ADTKD, patients often present with polyuria and accompanying polydipsia due to impaired sodium reabsorption in the kidney and tubular-interstitial damage, increasing fluid loss. The presence of hyposthenuria and polyuria in our patient confirms a reduced capacity of the kidney to concentrate urine.[19] The cause of anemia in these patients is also due to decreased renin production, which leads to decreased erythropoietin levels.[10] The renin-angiotensin system is involved in erythropoiesis, mainly through angiotensin II, which can stimulate erythroid progenitor cells and increase erythropoietin production. Angiotensin II also directly interacts with hypoxia-inducible factor 1, which regulates erythropoiesis, further stimulating erythropoietin secretion. As a result, the hemoglobin levels are typically between 8 and 11 g/dL in the case of the *REN* mutation.[20]

Early diagnosis of ADTKD caused by *REN* mutations is crucial for implementing targeted interventions that may prevent or delay the progression of CKD. Controlling hyperkalemia, hyperuricemia, metabolic acidosis, correcting anemia, and carefully monitoring kidney function can slow disease progression and preserve kidney function in the long term.[21] In our patient, the diagnosis was made at an early age of 1.7 years. Even after a follow-up period of 9 months, appropriate management has already resulted in improved kidney function, demonstrating the benefits of early and targeted intervention. Unlike most other forms of CKD, individuals with *REN* gene mutations should not be put on a low-sodium diet. Increased sodium intake helps to improve blood pressure and lower serum potassium levels. In addition, fludrocortisone can alleviate the clinical effects of hypoaldosteronism, such as hyperkalemia and acidemia.[22] Anemia responded well to erythropoietin treatment, not to iron therapy.[23] Although hyperuricemia begins in the infantile period, it can occur in adolescence in untreated

cases of gout, which can be easily prevented by daily allopurinol administration.[20,22] Since our patient had not yet developed marked hypotension or hyperkalemia, a high-sodium diet or fludrocortisone therapy was not initiated at this stage. Allopurinol, oral sodium bicarbonate and erythropoietin therapies were initiated after the diagnosis. The family was appropriately informed about the chronic nature of the kidney disease, and a lifelong follow-up strategy was established.

This case report has several limitations. The most important of these is that the *REN* variant identified in the genetic analysis is classified as a VUS. Its pathogenicity has not been definitively established, and there are no functional studies confirming its clinical effect. According to gnomAD, this variant is ultra-rare, with a total allele frequency of 0.00025%, and no homozygous cases have been reported.[24] The detailed clinical and laboratory data of this young patient, who is highly consistent with ADTKD, provide valuable information. The high concordance of the patient's clinical and laboratory features with ADTKD and the ultra-rare nature of the variant strongly suggest that this *REN* variant may have pathogenic potential. Furthermore, as the report describes a single patient, the findings may not be generalizable to all individuals with the *REN* mutation. Also, our patient was diagnosed at our center nine months ago and is still being followed; therefore, the relatively short follow-up period limits the assessment of the long-term progression of CKD.

In conclusion, this case underscores the importance of a multidisciplinary approach in the evaluation of infants presenting with persistent anemia and subtle signs of systemic involvement. The coexistence of anemia, hyperuricemia, and elevated serum creatinine—despite an initially unremarkable workup—highlights the multisystemic nature of certain hereditary kidney disorders. Early collaboration between pediatric hematology, nephrology, and genetics was instrumental in establishing the diagnosis, thereby avoiding unnecessary invasive procedures such as a kidney biopsy. Prompt recognition and genetic confirmation of conditions like ADTKD not only allow for targeted interventions—including erythropoietin therapy and uric acid–lowering agents—but also enable appropriate family counseling and long-term disease monitoring. This case illustrates how early and coordinated evaluation across specialties can lead to timely diagnosis, improved clinical outcomes, and a better understanding of disease progression in complex pediatric presentations.

## Ethical approval

Informed consent was obtained from the parents of the patient included in the study for the use of patient data for scientific and academic purposes, provided that the identity information of the patients remained confidential.

## Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: EL, AŞ, HNK; data collection: EL, AŞ; analysis and interpretation of results: EL, AŞ, HNK; draft manuscript preparation: EL, AŞ. All authors reviewed the results and approved the final version of the manuscript.

## Source of funding

The authors declare the study received no funding.

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES

1. Raleigh MF, Yano AS, Shaffer NE. Anemia in infants and children: evaluation and treatment. Am Fam Physician 2024; 110: 612-620.

2. World Health Organization Regional Office for the Eastern Mediterranean. Monitoring health and health system performance in the Eastern Mediterranean Region: core indicators and indicators on the health-related sustainable development goals 2019. Cairo, Egypt: World Health Organization; 2020. Available at: https://applications.emro.who.int/docs/EMHST245E.pdf

3. Gedfie S, Getawa S, Melku M. Prevalence and associated factors of iron deficiency and iron deficiency anemia among under-5 children: a systematic review and meta-analysis. Glob Pediatr Health 2022; 9. https://doi.org/10.1177/2333794X221110860

4. Liu Y, Ren W, Wang S, Xiang M, Zhang S, Zhang F. Global burden of anemia and cause among children under five years 1990-2019: findings from the global burden of disease study 2019. Front Nutr 2024; 11: 1474664. https://doi.org/10.3389/fnut.2024.1474664

5. Libudzic-Nowak AM, Cachat F, Pascual M, Chehade H. Darbepoetin alfa in young infants with renal failure: single center experience, a case series and review of the literature. Front Pediatr 2018; 6: 398. https://doi.org/10.3389/fped.2018.00398

6. Endrias EE, Geta T, Israel E, Belayneh Yayeh M, Ahmed B, Moloro AH. Prevalence and determinants of anemia in chronic kidney disease patients in Ethiopia: a systematic review and meta-analysis. Front Med (Lausanne) 2025; 12: 1529280. https://doi.org/10.3389/fmed.2025.1529280

7. García Díaz FJ, Moreno Ortega M, Beth Martín L, Delgado Pecellín I. Severe anemia in infants: don't miss the clues. Clin Pediatr (Phila) 2023; 62: 1449-1451. https://doi.org/10.1177/00099228231160673

8. Shefer Averbuch N, Steinberg-Shemer O, Dgany O, et al. Targeted next generation sequencing for the diagnosis of patients with rare congenital anemias. Eur J Haematol 2018; 101: 297-304. https://doi.org/10.1111/ejh.13097

9. Boer DP, de Rijke YB, Hop WC, Cransberg K, Dorresteijn EM. Reference values for serum creatinine in children younger than 1 year of age. Pediatr Nephrol 2010; 25: 2107-2113. https://doi.org/10.1007/s00467-010-1533-y

10. Wilcox WD. Abnormal serum uric acid levels in children. J Pediatr 1996; 128: 731-741. https://doi.org/10.1016/s0022-3476(96)70322-0

11. Farid S, Latif H, Nilubol C, Kim C. Immune checkpoint inhibitor-induced fanconi syndrome. Cureus 2020; 12: e7686. https://doi.org/10.7759/cureus.7686

12. Devuyst O, Olinger E, Weber S, et al. Autosomal dominant tubulointerstitial kidney disease. Nat Rev Dis Primers 2019; 5: 60. https://doi.org/10.1038/s41572-019-0109-9

13. Živná M, Kidd KO, Barešová V, Hůlková H, Kmoch S, Bleyer AJ. Autosomal dominant tubulointerstitial kidney disease: a review. Am J Med Genet C Semin Med Genet 2022; 190: 309-324. https://doi.org/10.1002/ajmg.c.32008

14. Živná M, Kidd K, Zaidan M, et al. An international cohort study of autosomal dominant tubulointerstitial kidney disease due to REN mutations identifies distinct clinical subtypes. Kidney Int 2020; 98: 1589-1604. https://doi.org/10.1016/j.kint.2020.06.041

15. Schaeffer C, Olinger E. Clinical and genetic spectra of kidney disease caused by REN mutations. Kidney Int 2020; 98: 1397-1400. https://doi.org/10.1016/j.kint.2020.08.013

16. Clissold RL, Clarke HC, Spasic-Boskovic O, et al. Discovery of a novel dominant mutation in the REN gene after forty years of renal disease: a case report. BMC Nephrol 2017; 18: 234. https://doi.org/10.1186/s12882-017-0631-5

17. Ma J, Hu Z, Liu Q, Li J, Li J. Case report: a potentially pathogenic new variant of the REN gene found in a family experiencing autosomal dominant tubulointerstitial kidney disease. Front Pediatr 2024; 12: 1415064. https://doi.org/10.3389/fped.2024.1415064

18. Cianciolo G, Provenzano M, Hu L, et al. RAASi, MRA and FGF-23 in CKD progression: the usual suspects? Minerva Urol Nephrol 2025; 1-12. https://doi.org/10.23736/S2724-6051.25.06082-3

19. Schaeffer C, Izzi C, Vettori A, et al. Autosomal dominant tubulointerstitial kidney disease with adult onset due to a novel renin mutation mapping in the mature protein. Sci Rep 2019; 9: 11601. https://doi.org/10.1038/s41598-019-48014-6

20. Zivná M, Hůlková H, Matignon M, et al. Dominant renin gene mutations associated with early-onset hyperuricemia, anemia, and chronic kidney failure. Am J Hum Genet 2009; 85: 204-213. https://doi.org/10.1016/j.ajhg.2009.07.010

21. Econimo L, Schaeffer C, Zeni L, et al. Autosomal dominant tubulointerstitial kidney disease: an emerging cause of genetic CKD. Kidney Int Rep 2022; 7: 2332-2344. https://doi.org/10.1016/j.ekir.2022.08.012

22. Bleyer AJ, Zivná M, Hulková H, et al. Clinical and molecular characterization of a family with a dominant renin gene mutation and response to treatment with fludrocortisone. Clin Nephrol 2010; 74: 411-422. https://doi.org/10.5414/cnp74411

23. Beck BB, Trachtman H, Gitman M, et al. Autosomal dominant mutation in the signal peptide of renin in a kindred with anemia, hyperuricemia, and CKD. Am J Kidney Dis 2011; 58: 821-825. https://doi.org/10.1053/j.ajkd.2011.06.029

24. Global Core Biodata Resource. Genome aggregation database (gnomAD™). SNV:1-204154978-A-G(GRCh38). Available at: https://gnomad.broadinstitute.org/variant/1-204154978-A-G?dataset=gnomad_r4 (Accessed on Sep 14, 2025).

The Turkish Journal of Pediatrics 2025; 67: 912-919
https://doi.org/10.24953/turkjpediatr.2025.6246

Case Report

# Hypomagnesemia as a primary clue for the diagnosis of 17q12 deletion syndrome associated with spinal syringomyelia: a case report

Yeşim Özdemir Atikel[1], Ayça Kocaağa[2], Kenan Delil[3], Duygu İskender Mazman[4], Meltem Didem Çakır[5], Sevgi Yimenicioğlu[6]

*[1]Department of Pediatric Nephrology, Eskişehir City Hospital, Health Practice and Research Center, University of Health Sciences, Eskişehir, Türkiye; [2]Department of Medical Genetics, Eskişehir City Hospital, Health Practice and Research Center, University of Health Sciences, Eskişehir, Türkiye; [3]Department of Medical Genetics, Ahenk Laboratory Genetic Diseases Evaluation Center, Türkiye; [4]Department of Pediatric Gastroenterology, Eskişehir City Hospital, Eskişehir, Türkiye; [5]Department of Pediatric Endocrinology, Eskişehir City Hospital, Eskişehir, Türkiye; [6]Department of Pediatric Neurology, Eskişehir City Hospital, Health Practice and Research Center, University of Health Sciences, Eskişehir, Türkiye.*

## ABSTRACT

**Background.** Inherited renal hypomagnesemia is rare but may indicate an underlying genetic condition, and it should be considered when evaluating unexplained hypomagnesemia. 17q12 deletion syndrome, a recurrent microdeletion including *HNF1B* (hepatocyte nuclear factor 1 beta) and neighboring genes such as *LHX1* (LIM homeobox 1) and *ACACA* (acetyl-CoA carboxylase alpha), is associated with renal magnesium wasting, neurodevelopmental deficits, and multi-organ involvement. However, spinal cord anomalies, particularly syringomyelia, have not been reported to date.

**Case Presentation.** A 12-year-old girl was referred to our pediatric nephrology department with frequent urination. Her medical history included neurodevelopmental delay, scoliosis, and behavioral abnormalities. Laboratory tests showed a serum magnesium level of 1.5 mg/dL and an elevated fractional urine magnesium excretion of 4.1%. Serum glucose, aspartate transaminase, and alanine transaminase were mildly elevated. There were no structural anomalies on urinary ultrasound and cranial magnetic resonance imaging (MRI). Radiological investigations revealed thoracolumbar scoliosis on spinal X-ray and a central syrinx extending from T3 to T6 levels on thoracic spinal MRI. Chromosomal microarray analysis identified a 1.4 Mb deletion at chromosome 17q12, which contains the *HNF1B* gene, confirming the diagnosis of 17q12 deletion syndrome. Oral magnesium supplementation was initiated, and the patient was referred to a multidisciplinary care team.

**Conclusions.** This case highlights the importance of considering genetic etiologies, particularly 17q12 deletion syndrome, in children presenting with persistent hypomagnesemia and neurodevelopmental delay. Recognizing electrolyte imbalances, despite the absence of renal structural abnormalities, and identifying coexisting spinal cord anomalies, such as syringomyelia, may guide timely genetic evaluation and enable earlier diagnosis.

**Key words:** 17q12 deletion, case report, *HNF1B*, hypomagnesemia, syringomyelia.

Hypomagnesemia is commonly observed in hospitalized patients, typically due to inadequate dietary intake or excessive gastrointestinal or renal losses. Although inherited forms of renal hypomagnesemia are rare, they are clinically significant and should be considered in cases of persistent and unexplained hypomagnesemia.[1] One such

✉ Yeşim Özdemir Atikel ▪ yesozdemir@gmail.com

Received 1st May 2025, revised 8th Sep 2025, accepted 11th Nov 2025.

genetic cause is 17q12 deletion syndrome, involving haploinsufficiency of the *HNF1B* (hepatocyte nuclear factor 1 beta) gene.[2]

Several mechanisms have been proposed to explain *HNF1B*-related renal magnesium wasting. *HNF1B* is known to regulate the expression of key distal convoluted tubule transporters and channels involved in magnesium reabsorption, including *TRPM6* (transient receptor potential melastatin 6), *FXYD2* (FXYD domain-containing ion transport regulator 2), and *KCNJ16* (potassium inwardly rectifying channel subfamily J member 16). Reduced transcriptional activity of these genes due to *HNF1B* haploinsufficiency impairs active magnesium transport, leading to renal magnesium loss and hypomagnesemia.[2]

Beyond *HNF1B*, the recurrent 17q12 microdeletion typically includes 15–20 protein-coding genes, such as *LHX1* (LIM homeobox 1), *ACACA* (acetyl-CoA carboxylase alpha), and *ZNHIT3* (zinc finger HIT-type containing 3). The combined loss of these neighboring genes may contribute to the neurodevelopmental and other extrarenal features of the syndrome.[2-4]

The clinical phenotype of 17q12 deletion syndrome is markedly heterogeneous. It may involve structural or functional abnormalities of the kidney and urinary tract (including hydronephrosis, polycystic kidney disease, or hypoplastic/dysplastic kidneys), metabolic disturbances such as hyperuricemia and maturity-onset diabetes of the young type 5 (MODY5), genital anomalies, hepatic disorders (e.g., liver cysts, hypertransaminasemia), and various neurodevelopmental or neuropsychiatric disorders, including developmental delay, intellectual disability, autism spectrum disorder (ASD), attention-deficit/hyperactivity disorder (ADHD), and other psychiatric conditions.[2,3]

While renal, hepatic, and neurodevelopmental abnormalities are well-established in 17q12 deletion syndrome, spinal cord anomalies, particularly syringomyelia, have not been reported to date. Here, we describe a pediatric patient with hypomagnesemia and neurodevelopmental delay, who was ultimately diagnosed with 17q12 deletion syndrome, presenting with coexisting thoracolumbar scoliosis and spinal syringomyelia.

## Case Presentation

A 12-year-old girl was referred to our pediatric nephrology department in December 2023 due to frequent urination. She had no polyuria, polydipsia, urinary tract infections, urine incontinence, or constipation. Her medical history included intellectual disability and autism spectrum disorder, with ongoing care and treatment from pediatric psychiatry and neurology, in which she was prescribed sertraline and risperidone.

She was born at term by cesarean section, with a birth weight of 2300 g, and did not require neonatal critical care. There was no parental consanguinity, and the family history was unremarkable. She started walking at 12 months and spoke her first words at 18 months; however, by the age of 4 years, she was unable to build meaningful sentences.

On physical examination, she exhibited developmental delay with cognitive and social deficits compared to her age-matched peers. No apparent dysmorphic features were noted other than prominent auricles. Her weight was 34 kg (SD score: -1.56) and height was 150 cm (SD score: -0.48), corresponding to a body mass index (BMI) of 15.1 kg/m$^2$ (SD score: -1.75). Blood pressure was 105/62 mmHg (systolic 53rd percentile, diastolic 51st percentile). Thoracolumbar scoliosis was observed. Neurological, cardiovascular, and abdominal examinations were otherwise unremarkable.

Laboratory findings revealed a serum glucose level of 141 mg/dL (postprandial), creatinine 0.55 mg/dL, sodium 137 mmol/L, potassium 3.7 mmol/L, chloride 100 mmol/L, calcium 9.1 mg/dL, phosphorus 3.8 mg/dL, and magnesium 1.5 mg/dL (reference range, 1.6–2.1 mg/dL). Blood

gas analysis demonstrated a pH of 7.46 and a bicarbonate level of 26.2 mmol/L. Complete blood count was within normal limits except for a mildly decreased platelet count ($131\times10^3$/μL). Liver enzymes were mildly elevated with aspartate transaminase (AST) 28 IU/L and alanine transaminase (ALT) 37 IU/L. Urinalysis showed a pH of 5.5, specific gravity of 1.006, and no evidence of hematuria, proteinuria, or sediment abnormalities. Spot urine analysis revealed an elevated fractional excretion of magnesium (FeMg) of 4.1% and a normal calcium-to-creatinine ratio of 0.23 mg/mg.

The patient, who had been previously followed by pediatric neurology, was noted to have borderline serum magnesium levels (1.7 mg/dL) approximately one year earlier, along with mildly elevated AST (51 IU/L) and ALT (45 IU/L) levels. Thyroid function tests; vitamin B12, folic acid, and 25-hydroxyvitamin D levels; carnitine-acylcarnitine analysis by tandem mass spectrometry; urinary organic acid analysis; and serum lactate, pyruvate, and ammonia levels were all within normal limits. Urinary, hepatobiliary, and suprapubic pelvic ultrasonography findings were normal. Scoliosis radiography confirmed thoracolumbar scoliosis (Fig. 1a). Brain MRI showed no abnormalities. Cervical MRI revealed loss of cervical lordosis, while thoracic MRI demonstrated a central syrinx in the spinal cord extending from the T3 to T6 levels (Fig. 1b).

She was started on oral magnesium supplementation and referred to the medical genetics department for further evaluation. Chromosomal microarray analysis was performed on genomic DNA extracted from peripheral blood samples using the Qiagen DNA extraction kit (Qiagen, Hilden, Germany). Variant annotation was based on the GRCh38 (hg38) human genome reference, and interpretation followed the American College of Medical Genetics and Genomics (ACMG) standards. The analysis identified a heterozygous 1.4-Mb deletion at 17q12 (arr[GRCh38] 17q12(36459737_37889808) x1), encompassing several genes including *HNF1B*, *PIGW* (phosphatidylinositol-glycan biosynthesis class W protein), *ACACA*, *LHX1* and *ZNHIT3*. The microarray profile is shown in Fig. 2a, and the schematic representation of all genes within this region is displayed in Fig. 2b.

Following the confirmation of 17q12 deletion syndrome, pediatric endocrinology and gastroenterology consultations were added to her ongoing multidisciplinary follow-up

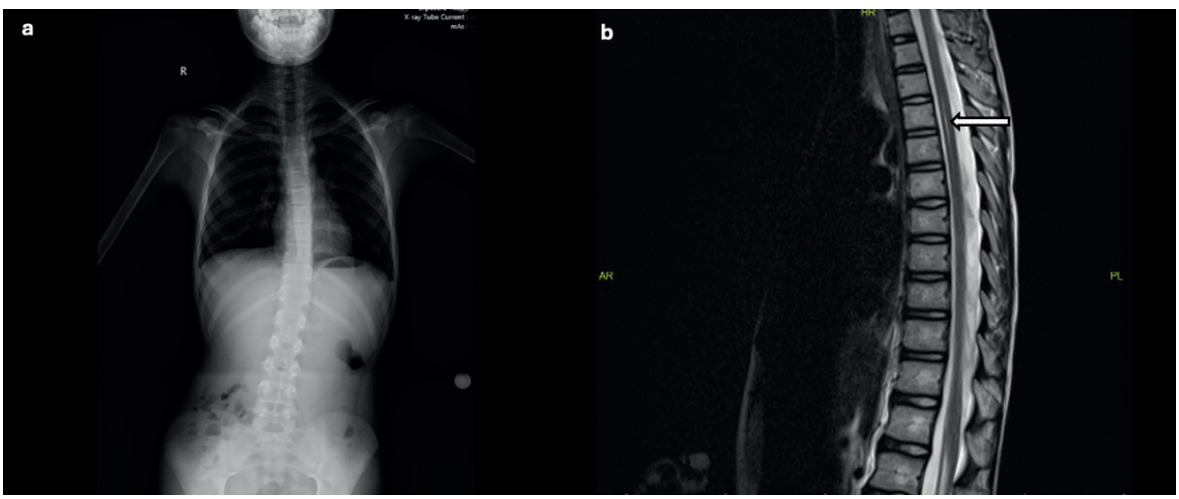



**Fig. 1. a)** Anteroposterior scoliosis radiograph demonstrating right-convex thoracolumbar curvature. **b)** Mid-sagittal T2-weighted thoracic magnetic resonance imaging, showing a central syrinx (arrow) extending from the T3 to T6 vertebrae.

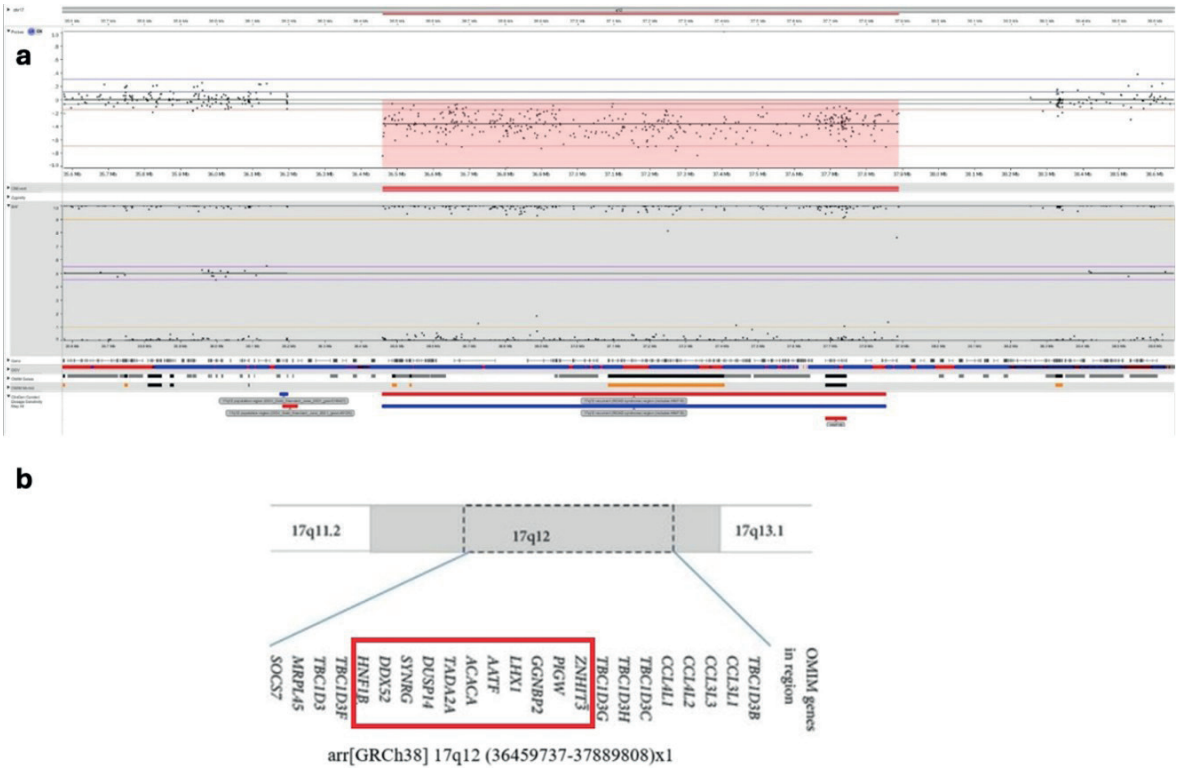



**Fig. 2. a)** Chromosomal microarray analysis revealing a heterozygous 1.4-Mb deletion at 17q12 (arr[GRCh38] 17q12(36459737–37889808)x1). The deleted region is marked in red and includes the *HNF1B*, *PIGW*, *ACACA*, *LHX1* and *ZNHIT3* genes. The log2 ratio plot (top panel) shows a deviation indicative of copy number loss, and the B-allele frequency plot (middle panel) confirms the heterozygous deletion. **b)** Schematic presentation of the 17q12 chromosomal region. The deleted segment in the patient (arr[GRCh38] 17q12 (36459737–37889808)x1) is shown in red and contains key OMIM genes, including *HNF1B, PIGW, ACACA, LHX1* and *ZNHIT3*. Other adjacent genes within this region are also illustrated schematically to indicate the broader genomic context.

under pediatric neurology, psychiatry, and nephrology. At the most recent follow-up visit in April 2025, her weight was 40 kg (SD score: -2.44), height was 154 cm (SD score: -1.18), and BMI was 16.8 kg/m$^2$ (SD score: -1.95). Blood pressure was 110/65 mmHg. Laboratory tests demonstrated a serum glucose of 104 mg/dL (fasting), insulin level of 6 μIU/mL, and a HOMA-IR index of 1.54. Serum creatinine was 0.61 mg/dL, uric acid 5.6 mg/dL, and magnesium 1.9 mg/dL. Other electrolytes, as well as blood pH and bicarbonate levels, were within normal limits. Liver function tests showed mildly increased levels of AST (47 IU/L), ALT (59 IU/L), and gamma-glutamyl transferase (GGT: 104 IU/L). The lipid profile, including total cholesterol, high-density lipoprotein (HDL), low density lipoprotein (LDL), and triglycerides, was normal (151/70/71/48 mg/dL, respectively). Urinalysis showed a pH of 5.5, a specific gravity of 1.022, no proteinuria, no glucosuria, and 5 erythrocytes per high-power field. A summary of the initial and follow-up laboratory findings is presented in Table I.

Written informed consent was obtained from the patient's legal guardians for publication of this case report and accompanying images.

## Discussion

The 17q12 deletion syndrome is a recurrent multigenic microdeletion encompassing approximately 15 protein-coding genes including *HNF1B*, *PIGW*, *ACACA*, *LHX1* and *ZNHIT3*.[3,4] This chromosomal abnormality

**Table I.** Summary of initial and follow-up laboratory findings.

| Parameter | Initial result | Follow-up result |
|---|---|---|
| Serum magnesium (mg/dL) | 1.5 | 1.9 |
| Fractional excretion of magnesium (%) | 4.1 | — |
| Serum potassium (mmol/L) | 3.7 | 4.2 |
| Serum calcium (mg/dL) | 9.1 | 10.0 |
| Serum phosphorus (mg/dL) | 3.8 | 3.5 |
| Serum creatinine (mg/dL) | 0.55 | 0.61 |
| Serum glucose (mg/dL) | 141 (postprandial) | 104 (fasting) |
| AST / ALT (IU/L) | 28 / 37 | 47 / 59 |
| GGT (IU/L) | — | 104 |
| Serum sodium (mmol/L) | 137 | 140 |
| Serum chloride (mmol/L) | 100 | 105 |
| Blood pH | 7.46 | 7.43 |
| Serum bicarbonate (mmol/L) | 26.2 | 24.3 |
| Serum uric acid (mg/dL) | — | 5.6 |
| Urine calcium / creatinine ratio (mg/mg) | 0.23 | — |

ALT, alanine transaminase; AST, aspartate transaminase; GGT, gamma-glutamyl transferase.

results in a heterogeneous phenotype involving renal, urogenital, metabolic, neurodevelopmental and psychiatric abnormalities. In our patient, the two main clinical findings, persistent hypomagnesemia and neurodevelopmental delay, reflect this dual renal and neurological involvement.[3]

In 17q12 deletion syndrome, prenatal imaging may reveal renal anomalies such as increased echogenicity and poor corticomedullary differentiation, whereas postnatal findings may identify renal hypoplasia, dysplasia, multicystic dysplastic kidney, or renal agenesis.[3] Other urinary tract anomalies reported in this syndrome include horseshoe kidney, ureteropelvic junction obstruction, isolated hydronephrosis, and hydroureter. Tubulointerstitial disease may manifest with impaired urine-concentrating capacity, unremarkable urinary sediment, minimal proteinuria, hyperuricemia, hypomagnesemia and hypokalemia.[3] In our patient, renal ultrasonography revealed no structural abnormalities; however, persistent hypomagnesemia and elevated urinary magnesium excretion suggested functional tubular impairment. This finding is consistent with previous observations in *HNF1B*-related disease, where renal magnesium wasting has been reported in up to 62% of patients despite normal renal imaging.[5]

Even in the absence of renal malformations, 17q12 deletion syndrome should be considered as a possible etiology in children presenting with neurodevelopmental disorders and electrolyte disturbances. Hypomagnesemia, in particular, served as the biochemical marker that prompted genetic investigation in our patient. We initially suspected Gitelman syndrome based on the presence of hypomagnesemia and mild metabolic alkalosis; however, the lack of hypocalciuria and only borderline hypokalemia pointed to an alternative diagnosis. *HNF1B* haploinsufficiency is known to impair distal tubular magnesium reabsorption, leading to renal magnesium wasting, mild hypokalemia, and metabolic alkalosis, often resembling a Gitelman-like tubulopathy.[6] Although classical findings such as severe hypokalemia or obvious metabolic alkalosis were not prominent in our patient, persistent hypomagnesemia and elevated fractional renal magnesium excretion

were suggestive of distal tubular dysfunction, ultimately leading to the diagnosis of 17q12 deletion syndrome. Psychotropic medications such as sertraline and risperidone have been reported to influence magnesium homeostasis, but current evidence suggests they do not cause clinically relevant magnesium depletion. Experimental and clinical data indicate that these agents may even increase intracellular magnesium concentrations and support neurochemical stability through modulation of glutamate and GABAergic pathways.[7,8] In our patient, the persistence of hypomagnesemia with elevated FeMg, despite ongoing psychotropic therapy and in the absence of other implicated drugs, supports a renal magnesium-wasting mechanism secondary to *HNF1B* haploinsufficiency rather than a medication effect. Although higher fractional excretion of magnesium (FeMg) values have been reported in severe renal magnesium-wasting states, milder elevations, such as the 4.1% observed in our patient, can still indicate renal loss, particularly in early or partial distal convoluted tubule dysfunction. The persistently low serum magnesium and absence of extrarenal losses further support a renal etiology. Nutritional status was also reviewed to exclude dietary magnesium deficiency; the patient had no signs of malnutrition or poor intake, making inadequate magnesium intake an unlikely cause.

Neurodevelopmental and neuropsychiatric manifestations are also common in 17q12 deletion syndrome and include developmental delay, intellectual disability, ASD, ADHD, and, less frequently, anxiety, schizophrenia, and bipolar disorder.[3] Our patient exhibited moderate neurodevelopmental delay and ASD features beginning in early childhood, requiring ongoing evaluation by pediatric psychiatry and neurology. Although brain MRI findings were normal, her clinical features are consistent with previous reports indicating that functional or ultrastructural brain alterations, rather than major anatomic defects, may underlie the neurological manifestations of 17q12 deletion syndrome.[9] Milone et al.[9] described nonspecific brain abnormalities in affected patients, including ventricular dilatation, white-matter signal changes, hippocampal atrophy, and corpus callosum agenesis or thinning. These reports support that normal neuroimaging does not exclude neurocognitive dysfunction in 17q12 deletion syndrome. The loss of other genes within the 17q12 interval, particularly *LHX1* and *ACACA*, may underlie the neurodevelopmental and extrarenal manifestations through disruption of neuronal differentiation and metabolic pathways.[4] *LHX1* encodes a LIM-homeodomain transcription factor essential for neuronal differentiation and early embryonic patterning, whereas *ACACA* participates in fatty-acid metabolism and neuronal energy homeostasis.[4] Haploinsufficiency of these genes likely contributes to the neurodevelopmental phenotype observed in our patient, supporting a multigenic rather than a single-gene pathogenesis.

Neuroimaging findings in our patient included loss of cervical lordosis and a thoracic spinal cord syrinx extending from T3 to T6. Although such findings have not been routinely reported in association with 17q12 deletion syndrome, they may represent underrecognized aspects of its broader neurodevelopmental phenotype. Existing literature has focused mainly on brain malformations. The coexistence of spinal syringomyelia in our patient therefore may reflect an underrecognized manifestation of the broader neurodevelopmental phenotype associated with 17q12 deletion syndrome. Several mechanisms may be proposed: (1) loss of neurodevelopmentally relevant genes within the deleted 17q12 interval, such as *LHX1*, which plays a role in neuronal patterning and differentiation; (2) altered cerebrospinal fluid dynamics due to subtle maldevelopment of the spinal canal or hindbrain structures, even in the absence of Chiari malformation; or (3) an incidental finding unrelated to the microdeletion. Given the single-case nature

of this report, causality cannot be established, and further studies are required to clarify this potential association. Our case and these hypotheses highlight the potential importance of performing spinal imaging in selected patients with 17q12 deletions, especially in those with neuromotor or postural anomalies such as scoliosis.

Beyond the renal phenotype, *HNF1B* acts as a key transcriptional regulator during organogenesis of the pancreas, liver, and genitourinary tract.[2,3] Its haploinsufficiency may disrupt pancreatic β-cell differentiation and hepatobiliary development, leading to mild hyperglycemia, elevated liver enzyme levels, and, in some cases, reproductive tract anomalies. These mechanisms account for the broad systemic manifestations observed in 17q12 deletion syndrome.[2,3] Mildly elevated glucose and liver enzyme levels in our patient necessitated consultation with endocrinology and gastroenterology teams, and inclusion in a structured multidisciplinary monitoring program to anticipate future endocrine and metabolic complications, including the development of MODY5.

In conclusion, this case highlights the importance of considering 17q12 deletion syndrome in children presenting with hypomagnesemia and neurodevelopmental delay, even in the absence of structural renal or cerebral anomalies. The coexistence of thoracolumbar scoliosis and spinal syringomyelia in our patient further emphasizes the phenotypic diversity of this syndrome. Early recognition of 17q12 deletion syndrome enables effective management, genetic counseling, and coordinated multidisciplinary follow-up. Serum magnesium measurement should be included in the diagnostic assessment of children with developmental delays, cognitive impairment, or autism spectrum disorder, especially when accompanied by subtle electrolyte abnormalities. Clinicians should remain alert for persistent hypomagnesemia as a potential early clue to underlying syndromic conditions such as 17q12 deletion syndrome.

**Ethical approval**

Written informed consent was obtained from the patient's legal guardians for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

**Author contribution**

The authors confirm contribution to the paper as follows: Case report conception and design: YÖA; data collection: YÖA, AK, KD, DİM, MDÇ, SY; draft manuscript preparation: YÖA. All authors reviewed the results and approved the final version of the manuscript.

**Source of funding**

The authors declare the study received no funding.

**Conflict of interest**

The authors declare that there is no conflict of interest.

**REFERENCES**

1. Knoers NV. Inherited forms of renal hypomagnesemia: an update. Pediatr Nephrol 2009; 24: 697-705. https://doi.org/10.1007/s00467-008-0968-x
2. Sánchez-Cazorla E, Carrera N, García-González MÁ. HNF1B transcription factor: key regulator in renal physiology and pathogenesis. Int J Mol Sci 2024; 25: 10609. https://doi.org/10.3390/ijms251910609
3. Mitchel MW, Moreno-De-Luca D, Myers SM, et al. 17q12 Recurrent Deletion Syndrome. 2016 Dec 8 [Updated 2025 Aug 14]. In: Adam MP, Bick S, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2025. Available from: https://www.ncbi.nlm.nih.gov/books/NBK401562/
4. Clissold RL, Hamilton AJ, Hattersley AT, Ellard S, Bingham C. HNF1B-associated renal and extra-renal disease-an expanding clinical spectrum. Nat Rev Nephrol 2015; 11: 102-112. https://doi.org/10.1038/nrneph.2014.232

5. Faguer S, Decramer S, Chassaing N, et al. Diagnosis, management, and prognosis of HNF1B nephropathy in adulthood. Kidney Int 2011; 80: 768-776. https://doi.org/10.1038/ki.2011.225

6. Adalat S, Hayes WN, Bryant WA, et al. HNF1B mutations are associated with a Gitelman-like tubulopathy that develops during childhood. Kidney Int Rep 2019; 4: 1304-1311. https://doi.org/10.1016/j.ekir.2019.05.019

7. Nechifor M. Interactions between magnesium and psychotropic drugs. Magnes Res 2008; 21: 97-100.

8. Nechifor M. Magnesium in psychoses (schizophrenia and bipolar disorders). In: Vink R, Nechifor M, editors. Magnesium in the Central Nervous System. Adelaide (AU): University of Adelaide Press; 2011. https://doi.org/10.1017/UPO9780987073051.023

9. Milone R, Tancredi R, Cosenza A, et al. 17q12 recurrent deletions and duplications: description of a case series with neuropsychiatric phenotype. Genes (Basel) 2021; 12: 1660. https://doi.org/10.3390/genes12111660

The Turkish Journal of Pediatrics 2025; 67: 920-926
https://doi.org/10.24953/turkjpediatr.2025.6622

Case Report

# Pediatric inflammatory myofibroblastic tumor of the urinary bladder: a rare case report and treatment approach

Kübra Ozturk Yuzdemir[1], Idil Rana User[1], H. Nursun Ozcan[2], Diclehan Orhan[3], Ali Varan[4], Ibrahim Karnak[1], Burak Ardicli[1]

[1]*Department of Pediatric Surgery, Faculty of Medicine, Hacettepe University, Ankara, Türkiye;* [2]*Department of Pediatric Radiology, Faculty of Medicine, Hacettepe University, Ankara, Türkiye;* [3]*Department of Pediatric Pathology, Faculty of Medicine, Hacettepe University, Ankara, Türkiye;* [4]*Department of Pediatric Oncology, Faculty of Medicine, Hacettepe University, Ankara, Türkiye.*

## ABSTRACT

**Background.** Inflammatory myofibroblastic tumor (IMT) is a rare mesenchymal neoplasm of intermediate malignant potential, commonly arising in the lungs and intra-abdominal organs. Involvement of the urinary bladder is exceptionally rare, particularly in children, and may clinically and radiologically mimic malignant tumors.

**Case Presentation.** We report the case of a 10-year-old girl who presented with painless macroscopic hematuria and syncope, necessitating blood transfusion. Initial imaging revealed a bladder mass, and biopsy initially suggested rhabdomyosarcoma. Definitive histopathological evaluation, however, confirmed IMT. Partial cystectomy was performed, but due to positive surgical margins and recurrent hematuria, targeted therapy with crizotinib was initiated based on anaplastic lymphoma kinase (ALK) positivity. At 12-month follow-up, the patient remained symptom-free with no evidence of recurrence on imaging.

**Conclusion.** Pediatric IMT of the bladder is a rare but important differential diagnosis for bladder masses. Accurate histological diagnosis is essential, as this tumor may mimic malignancy and influence the treatment plan. Complete surgical excision remains the cornerstone of treatment, while targeted therapies such as ALK inhibitors offer valuable options in cases with residual disease or risk of recurrence. This case highlights the importance of a multidisciplinary approach involving surgery, pathology, and oncology. Further pediatric-focused studies are warranted to refine treatment strategies and define long-term outcomes.

**Key words:** soft tissue neoplasm, inflammatory myofibroblastic tumor, urinary bladder mass, children, crizotinib.

Inflammatory myofibroblastic tumor (IMT) is a rare mesenchymal neoplasm primarily affecting children and young adults, with intermediate malignant potential and variable histology and behavior.[1] Because IMT can arise at multiple sites and shows heterogeneous histologic and radiologic features – with behavior ranging from spontaneous regression to local recurrence and rare metastasis – it remains incompletely understood.[2] It can occur anywhere in the body, but it is most commonly detected in the lung, retroperitoneum, and gastrointestinal tract.[3] Approximately 9.5% of extrapulmonary IMTs arise in the genitourinary system, most commonly in the bladder.[4]

✉ Kübra Öztürk Yüzdemir ▪ kubraozturk.610@gmail.com

Received 12th Feb 2025, revised 27th Sep 2025, 22nd Nov 2025, accepted 24th Nov 2025.

This manuscript has been previously published as a preprint on the Authorea preprint platform. https://doi.org/10.22541/au.174228171.18055562/v1

Symptoms vary depending on the location and size. It is estimated that one-third of IMT patients present with systemic symptoms such as fever, fatigue, growth retardation, or anemia. Definitive management generally involves complete resection; however, when complete excision is not feasible, systemic therapies may be considered. Over the past decade, advances in tumor biology and molecular genetics have refined the characterization of IMT and enabled the development of targeted therapies. However, important uncertainties persist regarding optimal management.

Masses of the urinary bladder are rarely seen in pediatric age groups, which often poses significant diagnostic challenges and necessitates histological differentiation and confirmation. This case report contributes to the limited literature on pediatric IMTs of the bladder by describing a rare pediatric case successfully treated with partial cystectomy and targeted anaplastic lymphoma kinase (ALK) inhibition, highlighting the role of a multidisciplinary approach and the potential of organ-sparing strategies. We present a case of a 10-year-old girl with painless gross hematuria caused by an IMT of the bladder (IMTB).

## Case Presentation

A 10-year-old girl was admitted to another hospital presenting with painless gross hematuria that led to syncope and required transfusion. Ultrasonography (US) revealed a well-circumscribed mass located at the base of the bladder. Due to uncontrollable hematuria, a palliative laparotomy was performed at another hospital. A biopsy of the bladder mass, initially suspected to represent rhabdomyosarcoma, was subsequently diagnosed as an IMT upon histopathological evaluation. The patient was then referred to our institution for further evaluation and management. The patient's medical history was otherwise unremarkable, and both physical examination and hematologic evaluations—including complete blood count, coagulation profile, and basic serum chemistry—were within normal limits. Abdominal US showed a well-defined hypoechoic lesion measuring 30×20 mm at the bladder base, without calcification. Color Doppler imaging demonstrated mild vascularization within the lesion. Magnetic resonance imaging (MRI) revealed a contrast-enhanced solid mass measuring 20×23×30 mm in the left posterolateral bladder wall, near the bladder neck (Fig. 1). On cystoscopy, the right ureter orifice was visualized, but the left orifice was obscured by a protruding mass on the left bladder wall extending toward the bladder neck.

The patient underwent a partial cystectomy, preserving both ureters and the trigone. Because the diagnosis had already been established by biopsy, surgical excision was performed with the goal of preserving bladder function and

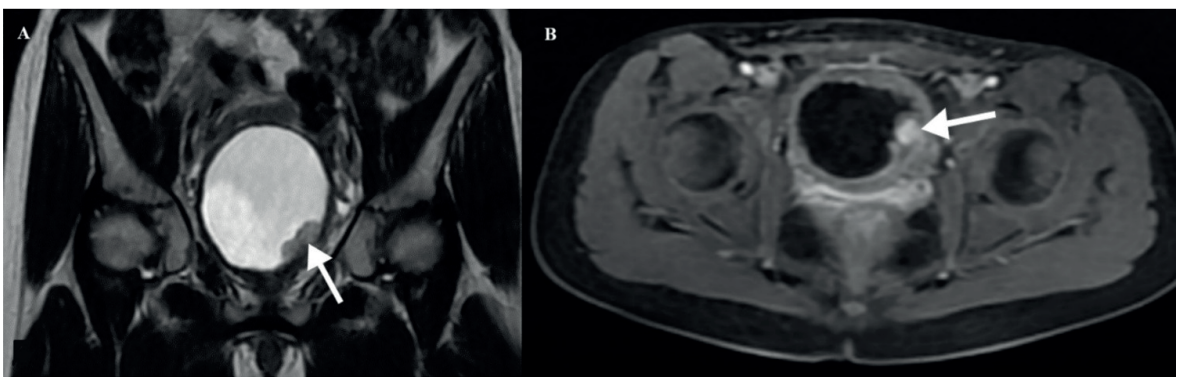



**Fig. 1.** Coronal T2-weighted (a) and post-contrast axial T1-weighted (b) MRI show a polypoid mass lesion (arrows) with lobulated margins.

the left ureter. The postoperative course was uneventful, with no complications.

The diagnosis of IMT was confirmed by histopathological examination. Surgical margins were positive. Immunohistochemical analysis showed patchy staining with smooth muscle actin (SMA), focal weak ALK positivity, and negative staining for desmin. Immunostaining for myogenic differentiation 1 (MyoD1) and myogenin was non-specific. The Ki-67 proliferation index was approximately 5% (Fig. 2).

The patient was followed for six months without additional treatment. She later developed recurrent hematuria, prompting a repeat MRI. Minimal nonspecific bladder wall thickening was observed. There was no evidence of recurrence. Crizotinib therapy was initiated due to persistent symptoms and positive surgical margins. She received crizotinib for ten months. Hematuria resolved with crizotinib and after one year follow-up there was no evidence of disease recurrence.

Written informed consent was obtained from the patient's legal guardian for publication of this case and accompanying images.

## Discussion

IMTs are rare mesenchymal neoplasms, most commonly occurring in the lungs and intra-abdominal organs, though they can also arise in genitourinary structures such as the bladder. In children, IMTB is exceptionally rare and requires a multidisciplinary diagnostic and

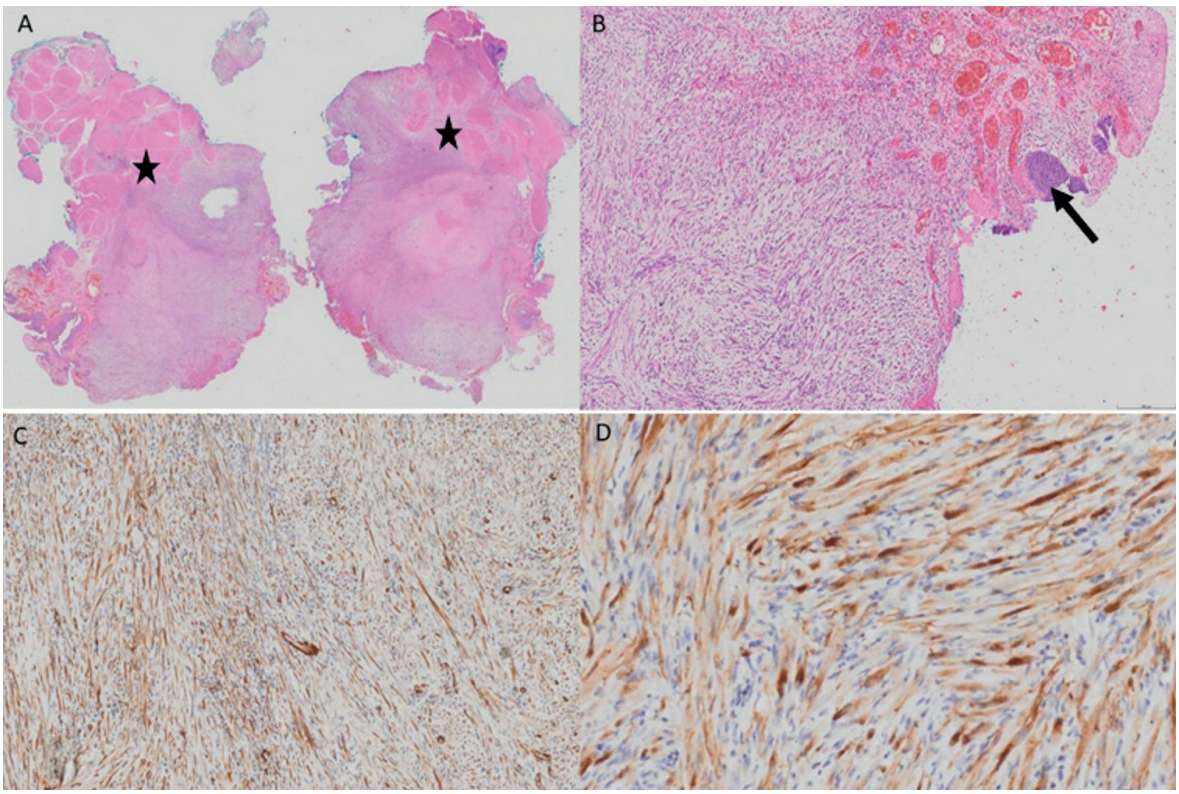



**Fig. 2.** Histopathological characteristics of the tumor. a) Tumor infiltrating the muscle tissue of the bladder (area between star shapes). Tumor cells are in solid growth pattern with storiform areas (H&E). b) Inflammatory myofibroblastic tumor composed of spindle shaped, fibroblast- or smooth muscle cell-like neoplastic cells which are forming bundles, and of inflammatory cells. Bladder mucosa is shown with arrow (H&E). c) Immunohistochemical smooth muscle actin positivity (brown cytoplasmic staining) of neoplastic cells. d) Nuclear and cytoplasmic ALK positivity (brown staining) in tumor cells.

therapeutic approach. Their unpredictable clinical behavior - ranging from benign to locally aggressive forms - underscores the importance of comprehensive diagnostic and therapeutic strategies.

The clinical presentation of IMT varies according to tumor site. In IMTB, the most common symptoms are hematuria, dysuria, and obstructive voiding complaints. Because these symptoms can mimic both benign and malignant bladder lesions, imaging and histopathological confirmation are essential. In our case, the patient presented with painless gross hematuria resulting in syncope and requiring transfusion, a rare and severe manifestation of IMTB.

Definitive diagnosis is established through histopathologic examination and immunohistochemical analysis. The hallmark histologic features of IMT include spindle cell proliferation accompanied by a prominent inflammatory infiltrate composed of plasma cells, lymphocytes, and eosinophils.[5] Immunohistochemical staining for ALK, smooth muscle actin, and desmin helps differentiate IMT from other spindle cell neoplasms. Our case showed patchy SMA positivity, focal weak ALK positivity, and negative staining for desmin, with a Ki-67 proliferation index of approximately 5%, findings consistent with previously reported cases of IMTB.[5] The underlying etiology of IMTB remains unclear. Proposed contributing factors include infection, prior instrumentation, trauma, and immunosuppression, which may induce an exaggerated inflammatory response leading to myofibroblastic proliferation.[2,4] Genetic alterations are also thought to play a key role in the pathogenesis of IMT. Recent molecular studies have shown that IMTs are associated with ALK gene rearrangements in approximately 50-70% of cases.[6] In pediatric IMTBs, ALK-positivity serves as an important biomarker that guides treatment decisions. Conversely, ALK-negative tumors, tend to follow a more aggressive course and may require alternative therapeutic strategies. Other genetic alterations, including ROS1 and platelet-derived growth factor receptor beta (PDGFRB) rearrangements, have also been identified in a subset of IMT, representing potential targets for novel therapies.[7]

The surgical management of IMTB differs from that of malignant bladder tumors, in which radical or extensive resections are often required. IMTB is generally regarded as a tumor of intermediate malignant potential, characterized by a propensity for local recurrence and rare metastatic spread. Consequently, organ-sparing surgery is typically favored whenever feasible. Preoperative or intraoperative biopsy plays a pivotal role in establishing the diagnosis, enabling the surgical team to tailor the procedure and avoid unnecessary radical interventions. Early histological confirmation facilitates a conservative, function-preserving approach focused on complete local excision, which is particularly important in pediatric patients, for whom bladder preservation and minimizing morbidity are key priorities.[4,8,9] Surgical resection remains the primary treatment option for patients with localized IMT. Although IMT is generally considered a tumor of intermediate malignant potential, distant metastases and local recurrences have been reported in some cases. Reported local recurrence rates in large pediatric series range from 15% to 37%.[8] However, as demonstrated in the present case partial excision can be appropriate option when organ involvement may compromise vitality, quality of life, and function. When surgical margins are positive or complete resection is not feasible, additional therapeutic strategies may be required. Anaplastic lymphoma kinase inhibitors such as crizotinib and alectinib have shown promising results in ALK-positive IMT, offering an effective organ-sparing, non-surgical alternative.[10] While surgical resection remains the cornerstone of treatment for IMTB, avoiding unnecessary radical or morbid procedures is of particular importance in pediatric patients. In recent years, systemic therapy with ALK inhibitors has emerged as a valuable adjunct, especially in patients with ALK-positive tumors. Preoperative use of ALK inhibitors can reduce

tumor burden, facilitate less invasive, organ-sparing surgery, and minimize perioperative morbidity. This strategy has been reported to achieve good local control and preserve bladder function in selected cases.[8,10,11]

In pediatric IMTB, there is a growing trend toward organ-sparing surgical strategies supported by molecularly targeted therapies, rather than extensive resections that may lead to long-term morbidity. Pediatric case series have demonstrated that limited surgery combined with close follow-up or ALK inhibitor therapy, when indicated, can provide excellent local control and preserve bladder function. The preoperative or primary use of ALK inhibitors in selected ALK-positive cases may reduce tumor size, facilitate conservative surgery, and help avoid radical interventions. This underscores the importance of a multidisciplinary treatment approach that integrates pediatric surgery, pathology, radiology, and oncology to achieve optimal outcomes in this rare disease.[8-11]

Our patient initially underwent postoperative surveillance but subsequently developed recurrent hematuria, prompting an MRI evaluation, which revealed minimal nonspecific bladder wall thickening without evidence of recurrence. Owing to her persistent symptoms and positive margins, crizotinib therapy was initiated, resulting in complete resolution of hematuria and no evidence of disease recurrence at one-year follow-up. This observation is consistent with previous reports demonstrating the efficacy of crizotinib in achieving remission in ALK-positive IMTs.[10]

Although Ki-67 is not a validated or standardized criterion for guiding treatment decisions in IMTB, the proliferative index can provide valuable adjunctive information regarding tumor biology. In IMT, lower Ki-67 levels have been associated with a more indolent clinical course, whereas higher proliferative activity has been reported in more aggressive cases.[12,13] Similar trends have been robustly demonstrated across multiple solid tumors: in renal cell carcinoma, prostate cancer, and bladder cancer, elevated Ki-67 expression correlates with adverse pathological features and significantly worse survival outcomes.[14-16] These data collectively support the use of Ki-67 as an adjunctive marker of biological behavior, while emphasizing that it should not be used as a stand-alone criterion to guide management decisions.[7,9]

In addition to our case, several ALK-positive pediatric IMTB have been reported in the literature, providing valuable insights into their clinical course and therapeutic options. Beland et al. reported a three-case pediatric series in which patients demonstrated favorable responses to either targeted ALK inhibition or surgical resection, with durable disease control during follow-up.[9] Similarly, Fujiki et al. reported a pediatric patient with a fibronectin 1–ALK fusion who achieved complete remission following treatment with the ALK inhibitor alectinib.[11] Earlier molecular studies by Antonescu et al. identified ALK gene rearrangements as the most common molecular driver in IMTs, supporting the rationale for targeted therapy in selected cases.[7] Collectively, these reports indicate that ALK-positive IMTB typically presents with localized disease and shows responses to ALK inhibitors, particularly in cases with residual or unresectable tumors, offering a potentially effective organ-sparing alternative to radical surgery.

The optimal duration of crizotinib therapy in pediatric IMTB remains undefined. Previous reports have demonstrated treatment durations ranging from 12 to 24 months, with most patients achieving clinical and radiological responses within the first few months of therapy.[9,10,17] In our case, the patient achieved sustained remission after 10 months of treatment, consistent with previously reported outcomes.

While IMTs generally have a favorable prognosis, recurrence rates vary depending on tumor localization, histological features, and completeness of resection. A study on

recurrent IMT reported that IMTB may recur after transurethral resection, particularly in the presence of positive surgical margins or incomplete excision, underscoring the need for long-term follow-up.[18] Close post-treatment surveillance with periodic imaging is recommended to detect early recurrence and to guide subsequent management. Incorporating molecular profiling into treatment planning allows for a more personalized therapeutic approach, potentially optimizing outcomes for pediatric patients with IMT.

This case report is limited by its single-case nature and the relatively short follow-up duration of 12 months, which may not allow for a comprehensive assessment of recurrence risk. Extended clinical and radiologic surveillance will be important to detect late recurrences and evaluate the long-term efficacy of targeted therapy.

### *Conclusion*

Pediatric IMTBs are rare tumors that require thorough clinical and pathological characterization to inform appropriate treatment strategies. The presence of ALK mutations significantly impacts therapeutic decision-making, underscoring the importance of targeted therapies in conjunction with surgical interventions. When feasible, complete excision should be pursued, otherwise, organ- and function-sparing approaches may be considered. This case highlights the critical role of early diagnosis, tailored surgical management, and targeted therapy in the setting of residual disease, illustrating the potential of crizotinib to achieve durable disease control. Advances in molecular diagnostics and targeted therapies offer promising opportunities for optimizing management, particularly in recurrent or unresectable cases. Future studies with larger patient cohorts are needed to further clarify disease pathogenesis, refine molecular classification, and define evidence-based treatment algorithms for pediatric IMTs.

### Ethical approval

Written informed consent was obtained from the patient's legal guardians.

### Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: IK, BA; data collection: KOY, DO, AV, HNO; interpretation of results: IRU, HNO, DO, AV, IK, BA; draft manuscript preparation: KOY, BA, IRU. All authors reviewed the results and approved the final version of the manuscript.

### Source of funding

The authors declare the study received no funding.

### Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES

1. Rich BS, Fishbein J, Lautz T, et al. Inflammatory myofibroblastic tumor: a multi-institutional study from the Pediatric Surgical Oncology Research Collaborative. Int J Cancer 2022; 151: 1059-1067. https://doi.org/10.1002/ijc.34132
2. Zhao JJ, Ling JQ, Fang Y, et al. Intra-abdominal inflammatory myofibroblastic tumor: spontaneous regression. World J Gastroenterol 2014; 20: 13625-13631. https://doi.org/10.3748/wjg.v20.i37.13625
3. Inamura K, Kobayashi M, Nagano H, et al. A novel fusion of HNRNPA1-ALK in inflammatory myofibroblastic tumor of urinary bladder. Hum Pathol 2017; 69: 96-100. https://doi.org/10.1016/j.humpath.2017.04.022

4. Montgomery EA, Shuster DD, Burkart AL, et al. Inflammatory myofibroblastic tumors of the urinary tract: a clinicopathologic study of 46 cases, including a malignant example inflammatory fibrosarcoma and a subset associated with high-grade urothelial carcinoma. Am J Surg Pathol 2006; 30: 1502-1512. https://doi.org/10.1097/01.pas.0000213280.35413.1b

5. Jang EJ, Kim KW, Kang SH, Pak MG, Han SH. Inflammatory myofibroblastic tumors arising from pancreas head and peri-splenic area mimicking a malignancy. Ann Hepatobiliary Pancreat Surg 2021; 25: 287-292. https://doi.org/10.14701/ahbps.2021.25.2.287

6. Lowe E, Mossé YP. Podcast on emerging treatment options for pediatric patients with ALK-positive anaplastic large cell lymphoma and inflammatory myofibroblastic tumors. Oncol Ther 2024; 12: 247-255. https://doi.org/10.1007/s40487-024-00275-6

7. Antonescu CR, Suurmeijer AJH, Zhang L, et al. Molecular characterization of inflammatory myofibroblastic tumors with frequent ALK and ROS1 gene fusions and rare novel RET rearrangement. Am J Surg Pathol 2015; 39: 957-967. https://doi.org/10.1097/PAS.0000000000000404

8. Nagumo Y, Maejima A, Toyoshima Y, et al. Neoadjuvant crizotinib in ALK-rearranged inflammatory myofibroblastic tumor of the urinary bladder: a case report. Int J Surg Case Rep 2018; 48: 1-4. https://doi.org/10.1016/j.ijscr.2018.04.027

9. Beland LE, Van Batavia JP, Mittal S, Kolon TF, Surrey LF, Long CJ. Inflammatory myofibroblastic tumor of the bladder in childhood: a three case series. Urology 2025; 200: e72-e75. https://doi.org/10.1016/j.urology.2025.02.045

10. Kaino A, Niizuma H, Katayama S, et al. Two-year crizotinib monotherapy induced durable complete response of pediatric ALK-positive inflammatory myofibroblastic tumor. Pediatr Blood Cancer 2023; 70: e30330. https://doi.org/10.1002/pbc.30330

11. Fujiki T, Sakai Y, Ikawa Y, et al. Pediatric inflammatory myofibroblastic tumor of the bladder with ALK-FN1 fusion successfully treated by alectinib. Pediatr Blood Cancer 2023; 70: e30172. https://doi.org/10.1002/pbc.30172

12. Dobrosz Z, Ryś J, Paleń P, Własczczuk P, Ciepiela M. Inflammatory myofibroblastic tumor of the bladder - an unexpected case coexisting with an ovarian teratoma. Diagn Pathol 2014; 9: 138. https://doi.org/10.1186/1746-1596-9-138

13. Buccoliero AM, Ghionzoli M, Castiglione F, et al. Inflammatory myofibroblastic tumor: clinical, morphological, immunohistochemical and molecular features of a pediatric case. Pathol Res Pract 2014; 210: 1152-1155. https://doi.org/10.1016/j.prp.2014.03.011

14. Xie Y, Chen L, Ma X, et al. Prognostic and clinicopathological role of high Ki-67 expression in patients with renal cell carcinoma: a systematic review and meta-analysis. Sci Rep 2017; 7: 44281. https://doi.org/10.1038/srep44281

15. Berlin A, Castro-Mesta JF, Rodriguez-Romo L, et al. Prognostic role of Ki-67 score in localized prostate cancer: a systematic review and meta-analysis. Urol Oncol 2017; 35: 499-506. https://doi.org/10.1016/j.urolonc.2017.05.004

16. Tian Y, Ma Z, Chen Z, et al. Clinicopathological and prognostic value of Ki-67 expression in bladder cancer: a systematic review and meta-analysis. PLoS One 2016; 11: e0158891. https://doi.org/10.1371/journal.pone.0158891

17. Schöffski P, Kubickova M, Wozniak A, et al. Long-term efficacy update of crizotinib in patients with advanced, inoperable inflammatory myofibroblastic tumour from EORTC trial 90101 CREATE. Eur J Cancer 2021; 156: 12-23. https://doi.org/10.1016/j.ejca.2021.07.016

18. Alene AT, Tamir KT, Melaku A, Teka MD, Desalew ED. Recurrent inflammatory myofibroblastic tumors (IMTs) of bladder managed with transurethral resection; case report. Int J Surg Case Rep 2025; 128: 110978. https://doi.org/10.1016/j.ijscr.2025.110978

The Turkish Journal of Pediatrics 2025; 67: 927-928
https://doi.org/10.24953/turkjpediatr.2025.7318



# Expanding the health-related behavior perspective on problematic internet use in adolescents

Eylem Şerife Kalkan[1]

[1]*Department of Pediatrics, Yenimahalle Training and Research Hospital, Ankara, Türkiye.*

To the Editor,

I read the article entitled ''Association of problematic internet use with health-related daily habits in adolescents: evidence from a school-based survey'' by Çelik et al., with great interest.[1] The authors provide valuable insights into the prevalence (12.1%) and health behavior correlates of problematic internet use (PIU) among Turkish high school students, identifying significant associations with weekday internet usage time ≥ 2 hours, sleep problem, having infrequent breakfast, frequent salty snacks and frequent sugary-carbonated drinks. I appreciate the authors for addressing a growing public health concern and for their comprehensive evaluation of internet use, sleep, diet and physical activity within the same analytic model.

The study successfully demonstrates the association of PIU with several modifiable health-related behaviors, contributing to our understanding of how adolescents' daily routines may interact with emerging digital habits. However, a broader clinical and public health perspective could further enrich these findings and help guide more targeted interventions.

Beyond measuring internet time, it is essential to investigate why adolescents engage in internet use and how these motivations relate to developmental and psychosocial stressors during this critical stage of growth. Identifying what adolescents do online could improve risk estimates. Merely quantifying screen time provides an incomplete picture; the content, context, and emotional purpose of online engagement may be even more influential than the duration itself. Different digital behaviors, such as gaming, social networking, exploring or watching videos, carry different psychosocial and neurobehavioral impacts. For example, adolescents who spend long hours online for academic or social support purposes may experience very different psychosocial outcomes.[2] Integrating brief platform-specific questions or validated scales for gaming, social media, or other content domains could enhance both the precision and interpretive depth of adolescent PIU research.

Another point is that while the authors report no association between PIU and sleep duration, but a significant increase with ''at least one type of sleep problem'', the sleep construct combines diverse symptoms such as difficulty falling asleep, frequent interruption of sleep, difficulty waking up in the morning, feeling sleepy in the morning. Disaggregating these components or including validated tools like the Pittsburgh Sleep Quality Index could reveal symptom-specific associations, consistent with evidence linking PIU to delayed bedtimes and wake-up times, insomnia and excessive daytime sleepiness.[3] Additionally, the absence of standardized tools for measuring physical

✉ Eylem Şerife Kalkan ▪ eylemkaymaz@gmail.com

Received 25th Oct 2025, accepted 8th Dec 2025.

activity, and nutrition also weakens the precision of behavioral assessment.

Considering both internet use and other health behaviors as interconnected, rather than isolated, may help future studies identify shared mechanisms such as self-regulation difficulties and circadian misalignment, that underlie both digital overuse and unhealthy health behaviors. Additionally, the cross-sectional nature of the study precludes any causal inference between PIU and health-related behaviors. Adolescents with disturbed sleep or poor dietary habits may be more vulnerable to excessive internet use, rather than the reverse.

Despite these limitations, Çelik et al.[1] provide valuable evidence highlighting PIU as a prevalent condition intertwined with modifiable health behaviors. Future research, expanding internet use to include specific online activity types and a holistic examination of a key health behavior, sleep problems, would help clarify mechanisms and guide more nuanced interventions.

**Source of funding**

The authors declare the study received no funding.

**Conflict of interest**

The authors declare that there is no conflict of interest.

## REFERENCES

1. Çelik E, Oflu A, Bükülmez A. Association of problematic internet use with health-related daily habits in adolescents: evidence from a school-based survey. Turk J Pediatr 2025; 67: 473-482. https://doi.org/10.24953/turkjpediatr.2025.5850
2. Moreno M, Riddle K, Jenkins MC, Singh AP, Zhao Q, Eickhoff J. Measuring problematic internet use, internet gaming disorder, and social media addiction in young adults: cross-sectional survey study. JMIR Public Health Surveill 2022; 8: e27719. https://doi.org/10.2196/27719
3. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res 1989; 28: 193-213. https://doi.org/10.1016/0165-1781(89)90047-4

The Turkish Journal of Pediatrics 2025; 67: 929-930
https://doi.org/10.24953/turkjpediatr.2025.7607



# Response to the letter to the editor: "Expanding the health-related behavior perspective on problematic internet use in adolescents"

Esra Çelik[1], Ayşe Oflu[1], Ayşegül Bükülmez[1]

[1]*Department of Pediatrics, Faculty of Medicine, Afyonkarahisar Health Sciences University, Afyonkarahisar, Türkiye.*

To the Editor,

We sincerely thank the author for the constructive comments[1] on our article titled "Association of problematic internet use with health-related daily habits in adolescents: evidence from a school-based survey".[2] We believe that the presented criticisms will shed light on future studies on similar topics.

The author emphasizes the importance of assessing not only the duration of use but also the reason adolescents use the internet and the activities they engage in. The specific purpose of focusing on internet usage duration in the current study was that we anticipated increased usage duration as the primary problem. Considering this shortcoming of our study, we emphasized the importance of examining variables such as adolescents' internet use purposes, motivations, and which devices they prefer in the limitations section. Although a recent study found that those who primarily spent time on social media or games reported significantly higher levels of problematic internet use compared to their peers who used the internet primarily for academic purposes[3], cohort studies are needed to establish a causal relationship between problematic internet use (PIU) and both usage duration and content.

We find the author's recommendation to use standardized measurement tools to assess daily health habits warranted. We previously discussed this issue in the limitations section of our study. In the current study, sleep problems were assessed with symptom-based items; however, future research could obtain more precise and standardized data by employing validated instruments such as the Pittsburgh Sleep Quality Index (PSQI).[4]

Furthermore, it is plausible to speculate that PIU and other health behaviors may arise from the disruption of common underlying mechanisms, such as impaired self-regulation or circadian misalignment. Our findings support the idea that PIU often co-occurs with multiple lifestyle disorders rather than existing alone. We also agree with the author regarding the limitations of our study's cross-sectional nature regarding causation. While our study findings suggest an association between PIU and health-related daily habits, causality cannot be inferred. Longitudinal studies are needed to clarify these bidirectional relationships, such as whether PIU triggers these negative habits or whether the presence of these habits leads to PIU.

We appreciate both the author's methodological considerations and insightful suggestions for future research. A detailed evaluation of internet and other digital addictions, not only in terms of duration but also in terms of content, and examining health-related variables with

✉ Ayşe Oflu ▪ ayseoflu@gmail.com

Received 3rd Dec 2025, accepted 8th Dec 2025.

validated measurement tools, will contribute to a more comprehensive understanding of PIU among adolescents.

**Source of funding**

The authors declare the study received no funding.

**Conflict of interest**

The authors declare that there is no conflict of interest.

**REFERENCES**

1. Kalkan EŞ. Expanding the health-related behavior perspective on problematic internet use in adolescents. Turk J Pediatr 2025; 67: 927-928. https://doi.org/10.24953/turkjpediatr.2025.7318
2. Çelik E, Oflu A, Bükülmez A. Association of problematic internet use with health-related daily habits in adolescents: evidence from a school-based survey. Turk J Pediatr 2025; 67: 473-482. https://doi.org/10.24953/turkjpediatr.2025.5850
3. Osser B, Toth C, Nistor-Cseppento CD, Cevei M, Aur C, Orodan M, Fazakas R, Bondar LI. Predictors of problematic internet use among Romanian high school students. Children (Basel) 2025; 12: 1292. https://doi.org/10.3390/children12101292
4. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res 1989; 28: 193-213. https://doi.org/10.1016/0165-1781(89)90047-4

# TRIBUTE TO REVIEWERS FOR VOLUME 67 (2025)

Turkish Journal of Pediatrics relies on reviewers for their generous donations of time, effort and expertise. We thank them for their great contribution to maintaining the high standards of the Journal.

Banu Acar
Tuğba Acer Demir
Dilber Ademhan Tural
Ayşe Ağbaş
Merve Aka
Selin Akbaş Aliyev
İzzet Türkalp Akbaşlı
Özlem Akbulut
Abdullah Barış Akcan
Elif Akcay
Devrim Akdemir
Zülfikar Akelma
Hediye Seval Akgün
Ayşe Aksoy
Özlem Yüksel Aksoy
Tekin Aksu
Yakut Akyön Yılmaz
Meryem Albayrak
Bilge Aldemir Kocabaş
Didem Aliefendioğlu
Derya Altay
Cansu Altuntaş
Nilgün Altuntaş
Murat Anıl
Banu Anlar
Deniz Anuk İnce
Didem Ardıçlı
Iskandar Arfan
Ebru Arhan
Emre Arı
Hatice Feray Arı
Zeynep Arıkan Ayyıldız
Duran Arslan
Melike Arslan
Nur Arslan
İlknur Arslanoğlu
Seçil Arslansoyu Çamlar
Deniz Aslan
Fatma Pınar Atasayar
Nursel Atay Ünal
Bahriye Atmış
Pınar Özge Avar Aydın
Gülhadiye Avcu
Canan Ayabakan
Yusuf Aydemir
Beril Aydın
Orkun Aydın
Yeşim Aydınok
Seda Aydoğan
Canan Aygün
Fatma Deniz Aygün
Demet Aygün Arı
Kübra Aykaç
Hayrettin Hakan Aykan
Ebru Aypar
Emine Azak
Müjdem Nur Azılı
Özlem Bağ
Necati Balamtekin
Demet Baltu
Zeren Barış
Kenan Barut
Kübra Baskın
Ahmet Yağmur Baş
Ahmet Baştürk
Ezgi Deniz Batu
Turan Bayhan
Nevzat Aykut Bayrak
Tuğba Bedir Demirdağ
Ayşen Bingöl
Emine Bahar Bingöller Pekcici
Koray Boduroğlu
Mehmet Boşnak
Özlem Boybeyi Türer
Ceyhun Bozkurt
Murat Fani Bozkurt
Elif Böncüoğlu
Selda Fatma Bülbül
Ayşe Büyükcam
Bahar Büyükkaragöz
Gönül Büyükyılmaz
Mert Canbaz
Fuat Emre Canpolat
Özlem Cavkaytar
Tülin Tıraje Celkan
Ali Bülent Cengiz
Burcu Ceylan Cura Yayla
Yeliz Çağan Appak
Mustafa Çakan
Özlem Çakıcı
Selen Ceren Çakmak
Gönül Çaltepe
Solmaz Çelebi
Mehmet Çeleğen
Tuba Çelen Yoldaş
Hasan Tolga Çelik
Melda Çelik
Yusuf Selman Çelik
İbrahim İlker Çetin
Merih Çetinkaya
Merve Çıkılı Uytun
Güle Çınar
Tuğrul Çiçek
Nazan Çobanoğlu

Cem Çöteli
Bahar Çuhacı Çakır
Hüseyin Dağ
Aydan Değerliyurt
Arzu Meltem Demir
Dilan Demir
Ercan Demir
Osman Oğuz Demir
Selcan Demir
Metin Demirkaya
Demet Demirkol
Kaan Demirören
Duygu Demirtaş
Uğur Deveci
İlker Devrim
Özlem Dikmetaş Sandıkçı
Berat Dilek Demirel
Dilek Dilli
Handan Dinçaslan
H. Anıl Dinçer
Meltem Direk
Emre Divarcı
Eser Doğan
Güzide Doğan
Hasan Serkan Doğan
Yücel Duman
İbrahim Ece
Öznur Ege Akdi
Ödül Eğritaş Gürkan
İbrahim Eker
Murat Elli
Dicle Emet
Tuba Eminoğlu
Nagehan Emiralioğlu
Yeşim Er Öztaş
Ela Erdem Eralp
İlkay Erdoğan
Tuğba Erener Ercan
Ebru Ergenekon
Yakup Ergül
Burcu Ersöz Alan
Ayşegül Ertuğrul
İlker Ertuğrul
Makbule Esen Öksüzoğlu
Saliha Esenboğa
Şükran Gülin Evinç
Ali Fettah
Zeynep Gökçe Gayretli Aydın
Dildar Bahar Genç
Mustafa Gençeli
Pınar Gençpınar
İlknur Girişgen
Saniye Girit
Gökhan Göçmen
Zehra Gök Metin
Gülden Gökçay
Yasemin Gökdemir
Bora Gülhan
Ayşe Gültekingil
Abdülgani Gülyüz
Ersin Gümüş
Yasemin Dere Günal
Ceren Günbey
Mehmet Gündüz
Nilay Güneş
Mesut Güngör
Ayla Günlemez
Cüneyt Günşar
Ayşenur Gür
Pınar Gürkaynak
Dilek Gürlek Gökçebay
Semra Gürsoy
Burcu Güven
Serçin Güven
Göknur Haliloğlu
Selda Hançerli Törün
Melih Hangül
Zehra Şule Haskoloğlu
Volkan Hazar
Sevinç Hepkarşı
Hayriye Hızarcıoğlu Gülşen
İbrahim Murat Hirfanoğlu
Tuğba Hirfanoğlu
Ayşe Hitay İnan
Fatma Ilgaz
Emel Isıyel
Özkan İlhan
Zeynep İnce
Sonay İncesoy Özdemir
Ahmet Çağkan İnkaya
Mihriban İnözü
İrem İyigün
Candaş Kafalı
Eylem Şerife Kalkan
Sema Kalkan
Emine Serra Kamer
Aydan Kansu
Bülent Kara
Cengiz Kara
Mahmut Zabit Kara
Manolya Kara
Soner Sertan Kara
Betül Karaatmaca
Mehmet Karacan
Kıvılcım Karadeniz Cerit
Heves Karagöz
Güngör Karagüzel
Taliha Karakök
Cemşit Karakurt
Ayşe Karaman
Serap Karaman
Yiğitcan Karanfil

Kenan Karbeyaz
Asuman Nur Karhan
Gökçen Kartal Öztürk
Aslı Kavaz Tufan
Hamide Kaya
Zühre Kaya
Ümmüşen Kaya Akca
Gülşah Kaya Aksoy
Rejin Kebudi
Mehmet Keskin
Shrayash Khare
Buket Kılıç
Mustafa Kılıç
Gonca Kılıç Yıldırım
Ayşe Ayzıt Kılınç Sakallı
Ezgi Kıran Taşçı
Serkan Kırık
Merve Kişioğlu
Ülker Koçak
Meltem Koloğlu
Meda Kondolot
Bahadır Konuşkan
Muammer Osman Köksal
Yavuz Köksal
Mustafa Kömür
Büşra Köseoğlu
Zarife Kuloğlu
Eda Didem Kurt Şükür
Aylin Irmak Kuruç
Arif Kut
Nurettin Onur Kutlu
Semanur Kuyucu
Osman Küçükosmanoğlu
Serhan Küpeli
İncilay Lay
Emre Leventoğlu
Balahan Makay
Ece Mekik
Ayşe Mete Yeşil
Ayşe Metin
Cem Mocan
Akmer Mutlu
Kevser Nalbant
Halime Nayır Büyükşahin
Melike Ocak
Süheyla Ocak
Berna Oğuz
Pelin Oğuzkurt
Arzu Okur
Lale Olcay
Nihal Olgaç Dündar
Diclehan Orhan
Hüsnü Fahri Ovalı
Yeşim Oymak
Alkım Öden Akman
Hakan Öğütlü
Lütfiye Öksüz
Emel Ömercioğlu
Yurday Öncül
Hale Ören
Emel Örün
Utku Arman Örün
Gökçen Öz Tunçer
Ezgi Özalp Akın
Deniz Özalp Kızılay
Fatih Özaltın
Nazan Özbarlas
Hatice Nursun Özcan
Rahşan Özcan
Lütfiye Hilal Özcebe
Deniz Özçeker
Hayriye Uğur Özçelik
Semanur Özdel
Ali Özdemir
Cevdet Özdemir
Gül Nihal Özdemir
Gülşah Özdemir
Özmert Özdemir
Yusuf Emre Özdemir
Esra Özek Yücel
Bilge Özgör
Rıza Köksal Özgül
Senem Özgür
Özlem Özgür Gündeşlioğlu
Hatice Asuman Özkara
Canan Özlü
Zeynep Alev Özön
Hakan Öztürk
Kübra Öztürk
Zeynel Abidin Öztürk
Gökhan Özyiğit
Derya Özyörük
Ayşenur Paç Kısaarslan
Ömür Mustafa Parkan
Oytun Portakal
Şükran Poyrazoğlu
Cansın Saçkesen
Hakan Salman
İnci Nur Saltık Temizel
Sinan Sarı
Serdar Ümit Sarıcı
Özlem Sarıtaş Nakip
Seha Kamil Saygılı
Eylem Ulaş Saz
Esra Serdaroğlu
Oğuzhan Serin
Ahmet Soysal
Fatma Müjgan Sönmez
Oğuz Söylemezoğlu
Ramona Florina Stroescu
Hilal Susam Şen
Serra Sürmeli Döven

Özge Sürmeli Onay
Fetih Furkan Şahin
Murat Şahin
Nihal Şahin
Sezgin Şahin
Bülent Enis Şekerel
Nesrin Şenbil
Aydın Şencan
Gülser Şenses Dinç
Zeynep Şıklar
Pelin Özlem Şimşek Kiper
Tuğba Şişmanlar Eyüboğlu
Yılmaz Tabel
Nurdan Taçyıldız
Cansaran Tanıdır
Sevgen Tanır Başaranoğlu
Elif Seren Tanrıverdi
Hikmet Gülşah Tanyıldız
Demet Taş
Zihni Ekim Taşkıran
Tuğba Taştemel Öztürk
Hande Taylan Şekeroğlu
Serap Teber
Kadir Şerafettin Tekgündüz
Leman Tekin Orgun
Abdulkerim Temiz
Sibel Tiryaki
Haluk Topaloğlu
Hacer Topcu
Ali Evren Tufan
Deniz Tuğcu
Varol Tunalı
Özden Turan
Serap Turan
Özlem Tüfekçi
Canan Türkyılmaz
Filiz Tütüncüler Kökenli
Zeynep Tüzün Gün
Berna Uluğ
Çiğdem Ulukaya Durakbaşa
Ayşen Uncuoğlu
Nurdan Uraş
İdil Rana User
Nuray Uslu Kızılkan
Canan Aslı Utine Yıldırım
Hatice Uygun
Sabide Duygu Uygun
Metin Uysalol
Ekrem Ünal
Fatih Ünal
Sezin Ünal
Özlem Ünal Uzun
Aysel Ünlüsoy Aksu
Yağmur Ünsal
Ayşegül Ünüvar
Gizem Ürel Demir
Zeynep Üstünyurt
Ali Varan
Birgül Varan
Ganiye Begül Yağcı
Yusuf Yakupoğulları
Mehmet Yalaz
Bilgehan Yalçın
Elmas Ebru Yalçın
Hatice Sonay Yalçın Cömert
Hüsniye Neşe Yaralı
Coşkun Yarar
Sevgi Yaşar Durmuş
Güliz Fatma Yavaş
Sinan Yavuz
Nalan Yazıcı
Sibel Yel
Edanur Yeşil
Osman Yeşilbaş
Miraç Yıldırım
Ülkü Miray Yıldırım
Işıl Yıldırım Baştuhan
Adalet Elçin Yıldız
Mehmet Yıldız
Melek Yıldız
Dinçer Yıldızdaş
Arzu Yılmaz
Gonca Yılmaz
Kutluhan Yılmaz
Özge Yılmaz
Resul Yılmaz
Sine Yılmaz
Songül Yılmaz
Şebnem Yılmaz
Deniz Yılmaz Karapınar
Şule Yiğit
Murat Yurdakök
Ali Yurtseven
Aylin Yücel
Hasan Yüksel
Selçuk Yüksel
Emine Zengin

## SHORT COMMUNICATION



## CASE REPORTS



## LETTERS TO THE EDITOR




Printed by METEKSAN in Ankara, Turkey