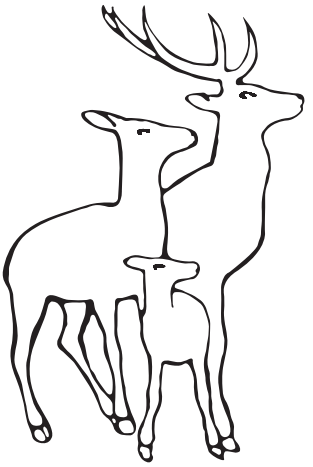
ISSN 0041-4301
Online ISSN 2791-6421
www.turkishjournalpediatrics.org

# THE TURKISH JOURNAL OF PEDIATRICS

ORIGINAL ARTICLES



volume 66
issue 5
September-October 2024



# THE TURKISH JOURNAL OF PEDIATRICS

www.turkjpediatr.org

**Volume 66 ▪ Issue 5**
**September-October 2024**

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

# THE TURKISH JOURNAL OF PEDIATRICS

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

Cilt: 66 Sayı: 5, Eylül-Ekim 2024



**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:** XX.XX.2024

**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: 66 Issue: 5, September-October 2024



**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:** XX.XX.2024

**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, CABI Abstracts, ProQuest, EBSCOhost, BIOSIS Previews, Türkiye Citation Index and TR-Index.

# CONTENTS

VOLUME: 66 ISSUE: 5 SEPTEMBER-OCTOBER 2024

ORIGINAL ARTICLES

# CONTENTS

VOLUME: 66 ISSUE: 5 SEPTEMBER-OCTOBER 2024

## SHORT COMMUNICATION



## COMMENTARY



## CASE REPORTS

The Turkish Journal of Pediatrics 2024; 66: 511-524
https://doi.org/10.24953/turkjpediatr.2024.5300

**Original Article**

# Differences in physical activity, sedentary time, and anthropometric variables among children and adolescents: The TUBON project

Necip Demirci[1], Ayda Karaca[1], Emine Çağlar[2], Pelin Aksen[3], Nigar Küçükkubaş[4], Mehmet Mesut Çelebi[5], Erdem Karabulut[6], Gıyasettin Demirhan[7], Sadettin Kirazcı[8], Elif Nursel Özmert[9]

*[1]Division of Physical Activity and Health, Department of Recreation, Faculty of Sport Sciences, Hacettepe University, Ankara; [2]Division of Physical Education and Sports for the Disabled, Department of Physical Education and Sports Teaching, Faculty of Sport Sciences, Hacettepe University, Ankara; [3]Division of Movement and Training Sciences, Department of Coaching Education, Faculty of Sport Sciences, Kırıkkale University, Kırıkkale; [4]Department of Recreation, Faculty of Sport Sciences, Yalova University, Yalova; [5]Department of Sports Medicine, Faculty of Medicine, Ankara University, Ankara; [6]Department of Biostatistics, Faculty of Medicine, Hacettepe University, Ankara; [7]Division of Physical Education and Sports, Department of Physical Education and Sports Teaching, Faculty of Sport Sciences, Hacettepe University, Ankara; [8]Department of Physical Education and Sports, Faculty of Education, Middle East Technical University, Ankara; [9]Division of Developmental Pediatrics, Department of Pediatrics, Faculty of Medicine, Hacettepe University, Ankara, Türkiye.*

## ABSTRACT

**Background.** Although physical inactivity may lead to increasing obesity prevalence, research on anthropometric variables changes based on physical activity (PA) in children and adolescents is limited. PA decreases with age, while sedentary behavior increases. The study aimed to examine differences in objectively measured sedentary time, light-intensity physical activity (LPA), and moderate-to-vigorous intensity physical activity (MVPA) between children and adolescents, and the differences in the percentiles of anthropometric variables between physically active and inactive groups according to World Health Organization PA recommendations.

**Methods.** A total of 759 participants aged 6-17 years (boys, n=358; girls, n=401) were included in the study. The ActiGraph wGT3x-BT accelerometer was used to measure sedentary time, LPA, and MVPA. Height, weight, waist circumference (WC), triceps skinfold thickness (T-SFT), and medial-calf skinfold thickness (M-SFT) were measured. Body fat percentage (BF%) and body mass index (BMI) were calculated, and the percentiles of anthropometric variables were categorized.

**Results.** The findings showed that children had less sedentary time and a higher LPA than adolescents for both genders ($p<0.05$). Children had a higher MVPA than adolescents in girls ($p<0.05$), but the difference was insignificant in boys ($p>0.05$). In boys, physically active children were in lower percentiles for T-SFT and BF% than those who did not ($p<0.05$). In boys, adolescents who were physically inactive were in higher percentiles for BMI, T-SFT, M-SFT, and BF% ($p<0.05$). In addition, in girls, adolescents who were physically active were in lower percentiles of BMI, M-SFT, and BF%, whereas children who were physically active were in lower percentiles of M-SFT and BF% ($p<0.05$).

**Conclusion.** Sedentary time increases while PA decreases with age. Children and adolescents who met the WHO PA recommendation had lower percentiles of anthropometric variables, indicating the importance of PA in preventing obesity in these age groups.

**Key words:** physical activity, sedentary time, anthropometry, children, adolescents.

✉ Necip Demirci ▪ necip.dmrc@gmail.com

Received 8th Sep 2024, revised 21st Oct 2024, accepted 29th Oct 2024.

Children and adolescents should do at least 60 minutes per day of moderate-to-vigorous intensity physical activity and bone-strengthening activities at least three days a week.[1] Also, replacement of sedentary time with light-intensity physical activity and moderate-to-vigorous intensity physical activity is recommended for health benefits.[1] Sedentary behavior is any activity performed while sitting, lying down, or reclining and characterized by an energy expenditure of 1.5 metabolic equivalents (METs) or less. Light-intensity physical activity is between 1.5 and 3 METs, while moderate-to-vigorous intensity physical activity includes intensities above 3 METs.[1] Physical activity is crucial for improving cardiometabolic and bone health, and body composition in children and adolescents.[2,3] Moderate-to-vigorous intensity physical activity may reduce body fat in individuals with high body fat[4], potentially preventing obesity in children and adolescents.[1] Sedentary time is positively associated with overweight and obesity.[5] Increased sedentary time negatively impacts body mass index (BMI), waist circumference (WC), and fat mass index.[6,7] Strategies to prevent excessive adiposity in children and adolescents include increasing light-intensity physical activity with reduced sedentary time.[8] Research has highlighted light-intensity physical activity due to its role in reducing sedentary behavior and increasing physical activity levels.[9,10] Light-intensity physical activity is an important part of daily physical activity and is characterized by the borderline of sedentary time and moderate-to-vigorous intensity physical activity. Although light-intensity physical activity accounts for the majority of daily physical activity and contributes to an increase in daily energy expenditure, more research is needed on its beneficial effects on health.[9]

Jiménez-Pavón et al.[11] showed that higher physical activity reduced fat mass in adolescents. Conversely, conflicting results exist regarding the effects of physical activity on obesity indicators such as BMI and fat mass.[12] A review by Janssen and Leblanc[13] found weak associations between physical activity and obesity in school-aged children. Additionally, studies on changes in anthropometric variables based on objectively measured physical activity and sedentary time in prepubertal children are limited.[6] Further research is needed to understand the difference in anthropometric variables based on physical activity. Current physical activity guidelines have several limitations, such as self-reported assessment and inadequate addressing of cardiovascular disease risk markers.[14] Accordingly, there is insufficient evidence to fully define dose-response relationships between physical activity and health outcomes.[15] To the best of our knowledge, few studies have compared anthropometric variables in children based on physical activity recommendations. Further research is needed also on the percentiles of anthropometric variables relative to physical activity guidelines.

Previous studies suggest a decline in physical activity and an increase in sedentary behavior from childhood to adolescence.[16,17] Although it is suggested that the most significant decline occurs during adolescence, evidence indicates that it may occur earlier.[4] Identifying adolescents based on biological maturation is critical, but age-based identification is often used. The World Health Organization (WHO) classifies adolescents as those aged 10 to 19 within the broader category of children aged 5 to 19 years.[18] However, discrepancies between adolescent and childhood age ranges in physical activity and sedentary time research[16,19] suggest the need for further studies using age definitions of the WHO. Taken together with this rationale, it seems important to compare obesity indicators according to whether the physical activity recommendation is met or not, which is limited in the literature. Therefore, the study aimed to compare objectively measured sedentary time and physical activity levels according to the age groups of children and adolescents and to compare the percentiles of anthropometric variables according to whether they met physical activity recommendations in children and adolescents.

## Materials and Methods

### *Participants*

The study included 891 healthy children and adolescents aged 6 to 17 years from the provinces of Ankara, Kırıkkale, Bartın, Ordu, Eskişehir, Antalya, İzmir, İstanbul, Batman, Mardin, Van, and Ağrı, representing 11 of the 12 regions of Türkiye from 27th September 2022, through 3rd June 2023, according to the Nomenclature of Territorial Units for Statistics Level 1 (NUTS-1). However, 132 participants who did not meet the wearing criteria for accelerometer data were excluded; therefore, the present study included 759 healthy children and adolescents. Participants with physical, visual, hearing, or intellectual disabilities, chronic illnesses, and an electronic or other medical implant in their body were excluded from the study. The data for this study were obtained from the TUBON project (see https://tubon-projesi.hacettepe.edu.tr/tr) funded by the Scientific and Technological Research Council of Türkiye (TÜBİTAK, project number: SBAG 120S408). The sample of TUBON project was randomly selected from twelve provinces, including primary, secondary, and high schools affiliated to the Ministry of National Education of the Republic of Türkiye. In the sampled schools, the classes for each grade were listed. From each grade, the classes were randomly selected using simple random sampling. A list of students in the selected classes was prepared, and 10% of the list of participants recruited by stratified random sampling by gender was randomly selected. The accelerometers were only worn by approximately a 10% subgroup (n=759) of the TUBON project, not the whole sample (n=7659), due to the high cost of the ActiGraph wGT3x-BT. Therefore, the sample of this study consists of the accelerometer data collected from approximately 10% of the sample of the TUBON project. The study was conducted in accordance with the ethical standards of the Declaration of Helsinki, and participants and their parents signed an informed consent form. This study was approved by the Non-Interventional Clinical Research Ethics Board of Hacettepe University.

### *Data collection*

The researchers provided participants with consent and demographic information forms. Participants who agreed to participate and had parental consent completed the health information forms. Then participants were asked to wear the accelerometers. Researchers explained the purpose of the study and tests, and the physician examined the participants before conducting anthropometric measurements.

### *Demographic and health information forms*

Demographic information was collected from participants and their parents. The parents filled out demographic information forms for themselves. Parents also filled out the form for their 6 and 10-year-old children, and children over 10 filled it out for themselves. The form asked about the participants' history of cyanosis, palpitations, and dizziness during physical activity and any family history of sudden death under the age of 45.

### *Anthropometric measurements*

Participants were instructed to wear light, comfortable clothes before the anthropometric measurements. Height, sitting height, and body weight were measured twice, with the highest value used for analysis. Skinfold thickness measurements were also performed twice, with a third measurement if the difference between two measurements was greater than 10%.

Height and sitting height were measured with a portable stadiometer (SECA 217, Germany) to 0.01 m accuracy. Height was measured with subjects standing without shoes, after a deep inhalation, and with the head in the Frankfort plane.[20] Body weight was measured to the nearest 0.1 kg without shoes using bioelectrical impedance analysis (Tanita MC580, Japan).[20] BMI was calculated (kg/m$^2$) based on height and weight.

The study utilized a Holtain skinfold caliper (Holtain Ltd, Crymych, United Kingdom) to measure triceps skinfold thickness (T-SFT) and medial-calf skinfold thickness (M-SFT), with each measurement taken twice and averaged. T-SFT was assessed at the midline of the upper arm, between the acromion and olecranon processes.[21] For M-SFT, the medial (inside) of the calf was marked for the maximal circumference measurement. A vertical skinfold was grabbed and measured with a caliper approximately 1 cm proximal to the specified point.[20,22] Body fat percentage (BF%) was estimated using equations by Slaughter et al.[23] for children aged 8–18 years and Dezenberg et al.[24] for children under eight years.

The equations are as follows:

For children aged 8–18 years:

[*Males* = 0.735 *(triceps+calf)*+1.0*), Females* = 0.610 *(triceps+calf)*+5.1]

For children under eight years of age:

(0.342**body weight*+0.256* *triceps*+0.837**gender*-7.388)

WC was measured to the nearest 0.1 cm at the narrowest part of the waist using a Gullick meter and recorded in centimeters.[20,21] WC were performed twice, and the average of the two measurements was used in the statistical analysis.

In this study, BMI and WC percentiles were derived from the Centers for Disease Control and Prevention's (CDC) Anthropometric Reference Data for Children.[25] T-SFT and M-SFT percentiles were based on the study reference by Cicek et al.[26] and Kuhle et al.[27], respectively. BF% percentiles were based on Soylu et al.'s data for Turkish children and adolescents.[28] BMI was categorized as underweight (<5th percentile), healthy weight (5th-84th percentile), overweight (85th-94th percentile), and obese (≥95th percentile)[29], but overweight and obese combined into ≥85th percentile. WC, T-SFT, and BF% were categorized as <5th, 5th-84th, and ≥85th percentiles, while M-SFT was categorized into <3rd, 10th-74th, and ≥75th percentiles.

### *Determination of physical activity and sedentary behavior*

Participants' physical activity and sedentary time were objectively assessed using ActiGraph wGT3x-BT triaxial accelerometers (ActiGraph LLC, Pensacola, FL, USA). Children and adolescents were asked to wear the ActiGraph wGT3x-BT for seven consecutive days and were also instructed to wear an accelerometer attached to an elastic belt on the right hip. Participants and their parents were informed that the accelerometer should not be removed except for bathing and swimming. However, participants who did not want to wear the accelerometer during sleep were allowed to remove it. The ActiGraph wGT3X-BT was set to collect raw acceleration data at 30 Hz using ActiLife software (version 6.13.3).

Accelerometer data were analyzed using 15-second epochs. A valid day (wear time) was defined as ≥480 min-day-1 (8 h-day$^{-1}$). Accelerometer data from children and adolescents that included at least three valid weekdays and one valid weekend day were eligible for inclusion in the study.[30] Non-wear time was defined as a minimum of 60 consecutive minutes of zero counts, allowing for 2 minutes of counts between 0 and 100.[31] The cut-off points of Evenson et al.[32] were chosen to define sedentary time as <100 counts per minute (cpm), light physical activity as 101–2295 cpm, moderate physical activity as 2296–4011 cpm, and vigorous physical activity as ≥4012 cpm. As a result of data processing, sedentary time per day, light-intensity physical activity, and moderate-to-vigorous intensity physical activity were obtained for this study.

### *Statistical analyses*

Results are presented as percentages, counts, and means ± standard deviations. Normality was assessed using skewness and kurtosis tests, and variance equality was evaluated with Levene's test. According to WHO guidelines, adolescents are defined as individuals aged 10-19 years.[33] Therefore, participants aged

6-9 years were categorized as children and those aged 10-17 years as adolescents. An independent-sample t-test was used to compare sedentary time, light-intensity physical activity, and moderate-to-vigorous intensity physical activity durations between age groups for both genders, following validation of normality and homogeneity assumptions. The significance level was set at $p<0.05$. Effect sizes were calculated using Cohen's d. Effect sizes (Cohen's d) are classified as small (d = 0.2), medium (d = 0.5), and large (d ≥ 0.8).[34] The missing data for anthropometric variables (26 participants) is attributable to those who did not participate in the anthropometric measurements among the 759 participants wearing accelerometers. For children and adolescents aged 5-17, a daily 60-minute moderate-to-vigorous intensity physical activity is recommended.[1] Participants were classified into two groups: those who met the 60-minute moderate-to-vigorous intensity physical activity guideline (active) and those who did not (inactive). A chi-square test was used to compare the percentiles of anthropometric variables between those meeting and not meeting the moderate-to-vigorous intensity physical activity guideline for both genders.

## Results

Participant characteristics, including age, the percentiles of anthropometric variables, sedentary time, and physical activity, are presented in Table I. In addition, the comparison of sedentary time and physical activity levels between children and adolescents (different age groups) is presented in Fig. 1 for both genders. The comparison of anthropometric variables' percentiles according to whether or not they meet the physical activity recommendation in both boys and girls is shown in Tables II and III, respectively.

There were statistical differences in sedentary time ($p$=0.001; Cohen's $d$=0.99) and light-intensity physical activity ($p$=0.001; Cohen's $d$=0.13) between children in the 6-9 age group and adolescents in the 10-17 age group in boys (Fig. 1). The children's group had a significantly lower sedentary time than the adolescents for both genders, while they had a significantly higher light-intensity physical activity than adolescents (Fig. 1). No significant differences in moderate-to-vigorous intensity physical activity were found between children and adolescent boys. There were significant differences between children and adolescents in sedentary time ($p$=0.001; Cohen's $d$=0.85), light-intensity physical activity ($p$=0.001; Cohen's $d$=0.15), and moderate-to-vigorous intensity physical activity ($p$=0.001; Cohen's $d$=0.36) for girls (Fig. 1). Children had a significantly lower sedentary time and a higher level of light-intensity physical activity and moderate-to-vigorous physical activity than adolescents (Fig. 1).

Chi-square analysis showed that boys aged 6-9 years who did less than 60 minutes of moderate-to-vigorous intensity physical activity had a higher percentage of ≥85th percentiles for T-SFT and BF% than those who did 60 minutes or more, whereas those who did 60 minutes or more of moderate-to-vigorous intensity physical activity had a higher percentage of <5th and 5th-84th percentiles for T-SFT and BF% than those who did less than 60 minutes of moderate-to-vigorous intensity physical activity, ($p$<0.05, Table II). Conversely, the difference was insignificant for BMI, WC, and M-SFT percentiles. Among boys aged 10-17 years, those who did less than 60 minutes of moderate-to-vigorous intensity physical activity had a higher percentage of ≥85th percentile for BMI, T-SFT, M-SFT, and BF% than those who did 60 minutes or more, while those who did 60 minutes or more had a higher percentage of <5th and 5th-84th percentiles for BMI, T-SFT, M-SFT, and BF% than those who did less than 60 minutes of moderate-to-vigorous intensity physical activity ($p$<0.05, Table II).

Girls aged 6-9 years who did less than 60 minutes of moderate-to-vigorous intensity physical activity had a higher percentage of 5th-84th and ≥85th percentiles for BF% than

**Table I.** Descriptive characteristics of the participants.

| Variables | Boys | | | | Girls | | | |
|---|---|---|---|---|---|---|---|---|
| | Children (age 6-9) | | Adolescents (age 10-17) | | Children (age 6-9) | | Adolescents (age 10-17) | |
| | n | Mean±SD | n | Mean±SD | n | Mean±SD | n | Mean±SD |
| Height (cm) | 122 | 131.44±8.29 | 213 | 163.06±13.99 | 114 | 130.66±7.89 | 266 | 155.86±9.24 |
| Body weight (kg) | 124 | 30.70±9.24 | 218 | 55.69±16.48 | 115 | 28.74±6.72 | 274 | 50.19±11.75 |
| Age (year) | 128 | 8.32±1.02 | 230 | 13.84±2.28 | 119 | 8.31±1.05 | 282 | 13.90±2.27 |
| BMI percentiles | | | | | | | | |
| <5th | 11 | 13.58±0,59 | 13 | 14.33±1.94 | 15 | 13.19±0.81 | 31 | 15.35±1.64 |
| 5th-84th | 93 | 16.88±2.20 | 174 | 20.31±2.94 | 87 | 16.71±1.87 | 227 | 20.88±2.90 |
| ≥85th | 17 | 24.12±4.56 | 22 | 28.21±3.56 | 10 | 22.34±1.94 | 6 | 30.26±3.95 |
| WC percentiles | | | | | | | | |
| <5th | 19 | 49.46±2.68 | 29 | 58.04±4.98 | 30 | 49.01±3.01 | 110 | 59.40±4.70 |
| 5th-84th | 103 | 59.54±6.92 | 184 | 70.80±7.09 | 84 | 57.86±5.28 | 159 | 68.29±6.46 |
| ≥85th | 3 | 74.07±4.60 | 6 | 93.43±5.93 | 3 | 74.52±9.69 | 2 | 95.53±4.00 |
| T-SFT percentiles | | | | | | | | |
| <5th | 6 | 4.58±0.77 | 7 | 4.63±0.84 | 3 | 5.63±0.55 | 7 | 6.74±0.54 |
| 5th-84th | 70 | 9.16±2.26 | 146 | 9.62±2.96 | 66 | 10.94±2.58 | 196 | 14.68±3.87 |
| ≥85th | 49 | 19.54±4.62 | 66 | 23.17±4.71 | 47 | 19.87±4.19 | 69 | 25.62±4.03 |
| M-SFT percentiles | | | | | | | | |
| <3rd | 10 | 4.60±0.50 | 24 | 5.52±1.93 | 7 | 5.46±0.56 | 19 | 7.20±1.01 |
| 10th-74th | 72 | 8.61±2.15 | 126 | 9.73±4.22 | 62 | 10.03±2.04 | 155 | 13.79±3.37 |
| ≥75th | 43 | 19.85±4.57 | 69 | 23.82±5.59 | 45 | 18.24±4.90 | 97 | 24.64±4.16 |
| BF% percentiles | | | | | | | | |
| <5th | 58 | 5.43±3.21 | 26 | 8.99±1.32 | 40 | 5.64±2.45 | 0 | 0±0 |
| 5th-84th | 39 | 17.47±3.58 | 116 | 14.86±3.30 | 51 | 19.05±3.62 | 108 | 18.91±3.14 |
| ≥85th | 28 | 32.74±5.55 | 77 | 34.40±7.35 | 24 | 30.69±5.42 | 163 | 30.90±5.85 |
| Sedentary time (min/day) | 128 | 594.91±178.91 | 230 | 784.08±202.77 | 119 | 636.45±184.47 | 282 | 801.23±202.15 |
| LPA (min/day) | 128 | 283.69±51.83 | 230 | 215.92±52.92 | 119 | 288.95±58.11 | 282 | 208.09±51.88 |
| MVPA (min/day) | 128 | 63.58±20.85 | 230 | 65.43±25.14 | 119 | 48.16±17.02 | 282 | 41.91±18.18 |
| Wear time (min/day) | 128 | 941.25±199.47 | 230 | 1061.68±208.91 | 119 | 974.45±209.15 | 282 | 1045.97±218.23 |

BF%, body fat percentage; BMI, body mass index; LPA, light-intensity physical activity; M-SFT, medial-calf skinfold thickness; MVPA, moderate-to-vigorous intensity physical activity; SD, standard deviation; T-SFT, triceps skinfold thickness; WC, waist circumference.

those who did 60 minutes or more. Those who did 60 minutes or more had a higher percentage of <5th percentile for BF% than those who did less than 60 minutes of moderate-to-vigorous intensity physical activity ($p$<0.05, Table III). Conversely, the difference was insignificant for BMI, WC, T-SFT, and M-SFT percentiles. Girls aged 10-17 years who did less than 60 minutes of moderate-to-vigorous intensity physical activity had a lower percentage of <5th and ≥85th percentiles for BMI than those who did 60 minutes or more, while those who did 60 minutes or more had a lower percentage of 5th-84th percentiles for BMI than those who did less than 60 minutes of moderate-to-vigorous intensity physical activity ($p$<0.05, Table III). Those who did less than 60 minutes of moderate-to-vigorous intensity physical activity had a higher percentage of ≥85th percentile for M-SFT and BF% than those who did 60 minutes or more. Additionally, those who did 60 minutes or more had a higher percentage of <5th (except

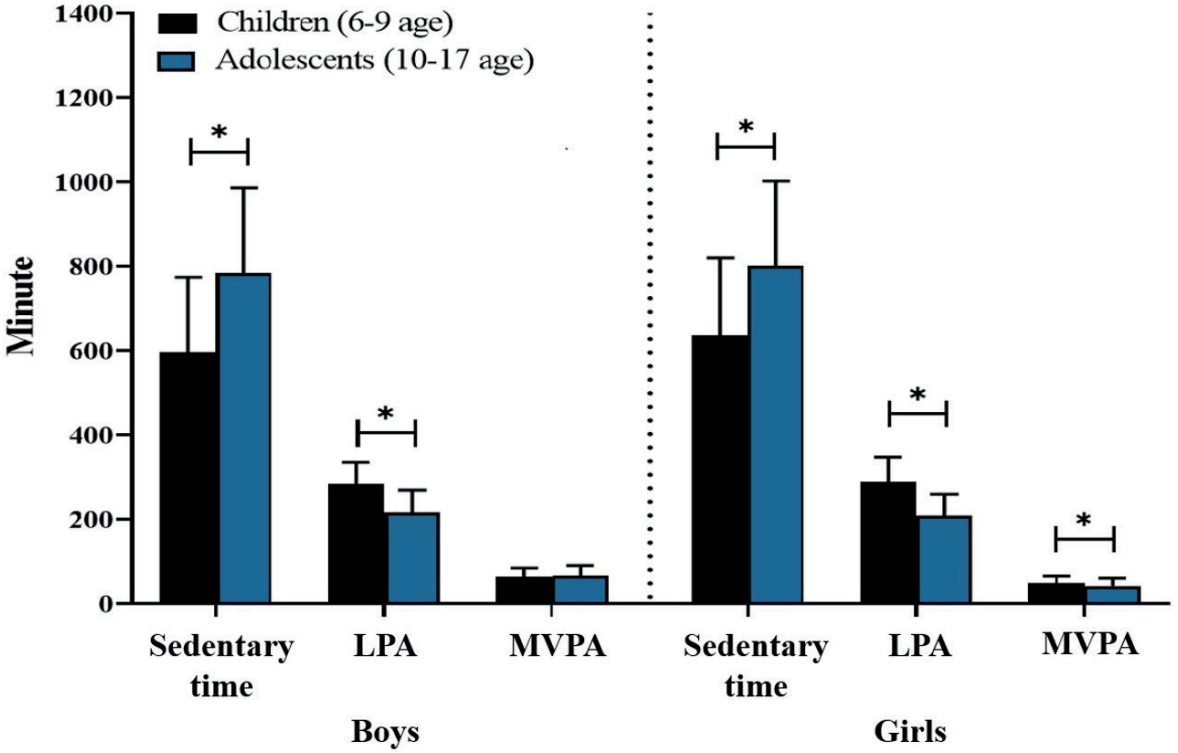



**Fig. 1.** The comparison of sedentary time and physical activity levels between children and adolescents in boys (*n=358*) and girls (*n=401*).

Sedentary time, light-intensity physical activity (LPA), and moderate-to-vigorous intensity physical activity (MVPA) values for boys and girls with different age groups.
*p<0.0.5

M-SFT) and 5th-84th percentiles for M-SFT and BF% than those who did less than 60 minutes of moderate-to-vigorous intensity physical activity, but the difference was insignificant for WC and T-SFT ($p<0.05$, Table III).

## Discussion

The present study investigated the difference in sedentary time and physical activity levels between the age groups of children and adolescents and the difference in the percentiles of the anthropometric variables between those who met and those who did not meet the physical activity recommendation of the WHO for children and adolescents in both genders. The present study found that adolescents had higher sedentary time than children among boys, while light-intensity physical activity was lower. Among girls, sedentary time was higher in adolescents, but both light-intensity physical activity and moderate-to-vigorous intensity physical activity were higher in children. Physical activity generally decreases from childhood to adolescence, while sedentary time increases in these transition periods.[16] A systematic review by Pearson et al.[35] reported a 10-20 minute daily increase in sedentary time during the transition from primary/middle to secondary/high school in both genders. Studies have also shown an increase in sedentary time with age.[36-38] A study from the International Children's Accelerometer Database found a 4.2% decrease in total physical activity with age, mainly due to decreased light-intensity physical activity.[37] These studies are consistent with our study, which found higher sedentary time but lower light-intensity physical activity in adolescents than in children. Additionally, the average moderate-to-vigorous intensity

**Table II.** Percentiles of anthropometric variables according to meeting physical activity recommendations of the World Health Organization (≥ 60 min of MVPA per day) in boys.

| | Variables | < 60 min of MVPA per day (n=56) | | ≥ 60 min of MVPA per day (n=69) | | $\chi^2$ | p |
|---|---|---|---|---|---|---|---|
| | | n | % | n | % | | |
| Children (age 6-9) (n=125) | BMI percentile | | | | | | |
| | <5th | 5 | 9.1 | 6 | 9.1 | | |
| | 5th-84th | 42 | 76.4 | 51 | 77.3 | 0.021 | 0.990 |
| | ≥85th | 8 | 14.5 | 9 | 13.6 | | |
| | WC percentiles | | | | | | |
| | <5th | 9 | 16.1 | 10 | 14.5 | | |
| | 5th-84th | 44 | 78.6 | 59 | 85.5 | 3.535 | 0.167 |
| | ≥85th | 3 | 5.4 | 0 | 0.0 | | |
| | T-SFT percentiles | | | | | | |
| | <5th | 1 | 1.8 | 5 | 7.2 | | |
| | 5th-84th | 26 | 46.4 | 44 | 63.8 | 7.424 | 0.021 |
| | ≥85th | 29 | 51.8 | 20 | 29.0 | | |
| | M-SFT percentiles | | | | | | |
| | <3rd | 2 | 3.6 | 8 | 11.6 | | |
| | 10th-74th | 32 | 57.1 | 40 | 58.0 | 3.089 | 0.202 |
| | ≥75th | 22 | 39.3 | 21 | 30.4 | | |
| | BF% percentiles | | | | | | |
| | <5th | 21 | 37.5 | 37 | 53.6 | | |
| | 5th-84th | 17 | 30.4 | 22 | 31.9 | 6.054 | 0.048 |
| | ≥85th | 18 | 32.1 | 10 | 14.5 | | |
| | Variables | < 60 min of MVPA per day (n=105) | | ≥ 60 min of MVPA per day (n=114) | | $\chi^2$ | p |
| | | n | % | n | % | | |
| Adolescents (age 10-17) (n=219) | BMI percentiles | | | | | | |
| | <5th | 7 | 6.9 | 6 | 5.6 | | |
| | 5th-84th | 77 | 76.2 | 97 | 89.8 | 8.697 | 0.013 |
| | ≥85th | 17 | 16.8 | 5 | 4.6 | | |
| | WC percentiles | | | | | | |
| | <5th | 13 | 12.4 | 16 | 14.0 | | |
| | 5th-84th | 87 | 82.9 | 97 | 85.1 | 2.985 | 0.228 |
| | ≥85th | 5 | 4.8 | 1 | 0.9 | | |
| | T-SFT percentiles | | | | | | |
| | <5th | 1 | 1 | 6 | 5.3 | | |
| | 5th-84th | 61 | 58.1 | 85 | 74.6 | 13.056 | 0.001 |
| | ≥85th | 43 | 41.0 | 23 | 20.2 | | |
| | M-SFT percentiles | | | | | | |
| | <3rd | 5 | 4.8 | 19 | 16.7 | | |
| | 10th-74th | 56 | 53.3 | 70 | 61.4 | 14.609 | 0.001 |
| | ≥75th | 44 | 41.9 | 25 | 21.9 | | |
| | BF% percentiles | | | | | | |
| | <5th | 8 | 7.6 | 18 | 15.8 | | |
| | 5th-84th | 47 | 44.8 | 69 | 60.5 | 14.543 | 0.001 |
| | ≥85th | 50 | 47.6 | 27 | 23.7 | | |

BF%, body fat percentage; BMI, body mass index; M-SFT, medial-calf skinfold thickness; MVPA, moderate-to-vigorous intensity physical activity; SD, standard deviation; T-SFT, triceps skinfold thickness; WC, waist circumference.

**Table III.** Percentiles of anthropometric variables according to meeting physical activity recommendations of the World Health Organization (≥ 60 min of MVPA per day) in girls..

| | Variables | < 60 min of MVPA per day (n=94) | | ≥ 60 min of MVPA per day (n=23) | | $\chi^2$ | p |
|---|---|---|---|---|---|---|---|
| | | n | % | n | % | | |
| Children (age 6-9) (n=117) | BMI percentile | | | | | | |
| | <5th | 13 | 14.3 | 2 | 9.5 | | |
| | 5th-84th | 70 | 76.9 | 17 | 81.0 | 0.335 | 0.846 |
| | ≥85th | 8 | 8.8 | 2 | 9.5 | | |
| | WC percentiles | | | | | | |
| | <5th | 23 | 24.5 | 7 | 30.4 | | |
| | 5th-84th | 69 | 73.4 | 15 | 65.2 | 1.343 | 0.483 |
| | ≥85th | 2 | 2.1 | 1 | 4.3 | | |
| | T-SFT percentiles | | | | | | |
| | <5th | 3 | 3.2 | 0 | 0.0 | | |
| | 5th-84th | 53 | 57.0 | 13 | 56.5 | 0.805 | 0.669 |
| | ≥85th | 37 | 39.8 | 10 | 43.5 | | |
| | M-SFT percentiles | | | | | | |
| | <3rd | 4 | 4.3 | 3 | 13.6 | | |
| | 10th-74th | 53 | 57.6 | 9 | 40.9 | 3.672 | 0.132 |
| | ≥75th | 35 | 38.0 | 10 | 45.5 | | |
| | BF% percentiles | | | | | | |
| | <5th | 27 | 29.3 | 13 | 56.5 | | |
| | 5th-84th | 45 | 48.9 | 6 | 26.1 | 5.893 | 0.049 |
| | ≥85th | 20 | 21.7 | 4 | 17.4 | | |
| | Variables | < 60 min of MVPA per day (n=229) | | ≥ 60 min of MVPA per day (n=43) | | $\chi^2$ | p |
| | | n | % | n | % | | |
| Adolescents (age 10-17) (n=272) | BMI percentiles | | | | | | |
| | <5th | 20 | 9.0 | 11 | 26.8 | | |
| | 5th-84th | 198 | 88.8 | 29 | 70.7 | 10.728 | 0.005 |
| | ≥85th | 5 | 2.2 | 1 | 2.4 | | |
| | WC percentiles | | | | | | |
| | <5th | 92 | 40.4 | 18 | 41.9 | | |
| | 5th-84th | 134 | 58.8 | 25 | 58.1 | 0.400 | 0.819 |
| | ≥85th | 2 | 0.9 | 0 | 0.0 | | |
| | T-SFT percentiles | | | | | | |
| | <5th | 5 | 2.2 | 2 | 4.7 | | |
| | 5th-84th | 162 | 70.7 | 34 | 79.1 | 3.167 | 0.193 |
| | ≥85th | 62 | 27.1 | 7 | 16.3 | | |
| | M-SFT percentiles | | | | | | |
| | <3rd | 14 | 6.1 | 5 | 11.6 | | |
| | 10th-74th | 127 | 55.7 | 28 | 65.1 | 4.341 | 0.047 |
| | ≥75th | 87 | 38.2 | 10 | 23.3 | | |
| | BF% percentiles | | | | | | |
| | <5th | 0 | 0.0 | 0 | 0.0 | | |
| | 5th-84th | 81 | 35.5 | 27 | 62.8 | 11.219 | 0.001 |
| | ≥85th | 147 | 64.5 | 16 | 37.2 | | |

BF%, body fat percentage; BMI, body mass index; M-SFT, medial-calf skinfold thickness; MVPA, moderate-to-vigorous intensity physical activity; SD, standard deviation; T-SFT, triceps skinfold thickness; WC, waist circumference.

of physical activity decreased in girls by 41%, compared to 7% in boys.[19] Several studies have reported a decline in moderate-to-vigorous intensity physical activity during early adolescence, particularly in girls.[39,40] Farooq et al.[4] revealed that annual moderate-to-vigorous intensity physical activity decreased from age 6 in girls and 9 in boys. The annual decline in moderate-to-vigorous intensity physical activity from age 9 was 7.8% for boys and 10.2% for girls, with moderate-to-vigorous intensity physical activity generally decreasing with age in both genders.[4] However, in our study, moderate-to-vigorous intensity physical activity did not significantly decrease in boys aged 11-17 compared to ages 6-9. Early maturation, which is associated with increased height, body weight, and lean mass in boys, indicates a favorable physical structure, especially in types of physical activity that require speed, strength, and power.[41] Thus, the favorable physical structure may lead to an increase in moderate-to-vigorous intensity physical activity during early maturation.[41] Considering that those with early maturation in the present study are in the adolescent age groups, this may explain why moderate-to-vigorous intensity physical activity was not significantly higher in children, as moderate-to-vigorous intensity physical activity time may increase in adolescents.

Another finding of the present study was that the percentage of boys aged 6-9 years who were physically active were lower in the upper percentiles for BF% and T-SFT than those who were not. The percentage of boys aged 10-17 years who were physically inactive were higher in the upper percentiles for BMI, T-SFT, M-SFT, and BF% than in those who were. Girls aged 10-17 years who were physically inactive were higher in the upper percentiles of M-SFT and BF%, but they were not in the upper percentiles of BMI. A study by Mateo-Orcajada et al.[42] found that regular physical activity among adolescents resulted in lower BF%, fat mass, and fat mass index compared to physically inactive individuals. Other studies also provide evidence that SFT was higher in adolescents who engaged in regular physical activity.[43,44] Although the current study is consistent with the findings of these studies, it differs from them in that participants were divided into active or inactive groups according to WHO recommendations and physical activity was measured objectively.

Füssenich et al.[14] found that children meeting the physical activity recommendation of the WHO had a lower BF%. Chaput et al.[45] reported that children not meeting the physical activity guidelines were more likely to be overweight or obese. Studies indicate that BMI decreases as moderate-to-vigorous intensity physical activity increases in both boys and girls.[45-47] Higher moderate-to-vigorous intensity physical activity was associated with lower BMI and WC Z-scores at the 10th percentiles.[48] Thus, reducing childhood obesity prevalence could be achieved by shifting the upper percentiles of BMI and WC distributions to lower values.[48] These findings align with our results; however, in the present study, the percentage of adolescents meeting the physical activity recommendations was lower in the upper BMI percentiles among girls. This may be attributed to the limited sample size in these higher percentiles. A cross-sectional study of 225 children aged 7.9-11.1 years showed that moderate-to-vigorous intensity physical activity was not associated with BF% in Swedish children.[49] A number of studies have shown that physical activity was not associated with WC or BMI in both genders in children and adolescents.[5,50] Thus, the inconsistent findings suggest that more research is needed to determine the role of moderate-to-vigorous intensity physical activity on anthropometrics such as adiposity and BMI.[51]

A notable finding in the present study was that significant differences were found between the physically active and inactive groups in more anthropometric variables in adolescents of both genders compared with children. A United Nations large sample study found that the most statistically significant changes in adiposity, such as BMI and fat mass, occurred in adolescents aged 12-15 years.[52] Regular physical

activity may be a critical factor in reducing obesity in late childhood and early adolescence, when physical activity declines significantly and obesity incidence is high.[53] Therefore, the role of physical activity in the significant changes in body composition that occur during adolescence may be more pronounced than in childhood. This may explain the difference between the active and inactive groups in more anthropometric variables in adolescents compared to children. Furthermore, in the present study, sedentary time was statistically lower in children than in adolescents for both girls and boys. In this respect, children in the active and inactive groups may have had a low daily sedentary time, which may have limited the role of physical activity. Limiting the role of physical activity could be another explanation for the difference in fewer anthropometric variables between the active and inactive groups in children.

The strengths of this study include being the first to objectively measure sedentary time and physical activity using accelerometers, with a large sample size representing 11 of the 12 regions of Türkiye. Accordingly, the analysis of physical activity and sedentary time of children from regions with different geographical and cultural characteristics is also an important strength of the study. As there is a limited number of health-related studies on light-intensity physical activity in the literature, the investigation of light-intensity physical activity is another strength of this study. The study also has several limitations. The anthropometric variables may have been influenced by nutritional status being potentially confounding factors, as sedentary time and physical activity may be associated with energy-inducing foods such as junk food. Another of the study's limitations is that although this study is a large study representing 11 regions of Türkiye, the number of participants for children and adolescents in the group 85th percentile or above is relatively low. In addition, the SFT measurements could have included more body sites. Additionally, another drawback is the lack of biological maturation determined by the Tanner stage. Biological maturation may significantly influence anthropometric variables associated with physical activity, suggesting it is an important confounding factor. Other limitations of the study include that the sample distributions of active and inactive children and adolescents were not close, especially for girls. Recent global data show that the majority (81%) of boys and girls aged 11-17 years do not meet physical activity recommendations. We believe that the higher number of participants not meeting physical activity recommendations in our sample is due to the high prevalence of inactivity worldwide.

In conclusion, our study revealed that both boys and girls had lower sedentary time than adolescents, while children had higher levels of light-intensity physical activity. There was no difference in moderate-to-vigorous intensity physical activity between children and adolescents in boys, but children had higher moderate-to-vigorous intensity physical activity than adolescents in girls. Children who met the physical activity recommendation were involved in lower percentiles for T-SFT and BF% (only BF% in girls) than those who did not. The study found that adolescents who met the physical activity recommendation were in lower percentiles of anthropometric variables than those who did not, highlighting the role of meeting physical activity recommendations in reducing the risk of obesity, especially in adolescents compared with children.

**Acknowledgments**

We would like to acknowledge the measurement team involved in the project measurements, the administrative team of schools, and the families who consented to their children's participation in the present study.

**Ethical approval**

This study was approved by the Non-Interventional Clinical Research Ethics Board of Hacettepe University (date: 16.07.2019, number: GO 19/713).

## Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: ND, AK, EC, PA, NK, MMC, EK, GD, SK, and ENÖ; data collection: ND, AK, EC, PA, NK, and MMC; analysis and interpretation of results: ND, AK, EC, EK; draft manuscript preparation: ND, AK. All authors reviewed the results and approved the final version of the article.

## Source of funding

The authors declare that the study is funded by the Scientific and Technological Research Council of Türkiye (grant number: SBAG-120S408) and Hacettepe University Scientific Research Projects Coordination Unit (grant number: THD-2022-20266).

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES

1. World Health Organization (WHO). WHO guidelines on physical activity and sedentary behaviour. 2020. Available at: https://www.who.int/publications/i/item/9789240015128 (Accessed on May 22, 2022).
2. Tan VP, Macdonald HM, Kim S, et al. Influence of physical activity on bone strength in children and adolescents: a systematic review and narrative synthesis. J Bone Miner Res 2014; 29: 2161-2181. https://doi.org/10.1002/jbmr.2254
3. Hills AP, Andersen LB, Byrne NM. Physical activity and obesity in children. Br J Sports Med 2011; 45: 866-870. https://doi.org/10.1136/bjsports-2011-090199
4. Farooq A, Martin A, Janssen X, et al. Longitudinal changes in moderate-to-vigorous-intensity physical activity in children and adolescents: a systematic review and meta-analysis. Obes Rev 2020; 21: e12953. https://doi.org/10.1111/obr.12953
5. Marques A, Minderico C, Martins S, Palmeira A, Ekelund U, Sardinha LB. Cross-sectional and prospective associations between moderate to vigorous physical activity and sedentary time with adiposity in children. Int J Obes (Lond) 2016; 40: 28-33. https://doi.org/10.1038/ijo.2015.168
6. Riso EM, Kull M, Mooses K, Hannus A, Jürimäe J. Objectively measured physical activity levels and sedentary time in 7-9-year-old Estonian schoolchildren: independent associations with body composition parameters. BMC Public Health 2016; 16: 346. https://doi.org/10.1186/s12889-016-3000-6
7. Steele RM, van Sluijs EM, Cassidy A, Griffin SJ, Ekelund U. Targeting sedentary time or moderate- and vigorous-intensity activity: independent relations with adiposity in a population-based sample of 10-y-old British children. Am J Clin Nutr 2009; 90: 1185-1192. https://doi.org/10.3945/ajcn.2009.28153
8. Loprinzi PD, Cardinal BJ, Lee H, Tudor-Locke C. Markers of adiposity among children and adolescents: implications of the isotemporal substitution paradigm with sedentary behavior and physical activity patterns. J Diabetes Metab Disord 2015; 14: 46. https://doi.org/10.1186/s40200-015-0175-9
9. Moura BP, Rufino RL, Faria RC, Amorim PRS. Effects of isotemporal substitution of sedentary behavior with light-intensity or moderate-to-vigorous physical activity on cardiometabolic markers in male adolescents. PLoS One 2019; 14: e0225856. https://doi.org/10.1371/journal.pone.0225856
10. Volpato LA, Fernandes DZ, Correa RC, et al. Light-intensity physical activity patterns and associated factors in adolescents. Mot Rev Educ Fis 2021; 27: e1021020172. https://doi.org/10.1590/s1980-65742021017220
11. Jiménez-Pavón D, Fernández-Vázquez A, Alexy U, et al. Association of objectively measured physical activity with body components in European adolescents. BMC Public Health 2013; 13: 667. https://doi.org/10.1186/1471-2458-13-667
12. Schwarzfischer P, Weber M, Gruszfeld D, et al. BMI and recommended levels of physical activity in school children. BMC Public Health 2017; 17: 595. https://doi.org/10.1186/s12889-017-4492-4
13. Janssen I, Leblanc AG. Systematic review of the health benefits of physical activity and fitness in school-aged children and youth. Int J Behav Nutr Phys Act 2010; 7: 40. https://doi.org/10.1186/1479-5868-7-40
14. Füssenich LM, Boddy LM, Green DJ, et al. Physical activity guidelines and cardiovascular risk in children: a cross sectional analysis to determine whether 60 minutes is enough. BMC Public Health 2016; 16: 67. https://doi.org/10.1186/s12889-016-2708-7
15. Chaput JP, Willumsen J, Bull F, et al. 2020 WHO guidelines on physical activity and sedentary behaviour for children and adolescents aged 5-17 years: summary of the evidence. Int J Behav Nutr Phys Act 2020; 17: 141. https://doi.org/10.1186/s12966-020-01037-z

16. Ortega FB, Konstabel K, Pasquali E, et al. Objectively measured physical activity and sedentary time during childhood, adolescence and young adulthood: a cohort study. PLoS One 2013; 8: e60871. https://doi.org/10.1371/journal.pone.0060871

17. Troiano RP, Berrigan D, Dodd KW, Mâsse LC, Tilert T, McDowell M. Physical activity in the United States measured by accelerometer. Med Sci Sports Exerc 2008; 40: 181-188. https://doi.org/10.1249/mss.0b013e31815a51b3

18. World Health Organization (WHO). Adolescent health. 2024. Available at: https://www.who.int/health-topics/adolescent-health#tab=tab_1 (Accessed on July 5, 2024).

19. Sember V, Jurak G, Kovač M, Đurić S, Starc G. Decline of physical activity in early adolescence: a 3-year cohort study. PLoS One 2020; 15: e0229305. https://doi.org/10.1371/journal.pone.0229305

20. Gordon CC, Chumlea WC, Roche AF. Stature, recumbent length, and weight. In: Lohman T, Roche A, Martorell R, editors. Measurement Descriptions and Techniques. Anthropometric standardization reference manual. Champaign, IL: Human Kinetics Books; 1988: 3-8.

21. Callaway CW, Chumlea WC, Bouchard C, et al. Circumferences. In: Lohman T, Roche A, Martorell R, editors. Anthropometric standardization reference manual. Champaign, IL: Human Kinetics Books; 1988: 39-54.

22. Surendar J, Indulekha K, Deepa M, Mohan V, Pradeepa R. Association of adiposity, measured by skinfold thickness, with parental history of diabetes in a South Indian population: data from CURES-114. Postgrad Med J 2016; 92: 379-385. https://doi.org/10.1136/postgradmedj-2015-133363

23. Slaughter MH, Lohman TG, Boileau RA, et al. Skinfold equations for estimation of body fatness in children and youth. Hum Biol 1988; 60: 709-723.

24. Dezenberg CV, Nagy TR, Gower BA, Johnson R, Goran MI. Predicting body composition from anthropometry in pre-adolescent children. Int J Obes Relat Metab Disord 1999; 23: 253-259. https://doi.org/10.1038/sj.ijo.0800802

25. Fryar CD, Carroll MD, Gu Q, Afful J, Ogden CL. Anthropometric reference data for children and adults: United States, 2015-2018. Vital Health Stat 3 2021; 36: 1-44.

26. Çiçek B, Öztürk A, Mazıcıoğlu MM, Kurtoğlu S. Arm anthropometry indices in Turkish children and adolescents: changes over a three-year period. J Clin Res Pediatr Endocrinol 2014; 6: 216-226. https://doi.org/10.4274/Jcrpe.1574

27. Kuhle S, Ashley-Martin J, Maguire B, Hamilton DC. Percentile curves for skinfold thickness for Canadian children and youth. PeerJ 2016; 4: e2247. https://doi.org/10.7717/peerj.2247

28. Soylu M, Şensoy N, Doğan İ, Doğan N, Mazıcıoğlu MM, Öztürk A. Four-site skinfolds thickness percentiles of schoolchildren and adolescents in Turkey. Public Health Nutr 2021; 24: 5414-5425. https://doi.org/10.1017/S1368980021003323

29. Centers for Disease Control and Prevention (CDC). Child and teen BMI categories. 2024. Available at: https://www.cdc.gov/bmi/child-teen-calculator/bmi-categories.html (Accessed on May 25, 2024).

30. Cain KL, Sallis JF, Conway TL, Van Dyck D, Calhoon L. Using accelerometers in youth physical activity studies: a review of methods. J Phys Act Health 2013; 10: 437-450. https://doi.org/10.1123/jpah.10.3.437

31. Colley R, Connor Gorber S, Tremblay MS. Quality control and data reduction procedures for accelerometry-derived measures of physical activity. Health Rep 2010; 21: 63-69.

32. Evenson KR, Catellier DJ, Gill K, Ondrak KS, McMurray RG. Calibration of two objective measures of physical activity for children. J Sports Sci 2008; 26: 1557-1565. https://doi.org/10.1080/02640410802334196

33. World Health Organization (WHO). The Global Action for Measurement of Adolescent health (GAMA). 2024. Available at: https://www.who.int/groups/the-global-action-for-measurement-of-adolescent-health (Accessed on July 8, 2024).

34. Cohen J. Statistical power analysis for the behavioral sciences. 2nd ed. Hillsdale, NJ: Lawrence Erlbaum Associates; 1988.

35. Pearson N, Haycraft E, P Johnston J, Atkin AJ. Sedentary behaviour across the primary-secondary school transition: a systematic review. Prev Med 2017; 94: 40-47. https://doi.org/10.1016/j.ypmed.2016.11.010

36. Colley RC, Garriguet D, Janssen I, et al. The association between accelerometer-measured patterns of sedentary time and health risk in children and youth: results from the Canadian Health Measures Survey. BMC Public Health 2013; 13: 200. https://doi.org/10.1186/1471-2458-13-200

37. Cooper AR, Goodman A, Page AS, et al. Objectively measured physical activity and sedentary time in youth: the International Children's Accelerometry Database (ICAD). Int J Behav Nutr Phys Act 2015; 12: 113. https://doi.org/10.1186/s12966-015-0274-5

38. Spittaels H, Van Cauwenberghe E, Verbestel V, et al. Objectively measured sedentary time and physical activity time across the lifespan: a cross-sectional study in four age groups. Int J Behav Nutr Phys Act 2012; 9: 149. https://doi.org/10.1186/1479-5868-9-149

39. Corder K, Sharp SJ, Atkin AJ, et al. Change in objectively measured physical activity during the transition to adolescence. Br J Sports Med 2015; 49: 730-736. https://doi.org/10.1136/bjsports-2013-093190

40. Young DR, Cohen D, Koebnick C, et al. Longitudinal associations of physical activity among females from adolescence to young adulthood. J Adolesc Health 2018; 63: 466-473. https://doi.org/10.1016/j.jadohealth.2018.05.023

41. Adami F, Benedet J, Takahashi LAR, da Silva Lopes A, da Silva Paiva L, de Vasconcelos FAG. Association between pubertal development stages and body adiposity in children and adolescents. Health Qual Life Outcomes 2020; 18: 93. https://doi.org/10.1186/s12955-020-01342-y

42. Mateo-Orcajada A, González-Gálvez N, Abenza-Cano L, Vaquero-Cristóbal R. Differences in physical fitness and body composition between active and sedentary adolescents: a systematic review and meta-analysis. J Youth Adolesc 2022; 51: 177-192. https://doi.org/10.1007/s10964-021-01552-7

43. Sivrikaya K, Ziyagil MA, Çebi M. Relationship between body mass index and skinfold thickness in exercised and sedentary boys and girls. Univers J Educ Res 2019; 7: 48-54. https://doi.org/10.13189/ujer.2019.070107

44. Agata K, Monyeki MA. Association between sport participation, body composition, physical fitness, and social correlates among adolescents: the PAHL study. Int J Environ Res Public Health 2018; 15: 2793. https://doi.org/10.3390/ijerph15122793

45. Chaput JP, Lambert M, Mathieu ME, Tremblay MS, O' Loughlin J, Tremblay A. Physical activity vs. sedentary time: independent associations with adiposity in children. Pediatr Obes 2012; 7: 251-258. https://doi.org/10.1111/j.2047-6310.2011.00028.x

46. Gába A, Mitáš J, Jakubec L. Associations between accelerometer-measured physical activity and body fatness in school-aged children. Environ Health Prev Med 2017; 22: 43. https://doi.org/10.1186/s12199-017-0629-4

47. Velde GT, Plasqui G, Willeboordse M, Winkens B, Vreugdenhil A. Associations between physical activity, sedentary time and cardiovascular risk factors among Dutch children. PLoS One 2021; 16: e0256448. https://doi.org/10.1371/journal.pone.0256448

48. Mitchell JA, Dowda M, Pate RR, et al. Physical activity and pediatric obesity: a quantile regression analysis. Med Sci Sports Exerc 2017; 49: 466-473. https://doi.org/10.1249/MSS.0000000000001129

49. Dencker M, Thorsson O, Karlsson MK, Lindén C, Wollmer P, Andersen LB. Daily physical activity related to aerobic fitness and body fat in an urban sample of children. Scand J Med Sci Sports 2008; 18: 728-735. https://doi.org/10.1111/j.1600-0838.2007.00741.x

50. Zheng Y, Liang J, Zeng D, et al. Association of body composition with pubertal timing in children and adolescents from Guangzhou, China. Front Public Health 2022; 10: 943886. https://doi.org/10.3389/fpubh.2022.943886

51. Reid RE, Fillon A, Thivel D, et al. Can anthropometry and physical fitness testing explain physical activity levels in children and adolescents with obesity? J Sci Med Sport 2020; 23: 580-585. https://doi.org/10.1016/j.jsams.2019.12.005

52. Stierman B, Ogden CL, Yanovski JA, Martin CB, Sarafrazi N, Hales CM. Changes in adiposity among children and adolescents in the United States, 1999-2006 to 2011-2018. Am J Clin Nutr 2021; 114: 1495-1504. https://doi.org/10.1093/ajcn/nqab237

53. Basterfield L, Reilly JK, Pearce MS, et al. Longitudinal associations between sports participation, body composition and physical activity from childhood to adolescence. J Sci Med Sport 2015; 18: 178-182. https://doi.org/10.1016/j.jsams.2014.03.005

The Turkish Journal of Pediatrics 2024; 66: 525-533
https://doi.org/10.24953/turkjpediatr.2024.4900

**Original Article**

# Evaluation of lead levels in children with chronic constipation

Abdurrahman Zarif Güney[1], Güzide Doğan[2], Ali Toprak[1]

*[1]Department of Pediatrics, Faculty of Medicine, Bezmialem Vakıf University, İstanbul; [2]Department of Pediatric Gastroenterology Hepatology and Nutrition, Faculty of Medicine, Biruni University, İstanbul, Türkiye.*

## ABSTRACT

**Objectives.** This study aimed to assess blood and hair lead levels (BLL and HLL) in children with chronic constipation and compare them to healthy children; and investigated lead exposure's role in the etiology of constipation. It also explored the correlation between BLL and HLL.

**Study Design.** The study included 84 constipated children aged 3-18 years as the case group and an equal number of constipation-free children as controls. Organic diseases were ruled out through history-taking, physical exams and laboratory tests. Blood and hair samples were collected and analyzed for lead levels using standardized methods.

**Results.** The constipated children group had significantly higher BLL (3.66 μg/dL) compared to the control group (1.61 μg/dL) with no significant HLL difference. Additionally, 48.8% of constipated children exceeded the reference value of 3.5 μg/dL, in contrast to 4.8% of the control group. BLL was unaffected by gender and age, while HLL were higher in girls and low ages. No significant correlation existed between BLL and HLL. The age of the housing showed a positive correlation with higher BLL and HLL. Lead exposure sources like drinking water, home renovation history, parental smoking, or nearby industrial facilities showed no significant relationships with lead levels.

**Conclusions.** Understanding the constipation-lead exposure link is crucial for prevention and intervention. HLL may vary with gender and age due to external lead particles, which is why BLL continues to be a more reliable measure. Healthcare providers should remember to investigate lead exposure risk factors in constipation patients and test BLL when necessary.

**Key words:** constipation, lead, blood lead level, hair lead level.

Constipation is defined as infrequent or difficult bowel movements, diagnosed according to the ROME IV criteria. Constipation accounts for 3% of general pediatric clinic admissions and 25% of pediatric gastroenterology and hepatology clinic admissions.[1,2] Only 5% of constipated children have an organic cause, while no organic cause can be found in 95% of cases.[3] Heavy metal exposure, particularly lead, has emerged as a significant factor among the many potential organic contributors to constipation.[4-6] Lead exposure was primarily attributed to the use of leaded gasoline from the 1920s until the present. Leaded gasoline is no longer available worldwide as of 2021 due to the depletion of the last leaded gasoline refinery in Algeria. Thus the world has bid farewell to this source of lead contamination.[7,8] Other routes of exposure

✉ Abdurrahman Zarif Güney ▪ a.zarifguney@hotmail.com

Received 29th Jun 2024, revised 3rd Oct 2024, accepted 20th Oct 2024.

This study was presented as a poster presentation at the 56th Annual Meeting of the European Society of Pediatric Gastroenterology, Hepatology and Nutrition in Milan in May 2024.

include water distribution systems, dietary sources, medications, toys, and jewelry.[9]

The adverse effects of lead extend across various body systems, impacting the nervous system, gastrointestinal system, as well as the cardiovascular, genitourinary, and hematopoietic systems. In the gastrointestinal system, abdominal pain, cramps, constipation, nausea, vomiting, bloating, and weight loss may occur.[10,11] It is hypothesized that lead causes constipation via direct inhibitory effects on the neural plexus of the intestine and also on smooth muscle contractions.[12] Additionally, metabolic alterations, such as the accumulation of δ-aminolevulinic acid (a porphyrin precursor), resulting from lead-induced porphyrinopathy, also diminish intestinal motility. Moreover, lead alters the stimulation of the cell membrane through changes in the activity of cell membrane channels, which is also implicated in the occurrence of constipation.[13]

Measuring lead levels in whole blood is the most used and considered most reliable method for demonstrating, monitoring, determining the treatment method when necessary, taking protective measures, and performing screening programs for lead exposure. On May 14, 2021, the Lead Exposure Prevention and Advisory Committee (LEPAC) updated the reference value for children's blood lead levels (BLL) from 5 μg/dL to 3.5 μg/dL.[14]

Although measuring lead levels in blood is the gold standard test, it is not possible to distinguish between long-term exposure to lead and short-term exposure to high levels of lead by measuring BLL. Moreover, the lead level in the blood represents only a small fraction of the total lead burden in the body and may not be a favorable reflection of the total lead load. Therefore, there is a need to measure lead levels from different samples, such as hair in addition to blood.[15-17]

This study aimed to assess BLL and hair lead levels (HLL) in children with chronic constipation and compare them to healthy children and investigated lead exposure's role in constipation etiology. It also explored the correlation between BLL and HLL.

## Materials and Methods

The study included 168 participants divided into two groups, each with 84 children. The case group comprised children aged 3 to 18 who visited our clinic with complaints of constipation between February 2022 and July 2023. The study was conducted in Istanbul a densely populated urban area. The control group consisted of 84 children matched for age and gender, who were scheduled for blood tests in the outpatient clinic for various reasons, had no complaints of constipation, and had no chronic illnesses. In this study, constipation was defined as infrequent or difficult defecation lasting for two months or longer. A rigorous selection process was implemented for both groups to ensure the accuracy of the study. This process included comprehensive medical history evaluations, physical examinations, and laboratory tests. We excluded any organic diseases as the potential cause of constipation in all participants. In the course of our investigation, children who were fed solid and low-fiber foods and had inadequate fluid intake were excluded from the study. Accordingly, both groups underwent a standardization process in terms of dietary habits. Written informed consent was obtained from the parents of all participating children. Exclusion criteria included refusal to participate in the study or provide blood and hair samples. Demographic information forms were completed by the parents, which included details such as the age of their homes, history of home renovations, method of obtaining drinking water, presence of nearby factories, and family history of smoking.

The study was approved by the local ethics committee on January 13, 2022, under decision number E-46716. Additionally, it was supported by the Bezmialem Vakıf University Scientific Research Projects Coordination Unit under project number 20220201, as decided on

February 24, 2022, through decision number E-18245212-108-99-53026.

### *Blood sample collection and blood lead level measurement*

After obtaining written informed consent from their parents, blood samples were collected from the participants. To maintain sterility, the relevant skin area was cleaned with ethyl alcohol before blood collection. The blood was collected using single-use vacuum needle tips into EDTA-containing tubes, ensuring a minimum volume of 2 mL. The tubes were then stored in a refrigerator at +4°C until the day of testing.

Standard solutions were prepared by diluting the lead stock standard solution of 1000 μg/mL in blood samples. These solutions were appropriately vortexed, and 10 μL of each solution were injected into the graphite furnace using an automatic sampler. The electrothermal graphite furnace atomic absorption spectrophotometry method was employed to measure BLLs, creating an automatic calibration curve. The results were reported in μg/dL.

### *Hair sample collection and hair lead level measurement*

Hair samples were collected from the upper occipital region of the scalp based on a minimum weight criterion of 0.3 grams. The samples were obtained from the hair root using scissors and stored in plastic bags in a dry environment until the day of testing.

Lead levels in the hair samples were measured using ICP-MS (Inductively Coupled Plasma – Mass Spectrometer) Thermo/X series 2 equipment. The analytical method for detecting heavy metals in these samples followed the chemical analysis clinical study guide of the Hewlett Packard Company Group. A 0.25-gram hair sample was dissolved in 5 mL of HNO3 using a microwave (Cem MARS 5). The dissolution process was conducted under conditions of 600 psi pressure and 200°C. After cooling, the sample was diluted with distilled water to a final volume of 25 mL, transferred to polyethylene tubes, and placed in the ICP automatic sampler for measurements.

### *Statistical analysis*

The study included 84 patients in each group, based on previous research with a 95% confidence level and 80% power, as recommended by the literature. The mean difference was 1.5 units, with a standard deviation of 3.5 units. The data were analyzed using IBM SPSS Statistics 22.0 software. Continuous variables were assessed using the t-test, while the chi-square test was employed to analyze categorical variables between groups. The study presented descriptive statistics, including mean ± standard deviation, frequency, and percentage values. The level of statistical significance was determined by a p-value of less than 0.05. For the correlation assessment between BLL, HLL, children's age, and building age, Spearman's rho was utilized. This nonparametric test is appropriate for evaluating the strength and direction of the associations between ranked variables. Correlation coefficients ($r_s$) were calculated to determine the relationships, with significance levels set at $p < 0.05$ for moderate correlations and $p < 0.01$ for strong correlations.

## Results

The study involved 168 children aged 3 to 18 years. The case group was comprised of 46 boys (54.8%) and 38 girls (45.2%), while the control group consisted of 45 boys (53.6%) and 39 girls (46.4%). The mean age of the case group was 7.21 years, and the mean age of the control group was 7.23 years. The study revealed no statistically significant differences between the case and control groups with regard to the age of the building, history of home renovation, type of drinking water and children whose parents smoked. Consequently, the case and control groups were statistically matched in terms of age, gender and other characteristics that may affect lead levels (Table I).

**Table I.** Characteristics of children with constipation and controls.

| Parameter | | Case group (n=84) | Control group (n=84) | p* |
|---|---|---|---|---|
| Sex | Male, n (%) | 46 (54.8%) | 45 (53.6%) | 0.877 |
| | Female, n (%) | 38 (45.2%) | 39 (46.4%) | |
| Age, yr | Mean±SD | 7.21±4.15 | 7.23±4.20 | 0.971 |
| | Median (Q1-Q3) | 6 (4-10) | 6 (4-9) | |
| Building age, yr | Mean±SD | 20.44±11.55 | 17.79±12.07 | 0.087 |
| | Median (Q1-Q3) | 20 (10-30) | 15 (9.2-25) | |
| House renovation, n (%) | Yes | 28 (33.3%) | 23(27.4%) | 0.401 |
| | No | 56 (66.7%) | 61(72.6%) | |
| Drinking water, n (%) | Tap water | 6 (7.1%) | 6 (7.1%) | 0.625 |
| | Tap water with filter | 47 (56%) | 41 (48.8%) | |
| | Bottled water | 31(36.9%) | 37 (54.4%) | |
| Smoking parent, n (%) | Yes | 40 (47.6%) | 43 (51.2%) | 0.643 |
| | No | 44 (52.4%) | 41(48.8%) | |

In this study, we examined the lead levels of children with and without constipation. The highest BLL recorded was 9.1 μg/dL, and the highest HLL was 9.83 μg/g. The mean BLL was 2.63 μg/dL, and the mean HLL was 1.07 μg/g in the 168 children (Table II).

Children with constipation in the case group had an average BLL of 3.66 μg/dL, which was significantly higher than the control group's average of 1.61 μg/dL (p<0.001, Table II).

The mean HLL of children in the case group with constipation was 1.26 μg/g, while the mean HLL of children in the control group was 0.88 μg/g. However, there was no statistically significant difference in HLL between the two groups (p=0.801, Table II).

The BLL of 48.8% of children with constipation exceeded the reference value of 3.5 μg/dL, whereas only 4.8% of children in the control group exceeded this reference value. This indicates that the likelihood of BLL being above the reference value in children with constipation was ten times higher than in the control group.

The mean BLL for boys was 2.54 μg/dL, while for girls it was 2.74 μg/dL. There was no statistically significant difference in BLL between the two groups (p=0.508, Table III). When comparing the HLL of male and female children in our study, we found that boys had a mean HLL of 0.88 μg/g, while girls had a significantly higher mean HLL of 2.74 μg/g (p<0.001, Table III).

There was no significant relationship between BLL and the ages of children (p=0.464). However, a significant negative correlation was found between HLL and the ages of children, indicating a low-degree negative relationship (p<0.001, $r_s$=0.3).

When statistically analyzing the relationship between the levels of lead in the blood and hair, no significant correlation was found between the two groups (p=0.414, $r_s$=0.063).

**Table II.** Blood and hair lead levels in constipation and control groups.

| Lead levels | Group | Min-Max | Mean±SD | Median (Q1-Q3) | P* |
|---|---|---|---|---|---|
| Blood (μg/dL) | Case | 1 – 9.1 | 3.66±1.90 | 3.4 (2.2-4.97) | <0.001 |
| | Control | 0-5 | 1.61±1.13 | 1.45 (1-2.27) | |
| Hair (μg/g) | Case | 0.02 – 9.83 | 1.26±1.67 | 0.72 (0.3-1.61) | 0.801 |
| | Control | 0.04 – 2.81 | 0.88 ± 0.58 | 0.8 (0.33-1.38) | |

Analyzing the correlation between the age of the building and lead levels in children, we discovered a statistically significant positive correlation between the age of the building and both blood ($p<0.001$; $r_s=0.28$) and hair ($p=0.02$, $r_s=0.179$) lead levels. We found no statistically significant difference between individuals with and without a history of home renovation in terms of BLL (p=0.372) and HLL (p=0.628) The study found no statistically significant relationship between the type of drinking water and blood and HLL (p=0.683 for BLL; p=0.656 for HLL) We found no statistically significant difference in lead levels between children whose parents smoked and those whose did not (p=0.354 for BLL; p=0.655 for HLL, Table III).

When examining the effect of factories or industrial establishments in close proximity to homes on their lead levels, we found that the mean BLL was 2.63 μg/dL and the mean HLL was 1.10 μg/g for those without such establishments nearby. However, individuals living near factories had a significantly higher mean BLL of 2.66 μg/dL and mean HLL of 0.89 μg/g. Our study found no statistically significant difference in lead levels between the two groups: those with and without a factory near their homes (p=0.792 for BLL; p=0.522 for hair lead, Table III).

## Discussion

Lead is the most implicated heavy metal in constipation, and lead exposure is one of the preventable causes of constipation. Therefore, in our study, BLL and HLL of constipated and healthy children were evaluated to elucidate the etiology of chronic constipation. On May 14, 2021, the CDC's LEPAC updated the children's blood lead reference value from 5 μg/dL to 3.5 μg/dL (0.17 μmol/L). The updated value reflects the latest scientific evidence on the harmful effects of lead exposure on children, but the CDC emphasizes that there is no safe BLL and that even very low lead levels can cause neurodevelopmental effects. In our study, the mean BLL in the constipation group was above the reference value of 3.5 μg/dL, while the mean BLL in the control group was below this reference value. When the BLLs of

**Table III.** A comparison of blood and hair lead levels according to the distinctive characteristics of the patients.

| | Parameters | | n | Mean±SD | Median (Q1-Q3) | p* |
|---|---|---|---|---|---|---|
| Blood lead levels (μg/dL) | Sex | Male | 91 | 2.54±1.76 | 2.1 (1.2-3.3) | 0.508 |
| | | Female | 77 | 2.74±1.99 | 2.5 (1.2-4.1) | |
| | House renovation | No | 117 | 2.52±1.76 | 2.1 (1.3-3.45) | 0.372 |
| | | Yes | 51 | 2.89±2.08 | 2.5 (1.2-3.9) | |
| | Drinking water | Tap water | 12 | 2.77±1.26 | 2.5 (2.02-3.5) | 0.683 |
| | | Tap water with filter | 88 | 2.72±2.07 | 2.35 (1.1-3.75) | |
| | | Bottled water | 68 | 2.50±1.69 | 2.1 (1.2-3.45) | |
| | Smoking parent | No | 85 | 2.79±1.89 | 2.1 (1.3-3.85) | 0.354 |
| | | Yes | 83 | 2.47±1.84 | 2.3 (1.1-3.5) | |
| Hair lead levels (μg/g) | Sex | Male | 91 | 0.88±1.21 | 0.47 (0.24-1.29) | <0.001 |
| | | Female | 77 | 1.29±1.28 | 1.14 (0.57-1.54) | |
| | House renovation | No | 117 | 1.08±1.19 | 0.81 (0.33-1.44) | 0.628 |
| | | Yes | 51 | 1.03±1.42 | 0.64 (0.3-1.44) | |
| | Drinking water | Tap water | 12 | 1.15±1.21 | 0.77 (0.28-1.7) | 0.656 |
| | | Tap water with filter | 88 | 1.10±1.15 | 0.63 (0.28-1.43) | |
| | | Bottled water | 68 | 1.02±0.78 | 0.87 (0.35-1.42) | |
| | Smoking parent | No | 85 | 0.95±0.9 | 0.81 (0.31-1.44) | 0.655 |
| | | Yes | 83 | 1.19±1.54 | 0.71 (0.32-1.44) | |

the two groups were compared, it was found that the BLL of the group with constipation was statistically significantly higher than that of the group without constipation. In the constipation group, 48.8% of children had BLL above the reference value, while only 4.8% of children in the control group had elevated levels. These results suggest that children with constipation are at a ten times higher risk of lead exposure than those without constipation. In February 2023, Zamani et al. conducted a study on 237 children with chronic constipation. The study found that 20.67% of patients with constipation had BLL ≥ 5 μg/dL and the mean BLL in children with chronic constipation was 3.51 μg/dL.[12] These results indicate that BLL in children with chronic constipation are above the reference value, which is consistent with our study. This emphasizes the importance of lead as a potential cause of constipation and highlights the necessity of investigating lead exposure in its etiology.

According to the CDC's LEPAC, it is recommended that children be screened for lead levels twice, at 12 and 24 months of age. If a child is not screened at 24 months of age, it is recommended that they be screened at least once before they reach 72 months of age. For those who cannot be screened, it is important and recommended to identify high risk groups and check BLLs.[12] As there is currently no screening program in our country, the children in the study had not been previously screened for lead. Our study analyzed children aged 3-18 years, which is the upper limit of the age range covered by the screening program recommended by the CDC. The findings of our study are important for contributing to research on the effectiveness of screening programs by comparing them with other countries that implement screening programs.

Our study shows that the average BLL in our country are higher than in the United States.[9] This can be attributed to the continued use of lead-based paints and the absence of a screening program.

Research on lead in our country has been limited in scope, primarily involving small numbers of individuals, and remaining regional. However, studies conducted after the removal of leaded gasoline have shown a significant decrease in BLLs in children, especially in areas with heavy traffic. For example, a study conducted in İstanbul in 2013 found that the average BLL in children decreased from 8.4 μg/dl in 2000 to 1.84 μg/dL. Another study from our team, found that the average BLL of healthy children in İstanbul was 1.61 μg/dL, indicating a decrease since 2013.[18,19]

The available studies on lead levels in children with constipation are limited. Sevinc et al. found that BLLs were 5.12 μg/dL in the functional gastrointestinal disease group, 12.29 μg/dL in the functional constipation group, and 1.77 μg/dL in the control group.[20] Consistent with our study, it was observed that BLLs were significantly higher in the constipation group than in the control group. However, Sevinc et al.'s study on children in Karabük found higher lead levels in both the control group and the constipation group compared to our study. This difference may be explained by the higher density of industrial establishments and factories in Karabük compared to İstanbul, but more comprehensive studies are needed.

HLL can be measured as an alternative to BLL due to the ease of collection and storage, low cost and transport to the laboratory. In this study, the mean HLL of children in the control group was 0.88 μg/g, while the mean HLL of children with constipation was 1.26 μg/g. Adams et al. conducted a study in 2006 comparing the mean HLL of healthy children and children with autism spectrum disorder.[21] The results showed that the mean HLL of healthy children was 0.81 μg/g, while that of children with autism spectrum disorder was 0.62 μg/g. These results are similar to the HLL found in healthy children in our study. Although there have been many studies on HLL in children, a reference level for HLL in healthy children has not yet been established.[22] Our study could serve

as a resource for future research to establish reference values for HLL.

This is the first study to evaluate HLL and BLL together in children with and without constipation. The results indicate that there is no statistically significant difference in HLL between constipated and non-constipated children. This lack of difference may be due to the fact that it takes years for lead to accumulate in the hair or that external lead particles adhere to it, affecting the measured lead levels.

No significant correlation was found ($p=0.41$, $r_s=0.06$) when analyzing the relationship between blood and HLL in our study. According to the current literature review, Iaquinta et al. also found no correlation between hair and blood in their study conducted in January 2024.[23] The Toxicological Profile for Lead guideline of ATSDR, published in August 2020, states that HLL measurement is a relatively poor predictor, which is consistent with our findings.[24] The lack of correlation between lead particles and hair can be attributed to the adherence of exogenous lead particles to the hair. These particles adhere to the hair via dust, and there is currently no gold standard washing method to completely remove them. The study by Morton et al. demonstrated that the hair washing method recommended by the International Atomic Energy Agency is insufficient to completely remove exogenous lead from hair.[25] According to a study by Renshaw et al. exogenous lead particles tend to accumulate more in the distal part of the hair, resulting in a higher concentration of lead in the distal part compared to the proximal part.[26] Several studies have shown that various factors, such as age, gender, hair color, smoking, and ecological factors (geographical, racial/ethnic), can influence lead levels in hair. Schuhmacher et al. found that girls had higher HLL than boys, and that HLL decreased with age.[27] Finally, in 2020, Vigeh et al. conducted a study that found a negative correlation between lead levels in the hair of Iranian children and their weight.[28] The study also found that HLLs were higher in girls than boys. In our study, in agreement with previous studies, HLLs were higher in girls than in boys, but no significant difference in BLLs was observed between the sexes. This may be explained by the fact that exogenous lead particles adhere more to the hair of girls because their hair is longer. In this study, we found that BLLs did not vary with age, but we observed a negative correlation between age and HLLs. This tendency for HLL to decrease with age has been observed in numerous studies, including our own.[27,29] This may be attributed to exogenous particles adhering to the hair. It is possible that young children have more contact with lead dust on the ground, leading to increased exposure to exogenous lead particles and higher lead levels. Our study concludes that hair is not a reliable material to measure endogenous lead exposure due to the influence of exogenous lead particles on lead levels. However, further research is needed to investigate the correlation between lead levels in hair and exogenous lead particles.

In our study, we observed that as the age of the homes in which children lived increased, so did the lead levels. This data supports the information provided by the CDC that children living in older homes are at higher risk of lead exposure.[8] This may be due to the greater use of lead-based paint in older homes and the increased exposure of children to paint particles.

The CDC also lists drinking water used in homes as a source of lead exposure and lead pipes that deliver water to homes are blamed.[8] In our study, children were categorized according to the type of drinking water they used, but no statistically significant association was found between the type of drinking water and BLL and HLL. This finding may be due to the reduced use of lead pipes in plumbing systems in our country.

Smoking and exposure to cigarette smoke have been linked to lead exposure.[8] Wolfsperger et al. found that HLL were higher in young healthy adult smokers than in nonsmokers.[30] In our study, we compared the BLL and HLL of children with parents who smoke to those with non-smoking parents and found no significant

difference. The absence of a correlation may be due to parents refraining from smoking around their children and reducing their direct exposure to cigarette smoke. However, further extensive research is required to investigate this matter.

Finally, understanding the constipation-lead exposure link is crucial for prevention and intervention. Healthcare providers should remember to investigate lead exposure risk factors in constipation patients and test BLL when necessary. Few studies have looked at BLL and HLL together in children, and none have looked at their association with constipation. This study is the first of its kind, which makes it important. However, it is limited by the relatively small number of children included, so more comprehensive studies are needed to better understand the role of lead levels in the etiology of chronic constipation and to assess the impact of different methods of measurement other than blood, such as hair.

## Ethical approval

This study was approved by the Bezmialem Vakıf University Clinical Research Ethics Committee on January 13, 2022 (Decision number E-46716).

## Author contribution

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

## Source of funding

This study was supported by the Bezmialem Vakıf University Scientific Research Projects Coordination Unit (Project No: 20220201).

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES

1. Aziz I, Whitehead WE, Palsson OS, Törnblom H, Simrén M. An approach to the diagnosis and management of Rome IV functional disorders of chronic constipation. Expert Rev Gastroenterol Hepatol 2020; 14: 39-46. https://doi.org/10.1080/17474124.2020.1708718
2. Benninga M, Candy DC, Catto-Smith AG, et al. The Paris Consensus on Childhood Constipation Terminology (PACCT) Group. J Pediatr Gastroenterol Nutr 2005; 40: 273-275. https://doi.org/10.1097/01.mpg.0000158071.24327.88
3. Inan M, Aydiner CY, Tokuc B, et al. Factors associated with childhood constipation. J Paediatr Child Health 2007; 43: 700-706. https://doi.org/10.1111/j.1440-1754.2007.01165.x
4. Tabbers MM, Boluyt N, Berger MY, Benninga MA. Clinical practice: diagnosis and treatment of functional constipation. Eur J Pediatr 2011; 170: 955-963. https://doi.org/10.1007/s00431-011-1515-5
5. Nurko S, Zimmerman LA. Evaluation and treatment of constipation in children and adolescents. Am Fam Physician 2014; 90: 82-90.
6. Bushnell PJ, Jaeger RJ. Hazards to health from environmental lead exposure: a review of recent literature. Vet Hum Toxicol 1986; 28: 255-261.
7. World Health Organization Regional Office for Europe European Environment and Health Information System (ENHIS). Exposure of children to chemical hazards in food. 2007. Available at: https://iris.who.int/bitstream/handle/10665/370951/WHO-EURO-2007-6412-46178-66793-eng.pdf?sequence=1 (Accessed on January 1, 2024).
8. National Geographic. Finally, the end of leaded gas. 2021. Available at: https://www.nationalgeographic.com/environment/article/finally-the-end-of-leaded-gas (Accessed on January 1, 2024).
9. Centers for Disease Control and Prevention (CDC). Childhood lead poisoning prevention. Sources of lead exposure. 2023. Available at: https://www.cdc.gov/lead-prevention/prevention/index.html (Accessed on January 1, 2024).
10. Surkan PJ, Zhang A, Trachtenberg F, Daniel DB, McKinlay S, Bellinger DC. Neuropsychological function in children with blood lead levels <10 microg/dL. Neurotoxicology 2007; 28: 1170-1177. https://doi.org/10.1016/j.neuro.2007.07.007

11. Dietrich KN, Krafft KM, Bornschein RL, et al. Low-level fetal lead exposure effect on neurobehavioral development in early infancy. Pediatrics 1987; 80: 721-730. https://doi.org/10.1542/peds.80.5.721

12. Zamani N, Hosseini A, Farnaghi F, et al. Blood lead level evaluation in children presenting with chronic constipation in Tehran-Iran: a cross-sectional study. Sci Rep 2023; 13: 2301. https://doi.org/10.1038/s41598-023-29487-y

13. Shabani M, Hadeiy SK, Parhizgar P, et al. Lead poisoning; a neglected potential diagnosis in abdominal pain [published correction appears in BMC Gastroenterol 2021; 21: 411.]. BMC Gastroenterol 2020; 20: 134. https://doi.org/10.1186/s12876-020-01284-1

14. Ruckart PZ, Jones RL, Courtney JG, et al. Update of the blood lead reference value - United States, 2021. MMWR Morb Mortal Wkly Rep 2021; 70: 1509-1512. https://doi.org/10.15585/mmwr.mm7043a4

15. Barbosa F Jr, Tanus-Santos JE, Gerlach RF, Parsons PJ. A critical review of biomarkers used for monitoring human exposure to lead: advantages, limitations, and future needs. Environ Health Perspect 2005; 113: 1669-1674. https://doi.org/10.1289/ehp.7917

16. Rabinowitz M, Leviton A, Needleman H. Lead in milk and infant blood: a dose-response model. Arch Environ Health 1985; 40: 283-286. https://doi.org/10.1080/00039896.1985.10545933

17. Omokhodion FO, Crockford GW. Lead in sweat and its relationship to salivary and urinary levels in normal healthy subjects. Sci Total Environ 1991; 103: 113-122. https://doi.org/10.1016/0048-9697(91)90137-4

18. World Health Organization (WHO). Legally-binding controls on lead paint. 2023. Available at: https://www.who.int/data/gho/data/themes/topics/indicator-groups/legally-binding-controls-on-lead-paint (Accessed on January 10, 2024).

19. Dikme G, Arvas A, Gur E. The relation between blood lead and mercury levels and chronic neurological diseases in children. Turk Ach Pediatr 2013; 48: 221-225. https://doi.org/10.4274/tpa.296

20. Sevinc N, Bilici N, Sevinc E, Dogan E. Blood and faecal lead levels in children with various functional gastrointestinal disorders. An Pediatr (Engl Ed) 2022; 96: 35-42. https://doi.org/10.1016/j.anpede.2021.02.001

21. Adams JB, Holloway CE, George F, Quig D. Analyses of toxic metals and essential minerals in the hair of Arizona children with autism and associated conditions, and their mothers. Biol Trace Elem Res 2006; 110: 193-209. https://doi.org/10.1385/BTER:110:3:193

22. Guo BQ, Li HB, Liu YY. Association between hair lead levels and autism spectrum disorder in children: a systematic review and meta-analysis. Psychiatry Res 2019; 276: 239-249. https://doi.org/10.1016/j.psychres.2019.05.022

23. Iaquinta F, Machado I. Biomonitoring of arsenic, lead, manganese and mercury in hair from a presumably exposed Uruguayan child population. Bioanalysis 2024; 16: 107-116. https://doi.org/10.4155/bio-2023-0157

24. Agency for Toxic Substances and Disease Registry. Toxicological profile for lead. 2020. Available at: https://www.atsdr.cdc.gov/toxprofiles/tp13.pdf

25. Morton J, Carolan VA, Gardiner PHE. Removal of exogenously bound elements from human hair by various washing procedures and determination by inductively coupled plasma mass spectrometry. Anal Chim Acta 2002; 455: 23-34. https://doi.org/10.1016/S0003-2670(01)01578-1

26. Renshaw GD, Pounds CA, Pearson EF. Variation in lead concentration along single hairs as measured by non-flame atomic absorption spectrophotometry. Nature 1972; 238: 162-163. https://doi.org/10.1038/238162a0

27. Schuhmacher M, Domingo JL, Llobet JM, Corbella J. Lead in children's hair, as related to exposure in Tarragona province, Spain. Sci Total Environ 1991; 104: 167-173. https://doi.org/10.1016/0048-9697(91)90070-u

28. Vigeh M, Yokoyama K, Matsukawa T, Shinohara A, Ohtani K, Shariat M. Hair metal levels and childhood weight gain. Iran J Public Health 2020; 49: 1510-1519. https://doi.org/10.18502/ijph.v49i8.3895

29. Tippairote T, Temviriyanukul P, Benjapong W, Trachootham D. Prevalence and factors associated with high levels of aluminum, arsenic, cadmium, lead, and mercury in hair samples of well-nourished Thai children in Bangkok and perimeters. Biol Trace Elem Res 2019; 188: 334-343. https://doi.org/10.1007/s12011-018-1435-6

30. Wolfsperger M, Hauser G, Gössler W, Schlagenhaufen C. Heavy metals in human hair samples from Austria and Italy: influence of sex and smoking habits. Sci Total Environ 1994; 156: 235-242. https://doi.org/10.1016/0048-9697(94)90190-2

The Turkish Journal of Pediatrics 2024; 66: 534-543
https://doi.org/10.24953/turkjpediatr.2024.4691

**Original Article**

# Adaptation of the ''Food Allergy Self-Efficacy Scale for Parents''(FASE-P) to Turkish: a validity and reliability study

Nilay Çalışkan[1], Şeyma Genç[2], Güler Yıldırım[1], Hamit Bologur[1], Hilal Güngör[1], Merve Karaca Şahin[1], Muhammed Fatih Erbay[1], Şefika İlknur Kökcü Karadağ[1], Özlem Terzi[3], Deniz Özçeker[1]

*[1]Department of Pediatric Allergy and Immunology, Prof. Dr. Cemil Taşçıoğlu City Hospital, İstanbul; [2]Republic of Türkiye Ministry of Health İlkadım District Health Directorate, Samsun; [3]Department of Public Health, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Türkiye.*

## ABSTRACT

**Background.** Food allergy is a public health issue that has a significant impact on the lives of families. Parental self-efficacy/confidence is important in managing food allergies. The aim of this study is to validate the "Food Allergy Self-Efficacy Scale for Parents" (FASE-P) and assess parental self-efficacy in managing their child's food allergy.

**Methods.** Turkish version of the FASE-P (T-FASE-P) was administered to 347 parents of children aged 0-18 who had been followed for at least one month due to food allergy at the Pediatric Allergy Clinic of Prof. Dr. Cemil Taşcıoğlu City Hospital between September 1 and December 31, 2023, through face-to-face interviews and online surveys for parents of children with food allergies from the general population. Content validity, exploratory factor analysis (EFA), and confirmatory factor analysis (CFA) were conducted to evaluate the validity of the scale. General Self-Efficacy Scale (GSES) was used for concurrent criterion validity. Internal consistency analysis, test-retest application, and item analysis were conducted to assess its reliability.

**Results.** T-FASE-P scale initially contained 21 items, and the Cronbach's alpha coefficient ($\alpha$) calculated in this form was found to be 0.89. Later, when 4 items were excluded, the 17-item version of the scale was calculated as $\alpha$=0.90. The intra-class correlation coefficient between the test and re-test was found to be 0.972. The content validity index value of the scale was calculated as 0.99, indicating that the content validity was at a sufficient level. In the EFA, it was determined that the scale formed a three-factor structural model and that this model explained 60.82% of the total variance. As a result of the CFA, the fit indices were calculated as $\chi$2/df=2.341, GFI=0.919, TLI=0.950, indicating a good level of fit. Based on the analysis results, T-FASE-P consists of 17 items and three subscales.

**Conclusion.** T-FASE-P scale is a valid and reliable measurement tool that can be used to determine the food allergy self-efficacy of Turkish parents.

**Key words:** children, food allergy, parents, self-efficacy.

Food allergy is one of the rapidly increasing allergic diseases worldwide and is a public health problem with a significant impact on the lives of families.[1] There is no definitive treatment for food allergy. The goal is to avoid allergens and administer emergency treatments

✉ Nilay Çalışkan ▪ nilaykadakal@gmail.com

Received 30th Apr 2024, revised 3rd Jul 2024, 2nd Sep 2024, accepted 30th Sep 2024.

This study was presented as an oral presentation at the 18th International Children's Allergy Immunology and Asthma Congress on April 26, 2024 in the Turkish Republic of Northern Cyprus.

in accidental encounters.[2] Symptoms can range from mild to moderate, such as hives, to life-threatening reactions.[3] Since children are mostly diagnosed before the age of 5, parents are largely responsible for managing food allergies, including avoiding allergens, checking food labels, preventing accidental exposures, and carrying an adrenaline auto-injector.[4,5] Therefore, managing food allergies can increase stress and anxiety in parents, particularly mothers, affecting their daily and social activities, and reducing their quality of life.[6-8]

Studies evaluating the quality of life and parental burden of food allergy have found associations between decreased quality of life and factors such as using epinephrine, having multiple food allergies, experiencing anaphylaxis, being younger at the time of reaction, and having milk and egg allergies.[9,10]

In a recent study conducted in our country, mothers of children with food allergies had higher levels of depression and anxiety than mothers in the control group.[11]

Another study recently reported in our country determined that breastfeeding mothers of babies with food allergies are more anxious and prone to depression, and the need for social support for caregivers was emphasized. These findings make us think that we need measurement tools for our country that will identify areas where parents feel inadequate in their social lives.[12]

Informing parents about food allergies is important for protecting the patient from life-threatening reactions, preventing unnecessary eliminations leading to nutritional deficiencies, and preserving the quality of life of the patient/parent.[13]

When other studies in this context are examined, it is found that the lack of knowledge about preventing accidental ingestion of allergens and applying emergency treatment approaches increases anxiety.[14] Most parents report being worried about their children having an anaphylactic reaction and not knowing what to do during anaphylactic shock.[15]

Therefore, parental self-efficacy/confidence is important in managing food allergies and is associated with better parental quality of life.[16]

Self-efficacy is defined as the belief in one's ability to organize and execute actions necessary to manage situations effectively.[17] Developing self-efficacy helps individuals feel more capable of overcoming challenging problems. Enhancing self-efficacy in children with chronic illnesses and their parents can improve their quality of life.[18,19]

Food allergies require constant attention to prevent accidental exposure and potentially life-threatening symptoms, impacting the anxiety levels and quality of life of families.[20]

Existing quality of life scales are good at determining the impact of food allergies on various aspects of life but are insufficient in identifying areas where there is a lack of confidence in managing them. The widely used General Self-Efficacy Scale (GSES) aims to predict coping with daily challenges but does not encompass issues related to managing food allergies. Therefore, there is a need for guiding studies to identify areas of low self-efficacy in managing food allergies. This study will contribute to the literature by adapting and validating the "Food Allergy Parental Self-Efficacy Scale" into Turkish to measure parental self-efficacy in managing food allergies.

## Materials and Methods

### *Study population and design*

It is recommended to reach a sample size that is at least 2-10 times the number of items in the scale when adapting a scale to another culture.[18] It was decided to include at least 210 participants, based on a sample size that is 10 times the number of scale items. However, considering potential data losses, 347 volunteers meeting the criteria and in follow-up were included

in the study. Between September 1, 2023, and December 31, 2023, 347 parents of children aged 0-18 who had been followed for at least one month due to food allergy at the Pediatric Allergy Clinic of Prof. Dr. Cemil Taşcıoğlu City Hospital were included in the study. The study aimed to evaluate the reliability and validity of the scale on a large sample through an online survey of parents of children with food allergies from the general population.

### *Data collection tools*

The data collection tools included an introductory information form, the Turkish-translated Food Allergy Self-Efficacy Scale for Parents (T-FASE-P), and the GSES. Participants provided verbal consent, and survey forms were administered face-to-face, online, or over the telephone. It took an average of 8-10 minutes to complete the forms.

### *FASE-P scale*

The scale consists of 5 subscales and 21 items: " Precaution & Prevention" (item numbers 1-6), "Allergic Treatment" (#7-8),"Food Allergy Identification" (#9-11), "Seeking Information About Food Allergy" (#12-15) and "Managing Social Activities" (#16-21). Responses on the scale are collected and then divided by 21 to obtain the total average score. The resulting score has a range of 0-100. Similarly, the items in each subscale are summed and divided by the number of items in that subscale. Each item in the FASE-P is scored on a 100-point visual analog scale; higher scores indicate greater selfefficacy for managing food allergies.[21]

### *General self-efficacy scale (GSES)*

It consists of 10 questions that assess parents' general self-efficacy regardless of the underlying disease. The adaptation, validity, and reliability study of the GSES developed by Sherer and colleagues (1982) into Turkish was conducted by Yıldırım and İnan[17] The scale's internal consistency (Cronbach's alpha) value was found to be 0.80. It is a valid and reliable tool for measuring the general self-efficacy of individuals aged 18 and over who have at least completed primary school. The total score of the scale can range from 17 to 85; an increase in score indicates an increase in self-efficacy beliefs.

### *Translation process*

The adaptation of the scale into Turkish was conducted with permission from one of the authors who developed the scale. The English version of the FASE-P was translated into Turkish following the steps outlined by the World Health Organization guidelines, including: 1) Forward translation into Turkish was conducted by two independent individuals who are native Turkish speakers and fluent in English. Easily understandable words or phrases that convey the same concept as in the original text were selected for translation. 2) The reconciled version was designed by two independent Turkish pediatricians who are fluent in English. 3) The final version was re-translated into English by an independent bilingual translator whose native language is English. The back-translated version was checked by an independent supervisor. 4) Translated questionnaires were pretested on 15 parents to confirm clarity and comprehensibility. Participants provided feedback on whether the terms were difficult to understand or if the questions were ambiguous. Except for a few questions, no question was misunderstood or misinterpreted by the parents at this stage.

### *Content validity*

To evaluate the content validity of the scale, the final version of the translated scale was emailed to 10 experts including 3 pediatrician, 1 psychiatrist, 6 pediatric allergy subspecialists from different institutions (public and private sectors) for their opinions, and the content validity indexes (CVIs) were calculated for each item. Modifications were made to the items based on the experts' suggestions, and the final version of the scale was provided.

### *Reliability*

To determine the internal consistency and reliability of the T-FASE-P, Cronbach's alpha coefficient was measured for each age group and each subscale. A test-retest analysis was conducted to test its stability over time. Test-retest reliability was determined using the intraclass correlation coefficient (ICC) for two surveys conducted with parents whose child's allergy status remained the same between two visits, with a two-week interval.

### *Construct validity*

To determine the factor structure to which the items in the scale are connected, an exploratory factor analysis (EFA) was conducted. Factor analysis is an analytic technique that permits the reduction of a large number of correlated variables to a smaller number of latent dimensions. The goal of factor analysis is to achieve parsimony by using the smallest number of explanatory concepts to explain the maximum amount of common variance in a correlation matrix.[22] Varimax-axis rotation method was used to calculate the factor loads, and confirmatory factor analysis (CFA) was performed to test the compatibility with the original study. Goodness of fit values (adjusted goodness-of-fit index [AGFI], comparative fit index [CFI], goodness-of-fit index [GFI], incremental fit index [IFI], root mean square error of approximation [RMSEA], chi square [$\chi 2/df$], Tucker-Lewis index [TLI]) were examined for CFA.

### *Concurrent criterion validity*

In this study, the GSES was used to determine criterion validity. The scores of parents on the T-FASE-P and GSES were tested using Pearson correlation analysis.

### *Statistical analyses*

The data analyses were conducted using SPSS (Statistical Package for Social Sciences) 23 (SPSS Inc., Chicago, IL, USA) and AMOS (Analysis of Moment Structures) 26 programs. The study employed statistical analyses for validity and reliability (content validity, internal consistency analysis, EFA, CFA, etc.). Descriptive statistics were used to express continuous variables: mean ± standard deviation (SD), minimum and maximum values. Frequency data were presented as counts and percentages (%). Normal distribution of continuous variables was assessed using the Kolmogorov-Smirnov test. Chi-square test was used for comparison of frequency data. Non-parametric tests were used for group comparisons of continuous variables when they did not follow a normal distribution. The Mann-Whitney U test was conducted for continuous variable comparisons between two groups. A significance level of $p < 0.05$ was considered for all statistical comparisons.

### *Ethical committee approval*

The ethical approval for the reliability and validity phase of this study was obtained from the Prof. Dr. Cemil Taşcıoğlu City Hospital Clinical Research Ethics Committee with decision number 2023/214 dated 13/07/2023. Informed consent was obtained from all parents.

## Results

The study included 347 parents of children aged 0-18 diagnosed with food allergies. The average age of the children was 48.4±41.8 months, with 64.3% being female. The most common allergies were cow's milk (59.7%), eggs (55.9%), walnuts (26.5%), hazelnuts (25.9%), and peanuts (25.6%). The average age of the parents was 33.6±5.8 years, with 90.8% being mothers and 9.2% fathers. 62.9% of the mothers and 68.8% of the fathers were university graduates. There was no statistically significant difference between the parents in terms of age and education level ($p=0.13$ and $p=0.51$, Table I).

### *Validity study findings*

### *Content validity*

In this study, content validity was established by consulting expert opinions. For this purpose,

**Table I.** Descriptive characteristics of parents and their food-allergic children.

| | |
|---|---|
| Parents, n | 347 |
| Mother / father, n (%) | 315 / 32 (90.8% / 9.2%) |
| Age, yr, mean±SD (min-max) | 33.6±5.8 (18-59) |
| Education level of mother / father, n (%) | |
| Primary or high school | 117 / 10 (37.1% / 31.2%) |
| University | 198 / 22 (62.9% / 68.8%) |
| Children | |
| Age, mo, mean±SD (min-max) | 48.4±41.8 (1-120) |
| Female / male, n (%) | 223 (64.3%) / 123 (35.4%) |
| Common food allergies, n (%) | |
| Cow milk | 207 (59.7%) |
| Hen egg | 194 (55.9%) |
| Walnut | 92 (26.5%) |
| Hazelnut | 90 (25.9%) |
| Peanut | 89 (25.6%) |

SD: standard deviation

the opinions of 9 experts were sought. The Davis method was used to consult expert opinions. According to this approach, experts were asked to express their opinions using four different rating options: (a) "item appropriate," (b) "item needs slight revision," (c) "item needs substantial revision," and (d) "item not appropriate." Then, the numbers of experts who selected (a) "item appropriate" and (b) "item needs slight revision" were totaled and divided by the total number of experts to calculate Content Validity Index (CVI).

In the study, the CVI of the scale was calculated as 0.99, indicating that the content validity of the scale was determined to be at a sufficient level.

### *Concurrent criterion validity*

GSES was used to determine criterion validity. It was found that the correlation between the T-FASE-P and the GSES was positive, moderate in strength, and statistically highly significant ($r=0.27$, $p<0.001$).

GSES score was found to have statistically significant weak positive correlations with subscale 1 score (r: 0.24, $p<0.001$), subscale 2 score (r: 0.20, $p<0.001$), and subscale 3 score (r: 0.19, $p<0.001$).

### *Construct validity*

The Kaiser-Meyer-Olkin Measure of Sampling Adequacy (KMO) coefficient was calculated as 0.86 to assess the suitability of the data for EFA. It was determined that the sample adequacy was at a very good level. Additionally, the result of Bartlett's test of sphericity yielded an approximate chi-square value of 3730.19 ($p<0.001$), indicating that the data in the study were suitable for factor analysis.

When evaluating the results of the factor analysis conducted using the Varimax rotation method, the criterion was considered that items with factor loadings greater than 0.30 and positive should be included in the factor.

In the analysis to determine the items to be included in the factor, when items appeared in more than one factor, if the difference in the loadings they gave to these factors was less than 0.10, the item was eliminated. The factor loadings of the scale range from 0.574 to 0.849 (Table II). Items 4, 16, 17, 18, and 19 were found to be under two factors. It was decided that these items should remain under the factors to which they had higher factor loadings. Therefore, "item 4" was evaluated under Factor 2, and Items 16, 17, 18, and 19 were evaluated

**Table II.** Exploratory factor analysis results for the T-FASE-P cale.

| Items | Factor 1 | Factor 2 | Factor 3 |
|---|---|---|---|
| Item 1 | | 0.576 | |
| Item 2 | | 0.693 | |
| Item 3 | | 0.722 | |
| Item 4 | 0.386 | 0.680 | |
| Item 5 | | 0.708 | |
| Item 6 | | 0.691 | |
| Item 7 | | | 0.670 |
| Item 8 | | | 0.574 |
| Item 9 | | | 0.790 |
| Item 10 | | | 0.740 |
| Item 11 | | | 0.690 |
| Item 16 | 0.706 | 0.443 | |
| Item 17 | 0.705 | 0.428 | |
| Item 18 | 0.792 | 0.312 | |
| Item 19 | 0.777 | 0.345 | |
| Item 20 | 0.849 | | |
| Item 21 | 0.848 | | |
| Eigenvalues | 6.714 | 2.330 | 1.297 |
| Variance % | 24.090 | 20.949 | 15.785 |
| Cumulative Variance% | 24.090 | 45.039 | 60.824 |

T-FASE-P: Turkish version of the Food Allergy Self-Efficacy Scale for Parents

under Factor 1. As a result of the analysis, a three-factor structural model with eigenvalues greater than 1 was observed to emerge. It was found that the factor loadings, which were positive for the 6 items (#16-21) under Factor 1 of the T-FASE-P scale, ranged from 0.705 to 0.849.

There are 6 items (#1-6) under Factor 2, and it was observed that the factor loadings, which were positive, ranged from 0.576 to 0.722. Factor 3 consists of 5 items (#7-11), and it was observed that the factor loadings, which were positive, ranged from 0.574 to 0.790. Factor 1 accounted for 24.09% of the total variance, Factor 2 accounted for 20.95%, and Factor 3 accounted for 15.78%. When all factors were considered together, it was determined that the scale explained 60.82% of the total variance (Table II).

The 17-item, 3-factor structure resulting from EFA was tested using CFA. When examining the fit indices, the following values were obtained: $\chi 2/df=2.341$, RMSEA=0.062, GFI=0.919, CFI=0.958, AGFI=0.891, TLI=0.950, and IFI=0.959. GFI, TLI, IFI, and $\chi 2/df$ indicated good fit, while RMSEA, CFI, and AGFI showed an acceptable fit (Table III). The path diagram of the model for the scale is shown in Fig. 1.

**Table III.** Results of confirmatory factor analysis.

| Model fit | Score |
|---|---|
| $\chi 2/df$ | 2.341 |
| RMSEA | 0.062 |
| GFI | 0.919 |
| CFI | 0.958 |
| AGFI | 0.891 |
| TLI | 0.950 |
| IFI | 0.959 |

AGFI, adjusted goodness-of-fit Index; CFI, comparative fit index; GFI, goodness-of-fit index; IFI, incremental fit index; RMSEA, root mean square error of approximation; $\chi 2/df$, chi-square, TLI, Tucker-Lewis index.

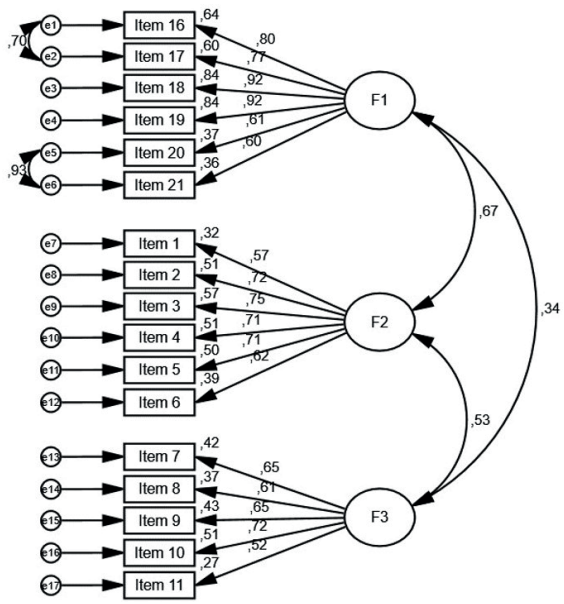



**Fig. 1.** Path diagram of the T-FASE-P scale.

T-FASE-P: Turkish version of the Food Allergy Self-Efficacy Scale for Parents

**Table IV.** The results of the internal consistency analysis.

| | Corrected item-total correlation | Cronbach's alpha if item deleted |
|---|---|---|
| Item 16 | 0.730 | 0.878 |
| Item 17 | 0.704 | 0.879 |
| Item 18 | 0.721 | 0.878 |
| Item 19 | 0.746 | 0.877 |
| Item 20 | 0.599 | 0.883 |
| Item 21 | 0.598 | 0.883 |
| Item 1 | 0.501 | 0.886 |
| Item 2 | 0.608 | 0.882 |
| Item 3 | 0.633 | 0.881 |
| Item 4 | 0.643 | 0.881 |
| Item 5 | 0.607 | 0.882 |
| Item 6 | 0.514 | 0.885 |
| Item 7 | 0.402 | 0.888 |
| Item 8 | 0.472 | 0.886 |
| Item 9 | 0.273 | 0.890 |
| Item 10 | 0.360 | 0.890 |
| Item 11 | 0.273 | 0.890 |
| Item 12 | 0.060 | 0.892 |
| Item 13 | 0.095 | 0.892 |
| Item 14 | 0.097 | 0.892 |
| Item 15 | 0.051 | 0.893 |

### *Findings regarding the reliability study*

#### *Internal consistency analysis*

According to the results of the internal consistency analysis, it was observed that the item-total score correlation coefficients ranged from 0.051 to 0.746 (Table IV). The corrected item-total correlations for the 4 items (#12-15) questioning information acquisition from family doctors or nurses, hospital pediatricians or pediatric allergy specialists, food sellers, and websites regarding food allergies were found to be less than 0.20. Therefore, it was considered that these items didn't move in the same direction as the whole scale. It was decided that these items should be removed from the scale as the Cronbach's alpha value of the scale significantly increased when these items were removed.

**Table V.** Cronbach's alphas for the T-FASE-P scale and subscales.

| T-FASE-P | Cronbach's alfa |
|---|---|
| Total scale | 0.90 |
| Subscales | |
| Precaution & prevention | 0.83 |
| Allergic treatment / Food allergen identification | 0.74 |
| Managing social activities | 0.91 |

*Turkish version of the Food Allergy Self-Efficacy Scale for Parents.

The T-FASE-P scale initially contained 21 items, and the Cronbach's alpha coefficient ($\alpha$) calculated for this version was found to be 0.89. After removing 4 items (#12-15) the 17-item version of the scale was calculated to have an $\alpha$ of 0.90. These results indicate that the scale and its subscales have a high level of reliability. The evaluation in the subsequent stages of the study continued with the 17-item version. The survey questions used in this study are listed in the Supplementary Materials.

The alpha values for the final version of the scale were found to be $\alpha$=0.83 for the "precaution & prevention" subscale, $\alpha$=0.74 for the "allergic treatment & food allergen identification" subscale, and $\alpha$=0.91 for the "managing social activities" subscale (Table V).

#### *Test-retest reliability*

For the re-test application, 30 volunteers were asked to respond to the same form using the same method as the initial test. Re-tests were conducted two weeks apart, with the condition that allergy status remained consistent between the two visits. The intraclass correlation coefficient between the first test and retest was found to be 0.97. The intraclass correlation coefficients for subscales 1, 2, and 3 were 0.78 ($p<0.001$), 0.97 ($p<0.001$), and 0.75 ($p<0.001$), respectively.

## Discussion

In this study, we aimed to evaluate the reliability and validity of the T-FASE-P. Initially, linguistic equivalence with the original form was achieved through the back-translation method. This result indicated that the process of translating the scale into Turkish was successfully completed.

After the linguistic equivalence study, we examined the psychometric properties of the scale through exploratory and confirmatory factor analysis, test-retest reliability, calculation of internal consistency coefficients, and criterion-related validity methods. We found that T-FASE-P is a valid and reliable scale that can be used in the Turkish population.

According to the reliability analysis, it was observed that the overall alpha value of the scale (0.90) was almost the same as the original scale's value of 0.88, indicating that it has excellent internal consistency.[21]

Prior to factor analysis, the Kaiser-Meyer-Olkin (KMO) test and Bartlett's test of sphericity were conducted to assess whether the sample size was adequate for factor analysis. The evaluation indicated that the KMO test was above 0.60 and the result of Bartlett's test of sphericity was statistically significant, indicating that the data were suitable for factor analysis and the sample adequacy was at a very good level.

To determine the factor structure, exploratory and confirmatory factor analyses were conducted. According to the EFA, a three-factor structure was obtained, explaining 60.82% of the total variance. The factor loadings of the 17 items under these three factors ranged from 0.574 to 0.849, and those items with factor loadings below 0.30 were removed from the scale. Similar to our results, the total variance of the original scale was found to be 59.8%, with factor loadings ranging from 0.408 to 0.849.[21] It was determined that the five-factor structure in its original form was reduced to a three-factor structure in the sample of Turkish parents, and the goodness-of-fit indices maintained its appropriateness through the evaluations.

To demonstrate the factor structure of the scale and how well the measurement model fits the data, fit indices were calculated. It is recommended to use multiple indices when evaluating fit.[17,23] Therefore, in the CFA conducted for our scale, the fit indices were calculated as follows: $\chi 2/df=2.341$, GFI=0.919, TLI=0.950, RMSEA=0.062. All indices were evaluated according to the standard criteria recommended by Schermelleh-Engel et al., indicating that the model fit well.[24]

The study found a strong and significant correlation ($p<0.001$) between T-FASE-P and GSES, supporting the criterion validity of the scale. However, the correlation with GSES was lower than expected, possibly because GSES does not specifically address issues relevant to managing food allergies. Previous studies have also found only moderate correlations between general and parental self-efficacy, indicating that generalized self-efficacy may not be sufficiently sensitive in measuring behavior-specific self-efficacy.[25]

## Conclusion

The collaboration between allergy specialists and psychologists, as well as education and public health measures supporting food allergy self-efficacy in families, are crucial for the future success of managing food allergies. Identifying areas of insufficient parental self-efficacy may be important for managing food allergies in children and improving their quality of life. Additionally, the T-FASE-P may vary depending on cultural dietary habits and lifestyles, so it is necessary to validate the questionnaire in different societies.

## Supplementary materials

Supplementary materials for this article are available online at https://doi.org/10.24953/turkjpediatr.2024.4691

## Ethical approval

The ethical approval for the reliability and validity phase of this study was obtained from the Ondokuz Mayıs University Clinical Research Ethics Committee (number 2023/214 date 13/07/2023).

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: NÇ, DÖ; data collection: GY, HG, MFE, ŞİKK, HB, MKŞ; analysis and interpretation of results: ŞG, ÖT; draft manuscript preparation: NÇ, HG, DÖ. All authors reviewed 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. Peters RL, Krawiec M, Koplin JJ, Santos AF. Update on food allergy. Pediatr Allergy Immunol 2021; 32: 647-657. https://doi.org/10.1111/pai.13443
2. Seth D, Poowutikul P, Pansare M, Kamat D. Food allergy: a review. Pediatr Ann 2020; 49: e50-e58. https://doi.org/10.3928/19382359-20191206-01
3. Boyce JA, Assa'ad A, Burks AW, et al. Guidelines for the diagnosis and management of food allergy in the United States: summary of the NIAID-sponsored expert panel report. Nutr Res 2011; 31: 61-75. https://doi.org/10.1016/j.nutres.2011.01.001
4. Brown D, Negris O, Gupta R, et al. Food allergy-related bullying and associated peer dynamics among black and white children in the FORWARD study. Ann Allergy Asthma Immunol 2021; 126: 255-263.e1. https://doi.org/10.1016/j.anai.2020.10.013
5. Pappalardo AA, Herbert L, Warren C, et al. Self-efficacy among caregivers of children with food allergy: a cohort study. J Pediatr Psychol 2022; 47: 674-684. https://doi.org/10.1093/jpepsy/jsab137
6. King RM, Knibb RC, Hourihane JO. Impact of peanut allergy on quality of life, stress and anxiety in the family. Allergy 2009; 64: 461-468. https://doi.org/10.1111/j.1398-9995.2008.01843.x
7. Bollinger ME, Dahlquist LM, Mudd K, Sonntag C, Dillinger L, McKenna K. The impact of food allergy on the daily activities of children and their families. Ann Allergy Asthma Immunol 2006; 96: 415-421. https://doi.org/10.1016/S1081-1206(10)60908-8
8. Cummings AJ, Knibb RC, King RM, Lucas JS. The psychosocial impact of food allergy and food hypersensitivity in children, adolescents and their families: a review. Allergy 2010; 65: 933-945. https://doi.org/10.1111/j.1398-9995.2010.02342.x
9. Ward CE, Greenhawt MJ. Treatment of allergic reactions and quality of life among caregivers of food-allergic children. Ann Allergy Asthma Immunol 2015; 114: 312-318.e2. https://doi.org/10.1016/j.anai.2014.12.022
10. Howe L, Franxman T, Teich E, Greenhawt M. What affects quality of life among caregivers of food-allergic children? Ann Allergy Asthma Immunol 2014; 113: 69-74.e2. https://doi.org/10.1016/j.anai.2014.04.016
11. Kılıç N, Kaya Ş, Taşçı G, Özsoy F, Kılıç M. Quality of life in children with food allergies, psychiatric symptoms, and caregiving burden of their mothers. Allergol Immunopathol (Madr) 2023; 51: 48-56. https://doi.org/10.15586/aei.v51i5.860
12. Yilmaz O, Kacar AS, Gogebakan E, et al. The relationship between dietary elimination and maternal psychopathology in breastfeeding mothers of infants with food allergy. Pediatr Allergy Immunol 2022; 33: e13670. https://doi.org/10.1111/pai.13670
13. Altıntaş DU, Büyüktiryaki AB, Çağdaş Ayvaz DN, et al. Besin Alerjisi: Türk Ulusal Rehberi 2017. Astım Allerji İmmünoloji 2017; 15: S81.
14. Mandell D, Curtis R, Gold M, Hardie S. Anaphylaxis: how do you live with it? Health Soc Work 2005; 30: 325-335. https://doi.org/10.1093/hsw/30.4.325
15. Akeson N, Worth A, Sheikh A. The psychosocial impact of anaphylaxis on young people and their parents. Clin Exp Allergy 2007; 37: 1213-1220. https://doi.org/10.1111/j.1365-2222.2007.02758.x
16. Knibb RC, Barnes C, Stalker C. Parental self-efficacy in managing food allergy and mental health predicts food allergy-related quality of life. Pediatr Allergy Immunol 2016; 27: 459-464. https://doi.org/10.1111/pai.12569
17. Yildirim F, Ilhan IO. The validity and reliability of the general self-efficacy scale-Turkish form. Turk Psikiyatri Derg 2010; 21: 301-308.

18. Bravo L, Killela MK, Reyes BL, et al. Self-management, self-efficacy, and health-related quality of life in children with chronic ıllness and medical complexity. J Pediatr Health Care 2020; 34: 304-314. https://doi.org/10.1016/j.pedhc.2019.11.009

19. Kim Y, Park HR. Structural equation model of health-related quality of life in school age children with asthma. J Korean Acad Nurs 2018; 48: 96-108. https://doi.org/10.4040/jkan.2018.48.1.96

20. Çapık C, Gözüm S, Aksayan S. Intercultural scale adaptation stages, language and culture adaptation: updated guideline. Florence Nightingale J Nurs 2018; 26: 199-210. https://doi.org/10.26650/FNJN397481

21. Knibb RC, Barnes C, Stalker C. Parental confidence in managing food allergy: development and validation of the Food Allergy Self-Efficacy Scale for Parents (FASE-P). Clin Exp Allergy 2015; 45: 1681-1689. https://doi.org/10.1111/cea.12599

22. Worthington RL, Whittaker TA. Scale development research: a content analysis and recommendations for best practices. The Counseling Psychologist 2006; 34: 806–838. https://doi.org/10.1177/0011000006288127

23. Büyüköztürk S. Veri analizi el kitabi. Ankara: Pegem A Yayıncılık; 2004.

24. Schermelleh-Engel K, Moosbrugger H, Müller H. Evaluating the fit of structural equation models: test of significance and descriptive goodness-of-fit measures. Methods of Psychological Research Online 2003; 8: 23-74.

25. Coleman PK, Karraker KH. Parenting self-efficacy among mothers of school-age children: conceptualization, measurement, and correlates. Family Relations 2000; 49: 13-24. https://doi.org/10.1111/j.1741-3729.2000.00013.x

The Turkish Journal of Pediatrics 2024; 66: 544-555
https://doi.org/10.24953/turkjpediatr.2024.4769

**Original Article**

# Sleep in hospitalized children with cancer: relationship with psychiatric disorders and hospital conditions

Burcu Güneydaş Yıldırım[1], Hasan Cem Aykutlu[2], Tuba Eren[3]

*[1]Child and Adolescent Psychiatry Unit, Kırklareli Training and Research Hospital, Kırklareli; [2]Department of Child and Adolescent Psychiatry, Trakya University Hospital, Edirne; [3]Department of Child Health and Diseases, Trakya University Hospital, Edirne, Türkiye.*

## ABSTRACT

**Background.** Children with cancer often undergo prolonged and recurrent hospitalization, which leads to an increased incidence of sleep disruptions and psychiatric disorders. This study aimed to objectively quantify the prevalence of sleep disruptions in hospitalized pediatric oncology patients and to determine the effects of psychiatric disorders, treatment regimens, and hospital conditions on sleep patterns.

**Method.** This cross-sectional study included 39 children who were undergoing treatment and monitoring in the pediatric oncology inpatient service. Parents completed questionnaires providing information about their child's sleep patterns, quality of life, and hospital conditions. The children were monitored for five days using actigraphy to record sleep parameters. They were evaluated with a semi-structured interview form (Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version-DSM 5-Turkish Adaptation) for psychiatric diagnoses.

**Results.** Sleep disruptions were identified in 27 (69.2%) children with cancer. In addition to adjustment disorder and anxiety disorder psychiatric diagnoses, behavioral problems and emotional symptoms were more common in the group with sleep disruptions. Actigraphy measurements indicated that poor sleep was associated with younger age, recent cancer diagnosis, specific phobias, depression, daytime napping, and frequent vital sign assessments.

**Conclusion.** Sleep problems in hospitalized children with cancer are linked to psychiatric comorbidities, treatment routines, and hospital conditions. By recognizing psychiatric symptoms and optimizing hospital conditions that affect sleep, healthcare providers can enhance the quality of sleep for these children.

**Key words:** cancer, sleep, actigraphy, child psychiatry, Schedule for Affective Disorders and Schizophrenia for School-Age Children - Present and Lifetime Version, K-SADS-PL.

Cancer refers to a group of diseases characterized by the uncontrolled proliferation and spread of abnormal cells.[1] With advancements in early diagnosis and treatment, five-year survival rates have reached 80%, emphasizing the importance of quality of life and psychosocial approaches in the care of cancer patients.[2]

Adequate restorative sleep is crucial for children's pain perception, glucose regulation, neuroendocrine function, and immune system function.[3] However, treatment side effects, hospital environmental factors (such as noise and light), and frequent monitoring exacerbate sleep problems in pediatric cancer patients.[4,5] Sleep disruptions affect 30-75% of newly diagnosed or recently treated cancer patients, which is nearly double the rate seen in the general population.[6] Previous studies have shown that 60% of children with cancer

✉ Burcu Güneydaş Yıldırım ▪ burcuguneydas@gmail.com

Received 23rd May 2024, revised 23rd Jul 2024, 17th Sep 2024, accepted 21st Oct 2024.

experience excessive daytime sleepiness, 30-45% suffer from frequent sleep interruptions, and 66% have insufficient total sleep duration.[3,4,7]

In addition to sleep disruptions, children with cancer commonly experience depression, anxiety, adjustment disorder, post-traumatic stress disorder (PTSD), and behavioral problems. The rate of psychiatric diagnoses in this population is 2-2.5 times higher than that of the general population.[8-10] PTSD, anxiety and depressive symptoms persist in a significant proportion of children undergoing treatment and adolescent cancer survivors.[11] Notably, 50-80% of patients with psychiatric disorders experience sleep disturbances[12], suggesting that sleep issues may be related not only to cancer treatment and hospital conditions but also to comorbid psychiatric disorders.

Recent literature on sleep and cancer has highlighted a lack of studies on this topic and a dearth of objective sleep measurements in evaluating sleep disruptions.[4] This study aims to objectively assess sleep disruptions in pediatric oncology patients and investigate the impact of psychiatric disorders, treatments, and hospital conditions on sleep patterns during hospitalization. Specifically, we seek to answer the following questions: (1) What is the prevalence of sleep disruptions in hospitalized children with cancer based on objective measurements (actigraphy) and subjective scales? (2) Is the frequency of psychiatric comorbidities higher in the group with sleep disruptions compared to the group without sleep disruptions? (3) What hospital and treatment conditions are associated with sleep disruptions?

## Materials and Methods

### *Sample*

The study included 39 cancer patients who were being treated and followed up as in-patients in the Pediatric Hematology-Oncology Department between June 2021 and March 2022. The inclusion criteria were: (1) Age between 3 and 18 years, (2) Presence of a literate parent throughout the treatment, (3) Absence of autism spectrum disorder[13] or intellectual disability[14], as these conditions could affect data collection (i.e., actigraphy), (4) Informed consent provided by the parent or legal guardian. The Ethics Committee of Trakya University approved the study on June 14, 2021.

### *Data collection tools*

Psychiatric evaluations were conducted by two experienced child and adolescent psychiatrists (B.G.Y. and H.C.A.) using the Schedule for Affective Disorders and Schizophrenia for School-Age Children - Present and Lifetime Version -DSM 5 - Turkish Adaptation (K-SADS-PL-DSM-5-T). In addition to the K-SADS-PL-DSM-5-T, Revised Child Anxiety and Depression Scale (RCADS), Preschool Anxiety Scale (PAS), Strengths and Difficulties Questionnaire-parent form, Pediatric Quality of Life Inventory (PedsQL), and Children's Sleep Habits Questionnaire (CSHQ) were employed to assess specific aspects of mental health, quality of life and sleep. Parents also completed a comprehensive sleep diary adapted for hospitalized children with cancer. Objective sleep data were collected using actigraphy.

*Sociodemographic Data Form:* This form was developed based on relevant literature to collect information about the children's sociodemographic characteristics, cancer type, age at diagnosis, treatment duration, treatment type, and prognosis.

*Schedule for Affective Disorders and Schizophrenia for School-Age Children - Present and Lifetime Version - DSM 5 -Turkish Adaptation- (K-SADS-PL-DSM-5-T):* This semi-structured interview tool developed by Kaufman et al.[15] comprises three parts: an unstructured interview gathering sociodemographic and developmental information, a structured screening of over 200 specific symptoms, and a diagnostic evaluation based on DSM-5 criteria. The assessment integrates information from child interviews, parent interviews, and clinical observations

to determine the presence and severity of psychiatric symptoms and diagnoses. Ünal et al.[16] conducted the validity and reliability study of the DSM-5 updated version in Turkish.

*Strengths and Difficulties Questionnaire (SDQ):* Designed by Goodman et al.[17] to screen for mental disorders in children and adolescents. It consists of 25 items with 5 subscales of behavioral problems, emotional symptoms, hyperactivity, peer problems, and prosocial behavior. Cut off values and interpretation are described elsewhere.[18] Güvenir et al.[19] conducted the validity and reliability studies of the Turkish version.

*Pediatric Quality of Life Inventory (PedsQL):* This scale was developed by Varni et al.[20] in 1999, with validity and reliability studies of the Turkish version performed by Memik et al.[21,22] The scale yields a total score, physical health score, and psychosocial health score, ranging from 0 to 100. Higher scores indicate better health-related quality of life.

*Pre-school Anxiety Scale (PAS):* Revised by Edwards et al. in 2018, with the Turkish version validated by Uğraş et al.[23] This 29-item scale measures social phobia, separation anxiety disorder, specific phobia, and generalized anxiety disorder symptoms in children aged 2 to 5 years. A score of ≥34 indicates an anxiety disorder.[24]

*Revised Child Anxiety and Depression Scale (RCADS):* Revised by Chorpita et al. from the Spence Anxiety Scale for Children[25] it includes 47 items across 6 subscales based on DSM-IV criteria. Gormez et al. conducted a validity and reliability study of the Turkish version.[26] Age and gender-specific scores were evaluated according to reference values.[25]

*Sleep Diary:* Adapted from the Sleep in a Children's Hospital–Parent Version (SinCH-P) and the Sleep at Memorial Sloan Kettering Cancer Center (SAM) questionnaires[4,27], it includes 12 items completed by parents,covering various aspects of sleep patterns and disturbances specific to hospitalized children with cancer (e.g. bed time, medication requirements, and vital signs measurement) (Supplementary Materials).

*Children's Sleep Habits Questionnaire (CSHQ):* Developed by Owens et al.[28] to investigate children's sleep habits and problems Perdahlı Fiş et al. conducted the validity and reliability study of the Turkish version in 2010.[29] A score of ≥41 is considered 'clinically significant'. Although originally designed for children aged 4 to 10 years[28], it has been found useful for screening sleep problems in toddlers[30] and has been used in studies with adolescents.[31,32]

*Actigraphy:* The Actiwatch 2 device (Actiwatch 2®, Philips Respironics, Murrysville, PA, USA) was used to measure rest-activity patterns, detect light and motor movements, and monitor sleep-wake cycles. It was placed on the non-dominant wrist of each patient for five days. Sleep parameters were analyzed according to age-specific reference values.[3,33,34] In line with previous research the following criteria were applied for sleep parameters: Sleep onset latency exceeding 20 minutes was considered prolonged. The reference values for total sleep duration varied by age group: 10-13 hours for ages 3-5 years, 9-11 hours for ages 6-11 years, and 8-10 hours for ages 12-17 years.[34] Sleep efficiency below 80% was regarded as low.[3,34] Bedtime was considered delayed if it was later than 22:00 for the 3 to 5 years age group, 23:00 for the 6 to 11 years age group, and 24:00 for the 12 to 17 years age group. Similarly, a wake-up time later than 9:00 for all age groups was considered a delayed wake-up time.[3,33,34]

### *Data analysis*

Data were analyzed using SPSS version 22.0 software. Descriptive statistics were reported as mean ± standard deviation (SD), median, minimum, maximum values, or number (n) and percentage (%). The one sample Kolmogorov-Smirnov test was used to evaluate the normality of distribution. Independent samples t-test and Mann-Whitney U-test were applied for quantitative data analysis. Relationships

between quantitative variables were examined using Pearson or Spearman correlation analysis. Pearson, Yates, or Fisher exact chi-square tests were used for categorical data. A p-value <0.05 was considered statistically significant.

## Results

The study evaluated 39 patients, consisting of 71.8% males and 28.2% females, with a mean age of 10.1 ± 5.2 years (median: 11 years). Of these, 51.3% (n=20) were in the pediatric age group (3-11 years) and 48.7% (n=19) in the adolescent age group (12-17 years). Eleven children in the pediatric group were under 6 years of age. Patients were monitored for hematological tumors (53.8%), central nervous system (CNS) tumors (7.7%), and other solid tumors (38.5%). Of the total patients, 61% were hospitalized for initial diagnosis, 30.8% for relapse diagnosis, and 7.7% for routine follow-up (Table I).

According to the CSHQ, 69.2% of all patients and 81.8% of patients under 6 years of age had sleep disturbances. Actigraphy revealed that 74.4% of patients exhibited prolonged sleep onset latency (Table I). No significant

**Table I.** Sociodemographic, psychiatric and sleep data of patients with cancer.

| | | n | % |
|---|---|---|---|
| Age (years) | 3-11 | 20 | 51.3 |
| | 12-17 | 19 | 48.7 |
| Gender | Male | 28 | 71.8 |
| | Female | 11 | 28.2 |
| CSHQ Sleep disruptions | Yes | 27 | 69.2 |
| | No | 12 | 30.8 |
| Actigraphy Sleep onset latency (>20 minutes) | Yes | 29 | 74.4 |
| | No | 10 | 25.6 |
| K-SADS Psychiatric diagnosis | Yes | 25 | 64.1 |
| | No | 14 | 35.9 |
| Tic disorder | Yes | 1 | 2.6 |
| Encopresis | Yes | 1 | 2.6 |
| Enuresis | Yes | 2 | 5.1 |
| Specific phobia | Yes | 3 | 7.7 |
| Social phobia | Yes | 5 | 12.8 |
| Separation anxiety | Yes | 8 | 20.5 |
| Generalized anxiety | Yes | 3 | 7.7 |
| Disruptive mood dysregulation disorder | Yes | 2 | 5.1 |
| Depression | Yes | 3 | 7.7 |
| Adjustment disorder | Yes | 8 | 20.5 |
| Attention deficit hyperactivity disorder | Yes | 7 | 17.9 |
| Oppositional defiant disorder | Yes | 2 | 5 |
| Cancer type | Hematological tumor | 21 | 53.8 |
| | CNS tumor | 3 | 7.7 |
| | other solid tumors | 15 | 38.5 |
| Diagnosis | Initial diagnosis | 24 | 61.5 |
| | Relapse | 12 | 30.8 |
| | Remission | 3 | 7.7 |

CSHQ, Children's Sleep Habits Questionnaire; CNS, central nervous system; K-SADS, Schedule for Affective Disorders and Schizophrenia for School-Age Children.

differences between genders were observed regarding sleep disruptions. Younger patients experienced significantly higher numbers of awakenings (p=0.043) and wakening after sleep onset (WASO, p=0.040). WASO was also higher in patients who napped during the day (p=0.049) and those with initial diagnosis (p=0.015), but lower in patients with relapsed diagnosis (p=0.039). Sleep efficiency was lower in patients who napped during the day (p=0.001), and those with an initial diagnosis (p=0.009), but higher in patients with relapse (p=0.022). Sleep onset latency was higher in patients who napped during the day (p=0.027, Table II).

According to the K-SADS-PL, 64.1% of all these patients with cancer had a psychiatric diagnosis (Table I). The most common diagnoses were anxiety disorders, trauma-related disorders, attention deficit hyperactivity disorder (ADHD), depression disorders, externalizing disorders, oppositional defiant disorder (ODD), and tic disorders.

The presence of any psychiatric diagnosis (77.8% vs 23.3%, p=0.012) and adjustment disorder diagnosis (29.6% vs 0%, p=0.034) were higher in the group with sleep disorders compared to the other group. In the pre-school age group with sleep disruptions, the rate of anxiety disorder diagnosis was higher (100% vs 0%, p=0.018, Table III). A negative correlation was found between the specific phobia scores on the PAS administered to children under age 6 and total sleep duration (r=-0.632, p=0.037, Table II). Sleep efficiency was lower in patients with depression (p=0.031). Onset latency was higher in patients who were diagnosed with depression (p=0.029, Table II).

Psychosocial health score was found to be lower in the group with sleep disruptions (p = 0.048). In addition to general difficulty (p=0.006), when the SDQ was evaluated, emotional symptoms (p=0.017) and behavioral problems (p=0.001) were found to be more prevalent among patients with sleep disruptions (Table III).

**Table II.** Examination of factors associated with the actigraphy data of the patients.

| | | Time in bed | Total sleep time | Onset latency | Sleep efficiency | WASO | Number of awakenings |
|---|---|---|---|---|---|---|---|
| Age | r (n=25) | -0.144 | -0.187 | 0.046 | 0.095 | -0.330 | -0.326 |
| | p | 0.383[s] | 0.255* | 0.779[s] | 0.564* | ***0.040**** | ***0.043**** |
| Initial diagnosis | Yes (n=3) | 563.7±105.8 | 448.4±96 | 14.2±9.9 | 80.2±5.8 | 75.1±21.5 | 29.3±9.1 |
| | No (n=36) | 596.4±126.4 | 482.8±110.8 | 13.5±13.3 | 84.9±16.8 | 59.4±16.8 | 26.2±8.8 |
| | p[m] | 0.784 | 0.386 | 0.355 | ***0.009*** | ***0.015*** | 0.284 |
| Relapse | Yes (n=8) | 568.1±79.7 | 481.6±64.9 | 12.2±11.3 | 85.0±3.4 | 59.1±14.3 | 26.5±8.1 |
| | No (n=31) | 565.0±125.8 | 452.7±114.6 | 14.7±11.2 | 80.6±5.9 | 73.5±22.2 | 28.9±9.4 |
| | p[m] | 0.915 | 0.191 | 0.247 | ***0.022*** | ***0.039*** | 0.447 |
| K-SADS depression | Yes (n=3) | 566.3±57.7 | 418.3±69.3 | 29.7±3.3 | 73.7±6.4 | 88.7±22.9 | 31.5±4.8 |
| | No (n=36) | 565.9±116.5 | 465.2±104.1 | 12.6±10.6 | 82.7±5.1 | 67.4±20.3 | 27.8±9.3 |
| | p[m] | 0.979 | 0.370 | ***0.029*** | ***0.031*** | 0.155 | 0.370 |
| PAS-specific phobia | r (n=11) | -0.577 | -0.632 | 0.132 | -0.373 | -0.042 | -0.237 |
| | p* | 0.063 | ***0.037*** | 0.699 | 0.259 | 0.903 | 0.484 |
| Napping during the day | Yes (n=7) | 566.29±103.4 | 454±93.4 | 15.9±11.6 | 80.7±5.6 | 71.6±21.6 | 28.3±9.5 |
| | No (n=32) | 564.7±151.5 | 491.3±133.2 | 6.4±3.9 | 86.9±1.8 | 59.1±16.1 | 27.6±7.5 |
| | p[m] | 0.808 | 0.434 | ***0.027*** | ***0.001*** | ***0.049*** | 0.945 |

K-SADS, Schedule for Affective Disorders and Schizophrenia for School-Age Children; PAS, Pre-School Anxiety Scale; r, correlation coefficient; WASO, wakening after sleep onset.
[m] Mann-Whitney U test, *Pearson correlation analysis, [s] Spearman correlation analysis

**Table III.** Comparisons of PedsQL, SDQ, K-SADS-PL and PAS in patients with and without sleep disruptions according to the CSHQ.

| | Sleep disruptions (+) | | Sleep disruptions (-) | | P |
|---|---|---|---|---|---|
| | Mean±SD | | Mean±SD | | |
| PedsQL: Psychosocial health total score | 62.0 ± 19.7 | | 74.9 ± 15.2 | | ***0.048***[m] |
| | n | % | n | % | |
| SDQ: General difficulty | 12 | 44.4 | 0 | 0 | ***0.006***[X²] |
| SDQ: Emotional symptoms score | 13 | 48.1 | 1 | 8.3 | ***0.017***[X²] |
| SDQ: Behavioral problems score | 16 | 59.3 | 0 | 0 | ***0.001***[X²] |
| K-SADS: Presence of diagnosis | 21 | 77.8 | 4 | 23.3 | ***0.012***[X²] |
| Adjust K-SADS: ment disorder | 8 | 29.6 | 0 | 0 | ***0.034***[X²] |
| PAS: Anxiety disorder | 9 | 100 | 0 | 0 | ***0.018***[X²] |

CSHQ, Children's Sleep Habits Questionnarie; K-SADS, Schedule for Affective Disorders and Schizophrenia for School-Age Children; PAS, Pre-School Anxiety Scale; PedsQL, Pediatric Quality of Life Inventory; SD, standard deviation; SDQ, Strengths and Difficulties Questionnaire.
[m] Mann Whitney U test, [X²] Chi-square test (Fisher exact test).

In the hospital conditions evaluations, there was no relationship between the sleep disturbance and need for sleep medication, pain during sleep, noise-light in the room, presence and type of cancer treatment (Table IV). However, there was a difference in normal bed time and late bedtime according to the frequent vital sign assessments (≥3) (36.4% vs 63.6%, p=0.06).

## Discussion

Sleep issues as well as psychiatric symptoms and disorders are prevalent in children with cancer, negatively impacting their quality of life.[4] This study demonstrates that children with cancer who experience sleep problems are more likely to have psychiatric issues compared to those who sleep well. Adjustment disorder diagnosis across all ages and anxiety disorders in pre-schoolers were more common in those with sleep disruptions. Patients with depression exhibited decreased sleep efficiency. Furthermore, frequent vital sign assessments were found to delay bedtime in these hospitalized children.

According to the CSHQ, 69.2% of the children with cancer in this study were diagnosed with sleep disruptions, more than twice the rate in the general pediatric population (20-30%).[35] This aligns with Traube et al.'s findings, where 66% of hospitalized children with cancer had inadequate sleep.[4] In an adult study, 30-75%

**Table IV.** Comparisons of hospital conditions and types of treatment in patients with and without sleep disruptions according to the CSHQ.

| | Sleep disruptions present | | No sleep disruptions | | P |
|---|---|---|---|---|---|
| | n | % | n | % | |
| Need for sleep medication | 3 | 11.1 | 0 | 0 | ***0.539***[f] |
| Pain during sleep | 4 | 14.8 | 2 | 16.7 | ***1.000***[f] |
| Noise, light in the room | 4 | 14.8 | 1 | 8.3 | ***1.000***[f] |
| Surgical Treatment | 2 | 7.4 | 0 | 0 | ***1.000***[f] |
| Radiotherapy | 2 | 7.4 | 0 | 0 | ***1.000***[f] |
| Chemotherapy | 11 | 40.7 | 5 | 41.7 | ***0.957***[f] |
| Cancer treatment | 15 | 55.6 | 5 | 41.7 | ***0,423***[f] |

CSHQ: Children's Sleep Habits Questionnaire, [f] Fisher exact test.

of newly diagnosed or recently treated cancer patients reported sleep disruptions, nearly double the rate of the general population.[6]

As a result of the analysis a significant correlation between actigraphy data and age was revealed, with younger children exhibiting an increased number of awakenings after sleep onset and longer total time spent awake. These findings align with previous research on sleep patterns in hospitalized pediatric populations. For instance, Meltzer et al. observed that school-age children experienced more sleep disturbances compared to adolescents in a hospital setting.[27] Similarly, Stremler et al. reported the most substantial decrease in sleep duration among the youngest age group of hospitalized pediatric patients.[5] These consistent results suggest that younger children may find hospitalization more distressing and struggle to cope with unfamiliar environments.

Le Guen et al.'s study on cancer patients revealed disrupted nocturnal sleep patterns, including difficulty initiating sleep, frequent awakenings, and reduced sleep efficiency.[36] Similarly, our study demonstrated that patients who napped during the day exhibited prolonged sleep latency, decreased sleep efficiency, and increased WASO. These results suggest a vicious cycle in which interrupted nighttime sleep leads to daytime sleepiness, further exacerbating nighttime sleep problems.

Our study revealed that patients in the newly diagnosed group exhibited low sleep efficiency and high WASO values. Similarly, Le Guen et al. reported similar patterns of low sleep efficiency and high WASO in newly diagnosed cancer patients compared to a control group.[36] Additionally, Chang et al. observed poorer sleep quality in adult patients with newly diagnosed lung cancer relative to healthy controls.[37] Collectively, these results suggest that the uncertainties and stress associated with diagnosis and treatment initiation may significantly disrupt sleep.

Interestingly, our study found that patients with recurrent hospitalizations due to relapse exhibited shorter sleep latency, higher sleep efficiency, and lower WASO, which is contrary to some previous findings.[38] This may be due to better disease interpretation and adaptation to hospital routines in older, experienced patients.[39] Our findings suggest that older patients with recurrent relapses may have a more sophisticated understanding of their condition, potentially easing adaptation to hospital routines. This familiarity, combined with consistent care, may facilitate quicker adjustment to hospital environments, including sleep patterns, potentially preventing sleep disorders in this population.

While the prevalence of psychiatric diagnoses in the general child and adolescent population is estimated to be approximately 20%, studies have demonstrated that this rate increases 2 to 2.5-fold in children and adolescents diagnosed with chronic diseases such as cancer, compared to their healthy peers.[8,10,40] Consistent with these findings, the current study observed a prevalence of psychiatric diagnoses in children with cancer of 64.1%.

The group with sleep disruptions exhibited a significantly higher prevalence of psychiatric diagnoses, with adjustment disorder being the most common diagnosis at the rate of 29.6%. This finding aligns closely with a study by Rait et al., which reported a 31% rate of adjustment disorder with depressive features in children with cancer.[41] Importantly, Kouros et al. highlighted a bidirectional relationship between sleep disorders and adjustment disorders, suggesting that these conditions may influence each other through mood alterations such as tension and anger.[42] This interplay is particularly relevant in our context, as sleep disturbances, including frequent awakenings and reduced sleep duration, may exacerbate adjustment disorders by elevating perceived stress levels and diminishing coping abilities in children. Conversely, symptoms associated with

adjustment problems, such as pain and fatigue, may also contribute to sleep disorders, creating a potential cycle of mutual reinforcement.

Roy-Byrne et al. reported that the prevalence of any anxiety disorder in cancer patients ranges from 6% to 33%.[43] In the present study, anxiety disorders were the most common psychiatric diagnosis among children with cancer, occurring in 33.3% of cases. Previous literature has indicated that symptoms related to sleep disturbance can affect up to 80-90% of children diagnosed with anxiety disorders.[38,40] Thus, the high rates of sleep disruptions observed in our study sample may be associated with the increased prevalence of anxiety disorder diagnoses.

This study revealed that children younger than 6 years who experienced sleep disruptions exhibited a higher prevalence of anxiety disorders and elevated scores for specific phobia associated with shorter total sleep duration, an association not observed in children older than 6 years. The concurrent diagnosis of sleep disruptions and anxiety disorders in young children may be attributed to their heightened sensitivity to diagnostic procedures and treatment side-effects, as well as their increased vulnerability to the distress of hospitalization.[39,44,45] Furthermore, sleep disturbances in this age group may be exacerbated by fear associated with intensive interventional procedures and frightening thoughts or visual images related to themes such as darkness and sleeping alone.

Depression significantly impacts sleep patterns, affecting both quality and quantity. Depressed individuals often experience delayed sleep onset, reduced deep sleep, frequent awakenings, and daytime drowsiness.[46] This relationship is particularly evident in adolescents, with 88% of depressive disorders accompanied by sleep issues. Chronic insomnia is associated with a 2-3 fold increased risk of depression.[47,48] Our study corroborates existing research, demonstrating that depressed patients exhibit longer sleep onset latency and decreased sleep efficiency compared to non-depressed counterparts. These findings highlight the importance of sleep regulation, especially for vulnerable groups like cancer patients. The link between sleep quality, depression recurrence, and suicidal behavior underscores the critical nature of this relationship.[49]

Our study revealed higher rates of affective and behavioral problems and lower psychosocial subscale scores on the PedsQL in the group with sleep disturbances. These findings corroborate previous research demonstrating increased affective and behavioral problems in children with sleep issues[50,51] and diminished psychosocial subscale scores on the PedsQL among adolescents undergoing chemotherapy.[52] These results further underscore the detrimental effects of sleep disruption in this population.

Previous research has demonstrated that frequent nighttime vital sign assessments are associated with sleep disturbance in hospitalized children with cancer.[4] Our study corroborates and extends these findings, revealing that such assessments were linked to delayed bedtimes and reduced total sleep time. Interestingly, while existing literature has emphasized the role of environmental factors (e.g., noise and light), cancer treatment modalities, and pain levels in disrupting sleep for these children[3-5,53], our results did not show significant effects of these variables. This discrepancy may be attributed to our study's limited sample size, which potentially attenuated the detectable impact of these factors.

Our study's primary strength lies in its comprehensive approach to sleep assessment in pediatric cancer patients, employing both objective and subjective measures. The use of 5-day actigraphy records under controlled hospital conditions minimized environmental variability and ensured consistent data collection. Furthermore, the integration of detailed psychiatric evaluations, including semi-structured interviews and standardized scales, distinguishes this research from previous studies in the field.

However, several limitations warrant consideration. The study was conducted during the COVID-19 pandemic, potentially influencing children's sleep patterns. The absence of pre-hospitalization sleep data and the heterogeneity of cancer stages among participants limit our ability to draw definitive conclusions about cancer-specific sleep disruptions. Additionally, the lack of information on hospitalization duration and comorbidities constrains the depth of our analysis. Finally, the small sample size and limited representation in ceratin subgroups restrict the generalizability of our findings. Future research should address these limitations by employing larger, more homogeneous samples and accounting for pre-existing sleep patterns and comorbidities.

Pediatric cancer patients undergoing inpatient treatment experience a markedly higher prevalence of sleep disturbances compared to healthy children. These disruptions can significantly impact psychiatric symptoms, treatment adherence, and hospitalization duration. However, sleep disorders and associated psychiatric conditions in this population are often overlooked, being mistakenly attributed solely to cancer diagnosis and treatment. To address this issue, a comprehensive approach is crucial. This includes providing targeted sleep education, optimizing hospital environments, increasing awareness among healthcare providers about the sleep-psychiatric symptom relationship, and facilitating timely child psychiatry consultations. Implementation of these strategies can lead to earlier detection and management of sleep problems, ultimately enhancing the quality of life for pediatric cancer patients.

## Ethical approval

The study was approved by Ethics Committee of Trakya University (date: 14.06.2021, number: 2021-278).

## Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: BGY, HCA, TE; data collection: BGY; analysis and interpretation of results: BGY, HCA; critically reviewing the work for important intellectual content: BGY, HCA, TE; draft manuscript preparation: BGY. All authors reviewed the results and approved the final version of the article.

## Supplementary materials

Supplementary materials for this article are available online at https://doi.org/10.24953/turkjpediatr.2024.4769

## 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. Ward E, DeSantis C, Robbins A, Kohler B, Jemal A. Childhood and adolescent cancer statistics, 2014. CA Cancer J Clin 2014; 64: 83-103. https://doi.org/10.3322/caac.21219
2. Geçkalan Soysal D, Yazıcı N, Erbay A. Epidemiology and survival analysis of children with malignant tumors. Cukurova Medical Journal 2020; 45: 1042-1050. https://doi.org/10.17826/cumj.685250
3. Linder LA, Christian BJ. Nighttime sleep characteristics of hospitalized school-age children with cancer. J Spec Pediatr Nurs 2013; 18: 13-24. https://doi.org/10.1111/jspn.12005
4. Traube C, Rosenberg L, Thau F, et al. Sleep in hospitalized children with cancer: a cross-sectional study. Hosp Pediatr 2020; 10: 969-976. https://doi.org/10.1542/hpeds.2020-0101

5. Stremler R, Micsinszki S, Adams S, Parshuram C, Pullenayegum E, Weiss SK. Objective sleep characteristics and factors associated with sleep duration and waking during pediatric hospitalization. JAMA Netw Open 2021; 4: e213924. https://doi.org/10.1001/jamanetworkopen.2021.3924

6. Kaleyias J, Manley P, Kothare SV. Sleep disorders in children with cancer. Semin Pediatr Neurol 2012; 19: 25-34. https://doi.org/10.1016/j.spen.2012.02.013

7. Rosen G, Brand SR. Sleep in children with cancer: case review of 70 children evaluated in a comprehensive pediatric sleep center. Support Care Cancer 2011; 19: 985-994. https://doi.org/10.1007/s00520-010-0921-y

8. Perrin JM, Gnanasekaran S, Delahaye J. Psychological aspects of chronic health conditions. Pediatr Rev 2012; 33: 99-109. https://doi.org/10.1542/pir.33-3-99

9. Varni JW, Limbers CA, Burwinkle TM. Impaired health-related quality of life in children and adolescents with chronic conditions: a comparative analysis of 10 disease clusters and 33 disease categories/severities utilizing the PedsQL 4.0 Generic Core Scales. Health Qual Life Outcomes 2007; 5: 43. https://doi.org/10.1186/1477-7525-5-43

10. Çöp E, Şenses Dinç G, Çengel Kültür SE. Relationship with psychiatric symptoms: a preliminary report coping styles of mothers of children with chronic diseases and their. Turkish Journal of Pediatric Disease 2016; 10: 170-176.

11. Kosir U, Wiedemann M, Wild J, Bowes L. Psychiatric disorders in adolescent cancer survivors: a systematic review of prevalence and predictors. Cancer Rep (Hoboken) 2019; 2: e1168. https://doi.org/10.1002/cnr2.1168

12. Ford DE, Kamerow DB. Epidemiologic study of sleep disturbances and psychiatric disorders. An opportunity for prevention? JAMA 1989; 262: 1479-1484. https://doi.org/10.1001/jama.1989.03430110069030

13. Richdale AL, Schreck KA. Sleep problems in autism spectrum disorders: Prevalence, nature, & possible biopsychosocial aetiologies. Sleep Med Rev 2009; 13: 403-411. https://doi.org/10.1016/j.smrv.2009.02.003

14. van de Wouw E, Evenhuis HM, Echteld MA. Prevalence, associated factors and treatment of sleep problems in adults with intellectual disability: a systematic review. Res Dev Disabil 2012; 33: 1310-1332. https://doi.org/10.1016/j.ridd.2012.03.003

15. Kaufman J, Birmaher B, Brent D, et al. Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): initial reliability and validity data. J Am Acad Child Adolesc Psychiatry 1997; 36: 980-988. https://doi.org/10.1097/00004583-199707000-00021

16. 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). Turk Psikiyatri Derg 2019; 3: 42-50. https://doi.org/10.5080/u23408

17. Goodman R. The strengths and difficulties questionnaire: a research note. J Child Psychol Psychiatry 1997; 38: 581-586. https://doi.org/10.1111/j.1469-7610.1997.tb01545.x

18. Bourdon KH, Goodman R, Rae DS, Simpson G, Koretz DS. The Strengths and Difficulties Questionnaire: U.S. normative data and psychometric properties. J Am Acad Child Adolesc Psychiatry 2005; 44: 557-564. https://doi.org/10.1097/01.chi.0000159157.57075.c8

19. Güvenir T, Özbek A, Baykara B, Arkar H, Şentürk B, İncekaş S. Psychometric properties of the Turkish version of the Strengths and Difficulties Questionnaire (SDQ). Turkish Journal of Child and Adolescent Mental Health 2008; 15: 65-74.

20. Varni JW, Seid M, Rode CA. The PedsQL: measurement model for the pediatric quality of life inventory. Med Care 1999; 37: 126-139. https://doi.org/10.1097/00005650-199902000-00003

21. Uneri OS, Agaoglu B, Coskun A, Memik NC. Validity and reliability of Pediatric Quality of Life Inventory for 2- to 4-year-old and 5- to 7-year-old Turkish children. Qual Life Res 2008; 17: 307-315. https://doi.org/10.1007/s11136-007-9303-4

22. Memik NÇ, Ağaoğlu B, Coşkun A, Üneri ÖŞ, Karakaya I. The validity and reliability of the Turkish Pediatric Quality of Life Inventory for Children 13-18 years old. Turk Psikiyatri Derg 2007; 18: 353-363.

23. Uğraş S, Koşe Demiray Ç, Mutluer T, Coşkun M. The reliability and validity of Turkish version of Preschool Anxiety Scale. Turkish Journal of Child and Adolescent Mental Health 2018; 25: 63-76.

24. Edrissi F, Havighurst SS, Aghebati A, Habibi M, Arani AM. A pilot study of the tuning in to kids parenting program in Iran for reducing preschool children's anxiety. Journal of Child and Family Studies 2019; 28: 1695-1702. https://doi.org/10.1007/s10826-019-01400-0

25. Chorpita BF, Ebesutani C, Spence SH. Revised Children's Anxiety and Depression Scale: User's Guide. Jan 25, 2022. Available at: https://www.childfirst.ucla.edu/wp-content/uploads/sites/163/2022/01/RCADSUsersGuide20220125.pdf

26. Gormez V, Kılınçaslan A, Orengul AC, et al. Psychometric properties of the Turkish version of the Revised Child Anxiety and Depression Scale – Child Version in a clinical sample. Psychiatry and Clinical Psychopharmacology 2017; 27: 84-92. https://doi.org/10.1080/24750573.2017.1297494

27. Meltzer LJ, Davis KF, Mindell JA. Patient and parent sleep in a children's hospital. Pediatr Nurs 2012; 38: 64-72.

28. 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.

29. Perdahlı Fiş N, Arman A, Ay P, et al. The validity and the reliability of Turkish version of Children's Sleep Habits Questionnaire. Alpha Psychiatry 2010; 11: 151-160.

30. Goodlin-Jones BL, Sitnick SL, Tang K, Liu J, Anders TF. The Children's Sleep Habits Questionnaire in toddlers and preschool children. J Dev Behav Pediatr 2008; 29: 82-88. https://doi.org/10.1097/dbp.0b013e318163c39a

31. Shui AM, Richdale AL, Katz T. Evaluating sleep quality using the CSHQ-Autism. Sleep Med 2021; 87: 69-76. https://doi.org/10.1016/j.sleep.2021.08.022

32. Ekinci O, Isik U, Gunes S, Ekinci N. Understanding sleep problems in children with epilepsy: associations with quality of life, attention-deficit hyperactivity disorder and maternal emotional symptoms. Seizure 2016; 40: 108-113. https://doi.org/10.1016/j.seizure.2016.06.011

33. Teicher MH. Actigraphy and motion analysis: new tools for psychiatry. Harv Rev Psychiatry 1995; 3: 18-35. https://doi.org/10.3109/10673229509017161

34. McLaughlin Crabtree V, Williams NA. Normal sleep in children and adolescents. Child Adolesc Psychiatr Clin N Am 2009; 18: 799-811. https://doi.org/10.1016/j.chc.2009.04.013

35. van Litsenburg RRL, Waumans RC, van den Berg G, Gemke RJBJ. Sleep habits and sleep disturbances in Dutch children: a population-based study. Eur J Pediatr 2010; 169: 1009-1015. https://doi.org/10.1007/s00431-010-1169-8

36. Le Guen Y, Gagnadoux F, Hureaux J, et al. Sleep disturbances and impaired daytime functioning in outpatients with newly diagnosed lung cancer. Lung Cancer 2007; 58: 139-143. https://doi.org/10.1016/j.lungcan.2007.05.021

37. Chang WP, Lin CC. Relationships of salivary cortisol and melatonin rhythms to sleep quality, emotion, and fatigue levels in patients with newly diagnosed lung cancer. Eur J Oncol Nurs 2017; 29: 79-84. https://doi.org/10.1016/j.ejon.2017.05.008

38. Üstün G, Akan B, Küçük L. Examining the relationship between psychosocial symptoms in hospitalized children and the anxiety levels of their mothers. E-Journal of Dokuz Eylul University Nursing Faculty 2021; 14: 207-216. https://doi.org/10.46483/deuhfed.832698

39. Crisp J, Ungerer JA, Goodnow JJ. The impact of experience on children's understanding of illness. J Pediatr Psychol 1996; 21: 57-72. https://doi.org/10.1093/jpepsy/21.1.57

40. Barlow JH, Ellard DR. The psychosocial well-being of children with chronic disease, their parents and siblings: an overview of the research evidence base. Child Care Health Dev 2006; 32: 19-31. https://doi.org/10.1111/j.1365-2214.2006.00591.x

41. Rait DS, Jacobsen PB, Lederberg MS, Holland JC. Characteristics of psychiatric consultations in a pediatric cancer center. Am J Psychiatry 1988; 145: 363-364. https://doi.org/10.1176/ajp.145.3.363

42. Kouros CD, El-Sheikh M. Daily mood and sleep: reciprocal relations and links with adjustment problems. J Sleep Res 2015; 24: 24-31. https://doi.org/10.1111/jsr.12226

43. Roy-Byrne PP, Davidson KW, Kessler RC, et al. Anxiety disorders and comorbid medical illness. Gen Hosp Psychiatry 2008; 30: 208-225. https://doi.org/10.1016/j.genhosppsych.2007.12.006

44. Akyay A, Olcay L. Early and late side effects of acute lymphoblastic leukemia therapy in children. Turkish Journal of Pediatric Disease 2014; 1: 46-54.

45. Wilkinson SR. Germs. Nursery school children's views on the causality of illness. Clin Pediatr (Phila) 1987; 26: 465-469. https://doi.org/10.1177/000992288702600906

46. Riemann D, Schnitzler M, Hohagen F, Berger M. Depression and sleep-the status of current research. Fortschr Neurol Psychiatr 1994; 62: 458-478. https://doi.org/10.1055/s-2007-1002303

47. Ersöz Alan B, Akdemir D. Psikiyatrik Hastalıklar ve Uyku Bozuklukları. In: Özçelik U, Aslan AT, Erdem Eralp E, editors. Çocuklarda Uyku Bozuklukları. İstanbul: Logos Yayıncılık; 2022: 574-588.

48. Nelson CJ, Cho C, Berk AR, Holland J, Roth AJ. Are gold standard depression measures appropriate for use in geriatric cancer patients? A systematic evaluation of self-report depression instruments used with geriatric, cancer, and geriatric cancer samples. J Clin Oncol 2010; 28: 348-356. https://doi.org/10.1200/JCO.2009.23.0201

49. Nutt D, Wilson S, Paterson L. Sleep disorders as core symptoms of depression. Dialogues Clin Neurosci 2008; 10: 329-336. https://doi.org/10.31887/DCNS.2008.10.3/dnutt

50. Smedje H, Broman JE, Hetta J. Associations between disturbed sleep and behavioural difficulties in 635 children aged six to eight years: a study based on parents' perceptions. Eur Child Adolesc Psychiatry 2001; 10: 1-9. https://doi.org/10.1007/s007870170041

51. Liu G, Fang L, Pan Y, Zhang D. Media multitasking and adolescents' sleep quality: the role of emotional-behavioral problems and psychological suzhi. Children and Youth Services Review 2019; 100: 415-421. https://doi.org/10.1016/j.childyouth.2019.03.026

52. Erickson JM, Beck SL, Christian BR, et al. Fatigue, sleep-wake disturbances, and quality of life in adolescents receiving chemotherapy. J Pediatr Hematol Oncol 2011; 33: e17-e25. https://doi.org/10.1097/MPH.0b013e3181f46a46

53. Sheikh IN, Roth M, Stavinoha PL. Prevalence of sleep disturbances in pediatric cancer patients and their diagnosis and management. Children (Basel) 2021; 8: 1100. https://doi.org/10.3390/children8121100

The Turkish Journal of Pediatrics 2024; 66: 556-566
https://doi.org/10.24953/turkjpediatr.2024.4830

**Original Article**

# Sedation - analgesia - muscle relaxant - withdrawal and delirium practices in pediatric intensive care units in Türkiye

Esra Koçkuzu[1], Ali Korulmaz[2], Ümit Altuğ[3], Gürkan Bozan[4], Dinçer Yıldızdaş[5]

*[1]Department of Pediatric Intensive Care Unit, Ankara Bilkent City Hospital, Ankara; [2]Clinic of Pediatric Intensive Care Unit, Kocaeli City Hospital, Kocaeli; [3]Clinic of Pediatric Intensive Care Unit, Pamukkale University Faculty of Medicine, Denizli; [4]Department of Pediatric Intensive Care Unit, Eskisehir University Faculty of Medicine, Eskisehir; [5]Department of Pediatric Intensive Care Unit, Cukurova University Faculty of Medicine, Adana, Türkiye.*

## ABSTRACT

**Background.** Pain and sedation management is an integral part of pediatric intensive care practice. Sedoanalgesia management must be balanced in order to optimize comfort and avoid complications. In order to achieve this balance, sedoanalgesia management needs to be clarified in pediatric intensive care units (PICU). With this study, we aimed to investigate sedation, analgesia, withdrawal and delirium practices, pharmacologic agent preferences, and current experiences and practices in scoring systems in PICUs in Türkiye.

**Method.** A questionnaire consisting of 57 questions was sent via e-mail to the 'Pediatric Intensive Care and Emergency' group, which includes all intensive care specialists, subspecialty students and lecturers in Türkiye.

**Results.** Our study involved 36 pediatric intensive care physicians working in PICUs in Türkiye. Among the PICU specialists who participated in the study, 83.3% stated that they performed routine assessments of sedation efficacy. While dexmedetomidine was the most commonly used sedative agent in patients undergoing noninvasive mechanical ventilation, benzodiazepines were the most preferred pharmacologic agent for sedation during mechanical ventilation. Of the pediatric intensivists who participated in the study, 94.4% stated that they performed routine pain assessments in their units. Of the PICU specialists who participated in the study, 69.4% stated that muscle relaxants were most commonly used to prevent patient-ventilator incompatibility during mechanical ventilation. Of the participants, 88.8% made withdrawal assessments when discontinuing sedo-analgesic agents. Delirium assessment was routinely performed by 58.3% of the participants.

**Conclusions.** This study showed that the practices in sedoanalgesia management in PICUs in Türkiye are in parallel with recommendations of the sedation guideline. Despite the increased sensitivity in sedoanalgesia management, awareness in the management of delirium and withdrawal syndrome is not at the desired level. Therefore, there is a need to develop guidelines, raise awareness and increase training on these issues in our Türkiye.

**Key words:** pediatric intensive care, sedation, analgesia.

Optimal sedation-analgesia management is one of the most important issues in pediatric intensive care units (PICU). Underlying disease, unfamiliar environments, noise and crowd, disruption of the day-night cycle, separation from parents, mechanical ventilation (MV), repetitive invasive interventions are factors that cause fear, stress, anxiety and pain in children who are being monitored in the PICU.[1] Pain and sedation management is an integral part

✉ Esra Koçkuzu ▪ esrakockz@gmail.com

Received 10th Jun 2024, revised 26th Aug 2024, accepted 12th Sep 2024.

of PICU practice. Sedoanalgesia management must be performed with a balance, to ensure optimal comfort and avoid complications. While inadequate sedation may cause unnecessary psychological and physical stress as well as accidental extubation, excessive sedation may lead to prolonged MV, prolonged stay in the PICU, iatrogenic withdrawal syndrome and delirium.[2,3] To date, only a few international and national clinical practice guidelines for the management of pain and sedation in children have been published.[4,5] Therefore, the need for studies on sedoanalgesia management in PICUs still remains.

With this study, we aimed to investigate sedation, analgesia, withdrawal and delirium practices, pharmacologic agent preferences, and current experiences and practices in scoring systems in PICUs in Türkiye.

## Materials and Methods

Our study was announced by sending e-mails to the 'Pediatric Intensive Care and Emergency' group, which includes all intensive care specialists, subspecialty students and lecturers in Türkiye. The e-mail included a link to the survey titled "Approaches to Sedation-Analgesia-Muscle Relaxant-Withdrawal and Delirium Practices in the PICU" consisting of 57 questions. Pediatric intensive care specialists, subspecialty students receiving training in this field and faculty members providing education were invited to the study. After the announcement of the study, four weeks were given to participate in the survey. At the end of the period, the responses received were saved in a Microsoft Excel 2018 file. The study was approved by Çukurova University Faculty of Medicine Ethics Committee (13-10-2023).

### *Survey content*

The survey consisted of 57 questions about general information, sedation, analgesia, muscle relaxants, withdrawal syndrome and delirium, environmental arrangements and early mobilization. In the first part of the survey, there were descriptive questions including age, title, affiliation, and years of service. In the second part, there were common questions such as whether assessment scales related to sedation, analgesia, withdrawal and delirium were used, who performed the assessment, how often it was performed, and what was the most commonly used scale (with the statement that more than one option can be chosen). In addition, there were questions about the most common and primary sedative agent used in patients receiving high-flow nasal oxygen, patients undergoing noninvasive MV, intubated patients, and the most common and primary analgesic agent used in short-term medical interventions, trauma patients, postoperative surgical patients and postoperative cardiac surgery patients. There were questions about whether muscle relaxants were used, usage indications, whether brain activity measures electroencephalography (EEG), bispectral index (BIS) are used to assess patient wakefulness in units administering muscle relaxants, application methods related to early mobilization, participation of parents in treatment in the PICU, and the clinical approach to patients who develop delirium and withdrawal (with the statement that more than one option can be chosen).

### *Statistical analysis*

Only descriptive statistics were analyzed. Categorical variables were expressed as percentages (%) and continuous variables were calculated as the mean and standard deviation. Microsoft Excel 2018 was used to analyze the data.

## Results

### *General characteristics*

Thirty-six pediatric intensive care physicians working in PICUs in Türkiye participated in our study. Of the physicians who completed the questionnaire, 28 (77.7%) were 40 years of age or older. Seventeen (47.2%) physicians had been working in intensive care for 10 years

or more. Of the physicians who participated in the study, 11 (30.6%) were professors, 11 (30.6%) were subspecialists, six (16.7%) were assistant professors, five (13.9%) were associate professors, and three (8.3%) were subspecialty students.

### *Sedation practices in PICU*

Thirteen (36.1%) of the PICU specialists who participated in the study stated that they used a written sedation protocol for patients on MV. While dexmedetomidine was the most commonly used sedative agent in patients undergoing noninvasive MV, benzodiazepines were the most preferred pharmacologic agents for sedation during MV. In case of inadequate sedation, 29 (80.6%) participants preferred ketamine, 24 (66.7%) dexmedetomidine, 12 (33.3%) chloralhydrate, 4 (11.1%) propofol, and 1 (2.8%) thiopental. Daily waking up in sedated patients was reported to be done by 14 (38.9%) of the participants, while 15 (41%) of the participants reported that it was done according to the clinical condition of the patient. Routine assessment of sedation efficacy during MV follow-up was performed by 30 (83.3%) of the participants. The characteristics of the assessment of sedation efficacy (the frequency, the scales used, and who performed the assessment) are given in Table I.

### *Analgesic agent applications in PICU*

Thirty-four (94.4%) of the pediatric intensivists who participated in the study stated that they performed routine pain assessments in their units. For pain assessment, 27 (75%) of the participants did not use a written protocol. Table II presents the characteristics of the pain assessment, including the scales used, the person conducting the assessment, and the frequency of the assessment. While fentanyl was the most frequently preferred opiate as analgesic, ketamine was the most commonly used agent in short-term interventions (catheter insertion, performing biopsy, lumbar puncture, thoracic tube insertion, etc.) in extubated patients. The most preferred analgesic agents in various clinical situations (in patients with high-flow nasal cannula due to respiratory failure, in post-operative pediatric surgery patients, in trauma patients, in patients who underwent postoperative cardiac surgery) are listed in Table III.

### *Muscle relaxant applications in PICU*

It was reported that the PICU specialists who participated in the study most frequently used muscle relaxants during MV to prevent patient-mechanical ventilation asyncrony (69.4%). While 34 (94.4%) of PICU specialists frequently

**Table I.** Practices related to the assessment of sedation efficacy in pediatric intensive care units.

| | | N | % |
|---|---|---|---|
| Practitioner performing sedation assessment | Nurse | 27 | 74.3 |
| | Doctor | 9 | 25.7 |
| Frequency of sedation efficacy assessment | Once in every 2 hours | 6 | 17.1 |
| | Once in every 4 hours | 11 | 31.4 |
| | Once in every 6 hours | 4 | 11.4 |
| | Once in every 8 hours | 5 | 14.3 |
| | Once in every 12 hours | 6 | 17.1 |
| | Once in every 24 hours | 3 | 8.6 |
| Scales used in the assessment of sedation efficacy | COMFORT | 13 | 39.4 |
| | COMFORT Behavior (COMFORT-B) | 13 | 39.4 |
| | Ramsey Sedation Scale (RAS) | 11 | 33.3 |
| | State Behavioral Scale (SBS) | 6 | 18.2 |
| | The Brussels Sedation Assessment Scale | 4 | 12.1 |
| | Richmond Agitation and Sedation Scale (RASS) | 3 | 9.1 |

**Table II.** Practices related to pain assessment in pediatric intensive care units.

| | | N (35) | % |
|---|---|---|---|
| Practitioner performing the pain assessment | Nurse | 26 | 77.1 |
| | Doctor | 9 | 29.9 |
| Frequency of pain efficacy assessment | Once in every 2 hours | 4 | 11.8 |
| | Once in every 4 hours | 11 | 32.4 |
| | Once in every 6 hours | 9 | 26.5 |
| | Once in every 8 hours | 3 | 8.8 |
| | Once in every 12 hours | 4 | 11.8 |
| | Once in every 24 hours | 3 | 8.8 |
| Scales used in the assessment of pain efficacy in patients aged 6 years and older | Wong-Baker Faces Scale | 13 | 41.9 |
| | Visual Analog Scale (VAS) | 12 | 38.7 |
| | FLACC (Face, Legs, Activity, Cry, Consolability) | 11 | 35.5 |
| | COMFORT-B | 10 | 32.3 |
| | Numerical Rating Scales | 2 | 6.5 |
| | Multidimensional Assessment Pain Scale (MAPS) | 1 | 3.2 |
| Scales used in the assessment of pain efficacy in patients aged 6 years and younger | Wong-Baker Faces Scale | 17 | 50 |
| | FLACC (Face, Legs, Activity, Cry, Consolability) | 13 | 38.2 |
| | Visual Analog Scale (VAS) | 10 | 29.4 |
| | COMFORT-B | 10 | 29.4 |
| | Numerical rating scales | 1 | 2.9 |

**Table III.** The most commonly preferred analgesic agents in various clinical situations.

| | | n | % |
|---|---|---|---|
| Post-operative pediatric surgery patients | Acetaminophen | 29 | 80.6 |
| | Opiate | 26 | 72.2 |
| | Dexmedetomidine | 10 | 27.8 |
| | Ibuprofen | 4 | 11.1 |
| | Ketamine | 1 | 2.8 |
| | Other non-steroidal anti-inflammatory agents | 1 | 2.8 |
| In trauma patients | Opiate | 31 | 86.1 |
| | Acetaminophen | 26 | 72.2 |
| | Dexmedetomidine | 7 | 19.4 |
| | Ketamine | 4 | 11.1 |
| | Ibuprofen | 2 | 5.6 |
| | Other non-steroidal anti-inflammatory agents | 1 | 2.8 |
| In post-operative cardiac surgery patients | Opiate | 27 | 75 |
| | Dexmedetomidine | 23 | 63.9 |
| | Acetaminophen | 20 | 55.6 |
| | Ketamine | 6 | 16.7 |
| | Ibuprofen | 1 | 2.8 |
| | Other non-steroidal anti-inflammatory agents | 1 | 2.8 |
| High-flow nasal cannula in the PICU due to respiratory failure. | Dexmedetomidine | 21 | 58.3 |
| | Ketamine | 12 | 33.3 |
| | Midazolam | 2 | 5.6 |
| | No agent | 1 | 2.8 |

preferred rocuronium as muscle relaxant, 13 (36.1%) stated that they preferred vecuronium. Two (5.6%) of the participants used muscle relaxants as intermittent infusion, 8 (22.2%) as continuous infusion, and 26 (72.2%) as intermittent or continuous infusion depending on the situation. All participants applied eye closure and lubricant to prevent corneal abrasions in patients to whom they applied muscle relaxants. To assess the depth of sedation in patients receiving muscle relaxants, 17 (47.2%) of the participants used BIS monitoring instead of approved clinical scoring tools.

### *Withdrawal and delirium practices in PICU*

Thirty-two (88.8%) of the participants were making withdrawal assessments when discontinuing sedo-analgesic agents. Table IV presents practices related to withdrawal assessment, including who performs the assessment, the scales used, and the frequency of assessment. The PICU specialists who participated in the study stated that they applied the following strategies to patients with withdrawal symptoms: 23 (63.9%) increasing the dose of benzodiazepines and opiates, 8 (22.2%) ensuring frequent oral feeding, 22 (61.1%) bringing the family with the patient, 8 (22.2%) making the patient listen to music, 11 (30.6%) allowing the appropriate age group to use a television, tablet, telephone, 23 (63.9%) changing medication or adding new medication. Sixteen of the participants (16.7%) used phenobarbital, 29 (80.6%) dexmedetomidine, and thirteen (36.1%) chloralhydrate as drugs to help them with withdrawal.

Delirium assessment was being routinely performed by 21 (58.3%) of the participants. Practices regarding the assessment of delirium (who performed the assessment, the scales used, the frequency of assessment) are presented in Table V. Thirty (83.3%) of the participants were practicing environmental optimization to prevent the development of delirium. While haloperidol among the pharmacologic agents administered in patients with delirium was preferred by 30 (83.3%) participants, melatonin was preferred by 7 (19.4%), risperidone by 9 (25%), olanzapine by 4 (11.1%), quetiapine by 4 (11.1%), benzodiazepine by 1 (2.8%) participant. Thirty (83.3%) of the participants practiced early mobilization to prevent the development of delirium.

### *Methods applied for environment optimization in PICU*

The following were the practices performed by PICU specialists to provide patients with a day-night cycle and improve patients' sleep quality: 32 (88.9%) participants reduced lights at night, 10 (27.8%) participants used eye patches and 3 (8.3%) participants used earplugs in appropriate patients during sleep, 29 (80.6%) participants reduced invasive procedures in the evening and at night. All of the study participants stated that they did not allow parents to be present during invasive procedures such as central

**Table IV.** Practices related to withdrawal assessment in pediatric intensive care units.

| | | n | % |
|---|---|---|---|
| Practitioner conducting the withdrawal assessment | Doctor | 29 | 90.6 |
| | Nurse | 3 | 9.4 |
| Frequency of withdrawal assessment | Once in every 4 hours | 12 | 37.5 |
| | Once in every 6 hours | 7 | 21.9 |
| | Once in every 8 hours | 4 | 12.5 |
| | Once in every 12 hours | 9 | 28.1 |
| Scales used in the assessment of withdrawal | Withdrawal Assessment Tool version-1 (WAT-1) | 19 | 65.5 |
| | Sedation Withdrawal Score (SWS) | 5 | 17.2 |
| | Opioid and Benzodiazepine Withdrawal Score (OBWS) | 3 | 10.3 |
| | Sophia Observation withdrawal Symptoms Scale (SOS) | 1 | 3.4 |

**Table V.** Practices related to delirium assessment in pediatric intensive care units.

| | | N (35) | % |
|---|---|---|---|
| Practitioner performing the delirium assessment | Doctor | 15 | 71.4 |
| | Nurse | 6 | 28.6 |
| Frequency of delirium assessment | Once in every 8 hours | 8 | 38 |
| | Once in every 12 hours | 6 | 28.6 |
| | Once in every 24 hours | 7 | 33.4 |
| Scales used in the assessment of delirium | CAP-D (Cornell Assessment of Pediatric Delirium) | 13 | 61.9 |
| | pCAM-ICU (Pediatric Confusion Assessment Method-ICU) or psCAM-ICU Preschool | 7 | 33.3 |
| | Pediatric Anesthesia Emergence Delirium Scale (PAED) | 2 | 9.5 |
| | Confusion Assessment Method-ICU | 1 | 4.8 |

venous catheterization, endotracheal intubation and chest tube placement. PICU specialists reported that they implemented the following practices regarding parents: 21 (58.4%) allowed the parents to be with the patient at certain times during the day, 7 (19.4%) allowed the parents to be with the patient in the unit for 24 hours, 11 (30.6%) allowed the parents to be near the patient in an isolated room for 24 hours, 13 (36.1%) allowed the parents to be with the patient for a certain period of time only on visiting days. Participants stated their concerns about the impact of parents on patient care were as follows: 20 (55.6%) delay in intervening in a sudden and unexpected negative situation, 15 (41.6%) create risk of infection, 32 (88.9%) stated that parents would overreact to negative situations.

## Discussion

Assessment of pain and agitation in children is an ongoing challenge for PICU specialists. Therefore, the need for standardization has emerged to ensure an adequate level of sedation.[6] Over the past two decades, reports of the use of nurse-dependent sedation protocols have increased.[7-10] Although there are studies showing that applying a protocol in sedation decreases the duration of stay on the MV, there are also studies showing the contrary.[11,12] In our study, most of the participants (63.9%) stated that they did not use a written sedation protocol for patients on MV but performed routine assessments of sedation efficacy (83.3%). Similarly, we observed a high rate of pain assessment (94.4%) but a low use of a written protocol (25%). In a recent survey involving 215 PICUs from twenty-seven European countries, 71% reported using protocols for analgesia-sedation management. Daily assessment for pain (81%) and sedation (87%) was reported by most PICUs using the preferred validated FLACC scale (54%) and COMFORT Behavior (COMFORT-B) scale (48%), respectively. Both analgesia and sedation were mostly monitored by nurses.[13] In a survey study conducted in the United States, it was reported that most of the participants had a written sedation protocol and used scoring systems to evaluate sedation analgesia and the most common scoring tool used was the COMFORT score.[14] A survey conducted with 27 PICUs in Türkiye and published in 2020 reported that only 9 (33.3%) and 13 (48.1%) centers had a written protocol for analgesia and sedation, respectively. It was found that sedation efficacy was routinely evaluated in all PICUs and COMFORT (55.5%) and Ramsay (37%) sedation scales were the most commonly used scales for this purpose. The most commonly used rating scales for analgesia were reported to be the Wong-Baker FACES pain rating scale (51.8%) and the COMFORT behavior scale (44.4%). It was observed that nurses frequently made these assessments.[15] When our study is compared with these studies covering Europe, America and our country, it was observed that sedation and

analgesia assessment is routinely performed in our country, but the rate of written protocol implementation remains low compared to European and American countries, similar to the study[15] conducted in our country in 2020. This situation can be explained by the low number of trained personnel serving in PICUs compared to the pediatric population of our country and the excessive workload.

Similar to previous studies conducted in other countries, midazolam and fentanyl were found to be the most commonly used sedoanalgesic agents in intubated patients in PICUs in our country.[16-18] In cases of inadequate sedation, ketamine and then dexmedetomidine were the most commonly used agents. In retrospective and prospective studies of ketamine, it has shown good efficacy and a favorable safety profile in the short term, but data on long-term outcomes are not clear.[19,20] The conducted studies found that dexmedetomidine has similar sedation efficacy to benzodiazepine and reduces the need for opiates in PICU patients.[21-23] Studies have also shown that it reduces tachyarrhythmia and shortens the duration of MV exposure in postoperative cardiac surgery patients.[24] Benzodiazepine alone is a risk factor for delirium development.[25] For these reasons, dexmedetomidine is recommended in the first place in the guidelines for sedation in postoperative cardiac surgery patients hospitalized in the ICU and intubated patients hospitalized for other indications.[4] In the literature, its use has been described as a single agent with a continuous infusion in patients using noninvasive MV and as an adjuvant agent given simultaneously with benzodiazepines and opiates for sedation in patients undergoing postoperative cardiac surgery.[5] In our country, dexmedetomidine use ranks first in patients who are followed up with noninvasive MV and high-flow nasal cannula. In cases of inadequate sedation, it is often used after ketamine. Its use as first choice in intubated patients is low, which is consistent with the literature.[26,27]

In a survey study conducted in Canada, morphine was the most common analgesic agent used in intubated patients in PICUs, while acetaminophen and ibuprofen were the most commonly used adjuvant analgesics.[28] In our study, while opiates were the most commonly used analgesic agent in postoperative surgery patients, trauma patients and patients who underwent postoperative cardiac surgery, it was seen that the rates of acetaminophen and dexmedetomidine use were also high in these patient groups. In a survey involving anesthesiologists and emergency physicians except PICU specialists in the United States, the most commonly used sedoanalgesic agents were propofol, ketamine and fentanyl, respectively, in short-term procedures in extubated patients.[29] In our country, the preference of PICU specialists was ketamine, similar to the study conducted in 2020.[15]

Daily sedation interruption is one approach developed to avoid the negative effects of excessive sedation. Research demonstrates that daily sedation interruption decreases the duration of hospital stays and the number of days spent on MV in the adult population.[30] In a study comparing patients receiving daily sedation interruption with sick children receiving continuous sedation, daily sedation interruption led to improved clinical outcomes, including shorter MV and PICU stays.[31] In a survey conducted in Argentina, the rate of daily sedation interruption was 4%, whereas in our study, it was 38.9%.[26]

In our study, muscle relaxants were the most commonly used in intubated patients to prevent patient-mechanical ventilator asynchrony. The muscle relaxant of choice is often rocuronium. In the study by Twite et al. it was observed that the use of muscle relaxants could be preferred by 69% with similar indications, the rate of muscle relaxant use was 30%, the most commonly used agent was vecuronium, and hemodynamic findings (51.7%) were most frequently used as a sedation assessment tool in patients using muscle relaxants.[14] In a European centered study, it was observed that rocuronium was mostly preferred, the depth of sedation was mostly monitored through

hemodynamic findings (75%) in patients using muscle relaxants and the use of BIS (32%) was low.[13] The BIS monitor has been well validated in the pediatric anesthesiology literature for titrating depth of anesthesia in children and its use in PICUs is promising.[32,33] In our study, we observed that approximately half of the participants used BIS monitoring to assess the depth of sedation in patients receiving muscle relaxants. We observed an increase in the use of BIS monitors compared to previous years in our country, which was also higher than studies conducted in other countries.

Several prospective observational studies in PICUs have shown that delirium is frequently seen.[34] The biggest problem in PICUs today is the recognition of delirium. Assessment scales have been developed for this purpose. According to an international survey in 2014, 71% of PICU do not monitor delirium and the Pediatric Confusion Assessment Method (pCAM-ICU) is the only scale used in delirium assessment.[35] In a survey study conducted in Japan, it was observed that delirium was assessed at a rate of 21%, and the most frequently used scale was pCAM-ICU.[27] In our study, it was seen that delirium assessment increased compared to the past and the most commonly used assessment scale was the Cornell Assessment of Pediatric Delirium. Despite limited data, implementing environmental modifications, such as maintaining day/night cycles and ensuring healthy sleep conditions at night may affect the incidence and severity of delirium in children.[4] In our study, it was also found that environmental changes were frequently used to prevent the development of delirium. The use of atypical antipsychotic agents is preferred in resistant and severe delirium.[4] Although it was not asked in which condition it was used, the most frequently used medical agents were found to be haloperidol, risperidone and melatonin, respectively.

Prolonged and high dose sedoanalgesic use may lead to iatrogenic withdrawal syndrome.[36] There are some validated and reliable assessment tools for pediatric withdrawal syndrome. Studies have shown that the Withdrawal Assessment Tool version-1 (WAT-1) scale is effective for the assessment of withdrawal and its use has also been recommended by the sedation guide.[4,37,38] In our study, withdrawal assessment was performed by 88.8% of the participants and the most frequently used scale was WAT-1. There is no solid evidence on the prevention and management of withdrawal syndrome in critically ill children.The most current recommendation is to discontinue opiates and benzodiazepines in accordance with the protocol and to perform opiate and benzodiazepine replacement therapy when withdrawal symptoms occur.[5] In our study, it was observed that the most commonly used approach in patients with withdrawal was to increase the dose of the medication or to add new medication which is mostly dexmedetomidine and chloralhydrate. In addition to these, we observed that common approaches included allowing the patient's family to be present and encouraging them to listen to music.

To prevent stress and facilitate sleep in the PICU, it is important to promote an environment with adequate light and sound. Non-pharmacological comfort measures and sleep promotion are recommended by both adult and pediatric clinic guidelines[4,27] In our country, methods such as reducing lights at night, using eye patches for suitable patients, reducing blood collection and invasive interventions have been applied for this purpose. Parents are increasingly actively involved in decisions about children hospitalized in the PICU. It has been emphasized and suggested that parental involvement is important to make pediatric patients comfortable in an unfamiliar environment and to reduce anxiety and stress of parents, but there are limited studies on this subject.[4,5] All participants stated that they did not allow parents to be present by the patient during invasive interventions in our country. Only 19.4% of the PICU specialists allow the parents to stay with the patient permanently. This situation may be attributed to personnel and space limitations in our country, but we

believe that the primary cause is the violence patients' relatives inflict on healthcare workers. In fact, our study revealed that the most prevalent worry about parents' involvement in patient care was their tendency to overreact to potential negative circumstances and postpone intervention due to familial reactions.

### *Limitations*

The number of participants in the study was low considering the number of physicians working in the field of PICU in our country. However, since almost half of the physicians who participated in the study have been working in the PICU for more than 10 years and 61.1% of them were lecturers, we think that they adequately reflect sedation analgesia, withdrawal and delirium practices in PICUs in our country.

### *Conclusion*

This is the second survey study conducted on the management of sedation and analgesia in PICUs within Türkiye. This second survey revealed the implementation of practices similar to those in other countries in recent years. Although protocol usage rate related to sedation-analgesia remained low, it was found that the usage rate of assessment scales increased, and the assessment scale utilization and the sedoanalgesic agents used in various clinical scenarios were performed in parallel with the recommendations of the sedation guides. While awareness of delirium and withdrawal assessment has increased, it has not yet reached the desired level. Therefore, there is a need to develop guidelines, raise awareness, and increase training on these issues in our country.

## Ethical approval

The study was approved by the local ethics committee of Çukurova University Faculty of Medicine (Date: October 13, 2023; number: 137).

## Author contribution

The authors confirm contributions to the paper as follows: Study conception and design: EK, DY, AK, UA, GB; data collection: EK, DY, AK, UA, GB; analysis and interpretation of results: EK, DY; draft manuscript preparation: EK, DY 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. Karapınar B. Mekanik ventilasyon sırasında sedasyon. Karaböcüoğlu M, editor. Pediatrik Mekanik Ventilasyon. Çağdaş Medikal Kitabevi, Çapa Tıp Kitabevi; 2003: 211-225.
2. Ista E, van Dijk M, Gamel C, Tibboel D, de Hoog M. Withdrawal symptoms in critically ill children after long-term administration of sedatives and/or analgesics: a first evaluation. Crit Care Med 2008; 36: 2427-2432. https://doi.org/10.1097/CCM.0b013e318181600d
3. Choong K. Picu-acquired complications: the new marker of the quality of care. ICU Manag Pract 2019; 19: 85-88.
4. Smith HAB, Besunder JB, Betters KA, et al. 2022 Society of Critical Care Medicine clinical practice guidelines on prevention and management of pain, agitation, neuromuscular blockade, and delirium in critically ill pediatric patients with consideration of the ICU environment and early mobility. Pediatr Crit Care Med 2022; 23: e74-e110. https://doi.org/10.1097/PCC.0000000000002873
5. Mondardini MC, Sperotto F, Daverio M, Amigoni A. Analgesia and sedation in critically ill pediatric patients: an update from the recent guidelines and point of view. Eur J Pediatr 2023; 182: 2013-2026. https://doi.org/10.1007/s00431-023-04905-5

6. Vet NJ, Kleiber N, Ista E, de Hoog M, de Wildt SN. Sedation in critically ill children with respiratory failure. Front Pediatr 2016; 4: 89. https://doi.org/10.3389/fped.2016.00089

7. Deeter KH, King MA, Ridling D, Irby GL, Lynn AM, Zimmerman JJ. Successful implementation of a pediatric sedation protocol for mechanically ventilated patients. Crit Care Med 2011; 39: 683-688. https://doi.org/10.1097/CCM.0b013e318206cebf

8. Ista E, de Hoog M, Tibboel D, van Dijk M. Implementation of standard sedation management in paediatric intensive care: effective and feasible? J Clin Nurs 2009; 18: 2511-2520. https://doi.org/10.1111/j.1365-2702.2009.02836.x

9. Gaillard-Le Roux B, Liet JM, Bourgoin P, Legrand A, Roze JC, Joram N. Implementation of a nurse-driven sedation protocol in a PICU decreases daily doses of midazolam. Pediatr Crit Care Med 2017; 18: e9-e17. https://doi.org/10.1097/PCC.0000000000000998

10. Neunhoeffer F, Kumpf M, Renk H, et al. Nurse-driven pediatric analgesia and sedation protocol reduces withdrawal symptoms in critically ill medical pediatric patients. Paediatr Anaesth 2015; 25: 786-794. https://doi.org/10.1111/pan.12649

11. Blackwood B, Tume LN, Morris KP, et al. Effect of a sedation and ventilator liberation protocol vs usual care on duration of invasive mechanical ventilation in pediatric intensive care units: a randomized clinical trial. JAMA 2021; 326: 401-410. https://doi.org/10.1001/jama.2021.10296

12. Curley MAQ, Wypij D, Watson RS, et al. Protocolized sedation vs usual care in pediatric patients mechanically ventilated for acute respiratory failure: a randomized clinical trial. JAMA 2015; 313: 379-389. https://doi.org/10.1001/jama.2014.18399

13. Daverio M, von Borell F, Ramelet AS, et al. Pain and sedation management and monitoring in pediatric intensive care units across Europe: an ESPNIC survey. Crit Care 2022; 26: 88. https://doi.org/10.1186/s13054-022-03957-7

14. Twite MD, Rashid A, Zuk J, Friesen RH. Sedation, analgesia, and neuromuscular blockade in the pediatric intensive care unit: survey of fellowship training programs. Pediatr Crit Care Med 2004; 5: 521-532. https://doi.org/10.1097/01.PCC.0000144710.13710.2E

15. Ekinci F, Yildizdas D, Horoz OO, Aslan N; Society of Pediatric Emergency Intensive Care Medicine of Turkey-Sedoanalgesia Study Group. Sedation and analgesia practices in pediatric intensive care units: a survey of 27 centers from Turkey. J Pediatr Intensive Care 2020; 10: 289-297. https://doi.org/10.1055/s-0040-1716886

16. Rhoney DH, Murry KR. National survey on the use of sedatives and neuromuscular blocking agents in the pediatric intensive care unit. Pediatr Crit Care Med 2002; 3: 129-133. https://doi.org/10.1097/00130478-200204000-00007

17. Playfor SD, Thomas DA, Choonara I. Sedation and neuromuscular blockade in paediatric intensive care: a review of current practice in the UK. Paediatr Anaesth 2003; 13: 147-151. https://doi.org/10.1046/j.1460-9592.2003.00989.x

18. Long D, Horn D, Keogh S. A survey of sedation assessment and management in Australian and New Zealand paediatric intensive care patients requiring prolonged mechanical ventilation. Aust Crit Care 2005; 18: 152-157. https://doi.org/10.1016/s1036-7314(05)80028-1

19. Tessari A, Sperotto F, Pece F, et al. Is ketamine infusion effective and safe as an adjuvant of sedation in the PICU? Results from the Ketamine Infusion Sedation Study (KISS). Pharmacotherapy 2023; 43: 622-631. https://doi.org/10.1002/phar.2754

20. Heiberger AL, Ngorsuraches S, Olgun G, et al. Safety and utility of continuous ketamine infusion for sedation in mechanically ventilated pediatric patients. J Pediatr Pharmacol Ther 2018; 23: 447-454. https://doi.org/10.5863/1551-6776-23.6.447

21. Fagin A, Palmieri T, Greenhalgh D, Sen S. A comparison of dexmedetomidine and midazolam for sedation in severe pediatric burn injury. J Burn Care Res 2012; 33: 759-763. https://doi.org/10.1097/BCR.0b013e318254d48e

22. Tobias JD, Berkenbosch JW. Sedation during mechanical ventilation in infants and children: dexmedetomidine versus midazolam. South Med J 2004; 97: 451-455. https://doi.org/10.1097/00007611-200405000-00007

23. Grant MJC, Schneider JB, Asaro LA, et al. Dexmedetomidine use in critically ill children with acute respiratory failure. Pediatr Crit Care Med 2016; 17: 1131-1141. https://doi.org/10.1097/PCC.0000000000000941

24. Ghimire LV, Chou FS. Efficacy of prophylactic dexmedetomidine in preventing postoperative junctional ectopic tachycardia in pediatric cardiac surgery patients: a systematic review and meta-analysis. Paediatr Anaesth 2018; 28: 597-606. https://doi.org/10.1111/pan.13405

25. Mody K, Kaur S, Mauer EA, et al. Benzodiazepines and development of delirium in critically ill children: estimating the causal effect. Crit Care Med 2018; 46: 1486-1491. https://doi.org/10.1097/CCM.0000000000003194

26. Taffarel P, Bonetto G, Jorro Barón F, Meregalli C. Sedation and analgesia in patients on mechanical ventilation in pediatric intensive care units in Argentina. Arch Argent Pediatr 2018; 116: e196-e203. https://doi.org/10.5546/aap.2018.eng.e196

27. Koizumi T, Kurosawa H. Survey of analgesia and sedation in pediatric intensive care units in Japan. Pediatr Int 2020; 62: 535-541. https://doi.org/10.1111/ped.14139

28. Garcia Guerra G, Joffe AR, Cave D, et al. Survey of sedation and analgesia practice among Canadian pediatric critical care physicians. Pediatr Crit Care Med 2016; 17: 823-830. https://doi.org/10.1097/PCC.0000000000000864

29. Srinivasan M, Bhaskar S, Carlson DW. Variation in procedural sedation practices among children's hospitals. Hosp Pediatr 2015; 5: 148-153. https://doi.org/10.1542/hpeds.2014-0090

30. Kress JP, Pohlman AS, O'Connor MF, Hall JB. Daily interruption of sedative infusions in critically ill patients undergoing mechanical ventilation. N Engl J Med 2000; 342: 1471-1477. https://doi.org/10.1056/NEJM200005183422002

31. Gupta K, Gupta VK, Jayashree M, Singhi S. Randomized controlled trial of interrupted versus continuous sedative infusions in ventilated children. Pediatr Crit Care Med 2012; 13: 131-135. https://doi.org/10.1097/PCC.0b013e31820aba48

32. Bannister CF, Brosius KK, Sigl JC, Meyer BJ, Sebel PS. The effect of bispectral index monitoring on anesthetic use and recovery in children anesthetized with sevoflurane in nitrous oxide. Anesth Analg 2001; 92: 877-881. https://doi.org/10.1097/00000539-200104000-00015

33. Berkenbosch JW, Fichter CR, Tobias JD. The correlation of the bispectral index monitor with clinical sedation scores during mechanical ventilation in the pediatric intensive care unit. Anesth Analg 2002; 94: 506-11; table of contents. https://doi.org/10.1097/00000539-200203000-00006

34. Traube C, Mauer EA, Gerber LM, et al. Cost associated with pediatric delirium in the ICU. Crit Care Med 2016; 44: e1175-e1179. https://doi.org/10.1097/CCM.0000000000002004

35. Kudchadkar SR, Yaster M, Punjabi NM. Sedation, sleep promotion, and delirium screening practices in the care of mechanically ventilated children: a wake-up call for the pediatric critical care community. Crit Care Med 2014; 42: 1592-1600. https://doi.org/10.1097/CCM.0000000000000326

36. Best KM, Boullata JI, Curley MAQ. Risk factors associated with iatrogenic opioid and benzodiazepine withdrawal in critically ill pediatric patients: a systematic review and conceptual model. Pediatr Crit Care Med 2015; 16: 175-183. https://doi.org/10.1097/PCC.0000000000000306

37. Harris J, Ramelet AS, van Dijk M, et al. Clinical recommendations for pain, sedation, withdrawal and delirium assessment in critically ill infants and children: an ESPNIC position statement for healthcare professionals. Intensive Care Med 2016; 42: 972-986. https://doi.org/10.1007/s00134-016-4344-1

38. Ávila-Alzate JA, Gómez-Salgado J, Romero-Martín M, Martínez-Isasi S, Navarro-Abal Y, Fernández-García D. Assessment and treatment of the withdrawal syndrome in paediatric intensive care units: systematic review. Medicine (Baltimore) 2020; 99: e18502. https://doi.org/10.1097/MD.0000000000018502

The Turkish Journal of Pediatrics 2024; 66: 567-577
https://doi.org/10.24953/turkjpediatr.2024.4527

**Original Article**

# Common complications in spinal muscular atrophy (SMA) type 1 after nusinersen treatment

Yiğithan Güzin[1], Osman Büyükşen[1], Pınar Gençpınar[2,3], Nihal Olgaç Dündar[2,3], Figen Baydan[1]

*[1]Department of Pediatric Neurology, Tepecik Training and Research Hospital, University of Health Sciences, İzmir; [2]Department of Pediatric Neurology, İzmir Katip Çelebi University, İzmir; [3]Neuroscience Research Center, İzmir Katip Çelebi University, İzmir, Türkiye.*

## ABSTRACT

**Background.** Spinal muscular atrophy (SMA) is an inherited disease with progressive muscle weakness and atrophy. Despite the new treatments developed recently, primary and secondary effects of muscle weakness in patients with SMA cause mortality and morbidity. The aim of this study is to identify common problems in the follow-up of patients after new treatment modalities and to examine the difficulties in management of these problems.

**Methods.** The study included 16 patients diagnosed with SMA type 1 according to clinical findings and genetic results between 2017 and 2022. The patients were divided into two groups as living and deceased, and complications were examined and compared between the groups.

**Results.** The patients comprised 8 (50%) females and 8 (50%) males with a median age at diagnosis of 3 months. The patients had a history of gastrointestinal problems, orthopedic problems, infection and sepsis, and especially respiratory distress. Death occurred in 8 (50%) patients during follow-up (median age 38 months). Mortality was higher in patients who needed tracheostomy and had gastroesophageal reflux. The survival rate was better in patients who received more nusinersen treatment and had a higher CHOP-INTEND score.

**Conclusions.** Despite new-generation treatments for SMA type 1, morbidity and mortality rates remain very high. As the survival rate in SMA type 1 increases, the incidence of complications similar to those frequently seen in SMA type 2 and type 3 patients also increases. The follow-up and treatment of patients with SMA should be undertaken by a multidisciplinary team.

**Key words:** Spinal muscular atrophy type 1, mortality, morbidity, nusinersen, *SMN1*.

Spinal muscular atrophy (SMA) is an inherited disease with progressive muscle weakness and atrophy with degeneration of spinal anterior horn cells and destruction of alpha motor neuron cells.[1] The incidence of SMA ranges from 4 to 10 per 100,000 live births.[2,3] 95% of the cases result from a homozygous deletion of *SMN1* at the chromosomal locus 5q13. Classification of SMA subtypes is determined by age at onset and clinical severity and life expectancy. An early onset of symptoms is associated with poor prognosis.[4]

In SMA type 1, limited head control, hypotonia, and areflexia are seen during the first six months of life. Type I SMA is defined as 'non-sitters'. Muscle weakness is

✉ Yiğithan Güzin ▪ yguzin@hotmail.com

Received 21st Oct 2023, revised 19th Apr 2024, 27th May 2024, accepted 30th Sep 2024.

the major cause of complications in SMA type 1 patients, and they are known to develop gastrointestinal, nutritional, orthopedic, and especially respiratory problems.[5] Weakness in the respiratory muscles leads to progressive respiratory failure. Feeding problems may lead to developmental delay and pulmonary problems secondary to aspiration.[6] Decreased muscle strength and impaired mobility can cause many musculoskeletal problems.[7]

Recently, promising disease-modifying therapies such as nusinersen, onasemnogene abeparvovec and risdiplam have been developed for patients with SMA.[8,9] At present, nusinersen is currently the only treatment option approved for SMA and reimbursed by the health authorities in Türkiye. Before the era of these new-generation treatments, most SMA type 1 patients died of respiratory failure under the age of two years.[1]

Despite newly developed treatments, the primary and secondary effects of muscle weakness in patients with SMA cause morbidity and mortality. The management of these complications has a direct effect on morbidity and mortality.[10,11]

The aim of this study was to review the problems that may be seen in the follow-up of SMA type 1 patients after nusinersen, one of the new-generation therapies, and to examine the difficulties encountered in the management of these problems.

## Materials and Methods

The files of 22 patients diagnosed with SMA type 1 between 2017 and 2022 were retrospectively analyzed. Three patients who received onasemnogene abeparvovecalan treatment and three patients who did not receive nusinersen treatment were excluded from the study. A total of 16 patients with SMA type 1 who received at least four loading doses of nusinersen were included in the study. The diagnosis of SMA type 1 was made according to clinical findings and genetic tests.[1,4] All patients presented with limited head control, hypotonia, and areflexia in the first six months of life. In genetic tests, homozygous deletion in the *SMN1* gene and two copies of the *SMN2* gene were observed in all patients.

Nusinersen is an antisense oligonucleotide against *SMN2*.[12] It is currently the only treatment option for the treatment of SMA patients in Türkiye, which is approved and reimbursed by the health authorities. Nusinersen was administered to patients as a maintenance dose every four months following the first four loading doses (day 0, day 14, day 28 and day 63).[13] Intrathecal treatment administration was performed by a pediatric neurologist. No administration-related side-effects were observed in patients.

The patients were evaluated with respect to age, gender, medical history and family history, respiratory problems, gastrointestinal problems, orthopedic problems, number and duration of hospitalization, use and dose of nusinersen, and current Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP INTEND) scores. In patients older than two years, the Hammersmith Functional Motor Scale Extended (HMFSE) test was used in addition to the CHOP-INTEND score for evaluation. The CHOP-INTEND and HMFSE tests were performed before each nusinersen treatment by the physiotherapist authorized to perform the tests at our neuromuscular center. Approval was obtained from the Turkish Medicines and Medical Devices Agency before each dose of nusinersen with the results of CHOP-INTEND and HMFSE tests.

Our neuromuscular center is one of four reference centers in Izmir province authorized to apply nusinersen. In our center, SMA patients are followed up and treated with a multidisciplinary approach in neurological, physical, cardiac, respiratory and gastrointestinal aspects.

The patients were divided into two groups of living and deceased patients. Student's

t-test (Mann-Whitney U if non-parametric), chi-square, or Fisher's exact test were used to evaluate whether the data of the living and deceased patient groups differed.

Approval for the study was obtained from the Non-Interventional Research Ethics Committee of Tepecik Training and Research Hospital (decision no: 2023/04-23, dated: 03/05/2023). Family consent was waived because the study was a retrospective study.

## Results

The study included 16 patients diagnosed with SMA type 1, comprising eight (50%) females and eight (50%) males with a mean age of 34.9±24.1 months (median 38 months). The median age at diagnosis was 3 months (min: 1 month – max: 7 months). Consanguineous marriage was present in eight (50%) patients. There was a history of SMA in the cousins of three (18.8%) patients. No patient with a family history of SMA was diagnosed by early screening.

When the medical history was analyzed, four (25%) patients had a history of hospitalization in the neonatal intensive care unit (NICU) with a diagnosis of transient tachypnea of the newborn thought to be related to cesarean delivery.

Complications seen in deceased and living patients are summarized in Table I. It was observed that two patients had respiratory problems during sleep, so they were monitored with non-invasive mechanic ventilation (NIV) and the respiratory problems disappeared in the 3rd month of follow-up.

There was a history of at least one hospitalization in the intensive care unit due to respiratory distress in 13 (81.3%) patients. All of the deceased patients were followed up at least once in the intensive care unit due to respiratory distress (Table I).

A history of tracheostomy was present in eight patients. All patients who underwent tracheostomy had a history of prolonged intubation in the intensive care unit. Mortality was higher in patients who underwent tracheostomy. The median month of insertion of the tracheostomy was 8 months (range: 6-9 months). The tracheostomy was closed in only one patient after 13 months because the respiratory findings improved.

The median age of death of the eight patients (50%) who died during follow-up was 38 months (range: 8–48 months).

Four patients died due to septic shock in the intensive care follow-up, and two patients died due to pneumonia. Two patients were found dead at home and the exact cause of death is unknown.

There was a history of constipation in seven (46.7%) and reflux in five (31.3%) patients. Proton pump inhibitor (PPI) treatment was given to all patients with gastroesophageal reflux (GER). It was observed that the reflux symptoms of all patients regressed after PPI treatment. All the patients with reflux were in the deceased patient group (p=0.026).

Dysphagia was present in 10 (62.5%) patients, of which four (25%) received nasogastric tube (NT) and three (18.8%) received percutaneous endoscopic gastrostomy (PEG) (Table I). Weight gain was observed to be better in the follow-up of the patients with PEG.

All the patients regularly took vitamin D and calcium supplements, vitamin D or calcium deficiency was not detected in patients. Scoliosis was detected in eight (50%) patients with a median age of 46 months (range: 21-96 months). More severe joint contractures, especially in the lower extremities, were observed in five patients (Table I).

One of the patients suffered from sudden increases and decreases in blood pressure and episodes of flushing and pallor of extremities. This condition was considered to be autonomic dysfunction since it was accompanied by sudden changes in pulse rate and could not be explained by any other cause. In one patient,

**Table I.** Clinical findings in SMA type 1 patients, n (%).

| | Living (n=8) | Deceased (n=8) | Total (n=16) | p |
|---|---|---|---|---|
| Pulmonary problems | | | | |
| History of hospitalization for pneumonia | 7 (87.5%) | 8 (100%) | 15 (93.7%) | 1.000 |
| History of hospitalization in the ICU | 5 (62.5%) | 8 (100%) | 13 (81.3%) | 0.200 |
| Tracheostomy | 1 (12.5%) | 7 (87.5%) | 8 (50.0%) | **0.010** |
| Non-invasive respiratory support | 2 (25.0%) | - | 2 (12.5%) | 0.467 |
| Gastrointestinal complications | | | | |
| Dysphagia | 3 (37.5%) | 7 (87.5%) | 10 (62.5%) | 0.119 |
| Constipation | 3 (37.5%) | 4 (50.0%) | 7 (43.8%) | 0.619 |
| Reflux | - | 5 (62.5%) | 5 (31.3%) | **0.026** |
| Nutrition and malnutrition | | | | |
| Use of enteral nutrition | 7 (87.5%) | 7 (87.5%) | 14 (87.5%) | 1.000 |
| Weight loss | 6 (75.0%) | 5 (62.5%) | 11 (68.8%) | 1.000 |
| Weight less than -2 SDS | 6 (75.0%) | 5 (62.5%) | 11 (68.8%) | 1.000 |
| Height less than - 2 SDS | 6 (75.0%) | 5 (62.5%) | 11 (68.8%) | 1.000 |
| Use of NT | - | 4 (50.0%) | 4 (25.0%) | 0.077 |
| Use of PEG | 1 (12.5%) | 2 (25.0%) | 3 (18.8%) | 1.000 |
| Orthopedic Problems | | | | |
| Joint contracture | 3 (37.5%) | 2 (25.0%) | 5 (31.3%) | 1.000 |
| Scoliosis | 4 (50.0%) | 5 (62.5%) | 8 (50.0%) | 1.000 |
| Sepsis | 1 (12.5 %) | 4 (50.0%) | 5 (31.3%) | 0.282 |
| Urinary tract infection | 2 (25.0%) | 5 (62.5%) | 7 (43.8%) | 0.315 |
| Other | | | | |
| Pressure ulcer | - | 1 (12.5%) | 1 (6.3%) | 1.000 |
| Autonomic dysfunction | - | 1 (12.5%) | 1 (6.3%) | 1.000 |

ICU, intensive care unit; NT, nasogastric tube; PEG, percutaneous endoscopic gastrostomy; SDS, standard deviation score.

bedsores were detected during long-term intensive care follow-up.

All patients presenting with fever in our center are tested for urinary tract infection. Urinary system infection was detected in seven (43.8%) patients in this study. Although not statistically significant, a history of urinary tract infection and sepsis was observed more frequently in patients who died (p=0.282).

The comparisons of the number and duration of follow-ups and treatment of the living and deceased patients are summarized in Table II. Mortality was found to be higher in patients who needed tracheostomy and had gastroesophageal reflux. The survival rate was better in patients who received more nusinersen and had a higher CHOP-INTEND score and HMFSE (Table II).

## Discussion

In SMA type 1, many complications develop as a result of progressive muscle weakness. However, since the introduction of new promising treatments such as nusinersen, life expectancy has increased.[14,15] Therefore, prolonged life expectancy leads to the emergence of problems similar to the complications frequently seen in SMA type 2 and type 3 patients. Since mortality is still very high in SMA type 1, correct management of these complications plays an important role in reducing morbidity and mortality. Therefore, it is of critical importance to know the complications in these patients.[16,17]

**Table II.** Comparisons of the number and duration of follow-ups and treatment of living and deceased patients.

| | Total (n=16) | Living (n=8) | Deceased (n=8) | p-value |
|---|---|---|---|---|
| Female gender, n (%) | 8 (50.0%) | 5 (62.5%) | 3 (37.5%) | 0.619 |
| Age at diagnosis, months | 3 | 3 | 4 | 0.330 |
| Age at onset of first symptoms, months | 2 | 2 | 2.5 | 0.318 |
| Follow-up period, months | 38 (6-96) | 32 (6-96) | 38 (8-48) | 0.717 |
| First CHOP-INTEND score | 18 (2-47) | 18 (11-47) | 16 (2-25) | 0.105 |
| Last CHOP-INTEND score | 41 (19-64) | 48 (29-64) | 37 (19-64) | **0.071** |
| First HMFSE score | 17.5 (2-37) | 23.5 (15-37)* | 5 (2-8)** | **0.025** |
| Last HMFSE score | 19 (0-38) | 25 (18-38)* | 4 (0-8) ** | **0.028** |
| Age at first nusinersen treatment, months | 6 (2-30) | 4 (2-30) | 7 (2-30) | 0.820 |
| Number of nusinersen doses | 6 (4-18) | 11 (4-18) | 5 (4-11) | **0.071** |

Data presented as median, followed by (min-max) if available, unless otherwise specified. *(n=4), **(n=2)
CHOP-INTEND, Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders; HMFSE, Hammersmith Functional Motor Scale Extended.

The aim of this study was to evaluate common problems encountered in the follow-up of patients with SMA type 1 receiving nusinersen treatment.

### *Pulmonary complications*

Pulmonary complications are an important cause of morbidity and mortality in patients with SMA type 1.[18] Respiratory problems often result from hypoventilation, weakness in the cough reflex, and difficulty in expelling secretions. The severity of pulmonary complications is closely related to the type of SMA. All children with SMA type 1 and approximately one-third of patients with type 2 experience respiratory problems. Therefore, respiratory complications should be monitored more closely.[19,20]

Decreased hospitalisation rates after nusinersen treatment have been reported in literature[21,22], while other studies have reported no change in hospital admissions.[23]

In the current study, 15 (93.8%) patients had a history of at least one hospitalization due to respiratory distress caused by pneumonia. Of these patients, 13 (81.3%) were followed up in the intensive care unit at least once. These results indicate that further multicentre studies with larger cohorts are needed.

With the progression of the disease, NIV support at night may be required in children with respiratory distress during sleep. Ventilatory support may also be needed later in the day if daytime hypercapnia becomes a problem.[24] In addition, the benefit of early ventilatory support has been shown to improve survival and life assurance.[25] In the current study, it was observed that two patients had respiratory problems during sleep, so they were monitored with NIV and their respiratory problems disappeared in the 3rd month of follow-up.

Tracheostomy may be required in patients who cannot tolerate NIV and have prolonged intubation during intensive care admission.[26] Tracheostomy and NIV increase survival. Despite being an invasive method, tracheostomy is known to significantly reduce hospitalization rates compared with NIV.[27]

Lavie et al. reported that assisted ventilation needs remained stable after nusinersen in most patients with SMA type 1. Two of the patients in that study were reported to have died from acute respiratory failure and one patient suffered severe brain damage.[28]

Tracheostomy (due to prolonged intubation) was performed in eight (50%) patients in the current study, and of these, seven died during follow-up. These findings differed from the

literature. It was thought that the increased mortality rate in patients who underwent tracheostomy may have been due to long hospital stays in the intensive care unit and complications such as infection.

### *Gastrointestinal complications*

Gastrointestinal complications are much more common in patients with SMA type 1 than previously thought. The most common problems are GER, delayed gastric emptying, and constipation. These complications may increase the risk of aspiration and pneumonia and cause morbidity.[29]

In a study by de-Andrés-Beltrán et al. investigating the clinical and phenotypic characteristics of SMA type 1 patients after new pharmacological treatments, 18 of 50 patients (36%) had a history of GER.[30] Similar to the literature, five (31.3%) of the current study patients had a history of GER. Gastroesophageal reflux can be treated with acid neutralisers and/or acid secretion inhibitors such as histamine blockers and PPI. In all of the current study patients, symptoms disappeared after PPI treatment.

Symptoms of gastaroinsteinal dysfunction, such as GER, constipation, delayed gastric emptying, are important determinants of morbidity and mortality.[31,32] In the current study, all the patients with GER were lost even if treated. This was attributed to possible chronic pulmonary side-effects of GER.

In 2023, Chacko et al. found abnormal swallowing characteristics in eight patients with SMA type 1 who were receiving disease-modified therapy.[33]

Unlike the literature, the rate of dysphagia in the current study was found to be 62.5%. This was attributed to the retrospective nature of the study and lacking methods such as swallowing status scale, swallow questionnaire, surface electromyography, and videofluoroscopic studies to evaluate dysphagia.

There is no consensus on when to refer patients for gastrostomy tube placement.[1] Insertion of the gastrostomy tube does not protect patients from aspiration of oropharyngeal secretions, although some studies have suggested moderate benefit from this approach.[34]

Lavie et al. showed that 94% of 20 SMA type 1 patients receiving nusinersen treatment were fed by invasive methods.[35] In the current study, this rate was lower than the literature. A NT was applied in four (25%) patients, and PEG was performed in three (18.8%) patients, and it was observed that the weight gain of the patients was better after PEG. These results once again emphasise the importance of methods such as NT and PEG.

### *Nutrition and malnutrition*

Malnutrition is common in SMA type 1 patients. The increased risk of malnutrition in SMA patients can lead to loss of lean body mass, which can weaken the strength of already weak muscles, especially the respiratory muscles.[36,37]

Feler et al. reported that malnutrition was frequently seen in patients with SMA and that patients who were started on nusinersen therapy showed more significant improvement in nutritional status after nutritional interventions.[38] Therefore, patients should be evaluated in terms of nutrition during their follow-up, and attention should be paid to malnutrition.[39]

The literature indicates that individuals with SMA type 1 exhibit lower median weight and height.[40] Enteral nutritional supplements were administered together with the formula to 14 (87.5%) patients because they did not gain sufficient weight. In 11 patients, both weight and height percentiles were below the -2 standard deviation score (SDS) for age.

It has been shown that bone mineral density tends to decrease with advancing age in SMA patients. Therefore, patients should take adequate vitamin D supplements.[41] All the current study patients received regular vitamin

D and calcium supplements, and no vitamin D or calcium deficiency was observed in patient.

### *Orthopedic problems*

The weakness and decreased range of motion seen in SMA type 1 results in a predisposition to numerous musculoskeletal problems.[1,42,43] Although scoliosis is mostly seen in almost all patients with SMA type 2 and type 3, data on this subject are limited in patients with type 1 diagnosis.[44] With the increase in life expectancy after new generation treatments, scoliosis has become a frequent complication in patients with SMA type 1 diagnosis.

It has been reported in the literature that the rate of scoliosis development in SMA type 1 patients receiving nusinersen treatment varies between 75% and 100%.[35,45] In the current study, scoliosis was detected in eight (50%) patients and was attributed to the limited number of cases.

The development of joint contractures due to muscle weakness and immobility is one of the most common complications in patients with SMA type 1.[46] Increased duration of immobility and loss of motor function may aid the development of contractures.[47] In the current study, joint contractures, which were more prominent especially in the lower extremities, were observed in five patients. Although there are few studies in the literature about the incidence of joint contracture after nusinersen, it is known that individual physical therapy programs and passive movement of the joints help to prevent joint contractures.[48]

### *Infection-related complications and mortality*

The natural course of SMA type 1 is poor. Patients usually die before the age of two years.[1] In a study by Thomas et al. in 1993, the median age of death in SMA type 1 patients was seven months, while in a study by Kolb et al. in 2017 it was eight months.[49,50] Even though mortality rates due to nusinersen treatment have decreased significantly, the mortality rate remains high in patients with SMA type 1.[51]

In the current study, mortality developed during follow-up in eight (50%) patients at a median age of 38 months.

SMA type 1 is known to be a common cause of sudden infant death syndrome.[52] In this study, sudden infant death of unknown cause was recorded for two patients. In the literature, cases have been reported of symptomatic bradycardia and unexpected cardiopulmonary arrest thought to be due to autonomic involvement in patients with SMA.[53,54] In patients with no determined etiology, the possibility that the cause of sudden unexpected death was related to autonomic dysfunction was considered.

Better survival of the current study patients was associated with nusinersen treatment. When the characteristics of the living and deceased patients were compared, it was found that the surviving patients had received a greater number of nusinersen doses than the patients who died, which was consistent with the findings in literature.[55]

Weakness of pelvic muscles and sphincter defects in individuals with SMA can lead to urinary retention.[56] It is known that urinary retention and urinary catheterization increase the risk of urinary tract infections and therefore urosepsis.[57]

Literature regarding the incidence of urinary tract infection in SMA type 1 patients is sparse. However, in a study by Gök et al. it was reported that the risk of nephrolithiasis was higher in patients with SMA type 1 and urinary tract infection was observed more frequently in these patients.[58]

Urinary system infection was detected in seven (43.8%) patients included in this study. It was also observed that patients who died had a more frequent history of urinary tract infection. Although it was not statistically significant, it was thought that this may have been related to hospital-acquired and resistant infections, which increase with a prolonged length of stay in the intensive care unit.

### *Other*

Autonomic system involvement may be seen in SMA.[59-61] Autonomic dysfunction is one of the rare complications that can be seen in patients with SMA. Varriable measurements of blood pressure and heart rate, hyperhidrosis, GER, constipation, cardiac conduction abnormalities and cold-induced vasodilation of the fingers may occur.[59,62] In the current study, sudden increases and decreases in blood pressure accompanied by flushing were observed in the follow-up of one patient.

Pressure ulcers are one of the most common problems in immobile patients. Patients with SMA are also at risk of pressure ulcers. In a multicentre study conducted in the Catalonia region, pressure ulcers were observed in 10 (26.3%) of 38 patients with SMA type 1 who did not receive treatment, whereas in the current study, only two (9%) patients had pressure ulcers.[63] The less frequent occurrence of pressure ulcers in this study was thought to be because our patients received nusinersen treatment and anti-bedsore mattresses were used in the follow-up of immobile patients.

### *Limitations of the study*

As this study was retrospective in design, standardized methods for dysphagia assessment could not be used, also the sample included deceased patients. These prevented the application of widely accepted tools such as swallowing status scale, swallowing questionnaire, surface electromyography and videofluoroscopic methods. Similarly, measurements of mid-arm circumference and triceps skinfold to determine malnutrition were also lacking in the available data set. These limitations should be taken into account when interpreting the results of the study and it should be emphasised that more comprehensive assessment methods should be used in future studies.

## Conclusion

Morbidity and mortality rates remain high in SMA type 1 patients despite new generation therapies. Follow-up and treatment processes should be managed with a multidisciplinary approach. Prediction of the natural course and risks, determination of appropriate treatment options and timely intervention are important. There is a need to understand the changes caused by new treatment regimens in patients with motor symptoms, especially scoliosis, hip dislocation, dysphagia and GER. Therefore, it is important that further large-scale and multicenter prospective studies are conducted.

## Ethical approval

Approval for the study was obtained from the Non-Pharmacological Clinical Research Ethics Committee of Tepecik Training and Research Hospital (Decision no: 2023/04-23 Date: 03/05/2023) Family consent was waived because the study was a retrospective study.

## Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: YG, FB; data collection: NOD, FB, YG, PGP; analysis and interpretation of results: YG, OB, NOD; draft manuscript preparation: MK. 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. Kolb SJ, Kissel JT. Spinal muscular atrophy. Neurol Clin 2015; 33: 831-846. https://doi.org/10.1016/j.ncl.2015.07.004
2. Mostacciuolo ML, Danieli GA, Trevisan C, Müller E, Angelini C. Epidemiology of spinal muscular atrophies in a sample of the Italian population. Neuroepidemiology 1992; 11: 34-38. https://doi.org/10.1159/000110905
3. Thieme A, Mitulla B, Schulze F, Spiegler AW. Epidemiological data on Werdnig-Hoffmann disease in Germany (West-Thüringen). Hum Genet 1993; 91: 295-297. https://doi.org/10.1007/BF00218278
4. Darras BT. Spinal muscular atrophies. Pediatr Clin North Am 2015; 62: 743-766. https://doi.org/10.1016/j.pcl.2015.03.010
5. Finkel RS, McDermott MP, Kaufmann P, et al. Observational study of spinal muscular atrophy type I and implications for clinical trials. Neurology 2014; 83: 810-817. https://doi.org/10.1212/WNL.0000000000000741
6. Iannaccone ST. Modern management of spinal muscular atrophy. J Child Neurol 2007; 22: 974-978. https://doi.org/10.1177/0883073807305670
7. Wang HY, Ju YH, Chen SM, Lo SK, Jong YJ. Joint range of motion limitations in children and young adults with spinal muscular atrophy. Arch Phys Med Rehabil 2004; 85: 1689-1693. https://doi.org/10.1016/j.apmr.2004.01.043
8. Ludolph AC, Wurster CD. Therapeutic advances in SMA. Curr Opin Neurol 2019; 32: 777-781. https://doi.org/10.1097/WCO.0000000000000738
9. Angilletta I, Ferrante R, Giansante R, et al. Spinal muscular atrophy: an evolving scenario through new perspectives in diagnosis and advances in therapies. Int J Mol Sci 2023; 24: 14873. https://doi.org/10.3390/ijms241914873
10. Arnold WD, Kassar D, Kissel JT. Spinal muscular atrophy: diagnosis and management in a new therapeutic era. Muscle Nerve 2015; 51: 157-167. https://doi.org/10.1002/mus.24497
11. Wang CH, Finkel RS, Bertini ES, et al. Consensus statement for standard of care in spinal muscular atrophy. J Child Neurol 2007; 22: 1027-1049. https://doi.org/10.1177/0883073807305788
12. Hoy SM. Nusinersen: first global approval. Drugs 2017; 77: 473-479. https://doi.org/10.1007/s40265-017-0711-7
13. Hoy SM. Nusinersen: a review in 5q spinal muscular atrophy. CNS Drugs 2021; 35: 1317-1328. https://doi.org/10.1007/s40263-021-00878-x
14. Veldhoen ES, Wijngaarde CA, Hulzebos EHJ, et al. Natural history of respiratory muscle strength in spinal muscular atrophy: a prospective national cohort study. Orphanet J Rare Dis 2022; 17: 70. https://doi.org/10.1186/s13023-022-02227-7
15. Lagae L, Proesmans M, Van den Hauwe M, Vermeulen F, De Waele L, Boon M. Respiratory morbidity in patients with spinal muscular atrophy-a changing world in the light of disease-modifying therapies. Front Pediatr 2024; 12: 1366943. https://doi.org/10.3389/fped.2024.1366943
16. Berglund A, Berkö S, Lampa E, Sejersen T. Survival in patients diagnosed with SMA at less than 24 months of age in a population-based setting before, during and after introduction of nusinersen therapy. Experience from Sweden. Eur J Paediatr Neurol 2022; 40: 57-60. https://doi.org/10.1016/j.ejpn.2022.07.005
17. Farrar MA, Park SB, Vucic S, et al. Emerging therapies and challenges in spinal muscular atrophy. Ann Neurol 2017; 81: 355-368. https://doi.org/10.1002/ana.24864
18. Cherchi C, Chiarini Testa MB, Deriu D, et al. All you need is evidence: what we know about pneumonia in children with neuromuscular diseases. Front Pediatr 2021; 9: 625751. https://doi.org/10.3389/fped.2021.625751
19. Araki S, Hayashi M, Tamagawa K, et al. Neuropathological analysis in spinal muscular atrophy type II. Acta Neuropathol 2003; 106: 441-448. https://doi.org/10.1007/s00401-003-0743-9
20. Simonds AK. Chronic hypoventilation and its management. Eur Respir Rev 2013; 22: 325-332. https://doi.org/10.1183/09059180.00003113
21. Hepkaya E, Kılınç Sakallı AA, Ülkersoy İ, et al. The effects of nusinersen treatment on respiratory status of children with spinal muscular atrophy. Pediatr Int 2022; 64: e15310. https://doi.org/10.1111/ped.15310
22. Chen KA, Widger J, Teng A, Fitzgerald DA, D'Silva A, Farrar M. Real-world respiratory and bulbar comorbidities of SMA type 1 children treated with nusinersen: 2-year single centre Australian experience. Paediatr Respir Rev 2021; 39: 54-60. https://doi.org/10.1016/j.prrv.2020.09.002
23. Gonski K, Chuang S, Teng A, et al. Respiratory and sleep outcomes in children with SMA treated with nusinersen - real world experience. Neuromuscul Disord 2023; 33: 531-538. https://doi.org/10.1016/j.nmd.2023.04.007
24. Nickol AH, Hart N, Hopkinson NS, Moxham J, Simonds A, Polkey MI. Mechanisms of improvement of respiratory failure in patients with restrictive thoracic disease treated with non-invasive ventilation. Thorax 2005; 60: 754-760. https://doi.org/10.1136/thx.2004.039388

25. Oskoui M, Levy G, Garland CJ, et al. The changing natural history of spinal muscular atrophy type 1. Neurology 2007; 69: 1931-1936. https://doi.org/10.1212/01.wnl.0000290830.40544.b9

26. Gilgoff IS, Kahlstrom E, MacLaughlin E, Keens TG. Long-term ventilatory support in spinal muscular atrophy. J Pediatr 1989; 115: 904-909. https://doi.org/10.1016/s0022-3476(89)80740-1

27. Bach JR. The use of mechanical ventilation is appropriate in children with genetically proven spinal muscular atrophy type 1: the motion for. Paediatr Respir Rev 2008; 9: 45-56. https://doi.org/10.1016/j.prrv.2007.11.003

28. Lavie M, Diamant N, Cahal M, et al. Nusinersen for spinal muscular atrophy type 1: real-world respiratory experience. Pediatr Pulmonol 2021; 56: 291-298. https://doi.org/10.1002/ppul.25140

29. Ionasescu V, Christensen J, Hart M. Intestinal pseudo-obstruction in adult spinal muscular atrophy. Muscle Nerve 1994; 17: 946-948. https://doi.org/10.1002/mus.880170816

30. de-Andrés-Beltrán B, Güeita-Rodríguez J, Palacios-Ceña D, Rodríguez-Fernández ÁL. Clinical and functional characteristics of a new phenotype of SMA type I among a national sample of Spanish children: a cross-sectional study. Children (Basel) 2023; 10: 892. https://doi.org/10.3390/children10050892

31. Dipasquale V, Morello R, Romano C. Gastrointestinal and nutritional care in pediatric neuromuscular disorders. World J Clin Pediatr 2023; 12: 197-204. https://doi.org/10.5409/wjcp.v12.i4.197

32. Moore GE, Lindenmayer AW, McConchie GA, Ryan MM, Davidson ZE. Describing nutrition in spinal muscular atrophy: a systematic review. Neuromuscul Disord 2016; 26: 395-404. https://doi.org/10.1016/j.nmd.2016.05.005

33. Chacko A, Marshall J, Taylor O, McEniery J, Sly PD, Gauld LM. Dysphagia and lung disease in children with spinal muscular atrophy treated with disease-modifying agents. Neurology 2023; 100: 914-920. https://doi.org/10.1212/WNL.0000000000206826

34. Birnkrant DJ, Pope JF, Martin JE, Repucci AH, Eiben RM. Treatment of type I spinal muscular atrophy with noninvasive ventilation and gastrostomy feeding. Pediatr Neurol 1998; 18: 407-410. https://doi.org/10.1016/s0887-8994(97)00227-0

35. Lavie M, Rochman M, Sagi L, et al. Nonrespiratory complications of nusinersen-treated spinal muscular atrophy type 1 patients. Pediatr Pulmonol 2022; 57: 686-694. https://doi.org/10.1002/ppul.25795

36. Poruk KE, Davis RH, Smart AL, et al. Observational study of caloric and nutrient intake, bone density, and body composition in infants and children with spinal muscular atrophy type I. Neuromuscul Disord 2012; 22: 966-973. https://doi.org/10.1016/j.nmd.2012.04.008

37. Li YJ, Chen TH, Wu YZ, Tseng YH. Metabolic and nutritional issues associated with spinal muscular atrophy. Nutrients 2020; 12: 3842. https://doi.org/10.3390/nu12123842

38. Yerushalmy-Feler A, Levy D, Sagi L, et al. Nutritional therapy in children with spinal muscular atrophy in the era of nusinersen. J Pediatr Gastroenterol Nutr 2021; 72: e154-e160. https://doi.org/10.1097/MPG.0000000000003055

39. Tilton AH, Miller MD, Khoshoo V. Nutrition and swallowing in pediatric neuromuscular patients. Semin Pediatr Neurol 1998; 5: 106-115. https://doi.org/10.1016/s1071-9091(98)80026-0

40. Darras BT, Guye S, Hoffart J, et al. Distribution of weight, stature, and growth status in children and adolescents with spinal muscular atrophy: an observational retrospective study in the United States. Muscle Nerve 2022; 66: 84-90. https://doi.org/10.1002/mus.27556

41. Kinali M, Banks LM, Mercuri E, Manzur AY, Muntoni F. Bone mineral density in a paediatric spinal muscular atrophy population. Neuropediatrics 2004; 35: 325-328. https://doi.org/10.1055/s-2004-830366

42. Tangsrud SE, Carlsen KC, Lund-Petersen I, Carlsen KH. Lung function measurements in young children with spinal muscle atrophy; a cross sectional survey on the effect of position and bracing. Arch Dis Child 2001; 84: 521-524. https://doi.org/10.1136/adc.84.6.521

43. Montes J, McDermott MP, Martens WB, et al. Six-minute walk test demonstrates motor fatigue in spinal muscular atrophy. Neurology 2010; 74: 833-838. https://doi.org/10.1212/WNL.0b013e3181d3e308

44. Haaker G, Fujak A. Proximal spinal muscular atrophy: current orthopedic perspective. Appl Clin Genet 2013; 6: 113-120. https://doi.org/10.2147/TACG.S53615

45. Al Amrani F, Amin R, Chiang J, et al. Scoliosis in spinal muscular atrophy type 1 in the nusinersen era. Neurol Clin Pract 2022; 12: 279-287. https://doi.org/10.1212/CPJ.0000000000001179

46. Dubowitz V. Very severe spinal muscular atrophy (SMA type 0): an expanding clinical phenotype. Eur J Paediatr Neurol 1999; 3: 49-51.

47. Salazar R, Montes J, Dunaway Young S, et al. Quantitative evaluation of lower extremity joint contractures in spinal muscular atrophy: implications for motor function. Pediatr Phys Ther 2018; 30: 209-215. https://doi.org/10.1097/PEP.0000000000000515

48. Bieniaszewska A, Sobieska M, Gajewska E. Prospective analysis of functional and structural changes in patients with spinal muscular atrophy-a pilot study. Biomedicines 2022; 10: 3187. https://doi.org/10.3390/biomedicines10123187

49. Thomas NH, Dubowitz V. The natural history of type I (severe) spinal muscular atrophy. Neuromuscul Disord 1994; 4: 497-502. https://doi.org/10.1016/0960-8966(94)90090-6

50. Kolb SJ, Coffey CS, Yankey JW, et al. Natural history of infantile-onset spinal muscular atrophy. Ann Neurol 2017; 82: 883-891. https://doi.org/10.1002/ana.25101

51. Krupa D, Czech M, Chudzyńska E, Koń B, Kostera-Pruszczyk A. Real world evidence on the effectiveness of nusinersen within the national program to treat spinal muscular atrophy in Poland. Healthcare (Basel) 2023; 11: 1515. https://doi.org/10.3390/healthcare11101515

52. Valencia B, Bach JR. Eighteen years with spinal muscular atrophy (SMA) type 1. Tanaffos 2013; 12: 70-73.

53. Bach JR. Medical considerations of long-term survival of Werdnig-Hoffmann disease. Am J Phys Med Rehabil 2007; 86: 349-355. https://doi.org/10.1097/PHM.0b013e31804b1d66

54. Kawashima H, Ishii C, Yamanaka G, et al. Myopathy and neurogenic muscular atrophy in unexpected cardiopulmonary arrest. Pediatr Int 2011; 53: 159-161. https://doi.org/10.1111/j.1442-200X.2010.03211.x

55. Belančić A, Strbad T, Kučan Štiglić M, Vitezić D. Effectiveness of nusinersen in type 1, 2 and 3 spinal muscular atrophy: Croatian real-world data. J Clin Med 2023; 12: 2839. https://doi.org/10.3390/jcm12082839

56. Destro F, Marinoni F, Russo T, Selvaggio G, Riccipetitoni G. Spinal muscular atrophy (SMA) from the urological point of view: assessment of the urinary function in 38 patients. 2020; 7: 1789397. https://doi.org/10.1080/2331205X.2020.1789397

57. Gatti JM, Perez-Brayfield M, Kirsch AJ, Smith EA, Massad HC, Broecker BH. Acute urinary retention in children. J Urol 2001; 165: 918-921.

58. Gök A, Saygılı SK, Kuruğoğlu S, Saltık S, Canpolat N. Children with type 1 spinal muscular atrophy are at increased risk for nephrolithiasis. Pediatr Neurol 2024; 150: 32-36. https://doi.org/10.1016/j.pediatrneurol.2023.10.006

59. Hachiya Y, Arai H, Hayashi M, et al. Autonomic dysfunction in cases of spinal muscular atrophy type 1 with long survival. Brain Dev 2005; 27: 574-578. https://doi.org/10.1016/j.braindev.2005.02.009

60. Shababi M, Lorson CL, Rudnik-Schöneborn SS. Spinal muscular atrophy: a motor neuron disorder or a multi-organ disease? J Anat 2014; 224: 15-28. https://doi.org/10.1111/joa.12083

61. Poets C, Heyer R, von der Hardt H, Walter GF. Acute respiratory insufficiency as the initial clinical manifestation of spinal muscular atrophy. Monatsschr Kinderheilkd 1990; 138: 157-159.

62. de Queiroz Campos Araujo AP, de Queiroz Campos Araujo IP, de Queiroz Campos Araujo A. Autonomic nervous system dysfunction in motor neuron diseases. J Rare Dis Res Treat. 2018;3:1–5. https://doi.org/10.29245/2572-9411/2018/1.1136

63. Darbà J. Management and current status of spinal muscular atrophy: a retrospective multicentre claims database analysis. Orphanet J Rare Dis 2020; 15: 8. https://doi.org/10.1186/s13023-019-1287-y

The Turkish Journal of Pediatrics 2024; 66: 578-587
https://doi.org/10.24953/turkjpediatr.2024.4571

**Original Article**

# Foreign body aspiration through the eyes of a pediatric pulmonologist: Is it possible to reduce the rate of negative rigid bronchoscopies?

Birce Sunman[1], Halime Nayır Büyükşahin[1], İsmail Güzelkaş[1], Didem Alboğa[1], Meltem Akgül Erdal[1], Havva İpek Demir[1], Raziye Atan[1], Burcu Çapraz Yavuz[1], Burak Ardıçlı[2], İdil Rana User[2], Özlem Tekşam[3], Nagehan Emiralioğlu[1], Ebru Yalçın[1], Deniz Doğru[1], Uğur Özçelik[1], Nural Kiper[1]

*[1]Division of Pulmonology, Department of Pediatrics, Faculty of Medicine, Hacettepe University, Ankara; [2]Department of Pediatric Surgery, Faculty of Medicine, Hacettepe University, Ankara; [3]Division of Pediatric Emergency Medicine, Department of Pediatrics, Faculty of Medicine, Hacettepe University, Ankara, Türkiye.*

## ABSTRACT

**Background.** Identifying a foreign body aspiration (FBA) still remains a diagnostic difficulty. Moreover, the indications for bronchoscopy in subjects of suspected foreign bodies are not clear. The aim of this study was to evaluate the effectiveness of pediatric pulmonologists in diagnosing FBA.

**Methods.** This was a retrospective, single-center study on children who underwent rigid bronchoscopy for suspected FBA. Data on the patients were obtained from the medical records. Patients who had foreign bodies (FB) identified during rigid bronchoscopy were classified as FB positive, and those in whom rigid bronchoscopy did not detect FB were defined as FB negative. Demographic data as well as consultation status with a pediatric pulmonologist were compared between these two groups. Furthermore, the patients were categorized into three groups based on their clinical scores that assessed the likelihood of the presence of FB: low risk, moderate risk, and high risk.

**Results.** Out of 474 rigid bronchoscopies, 232 (48.9%) detected FB. Consultation by a pediatric pulmonologist was not requested in 388 (81.8%). Out of these 388 patients, 206 (53%) were negative for FB. In terms of FB detection success, there was no difference between individuals who sought pulmonology consultation and those who did not (58.1% vs. 53.1% respectively, p=0.059). However, when the children were categorized based on their risk levels, the incidence of detecting FB among children in low-risk group was 42% when they received consultation from the pulmonology department, whereas this incidence dropped to 5.6% when pulmonology consultation was not sought (p<0.001).

**Conclusions.** Consulting a pediatric pulmonologist, particularly for low-risk individuals, might reduce the likelihood of performing unnecessary bronchoscopies. Given that rigid bronchoscopy is an intrusive technique, it is crucial to reduce the number of negative bronchoscopies in order to mitigate complications associated with it.

**Key words:** foreign body, pediatric pulmonologist, rigid bronchoscopy.

✉ Birce Sunman ▪ bircesunman@gmail.com

Received 30th Mar 2024, revised 23rd Jul 2024, accepted 30th Sep 2024.

Foreign body aspiration (FBA) can cause significant morbidity and mortality in the pediatric population. It is a serious condition that commonly manifests in young children and necessitates prompt intervention in order to prevent potential complications and the risk of long-term sequelae. Most of the cases occur in infants and toddlers, and only a few children show the classic triad of choking, coughing, and unilateral wheezing or decreased air entry.[1]

Currently, rigid bronchoscopy performed under general anesthesia is the accepted method for removing a foreign body (FB) from the airways in pediatric patients. The widespread use of this method for both diagnostic and therapeutic intentions resulted in a significant proportion of negative bronchoscopies, ranging from 10 to 61% as reported by different research groups.[2-4] In addition, complications are not uncommon during rigid bronchoscopy, especially when performed on small children. Unnecessary bronchoscopy not only exposes the child to the potential risks associated with anesthesia, but it can also lead to perioperative complications such as bronchospasm, desaturation, edema, and hemorrhage.[5]

The indications for bronchoscopy in patients with suspected FB are not clearly defined. Each centre usually follows its own procedures based on the patient's medical history, physical examination, and radiological results.[6-8] Various scores and algorithms have been suggested so far to predict the existence of an FB and reduce the rate of negative bronchoscopies.[7-11] Nevertheless, none of them have shown adequate specificity or sensitivity to definitively establish the diagnosis.[9] Because of the serious long-term consequences of missed FBA and the difficulty of a definite diagnosis, many children who undergo rigid bronchoscopy arrive unfortunately with negative results.[8] The aim of this study was to evaluate the effectiveness of pediatric pulmonologists in predicting FBA. To the best of our knowledge, this is the first study from our Türkiye identifying the role of pediatric pulmonologists in the approach of FBA diagnosis. The secondary outcome of interest was to comprehensively analyze the characteristics of rigid bronchoscopies conducted for suspicious cases of FBA at our center and to identify disparities between bronchoscopies with high and low diagnostic success, with the purpose of avoiding unnecessary procedures and mitigating associated risks.

## Materials and Methods

### *Study design and population*

In this retrospective, single-center, cross-sectional descriptive study, we reviewed the hospital operating room records of all children aged 0 to 18 years who underwent rigid bronchoscopy for suspected FBA at our tertiary academic center from January 2015 to October 2023. A confirmed FB in the esophagus or larynx was excluded from this group.

In our center, suspected FBA is one of the reasons for the admission to the pediatric emergency department. The standard practice is to refer all patients who are suspected of having FBA to a pediatric surgeon. After the clinical and radiological evaluation of the patient, it is decided whether or not to perform rigid bronchoscopy. Some patients are consulted by a pediatric pulmonologist upon the decision of the pediatric emergency physician or pediatric surgeon. In our center, the criteria for consulting a pediatric pulmonologist for children with suspected FB are not well defined. Pediatric pulmonologists, if they are consulted, make decisions through a comprehensive approach to the patient, including physical examination, imaging, and clinical information.

In this study, children were divided into two groups as positive for FB and negative for FB. Patients who had FB identified during rigid bronchoscopy were classified as FB positive and in those whom rigid bronchoscopy did not detect FB were defined as FB negative. Additionally, the most recent scoring system categorized children as either low, moderate, or high risk of having FB.[12] The following criteria,

which consisted of the patient's medical history, physical examination, and radiological findings, were evaluated: a choking episode (0- no, 2- suspected or observed), exposure to any foreign body (0- no, 1- yes), a sudden cough during or after the event (0- no, 1- yes), absence of fever (≥ 38 °C) along with absence of rhinorrhea (0- no, 1- yes), unilateral wheezing or decreased air entry on auscultation (0- no, 2- yes), the presence of stridor (0- no, 1- yes), and imaging findings suggestive of FBA on chest X-ray (0- no, 2- yes). Regardless of the other components of the score, radiopaque FB received a full 10 points. The cumulative score (from lowest to highest risk) was between 0 and 10. Each child was categorized into one of three risk categories, according to their total score: low risk (1-3 points), moderate risk (4-6 points), or high risk (7-10 points). For each risk group, whether patients were referred to pediatric pulmonology was extracted from the medical records in order to ascertain the role played by a pediatric pulmonologist in determining the necessity for rigid bronchoscopy when FBA was suspected.

Clinical history, vital signs, and physical examination at the time of presentation were accessed from electronic medical records for each patient. Age, gender, witness situation of the event, the time elapsed between suspected aspiration and admission, suspected FB type, the presenting symptoms and signs and location of FB within pulmonary branching, were additional extracted data. These data were compared between positive FB and negative FB groups.

At our institution, plain radiographs are not formally reported by a radiologist unless consulted. Since the radiographic interpretation by the pediatric surgeon was recorded in the system, these notes were retrieved retrospectively. In addition, chest computed tomography (CT) scans reported by radiologists were gathered. Furthermore, rigid bronchoscopy findings were examined paying particular attention to the foreign body's type and location. FBs were classified into two groups as organic and inorganic. The amount of time that elapsed between FBA and admission to the hospital was grouped as less than 24 hours, less than a week, more than a week and more than a month. The records were examined for underlying diseases as well as diseases that emerged during the follow-up period.

Parental written informed consent was obtained in clinical practice prior to conducting rigid bronchoscopy on pediatric patients. As this study was conducted retrospectively, the analysis of the data obtained from medical records did not necessitate obtaining consent. The research protocol for this study was approved by the Clinical Research Ethics Committee of Hacettepe University (Reference number: SBA 23/186).

### *Statistical analysis*

Statistical analyses were performed using SPSS statistical software, version 21 (IBM Corp., Armonk, NY, USA). Normally distributed continuous variables were analyzed using student t-test and expressed as mean ± standard deviation (SD). Non-normally distributed continuous variables were analyzed using Mann-Whitney U test and expressed as median (interquartile range - IQR). Shapiro-Wilk test was used to assess normality where appropriate. Categorical variables were presented as percentages (%) and analyzed using chi-square test or Fisher's exact test. Values of $p<0.05$ were considered statistically significant. To examine the relationships among multiple-choice categorical variables multiple chi-Square test was performed and Bonferroni method for p-value adjustment was applied.

## Results

Among a total of 483 rigid bronchoscopy procedures performed with a suspicion of FBA at our pediatric tertiary care center during the study period, 474 were included in the study. Nine children with a confirmed FB in the larynx or esophagus were excluded from the analysis.

The median age of all patients at presentation was 19.44 months (IQR: 13.5-28.8) with the majority of the patients (n=387, 81.6%) younger than 3 years of age. Out of 474 rigid bronchoscopies, 232 (48.9%) detected FB. Four patients underwent two rigid bronchoscopies each to control if any residual material exists.

Among patients diagnosed with FBA via rigid bronchoscopy, 85 (18%) were admitted to the hospital within the initial twenty-four hours of the inciting event. Out of all the patients, 98.6% were admitted to the children's emergency services, while the remaining patients were admitted to either pediatric surgery or pediatric pulmonology outpatient clinics. In a 6-year-old boy previously diagnosed with asthma, FB was detected via rigid bronchoscopy more than 1 year after the doubtful event. Asthma/bronchial hyperreactivity (n=19, 4%) was the most frequent underlying disease in children who underwent rigid bronchoscopy due to suspected FBA.

Foreign bodies were more commonly found in the right lung (n=123, 53%) compared to the left (n= 89, 38.3%). In five (2.1%) patients, FB was detected in both the left and right main bronchus with the remaining in the trachea or carina (n=15, 6.4%). Organic material represented 84.9% of these foreign bodies with sunflower seeds being the most commonly aspirated material followed by hazelnuts, walnuts and peanuts.

Radiopaque foreign bodies such as turban pins, toy parts and pen caps represented the majority of the aspirated inorganic material. Out of 232 patients diagnosed with FBA, a definite history of experiencing abrupt choking while holding an object in the mouth or chewing something was collected from 204 (87.9%) patients. Similarly, out of the 242 patients without FBA, 207 of them (85.5%) reported a definite history of FBA (p=0.449).

Cough that started after the witnessed event and subsequent rhonchi in physical examination were the most common findings with a rate of 36%. Other presenting symptoms included solely wheezing, solely coughing, choking, respiratory distress, chest pain and flushing or cyanosis.

Among vitals, median transcutaneous oxygen saturation level on room air at presentation was 96% (min-max: 70-100%) in children with FBA. On physical examination, breath sounds were normal in 63% and 14.2% of patients without FBA and with FBA, respectively (p<0.001). Decreased breath sounds and rhonchi were noted in 71% of children with FBA. FBA was absent in 82.1% of patients who had a normal physical examination, while it was present in 17.8% of patients with a normal physical examination.

At least one chest radiograph was performed on 472 patients as an essential component of their diagnostic evaluation. Since two of the children had CT scans at the initial center they were admitted, chest X-rays were not performed at our center. A total of 27 patients had low-dose CT scan imaging. CT scans of two patients were normal although rigid bronchoscopy detected FB in both patients.

The radiologic interpretation of chest radiographs by pediatric surgeons for 136 patients (n=28.7%) were not available in the notes. No radiologic abnormality in chest X-rays was detected in 10.8% of patients with FBA and in 27.6% of patients without FBA (p<0.05). Unilateral air trapping (n=125, 54.3%) was the most common finding in the chest radiograph of patients with FBA. Atelectasis and bilateral air trapping were the other radiologic features noted in both groups.

In two children who underwent rigid bronchoscopy, pulmonologists also performed flexible fiberoptic bronchoscopy (FFB). FFB was conducted because of recurrent pneumonia in one of these patients. FB was detected although physical examination and chest radiography yielded normal results, and it was removed by rigid bronchoscopy. The second patient with the diagnosis of asthma underwent rigid

bronchoscopy due to the history of suspected FB, but a FB was not detected.

Among diagnostic tests such as history of the suspected FBA, physical examination, and radiographic signs, physical examination had the highest level of specificity and accuracy (63%, 95% confidence interval [CI]: 56.39-68.92%; and 73.6%, 95% CI: 69.86-77.94%, respectively). The most sensitive diagnostic tool was the history of the suspected FBA (87.9%; 95% CI: 83.03-91.83%). Positive predictive value and negative predictive value of physical examination were the highest to suspect FB (69%, 95% CI: 65.06-72.42%; and 82%, 95% CI: 76.80-86.51%, respectively). However, the sensitivity of the evaluation of physical examination and radiographic signs together was higher (94.5%, 95% CI: 93.88-98.78%) than the assessment of history, physical examination or radiography alone. Demographic data of study subjects are shown in Table I.

**Table I.** Demographic data of study subjects

| | Total | Positive FBA | Negative FBA | p-value |
|---|---|---|---|---|
| Number of patients, n (%) | 474 (100.0) | 232 (48.9) | 242 (51.1) | |
| Age (months), median (IQR) | 19.44 | 20 (5.1-195.2) | 18.6 (3.2-201) | 0.059 |
| Male: Female ratio | 1.25:1 (264:210) | 1.6:1 (143:89) | 1:1 (121:121) | **0.013** |
| Time to presentation, n (%) | | | | |
| Unknown | 57 (11.8) | 22 (9.5) | 35 (14.4) | 0.07 |
| <24 hours | 165 (34.8) | 85 (36.6) | 80 (33.0) | 0.42 |
| ≥24 hours, <1 week | 180 (38.0) | 86 (37.1) | 94 (38.8) | 0.68 |
| ≥1 week, <1 month | 58 (12.2) | 28 (12.1) | 30 (12.3) | 0.92 |
| ≥1 month | 13 (2.7) | 10 (4.3) | 3 (1.2) | 0.02 |
| ≥1 year | 1 (0.2) | 1 (0.4) | 0 (0) | 0.31 |
| Underlying disease, n (%) | | | | |
| None | 416 (87.8) | 209 (90.1) | 207 (85.5) | 0.13 |
| Asthma/bronchial hyperreactivity | 19 (4.0) | 5 (2.2) | 14 (5.8) | 0.045 |
| Down syndrome | 4 (0.8) | 1 (0.4) | 3 (1.2) | 0.31 |
| Prematurity | 5 (1.1) | 3 (1.3) | 2 (0.8) | 0.61 |
| Others | 30 (6.3) | 14 (6.0) | 16 (6.6) | 0.76 |
| Physical examination findings, n (%) | | | | |
| Total | 474 | 232 | 242 | |
| Normal | 186 (39.2) | 33 (14.2) | 152 (63.0) | **<0.0025*** |
| Unilateral decrease in breath sounds | 135 (28.5) | 90 (38.7) | 45 (18.5) | **<0.0025*** |
| Rhonchi | 84 (17.7) | 61 (26.3) | 23 (9.5) | **<0.0025*** |
| Unilateral decrease in breath sounds + rhonchi | 15 (3.1) | 14 (6.0) | 1 (0.4) | **<0.0025*** |
| Stridor | 9 (1.9) | 5 (2.2) | 4 (1.6) | 0.68 |
| Stridor and rhonchi | 2 (0.4) | 2 (0.9) | 0 (0) | 0.16 |
| Rales | 14 (3.0) | 5 (2.2) | 9 (3.7) | 0.31 |
| Intubated at admission | 7 (1.4) | 7 (3.0) | 0 (0) | 0.04 |
| Unknown** | 23 (4.8) | 15 (6.5) | 8 (3.3) | 0.10 |
| Consultation with Pediatric Pulmonology, n (%) | | | | |
| Yes | 388 (81.9) | 206 (53.1) | 182 (46.9) | 0.059 |
| No | 86 (18.1) | 50 (58.1) | 36 (41.9) | |

FBA: foreign body aspiration; IQR: interquartile range.
*Significant Bonferroni-adjusted p-values.
**missing interpretation of the X-rays by the physician who decided to perform rigid bronchoscopy.

**Table I.** Continued

| | Total | Positive FBA | Negative FBA | p-value |
|---|---|---|---|---|
| Patients consulted with Pediatric Pulmonology, n (%) | | | | |
| Total | 86 | 50 (58.1) | 36 (41.9) | 0.191 |
| Low risk, n (%) | 38 (44.2) | 16 (42.1) | 22 (57.9) | **<0.001** |
| Moderate risk, n (%) | 29 (33.7) | 18 (62.1) | 11 (37.9) | 0.614 |
| High risk, n (%) | 19 (22.1) | 16 (84.2) | 3 (15.8) | 0.057 |
| Chest radiographs, n (%) | | | | |
| Total | 472 | 230 | 242 | |
| Normal | 92 (19.5) | 25 (11.6) | 67 (27.6) | **<0.0025*** |
| Bilateral air trapping | 6 (1.3) | 3 (1.3) | 3 (1.2) | 0.92 |
| Unilateral air trapping | 194 (41.0) | 125 (53.9) | 69 (28.5) | **<0.0025*** |
| Radiopaque objects | 6 (1.3) | 6 (2.6) | 0 (0) | 0.012 |
| Infiltration/consolidation | 11 (2.3) | 1 (0.4) | 10 (4.1) | 0.006 |
| Atelectasis | 20 (4.2) | 9 (3.9) | 11 (4.5) | 0.68 |
| Other | 7 (1.5) | 4 (1.7) | 3 (1.2) | 0.68 |
| Unknown** | 136 (28.7) | 57 (24.6) | 79 (32.6) | 0.05 |
| Computed tomography, n (%) | | | | |
| Total | 27 | 27 (11.6) | 0 (0) | |
| Rigid bronchoscopy findings, n (%) | | | | |
| Normal | 172 (36.2) | 0 (0) | 172 (71.1) | **<0.002*** |
| Foreign body | 232 (49.0) | 232 (100.0) | 0 (0.0) | **<0.002*** |
| Secretions | 43 (9.0) | 2 (0.9) | 41 (16.9) | **<0.002*** |
| Purulent secretions | 16 (33.7) | 1 (0.4) | 15 (6.2) | **<0.002*** |
| Granulation tissue | 4 (0.8) | 0 (0) | 4 (1.7) | 0.04 |
| Tracheal bronchus | 7 (1.4) | 2 (0.8) | 5 (2.0) | 0.61 |
| Bronchial compression/ Malacia | 4 (0.8) | 0 (0) | 4 (1.7) | 0.04 |
| Cast | 1 (0.2) | 0 (0) | 1 (0.4) | 0.31 |
| Foreign body location, n (%) | | | | |
| Trachea | 8 (1.6) | 8 (3.4) | - | |
| Carina | 7 (1.4) | 7 (3.0) | - | |
| Right main | 100 (21.0) | 100 (43.1) | - | |
| Right upper | 2 (0.4) | 2 (0.9) | - | |
| Right middle | 2 (0.4) | 2 (0.9) | - | |
| Right Lower | 19 (4.0) | 19 (8.2) | - | |
| Left main | 77 (16.2) | 77 (33.2) | - | |
| Left upper | 4 (0.8) | 4 (1.7) | - | |
| Left lower | 8 (1.6) | 8 (3.4) | - | |
| Both Right and Left Main | 5 (1.0) | 5 (2.2) | - | |
| Types of the foreign body, n (%) | | | | |
| Unknown | 21 (4.4) | 21 (9.1) | - | |
| Organic | 198 (41.7) | 198 (85.3) | - | |
| Inorganic | 12 (3.1) | 12 (5.2) | - | |
| Tooth | 1 (0.2) | 1 (0.4) | - | |

FBA: foreign body aspiration; IQR: interquartile range.
*Significant Bonferroni-adjusted p-values.
**missing interpretation of the X-rays by the physician who decided to perform rigid bronchoscopy.

**Table II.** Patient characteristics according to risk groups

| | Total | Positive FBA | Negative FBA | p-value |
|---|---|---|---|---|
| Low risk | | | | |
| Consulted with pediatric pulmonology, n (%) | 38 (19.2) | 16 (42.1) | 22 (57.9) | **<0.001*** |
| Not consulted with pediatric pulmonology, n (%) | 160 (80.8) | 9 (5.6) | 151 (94.4) | |
| Moderate risk | | | | |
| Consulted with pediatric pulmonology, n (%) | 29 (20) | 18 (62.1) | 11 (37.9) | 0.768 |
| Not consulted with pediatric pulmonology, n (%) | 116 (80) | 66 (56.9) | 50 (43.1) | |
| High risk | | | | |
| Consulted with pediatric pulmonology, n (%) | 19 (14.5) | 16 (84.2) | 3 (15.8) | 0.091 |
| Not consulted with pediatric pulmonology, n (%) | 112 (85.5) | 107 (95.5) | 5 (4.5) | |

FBA: foreign body aspiration.

Pediatric pulmonologist consultation was requested in 86 (18.2%) patients. FB was detected in 50 of 86 (58%) who were consulted by a pulmonologist. Out of the 388 patients who did not receive a prior consultation from a pediatric pulmonologist, 206 were negative for FB. In terms of FB detection rate, there was no difference between individuals who sought pulmonology consultation and those who did not (58.1% vs. 46.9% respectively, p=0.059). However, when the children were categorized based on their risk levels, the incidence of FBA among children with a low risk of FBA was 42% when they received consultation from the pulmonology department; in contrast, this incidence dropped to 5.6% when a pulmonology consultation was not sought (p<0.001). In the moderate and high-risk groups, statistically significant difference in terms of the success of FB detection was not found between those who were consulted with pediatric pulmonology and those who were not (Table II).

## Discussion

Given the vague signs and symptoms seen on presentation, identifying FBA remains a diagnostic challenge. The most striking result of this study is the presence of a 51% rate of negative rigid bronchoscopy. The primary cause for this is the ongoing lack of ability to establish a clinical parameter that can consistently and reliably predict the presence of foreign objects in the airway in all cases. This study holds significant importance as a negative bronchoscopy could put patients at risk associated with general anesthesia, as well as potential complications of rigid bronchoscopy both during and after the procedure. To the best of our knowledge, this study is the first in Türkiye to assess the approach of pediatric pulmonologists in determining whether to perform rigid bronchoscopy in children with suspected FBA.

The suspicion of an FBA, particularly in pediatric patients, poses a significant challenge owing to the absence of precise and sensitive diagnostic indicators of FB. The task of developing a consistent decision-making algorithm for children who come to the emergency department with suspected FBA has been challenging due to the lack of a worldwide agreement on how to handle these patients, and the different standards of medical centers for performing rigid bronchoscopy. A missed FBA can have long-term consequences, necessitating a high level of suspicion. Bronchoscopy, on the other hand, is an intrusive procedure with potential hazards. Therefore, various scoring systems have been developed to date in an attempt to accurately predict FBA and reduce unnecessary procedures.[7-9,13] In our study, consultation by a pediatric pulmonologist was not requested in 388 (81.8%) out of 474 patients. Out of 388 patients who did not receive a prior consultation from a pulmonologist, 206 (53%) were negative for FB. Our rate of negative rigid bronchoscopy was found to be higher compared

to the literature. The literature reports a negative rate of 18% to 43% for rigid bronchoscopy, with the majority falling around 20%.[14]

In a recent study from our center, the rate of negative rigid bronchoscopy was found to be similar to ours.[8] FBA was noted in 52.1% (n=375) of 720 cases. This study was planned to create a new scoring system. The existence of FB was significantly associated with positive findings in physical examination and imaging and total FBA score. However, the patient history did not have any statistical significance in predicting positive cases. In our study, similarly, a definite history of experiencing abrupt choking while holding an object in the mouth or chewing something was collected from 204 (87.9%) and 207 (85.5%) of patients in the FB positive and negative groups, respectively.

When the children were categorized based on their risk levels, the probability of detection of FB in the low-risk group when they received consultation from the pulmonology department was 42%. In contrast, this incidence dropped to 5.6% when pulmonology consultation was not sought. Thus, the involvement of pulmonologists became apparent, particularly in infants who were in a low-risk group for FBA. We believe this is based on the clinical experience of pediatric pulmonologists. Nevertheless, the precise count of patients assessed by pulmonologists in the emergency room but not subjected to rigid bronchoscopy remains undisclosed. Therefore, the number of patients who do not undergo unnecessary rigid bronchoscopy thanks to pediatric pulmonologists is not known.

Another retrospective cohort study about scoring of FBA in children is from Israel.[12] In this study, total score based on medical history, physical examination, and chest X-ray findings were evaluated for their predictability. The study had a total of 412 children, with 154 (37.4%) diagnosed with FBA and 258 (62.6%) without FBA. The rate of negative rigid bronchoscopy was high, similar to our study. Data of children with suspected FBA who were also diagnosed with FB on rigid bronchoscopy were compared to those without FB on bronchoscopy. The findings indicated that children who were exposed to seeds/nuts, experienced stridor, had unilateral auscultatory findings (reduced breath sounds/wheezing) and displayed suggestive findings on chest X-rays (unilateral hyperinflation/atelectasis) had a notable risk of FBA. Nevertheless, cases of choking, abrupt coughing and the lack of fever and rhinorrhea did not demonstrate statistical significance in predicting FB. There were no significant changes between the groups in terms of oxygen saturation, complete blood count values and C-reactive protein levels. On the other hand, the X-ray, indicating the presence of a FBA, was statistically significant and accurate in predicting the presence of a FB.

In our study, the combination of both physical examination and radiographic findings showed higher sensitivity (94.5%) for detecting an FB. Similarly, recent studies indicate that the identification of FB is better achieved by physical examination findings and imaging results rather than relying solely on the patient's medical history. In accordance with this, the X-ray, had a strong likelihood of accurately predicting the presence of FB in one of the recent studies.[12] A recent study demonstrated that B-lines, barcode signs, pleural line anomalies, and consolidation were observed findings in lung ultrasonography of patients with FBA. The authors concluded that the value of lung ultrasound was equivalent to that of chest radiography.[15] Given the expertise of pediatric pulmonologists in this area, it is reasonable to anticipate that consulting a pulmonologist will decrease the rate of unfavorable rigid bronchoscopy outcomes.

In a very recent study, the positive predictive value of chest X-rays in children hospitalized with suspected FBA was assessed by three disciplines: pediatric pulmonology, pediatric radiology, and pediatric residents.[16] In this study, chest X-ray was found to have a high positive predictive value, as an independent

parameter, in predicting FBA in children. However, it had different predictability, when interpreted by pediatric residents, pediatric radiologists, and pediatric pulmonologists, even among physicians from the same discipline. By evaluating a chest X-ray, pediatric pulmonologists had the highest positive predictive value of diagnosing FBA, followed by pediatric residents and pediatric radiologists.

Based on a comprehensive meta-analysis of more than 1000 studies conducted in various countries between 1978-2008, children under the age of 3 constituted a minimum of 60% of the FBA cases.[17] In line with the literature, the median age of children whose rigid bronchoscopy was positive for FB was 20 months in our study. Additionally, 81.6% of these children were under the age of 3.

A greater number of cases were detected in male patients, and the overwhelming majority of aspirated foreign bodies were composed of organic matter.[17] The frequency of detection of foreign bodies was greater in the right lung compared to the left, predominantly in the mainstem bronchi.[18] Consistent with the literature, organic substances constituted the most commonly detected FB in our patients, with a higher prevalence observed in the right lung.

In our study, consistent with the literature, a cough that started after the witnessed event and subsequent rhonchi were the most common findings in children with FBA. On physical examination, breath sounds were normal in 63% and 14.2% of patients without FBA and with FBA, respectively. Decreased breath sounds and rhonchi were noted in 71% of children with FBA. FB was absent in 82.1% of patients who had a normal physical examination, while FB was present in 17.8% of patients with a normal physical examination. As a result, the patients with FBA were found less likely to have a physical examination without any respiratory findings.

The most important limitation of our study was its retrospective nature. In addition, the process of history taking and physical examination were subject to the individual interpretation of the physician and certain findings like interpretation chest X-rays by the physicians were missing.

In conclusion, this study is significant because it highlights how various disciplines contribute to FB diagnosis. Pediatric pulmonologists play a significant role in decreasing the incidence of negative rigid bronchoscopies in patients with low risk of having FB. However, further studies, especially prospective randomised controlled trials, are necessary to develop new algorithms to avoid negative bronchoscopies.

**Acknowledgements**

We would like to thank all the nursing and technical personnel in the Pulmonology Laboratory of our Pediatric Pulmonology Department for continuous support throughout the duration of this study.

**Ethical approval**

The research protocol for this study was approved by the Clinical Research Ethics Committee of Hacettepe University (date: 03.10.2023, number: SBA 23/186).

**Author contribution**

The authors confirm contribution to the paper as follows: Study conception and design: BS, NK; data collection: BS, HNB, İG, DA, MAE, HİD, RA, BCY; analysis and interpretation of results: BA, İRU, OT, EY, NE, DD, UÖ, NK; draft manuscript preparation: BS, NK 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. Mathew RP, Liang TI, Kabeer A, Patel V, Low G. Clinical presentation, diagnosis and management of aerodigestive tract foreign bodies in the paediatric population: part 2. SA J Radiol 2021; 25: 2027. https://doi.org/10.4102/sajr.v25i1.2027
2. Cataneo AJ, Cataneo DC, Ruiz RL. Management of tracheobronchial foreign body in children. Pediatr Surg Int 2008; 24: 151-156. https://doi.org/10.1007/s00383-007-2046-z
3. Heyer CM, Bollmeier ME, Rossler L, et al. Evaluation of clinical, radiologic, and laboratory prebronchoscopy findings in children with suspected foreign body aspiration. J Pediatr Surg 2006; 41: 1882-1888. https://doi.org/10.1016/j.jpedsurg.2006.06.016
4. Viot A, Babin E, Bequignon A, Moreau S, Vadillo M, Valdazo A. Bronchial foreign bodies in children. Ann Otolaryngol Chir Cervicofac 2002; 119: 174-180.
5. Righini CA, Morel N, Karkas A, et al. What is the diagnostic value of flexible bronchoscopy in the initial investigation of children with suspected foreign body aspiration? Int J Pediatr Otorhinolaryngol 2007; 71: 1383-1390. https://doi.org/10.1016/j.ijporl.2007.05.012
6. Cohen S, Avital A, Godfrey S, Gross M, Kerem E, Springer C. Suspected foreign body inhalation in children: what are the indications for bronchoscopy? J Pediatr 2009; 155: 276-280. https://doi.org/10.1016/j.jpeds.2009.02.040
7. Fasseeh NA, Elagamy OA, Gaafar AH, et al. A new scoring system and clinical algorithm for the management of suspected foreign body aspiration in children: a retrospective cohort study. Ital J Pediatr 2021; 47: 194. https://doi.org/10.1186/s13052-021-01147-9
8. Özyüksel G, Arslan UE, Boybeyi-Türer Ö, Tanyel FC, Soyer T. New scoring system to predict foreign body aspiration in children. J Pediatr Surg 2020; 55: 1663-1666. https://doi.org/10.1016/j.jpedsurg.2019.12.015
9. Janahi IA, Khan S, Chandra P, et al. A new clinical algorithm scoring for management of suspected foreign body aspiration in children. BMC Pulm Med 2017; 17: 61. https://doi.org/10.1186/s12890-017-0406-6
10. Stafler P, Nachalon Y, Stern Y, et al. Validation of a computerized scoring system for foreign body aspiration: an observational study. Pediatr Pulmonol 2020; 55: 690-696. https://doi.org/10.1002/ppul.24632
11. Pozailov S, Goldbart A, Aviram M, et al. Foreign body aspiration score (FOBAS)-a prospectively validated algorithm for the management and prediction of foreign body aspiration in children. Eur J Pediatr 2024; 183: 815-825. https://doi.org/10.1007/s00431-023-05347-9
12. Raviv I, Pozailov S, Avraham S, et al. Evaluation of foreign body aspiration score (FOBAS) in children-a retrospective cohort study. Eur J Pediatr 2023; 182: 4205-4212. https://doi.org/10.1007/s00431-023-05095-w
13. Kadmon G, Stern Y, Bron-Harlev E, Nahum E, Battat E, Schonfeld T. Computerized scoring system for the diagnosis of foreign body aspiration in children. Ann Otol Rhinol Laryngol 2008; 117: 839-843. https://doi.org/10.1177/000348940811701108
14. Cavel O, Bergeron M, Garel L, Arcand P, Froehlich P. Questioning the legitimacy of rigid bronchoscopy as a tool for establishing the diagnosis of a bronchial foreign body. Int J Pediatr Otorhinolaryngol 2012; 76: 194-201. https://doi.org/10.1016/j.ijporl.2011.11.002
15. Aydin O, Birbilen AZ, User IR, Turer OB, Teksam O. Lung ultrasound findings in children with foreign body aspiration. J Clin Ultrasound 2023; 51: 447-451. https://doi.org/10.1002/jcu.23306
16. Avraham S, Aviram M, Farkash Novik E, et al. Comparison of chest X-ray interpretation by pediatric pulmonologists, pediatric radiologists, and pediatric residents in children with suspected foreign body aspiration-a retrospective cohort study. Eur J Pediatr 2023; 182: 3101-3109. https://doi.org/10.1007/s00431-023-04943-z
17. Foltran F, Ballali S, Rodriguez H, et al. Inhaled foreign bodies in children: a global perspective on their epidemiological, clinical, and preventive aspects. Pediatr Pulmonol 2013; 48: 344-351. https://doi.org/10.1002/ppul.22701
18. Aslan N, Yıldızdaş D, Özden Ö, Yöntem A, Horoz ÖÖ, Kılıç S. Evaluation of foreign body aspiration cases in our pediatric intensive care unit: single-center experience. Turk Pediatri Ars 2019; 54: 44-48. https://doi.org/10.14744/TurkPediatriArs.2019.60251

The Turkish Journal of Pediatrics 2024; 66: 588-598
https://doi.org/10.24953/turkjpediatr.2024.4535

**Original Article**

# Adaptation of the Problem Areas in Diabetes-Teen Scale into Turkish and examination of its psychometric properties: a validity and reliability study

Seda Aybüke Sarı[1], Ezgi Agadayı[2], Nurullah Çelik[3], Seher Karahan[2], Ayça Kömürlüoğlu[4], Esra Döğer[5]

*[1]Department of Child and Adolescent Psychiatry, Hatay Mustafa Kemal University Faculty of Medicine, Hatay; [2]Department of Medical Education, Sivas Cumhuriyet University Faculty of Medicine, Sivas; [3]Department of Pediatric Endocrinology, Sivas Cumhuriyet University Faculty of Medicine, Sivas; [4]Department of Pediatrics, Sivas Cumhuriyet University Faculty of Medicine, Sivas; [5]Department of Pediatric Endocrinology, Gazi University Faculty of Medicine, Ankara, Türkiye.*

## ABSTRACT

**Objective.** Management of type 1 diabetes (T1DM) is quite challenging for both adolescents and their families. In this study, we aimed to translate the 14-item Problem Areas in Diabetes-Teen (PAID-T) scale, which measures variables that influence diabetes distress, to Turkish and investigate the Turkish version's reliability and validity.

**Methods.** One hundred and ninety-four adolescents with T1DM participated in the study. PAID-T and forms for sociodemographic and diabetes characteristics were used for data collection. The scale's content validity was checked using the Davis technique. Cronbach's $\alpha$ was used to analyze the scale's internal reliability and the test-retest for the scale's reliability. Exploratory factor analysis (EFA) was utilized to examine the factor structure. The fit of the scale was assessed using confirmatory factor analysis (CFA).

**Results.** Of the participants, 54.6% (n=106) were girls. The content validity index values of the scale items ranged between 0.86 and 1.0. The PAID-T scores of girls and boys were similar. No significant difference was found between PAID-T scores with sociodemographic data and diabetes characteristics ($p>0.05$). The test-retest correlation coefficient of the scale was found to be 0.952. The three-factor (emotional burden, family and friend distress, and regimen-specific distress) model identified in EFA explained 61.8% of the common variance. Fit analysis was performed using CFA for the three-factor model, which did not show adequate fit ($x2/df = 2.402$, GFI = 0.822, CFI = 0.815, NFI = 0.727, NNFI = 0.772, RMSEA = 0.118). The Cronbach $\alpha$ value of the scale was 0.864.

**Conclusion.** The Turkish version of the 14-item PAID-T showed moderate validity and strong reliability. Accordingly, it can be used as a reliable measurement tool to assess diabetes stress in adolescents with T1DM.

**Key words:** Type 1 diabetes, adolescents, problem areas, scale, validity, reliability.

Type 1 diabetes (T1DM) is one of the most common most common endocrinological diseases of childhood and adolescence.[1] In 2021, it was reported that the estimated prevalence of T1DM is approximately 8.4 million people worldwide, and approximately 1.5 million of these are children and young people under the age of 20.[2] T1DM, a chronic disease with mostly adolescence-onset, requires daily activities and tasks such as monitoring blood sugar at regular intervals, administering exogenous insulin, regular physical exercise, and complying with a diet.[3] During adolescence, when physical, emotional, and social changes occur, peer

✉ Seda Aybüke Sarı ▪ aybuke_sari@hotmail.com

Received 30th Mar 2024, revised 10th Jun 2024, 2nd Jul 2024, 21st Aug 2024, accepted 30th Sep 2024.

connections and identity development come to the fore, and independence grows, managing a chronic illness that entails such a tight treatment regimen can be quite challenging.[4] Adolescents with diabetes experience embarrassment, alienation, and a lack of social connections. Also, living with diabetes may have a negative impact on academic performance and family functioning.[5] While parents expect their teens to take responsibility for managing and implementing diabetes-related daily care activities, adolescents try to push the parent's boundaries, which could lead to conflict.[6]

T1DM is associated with significant mental disorders and increased psychological morbidity. Adolescents with T1DM face higher levels of psychosocial stress and adjustment issues than those without the disease, making following a strict diabetes care routine more challenging.[7] This diabetes-related emotional stress refers to the negative emotions arising from the difficulties of living with and managing diabetes.[8] It comprises the negative feelings and fears inherent in living with diabetes, such as worrying about complications, out-of-range blood sugar, not being supported by family or friends, or feeling overwhelmed by the diabetes regimen.[9] Diabetes-related emotional stress has been linked to poor glycemic control, low quality of life, increased psychiatric disorders (such as depression and anxiety disorder), and less adherence to drug regimens.[10] A systematic review conducted by Hagger et al.[11] stated that one-third of adolescents with T1DM experienced severe diabetes-related emotional stress like adults with T1DM. Thus, it is crucial to assess diabetes-related emotional stress in adolescents with T1DM, which has a significant detrimental impact on the long-term health outcomes of diabetes.

The number of standardized scales developed to identify problem areas for adolescents with T1DM is low. The novelty of the studies will be further enhanced by using a simple self-report scale for adolescents to comprehend and complete, and evaluating the difficulties and emotional burdens of living with diabetes. The Problem Areas in Diabetes- Teen version (PAID-T) scale, which was first adapted from the adult Problem Areas in Diabetes (PAID) scale by Dr. Weissberg-Benchell and her colleague[12] and shortened by Shapiro et al.[13], appears to be a very convenient measurement tool to specifically assess diabetes-related emotional stress. Furthermore, to perform cross-cultural studies in various groups and better assist their patients, clinicians require trustworthy, validated assessment instruments in their native languages and cultures.[14] In Türkiye, there is no available scale in Turkish for assessing diabetes-related emotional stress among adolescents with T1DM. Therefore, in the present study, we aimed to translate the original version of the 14-item PAID-T scale into Turkish to evaluate its validity and reliability and to examine its psychometric properties to measure diabetes-related problems in adolescents with T1DM.

## Materials and Methods

### *Design*

The data was collected between February 2021 - February 2022. Inclusion criteria include adolescents aged between 12-18 years, diagnosed with T1DM for at least one year and receiving inpatient treatment or outpatient follow-up by a pediatric endocrinologist in a tertiary hospital, and fluent in Turkish to complete questionnaires.

Patients under the age of 12 years and over the age of 18 years, having T1DM for less than one year, being illiterate, and having a serious psychiatric or neurological disease that may interfere with reading comprehension were excluded from the study. A child and adolescent psychiatrist evaluated all participants. Participants with severe depression, anxiety disorder, or a psychopathology that would impair their ability to assess reality were not included in the study.

Taking samples 5-10 times the number of items is sufficient for scale validity. The recommended sample size for a psychometric study is between 100-200 individuals, especially if the factors are strong and specific.[15,16]

### *Measurements*

*Questionnaire form:* The form prepared by the researchers consists of two parts: sociodemographic information about adolescents and their families and information about the teens' diabetes. The socio-demographic information consisted of questions regarding the adolescent's age, gender, place of residence, parents' occupation and educational background, family structure, and monthly income. The duration of diabetes, the type of diabetes treatment currently being used (insulin injection or pump), the frequency of daily glucose measurements, the current HbA1c level, the presence or absence of severe diabetes complications necessitating hospitalization, and the existence of any other medical conditions were all included in the section pertaining to diabetes.

*Problem Areas in Diabetes - Teen Scale (PAID-T):* The PAID scale was first developed in 1995 to measure the emotional stress areas specific to diabetes in adults.[17] A 26-item PAID-T was first developed by Weissberg-Benchell and Antisdel-Lomaglio[12] to evaluate adolescents with T1DM and their families by considering the developmental characteristics of adolescents. Finally, in 2018, the scale was transformed into the final version (short form) with 14 items.[13] Last year, Lee et al.[18] translated the 26-item form into Chinese and carried out a validity and reliability study. The 14-item PAID-T has been utilized in studies but has not been translated into any foreign language other than the most current German translation by Saßmann et al.[19]

PAID-T is a 6-point Likert scale consisting of 14 questions that measure diabetes-specific distress in adolescents (12 to 18 years of age) with diabetes. Participants are asked to rate on a scale of "1=not a problem'' to "6=serious problem'' how much each scale item bothered them over the past month according to their diabetes-related stress. High scores indicate a greater level of distress. The PAID-T has no subscales. There is no reverse item on the scale. The maximum score obtained from the scale is 84, and the minimum is 14. The Cronbach's $\alpha$ value of PAID-T was 0.93.[13]

### *Procedure*

We received permission to translate the English version of PAID-T from Jill Weissberg-Benchell (one of the authors) by e-mail. The World Health Organization (WHO) recommendations and the literature research on this topic were used as a guide for the intercultural adaptation of the scale.[20,21] In the first step for scale adaptation, two independent bilingual Turkish translators translated the scale from English to Turkish. After completing the translations, our research team evaluated the items of both translations in terms of whether they contained complex sentences or simple translations that would distort the item's content and prepared a joint translation. In the second step, the original text and the translation version were sent to fifteen experts in the field to calculate the content validity of the scale according to the Davis Technique.[22] They were asked to evaluate the scale items one by one as "Appropriate," (b) "Needs minor revision," (c) "Needs major revision," and (d) " Not appropriate." The "content validity index" (CVI) for each item is obtained by dividing the number of experts who marked options (a) and (b) by the total number of experts, and >0.80 is accepted as the suitability criterion for the item.

In the third step, two independent translators blindly translated the Turkish text back into English. They forwarded it to the original scale developer (Dr. Weissberg-Benchell) to confirm any meaning shift. After debating the disparities between the back and forward translations with our team, a 14-item Turkish PAID-T was ultimately authorized. As a

pilot study, 15 adolescents with T1DM were administered the Turkish version. Then, forty-three adolescents with T1DM were tested and retested on the final Turkish PAID-T at 3-week intervals. Analysis of test-retest reliability was completed. Afterward, the demographic form and the scale were applied to 194 adolescents with T1DM for the research. This study was approved by the Ethics Committee of Sivas Cumhuriyet University (date 13.01.2021, number 2021-01/23). Adolescents and their legal guardians were informed of the study and gave their written consent before the study.

### *Statistical analysis*

SPSS for Windows Version 25 package program was used for statistical analysis in the research. A normal distribution was accepted if the numerical values were in the range of -1.5 to +1.5 skewness and kurtosis values.[23] Descriptive statistical analyses of sociodemographic data and the score of the scale were calculated. The independent samples t-test was used to compare numerical data between two categorical variables; when comparing numerical data between more than two categorical variables one-way ANOVA was employed. To test the validity of the scale, the content validity study was evaluated with the Davis Technique.[22] CVI above 0.80 indicates good content validity.[24] In the context of the scale's reliability examination, test-retest reliability was used to identify the scale's consistency, and Cronbach's alpha was used to assess the scale's internal consistency. A Cronbach's alpha value above 0.80 is an indicator of strong reliability.[25] Construct validity was tested with confirmatory factor analysis. The suitability of the scale for factor analysis was evaluated with the sphericity method of Kaiser-Meyer-Olkin (KMO) and Bartlett. A KMO value above 0.6 and a significant Barlett's test indicate that the data are suitable for factor analysis.[24] Confirmatory factor analysis was calculated with the IBM SPSS Amos 20 package program. Confirmatory factor analysis results were reported with total variance values and factor loadings, X2/df, comparative fit test (CFI), goodness fit test (GFI), normed fit index (NFI), non-normed fit index-Tucker Lewis index (NNFI-TLI) and root mean square error of approximate (RMSEA) values.[26] An x2/df value of 3 and below is an excellent indicator of model fit. GFI and CFI values between 0.80-0.90 indicate that the model is suitable for a good fit, while 0.90 and above means an adequate good fit. The RMSEA value is a measure of approximate fit in the population. An RMSEA value of 0.06 and below indicates a good fit, while a value greater than 0.1 indicates a poor fit.[23] A p-value of less than 0.05 was considered for statistical significance, with a 95% confidence interval (CI).

## Results

One hundred ninety-four adolescents with T1DM participated in the study. The mean age of the adolescents was 13.6±2.4 years. The mean HbA1c was 9.1±1.9% and the mean diabetes duration was 4.0±3.5 years. Table I shows sociodemographic data of adolescents and information about their diabetes.

The mean total score obtained from the scale was 34.8±15.4 (min 14, max 97). The average of the scores given to the scale items varied between minimum 2.0±1.7 (Item 8: ''Feeling like my parents don't trust me to care for my diabetes.'') and maximum 2.9±1.8 (Item 7: ''Feeling like my friends or family act like 'diabetes police' (e.g. repetitively reminding to maintain a healty diet, checking blood sugars, not trying hard enough). The comparison of the participants' PAID-T scores with sociodemographic data and characteristics of diabetes is given in Table II. No significant relation was found between PAID-T scores with diabetes-related parameters and demographic data. As expected, PAID-T scores tended to decrease as the mother's and father's educational level, and socioeconomic status increased, but the difference was insignificant (p=0.168, p=0.353; and p=0.855 respectively).

**Table I.** Adolescents' diabetes and demographic characteristics (N=194), n (%).

| | |
|---|---|
| Age (months)* | 13.6 ±2.4 |
| Gender | |
| Female | 106 (54.6%) |
| Male | 88 (45.4%) |
| Place of residence | |
| Center | 117 (60.4%) |
| Rural | 77 (39.6%) |
| Maternal education level | |
| Primary and secondary education | 138 (71.4%) |
| High school | 33 (17.3%) |
| University and above | 23(11.3%) |
| Paternal education level | |
| Primary and secondary education | 95 (49.0%) |
| High school | 53 (27.5%) |
| University and above | 46 (23.5%) |
| Maternal employment status | |
| Employed | 26 (13.3%) |
| Unemployed | 168 (86.7%) |
| Paternal employment status | |
| Employed | 188 (96.9%) |
| Unemployed | 6 (3.1%) |
| Family structure | |
| Nuclear family | 162 (83.7%) |
| Extended family | 22 (11.2%) |
| Single parent (divorced or dead) | 10 (5.1%) |
| Monthly family income | |
| Minimum wage and below | 73 (37.8%) |
| Above minimum wage | 121 (62.2%) |
| HbA1c (%)* | 9.1 ± 1.9 |
| Duration of diabetes (months)* | 4.0 ± 3.5 |
| Presence of severe hypoglycemia in the last month (<50 mg/dl) | |
| Yes | 13 (6.7%) |
| No | 181 (93.3%) |
| Number of glucose monitoring per day | |
| 0-4 | 81 (43.5%) |
| 5-8 | 75 (40.3%) |
| >8 | 30 (16.2%) |
| Insulin administration method | |
| Pump | 8 (4.1%) |
| Pen | 186 (95.9%) |
| Additional chronic disease in teen | |
| Yes | 36 (18.8%) |
| No | 158 (81.2%) |

*Presented as mean ± standard deviation

**Table II.** The comparison of the participants' PAID-T scores with their demographic data and diabetes characteristics (N=194), mean ± SD.

| | PAID-T score | p |
|---|---|---|
| Teen gender | | |
| Female | 34.8 ± 14.6 | 0.980 |
| Male | 34.7 ± 16.4 | |
| Living in | | |
| Center | 32.6 ± 14.1 | 0.160 |
| Rural | 37.3 ± 19.0 | |
| Education level of mothers | | |
| Primary and secondary education | 35.9 ± 16.2 | 0.168 |
| High school | 29.9 ± 14.7 | |
| University and above | 28.2 ± 14.4 | |
| Education level of fathers | | |
| Primary and secondary education | 35.5 ± 17.2 | 0.353 |
| High school | 34.8 ± 13.6 | |
| University and above | 29.8 ± 15.4 | |
| Employment status of mothers | | |
| Employed | 28.4 ± 10.9 | 0.179 |
| Unemployed | 34.9 ± 16.4 | |
| Employment status of fathers | | |
| Employed | 33.9 ± 16.1 | 0.340 |
| Unemployed | 38.3 ± 6.1 | |
| Family structure | | |
| Nuclear family | 34.6 ± 16.1 | 0.520 |
| Extended family | 28.9 ± 13.5 | |
| Single ± divorced or dead | 36.0 ± 18.8 | |
| Monthly family income | | |
| Minimum wage and below | 34.4 ± 13.6 | 0.855 |
| Above than minimum wage | 33.8 ± 17.2 | |
| Presence of severe hypoglycemia in the last month ± <50 mg/dl | | |
| Yes | 40.4 ± 15.1 | 0.173 |
| No | 34.3 ± 15.4 | |
| Insulin administration method | | |
| Pump | 26.8 ± 11.6 | 0.088 |
| Pen | 35.1 ± 15.5 | |
| Number of glucose monitoring per day | | |
| 0-4 | 35.5 ± 16.0 | 0.926 |
| 5-8 | 34.6 ± 15.5 | |
| >8 | 35.4 ± 14.6 | |
| Additional chronic disease in teen | | |
| Yes | 35.2 ± 15.5 | 0.712 |
| No | 33.6 ± 16.2 | |

PAID-T, problem areas in diabetes-teen; SD, standard deviation.

We also did not find any significant correlation between the PAID-T score and the adolescent's age, duration of diabetes diagnosis, or HbA1c level (r=-0.017, p=0.819; r=0.002, p=0.984; r=0.065, p=0.480, respectively).

### *Psychometric Properties of the Turkish Version of the PAID-T*

The evaluation results of fifteen experts who reviewed the PAID-T translation into Turkish were analyzed using the Davis Technique, and the CVI was determined. As per the Davis Technique, the items' CVI scores varied from 0.86 to 1.0 (Table III).

We used test-retest analysis to assess the scale's reliability. After applying the test to 50 adolescents, we reached 43 of them again three weeks later and applied the retest. The scale's item correlation coefficients varied from 0.652 to 0.942 (all p-values <0.001). In the literature, an effect size above 0.5 is interpreted as large.[27] All items and the scale's total score had a significant positive and large effect size between the two measurements. The overall scale's correlation coefficient was calculated as 0.952.

The literature suggests that EFA and CFA analyses should be studied on different data sets.[28] We randomly divided our data set into two in the SPSS program. We calculated EFA and CFA on two different datasets.

The factor structure of the 14 items was examined using EFA with oblique rotation. Principal component analysis and direct oblimin techniques were utilized for this. Since there were three factors in the original scale, the number of factors was fixed to three. When the number of factors was also calculated based on the Eigenvalue, the results were similar for three factors. KMO = 0.833, and Bartlett's sphericity test results were $\chi 2$ = 610.864, p<0.001. EFA analysis indicated three factors accounted for 61.80% of the variance. The contents of these three factors were examined, and the factors were named emotional burden (5 items), family and friend distress (5 items), and regimen-specific distress (4 items), like Shapiro's original scale. Rotation sums of squared loadings of these factors: emotional burden = 4.1, family and friend distress= 2.2, and regimen-specific distress = 2.2. Factor loadings for the emotional burden factor ranged from 0.718 to 0.907, the family and friends distress factor ranged from 0.457 to 0.770, and the regimen-specific distress factor from -0.737 to -0.473. The factor loads in Table IV are expressed by the 3-factor model's

**Table III.** The results of CVI on PAID-T using the Davis technique, expert N=15.

| | Appropriate | Needs minor revision | Needs major revision | Not appropriate | CVI |
|---|---|---|---|---|---|
| Item 1 | 15 | 0 | 0 | 0 | 1.00 |
| Item 2 | 13 | 0 | 2 | 0 | 0.86 |
| Item 3 | 12 | 2 | 1 | 0 | 0.93 |
| Item 4 | 13 | 1 | 1 | 0 | 0.93 |
| Item 5 | 11 | 2 | 2 | 0 | 0.86 |
| Item 6 | 13 | 1 | 1 | 0 | 0.93 |
| Item 7 | 15 | 0 | 0 | 0 | 1.00 |
| Item 8 | 15 | 0 | 0 | 0 | 1.00 |
| Item 9 | 15 | 0 | 0 | 0 | 1.00 |
| Item 10 | 13 | 1 | 1 | 0 | 0.93 |
| Item 11 | 12 | 2 | 1 | 0 | 0.93 |
| Item 12 | 12 | 1 | 2 | 0 | 0.86 |
| Item 13 | 15 | 0 | 0 | 0 | 1.00 |
| Item 14 | 11 | 3 | 1 | 0 | 0.93 |

CVI, content validity index; PAID-T: problem areas in diabetes-teen.

**Table IV.** Factor loads based on the PAID-T items' pattern matrix from the exploratory factor analysis (N=194).

| | Factor 1: Emotional burden | Factor 2: Family and friends distress | Factor 3: Regimen-specific distress |
|---|---|---|---|
| Item 1 | 0.907 | | |
| Item 4 | 0.852 | | |
| Item 2 | 0.827 | | |
| Item 3 | 0.819 | | |
| Item 6 | 0.718 | | |
| Item 11 | | 0.770 | |
| Item 7 | | 0.596 | |
| Item 8 | | 0.558 | |
| Item 14 | | 0.508 | |
| Item 12 | | 0.458 | |
| Item 9 | | | -0.737 |
| Item 10 | | 0.349 | -0.722 |
| Item 5 | | | -0.711 |
| Item 13 | | | -0.473 |
| KMO | | 0.833 | |
| Bartlett's Test $\chi 2$ | | 610.864 | |
| P value | | <0.001 | |

Principal components and direct oblimin methods fixed to 3 factors, compatible with the original scale, are used.
KMO, kaiser-meyer-olkin; PAID-T, problem areas in diabetes-teen.

pattern matrix outcome. The table does not include factor loading less than 0.30. When we look at the results, item 10 "Feeling that I am often failing with my diabetes regimen'' is included in both factor 2 and factor 3. Since the item content was compatible with factor 3 (regimen-specific distress), it was assigned to factor 3.

The fit analysis of the three-factor model we determined in EFA was evaluated with CFA. The results of this three-factor and also one-factor models did not show an adequate fit (three-factor's results: x2/df = 2.402, GFI = 0.822, CFI = 0.815, NFI = 0.727, NNFI = 0.772, RMSEA = 0.118). The single-factor model's CFA was computed as follows: x2/df = 2.799, GFI = 0.735, CFI = 0.752, NFI = 0.668, RMSEA = 0.139.

The Cronbach $\alpha$ value of the Turkish PAID-T, which tested the scale's internal reliability, was calculated at 0.864. The range of the item-total correlation coefficients was 0.388 to 0.653. All items' $\alpha$ value was greater than 0.80, suggesting that the scale's internal consistency and reliability were appropriate.[25] Table V displays the results of the scale's internal reliability analysis.

**Table V.** Cronbach reliability analysis of the Turkish version of PAID-T.

| | Item total correlation | Cronbach's $\alpha$ if the item deleted |
|---|---|---|
| Item 1 | 0.577 | 0.852 |
| Item 2 | 0.636 | 0.849 |
| Item 3 | 0.608 | 0.850 |
| Item 4 | 0.653 | 0.849 |
| Item 5 | 0.388 | 0.861 |
| Item 6 | 0.423 | 0.873 |
| Item 7 | 0.592 | 0.851 |
| Item 8 | 0.423 | 0.860 |
| Item 9 | 0.544 | 0.854 |
| Item 10 | 0.575 | 0.853 |
| Item 11 | 0.538 | 0.854 |
| Item 12 | 0.568 | 0.852 |
| Item 13 | 0.509 | 0.855 |
| Item 14 | 0.482 | 0.857 |

PAID-T, problem areas in diabetes-teen.

## Discussion

In this study, we performed a Turkish validity and reliability study of the 14-item PAID-T scale to assess diabetes-related emotional stress areas (such as diabetes management and emotional burdens of living with diabetes) experienced by Turkish adolescents with T1DM. We aimed to introduce the scale to the Turkish medical literature and make sure that the scale is easily accessible and used by healthcare professionals who work in this field to assess patients during routine clinical practice.

In our study, the mean age was lower than the studies by Shapiro et al.[13] and Saßmann et al.[19]; the proportion of girls to boys was higher, similar that by Shapiro et al. Unlike the other two validity studies, the total PAID-T scores of boys and girls were very close to each other with no significant difference. The lack of a significant correlation between the gender variable and PAID-T score could be attributed to several factors, including a smaller sample size than in the other two studies, the inclusion of adolescents from a single ethnic origin and similar sociocultural level, a younger average age of adolescents (since disease awareness rises with age, female adolescents may be more aware of it than male adolescents), and the absence of psychopathology in the sample. Several studies investigating diabetes-related emotional stress in adolescents have demonstrated that female adolescents scored significantly higher on the scales and reported higher levels of stress than males.[3,7,11,29,30] Similar to our results, in the review of Hagger et al.[11], a significant difference was found in only 3 of 14 studies examining the relationship between the gender variable and diabetes-related emotional stress, while no significant difference was found in 11 studies. Girls with T1DM reported more severe depressive symptoms and anxiety than boys. Several studies reported that adolescents with depressive symptoms and anxiety were found to have higher diabetes-related emotional stress and girls were more likely to be psychosocially vulnerable than boys.[3,11,13,19,29]

When comparing PAID-T scores with sociodemographic information and diabetes features, we could not find any significant differences. Although the PAID-T scores of adolescents living in rural areas, parents with lower educational status, unemployed parents, low family income, living in divorced families, using insulin injections, and having additional chronic diseases were higher than those without, but statistically insignificant. This could potentially be attributed to the limited size of our sample. To date, most of the studies measuring diabetes stress in adolescents have mostly evaluated race/ethnicity, age and gender as demographic variables, and parameters such as parental education, employment status, and family structure have not been examined.[3,7,31] Vesco et al.[31] examined the relationship between the use of technological methods for treatment and follow-up with diabetes stress and HbA1c level in adolescents with T1DM and evaluated diabetes stress with the 14-item PAID-T. They discovered that diabetic stress had a negative correlation with poor family income and low socioeconomic status and a positive correlation with age. They also showed that those who used continuous subcutaneous insulin infusion (pump) had a significantly lower PAID-T score than those who did not (insulin injection group).

We also found no significant relationship between PAID-T scores, duration of diabetes, number of daily glucose checks, and HbA1c levels. While Shapiro et al.[13] did not find a significant difference between the age and duration of diabetes and PAID-T scores of adolescents in the STePS study, they found a significant difference in the camp study. Accordingly, older adolescents with long-term diabetes reported significantly higher distress than younger adolescents with shorter durations of diabetes. In contrast to our findings, Saßmann et al.[19] found a positive correlation between PAID-T score and HbA1c level and a negative correlation with the number of daily glucose measurements. Although they did not find a significant correlation between the insulin administration method and PAID-T

score as we did, they found a small positive correlation between serious complications and PAID-T scores. Polish authors translated the English 14-item PAID-T into Polish and applied it to adolescents (without conducting a validity and reliability study), and similar to our research, they did not find a significant correlation between PAID scores with HbA1c levels and duration of diabetes.[30] In a recent study using the 14-item PAID-T, PAID-T scores were significantly associated with HbA1c levels, whereas no significant association was found with age and duration of diabetes.[7]

As determined by the Davis Technique, the Turkish PAID-T scale items' CVI varied from 0.86 to 1.00, which means the scale has sufficient content validity.[24] The Turkish PAID-T's reliability was assessed by test-retest correlation, item-total correlation, and Cronbach $\alpha$ analyses. The scale items' correlation coefficients ranged from 0.652 to 0.942 based on test-retest results. The overall scale's test-retest reliability was determined to be 0.952. We found that the item-total correlation coefficients ranged from 0.388 to 0.653. Saßmann et al.[19] found that each item to total score correlation ranged from 0.69 to 0.53. A value of 0.30 and above in the item-total correlation analysis indicates high item discrimination rates, which our results confirm.[24] Cronbach's alpha value for Turkish PAID-T was calculated at 0.864, which was 0.93 in the stuy by Shapiro et al.[13] and 0.91 in that by Saßmann et al.[19] Accordingly, all of the items of the Turkish PAID-T were above 0.80, indicating strong reliability.[25]

In the study by Shapiro et al.[13], a three-factor structure was identified in EFA, and these factors were named emotional burden, family and friends' distress, and regimen-specific distress. These three factors explained 64.3% of the total variance and provided an acceptable fit. In the German adaptation of the scale, Saßmann et al.[19] tried the three-factor model like Shapiro et al. and also found that it provided an adequate fit. We tested the adequacy of the sample size to perform factor analysis with KMO and Bartlett's sphericity test. Accordingly, we had a large enough sample size to do the factor analysis. Of the total variation, 61.80% was explained by the 3-factor model, according to the EFA results. We named the 3-factor structure an emotional burden, family and friends' distress, and regimen-specific distress, similar to Shapiro et al.'s[13] and Saßmann et al.'s[19] study. In our EFA results, the factors to which the items were assigned were similar to Saßmann's study. We applied CFA to test the model fit of this 3-factor structure. Our x2/df value is an excellent indicator of model fit. Our GFI and CFI values indicate that the model is suitable for a good fit. The RMSEA value is a measure of approximate fit in the population. In our results, the RMSEA value was above 0.1. Since the GFI and CFI values were also on the borderline, we interpreted that the three-factor model did not fit well. Therefore, as Shapiro et al.[13] suggested, we recommend disregarding sub-factors to measure diabetes stress but taking the total scale score as the basis.

### *Strengths and Limitations*

The important limitation of our study is its cross-sectional, single-center design, and the relatively small number of participants. Therefore, our findings do not reflect all adolescents with T1DM in Turkey. There is a need for further multicenter studies with large samples on this subject. Another important limitation is that in both the original scale study and the translation study, the authors had adolescents fill out additional scales (quality of life, depression, and anxiety scales, etc.) in addition to the PAID-T, whereas we did not provide other scales in our study. Instead, we only conducted face-to-face psychiatric interviews and excluded children with a psychopathology.

On the other hand, the strengths of our study are that we included adolescents with T1DM from all socioeconomic levels as much as possible. We questioned sociodemographic data and information about diabetes with face-to-face interviews and had the adolescents fill out the PAID-T in a face-to-face setting. Simultaneously with the questionnaires, we analyzed HbA1c

levels. Our study's strongest aspect is that, aside from a very recent German adaptation, it is, as far as we know, the second validity and reliability analysis of the original 14-item PAID-T in a foreign language. In this regard, we believe our research will significantly contribute to the field of pediatric diabetes, especially in the Turkish medical literature.

## Conclusion

Approximately one-third of adolescents with T1DM experience significant levels of diabetes distress. Diabetes distress in adolescents with T1DM is associated with increased psychiatric disorders, poor glycemic control, decreased quality of life, and suboptimal diabetes outcomes. Due to the possible detrimental impacts on the prognosis of the disease, specialists working in pediatric endocrinology should regularly monitor distress in adolescents with T1DM. The 14-item Turkish PAID-T showed significant reliability and moderate validity. The Turkish PAID-T can be utilized by physicians and nurses who specialize in pediatric endocrinology in Türkiye to assess teenagers with T1DM, spot potential issues, and implement the required treatments.

## Acknowledgement

The authors would like to thank Dr. Jill Weissberg-Benchel for giving permission to translate the 14-item PAID-T scale into Turkish.

## Ethical approval

This study was approved by the Ethics Committee of Sivas Cumhuriyet University (date 13.01.2021, number 2021-01/23).

## Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: SAS, EA, NÇ; Data collection: SAS, NÇ, AK; Analysis and interpretation of results: SAS, EA, SK, ED; Draft manuscript preparation: SAS, EA. 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. American Diabetes Association. 13. Children and Adolescents: Standards of Medical Care in Diabetes-2019. Diabetes Care 2019; 42(Suppl 1): S148-S164. https://doi.org/10.2337/dc19-S013
2. Gregory GA, Robinson TIG, Linklater SE, et al. Global incidence, prevalence, and mortality of type 1 diabetes in 2021 with projection to 2040: a modelling study. Lancet Diabetes Endocrinol 2022; 10: 741-760. https://doi.org/10.1016/S2213-8587(22)00218-2
3. Inverso H, LeStourgeon LM, Parmar A, et al. Demographic and glycemic factors linked with diabetes distress in teens with type 1 diabetes. J Pediatr Psychol 2022; 47: 1081-1089. https://doi.org/10.1093/jpepsy/jsac049
4. Datye K, Bonnet K, Schlundt D, Jaser S. Experiences of adolescents and emerging adults living with type 1 diabetes. Diabetes Educ 2019; 45: 194-202. https://doi.org/10.1177/0145721718825342
5. Delamater AM, de Wit M, McDarby V, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Psychological care of children and adolescents with type 1 diabetes. Pediatr Diabetes 2018; 19(Suppl 27): 237-249. https://doi.org/10.1111/pedi.12736
6. Lohiya NN, Kajale NA, Lohiya NN, Khadilkar VV, Gondhalekar K, Khadilkar A. Diabetes distress in Indian children with type 1 diabetes mellitus and their mothers. J Pediatr Endocrinol Metab 2020; 34: 209-216. https://doi.org/10.1515/jpem-2020-0339
7. Hong KMC, Glick BA, Kamboj MK, Hoffman RP. Glycemic control, depression, diabetes distress among adolescents with type 1 diabetes: effects of sex, race, insurance, and obesity. Acta Diabetol 2021; 58: 1627-1635. https://doi.org/10.1007/s00592-021-01768-w

8. Esbitt SA, Tanenbaum ML, Gonzalez JS. Disentangling clinical depression from diabetes-specific distress: making sense of the mess we've made. In: Lloyd CE, Pouwer F, Hermanns N, editors. Screening for depression and other psychological problems in diabetes: A practical guide. Springer-Verlag Publishing/Springer Nature; 2013: 27-46.

9. Fisher L, Polonsky WH, Hessler D. Addressing diabetes distress in clinical care: a practical guide. Diabet Med 2019; 36: 803-812. https://doi.org/10.1111/dme.13967

10. Beverly EA, Rennie RG, Guseman EH, Rodgers A, Healy AM. High prevalence of diabetes distress in a university population. J Am Osteopath Assoc 2019; 119: 556-568. https://doi.org/10.7556/jaoa.2019.099

11. Hagger V, Hendrieckx C, Sturt J, Skinner TC, Speight J. Diabetes distress among adolescents with type 1 diabetes: a systematic review. Curr Diab Rep 2016; 16: 9. https://doi.org/10.1007/s11892-015-0694-2

12. Weissberg-Benchell J, Antisdel-Lomaglio J. Diabetes-specific emotional distress among adolescents: feasibility, reliability, and validity of the problem areas in diabetes-teen version. Pediatr Diabetes 2011; 12: 341-344. https://doi.org/10.1111/j.1399-5448.2010.00720.x

13. Shapiro JB, Vesco AT, Weil LEG, Evans MA, Hood KK, Weissberg-Benchell J. Psychometric properties of the problem areas in diabetes: teen and parent of teen versions. J Pediatr Psychol 2018; 43: 561-571. https://doi.org/10.1093/jpepsy/jsx146

14. Sousa VD, Rojjanasrirat W. Translation, adaptation and validation of instruments or scales for use in cross-cultural health care research: a clear and user-friendly guideline. J Eval Clin Pract 2011; 17: 268-274. https://doi.org/10.1111/j.1365-2753.2010.01434.x

15. Tavşancıl E. Tutumların ölçülmesi ve SPSS ile veri analizi. 6th ed. Nobel Tıp; 2018.

16. Büyüköztürk Ş. Factor analysis: basic concepts and using to development scale. Educational Administration in Theory & Practice 2002; 32: 470-483.

17. Polonsky WH, Anderson BJ, Lohrer PA, et al. Assessment of diabetes-related distress. Diabetes Care 1995; 18: 754-760. https://doi.org/10.2337/diacare.18.6.754

18. Lee SL, Wu LM, Chou YY, Lai FC, Lin SY. Developing the Chinese version problem areas in diabetes-teen for measuring diabetes distress in adolescents with type 1 diabetes. J Pediatr Nurs 2022; 64: 143-150. https://doi.org/10.1016/j.pedn.2022.02.011

19. Saßmann H, Kim-Dorner SJ, Framme J, et al. Psychometric properties of the German teen and parent versions of the Problem Areas in Diabetes Scale (PAID). Psychol Assess 2023; 35: e31-e42. https://doi.org/10.1037/pas0001243

20. World Health Organization (WHO). Process of translation and adaptation of instruments. 2009. https://web.archive.org/web/20150710041316/http://www.who.int/substance_abuse/research_tools/translation/en/

21. Çapık C, Gözüm S, Aksayan S. Intercultural scale adaptation stages, language and culture adaptation: updated guideline. Florence Nightingale J Nurs 2018; 26: 199-210. https://doi.org/10.26650/FNJN397481

22. Davis LL. Instrument review: getting the most from a panel of experts. Appl Nurs Res 1992; 5: 194-197. https://doi.org/10.1016/S0897-1897(05)80008-4

23. Tabachnick BG, Fidell LS. Using multivariate statistics. 4th ed. Boston: Ally and Bacon; 2001.

24. Karagöz Y, Köstereliođlu İ. Developing evaluation scale of communication skills with factor analysis. Dumlupınar University Journal of Social Sciences 2008; 21: 81-98.

25. Tavakol M, Dennick R. Making sense of Cronbach's alpha. Int J Med Educ 2011; 2: 53-55. https://doi.org/10.5116/ijme.4dfb.8dfd

26. Brown TA. Confirmatory factor analysis for applied research. Guilford Press; 2015.

27. Cohen J. A power primer. Psychol Bull 1992; 112: 155-159. https://doi.org/10.1037//0033-2909.112.1.155

28. Schumacker RE, Lomax RG. A beginner's guide to structural equation modeling. 3rd ed. New York, NY: Routledge; 2010.

29. Hagger V, Hendrieckx C, Cameron F, Pouwer F, Skinner TC, Speight J. Diabetes distress is more strongly associated with HbA1c than depressive symptoms in adolescents with type 1 diabetes: results from Diabetes MILES Youth-Australia. Pediatr Diabetes 2018; 19: 840-847. https://doi.org/10.1111/pedi.12641

30. Mianowska B, Fedorczak A, Michalak A, et al. Diabetes related distress in children with type 1 diabetes before and during the COVID-19 lockdown in spring 2020. Int J Environ Res Public Health 2021; 18: 8527. https://doi.org/10.3390/ijerph18168527

31. Vesco AT, Jedraszko AM, Garza KP, Weissberg-Benchell J. Continuous glucose monitoring associated with less diabetes-specific emotional distress and lower A1c among adolescents with type 1 diabetes. J Diabetes Sci Technol 2018; 12: 792-799. https://doi.org/10.1177/1932296818766381

The Turkish Journal of Pediatrics 2024; 66: 599-607
https://doi.org/10.24953/turkjpediatr.2024.4797

**Original Article**

# Exploring the predictive factors in the gastrointestinal involvement of patients with immunoglobulin A vasculitis

Betül Öksel[1], Nihal Şahin[1], Hafize Emine Sönmez[1]

[1]*Department of Pediatrics, Department of Pediatric Rheumatology, Faculty of Medicine, Kocaeli University, Kocaeli, Türkiye.*

## ABSTRACT

**Background.** Immunoglobulin A vasculitis (IgAV), the most common systemic vasculitis in children, typically presents with gastrointestinal (GI) symptoms in about half of cases. This study aimed to analyze the clinical and laboratory findings of patients with IgAV regarding GI involvement.

**Methods.** We compared the GI involvement data of the patients diagnosed with IgAV.

**Results.** Of the 210 patients (60.5% female and 39.5% male), 101 had GI involvement, with abdominal pain being the predominant symptom (n=98). White blood cell, neutrophil, monocyte, and platelet counts, C-reactive protein, neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR), systemic immune-inflammation index (SII), and systemic inflammation response index (SIRI) were significantly elevated in patients with GI involvement ($p<0.001$, $p<0.001$, $p=0.01$, $p=0.005$, $p=0.002$, $p<0.001$, $p=0.03$, $p=0.001$, $p<0.001$, $p<0.001$, respectively). The cutoff values for SII (>1035.7), SIRI (>1.65), NLR (>2.73), and MLR (>0.28) were determined, yielding respective sensitivities of 46%, 59%, 47%, and 53%, specificities of 83.1%, 69.1%, 81.3%, and 71.9%. Corresponding areas under the curve were 0.658, 0.668, 0.649, and 0.634, respectively (all $p<0.001$).

**Conclusion.** Although IgAV is a self-limiting disease, GI involvement can lead to serious consequences. Systemic inflammatory indices such as SII and SIRI may be indicative in identifying patients with GI involvement.

**Key words:** immunoglobulin A vasculitis, gastrointestinal involvement, Henoch-Schönlein purpura.

Immunoglobulin A vasculitis (IgAV), formerly known as Henoch-Schönlein Purpura (HSP), is the most common systemic vasculitis in children with an incidence ranging from 3 to 27 per 100,000.[1] The disease is characterized by the deposition of immunoglobulin A (IgA) containing immune complexes and complement components in the small vessel walls.[1,2] While it can occur at any age, it's more commonly seen in childhood with the highest frequency between the ages of 4 and 6.[3] Diagnosis is made based on classical signs and symptoms.[4] For the diagnosis of IgAV, the mandatory criterion was purpura or petechiae that were prominent on the lower extremities and without thrombocytopenia or coagulopathy. Other criteria included abdominal pain, arthritis (acute in any joint), arthralgia, renal involvement (proteinuria, hematuria), and the presence of IgA deposition demonstrated by biopsy criteria.

Gastrointestinal (GI) symptoms are present in approximately half of the cases of IgAV. A spectrum of clinical presentations is observed, ranging from mild symptoms such as nausea,

✉ Betül Öksel ▪ betul.oksel@kocaeli.edu.tr

Received 31st May 2024, revised 23rd Aug 2024, accepted 30th Sep 2024.

The study was presented as an oral presentation at the 3rd Panoramic View to Rheumatology Symposium, in May 2024 in Sakarya, Türkiye.

abdominal pain, and vomiting to more severe findings including GI bleeding, intestinal ischemia, and intussusception.[5] In prior studies, various parameters including lymphocyte count, neutrophil count, platelet count, hemoglobin level, mean platelet volume (MPV), neutrophil-to-lymphocyte ratio (NLR), and mean platelet volume-to-platelet count ratio (MPR) have been assessed in patients exhibiting GI system (GIS) involvement.[6-8] Accordingly, inflammatory markers such as NLR, MLR, PLR, systemic inflammation response index (SIRI), and systemic immune-inflammation index (SII) have emerged as valuable predictors of vascular diseases.[6,9,10] Particularly in the context of cardiovascular pathologies, oncological pathologies, chronic kidney disease, and more recently in COVID-19 patients, these markers have demonstrated their predictive potential.[11,12] In our study, we have further investigated the predictive role of systemic inflammatory indices such as SII and SIRI regarding GIS involvement. This study aims to evaluate the clinical and laboratory features of patients with IgAV and assess the role of laboratory parameters in predicting the course of GIS involvement.

## Materials and Methods

This cross-sectional retrospective study was conducted between August 2020 and January 2024. A total of 211 patients aged between 0-18 with a diagnosis of IgAV were included. The diagnosis of IgAV was determined using the Ankara 2008 criteria endorsed by the European League Against Rheumatism, Pediatric Rheumatology International Trials Organization, and Pediatric Rheumatology European Society.[4]

Demographic information (age, gender, age at diagnosis), clinical findings (skin, musculoskeletal system, gastrointestinal system, renal, neurological, pulmonary involvement), laboratory findings (white blood cell count [WBC], absolute neutrophil count, absolute lymphocyte count, absolute monocyte count, hemoglobin level, platelet count, C-reactive protein [CRP] level, albumin level, complement 3 [C3] and complement 4 [C4] levels, anti-dsDNA, extractable nuclear antigen [ENA] panel, anti-nuclear antibody [ANA] values, and results of *MEFV* [Mediterranean FeVer] gene analysis) were retrospectively reviewed and recorded from patients' files. Systemic involvements were assessed up until the last visit of the patients.

Abdominal involvement was defined as the presence of any of the following symptoms: 1) abdominal pain, 2) vomiting, 3) hematemesis, 4) melena, 5) hematochezia, and 6) intussusception.

Patients were considered to have renal involvement if they exhibited at least one of the following findings: 1) hematuria (>5 red blood cells/high power field), 2) red blood cell casts in urinary sediment, 3) nephritic proteinuria (4-40 mg/m$^2$/hour), 4) hypertension (blood pressures ≥95th percentile for gender, age, and height), 5) nephrotic proteinuria (>40 mg/m$^2$/hour), 6) renal insufficiency.

Patients were classified as experiencing a relapse if they developed a new disease-related symptom after a period of at least 3 months without any symptoms.[13]

The NLR is determined by dividing the neutrophil count by the lymphocyte count, whereas the PLR is calculated by dividing the platelet (PLT) count by the lymphocyte count. Moreover, the MLR is derived by dividing the monocyte count by the lymphocyte count. The SII is computed by multiplying the neutrophil count by the platelet count and then dividing it by the lymphocyte count. Similarly, the SIRI is obtained by multiplying the neutrophil count by the monocyte count and then dividing it by the lymphocyte count. The study was approved by the Kocaeli University Ethics Committee.

### *Statistical analysis*

The database was constructed using SPSS 29.0 (IBM Corp., Armonk, NY, USA) and MedCalc 14.0 (MedCalc Software, Ostend, Belgium). The adequacy of the variables'

normal distribution was assessed both visually (through histograms and probability plots) and analytically (Kolmogorov-Smirnov Shapiro-Wilk tests). Quantitative data were presented as means ± standard deviation or median (range). Categorical variables were compared using either the chi-square test or Fisher's exact test. Continuous data were compared using either the Student's t-test or the Mann-Whitney U test. ROC analysis was employed to determine the discerning threshold (cut-off) values among significant parameters for GIS involvement in patients with IgAV. Area under the curve (AUC), sensitivity, specificity, and cut-off values were calculated by the receiver operating characteristic (ROC) analysis. Results were deemed statistically significant when the p-value was less than 0.05 (two-tailed).

## Results

### *Baseline characteristics of patients*

A total of 211 patients diagnosed with IgAV were screened. One patient was excluded from the study due to a concomitant diagnosis of hemolytic anemia. Furthermore, three patients were also omitted from the study due to the lack of baseline laboratory parameters. Finally, data from 207 patients were analyzed. Of these patients, 60.5% were female (n=127) and 39.5% were male (n=83). The median age at diagnosis for patients was 78.5 months (range: 19-202 months). Among them, 34.8% (n=73) presented in autumn, 28.6% (n=60) in winter, 21.9% (n=46) in spring, and 14.8% (n=31) in summer. Within the four weeks preceding the onset of the disease, 108 patients (51.4%) had a history of upper respiratory tract (URT) infection, while 8 patients (3.8%) had a history of acute gastroenteritis (AGE).

Baseline clinical findings are given in Table I. All patients presented with purpuric rashes, with bullae observed in 3 patients (1.4%). In 19.1% of the patients (n=41), only cutaneous involvement was noted. Gastrointestinal involvement was present in 48.1% (n=101), and renal involvement was detected in 16.7% (n=35) of cases. The most common symptom related to GIS involvement was abdominal pain in 98 patients (46.7%), followed by nausea and vomiting in 37 patients (17.6%), bowel wall edema in 23 patients (11%), intussusception in 8 patients (3.8%), hematochezia in 7 patients (3.3%), and melena in 1 patient (0.5%). No cases of perforation were identified.

Upon evaluation for renal involvement, 2 patients (1%) were found to have hypertension, while 25 patients had proteinuria. Among these patients, 19 (9%) exhibited proteinuria at nephritic levels, and 6 (2.9%) had proteinuria at nephrotic levels. Hematuria was detected in 15 patients, with microscopic hematuria in 13 patients (6.2%) and macroscopic hematuria in 2 patients (1%).

Five patients were diagnosed with relapsing HSP. One patient presented with purpuric rash and nephritic-level proteinuria six months after the initial diagnosis. During follow-up, her nephritic proteinuria regressed within six months. The remaining four patients presented solely with purpuric rashes at 7 months, 1 year, 6 years, and 8 years after the initial diagnosis, respectively.

The baseline laboratory parameters are summarized in Table II. Out of 39 patients, *MEFV* analysis was conducted. Among them, 3 patients carried the E148Q heterozygous variant, 2 had the V726A heterozygous variant, 2 had the M680I heterozygous variant, and 1 had the M694V heterozygous variant. One patient had the R408Q/P369S compound heterozygous mutation, and 1 patient had the M694V homozygous mutation.

### *Assessing the predicting factors in gastrointestinal involvement*

When comparing patients with and without GIS involvement, there were no significant differences observed regarding gender and the season of presentation (Table I). In terms of clinical findings other than arthralgia, there

**Table I.** Comparing clinical findings among patients with immunoglobulin A vasculitis based on gastrointestinal system involvement

| Clinical and demographic findings | All patients (n=207), n (%) | Patients with GIS involvement (n=100), n (%) | Patients without GIS involvement (n=107), n (%) | p values |
|---|---|---|---|---|
| Gender | | | | 0.77 |
| Female | 127 (60.5) | 60 (59.4) | 67 (61.5) | |
| Male | 83 (39.5) | 41 (40.6) | 42 (38.5) | |
| Seasonal pattern | | | | 0.2 |
| Spring | 46 (21.9) | 23 (22.8) | 23 (21.1) | |
| Summer | 31 (14.7) | 17 (16.8) | 14 (12.8) | |
| Fall | 73 (34.8) | 39 (38.6) | 34 (31.2) | |
| Winter | 60 (28.6) | 22 (21.8) | 38 (34.9) | |
| History of infection for the last 4 weeks | | | | |
| URT infection | 108 (51.4) | 36 (35.6) | 72 (66.1) | **<0.001** |
| AGE | 8 (3.4) | 7(6.9) | 1(0.9) | **0.03** |
| Location of skin lesions | | | | |
| Buttocks and lower extremities alone | 194 (92.4) | 92 (91.1) | 102 (93.6) | 0.67 |
| Buttocks, lower and upper extremities, and trunk | 16 (7.6) | 9 (8.9) | 7 (6.4) | 0.67 |
| General | 9 (4.3) | 5 (5) | 4 (3.7) | 0.64 |
| Joint manifestations | | | | |
| Arthralgia | 102 (48.6) | 37 (36.6) | 65 (59.6) | **0.001** |
| Arthritis | 58 (27.6) | 23 (22.8) | 35 (32.1) | 0.16 |
| Hand-foot dorsum edema | 91 (43.3) | 39 (38.6) | 52 (47.7) | 0.21 |
| Renal involvement | | | | 0.10 |
| Nephritic proteinuria | 19 (9) | 13 (12.9) | 6 (5.5) | |
| Nephrotic proteinuria | 6 (2.9) | 4 (4) | 2 (1.8) | |
| No proteinuria | 185 (88.1) | 84 (83.1) | 101 (92.7) | |
| Renal involvement | | | | 0.33 |
| Microscopic hematuria | 13 (6.2) | 6 (5.9) | 7 (6.4) | |
| Macroscopic hematuria | 2 (1) | 2 (2) | 0 (0) | |
| No hematuria | 195 (92.8) | 93 (92.1) | 102 (93.6) | |

AGE, acute gastroenteritis; GIS, gastrointestinal system; URT, upper respiratory tract infection.

were no differences. Patients without GIS involvement showed a significantly higher frequency of arthralgia (p=0.001). There was no association between the presence of proteinuria and/or hematuria and GIS involvement.

Regarding laboratory findings, patients with GIS involvement showed elevated levels of WBC, absolute neutrophil count, monocyte count, platelet count, and CRP (p<0.001, p<0.001, p=0.01, p=0.005, p=0.002, respectively). Moreover, parameters such as NLR, PLR, MLR, SII, and SIRI were also significantly higher in patients with GIS involvement (p<0.001, p=0.03, p=0.001, p<0.001, p<0.001, respectively). The capacity of NLR, PLR, MLR, SII, and SIRI to foresee the GIS involvement was analyzed using the ROC curve. The best cut-off value for SII level was >1035.7 (sensitivity 46%, specificity 83.18%, AUC=0.658, 95% confidence interval

**Table II.** Comparing laboratory parameters among patients with immunoglobulin A vasculitis based on gastrointestinal system involvement

| Laboratory parameters | All patients (n=207) | Patients with GIS involvement (n=100) | Patients without GIS involvement (n=107) | p values |
|---|---|---|---|---|
| White blood cell, ×10³/μL | 10.38 (4.10-36.12) | 11.81 (4.10–36.12) | 9.5 (4.81–21.74) | **<0.001** |
| Neutrophil count, ×10³/μL | 5.97 (1.43-33.38) | 7.0 (1.76-33.88) | 5.2(1.43-16.17) | **<0.001** |
| Lymphocyte count, ×10³/μL | 2.9 (1.00-11.41) | 2.74 (1.00-11.41) | 3.08(1.43-7.5) | 0.14 |
| Monocyte count, ×10³/μL | 0.74 (0.12-2.20) | 0.84 (0.12-2.1) | 0.71 (0.26-2.2) | **0.01** |
| Hemoglobin, g/dL | 12.37 ± 1.32 | 12.52 ± 1.44 | 12.23 ± 1.20 | 0.12 |
| Platelet count, × 10³/μL | 373 (194-681) | 406 (194-681) | 356 (197-681) | **0.005** |
| NLR, % | 2.00 (0.28-20.79) | 2.47 (0.6-20.79) | 1.72 (0.28-6.46) | **<0.001** |
| PLR, % | 126.61 (49.3-536.2) | 132.9 (49.3-536.2) | 116.88 (50.7-250.3) | **0.03** |
| MLR, % | 0.24(0.1-0.7) | 0.28(0.1-0.7) | 0.22(0.1-0.7) | **0.001** |
| C-reactive protein, mg/L | 7.4 (0.3-133) | 10.1 (0.3-133) | 5.45 (0.3-71.8) | **0.002** |
| Albumin, g/dL | 4.28 (2.50-6.52) | 4.2 (2.5-5.01) | 4.3 (3.5-6.52) | **0.02** |
| C3, g/L (n=77) | 1.34 ± 0.24 | 1.31 ± 0.26 | 1.36 ± 0.21 | 0.35 |
| C4, g/L (n=77) | 0.25 (0.12-0.43) | 0.25 (0.12-0.41) | 0.22 (0.12-0.43) | 0.9 |
| SII | 738.02 (72.5-13219.4) | 924.14 (196.7-13219.4) | 630.74 (72.5-3590.8) | **<0.001** |
| SIRI | 1.45 (0.1-15.7) | 1.97 (0.2-15.7) | 1.14 (0.1-9.4) | **<0.001** |

Data presented as mean ± standard deviation (for hemoglobin and C3, normally-distributing data), or median (min-max). C3, C3 complement; C4, C4 complement; GIS, gastrointestinal system; MLR, monocyte/lymphocyte ratio; NLR, neutrophil/ lymphocyte ratio; PLR, platelet/lymphocyte ratio; SII, neutrophil x platelet/ lymphocyte count; SIRI, neutrophil x monocyte/ lymphocyte count.

[CI] 0.589-0.723, p<0.0001), for SIRI was > 1.65 (sensitivity 59%, specificity 69.16%, AUC=0.668, CI 0.599-0.732, p<0.0001), for NLR was > 2.73 (sensitivity 47%, specificity 81.31%, AUC=0.649, CI 0.580-0.714, p=0.0001), for MLR was >0.28 (sensitivity 53%, specificity 71.96%, AUC=0.634, CI 0.564-0.699, p=0.0007) (Fig. 1).

Mild patients were treated with non-steroid anti-inflammatory drugs (NSAIDs), while those who did not respond to NSAIDs or required hospitalization received steroids. Among 101 patients with GIS involvement, 75 (74.3%) were treated with steroids, and the remaining 26 (25.7%) used only NSAIDs. The parameters were compared between patients treated with steroids and those not treated with steroids (Table III). Only SIRI was found to be significantly elevated in patients treated with steroids (2.26 vs. 1.51, p=0.02).

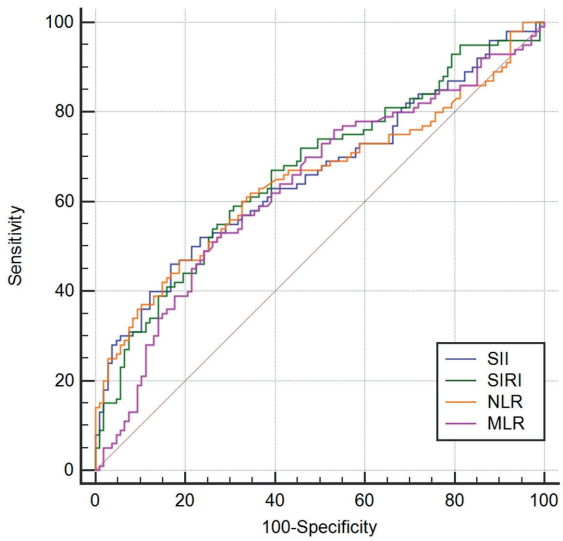



**Fig. 1.** Receiver operating characteristic curve of laboratory parameters. The best cut-off values were as follows: For SII level, >1035.7 (area under the curve [AUC] = 0.658); for SIRI, >1.65 (AUC = 0.668); for NLR, >2.73 (AUC = 0.649); and for MLR, >0.28 (AUC = 0.634).

MLR, monocyte/lymphocyte ratio; NLR, neutrophil/ lymphocyte ratio; SII, neutrophil x platelet/ lymphocyte count; SIRI, neutrophil x monocyte/ lymphocyte count.

**Table III.** Comparing parameters among patients treated with steroids and those not treated with steroids

| Laboratory parameters | Patients treated with steroids (n=75) | Patients treated without steroids (n=26) | P values |
|---|---|---|---|
| White blood cell, ×10³/μL | 12.22 (4.1-36.12) | 9.89 (4.77-19.0) | 0.06 |
| Neutrophil count, ×10³/μL | 7.82 (1.83-33.88) | 6.39 (1.76-16.06) | 0.09 |
| Lymphocyte count, ×10³/μL | 2.56 (1.0-11.41) | 2.81 (1.04-7.44) | 0.92 |
| Monocyte count, ×10³/μL | 0.86 (0.16-2.1) | 0.73 (0.12-1.64) | 0.17 |
| Hemoglobin (g/dL) | 12.51 ± 1.54 | 12.53 ± 1.10 | 0.95 |
| Platelet count, ×10³/μL | 410.5 (194-681) | 384.5 (267-584) | 0.43 |
| NLR, % | 2.68 (0.66-20.79) | 2.07 (0.6-9.73) | 0.12 |
| PLR, % | 133.22 (49.3-536.2) | 130.87 (63.4-355.8) | 0.83 |
| MLR, % | 0.29 (0.1-0.7) | 0.23 (0.1-0.6) | 0.06 |
| C-reactive protein, mg/L | 12.6 (0.3-133) | 7.7 (0.5-26.1) | 0.07 |
| Albumin, g/dL | 4.19 (2.5-5.01) | 4.33 (3.53-4.71) | 0.11 |
| SII | 1005.35 (196.7-13219.4) | 853.90 (200-4516.3) | 0.19 |
| SIRI | 2.26 (0.2-15.7) | 1.51 (0.2-7.2) | 0.02 |

Data presented as mean ± standard deviation (for hemoglobin, normally-distributing data), or median (min-max).
MLR, monocyte/lymphocyte ratio; NLR, neutrophil/lymphocyte ratio; PLR, platelet/lymphocyte ratio; SII, neutrophil x platelet/ lymphocyte count; SIRI, neutrophil x monocyte/ lymphocyte count.

## Discussion

Although IgAV is a self-limiting disease, GI and renal involvement may lead to serious consequences. In our study, we investigated markers that could predict GI involvement in patients with IgAV. Among the 207 patients examined, inflammatory markers at presentation were found to be higher in those with GI involvement. This study constitutes the initial examination of inflammatory biomarkers, notably SII and SIRI, concerning GI involvement in pediatric patients diagnosed with IgAV. Systemic inflammatory indices, such as SII and SIRI, exhibited significant elevation in patients with GI involvement, suggesting their potential utility in detecting such involvement.

IgAV shows a male predominance, with reported male-to-female ratios ranging from 1.2:1 to 1.8:1[1,14,15], although two studies[16,17] conducted in Korea have indicated a slight female predominance. Correspondingly, in our study, the female gender was more predominant (1.53:1). IgAV tends to occur especially in the autumn and winter months, which suggests a link with infections.[1,2] The incidence of URT infections before the onset of IgAV has been reported in the literature to range from 36% to 63%.[18-20] In the present study, a history of recent URT infections within the last month was present in 51.4% of patients, with 34.8% presenting in the autumn and 28.6% in the winter period. A study conducted in Taiwan documented a heightened incidence of GI involvement in children who did not have a history of URT infections before the onset of IgAV.[10] Likewise, in our study, individuals lacking GI involvement exhibited a greater prevalence of a history of URT infections ($p<0.001$). While earlier research has established a link between URT infections and nephritis, additional studies are warranted to elucidate the relationship between URT infections and GI involvement in these cases.[20]

Recent studies have investigated the correlation between organ involvement and various laboratory parameters in individuals diagnosed with IgAV. The neutrophil-to-lymphocyte ratio has been widely used to define the severity

of inflammation. In the study conducted by Gayret et al.[21] in which 119 HSP patients and 40 healthy children participated, NLR levels were significantly increased in IgAV patients, and PLR and platelet values were significantly higher in patients with GI bleeding compared to those without GI bleeding. Correspondingly, Makay et al.[6] observed a significant elevation in NLR among pediatric IgAV patients with GI bleeding compared to those without. In the aforementioned study, logistic regression analysis identified mean platelet volume (MPV) and NLR as two significant factors correlated with GI bleeding in patients. The optimal NLR threshold for predicting GI bleeding was determined to be 2.82, with a sensitivity of 81.0% and specificity of 76.0%. Another study has shown that IgAV patients with GI bleeding have significantly higher NLR compared to those without GI bleeding. The optimal cut off NLR for predicting GI bleeding was determined to be 2.05 with 93% sensitivity and 62% specificity.[22] In the present study, NLR and PLR values were found to be significantly higher in individuals with GIS involvement ($p<0.001$, $p=0.03$). The predictive threshold for NLR in anticipating GIS involvement was determined to be >2.73 (sensitivity 47%, specificity 81.3%, AUC=0.649, CI: 0.580-0.714, $p=0.0001$).

In a recent study, Suszek et al.[23] showed that the MLR serves as a valuable marker for evaluating the activity of systemic lupus erythematosus. Moreover, other studies[24,25] suggested that MLR could potentially serve as an indicator of disease activity in Takayasu arteritis and rheumatoid arthritis. Nevertheless, there is limited research examining the correlation between MLR and the severity of IgAV in pediatric patients. For instance, Yuan et al.[26] conducted a study involving 115 children diagnosed with IgAV and 95 healthy children. They found GI involvement in 29.5% of the patients and renal involvement in 10.4%. In children with IgAV, the neutrophil count, as well as the levels of NLR and MLR, were notably elevated in those with GI involvement compared to those without it. Logistic regression analysis identified the MLR as the sole significant risk factor for GI involvement among the parameters studied in this cohort of patients with IgAV. Moreover, a threshold MLR value of 0.245 effectively differentiated children with IgAV who had GI involvement from those who did not (AUC 0.694, with a sensitivity of 52.9% and specificity of 77.8%). The elevated MLR level could arise from an increase in monocyte counts or a decrease in lymphocyte counts. Monocytes serve as a fundamental source of pro-inflammatory mediators in various types of vasculitis. Lymphocytes have been demonstrated to play a broader role in regulating the inflammatory response at each stage of vasculitis. Furthermore, a decreased lymphocyte count has been linked to the progression of IgA vasculitis in rat and rabbit models.[27]

The utilization of SII and SIRI as a novel identifier has exhibited correlations with disease severity and prognosis in both cancer patients and individuals afflicted with inflammatory diseases. Some researchers contend that the SII offers a more comprehensive and balanced evaluation of the body's immune response and inflammation dynamics.[10,20] In a study conducted on children with Kawasaki Disease in China, a positive correlation between SII and the development of coronary artery lesions was observed. It has been demonstrated that elevated SII levels increase the risk of coronary artery lesions. Furthermore, a strong correlation between SII and CRP was found.[10] Lee et al.[9] have also examined the levels of SIRI in predicting the prognosis and mortality of ANCA-associated vasculitis. Patients with ANCA-associated vasculitis who had a SIRI ≥ 2847.9 $mm^3$ exhibited a significantly increased risk of mortality compared to those with a SIRI < 2847.9 $mm^3$.[9] In our study, for the first time, SII and SIRI parameters were assessed in patients with IgAV. It was observed that

these parameters were notably elevated in individuals with GI involvement. Additionally, SIRI was significantly higher in the group with severe GIS involvement. Further studies are required to establish the routine use of these novel biomarkers in clinical practice.

Our study had certain limitations including a retrospective design and a small sample size. However, the study is strengthened by the use of SII and SIRI parameters in patients with IgAV for the first time.

Consequently, organ involvement holds crucial significance in patients with IgAV. Utilizing simple and cost-effective markers can aid in its detection. This study aims to provide a clearer delineation of the demographic and clinical characteristics, as well as laboratory markers, associated with GI involvement in IgAV.

### Ethical approval

The study was approved by Kocaeli University Ethics Committee (date: 18.01.2024, number: 2024-24).

### Author contribution

Study conception and design: NŞ, HES; data collection: BÖ; analysis and interpretation of results: BÖ, NŞ, HES; draft manuscript preparation: BÖ, HES. 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. Trapani S, Micheli A, Grisolia F, et al. Henoch Schonlein purpura in childhood: epidemiological and clinical analysis of 150 cases over a 5-year period and review of literature. Semin Arthritis Rheum 2005; 35: 143-153. https://doi.org/10.1016/j.semarthrit.2005.08.007
2. Yang YH, Hung CF, Hsu CR, et al. A nationwide survey on epidemiological characteristics of childhood Henoch-Schönlein purpura in Taiwan. Rheumatology (Oxford) 2005; 44: 618-622. https://doi.org/10.1093/rheumatology/keh544
3. Oni L, Sampath S. Childhood IgA vasculitis (Henoch Schonlein Purpura)-advances and knowledge gaps. Front Pediatr 2019; 7: 257. https://doi.org/10.3389/fped.2019.00257
4. Ozen S, Pistorio A, Iusan SM, et al. EULAR/PRINTO/PRES criteria for Henoch-Schönlein purpura, childhood polyarteritis nodosa, childhood Wegener granulomatosis and childhood Takayasu arteritis: Ankara 2008. Part II: final classification criteria. Ann Rheum Dis 2010; 69: 798-806. https://doi.org/10.1136/ard.2009.116657
5. Chang WL, Yang YH, Lin YT, Chiang BL. Gastrointestinal manifestations in Henoch-Schönlein purpura: a review of 261 patients. Acta Paediatr 2004; 93: 1427-1431. https://doi.org/10.1080/08035250410020181
6. Makay B, Gücenmez ÖA, Duman M, Ünsal E. The relationship of neutrophil-to-lymphocyte ratio with gastrointestinal bleeding in Henoch-Schonlein purpura. Rheumatol Int 2014; 34: 1323-1327. https://doi.org/10.1007/s00296-014-2986-2
7. Hong SH, Kim CJ, Yang EM. Neutrophil-to-lymphocyte ratio to predict gastrointestinal bleeding in Henoch: Schönlein purpura. Pediatr Int 2018; 60: 791-795. https://doi.org/10.1111/ped.13652
8. Karadağ ŞG, Çakmak F, Çil B, et al. The relevance of practical laboratory markers in predicting gastrointestinal and renal involvement in children with Henoch-Schönlein Purpura. Postgrad Med 2021; 133: 272-277. https://doi.org/10.1080/00325481.2020.1807161
9. Lee LE, Pyo JY, Ahn SS, Song JJ, Park YB, Lee SW. Systemic inflammation response index predicts all-cause mortality in patients with antineutrophil cytoplasmic antibody-associated vasculitis. Int Urol Nephrol 2021; 53: 1631-1638. https://doi.org/10.1007/s11255-020-02777-4
10. Huang T, Peng Q, Zhang Y, Zhu Z, Fan X. The Systemic Immune-Inflammation Index (SII) and coronary artery lesions in Kawasaki disease. Clin Exp Med 2024; 24: 4. https://doi.org/10.1007/s10238-023-01265-0

11. Niculescu R, Russu E, Arbănași EM, et al. Carotid plaque features and inflammatory biomarkers as predictors of restenosis and mortality following carotid endarterectomy. Int J Environ Res Public Health 2022; 19: 13934. https://doi.org/10.3390/ijerph192113934

12. Russu E, Mureșan AV, Arbănași EM, et al. The predictive role of NLR and PLR in outcome and patency of lower limb revascularization in patients with femoropopliteal disease. J Clin Med 2022; 11: 2620. https://doi.org/10.3390/jcm11092620

13. Lei WT, Tsai PL, Chu SH, et al. Incidence and risk factors for recurrent Henoch-Schönlein purpura in children from a 16-year nationwide database. Pediatr Rheumatol Online J 2018; 16: 25. https://doi.org/10.1186/s12969-018-0247-8

14. Gardner-Medwin JM, Dolezalova P, Cummins C, Southwood TR. Incidence of Henoch-Schönlein purpura, Kawasaki disease, and rare vasculitides in children of different ethnic origins. Lancet 2002; 360: 1197-1202. https://doi.org/10.1016/S0140-6736(02)11279-7

15. Calviño MC, Llorca J, García-Porrúa C, Fernández-Iglesias JL, Rodriguez-Ledo P, González-Gay MA. Henoch-Schönlein purpura in children from northwestern Spain: a 20-year epidemiologic and clinical study. Medicine (Baltimore) 2001; 80: 279-290. https://doi.org/10.1097/00005792-200109000-00001

16. Shim JO, Han K, Park S, Kim GH, Ko JS, Chung JY. Ten-year nationwide population-based survey on the characteristics of children with Henoch-Schönlein purpura in Korea. J Korean Med Sci 2018; 33: e174. https://doi.org/10.3346/jkms.2018.33.e174

17. Hwang HH, Lim IS, Choi BS, Yi DY. Analysis of seasonal tendencies in pediatric Henoch-Schönlein purpura and comparison with outbreak of infectious diseases. Medicine (Baltimore) 2018; 97: e12217. https://doi.org/10.1097/MD.0000000000012217

18. Wang S, Tang H, Du W, Ding Y. Massive gastrointestinal hemorrhage caused by Henoch-Schoenlein purpura: a case report. Medicine (Baltimore) 2021; 100: e28240. https://doi.org/10.1097/MD.0000000000028240

19. Jauhola O, Ronkainen J, Koskimies O, et al. Clinical course of extrarenal symptoms in Henoch-Schonlein purpura: a 6-month prospective study. Arch Dis Child 2010; 95: 871-876. https://doi.org/10.1136/adc.2009.167874

20. Fest J, Ruiter R, Ikram MA, Voortman T, van Eijck CHJ, Stricker BH. Reference values for white blood-cell-based inflammatory markers in the Rotterdam study: a population-based prospective cohort study. Sci Rep 2018; 8: 10566. https://doi.org/10.1038/s41598-018-28646-w

21. Gayret OB, Erol M, Tekin Nacaroglu H. The relationship of neutrophil-lymphocyte ratio and platelet-lymphocyte ratio with gastrointestinal bleeding in Henoch-Schonlein purpura. Iran J Pediatr 2016; 26: e8191. https://doi.org/10.5812/ijp.8191

22. Yakut HI, Kurt T, Uncu N, Semsa Cayci F, Celikel Acar B. Predictive role of neutrophil to lymphocyte ratio and mean platelet volume in Henoch-Schönlein purpura related gastrointestinal and renal involvement. Arch Argent Pediatr 2020; 118: 139-142. https://doi.org/10.5546/aap.2020.eng.139

23. Suszek D, Górak A, Majdan M. Differential approach to peripheral blood cell ratios in patients with systemic lupus erythematosus and various manifestations. Rheumatol Int 2020; 40: 1625-1629. https://doi.org/10.1007/s00296-020-04669-3

24. Seringec Akkececi N, Yildirim Cetin G, Gogebakan H, Acipayam C. The C-reactive protein/albumin ratio and complete blood count parameters as indicators of disease activity in patients with Takayasu arteritis. Med Sci Monit 2019; 25: 1401-1409. https://doi.org/10.12659/MSM.912495

25. Du J, Chen S, Shi J, et al. The association between the lymphocyte-monocyte ratio and disease activity in rheumatoid arthritis. Clin Rheumatol 2017; 36: 2689-2695. https://doi.org/10.1007/s10067-017-3815-2

26. Yuan Y, Liu J, Zhou Y, et al. The relationship between monocyte-to-lymphocyte ratio and the risk of gastrointestinal system involvement in children with IgA vasculitis: a preliminary report. Adv Clin Exp Med 2021; 30: 999-1005. https://doi.org/10.17219/acem/138906

27. Li Y, Feng X, Huang L, et al. Hematologic and immunological characteristics of Henoch-Schönlein purpura in rat and rabbit models induced with ovalbumin based on type III hypersensitivity. Sci Rep 2015; 5: 8862. https://doi.org/10.1038/srep08862

The Turkish Journal of Pediatrics 2024; 66: 608-617
https://doi.org/10.24953/turkjpediatr.2024.5075

**Original Article**

# Comprehensive analysis of genotypic and phenotypic characteristics of biotinidase deficiency patients in the eastern region of Türkiye

Kısmet Çıkı[1,2], Ceren Alavanda[3], Emine İpek Ceylan[3,4], Tijen Tanyalçın[5], Sebile Kılavuz[1,6]

*[1]Division of Child Nutrition and Metabolism, Department of Pediatrics, Van Research and Training Hospital, Van; [2]Division of Child Nutrition and Metabolism, Department of Pediatrics, School of Medicine, Hacettepe University, Ankara; [3]Department of Medical Genetics, Van Research and Training Hospital, Van; [4]Department of Medical Genetics, Dr. Abdurrahman Yurtaslan Ankara Oncology Education and Research Hospital, Ankara; [5]Tanyalcin Medical Laboratory, Selective Screening and Metabolism Unit, İzmir; [6]Division of Child Nutrition and Metabolism, Department of Pediatrics, School of Medicine, Marmara University, İstanbul, Türkiye*

## ABSTRACT

**Background.** Biotin is a water-soluble vitamin that plays a key role in carboxylation. The formation of free biotin is impaired in biotinidase deficiency (BD), resulting in impaired biotin-dependent carboxylase functions. Based on the percentage of residual serum enzyme activity, BD is classified as partial and profound.

**Methods.** Retrospective data including gender, age, parental consanguinity, family history, biotinidase activity analyses, type of deficiency (partial-profound), physical examination, treatment, and genotypes were evaluated in patients diagnosed with biotinidase deficiency in a single center in the eastern region of Türkiye. Patients whose biotinidase enzyme activity was below 30% with biallelic variants in the *BTD* gene were diagnosed as BD.

**Results.** A total of 302 patients were included in the study. Parental consanguinity was present in 135 (44.7%) of them. Two hundred eighty-six (94.7%) were diagnosed by neonatal screening, 14 (4.6%) by family screening and two (0.06%) by clinical symptoms. Ninety-two (30.5%) of the patients were followed-up with profound deficiency and 210 (69.5%) with partial deficiency. A total of 306 variants were detected. Twenty different variants (3 novel - 3 rare) and 31 different genotypes were detected. The 3 most frequently detected variants were c.410G>A (p.Arg137His; 47.3%), c.1270G>C (p.Asp424His; 29.7%), and c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36; 15.3%). The 3 most frequently identified genotypes were c.410G>A (p.Arg137His) / c.1270G>C (p.Asp424His) compound heterozygous (32.4%), c.410G>A (p.Arg137His) homozygous (24.8%), and c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.1270G>C (p.Asp424His) compound heterozygous (12.2%). Patients with c.410G>A (p.Arg137His) homozygous variant, c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) homozygous variant and c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.410G>A (p.Arg137His) compound heterozygous variant were statistically significantly associated with profound deficiency. Compound heterozygosity of c.410G>A (p.Arg137His) / c.1270G>C (p.Asp424His) variants were significantly associated with partial deficiency.

**Conclusions.** The association between the *BTD* genotype and biochemical phenotype is not always consistent. Our study provides valuable data by adding variants with genotype-phenotype correlations to the literature and three novel variants, which can provide significant guidance in clinical follow-up.

**Key words:** biotin, biotinidase deficiency, *BTD*, genotype–phenotype correlation, partial, profound.

✉ Kısmet Çıkı ▪ dr.kismetciki@gmail.com

Received 9th Jul 2024, revised 2nd Sep 2024, 21st Oct 2024, accepted 25th Oct 2024.

Biotin, also known as vitamin B7 or Vitamin H, is a water-soluble vitamin that plays a key role in carboxylation reactions such as pyruvate carboxylase, propionyl-CoA carboxylase, methylcrotonyl-CoA carboxylase, and acetyl-CoA carboxylase. Therefore, biotin is required for gluconeogenesis, fatty acids biosynthesis, branched-chain amino acids catabolism and tricarboxylic acid cycle in which these carboxylase enzymes are involved. Biotin is released from these carboxylase enzymes by the enzyme biotinidase. Free biotin is then available for further carboxylation reactions. The formation of the free biotin is impaired in biotinidase deficiency (BD), resulting in impaired biotin-dependent carboxylase functions.[1,2]

Based on the percentage of residual serum enzyme activity, BD is classified as partial (enzyme activity 10–30%) and profound (enzyme activity <10%) deficiency. BD can occur at any age and may be asymptomatic for a long time. Symptoms include feeding difficulties, laryngeal stridor, apnea, alopecia, eczematous rash, conjunctivitis, lethargy, hypotonia, seizures, ataxia, muscle weakness, spastic paraparesis, global developmental delay, intellectual disability, optic atrophy, hearing loss, lactic acidosis, ketosis, hyperammonemia even coma, and death. The diagnosis is confirmed with the enzyme activity and the *BTD* gene analysis. The *BTD* gene is located at chromosome 3p25. The gene has four exons with sizes of 79, 265, 150, and 1,502 bp. More than 200 variants have been identified in the *BTD* gene so far. Genotype-phenotype correlation is still not well established.[1-4]

Biotin 5-20 mg/day is recommended for treatment. The onset of symptoms and the progression of the disease is mostly prevented with early treatment in various variants. However, optic atrophy, hearing loss and global developmental delay may be irreversible even if treatment is started or the treatment dose is increased. There is still no consensus on the follow-up and treatment protocol of the disease. The aim of this study was to investigate the clinical and genetic characteristics of our patients.[1-4]

## Materials and Methods

### *Patients*

Patients who were diagnosed with BD by low biotinidase activity (below 30%) and biallelic pathogenic / likely pathogenic variants in the BTD gene at the Pediatric Metabolism Department of Van Research and Training Hospital between January 2016 and December 2023 were enrolled in this study. This also included newborns screened in the Turkish newborn screening program and referred to the Nutrition and Metabolism unit for further evaluation with a biotinidase enzyme activity ≤ 65 microplate response units (MRU) in their dried blood spots. Enzyme activity and genetic analysis were performed in all patients. Gender, age, method of diagnosis (newborn screening, family screening or symptomatic), parental consanguinity, family history, enzyme levels, type of deficiency (partial vs. profound), clinical symptoms, treatment dose and genotypes were evaluated retrospectively.

### *Biotinidase activity measurement*

Biotinidase activity was measured by the modified method of Wolf in dried blood spots using microtiter plates. Following incubation of 3 mm punch with N-biotinyl p-aminobenzoate (Sigma-Aldrich Co., St. Louis, MO, USA) for 16 hours, enzymatic reaction was stopped by the gradual addition of trichloroacetic acid (Merck, Darmstadt, Germany). The clear solution free from blood spot and debris was mixed with sodium nitrite (Merck), ammonium sulfamate (Fluka Chemica GmbH, Buchs, Switzerland), and N-1-napthylethylenediamine dihydrochloride (Fluka analytical) in order; the absorbance of the developed color was measured using microplate reader named Stat Fax 3200; Awareness Technology INC PO

Drawer, Palm City, FL, USA, by 580/690 dual wavelength measurement using the software of the reader linear regression analysis: Y=Abs, X=Conc, against a blank with a six-point calibration curve with the enzyme unit=eu between the range 11.2–360 eu (median 254 eu).[5]

### *Molecular analysis*

After receiving written informed consent from the patients and their parents, genomic DNA was extracted from peripheral blood leukocytes using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. One hundred nanograms of total genomic DNA was used for library preparation with the Nextera DNA Library Preparation Kit (Illumina, San Diego, CA, USA). Next-generation sequencing (NGS) was performed via the MiSeq platform (Illumina, San Diego, CA, USA). The reference sequence NM_001370658.1 was used for the *BTD* gene. Variants were interpreted according to the American College of Medical Genetics and Genomics (ACMG) guidelines.[6] Verification of whether variants had been previously reported in the literature were assessed via ClinVar, or the Human Gene Variant Database (HGMD). Segregation analyses were also performed using the Miseq platform (San Diego, CA, USA).

### *Statistical analysis*

The data were analyzed using IBM SPSS 25 (IBM Inc., Armonk, NY, USA) program. Descriptive statistics (mean, standard deviation, median) were provided for numerical variables. The distribution of pathogenic variants among *BTD* genotypes was analyzed. If the p-value was <0.01, it was considered statistically significant.

### *Ethical approval*

The study was approved by the Ethics Committee of Van Research and Training Hospital on October 18th, 2023 (Approval No:2023/22-01). Informed consent was obtained from the legal guardians of the patients for the genetic analyses.

## Results

A total of 302 patients with a diagnosis of BD were included in the study. Of the patients, 141 (46.7%) were female and 161 (53.3%) were male. The mean age was 6.05 years (median: 4.73 years). Parental consanguinity was presented in 135 (44.7%) patients. Seventy-four patients (24.6%) had at least one member of their family with a BD. Two hundred eighty-six (94.7%) patients were diagnosed by neonatal screening, 14 (4.6%) by family screening and 2 (0.06%) by clinical symptoms. According to the percentages of biotinidase enzyme activity, 92 (30.5%) of the patients were followed up with profound deficiency and 210 (69.5%) with partial deficiency. The percentage of biotinidase enzyme activity was between 0-30 % (mean: 17.5%; median: 20%). One hundred seventy-eight patients (59%) received 5 mg, 113 patients 10 mg (37.4%), 8 patients 15 mg (2.6%), 1 patient 20 mg (0.3%) and 2 patients 25 mg biotin (0.7%) daily. Patients with clinical symptoms received high-dose therapy (usually >10 mg/day biotin).

Two patients who presented with clinical findings of BD were born before biotinidase deficiency screening was included in the Turkish newborn screening programme. Biotinidase enzyme activity were found to be 0, and a homozygous variant, c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36), was revealed in both patients through *BTD* gene analysis. One of the patients presented with seizures in the neonatal period, while the other exhibited developmental delay, seizures and hearing loss. In total, 16 patients (5%) had clinical symptoms. Two were symptomatic at the time of diagnosis whereas 14 patients developed symptoms during follow-up. Seven patients had seizures; four patients had dermatitis; four patients had hearing loss; three patients had global developmental delay; two patients had autism and one patient had optic atrophy. The most frequent variants revealed in symptomatic patients were c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36; 36%), c.410G>A (p.Arg137His; 30.5%) and

c.1270G>C (p.Asp424His; 13.8%). Clinical and laboratory findings of the symptomatic patients are given in Table I.

Fourteen patients (9 siblings and 5 parents) were diagnosed during family screening. Eight patients had profound deficiency, and 6 patients had partial deficiency. None of these patients presented with symptoms or metabolic decompensation. Subsequently, biotin treatment was initiated, and all patients are currently in routine follow-up.

*BTD* gene analysis was performed in all patients. Six hundred six variants were revealed in 302 patients. Twenty different variants (3 novel-3 rare) and 31 different genotypes were revealed. While 110 patients had homozygous variants, 192 patients had compound heterozygous variants. The 3 most

**Table I.** Clinical and laboratory findings of symptomatic patients with biotinidase deficiency.

| No | Sex | Age (yr) | Biotinidase enzyme activity, % | Clinical findings | *BTD* genotype |
|---|---|---|---|---|---|
| 1 | Male | 3.85 | 0 | Dermatitis | c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.896C>T (p.Ser299Phe) compound heterozygous |
| 2 | Male | 8.35 | 0 | Seizure | c.534_536delCGT (p.Val179del) homozygous |
| 3 | Female | 11.57 | 0 | Hearing loss, optic atrophy | c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) homozygous |
| 4 | Female | 15.42 | 0 | Dermatitis | c.410G>A (p.Arg137His) homozygous |
| 5 | Male | 15.69 | 0 | Seizure | c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) homozygous |
| 6 | Male | 16.75 | 0 | Global developmental delay, hearing loss, seizure | c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) homozygous |
| 7 | Male | 2.93 | 2 | Seizure | c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.410G>A (p.Arg137His) compound heterozygous |
| 8 | Male | 9.19 | 3 | Hearing loss, seizure | c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) homozygous |
| 9 | Male | 2.83 | 9 | Hearing loss, optic atrophy | c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.1270G>C (p.Asp424His) compound heterozygous |
| 10 | Female | 7.61 | 9 | Dermatitis | c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.410G>A (p.Arg137His) compound heterozygous |
| 11 | Male | 11.78 | 9 | Autism | c.410G>A (p.Arg137His) homozygous |
| 12 | Male | 4.00 | 12 | Autism | c.410G>A (p.Arg137His) homozygous |
| 13 | Female | 3.93 | 22 | Dermatitis | c.410G>A (p.Arg137His) / c.1270G>C (p.Asp424His) compound heterozygous |
| 14 | Female | 5.86 | 22 | Seizure | c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.1270G>C (p.Asp424His) compound heterozygous |
| 15 | Male | 3.68 | 28 | Global developmental delay | c.410G>A (p.Arg137His) / c.1270G>C (p.Asp424His) compound heterozygous |
| 16 | Male | 4.24 | 29 | Global developmental delay | c.410G>A (p.Arg137His) / c.1270G>C (p.Asp424His) compound heterozygous |

frequently revealed variants were c.410G>A (p.Arg137His; 47.3%), c.1270G>C (p.Asp424His; 29.7%) and c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36; 15.3%). The 3 most frequently revealed genotypes were c.410G>A (p.Arg137His) / c.1270G>C (p.Asp424His) compound heterozygous (32.4%), c.410 G>A (p.Arg137His) homozygous (24.8%), and c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.1270G>C (p.Asp424His) compound heterozygous (12.2%). Details of variants and genotype frequencies are given in Tables II and III. A statistical significance was found in patients with profound deficiency and c.410G>A (p.Arg137His) homozygous variant, c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) homozygous variant and c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.410G>A (p.Arg137His) compound heterozygous variants. Patients with c.410G>A (p.Arg137His) / c.1270G>C (p.Asp424His) compound heterozygous variant were significantly associated with partial deficiency.

## Discussion

BD, first described in 1983 by Wolf et al, is an autosomal recessive disorder.[1,7] Newborn screening for BD has been implemented in the USA since 1984, and in Türkiye since 2008.[8,9] The incidence of biotinidase deficiency varies between populations and is approximately 1/40,000 to 1/60,000 worldwide.[1] Türkiye is one of the countries with the highest incidence of BD and the incidence is approximately 1/7,116.[10] It was previously reported that the incidence of BD in southeastern provinces such as Diyarbakır and Şanlıurfa, where consanguineous marriages are common, was 1 in 2,359 and 1 in 1,177, respectively.[11] Van is a city located in the east of Türkiye. Our patients are from Van and neighbouring provinces such as Ağrı/Doğubeyazıt and Iğdır which

**Table II.** *BTD* variant analysis of patients.

| Variant | Total number | Partial BD | Profound BD |
|---|---|---|---|
| c.410G>A (p.Arg137His) | 287 | 176 | 111 |
| c.1270G>C (p.Asp424His) | 180 | 177 | 3 |
| c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) | 93 | 35 | 58 |
| c.175C>T (p.Arg59Cys) | 6 | 4 | 2 |
| c.333delC (p.Phe111Leufs*28) | 5 | 1 | 4 |
| c.534_536del (p.Val179del) | 5 | 3 | 2 |
| c.581A>G (p.Asn194Ser) | 5 | 5 | 0 |
| c.1535C>T (p.Thr512Met) | 4 | 4 | 0 |
| c.565C>T (p.Arg189Cys) | 3 | 3 | 0 |
| c.1273G>C (p.Gly425Arg)* | 3 | 1 | 2 |
| c.329A>C (p.Asp110Ala)* | 2 | 2 | 0 |
| c.497G>A (p.Cys166Tyr) | 2 | 0 | 2 |
| c.625C>T (p.Arg209Cys)** | 2 | 2 | 0 |
| c.896C>T (p.Ser299Phe) | 2 | 1 | 1 |
| c.1368A>C (p.Gln456His)** | 2 | 1 | 1 |
| c.908A>G (p.His303Arg) | 1 | 1 | 0 |
| c.1361A>G (p.Tyr454Cys)** | 1 | 0 | 1 |
| c.1350dupC (p.Cys451Leufs*13) | 1 | 1 | 0 |
| c.1466del p.(Pro489Leufs*13)* | 1 | 0 | 1 |
| c.1550G>A (p.Gly517Glu) | 1 | 0 | 1 |

BD, biotinidase deficiency.
* Novel variants likely pathogenic according to the ACMG classification.[6] ** Rare variants in the literature.

**Table III.** *BTD* genotypes identified in patients and their phenotypic characteristics.

| *BTD* genotype | Total number (%) | Partial BD (%) | Profound BD (%) | p |
|---|---|---|---|---|
| c.410G>A (p.Arg137His) / c.1270G>C (p.Asp424His) compound heterozygous | 98 (32.4%) | 98 (32.4%) | 0 (0%) | <0.001 |
| c.410G>A (p.Arg137His) homozygous | 75 (24.8%) | 35 (11.6%) | 40 (13.2%) | <0.001 |
| c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.1270G>C (p.Asp424His) compound heterozygous | 37 (12.2%) | 34 (11.2%) | 3 (1%) | 0.001 |
| c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.410G>A (p.Arg137His) compound heterozygous | 27 (8.9%) | 1 (0.3%) | 26 (8.6%) | <0.001 |
| c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) homozygous | 14 (4.6%) | 0 (0%) | 14 (4.6%) | <0.001 |
| c.1270G>C (p.Asp424His) homozygous | 14 (4.6%) | 14 (4.6%) | 0 (0%) | 0.010 |
| c.175C>T (p.Arg59Cys) / c.1270G>C (p.Asp424His) compound heterozygous | 4 (1.3%) | 4 (1.3%) | 0 (0%) | 0.175 |
| c.534_536del (p.Val179del) / c.1270G>C (p.Asp424His) compound heterozygous | 3 (1%) | 3 (1%) | 0 (0%) | 0.241 |
| c.565C>T (p.Arg189Cys) / c.1270G>C (p.Asp424His) compound heterozygous | 3 (1%) | 3 (1%) | 0 (0%) | 0.241 |
| c.410G>A (p.Arg137His) / c.1535C>T (p.Thr512Met) compound heterozygous | 3 (1%) | 3 (1%) | 0 (0%) | 0.241 |
| c.333delC (p.Phe111Leufs*28) homozygous | 2 (0.6%) | 0 (0%) | 2 (0,6%) | 0.034 |
| c.581A>G (p.Asn194Ser) homozygous | 2 (0.6%) | 2 (0.6%) | 0 (%0) | 0.340 |
| c.410G>A (p.Arg137His) / c.497G>A (p.Cys166Tyr) compound heterozygous | 2 (0.6%) | 0 (0%) | 2 (0.6%) | 0.034 |
| c.175C>T (p.Arg59Cys) / c.1361A>G (p.Tyr454Cys)/c.1368A>C (p.Gln456His) compound heterozygous | 1 (0.3%) | 0 (0%) | 1 (0.3%) | 0.136 |
| c.175C>T (p.Arg59Cys) / c.410G>A (p.Arg137His) compound heterozygous | 1 (0.3%) | 0 (%0) | 1 (0.3%) | 0.136 |
| c.393del (p.Phe131LeufsTer28) / c.1270G>C (p.Asp424His) compound heterozygous | 1 (0.3%) | 1 (0.3%) | 0 (0%) | 0.500 |
| c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.896C>T (p.Ser299Phe) compound heterozygous | 1 (0.3%) | 0 (0%) | 1 (0.3%) | 0.136 |
| c.329A>C (p.Asp110Ala) homozygous | 1 (0.3%) | 1 (0.3%) | 0 (0%) | 0.500 |
| c.534_536delCGT (p.Val179del) homozygous | 1 (0.3%) | 0 (0%) | 1 (0.3%) | 0.136 |
| c.410G>A (p.Arg137His) / c.625C>T (p.Arg209Cys) compound heterozygous | 1 (0.3%) | 1 (0.3%) | 0 (0%) | 0.500 |
| c.410G>A (p.Arg137His) / c.908A>G (p.His303Arg) compound heterozygous | 1 (0.3%) | 1 (0.3%) | 0 (0%) | 0.500 |
| c.410G>A (p.Arg137His) / c.1270G>C (p.Asp424His)/c.641A>G (p.Asn214Ser) compound heterozygous | 1 (0.3%) | 1 (0.3%) | 0 (0%) | 0.500 |
| c.410G>A (p.Arg137His) / c.1368A>C (p.Gln456His) compound heterozygous | 1 (0.3%) | 1 (0.3%) | 0 (0%) | 0.500 |
| c.410G>A (p.Arg137His) / c.1466del p.(Pro489Leufs*13) compound heterozygous | 1 (0.3%) | 0 (0%) | 1 (0.3%) | 0.136 |
| c.410G>A (p.Arg137His) / c.1550G>A (p.Gly517Glu) compound heterozygous | 1 (0.3%) | 0 (0%) | 1 (0.3%) | 0.136 |
| c.625C>T (p.Arg209Cys) / c.1270G>C (p.Asp424His) compound heterozygous | 1 (0.3%) | 1 (0.3%) | 0 (0%) | 0.500 |
| c.896C>T (p.Ser299Phe) / c.1270G>C (p.Asp424His) compound heterozygous | 1 (0.3%) | 1 (0.3%) | 0 (0%) | 0.500 |
| c.1270G>C (p.Asp424His)/c.1273G>C (p.Gly425Arg) compound heterozygous | 1 (0.3%) | 1 (0.3%) | 0 (0%) | 0.500 |
| c.1270G>C (p.Asp424His) / 1350dupC (p.Cys451Leufs*13) compound heterozygous | 1 (0.3%) | 1 (0.3%) | 0 (0%) | 0.500 |
| c.1270G>C (p.Asp424His) / c.1535C>T (p.Thr512Met) compound heterozygous | 1 (0.3%) | 1 (0.3%) | 0 (0%) | 0.500 |
| c.1273G>C (p.Gly425Arg) homozygous | 1 (0.3%) | 0 (0%) | 1 (0.3%) | 0.136 |

BD, biotinidase deficiency.

have a founder effect for BD. Unfortunately, consanguineous marriages are also common in the eastern provinces of Türkiye, similar to the cities in the south- eastern part of the country. Therefore, autosomal recessive diseases are more common in these regions.

Our study has the largest series of BD patients evaluated biochemically and genotypically in Türkiye including 302 patients. A significant proportion of our patients were diagnosed by neonatal screening (94.7%) similar to the literature.[2] In the study by Karaca et al., the majority of patients had profound deficiency.[9] In the study conducted by Kasapkara et al., equal numbers of partial and complete deficiency patients were seen.[12] Partial deficiency is more common in other studies reported from Türkiye similar to our study.[13-19] All patients (14 patients) diagnosed by family screening were asymptomatic which exhibits the importance of family screening even during adulthood. Diagnosis is essential to prevent severe deterioration in every stage of life. In the literature, in adult patients aged 19-63 years, presentation with impaired consciousness, oppositional paratonia (resistance to passive movement), bilateral optic atrophy and sensorineural hearing loss, scaly and erythematous diffuse rashes, bilateral horizontal nystagmus, ataxia, especially tetraparesis, spastic paraparesis, diplegia and peripheral neuropathy have been reported.[2] It is reasonable to start biotin treatment in patients with BD diagnosed by family screening, even if they are asymptomatic, to prevent these possible complications.

A meta-analysis published in 2023 reported that the most common finding in BD was neurological involvement, and the second most common was dermatological findings.[2] In Türkiye, studies have reported frequencies of clinical findings between 0.4% -15.4%.[9,12-19] In our study, the frequency of clinical findings was 5.2%. Neurological findings were common in our study. While neurological findings were reported to be more common in the studies by Karaca et al. and Sürücü Kara et al., dermatological findings were reported to be more common in the study by Öz et al.[9,13,17,19] The reason for this clinical difference is related to variants in the *BTD* gene. In our study, the c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) variant was more frequent compared to other studies. This variant is also associated with neurological findings.[9,17]

The most common variants reported in Turkish studies were c.1270G>C (p.Asp424His), c.410G>A (p.Arg137His), c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36), c.175C>T (p.Arg59Cys) and c.1535C>T (p.Thr512Met).[1,9,12-19] In our study, these variants constituted 93.8% of all detected variants.

Biotinidase is a very labile enzyme. For this reason, prematurity and cholestasis may cause false positivity. The time duration between the sample collection and the onset of the biochemical analyses, as well as inappropriate transportation conditions and temperature may affect the level of biotinidase enzyme activity.[20,21] Some research demonstrated that biotinidase activity may increase with age.[19,21] This is why results of the enzymatic and genetic tests should be evaluated together. Forny et al. recommended biotinidase activity should be re-performed at the age of 5 years in patients with partial BD, especially in those with the c.1270G>C (p.Asp424His) variant.[22]

The association between the *BTD* genotype and biochemical phenotype is not always consistent. Severe variants (deletions, insertions, or nonsense pathogenic variants) in homozygous or compound heterozygous individuals are associated with profound BD. Compound heterozygosity of c.1270G>C (p.Asp424His) with a severe variant is associated with partial BD.[3,20,23] However, in our study, the enzyme activity of 3 patients who had the compound heterozygous genotype c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) / c.1270G>C (p.Asp424His) was consistent with profound deficiency. Furthermore, in our study, the findings of some patients with the c.410G>A (p.Arg137His) homozygous variant were found to be

compatible with partial deficiency, while others were compatible with profound deficiency.

Patients with homozygous c.1270G>C (p.Asp424His) variant were reported to have 40-50% enzyme activity like carrier individuals. Moreover, it has been reported that these patients do not require treatment.[3,20] In our study, enzyme activities of 14 patients with c.1270G>C (p.Asp424His) homozygous variant were compatible with partial deficiency. In this study, none of the patients with profound deficiency were homozygous for the c.1270G>C (p.Asp424His) variant. Studies in the literature demonstrate that the c.1270G>C (p.Asp424His) variant is associated with partial deficiency. Although patients in the current study with the c.1270G>C (p.Asp424His) homozygous variant did not exhibit any symptoms, other studies with the same genotype have reported a varied clinical phenotype encompassing dermatitis, alopecia, hypotonia, seizure, hearing loss, speech delay, global developmental delay and autism.[13,15,17,19] Therefore, it is more appropriate to decide according to the biotinidase enzyme activity in patients with this genotype. According to the literature, the c.410G>A (p.Arg137His) and c.1270G>C (p.Asp424His) variants present mainly with cutaneous findings, whereas the c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) variant is mostly related to neurological findings which is an important demonstration of a severe clinical phenotype.[9,15,17] In our study, the most common variant in patients with neurological findings was c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) in accordance with the literature.

As a result of this study, the genotypic spectrum was expanded by adding three novel variants to the literature, namely c.1273G>C (p.Gly425Arg), c.329A>C (p.Asp110Ala), and c.1466del (p.Pro489Leufs*13). We also detected three variants rarely reported previously c.625C>T (p.Arg209Cys), c.1368A>C (p.Gln456His) and c.1361A>G (p.Tyr454Cys) (Tables II and III).[15,24,25]

In BD, 5-10 mg/day biotin is generally recommended. In symptomatic patients, dose escalation has been shown to improve symptoms or are associated with slow progression.[1,26] In our study, 11 patients received biotin treatment above 10 mg/day.

Wolf had previously drawn attention to the simplicity of this treatable disorder compared to other inherited metabolic disorders with the following sentence: "if you have to have an inherited metabolic disease, this is the one to have."[27] However, we believe this relatively simple disease is often a challenge for clinicians due to biochemical and genotypic discordance. Clinicians face challenges such as concerns about missed patients, overdiagnosis and unnecessary treatment. We presented biochemical and genetic results of a large cohort, and reported novel and rare variants related to BD in this study. To conclude, biochemistry and genotype may not always be compatible. Some patients with the same genotype may have a different biochemical phenotype. Unknown modified genes, environmental and hormonal factors may be the cause of this incompatibility. We recommend performing more than one measurement of biotinidase activity. The recurrent measurement of biotinidase activity and the genotype should be evaluated together and a decision should made based on the whole picture. Treatment is essential in patients diagnosed through family screening due to the possibility of variability of symptoms at any age. Ophthalmological and auditory examinations should be performed periodically especially for optic atrophy and hearing loss (yearly in profound BD, every two years in partial BD).[1] Patients with the c.38_44delGCGGCTGinsTCC (p.Cys13Phefs*36) variant might have a higher risk of developing severe symptoms.

**Acknowledgements**

We would like to thank the children and their parents who participated in this study.

## Ethical approval

Study was approved by the Ethics Committee of Van Research and Training Hospital on October 18th, 2023 (Approval No: 2023/22-01).

## Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: KÇ, CA; data collection: KÇ, CA; analysis and interpretation of results: KÇ, CA, EİC, TT, SK; draft manuscript preparation: KÇ, CA, EİC, TT, SK. 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. Canda E, Kalkan Uçar S, Çoker M. Biotinidase deficiency: prevalence, ımpact and management strategies. Pediatric Health Med Ther 2020; 11: 127-133. https://doi.org/10.2147/PHMT.S198656
2. Tankeu AT, Van Winckel G, Elmers J, et al. Biotinidase deficiency: what have we learned in forty years? Mol Genet Metab 2023; 138: 107560. https://doi.org/10.1016/j.ymgme.2023.107560
3. Wolf B. Biotinidase deficiency. In: Adam MP, Feldman J, Mirzaa GM, et al., editors. GeneReviews®. Seattle, WA: University of Washington; 1993–2024.
4. Kannan B, Navamani HK, Jayaseelan VP, Arumugam P. A rare biotinidase deficiency in the pediatrics population: genotype-phenotype analysis. J Pediatr Genet 2022; 12: 1-15. https://doi.org/10.1055/s-0042-1757887
5. Tanyalçın T, Aslan D. Use of big data for verification of decision levels for biotinidase deficiency and galactosemia. XXVII. Balkan Clinical Laboratory Federation Meeting BCLF 2019; XXX. National Congress of the Turkish Biochemical Society TBS 2019. Available at: http://www.tanyalcin.com/Data/GALTveBTD_REFERANS_DEGERLER.pdf
6. 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
7. Wolf B, Grier RE, Allen RJ, Goodman SI, Kien CL. Biotinidase deficiency: the enzymatic defect in late-onset multiple carboxylase deficiency. Clin Chim Acta 1983; 131: 273-281. https://doi.org/10.1016/0009-8981(83)90096-7
8. Wolf B, Heard GS, Jefferson LG, Proud VK, Nance WE, Weissbecker KA. Clinical findings in four children with biotinidase deficiency detected through a statewide neonatal screening program. N Engl J Med 1985; 313: 16-19. https://doi.org/10.1056/NEJM198507043130104
9. Karaca M, Özgül RK, Ünal Ö, et al. Detection of biotinidase gene mutations in Turkish patients ascertained by newborn and family screening. Eur J Pediatr 2015; 174: 1077-1084. https://doi.org/10.1007/s00431-015-2509-5
10. Baykal T, Hüner G, Sarbat G, Demirkol M. Incidence of biotinidase deficiency in Turkish newborns. Acta Paediatr 1998; 87: 1102-1103. https://doi.org/10.1080/080352598750031518
11. Toktaş İ, Sarıbaş S, Canpolat S, Erdem Ö, Özbek MN. Evaluation of patients diagnosed with phenylketonuria and biotinidase deficiency by the newborn screening program: a ten-year retrospective study. Turk J Pediatr 2022; 64: 985-992. https://doi.org/10.24953/turkjped.2022.467
12. Kasapkara ÇS, Akar M, Özbek MN, et al. Mutations in BTD gene causing biotinidase deficiency: a regional report. J Pediatr Endocrinol Metab 2015; 28: 421-424. https://doi.org/10.1515/jpem-2014-0056
13. Yılmaz B, Ceylan AC, Gündüz M, et al. Evaluation of clinical, laboratory, and molecular genetic features of patients with biotinidase deficiency. Eur J Pediatr 2024; 183: 1341-1351. https://doi.org/10.1007/s00431-023-05376-4

14. Seker Yilmaz B, Mungan NO, Kor D, et al. Twenty-seven mutations with three novel pathologenic variants causing biotinidase deficiency: a report of 203 patients from the southeastern part of Turkey. J Pediatr Endocrinol Metab 2018; 31: 339-343. https://doi.org/10.1515/jpem-2017-0406

15. Canda E, Yazici H, Er E, et al. Single center experience of biotinidase deficiency: 259 patients and six novel mutations. J Pediatr Endocrinol Metab 2018; 31: 917-926. https://doi.org/10.1515/jpem-2018-0148

16. Erdol S, Kocak TA, Bilgin H. Evaluation of 700 patients referred with a preliminary diagnosis of biotinidase deficiency by the national newborn metabolic screening program: a single-center experience. J Pediatr Endocrinol Metab 2023; 36: 555-560. https://doi.org/10.1515/jpem-2023-0003

17. Oz O, Karaca M, Atas N, Gonel A, Ercan M. BTD gene mutations in biotinidase deficiency: genotype-phenotype correlation. J Coll Physicians Surg Pak 2021; 31: 780-785. https://doi.org/10.29271/jcpsp.2021.07.780

18. Akgun A, Sen A, Onal H. Clinical, biochemical and genotypical characteristics in biotinidase deficiency. J Pediatr Endocrinol Metab 2021; 34: 1425-1433. https://doi.org/10.1515/jpem-2021-0242

19. Sürücü Kara İ, Köse E, Koç Yekedüz M, Eminoğlu FT. A different approach to the evaluation of the genotype-phenotype relationship in biotinidase deficiency: repeated measurement of biotinidase enzyme activity. J Pediatr Endocrinol Metab 2023; 36: 1061-1071. https://doi.org/10.1515/jpem-2023-0337

20. Sharma R, Kucera CR, Nery CR, Lacbawan FL, Salazar D, Tanpaiboon P. Biotinidase biochemical and molecular analyses: experience at a large reference laboratory. Pediatr Int 2024; 66: e15726. https://doi.org/10.1111/ped.15726

21. Borsatto T, Sperb-Ludwig F, Lima SE, et al. Biotinidase deficiency: genotype-biochemical phenotype association in Brazilian patients. PLoS One 2017; 12: e0177503. https://doi.org/10.1371/journal.pone.0177503

22. Forny P, Wicht A, Rüfenacht V, Cremonesi A, Häberle J. Recovery of enzyme activity in biotinidase deficient individuals during early childhood. J Inherit Metab Dis 2022; 45: 605-620. https://doi.org/10.1002/jimd.12490

23. Swango KL, Demirkol M, Hüner G, et al. Partial biotinidase deficiency is usually due to the D444H mutation in the biotinidase gene. Hum Genet 1998; 102: 571-575. https://doi.org/10.1007/s004390050742

24. Tanyalcin I, Stouffs K, Daneels D, et al. Convert your favorite protein modeling program into a mutation predictor: "MODICT". BMC Bioinformatics 2016; 17: 425. https://doi.org/10.1186/s12859-016-1286-0

25. Hsu RH, Chien YH, Hwu WL, et al. Genotypic and phenotypic correlations of biotinidase deficiency in the Chinese population. Orphanet J Rare Dis 2019; 14: 6. https://doi.org/10.1186/s13023-018-0992-2

26. Saleem H, Simpson B. Biotinidase deficiency. In: StatPearls. Treasure Island, FL: StatPearls Publishing; 2024. Available at: https://www.ncbi.nlm.nih.gov/books/NBK560607/

27. Wolf B. Biotinidase deficiency: "if you have to have an inherited metabolic disease, this is the one to have". Genet Med 2012; 14: 565-575. https://doi.org/10.1038/gim.2011.6

The Turkish Journal of Pediatrics 2024; 66: 618-624
https://doi.org/10.24953/turkjpediatr.2024.5188

**Short Communication**

# Sexually transmitted infections in sexually abused children: an audit project to implement PCR tests in a child advocacy center in Türkiye

Sıtkı Tıplamaz[1], Zeynep Ergenc[2,3], Murat Yaman[4], Tuğçe Kalaman[5], Elvan Sayın[4], Fatih Hitami Usluoğulları[1], Zeynep Arzu İlki[4], Nurver Ülger Toprak[4], Eda Kepenekli Kadayıfçı[2,6], Mehmet Akif İnanıcı[1]

*[1]Department of Forensic Medicine, Faculty of Medicine, Marmara University, İstanbul; [2]Department of Pediatrics, Division of Pediatric Infectious Diseases, Faculty of Medicine, Marmara University, İstanbul, Türkiye; [3]Department of Paediatric Infectious Diseases and Immunology, Great Ormond Street Hospital for Children, London, United Kingdom; [4]Department of Medical Microbiology, School of Medicine, Marmara University, İstanbul; [5]Council of Forensic Medicine, İstanbul; [6]Department of Pediatrics, Division of Pediatric Infectious Diseases, Faculty of Medicine, Bahçeşehir University, İstanbul, Türkiye.*

## ABSTRACT

**Background.** Sexual abuse in children can sometimes result in sexually transmitted infections (STIs), which can serve as crucial forensic evidence. Although PCR methods are now accepted as the gold standard for STI screening, they have not yet widely replaced traditional culture methods in Türkiye. This study aims to assess the necessity of implementing PCR-based STI testing at Child Advocacy Centers in Türkiye, where such testing is not routinely available.

**Methods.** Conducted between February and November 2023, this study included children who presented to the Child Advocacy Center of Marmara University Pendik Training and Research Hospital. High-risk victims were identified based on criteria including a history of penetrative sexual abuse and factors such as multiple perpetrators or significant age disparity. Serological tests and genital swabs were collected and analyzed using both bacterial culture methods and a comprehensive STI PCR panel.

**Results.** The study included 20 victims, with a median age of 16 years. STI PCR testing detected pathogens in 19 out of 21 samples, including *Chlamydia trachomatis* (20%) and *Neisseria gonorrhoeae* (5%). In contrast, culture methods identified no sexually transmitted pathogens.

**Conclusion.** PCR testing demonstrated significantly higher sensitivity for detecting STIs compared to traditional bacterial culture methods, as expected. Implementing PCR-based STI testing in Child Advocacy Centers is an urgent and essential need for providing an accurate diagnosis and robust forensic evidence, enhancing the care and legal protection of sexually abused children.

**Key words:** nucleic acid amplification test (NAAT), sexually transmitted disease, child monitoring center.

Sexual abuse encompasses a wide range of behaviors, ranging from inappropriate gestures to acts of penetration. Contrary to prevailing misconceptions, such abuse typically does not result in visible physical trauma on the victim's body. However, it has been observed that

✉ Sıtkı Tıplamaz ▪ tiplamaz@gmail.com

Received 6th Aug 2024, revised 12th Sep 2024, accepted 30th Sep 2024.

The pre-analysis findings of this study have been presented in the 42nd annual meeting of ESPID (European Society of Pediatric Infectious Diseases) as a poster presentation.

children who have undergone sexual abuse are increasingly susceptible to contracting sexually transmitted infections (STIs). These infections, which include *Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis, Treponema pallidum*, human immunodeficiency virus (HIV), and herpes simplex virus (HSV) type 2, present not only immediate health risks but also significant long-term morbidity and mortality if left untreated.[1] Moreover, their contagious nature renders them a pertinent public health concern. Importantly, the presence of such infections can serve as crucial evidence within legal proceedings of alleged instances of sexual abuse, sometimes constituting the sole substantiating evidence of the purported incident.

As healthcare professionals, our foremost priority is to identify and treat these infections, followed by investigating their origin, particularly inquiring about the possibility of sexual abuse. Furthermore, in instances where allegations of sexual abuse arise, it is incumbent upon us to elucidate the significance of these findings to the judicial system and, as appropriate, meticulously preserve pertinent samples as evidence for subsequent submission to law enforcement agencies. The identification of infections caused by *Neisseria gonorrhoeae, Chlamydia trachomatis, Trichomonas vaginalis*, HIV (in cases where transmission through blood or contaminated needles has been ruled out), and *Treponema pallidum* among individuals under the age of 16, particularly when perinatal transmission has been discounted, serves as compelling evidence of sexual abuse (the provisions outlined in the current Turkish Penal Code, which stipulates the age threshold at which a child can legally consent to sexual intercourse as 16 years.).[2-4]

The nucleic acid amplification test (NAAT), commonly known as the PCR test, has been accepted as the gold standard instead of the traditional culture method for diagnosing sexually transmitted infections, exhibits considerably higher sensitivity.[5] The Centers for Disease Control and Prevention (CDC) has embraced the NAAT as a diagnostic tool, supplemented by a confirmatory NAAT targeting distinct genetic regions within the bacterial strains, thereby supplanting the conventional reliance on culture-based methods.[6]

Child Advocacy Centers (CACs) in Türkiye currently lack available STI PCR testing for routine use with sexually abused children. With this audit Project, we aimed to assess the necessity of STI PCR tests in CACs in Türkiye.

## Materials and Methods

The study was performed according to the World Medical Association Declaration of Helsinki principles. It was approved by the local ethics committee of the Medical Faculty of Marmara University (Protocol No: 09.2023.196). The study included children who applied to the CAC of Marmara University Pendik Training and Research Hospital, Türkiye between February and November 2023. They were recruited with the informed consent of both the child and their legal guardian (usually one of the parents) before the anamnesis. The study involved high-risk victims to assess the need for incorporating STI PCR testing into routine procedures at CACs. Criteria to identify high-risk victims were established based on the literatüre.[2,7,8] Victims in the high-risk group are identified by a history of penetrative sexual abuse along with one or more of the following factors: multiple perpetrators, vaginal discharge after the incident, substance use, significant age disparity (more than 5 years), or lack of familiarity between the child and the perpetrator. The age of the participants, their habits (such as smoking and alcohol use), medication use, number of sexual partners or assailants, and physical examination findings were all assessed.

### *Serology*

Serology tests including Anti-HBC IgG, Anti-HBC IgM, Syphilis antibody, Anti-HBS, HBsAg, Anti-HIV1/2+p24 Ag, Anti-HCV, and VDRL-

RPR have been quantified from all victims who consented to give blood samples.

### *Molecular methods*

Genital samples from all participants were collected from the vagina using Dacron swabs (Bioeksen, İstanbul-Türkiye). Following collection, the samples were immediately transported and stored at -80°C to preserve them until molecular testing. On the day of the study, samples were retrieved from the -80°C freezer and thawed at room temperature for approximately 30 minutes. Each sample was then vortexed for 15 seconds to ensure homogeneity.

DNA extraction was automated using the rapid nucleic acid isolation kit (Bioeksen, Istanbul-Türkiye) on a Zybio EXM 3000 instrument. Subsequently, the Bio-Speedy Sexually Transmitted Infections RT-qPCR Panel Kit (Bioeksen, Istanbul-Türkiye) was employed to qualitatively assess the presence of specific pathogens in the extracted DNA. According to the manufacturer's instructions, this kit utilises reverse transcription and real-time multiplex PCR (RT-qPCR).

HSV-1, HSV-2, *Mycoplasma genitalium*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Treponema pallidum*, *Trichomonas vaginalis*, *Ureaplasma parvum/urealyticum, Mycoplasma hominis, Haemophilus ducreyi, Streptococcus agalactiae, Gardnerella vaginalis* were screened from vaginal swabs by PCR test.

### *Bacterial culture methods*

Swabs submerged in Amies medium (True Line, China) underwent bacterial culture and Gram stain preparation. All samples were inoculated onto three different media: Columbia agar with 5% sheep blood (bioMérieux, France) for general bacterial growth, MacConkey agar (bioMérieux, France) to differentiate lactose-fermenting from non-fermenting bacteria, and selective chocolate agar (PolyViteX VCAT3, bioMérieux, France) for fastidious organisms. Any identified growth was further analyzed using MALDI-TOF MS (VITEK MS, bioMérieux, France) for definitive bacterial identification.

Genital cultures, and specimens for STI PCR panels were collected from all children meeting the inclusion criteria on the day of admission to the CAC. Regardless of the test results, all victims were administered prophylactic treatment consisting of a single intramuscular dose of ceftriaxone 500 mg, oral doxycycline 100 mg twice daily for 7 days, and oral metronidazole twice daily for 7 days as per guidelines.[3]

## Results

During that period, 638 applications were submitted, and 219 of them were physically examined. A total of twenty victims, encompassing twenty-one separate assault events, were included in the study. The median age of the victims was 16 years (25th percentile: 14 years, 75th percentile: 17 years). Upon examination of the victims' medical histories, a history of smoking was identified in 65% (n=13) of cases, alcohol use in 55% (n=11), use of antidepressant/antipsychotic medications in 55% (n=11), and past or present use of substances in 35% (n=7). Multiple sexual intercourses were experienced with more than one partner before the examination in 90.5% (n=19) of the cases. An age disparity exceeding five years between victim and perpetrator was observed in 47.6% (n=10) of cases. Notably, in 81% (n=17) of the incidents, the perpetrator was unknown to the child. A history of incest was documented in two assaults. Four victims (20%) complained of vaginal discharge. Thirteen victims (65%) had a genital traumatic lesion.

Nineteen out of 20 victims consented to blood sampling. All tested participants exhibited negative test results except anti-HBs marker. Eleven participants had negative results for anti-HBs, prompting the scheduling of hepatitis B vaccination.

The median interval between the assault event and the admission was detected as 12 days (25p: 0 day, 75p: 49 days).

**Table I.** The summary of the results.

| Case number | Vaginal discharge | Medical findings | Serologic tests | Vaginal culture | Vaginal PCR |
|---|---|---|---|---|---|
| 1 | + | Healed hymenal transection | Anti-HBS + | *Candida albicans* + | *Gardnerella vaginalis, Haemophilus ducreyi, Mycoplasma hominis, Ureaplasma parvum/urealyticum* |
| 2 | + | Healed hymenal transection | Anti-HBS + | *Candida albicans* + | *Gardnerella vaginalis, Haemophilus ducreyi, Mycoplasma hominis, Ureaplasma parvum/urealyticum* |
| 3 | - | Healed hymenal transection | - | Normal flora | *Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma parvum/urealyticum* |
| 4 | - | - | Anti-HBS + | Normal flora | *Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma parvum/urealyticum* |
| 5 | - | Healed hymenal transection | Anti-HBS + | Leukocyte +, Normal flora | *Gardnerella vaginalis, Ureaplasma parvum/urealyticum* |
| 6 | + | - | - | Normal flora | ***Chlamydia trachomatis***, *Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma parvum/urealyticum* |
| 7 | - | Healed hymenal transection | - | Leukocyte +, *Candida albicans* + | ***Chlamydia trachomatis***, *Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma parvum/urealyticum* |
| 8 | - | Healed hymenal transection | - | Leukocyte +, Normal flora | *Gardnerella vaginalis, Haemophilus ducreyi, Ureaplasma parvum/urealyticum* |
| 9 | - | Healed hymenal transection | - | Normal flora | *Gardnerella vaginalis, Ureaplasma parvum/urealyticum* |
| 10 | - | Healed hymenal transection | - | Leukocyte +, Normal flora | *Gardnerella vaginalis* |
| 11 | - | - | Anti-HBS + | Normal flora | ***Neisseria gonorrhoeae***, *Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma parvum/urealyticum* |
| 12 | - | - | Anti-HBS + | Leukocyte +, Normal flora | *Ureaplasma parvum/urealyticum* |
| 13 | - | Healed hymenal transection | - | Normal flora | ***Chlamydia trachomatis***, *Gardnerella vaginalis, Ureaplasma parvum/urealyticum* |
| 14 | - | Healed hymenal transection | Anti-HBS + | Normal flora | - |
| 15 | + | - | - | Normal flora | *Gardnerella vaginalis, Ureaplasma parvum/urealyticum* |
| 16 | - | - | - | Normal flora | ***Chlamydia trachomatis***, *Gardnerella vaginalis, Mycoplasma hominis, Ureaplasma parvum/urealyticum* |
| 17 | - | Healed hymenal transection | Anti-HBS + | *Candida glabrata* + | *Ureaplasma parvum/urealyticum* |
| 18 | - | Healed hymenal transection | X | Normal flora | *Gardnerella vaginalis, Ureaplasma parvum/urealyticum* |
| 19 | - | Healed hymenal transection | - | *Staphylococcus aureus* + | *Gardnerella vaginalis, Ureaplasma parvum/urealyticum* |
| 20 | - | - | - | Normal flora | *Gardnerella vaginalis, Ureaplasma parvum/urealyticum* |
| 21 | - | - | Anti-HBS + | Normal flora | - |

Note: "+", detected; "-", not detected, "x", not taken. Case 4 and Case 11 are the same person but involved distinct incidents.

Genital swab culture yielded *Candida albicans* in 3 samples, *Candida glabrata in 1 sample,* and *Staphylococcus aureus* in 1 sample. However, no sexually transmitted infections were detected in any victim by culture.

PCR assays identified *Chlamydia trachomatis* in four victims with cycle threshold (CT) values of 19.2, 20.6, 23.5 and 24.0, respectively. *Haemophilus ducreyi* was detected in three victims with CT values of 17.6, 23.1, and 28.0, and *Neisseria gonorrhoeae* was identified in one victim with a CT value of 14.6. The overall results are summarized in Table I. Positive and negative control samples were run, and all results are shared in the supplementary data.

## Discussion

This study reaffirmed that the PCR method exhibits greater sensitivity and specificity compared to conventional culture methods. The incorporation of STI PCR testing is crucial in the management of sexually abused children and should be implemented in all CACs.

While no sexually transmitted pathogens were identified using cultures, PCR identified at least one pathogen in 19 out of 21 samples. This finding aligns with previous research.[5] These PCR-identified pathogens do not necessarily indicate an active infection. However, the CT threshold may inversely reflect the pathogen loads. Four cases (20%) had *C. trachomatis*, and one case (5%) had *N. gonorrhoeae*. Similar to other studies, *N. gonorrhoeae* prevalence was lower than *C. trachomatis*.[9] The detection of the STI pathogen rate in our study was higher than in other studies[10-12], which is thought to be due to participants having high-risk factors. We did not find a statistically significant difference in detecting STI pathogens between the group with traumatic genital findings and the group without. It is important to note that the sample size of this study was small for generalization, and all participants were already in the high-risk group for STIs.

Even though the participants were postpubertal children, the prepubertal children are admitted to the CAC. The prevalence of STIs is lower in prepubertal children compared to postpubertal. Standard culture methods lack sensitivity, and there is also a lack of data on the sensitivity and specificity of NAATs in the prepubertal age group.[13,14] These limitations pose extra challenges for accurate diagnosis and management of STIs in prepubertal children. We also should keep in mind that NAAT can detect bacteria without clear clinical significance, so cautious medical interpretation is required.[11]

A case involving two separate examinations is presented, prompted by a second criminal act committed three and a half months following the initial incident. The first PCR test for *N. gonorrhoeae* yielded a negative result, while the subsequent PCR test conducted during the second examination returned a positive result. Notably, the initial incident transpired three months before the first examination, while the second incident occurred only five days before the second examination. This finding has the potential to serve as evidence of sexual assault of the second crime, contingent upon a thorough investigation into the chronological details surrounding both incidents.

In three cases, despite the absence of any anogenital traumatic injury, PCR detected *C. trachomatis* in two cases and *N. gonorrhoeae* in one case. These infections constituted crucial physical evidence of alleged sexual abuse. Using culture methods alone would not have identified these infectious agents, underscoring the importance of STI screening at CACs. Given the potential legal ramifications of positive results, microbiology laboratories must retain both samples and isolates. Furthermore, authorities should establish and approve PCR screening and confirmatory tests, such as those endorsed by the FDA, if not already in place.[14]

Culture is not a diagnostic assay like PCR; hence, there exists a possibility of cultivating an agent in culture that may not be detected by the PCR test.[15,16] For this reason, and sensitivity

and specificity issues, the CACs must start to use PCR tests for STI, but also might continue to take culture samples along with PCR tests.

The updated guideline now recommends that STI testing should not be restricted solely to sites where penetration is described. This is due to the possibility of incomplete disclosures by the child and the potential for contiguous spread from the genitals to the anus, particularly in females. Confirmatory testing of positive results is recommended, particularly in situations where the findings may have legal significance, such as in children who are under 16 or sexually non-active (according to the Turkish Penal Code, it is currently in force). Pharyngeal *N. gonorrhea* and *C. trachomatis* have been added to the "Infections caused by sexual contact, if confirmed by appropriate testing, and perinatal transmission has been ruled out" section of the recent guideline.[2] Testing urine samples from boys is also recommended in the literature.[17] However, we exclusively collected vaginal samples from the children, as the PCR test kits utilized in this study are validated solely for vaginal swabs.

The major limitation of our study is the small sample size, which impeded the attainment of statistically significant results. Additionally, the absence of a control group consisting of non-sexually abused children further constrained the study's robustness. Another significant limitation was the lack of a confirmatory NAAT targeting different regions of the genetic material of the infectious agents, which could have validated the initial test results.

Our study reconfirmed that culture techniques are insufficient for the detection of STIs in sexually abused children. This audit highlights the imperative need for the implementation of PCR testing in CACs across Türkiye. Such measures not only enhance diagnostic accuracy but also provide robust forensic evidence for legal proceedings.

## Supplementary materials

Supplementary materials for this article are available online at https://doi.org/10.24953/turkjpediatr.2024.5188

## Ethical approval

The study was performed according to the principles of the World Medical Association Declaration of Helsinki. It was approved by the local ethics committee of the Medical Faculty of Marmara University (Protocol No: 09.2023.196).

## Author contribution

Study conception and design: ST, ZE, MY, ES, TK, FHU, ZAİ, NÜT, EKK, MAİ; data collection: ST, TK, MY, ES; analysis and interpretation of results: ST, ZE, TK, MY, ES; draft manuscript preparation: ST, ZE, TK. All authors reviewed the results and approved the final version of the article.

## Source of funding

The authors declare that the study received no funding. Bio-Speedy Sexually Transmitted Infections RT-qPCR Panel Kit (Bioeksen, Istanbul-Türkiye) was donated by Bioeksen Molecular Diagnostic Company.

## Conflict of interest

The authors declare that there is no conflict of interest.

## REFERENCES

1. Girardet RG, Lahoti S, Howard LA, et al. Epidemiology of sexually transmitted infections in suspected child victims of sexual assault. Pediatrics 2009; 124: 79-86. https://doi.org/10.1542/peds.2008-2947
2. Kellogg ND, Farst KJ, Adams JA. Interpretation of medical findings in suspected child sexual abuse: an update for 2023. Child Abuse Negl 2023; 145: 106283. https://doi.org/10.1016/j.chiabu.2023.106283

3. Kimberlin DW, Barnett ED, Lynfield R, Sawyer MH, editors. Red Book: 2021-2024 Report of the Committee on Infectious Diseases. American Academy of Pediatrics; 2021.

4. T.C. Resmi Gazete. Türk Ceza Kanunu. Available at: https://www.resmigazete.gov.tr/eskiler/2004/10/20041012.htm

5. Seña AC, Hsu KK, Kellogg N, et al. Sexual assault and sexually transmitted ınfections in adults, adolescents, and children. Clin Infect Dis 2015; 61(Suppl 8): S856-S864. https://doi.org/10.1093/cid/civ786

6. Centers for Disease Control and Prevention (CDC). Sexually Transmitted Infections Treatment Guidelines, 2021: Sexual Assault or Abuse of Children. Available at: https://www.cdc.gov/ std/ treatment-guidelines/sexual-assault-children. htm (Accessed on March 12, 2024).

7. Centers for Disease Control and Prevention (CDC). Sexually Transmitted Infections Treatment Guidelines, 2021: Sexual Assault and Abuse and STIs – Adolescents and Adults. Available at: https://www.cdc.gov/std/treatment-guidelines/sexual-assault-adults.htm (Accessed on March 12, 2024).

8. Okiring J, Getahun M, Gutin SA, et al. Sexual partnership concurrency and age disparities associated with sexually transmitted infection and risk behavior in rural communities in Kenya and Uganda. Int J Infect Dis 2022; 120: 158-167. https://doi.org/10.1016/j.ijid.2022.04.038

9. Kellogg ND, Melville JD, Lukefahr JL, Nienow SM, Russell EL. Genital and extragenital gonorrhea and chlamydia in children and adolescents evaluated for sexual abuse. Pediatr Emerg Care 2018; 34: 761-766. https://doi.org/10.1097/PEC.0000000000001014

10. Hernández Ragpa L, Valladolid Urdangaray A, Ferrero Benéitez OL, Díez Sáez C. Sexually transmitted infections in the context of a sexual assault. Enferm Infecc Microbiol Clin (Engl Ed) 2019; 37: 187-194. https://doi.org/10.1016/j.eimc.2018.11.004

11. Park JH, Kim N, Shin S, Roh EY, Yoon JH, Park H. Prevalence and correlated factors of sexually transmitted infections among women attending a Korean sexual assault center. J Forensic Leg Med 2020; 71: 101935. https://doi.org/10.1016/j.jflm.2020.101935

12. Skjælaaen K, Nesvold H, Brekke M, et al. Sexually transmitted infections among patients attending a sexual assault centre: a cohort study from Oslo, Norway. BMJ Open 2022; 12: e064934. https://doi.org/10.1136/bmjopen-2022-064934

13. Black CM, Driebe EM, Howard LA, et al. Multicenter study of nucleic acid amplification tests for detection of chlamydia trachomatis and neisseria gonorrhoeae in children being evaluated for sexual abuse. Pediatr Infect Dis J 2009; 28: 608-613. https://doi.org/10.1097/INF.0b013e31819b592e

14. Qin X, Melvin AJ. Laboratory diagnosis of sexually transmitted ınfections in cases of suspected child sexual abuse. J Clin Microbiol 2020; 58: e01433-e01419. https://doi.org/10.1128/JCM.01433-19

15. Folaranmi TA, Kretz CB, Kamiya H, et al. Increased risk for meningococcal disease among men who have sex with men in the United States, 2012-2015. Clin Infect Dis 2017; 65: 756-763. https://doi.org/10.1093/cid/cix438

16. Marchand-Senécal X, Bekal S, Pilon PA, Sylvestre JL, Gaudreau C. Campylobacter fetus cluster among men who have sex with men, Montreal, Quebec, Canada, 2014-2016. Clin Infect Dis 2017; 65: 1751-1753. https://doi.org/10.1093/cid/cix610

17. Workowski KA, Bachmann LH, Chan PA, et al. Sexually Transmitted Infections Treatment Guidelines, 2021. MMWR Recomm Rep 2021;70(No. RR-4):1–187. http://doi.org/10.15585/mmwr.rr7004a1

The Turkish Journal of Pediatrics 2024; 66: 625-629
https://doi.org/10.24953/turkjpediatr.2024.4895



# Parvovirus B19 infection in children: Is it more severe than expected?

Öznur Ege Akdi[1], Kübra Aykaç[1], Ali Bülent Cengiz[1], Yasemin Özsürekçi[1]

[1]*Department of Pediatric Infectious Diseases, Faculty of Medicine, Hacettepe University, Ankara, Türkiye.*

Human parvovirus B19 infection often manifests in the community as a mild exanthematous disease of childhood.[1] Complications such as headache, arthritis, and arthralgia occur less frequently in children compared to adults. However, certain populations including pregnant women, immunocompromised patients, and those with chronic hemolytic anemia, are at increased risk of developing serious complications due to parvovirus B19 infection.[2] It is of great importance to be aware of the potential outbreaks due to parvovirus B19 and the periodic increases in case numbers, especially in high-risk groups. Various outbreaks of parvovirus B19 have been reported worldwide at different times in non-tropical regions during winter and spring, typically lasting 3-6 months. Of the well-documented 30 erythema infectiosum outbreaks, 23 occurred between March and May, and these outbreaks affected 50 to 165 people. Furthermore, retrospective analysis of 50 years of data in North America has shown a cyclical pattern with increased disease activity approximately every 6 years, lasting for three years.[3,4]

In our center, cases identified have similarly occurred between March and May 2024, consistent with the literature (Table I). Outbreaks predominantly affect children aged 5-14 years and their close contacts, including parents and teachers.[5,6] Among the six patients, the age of the cases ranged from 15 months to 7 years. Although transmission primarily occurred via respiratory droplets, transmission through blood product transfusion, clotting factor concentrates, intravenous immunoglobulin (IVIG) infusion, and post-organ transplant donor transmission has also been reported.[7-9]

Parvovirus B19 infection can lead to erythema infectiosum, intrauterine infection with hydrops fetalis, transient aplastic crisis, myocarditis, vasculitis, hepatitis, and various neurological disorders.[10] Of our patients, two had erythema infectiosum, three had myocarditis, one had aplastic crisis, and subsequently developed hemophagocytic lymphohistiocytosis. All the patients, who presented to the clinic with various complaints between March 2024 and May 2024, required hospitalization for various reasons, and received a diagnosis of parvovirus B19 infection. There is no consensus on which symptoms warrant parvovirus PCR testing and when it is appropriate to perform this test. The patients underwent parvovirus PCR testing on the day of admission while the etiological investigations for their diagnosis were being planned.

One of the patients diagnosed with erythema infectiosum was a 5-year-old boy with T-cell acute lymphoblastic leukemia (T-ALL), presenting with fever persisting for 2 days, macular rash on the face and extremities, and swelling in the hands and feet. His parvovirus B19 PCR was markedly elevated at

✉ Yasemin Özsürekçi ▪ yas.oguz99@yahoo.com

Received 28th Jun 2024, revised 8th Aug 2024, 30th Sep 2024, accepted 21th Oct 2024.

640,200,000,000 IU/mL. He received 2 g/kg of IVIG over 5 days and was discharged after a 10-day hospital stay. Follow-up parvovirus PCR testing three months later was negative. The patient also exhibited pancytopenia, but it was difficult to distinguish whether it was related to the chemotherapy he received 5 days prior or secondary to parvovirus B19 infection. Another patient presented to the hospital during the exanthematous phase of the disease with a rash noticed on the cheeks. We learned that a week before admission, the patient had non-specific complaints such as weakness, headache, and subfebrile fever during the initial viremic phase of the illness. This patient, aged 6.5 years with a diagnosis of thalassemia intermedia, also had anemia, and their parvovirus PCR resulted in 120,700,000 IU/mL. They received 2 g/kg of IVIG and were discharged after a 5-day hospital stay. Both patients were hospitalized primarily due to the need for a transfusion.

Three other patients, initially diagnosed with myocarditis, were transferred from other hospitals and admitted directly to the intensive care unit (ICU) due to signs of heart failure. These children, who had no previous health conditions, presented with acute heart failure symptoms, elevated troponin levels, and abnormal echocardiographic findings, confirming myocarditis. Upon admission, all three had elevated troponin-I and brain natriuretic peptide levels and required inotropic support, IVIG, and steroid therapy (Table I).

- The first patient, a 15-month-old girl, exhibited nasal discharge and poor appetite for ten days, followed by facial swelling and decreased urine output. Her echocardiogram showed a reduced ejection fraction (EF) of 35%. A parvovirus PCR test confirmed the diagnosis at 152,000 IU/mL. She was discharged after 18 days in the hospital, including 10 days in ICU.
- The second patient, a 5-year-old, presented with a recent onset of poor appetite, restlessness, and nocturnal episodes of crying. An echocardiogram revealed a low

**Table I.** Characteristics of the patients with parvovirus B19 infection

| Patient no | Age | Sex | Underlying conditions | Presentation symptoms | Clinical diagnosis at admission | Parvovirus PCR (IU/mL) | Hematologic findings | Treatment | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 5 years | M | T-cell acute lymphoblastic leukemia | Fever, macular rash, swelling in hands and feet | Erythema infectiosum | 640,200,000,000 | Pancytopenia | IVIG | Recovery |
| 2 | 6.5 years | M | Thalassemia intermedia | Rash on the cheeks | Erythema infectiosum | 120,700,000 | Anemia | IVIG | Recovery |
| 3 | 15 months | F | None | Facial swelling, decreased urine output | Myocarditis | 152,000 | Normal | IVIG and steroid | Recovery |
| 4 | 5 years | M | None | Presyncope | Myocarditis | 6,600 | Normal | IVIG and steroid | Recovery |
| 5 | 2 years | F | None | Edema in hands and feet | Myocarditis | 27,480 | Normal | IVIG and steroid | Recovery |
| 6 | 7 years | M | Hereditary spherocytosis | Intermittent fever, palpitation | Aplastic crisis and HLH | 157,000 | Pancytopenia | IVIG and steroid | Recovery |

F, female; HLH, hemophagocytic lymphohistiocytosis; ICU, intensive care unit; IVIG, intravenous immunoglobulin; M, male; PCR, polymerase chain reaction.

EF, and a parvovirus PCR test was 6,600 IU/mL. This patient spent five days in the ICU and an additional 10 days in the general ward.

- The third patient, a 2-year-old, had a two-week history of cough and intermittent fever, later developing edema in the hands and feet. Imaging showed bilateral pleural effusion and cardiomegaly, with an EF of 30% and a parvovirus PCR of 27,480 IU/mL. Despite treatment with IVIG and steroids, the patient had a prolonged recovery, requiring approximately 1.5 months of hospitalization, including five days in the ICU.

It is important to note that while the presence of the virus in the blood does not necessarily indicate that the myocardium is infected by the same pathogen, the clustering of parvovirus PCR-positive cases without other identifiable causes cannot be disregarded. It should also be noted that since our hospital is a tertiary referral center, myocarditis patients were referred from surrounding provinces. Therefore, it is possible that we encountered more severe cases of parvovirus B19 myocarditis within a short timeframe of two months. Due to the lack of data on how many of the myocarditis cases previously diagnosed and followed in our clinic were parvovirus PCR positive, it has not been possible for us to make comparisons with previous years.

Lastly, a 7-year-old patient with hereditary spherocytosis was admitted with intermittent fever and palpitations. The patient's diagnostic workup, prompted by pancytopenia, revealed a parvovirus PCR of 157,000 IU/mL, suggesting an aplastic crisis. Although an aplastic crisis is a known complication of parvovirus B19 in patients with chronic hemolytic anemia, persistent fever, worsening pancytopenia, and elevated ferritin levels raised concerns about hemophagocytic lymphohistiocytosis (HLH). After initiating IVIG treatment based on a preliminary diagnosis, further HLH-specific testing and bone marrow aspiration confirmed HLH. Steroids were added due to the patient's resistant fever, and the patient was discharged after 15 days without the need for intensive care.

Due to having 6 patients who tested positive for parvovirus B19 with various clinical presentations in the past few months, we organized an online meeting on May 29, 2024, to discuss the situation nationwide and share our experiences with other clinicians. Shortly after this meeting, the European Centre for Disease Prevention and Control (ECDC) issued an evaluation drawing attention to reports of increased frequency of parvovirus B19 cases in several European countries as of June 5, 2024.[11] The report highlighted significant increases in the number of pregnant women infected with parvovirus B19 in Denmark by the end of 2023 and early 2024 (higher than the increase observed in 2017), rising rates of parvovirus B19 infection among both pediatric age groups and pregnant women as well as blood donors in France, and an increased number of erythema infectiosum cases identified among pediatric populations in the Netherlands, alongside frequent detections among blood donors.[12,13] According to the report, the risk posed by parvovirus B19-related illness has been assessed across four different populations. While the infection is considered low-risk for the general population, it is deemed to be of moderate to high risk for immunocompromised individuals (such as those using immunosuppressive drugs, HIV-infected persons, cancer patients, and organ transplant recipients) and patients with chronic hemolytic anemia. Consistent with this risk assessment, the infection in patients diagnosed with hereditary spherocytosis as in one of our cases, has resulted in a more severe clinical impact compared to other patients.

In this assessment, considerations were also made regarding measures to protect populations at risk of developing severe complications due to parvovirus B19 infection. Priority was given to ensuring that healthcare workers are aware of the increasing prevalence of parvovirus B19 infection. Recommendations also include

monitoring the immunity of pregnant women working in high-risk professions, such as healthcare and teaching, where there is a heightened risk of exposure to parvovirus B19.[11]

It is important to note the periodic increases in parvovirus B19 infections and exercise caution regarding potential complications in at-risk patient groups. In the post-pandemic era, it is essential to determine whether the recent rise in parvovirus B19 infections mirrors the intermittent patterns observed prior to the pandemic or if it displays distinct characteristics. Such evaluations should be integrated into the ongoing process of diagnosing and monitoring patients over time, allowing for timely intervention and better management of potential outbreaks.

The lack of knowledge regarding the number of cases with positive parvovirus B19 PCR from previous years, the hospitalization rates due to parvovirus B19 infection, and the absence of comparisons with previous years are limitations of this study.

### Ethical approval

The authors declare that they have obtained informed consent from the parents of the presented patients for their data to be included in this article.

### Author contribution

The authors confirm contribution to the paper as follows: Letter conception and design: ÖY; literature review: EAÖ; draft manuscript preparation: OY, EOA. 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. Anderson MJ, Lewis E, Kidd IM, Hall SM, Cohen BJ. An outbreak of erythema infectiosum associated with human parvovirus infection. J Hyg (Lond) 1984; 93: 85-93. https://doi.org/10.1017/s0022172400060964
2. Cherry JD, Schulte DJ. Human parvovirus B19. In: Cherry JD, Harrison GJ, Kaplan SL, Steinbach WJ, Hotez PJ, editors. Feigin and Cherry's Textbook of Pediatric Infectious Diseases. 8th ed. Vol 2. Philadelphia: Elsevier; 2019: 1330-1341.
3. Serjeant GR, Topley JM, Mason K, et al. Outbreak of aplastic crises in sickle cell anaemia associated with parvovirus-like agent. Lancet 1981; 2: 595-597. https://doi.org/10.1016/s0140-6736(81)92739-2
4. Edelson RN, Altman R. Erythema infectiosum: a statewide outbreak. J Med Soc N J 1970; 67: 805-809.
5. Gillespie SM, Cartter ML, Asch S, et al. Occupational risk of human parvovirus B19 infection for school and day-care personnel during an outbreak of erythema infectiosum. JAMA 1990; 263: 2061-2065.
6. Gonçalves G, Correia AM, Palminha P, Rebelo de Andrade H, Alves A. Outbreaks caused by parvovirus B19 in three Portuguese schools. Euro Surveill 2005; 10: 9-10. https://doi.org/10.2807/esm.10.06.00549-en
7. Hayakawa F, Imada K, Towatari M, Saito H. Life-threatening human parvovirus B19 infection transmitted by intravenous immune globulin. Br J Haematol 2002; 118: 1187-1189. https://doi.org/10.1046/j.1365-2141.2002.03741.x
8. Yee TT, Cohen BJ, Pasi KJ, Lee CA. Transmission of symptomatic parvovirus B19 infection by clotting factor concentrate. Br J Haematol 1996; 93: 457-459. https://doi.org/10.1046/j.1365-2141.1996.5161062.x
9. Yango A, Morrissey P, Gohh R, Wahbeh A. Donor-transmitted parvovirus infection in a kidney transplant recipient presenting as pancytopenia and allograft dysfunction. Transpl Infect Dis 2002; 4: 163-166. https://doi.org/10.1034/j.1399-3062.2002.01007.x
10. Anderson MJ, Higgins PG, Davis LR, et al. Experimental parvoviral infection in humans. J Infect Dis 1985; 152: 257-265. https://doi.org/10.1093/infdis/152.2.257

11. European Centre for Disease Prevention and Control. Risks posed by reported increased circulation of human parvovirus B19 in the EU/EEA. 2024. Available at: https://www.ecdc.europa.eu/en/publications-data/risks-posed-reported-increased-circulation-human-parvovirus-b19-eueea (Accessed on Jun 5, 2024).

12. Guillet M, Bas A, Lacoste M, et al. New atypical epidemiological profile of parvovirus B19 revealed by molecular screening of blood donations, France, winter 2023/24. Euro Surveill 2024; 29: 2400253. https://doi.org/10.2807/1560-7917.ES.2024.29.21.2400253

13. Nordholm AC, Trier Møller F, Fischer Ravn S, et al. Epidemic of parvovirus B19 and disease severity in pregnant people, Denmark, January to March 2024. Euro Surveill 2024; 29: 2400299. https://doi.org/10.2807/1560-7917.ES.2024.29.24.2400299

The Turkish Journal of Pediatrics 2024; 66: 630-636
https://doi.org/10.24953/turkjpediatr.2024.4856

Case Report

# Multipotent mesenchymal stromal cell therapy for a neonate with congenital diaphragmatic hernia and adhesive small bowel obstruction

Şükran Yıldırım[1], Aliye Kandırıcı[2]

[1]*Department of Neonatology, Prof. Dr. Cemil Taşcıoğlu City Hospital, University of Health Sciences, İstanbul;* [2]*Department of Pediatric Surgery, Prof. Dr. Cemil Taşcıoğlu City Hospital, University of Health Sciences, İstanbul, Türkiye.*

## ABSTRACT

**Background.** In the last decade, therapy using multipotent mesenchymal stromal cells (MSCs) has offered hope for regenerating the lungs of preterm babies with chronic lung disease. Due to similar disease mechanisms, it is logical to explore the potential impact of MSC therapy on pulmonary hypoplasia in congenital diaphragmatic hernia. Furthermore, MSCs may also contribute to the regeneration of the intestines affected by adhesive small bowel obstruction in patients with congenital diaphragmatic hernia.

**Case presentation.** A female newborn, delivered at 32 weeks and six days gestational age, was diagnosed with a left congenital diaphragmatic hernia. After surgical repair and respiratory/nutritional support for 39 days, she was still dependent on a ventilator and total parenteral nutrition. Two MSC treatments were given a week apart: 10 million cells/kg intratracheally and 5 million cells/kg intravenously. She was extubated, and her enteral nutrition improved after the treatment. No side effects were detected. We present the first documented case using MSCs derived from the umbilical cord to simultaneously treat pulmonary hypoplasia and adhesive small bowel obstruction of congenital diaphragmatic hernia.

**Conclusion.** Although MSC treatment is very promising for pulmonary hypoplasia and adhesive small bowel disease of congenital diaphragmatic hernia, much more needs to be learned about potential side effects, appropriate dosage, and the optimal method of administration.

**Key words:** mesenchymal stromal cell, congenital diaphragmatic hernia.

Congenital diaphragmatic hernia (CDH) is one of the most challenging diseases of the neonatal period, with a mortality rate of up to 60%.[1] Pulmonary hypertension and pulmonary hypoplasia, leading to respiratory failure, are the significant causes of death. Long-term ventilatory support adds diffuse alveolar damage, inflammation, and interstitial fibrois to pulmonary hypoplasia.[2] There is little to offer newborns after birth, aside from managing pulmonary hypertension, providing ventilatory support / extracorporeal membrane oxygenation (ECMO), and offering nutritional support[3,4], Multipotent mesenchymal stromal cell (MSC) therapy has emerged as a promising approach for the regeneration of hypoplastic lungs in the last decade in preterm babies.[5] MSC therapy has also been used for the treatment of hypoplastic lungs in CDH antenatally in animal models.[6-8] Nevertheless, limited experience is conveyed postnatally, particularly among humans.[9]

Another severe cause of mortality and morbidity in patients with CDH is adhesive small bowel obstruction (ASBO), which occurs mainly

✉ Şükran Yıldırım ▪ drsukranyildirim@yahoo.com

Received 18th Jun 2024, revised 1st Aug 2024, accepted 1st Oct 2024.

after midline laparotomy and patch repair.[10,11] Increased circulating adhesion molecules in CDH patients with pulmonary hypertension seem to contribute to the process.[12] An 11 to 20% incidence of ASBO has been reported in CDH survivors.[13] Occasionally, ASBO progresses to necrotizing enterocolitis (NEC).[13,14] NEC may occur even before surgery.[15] It is one of a neonate's most significant life-threatening diseases.[16] Also, severe morbidities, like strictures, adhesions, cholestasis, short bowel syndrome, and neurological sequelae, are possible. Conventional treatment is limited to supportive care and, at times, surgery intervention.[17] MSC treatment has gained interest in treating NEC like other neonatal disorders.[18]

We present a case of a newborn with CDH, pulmonary hypoplasia, ASBO, and NEC who showed improvement following MSC treatment.

## Case presentation

A female newborn born at 32 weeks six days gestational age with a birthweight of 2,000 grams was diagnosed with a CDH at 29 gestational weeks. She was intubated right after delivery and transferred to the neonatal intensive care unit. Her first chest X-ray is seen in Fig. 1. The abdominal and cranial ultrasounds, as well as the echocardiogram, showed no other associated anomalies. At four days old, she underwent surgery to repair a left diaphragmatic hernia. During the surgery, her stomach, spleen, cecum, ascending colon, transverse colon, and intestines were found in the left hemithorax. The left lung was hypoplastic, and a patch repair was performed. Following the surgery, she was placed on a high-frequency oscillator ventilator for eight days and then on synchronized intermittent mandatory ventilation (SIMV). A follow-up echocardiogram on the fourth day revealed a non-significant patent ductus arteriosus (PDA), which closed spontaneously after three days, and a patent foramen ovale (PFO). The pressure in the right ventricle, calculated from the tricuspid valve, was 40 mmHg. She had feeding intolerance for ten days and had ileal perforation (Fig. 2) on the 10th day, for which she had a second operation (primary repair). Two extubation attempts failed because of pulmonary hypoplasia, pneumothorax of the left lung, and ventilator-associated pneumonia. Minimal enteral feeding attempts failed, and an abdominal X-ray revealed pneumatosis intestinalis on the 31st day. The family was offered the option of MSC treatment. With the consent of the family and approval from the Republic of Türkiye Ministry of Health, Organ and Tissue Unit, MSCs were administered at doses of 10 million cells/kg intratracheally and 5 million cells/kg intravenously, twice on the 39th and 45th postnatal days. Fig. 3 illustrates the chest X-ray taken ten days after the initial treatment. The patient's respiratory, nutrition,

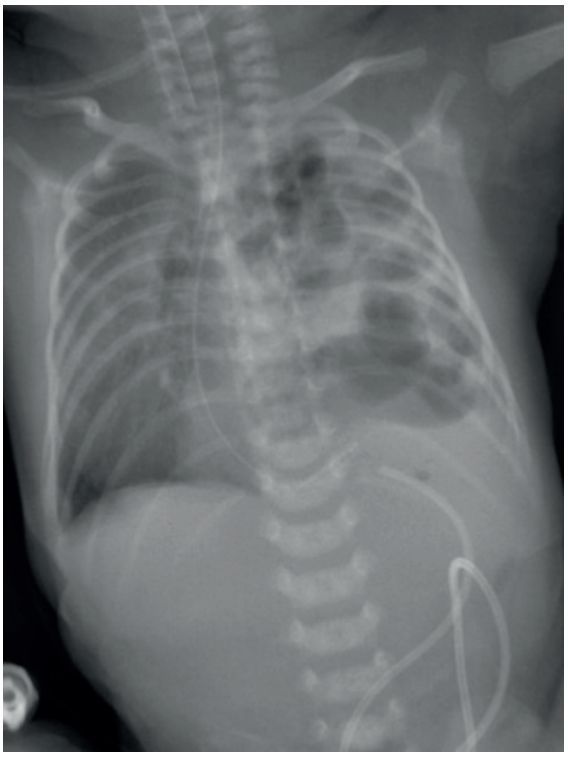


**Fig. 1.** The first radiographic imaging of the patient. The loops of the intestine are found in the left hemithorax, with the mediastinum shifted to the right. The umbilical catheter trace is displaced to the left due to the shifting of the abdominal organs. The abdominal cavity contains very little gas.

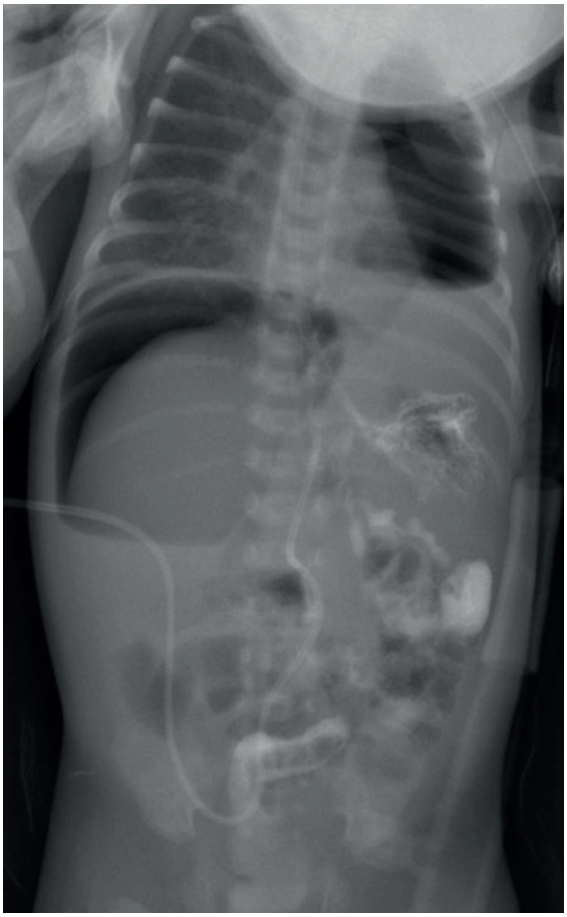


**Fig. 2.** The radiographic imaging of the patient on the tenth day. Subdiaphragmatic free air is present due to intestinal perforation. The left lung is hypoplastic, and the right lung appears hazy.

and clinical status before and after MSC treatment are seen in Fig. 4. The ventilation of both the left and right lung had improved, and the intestinal loops appeared normal. The patient was discharged on the 50th day.

Follow-up examinations at seven months revealed a minor delay in fine motor skills, and no tumors were found on a computed tomography (CT) scan of the chest or on abdominal ultrasound. The family provided written informed consent to publish this case report.

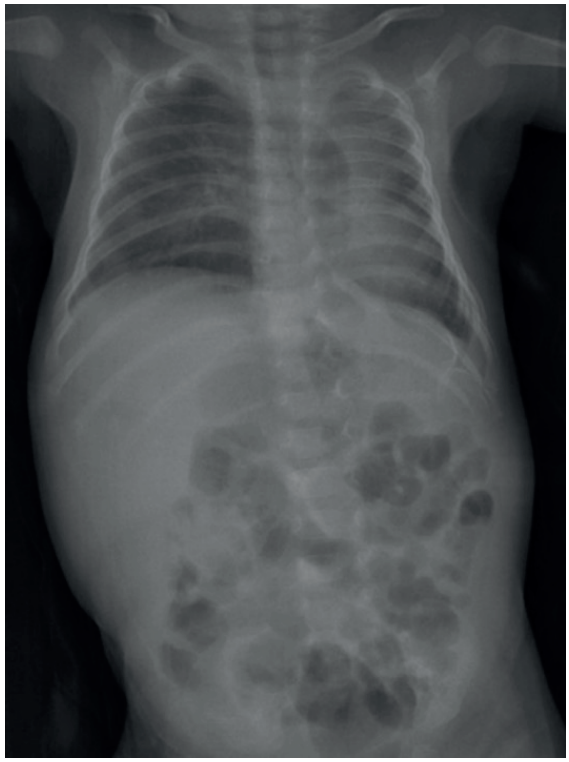


**Fig. 3.** The patient's radiographic imaging after ten days of the initial mesenchymal stromal cell treatment. The ventilation of both the left and right lung has improved. The appearance of the intestinal loops is normal.

## Discussion

Mesenchymal stromal cells (MSCs) hold tremendous potential in regenerative medicine. MSCs offer numerous benefits, including anti-inflammatory, anti-apoptotic, anti-oxidative, anti-fibrotic, pro-angiogenic, and anti-microbial effects. They can transform into damaged tissue cells and treat various medical conditions.[19] Mesenchymal stromal cells have broad biodistribution and poor homing efficiency to most target tissues because they are plastic adherent and entrapped in other organs' capillaries.[20] Their primary regeneration method involves releasing regenerative compounds such as cytokines, growth factors, and microRNAs, crucial to the

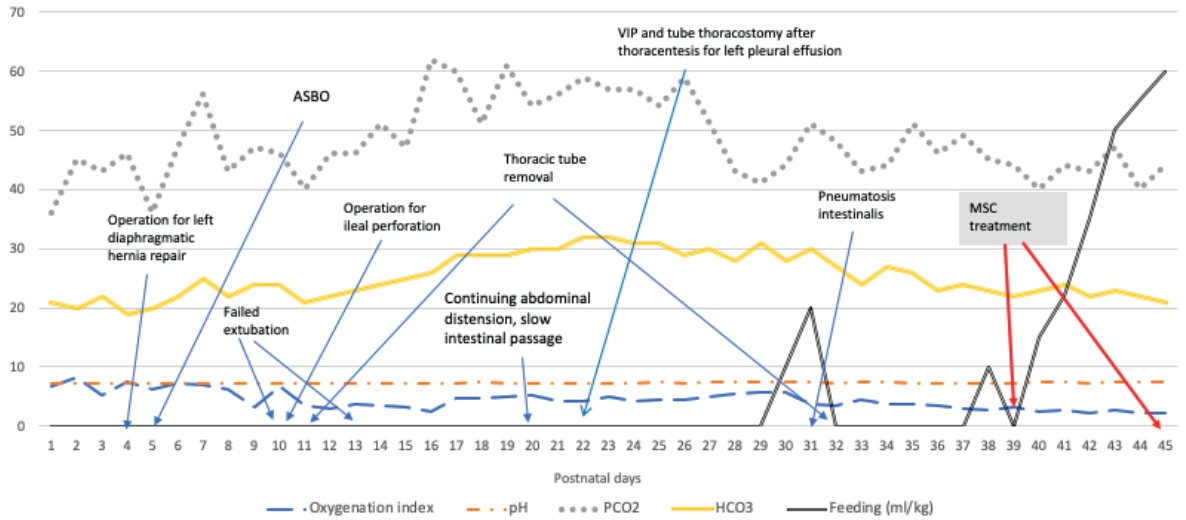



**Fig. 4.** The patient's respiratory, nutrition, and clinical status before and after MSC treatment.

ASBO, adhesive small bowel obstruction; CPAP, continuous positive airway pressure; $HCO_3$, bicarbonate (mEq/L); HFO, high-frequency oscillation; MSC, mesenchymal stromal cell; $PCO_2$, partial carbon dioxide pressure (mmHg); SIMV, synchronized mandatory ventilation; VAP, ventilator-associated pneumonia.

body's natural healing processes. So, despite poor engraftment, MSCs are still effective.[21] There are several ways to increase engraftment and improve the therapeutic effect, such as administering multiple doses, delivering MSCs close to the targeted organ, and priming MSCs with hypoxia or biochemical agents to increase homing.[22-25] Entrapment primarily occurs in the lungs[26], which is advantageous when the lung is the desired site.

Therefore, intravenous and endotracheal routes have proven successful in treating chronic lung disease in preterm babies such as bronchopulmonary dysplasia (BPD). In a phase II trial, MSC treatment for BPD successfully used a dose of 1x10 million cells/kg administered intratracheally. Clinical trials have shown that an IV dose of 1-10 million/kg MSC is also effective for treating BPD. Up to three doses have been used for the treatment of BPD.[5] A recent meta-analysis revealed that MSC therapy also shows promise for treating NEC. However, more data is needed regarding the optimal dose or timing of MSC treatment in NEC for humans.[18] In a specific case of a patient who was born preterm at 32 weeks and had been on a ventilator for 46 days, MSC treatment was the only option to treat both lung and small bowel conditions simultaneously. Patients with respiratory failure associated with pulmonary hypoplasia and pulmonary hypertension in CDH may also benefit from MSC treatment, given the shared pathophysiology of BPD and CDH.[27,28] Also, it is reasonable to explore the potential use of MSC treatment in treating ASBO progressing to NEC due to its promising results in treating NEC in animal studies.[18] As a result, we decided to administer 1x10 million cells/kg intratracheally and 5 million cells/kg intravenously to target both the lungs and intestines and overcome lung capillary entrapment.

The patient experienced prolonged difficulty tolerating feeding due to ASBO and was unable to be taken off the ventilator because of abdominal distension and pre-existing pulmonary hypoplasia. This led to a cycle of recurrent infections and pneumothoraces. Arterial blood gas tests revealed increasing bicarbonate levels, similar to those in patients with BPD. Following the initial MSC treatment, the patient's feeding tolerance improved. Subsequently, a gradual decline in bicarbonate levels was noted. After the second treatment, the patient was successfully weaned from the ventilator, and the total parenteral nutrition

was discontinued (Fig. 3). At seven months old, she weighs 8 kg with no residual respiratory or nutritional deficiencies. According to age-appropriate developmental standards, her results on the Denver neurodevelopmental test fall within the normal range except for a minor delay in fine motor skills. A follow-up computed tomography of the chest at seven months revealed well-expanded lungs. This is the first documented case of successfully treating pulmonary hypoplasia and ASBO/NEC simultaneously in CDH patients.

When considering potential side effects, it is essential to note that MSCs do not have major histocompatibility complex antigens, which helps to reduce concerns related to graft-versus-host disease. However, there have been reports of thromboembolism in adult patients and a microembolism phenomenon in a newborn after amnion-derived MSC transfusion.[29,30] Ongoing concerns persist regarding potential tumorigenicity, especially with repeated doses, even though MSC therapy is also used in tumor treatment.[31,32] However, human MSCs from the umbilical cord showed no signs of malignant transformation after up to 15 passages in vitro.[33] Umbilical cord-derived MSC therapy may also be a source of viral and mycoplasma infections, as it is a blood product.[34] To the best of our knowledge, no long-term side effects have been reported in newborns. In our specific case, no adverse effects were observed, and a chest CT scan at seven months of age did not reveal any tumors. The use of MSC exosomes (acellular therapy) for BPD has gained recent interest due to concerns regarding the potential adverse effects of MSCs and the observation that MSCs primarily exert their regenerative effects through paracrine signaling. Acellular therapy has been found to exhibit similar effects as MSCs.[35] We only had access to cellular MSC therapy, but acellular therapy may also benefit CDH and related complications. It is uncertain whether this particular patient would have been able to discontinue using the ventilator or TPN without the therapy. Additionally, the optimal dosage and the contribution of MSC therapy to long-term outcomes of CDH are currently unknown. Nevertheless, we advocate for further investigations of MSC therapy for CDH patients. Given the lack of data, we hope this case will encourage additional research.

## Ethical approval

The competent authority (Republic of Türkiye Ministry of Health, Organ and Tissue Unit) approved the stem cell therapy used in the patients presented in this article. The family provided written informed consent to publish this case report.

## Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: ŞY; data collection: ŞY; analysis and interpretation of results: ŞY, AK; draft manuscript preparation: ŞY, AK. 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. Wright JC, Budd JL, Field DJ, Draper ES. Epidemiology and outcome of congenital diaphragmatic hernia: a 9-year experience. Paediatr Perinat Epidemiol 2011; 25: 144-149. https://doi.org/10.1111/j.1365-3016.2010.01172.x
2. Sakurai Y, Azarow K, Cutz E, Messineo A, Pearl R, Bohn D. Pulmonary barotrauma in congenital diaphragmatic hernia: a clinicopathological correlation. J Pediatr Surg 1999; 34: 1813-1817. https://doi.org/10.1016/s0022-3468(99)90319-6

3. Terui K, Tazuke Y, Nagata K, et al. Weight gain velocity and adequate amount of nutrition for infants with congenital diaphragmatic hernia. Pediatr Surg Int 2021; 37: 205-212. https://doi.org/10.1007/s00383-020-04785-y

4. Williams E, Greenough A. Respiratory support of infants with congenital diaphragmatic hernia. Front Pediatr 2021; 9: 808317. https://doi.org/10.3389/fped.2021.808317

5. Razak A, Lei D, McDonald CA, Hunt RW, Miller SL, Malhotra A. Allogeneic Cell Therapy Applications in Neonates: A Systematic Review. Stem Cells Transl Med 2023; 12: 651–664. https://doi.org/10.1093/stcltm/szad048.

6. Yuniartha R, Alatas FS, Nagata K, et al. Therapeutic potential of mesenchymal stem cell transplantation in a nitrofen-induced congenital diaphragmatic hernia rat model. Pediatr Surg Int 2014; 30: 907-914. https://doi.org/10.1007/s00383-014-3576-9

7. Takayama S, Sakai K, Fumino S, et al. An intra-amniotic injection of mesenchymal stem cells promotes lung maturity in a rat congenital diaphragmatic hernia model. Pediatr Surg Int 2019; 35: 1353-1361. https://doi.org/10.1007/s00383-019-04561-7

8. Chalphin AV, Tracy SA, Lazow SP, Kycia I, Zurakowski D, Fauza DO. Congenital diaphragmatic hernia as a potential target for transamniotic stem cell therapy. J Pediatr Surg 2020; 55: 249-252. https://doi.org/10.1016/j.jpedsurg.2019.10.033

9. Karacanoğlu D, Çeleğen M, Yavuz S, Katlan B, Kesici S, Bayrakçı B. An inspiring horizon to congenital diaphragmatic hernia with mesenchymal stem cell therapy. Chest 2022; 161(Suppl): A548. https://doi.org/10.1016/j.chest.2022.04.082

10. Zahn KB, Franz AM, Schaible T, et al. Small bowel obstruction after neonatal repair of congenital diaphragmatic hernia-incidence and risk-factors identified in a large longitudinal cohort-study. Front Pediatr 2022; 10: 846630. https://doi.org/10.3389/fped.2022.846630

11. Macchini F, Raffaeli G, Amodeo I, et al. Recurrence of congenital diaphragmatic hernia: risk factors, management, and future perspectives. Front Pediatr 2022; 10: 823180. https://doi.org/10.3389/fped.2022.823180

12. Kobayashi H, Yamataka A, Okazaki T, Lane GJ, Puri P, Miyano T. Increased levels of circulating adhesion molecules in neonates with congenital diaphragmatic hernia complicated by persistent pulmonary hypertension. Pediatr Surg Int 2004; 20: 19-23. https://doi.org/10.1007/s00383-003-1072-8

13. Sakrani NF, Hamza E, Ahmed HA, Corbally MT. Surgical management of necrotizing enterocolitis following congenital diaphragmatic hernia repair. Bahrain Medical Bulletin 2015; 37: 253-255. https://doi.org/10.12816/0047415

14. Shanbhogue LK, Tam PK, Lloyd DA. Necrotizing enterocolitis following operation in the neonatal period. Br J Surg 1991; 78: 1045-1047. https://doi.org/10.1002/bjs.1800780906

15. Koh YH, Cooksey R, Prager P. Early onset necrotising enterocolitis in association with congenital diaphragmatic hernia in a term baby. BMJ Case Rep 2018; 2018: bcr2018224854. https://doi.org/10.1136/bcr-2018-224854

16. Markel TA, Underwood MA. Preface: The NEC Society. Semin Pediatr Surg 2018; 27: 1-2. https://doi.org/10.1053/j.sempedsurg.2017.11.001

17. Bazacliu C, Neu J. Necrotizing enterocolitis: long term complications. Curr Pediatr Rev 2019; 15: 115-124. https://doi.org/10.2174/1573396315666190312093119

18. Di SJ, Wu SY, Liu TJ, Shi YY. Stem cell therapy as a promising strategy in necrotizing enterocolitis. Mol Med 2022; 28: 107. https://doi.org/10.1186/s10020-022-00536-y

19. Merimi M, El-Majzoub R, Lagneaux L, et al. The therapeutic potential of mesenchymal stromal cells for regenerative medicine: current knowledge and future understandings. Front Cell Dev Biol 2021; 9: 661532. https://doi.org/10.3389/fcell.2021.661532

20. Krueger TEG, Thorek DLJ, Denmeade SR, Isaacs JT, Brennen WN. Concise review: mesenchymal stem cell-based drug delivery: the good, the bad, the ugly, and the promise. Stem Cells Transl Med 2018; 7: 651-663. https://doi.org/10.1002/sctm.18-0024

21. Chang C, Yan J, Yao Z, Zhang C, Li X, Mao HQ. Effects of mesenchymal stem cell-derived paracrine signals and their delivery strategies. Adv Healthc Mater 2021; 10: e2001689. https://doi.org/10.1002/adhm.202001689

22. Ramesh S, Daniel D, Götherström C, Madhuri V. Trophic effects of multiple administration of mesenchymal stem cells in children with osteogenesis imperfecta. Clin Transl Med 2021; 11: e385. https://doi.org/10.1002/ctm2.385

23. Gong C, Chang L, Sun X, et al. Infusion of two-dose mesenchymal stem cells is more effective than a single dose in a dilated cardiomyopathy rat model by upregulating indoleamine 2,3-dioxygenase expression. Stem Cell Res Ther 2022; 13: 409. https://doi.org/10.1186/s13287-022-03101-w

24. Caplan H, Olson SD, Kumar A, et al. Mesenchymal stromal cell therapeutic delivery: translational challenges to clinical application. Front Immunol 2019; 10: 1645. https://doi.org/10.3389/fimmu.2019.01645

25. Miceli V, Zito G, Bulati M, et al. Different priming strategies improve distinct therapeutic capabilities of mesenchymal stromal/stem cells: potential implications for their clinical use. World J Stem Cells 2023; 15: 400-420. https://doi.org/10.4252/wjsc.v15.i5.400

26. Fischer UM, Harting MT, Jimenez F, et al. Pulmonary passage is a major obstacle for intravenous stem cell delivery: the pulmonary first-pass effect. Stem Cells Dev 2009; 18: 683-692. https://doi.org/10.1089/scd.2008.0253

27. Sahni M, Bhandari V. Recent advances in understanding and management of bronchopulmonary dysplasia. F1000Res 2020; 9(Faculty Rev): 703. https://doi.org/10.12688/f1000research.25338.1

28. Nobuhara KK, Wilson JM. Pathophysiology of congenital diaphragmatic hernia. Semin Pediatr Surg 1996; 5: 234-242.

29. Wu Z, Zhang S, Zhou L, et al. Thromboembolism induced by umbilical cord mesenchymal stem cell infusion: a report of two cases and literature review. Transplant Proc 2017; 49: 1656-1658. https://doi.org/10.1016/j.transproceed.2017.03.078

30. Jung JW, Kwon M, Choi JC, et al. Familial occurrence of pulmonary embolism after intravenous, adipose tissue-derived stem cell therapy. Yonsei Med J 2013; 54: 1293-1296. https://doi.org/10.3349/ymj.2013.54.5.1293

31. Suzuki K, Sun R, Origuchi M, et al. Mesenchymal stromal cells promote tumor growth through the enhancement of neovascularization. Mol Med 2011; 17: 579-587. https://doi.org/10.2119/molmed.2010.00157

32. Harrell CR, Volarevic A, Djonov VG, Jovicic N, Volarevic V. Mesenchymal stem cell: a friend or foe in anti-tumor immunity. Int J Mol Sci 2021; 22: 12429. https://doi.org/10.3390/ijms222212429

33. Chen G, Yue A, Ruan Z, et al. Human umbilical cord-derived mesenchymal stem cells do not undergo malignant transformation during long-term culturing in serum-free medium. PLoS One 2014; 9: e98565. https://doi.org/10.1371/journal.pone.0098565

34. Baranovskii DS, Klabukov ID, Arguchinskaya NV, et al. Adverse events, side effects and complications in mesenchymal stromal cell-based therapies. Stem Cell Investig 2022; 9: 7. https://doi.org/10.21037/sci-2022-025

35. Zhang S, Mulder C, Riddle S, Song R, Yue D. Mesenchymal stromal/stem cells and bronchopulmonary dysplasia. Front Cell Dev Biol 2023; 11: 1247339. https://doi.org/10.3389/fcell.2023.1247339

The Turkish Journal of Pediatrics 2024; 66: 637-642
https://doi.org/10.24953/turkjpediatr.2024.4902

**Case Report**

# Changes in skin barrier integrity by electrical impedance spectroscopy during dupilumab treatment on a child with severe atopic dermatitis

Elif Yaycı[1], Işıl Ezelsoy[1], Beliz Su Gündoğdu[1], Betül Büyüktiryaki[2], Cansın Saçkesen[2]

*[1]School of Medicine, Koç University, İstanbul; [2]Department of Pediatrics, Division of Pediatric Allergy, School of Medicine, Koç University, İstanbul, Türkiye.*

## ABSTRACT

**Background.** Atopic dermatitis (AD) is a chronic inflammatory skin disease characterized by epidermal skin barrier dysfunction and altered immune response. Electrical impedance spectroscopy (EIS) has been used as a novel tool to detect skin barrier changes in AD. EIS is a non-invasive measure of the electrical impedance of tissue and is sensitive to cellular structure and extracellular environment.

**Case Presentation.** An 8-year-old girl presented with severe AD, starting at 3 years of age. She also had allergic rhinitis, food allergies, and sensitization to mites, eggs, and nuts. Unresponsive to other treatments, she was administered 300 mg of dupilumab, a monoclonal antibody inhibiting IL-4 and IL-13 activity. Patient's response to the treatment and skin barrier integrity was followed for 6 months: First at the baseline (before dupilumab) and then again at the 1st, 2nd, 3rd, and 5.5th month after dupilumab with SCORing Atopic Dermatitis (SCORAD), as well as measurements of moisture by MoistureMeterSC (Delfin®) and EIS by Nevisense® (SciBase) on the forearm and antecubital fossa of the same arm. At the end of 6 months, her SCORAD improved from 96 to 37. The moisture measurements were variable. The EIS by Z1 score in the forearm increased from 72 to 141 and EIS by MIX scores increased from 2.7 to 6.2. The correlation between SCORAD and forearm EIS by Z1 and MIX scores were significant: r=-0.913, (p=0.03) and r=-0.881, (p=0.049). The correlation between forearm MIX scores with sleeplessness and itching was significant: r=-0.956, (p=0.011), r=-0.942, (p=0.017).

**Conclusion.** As higher EIS scores reflect stronger barrier integrity, the increase in Z1 and MIX obtained from Nevisense® implies an improvement in the skin barrier integrity during dupilumab treatment. This report highlights the potential use of EIS in atopic dermatitis patients to evaluate treatment efficacy. We urge rapid and non-invasive use of EIS in pediatrics to be further investigated in clinical settings.

**Key words:** atopic dermatitis, children, electric impedance spectroscopy, epithelium, dupilumab.

Atopic dermatitis (AD) is a chronic inflammatory skin disorder with heterogeneous pathophysiology. Skin epithelial barrier dysfunction is one of the most important components of AD. Defects in the epithelial barrier increase transcutaneous water loss and allow microbial dysbiosis.[1-3] Th-2 mediated immune response is accentuated in AD, contributing to inflammatory changes. Th-2 cytokines interleukin (IL)-4 and IL-13 influence

✉ Cansın Saçkesen ▪ csackesen@ku.edu.tr

Received 30th Jun 2024, revised 12th Aug 2024, accepted 30th Sep 2024.

The study has been previously presented as a poster in World Immune Regulation Meeting XVII, 5-8 July 2023, Davos, Switzerland.

keratinocyte function and increase the epidermal barrier damage. They are also responsible for IgE production, T cell activation, and eosinophil recruitment.[1-3] While AD management depends on AD severity, inhibition of Th-2 cytokines via targeting the IL-4Ra receptor is introduced as one of the treatment models for AD. Dupilumab, a monoclonal antibody against IL-4Ra, has been well tolerated and shown to decrease AD severity significantly.[4] Furthermore, Berdyshev et al. reported improved skin barrier function by dupilumab with transepidermal water loss measurements and stratum corneum ceramide composition analysis in adults and adolescents.[5]

A new method proposed to evaluate epidermal integrity in atopic dermatitis is electrical impedance spectroscopy (EIS).[6-8] EIS is a non-invasive measure of a tissue's electrical resistance against alternating currents with different frequencies. Nevisense® (Scibase) device measures electrical impedance at 35 different frequencies along 1 kHz and 2.5 MHz intervals at 4 different depths in 10 permutations. It collects 700 data points. The applied voltage is 150 mV, and the current is 75 μA, which is undetectable by the patient.[6,7] EIS is sensitive to cell structure, compactness, and extracellular environment characteristics such as water and lipid content. EIS is approved for diagnosis and differentiation of skin cancers from benign lesions.[7] Rinaldi et al. used EIS to detect skin barrier defects of mice epidermal barrier damaged by proteases, cholera toxin, and tape stripping, where they confirmed the damage by RT-PCR and histological analysis.[8] Recent studies used EIS-based algorithms to differentiate between the skin of AD patients and healthy people in adults and pediatrics.[6,7] Consequently, we aimed to investigate the skin epidermal integrity during dupilumab treatment in a pediatric patient using EIS and moisture measurements.

## Case presentation

Here, we present an 8-year-old female patient with severe AD starting at the age of three years. She also has egg allergy, nut allergy, and allergic rhinitis with sensitization to mites. The patient is on an elimination diet for eggs and nuts and uses special measures to reduce mite exposure including bedsheets for the mite allergy. Patient history does not have recurrent infections, otitis, pneumonia, or abscesses, excluding the immunodeficiency diagnosis. In her initial laboratory tests, total IgE was 6006 IU/L, and eosinophil count was 1210 (14.6%). Due to high eosinophilia, IgE levels, and persistent severe atopic dermatitis, hypereosinophilia, hyper-IgE syndromes, and other immune regulation disorders with genetic mutations and IgG, IgA, IgM, vaccine serology, lymphocyte subset groups analysis by flow cytometry have all been investigated. However, these tests were negative. She was treated with regular topical corticosteroids (CS), topical calcineurin inhibitors (pimecrolimus and tacrolimus), and intermittent oral CS. Since she was unresponsive to the treatment, cyclosporine 4 mg/kg/day was started as an immunosuppressive therapy. However, severe AD persisted, and cyclosporine treatment was discontinued due to side effects (e.g., tremor and nausea). Following this, 300 mg/month of omalizumab, a monoclonal anti-IgE antibody, was administered. Omalizumab did not improve her AD lesions, and she experienced myalgia. After 2 months of omalizumab treatment, 300 mg dupilumab treatment was initiated, followed by a second dose 2 weeks later. Then, 300 mg of dupilumab was given monthly. During dupilumab treatment, the patient continued to use moisturizer creams daily. To ensure greater improvements in atopic dermatitis progression, topical corticosteroid was continued when there were atopic dermatitis flare-ups.

During six months of dupilumab treatment, the patient's response to treatment was followed by EIS and skin moisture measurements. The first measurement was collected before dupilumab treatment and recorded as the baseline. The other 4 measurements were obtained at the 1st, 2nd, 3rd, and 5.5th months of dupilumab treatment. To date, the patient has not experienced any adverse events with dupilumab treatment.

At each visit, SCORing Atopic Dermatitis (SCORAD), including self-reported sleeplessness and itching, were recorded for AD severity. Skin moisture measurements and EIS measurements were taken from 2 different sites each time. The first site, the forearm, is the clinically unaffected skin. Throughout the investigation, there was no active lesion in the forearm. In the second site, antecubital fossa, the patient had clinically active lesions. Triplicates of skin moisture measurement were taken from the volar forearm and antecubital fossa of the same arm using MoistureMeterSC® (Delfin Technologies, Kuopio, Finland). The average of triplicates was used as the final moisture measurement. Then, EIS measurements were collected in duplicates from the same areas using Nevisense®. To collect EIS measurements, the site was moistened with physiological saline for 30 seconds before applying the electrode. Z1 and MIX values obtained from Nevisense® were used as EIS scores. Z1, contact impedance, is the "mean value of all permutations for the amplitude at 1 kHz" whereas MIX is "the mean value of all permutations for the slope of the amplitude curve between 20-500 kHz".[9] Compared to Z1, the MIX score reflects the barrier function of deeper layers.[10] Importantly both scores are positively correlated with skin barrier function. The average of multiplicate measurements was used as final scores for Z1 and MIX.

After 6 months of dupilumab treatment, the pronounced improvement in her AD was marked by the reduction of her SCORAD score from 96.15 to 37.65. Her sleeplessness and itching scores reduced from 10 to 2 and 4, respectively. The average of skin moisture measurements in her volar forearm increased in earlier months of treatment but then returned to a baseline measurement (6.3) in the last (6.1) month. In the antecubital fossa, skin moisture measurements were 5.1 for the baseline and 13.3 for the final month. In the volar forearm, both final average Z1 (141.6) and final average MIX (6.2) scores were higher than baseline (Z1:72.5, MIX:2.7). All measurements are summarized in Table I.

Correlations between EIS scores, skin moisture scores, and SCORAD were evaluated by 2-tailed Pearson tests in SPSS. Forearm Z1 (r=-0.913, p=0.03) and forearm MIX scores (r=-0.881, p=0.049) had a significant inverse correlation with SCORAD values (Fig. 1a, 1b). The correlation between forearm MIX scores with sleeplessness and itching was significant: r=-0.956 (p=0.011), r=-0.942 (p=0.017) (Fig. 1c, 1d). However, no significant correlation was found between SCORAD and antecubital skin moisture measurements and antecubital EIS measurements. An informed consent was obtained from the parents for the publication of this case report.

**Table I.** Summary of all measurements.

| | SCORAD | Sleeplessness | Itching | Z1 (EIS) Forearm | MIX (EIS) Forearm | Skin moisture Forearm | Z1 (EIS) Antecubital fossa | MIX (EIS) Antecubital fossa | Skin moisture Antecubital fossa |
|---|---|---|---|---|---|---|---|---|---|
| Before dupilumab | 96.1 | 10 | 10 | 72.5 | 2.6 | 6.3 | 179.9 | 5.1 | 5.1 |
| 1st month of dupilumab | 57.3 | 4 | 5 | 93.5 | 5.8 | 17.4 | 169.6 | 10.1 | 11.0 |
| 2nd month of dupilumab | 63.1 | 4 | 5 | 99.6 | 6.5 | 11.5 | 176.0 | 8.7 | 16.0 |
| 3rd month of dupilumab | 47.2 | 3 | 6 | 113.2 | 6.2 | 6.3 | 63.6 | 6.0 | 8.9 |
| 5.5th month of dupilumab | 37.6 | 2 | 4 | 141.6 | 6.2 | 6.1 | 118.9 | 7.3 | 13.3 |

EIS, electrical impedence spectroscopy; SCORAD, SCORing Atopic Dermatitis.

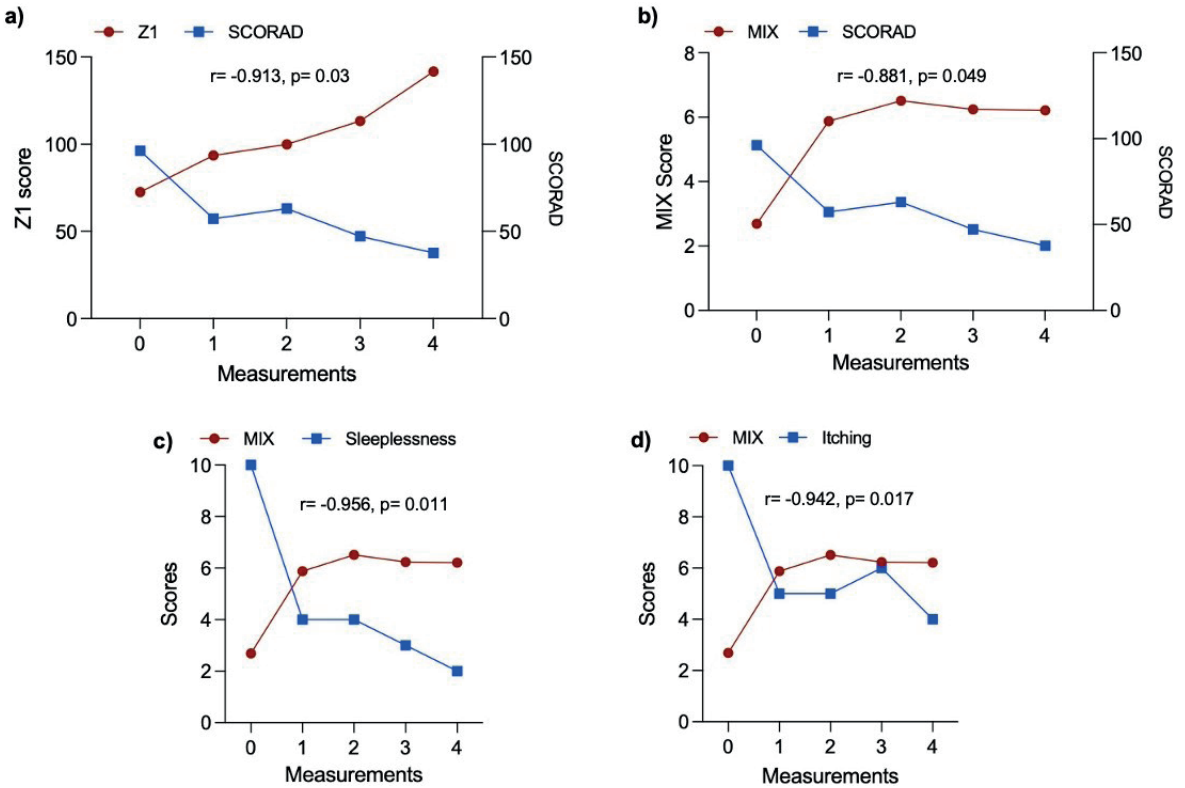



**Fig. 1.** Progression of forearm EIS scores with other severity score assessment during dupilumab treatment **a)** forearm EIS-Z1 scores with SCORAD, **b)** forearm EIS-MIX scores with SCORAD, **c)** forearm EIS-MIX scores with itching, and **d)** forearm EIS-MIX scores with sleeplessness. Measurement 0 represents the baseline, and measurements 1, 2, 3, and 4 represent the 1st, 2nd, 3rd, and 5.5th month of treatment, respectively. EIS, electrical impedence spectroscopy; SCORAD, SCORing Atopic Dermatitis.

## Discussion

Dupilumab efficacy in atopic dermatitis in children and adults has been well-acknowledged.[5,11,12] In accordance, the patient's SCORAD decreased by 56%. In the literature, Dupilumab has been found to improve the skin epithelial barrier by using skin biopsies. After dupilumab treatment, skin biopsies of atopic dermatitis patients have been found to reduce type-2 inflammation activity and increase the expression of epidermal differentiation barrier genes and lipid metabolism genes.[13] This report investigated the effect of dupilumab using non-invasive methods. Comparing the moisture, itchiness, sleeplessness, and SCORAD; the MIX score in the volar forearm showed the highest change in the first month of the dupilumab treatment. MIX score in the volar forearm noted a 223% increase, while the Z1 score increased by 129%. Since higher EIS scores (Z1 and MIX) reflect stronger skin barrier activity, an improving trend in EIS scores on the forearm may imply an improvement in skin epithelial barrier integrity during dupilumab treatment. Sasaki et al. demonstrated EIS's ability to differentiate between atopic dermatitis and healthy skin in children for the first time.[7] In another study, EIS measurements reflected skin barrier healing after 3 weeks of hospitalization for AD treatment in adult atopic dermatitis patients.[6] Yet, literature on the use of EIS in the evaluation of atopic dermatitis treatments in children is lacking. This case report highlights EIS's association with improved barrier function in a child after AD treatment for the first time.

The main variables EIS is influenced by are skin hydration, SC thickness, and cellular properties. EIS can be affected by the age of the participants.[10] While one study did not find any association between topical cream use and EIS measurement, a recent study investigating factors affecting EIS and transepidermal water loss measurements concluded that EIS is sensitive to the use of cream ointment and skin washing up to 90 minutes.[7,10] Sweating or prior exercise did not change EIS measurements.[10] In this patient, the differences in EIS scores in the volar forearm and antecubital fossa may be attributed to anatomic location and clinical severity of the skin. EIS measurements may vary according to the location of the measurement because of the different composition of the skin. Furthermore, Rinaldi et al. demonstrated significantly different EIS values in lesional areas compared to non-lesional measurements.[6] The patient had severe AD lesions in the antecubital fossa with lichenification, excoriation, and oozing. Because of these severe lesions in the antecubital area, EIS scores in the antecubital fossa are expected to be different from the scores obtained in the unaffected forearm area. Measurements from the same site may be affected by topical cream use and skin washing.[10] The patient used the topical creams on antecubital fossa variably which may have resulted in inconsistent progression of EIS scores in antecubital fossa along the dupilumab treatment.

While the use of scoring tools like SCORAD is well validated for clinical studies, the nature of scoring systems has its limitations due to intraobserver and interobserver variability.[14,15] EIS measurements can be standardized to have reliable and reproducible assessment of AD, supporting evidence-based medicine. Furthermore, SCORAD and other scoring tools aim to describe AD severity through present clinical symptoms while EIS differentiates between non-lesional skin of AD patients and healthy controls.[7] By evaluating the overall skin epithelial barrier status, EIS provides a more profound perspective for the assessment of treatment efficacy. To our knowledge, this is the first case to show skin barrier changes by dupilumab treatment using EIS changes in a pediatric patient. Rapid and non-invasive, EIS seems to be a potential objective tool to assess dupilumab efficacy at the very early stage of treatment by evaluating skin barrier dysfunction.

**Acknowledgements**

The authors gratefully acknowledge use of the services and facilities of the Koç University Research Center for Translational Medicine (KUTTAM), funded by the Republic of Turkey Ministry of Development. The content is solely the authors' responsibility and does not necessarily represent the official views of the Ministry of Development.

**Ethical approval**

Informed consent from parents of the patient is obtained for the publication of this case report.

**Author contribution**

The authors confirm contribution to the paper as follows: study conception and design: CS; data collection: EY, IE, BSG; data analysis and interpretation: EY, BB, CS; draft manuscript preparation: EY, BB, CS; critical review of the manuscript: IE, BSG, BB, CS. All authors reviewed the results and approved the final version of the article.

**Source of funding**

The authors declares the study received no funding.

**Conflict of interest**

The authors declares that there is no conflict of interest.

## REFERENCES


1. Weidinger S, Beck LA, Bieber T, Kabashima K, Irvine AD. Atopic dermatitis. Nat Rev Dis Primers 2018; 4: 1. https://doi.org/10.1038/s41572-018-0001-z
2. Peng W, Novak N. Pathogenesis of atopic dermatitis. Clin Exp Allergy 2015; 45: 566-574. https://doi.org/10.1111/cea.12495
3. David Boothe W, Tarbox JA, Tarbox MB. Atopic dermatitis: pathophysiology. Adv Exp Med Biol 2017; 1027: 21-37. https://doi.org/10.1007/978-3-319-64804-0_3
4. Harb H, Chatila TA. Mechanisms of dupilumab. Clin Exp Allergy 2020; 50: 5-14. https://doi.org/10.1111/cea.13491
5. Berdyshev E, Goleva E, Bissonnette R, et al. Dupilumab significantly improves skin barrier function in patients with moderate-to-severe atopic dermatitis. Allergy 2022; 77: 3388-3397. https://doi.org/10.1111/all.15432
6. Rinaldi AO, Korsfeldt A, Ward S, et al. Electrical impedance spectroscopy for the characterization of skin barrier in atopic dermatitis. Allergy 2021; 76: 3066-3079. https://doi.org/10.1111/all.14842
7. Sasaki M, Sundberg M, Frei R, et al. Electrical impedance spectroscopy detects skin barrier dysfunction in childhood atopic dermatitis. Allergy 2024; 79: 142-152. https://doi.org/10.1111/all.15895
8. Rinaldi AO, Morita H, Wawrzyniak P, et al. Direct assessment of skin epithelial barrier by electrical impedance spectroscopy. Allergy 2019; 74: 1934-1944. https://doi.org/10.1111/all.13824
9. SciBase. Nevisense Clinical Reference Guide. 2014. Article Number: 975-0009-04. Available at: https://scibase.com/wp-content/uploads/2017/11/Clinical-Reference-Guide-1.pdf
10. Huygen L, Thys PM, Wollenberg A, Gutermuth J, Krohn IK. Skin barrier function assessment: electrical impedance spectroscopy is less influenced by daily routine activities than transepidermal water loss. Ann Dermatol 2024; 36: 99-111. https://doi.org/10.5021/ad.23.052
11. Paller AS, Siegfried EC, Thaçi D, et al. Efficacy and safety of dupilumab with concomitant topical corticosteroids in children 6 to 11 years old with severe atopic dermatitis: a randomized, double-blinded, placebo-controlled phase 3 trial [published correction appears in J Am Acad Dermatol 2021; 84: 230]. J Am Acad Dermatol 2020; 83: 1282-1293. https://doi.org/10.1016/j.jaad.2020.06.054
12. Cork MJ, Thaçi D, Eichenfield LF, et al. Dupilumab safety and efficacy in a phase III open-label extension trial in children 6-11 years of age with severe atopic dermatitis. Dermatol Ther (Heidelb) 2023; 13: 2697-2719. https://doi.org/10.1007/s13555-023-01016-9
13. Guttman-Yassky E, Bissonnette R, Ungar B, et al. Dupilumab progressively improves systemic and cutaneous abnormalities in patients with atopic dermatitis. J Allergy Clin Immunol 2019; 143: 155-172. https://doi.org/10.1016/j.jaci.2018.08.022
14. de Bruin Weller MS, Knulst AC, Meijer Y, Bruijnzeel-Koomen CAFM, Pasmans SGM. Evaluation of the child with atopic dermatitis. Clin Exp Allergy 2012; 42: 352-362. https://doi.org/10.1111/j.1365-2222.2011.03899.x
15. Oranje AP, Glazenburg EJ, Wolkerstorfer A, de Waard-van der Spek FB. Practical issues on interpretation of scoring atopic dermatitis: the SCORAD index, objective SCORAD and the three-item severity score. Br J Dermatol 2007; 157: 645-648. https://doi.org/10.1111/j.1365-2133.2007.08112.x

The Turkish Journal of Pediatrics 2024; 66: 643-648
https://doi.org/10.24953/turkjpediatr.2024.5206

Case Report

# Challenging clinical management of a patient with Gaucher disease type IIIC homozygous for the D409H mutation, aortic valve calcification and porcelain aorta

Musa Öztürk[1], Ebru Aypar[1], Hülya Demir[2], Hayriye Hızarcıoğlu Gülşen[2], İlker Ertuğrul[1], Murat Güvener[3], Ergün Barış Kaya[4]

*[1]Department of Pediatric Cardiology, Hacettepe University Hospital, Ankara; [2]Department of Pediatric Gastroenterology, Hacettepe University Hospital, Ankara; [3]Department of Thoracic and Cardiovascular Surgery, Hacettepe University Hospital, Ankara; [4]Department of Cardiology, Hacettepe University Hospital, Ankara, Türkiye.*

## ABSTRACT

**Background.** Gaucher disease is a rare lysosomal storage disorder caused by glucocerebrosidase enzyme deficiency resulting in the cumulative deposition of glucocerebroside in macrophages, predominantly effecting bone marrow, liver and spleen. Gaucher disease type IIIC is a rare subtype that is characterized by cardiovascular involvement, eye-movement disorders, and late-onset neurological symptoms.

**Case presentation.** We present a 14-year-old adolescent boy diagnosed with Gaucher disease type IIIC at age four with a homozygous D409H mutation who developed severe aortic valve stenosis, extensive aortic calcification and a porcelain aorta despite enzyme replacement treatment since the diagnosis. Despite the challenges during the cardiac surgery, we successfully performed transcatheter aortic valve implantation (TAVI). The patient developed a complete atrioventricular block and required a pacemaker after the TAVI. He experienced further complications during the follow-up.

**Conclusion.** The case presents the challenges in the treatment of cardiovascular complications in patients with Gaucher disease and demonstrates the importance of individualized treatment approaches, as well as the potential advantages and complications of TAVI in difficult situations like this.

**Key words:** Gaucher disease type IIIC, D409H mutation, porcelain aorta, transcatheter aortic valve implantation (TAVI).

Gaucher disease is a rare autosomal-recessive lysosomal storage disorder caused by glucocerebrosidase enzyme deficiency.[1] Enzyme deficiency results in the cumulative deposition of glucocerebroside in macrophages, predominantly influencing the bone marrow, liver and spleen. It is classified into three main subtypes according to the patient's clinical presentation. Gaucher disease type IIIC (OMIM #231005) is a rare subtype and characterized by cardiovascular involvement, eye-movement disorders and late-onset neurological symptoms.[2] Cardiovascular involvement includes calcification in the aortic and mitral valves and the aorta.[3-6]

Porcelain aorta is characterized by nearly or completely circumferential calcification of the ascending aorta and/or aortic arch, poses significant challenges during cardiac surgeries such as aortic valve replacement and coronary artery bypass grafting. This extensive

✉ Ebru Aypar ▪ ebruaypar@gmail.com

Received 30th Jun 2024, revised 12th Aug 2024, accepted 30th Sep 2024.

calcification blocks safe aortic cross-clamping or cannulation, increasing the risk of complications like aortic embolization, dissection, or rupture.[7] Although a porcelain aorta is commonly associated with atherosclerosis in adult patients, it may also appear as non-atherosclerotic in states such as systemic inflammatory diseases, radiation-related and chronic renal failure.[8] We present an adolescent patient with Gaucher disease type IIIC who developed significant aortic valve stenosis (AS) and porcelain aorta due to calcifications. He was managed with various catheter interventions and cardiac surgery. To the best of our knowledge, our patient is the first Gaucher type IIIC patient to undergo a TAVI procedure for severe AS and porcelain aorta due to calcifications.

**Case presentation**

The patient was diagnosed with Gaucher disease type IIIC at 4 years old by low glucocerebrosidase enzyme levels and the presence of Gaucher cells in the bone marrow histopathological examination. The glucocerebrosidase enzyme level was 0.87 nmol/s/mgp (normal range, 5-13.5). A homozygous D409H genetic mutation was identified. During the follow-up, despite periodic enzyme replacement therapy since the diagnosis, he had progressive AS due to calcifications. Echocardiography at 14 years showed an aortic valve with thick and immobile leaflets, severe AS with a mean gradient of 38 mmHg, left ventricular (LV) hypertrophy and mild thickening of the mitral valve and mild mitral regurgitation. Percutaneous left heart catheterization showed that the LV systolic pressure was 210 mmHg, and a 97 mmHg systolic pressure gradient was measured at the level of the aortic valve. After discussion with the cardiac surgeons, it was determined that a surgical prosthetic aortic valve replacement would be the initial treatment. However, when the median sternotomy was performed and the pericardium was opened, it was observed that the patient had extensive calcifications in the ascending aorta and the aortic arch, therefore, aortic cross clamping could not be performed. As the coronary buttons were also calcified, the patient was considered inoperable. Further discussions with the adult cardiologists led to the decision to perform transcatheter aortic valve implantation (TAVI). Cardiac computed tomography (CT) before the intervention showed that the aortic annulus diameter was 24x20 mm (+0.2 z score) with a circumference of 70 mm, and a surface area of 3.8 $m^2$. The left main coronary artery was located 10 mm away, while the right coronary artery was 14 mm away from the aortic annulus, sinus of Valsalva diameter was 25x26x26 mm (-0.8 z score) and the ascending aorta diameter was 17x20 mm (-0.7 z score). During the procedure, using the femoral artery access. Boston Scientific Amplatz Super Stiff™ guidewire was introduced into the LV. A 23-mm self-expandable Medtronic the Evolut™ R valve was placed over this wire in the appropriate aortic valve position. After TAVI, echocardiography showed no pressure gradient at the aortic valve and mild aortic regurgitation.

The patient was followed-up with periodic echocardiographic evaluations. After 18 months following the TAVI procedure, he complained of episodic dizziness. Electrocardiogram (ECG) showed a complete atrioventricular (AV) block. 24-hour ECG monitoring (Holter) revealed episodic 2:1 AV complete block with rare 1:1 transmission and an escape rhythm characterized by narrow QRS complexes. The average heart rate was 43 beats, minimum heart rate was 36 beats, and the maximum heart rate was 83 beats per minute. A transvenous pacemaker was implanted. The left subclavian vein was catheterized via an extrapleural approach and a transcatheter 58 cm Medtronic SelectSecure™ Model 3830 lead was implanted in the septal region. A Vitatron™ G20A2 pacemaker battery was placed in the left pectoral area.

Two months post-pacemaker installation, he experienced persistent fever, and echocardiographic assessment revealed a vegetation on the mitral valve. During the

medical treatment for infective endocarditis (IE) with intravenous antibiotics, echocardiography showed mitral cord rupture due to IE and severe mitral regurgitation. He underwent surgery again, and a redo-sternotomy was performed. This time he had femoral cannulation due to the presence of a calcified porcelain aorta. The femoral artery, femoral vein, and superior vena cava were selectively cannulated. The heart was fibrillated at 28 °C and the left atrium was accessed by performing a right atriotomy on the beating heart. The mitral valve was found to be significantly deformed with two of the chordae in the anterior leaflet being ripped. The mitral valve was excised and replaced with a 25 mm St Jude™ mechanical prosthetic valve. The surgery was successfully completed without complications. Figure 1 displays the patient's cardiac images in chronological order.

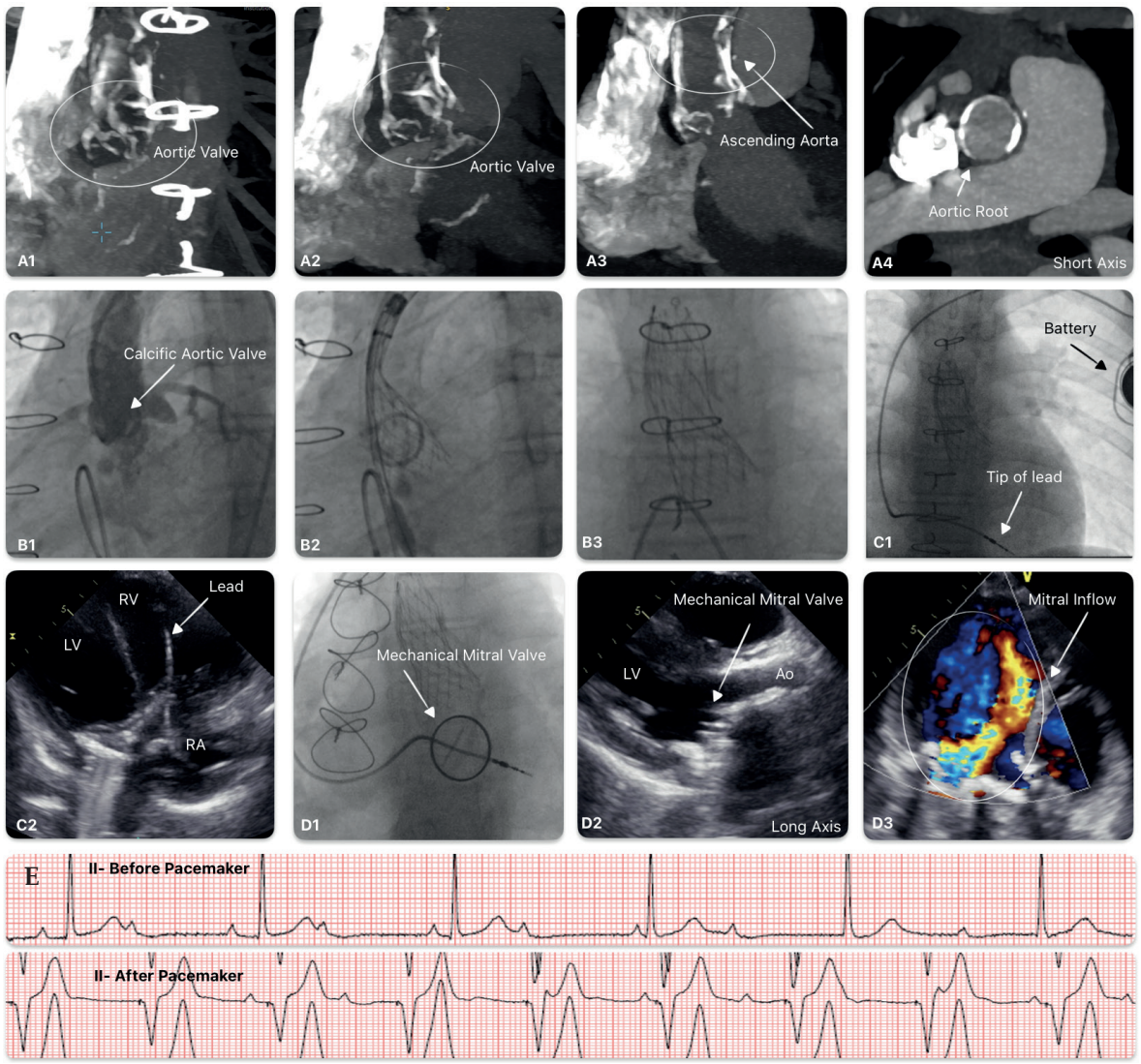



**Fig. 1.** Summary images of interventional procedures and imaging performed on the patient. **A:** Cardiac tomography images showing calcific aortic valve, aortic root and ascending aorta before the transcatheter aortic valve implantation (TAVI) procedure. **B:** Angiographic images of the TAVI procedure. **C:** Images showing the pacemaker implantation. **D:** Angiographic and echocardiographic image showing the prosthetic mitral valve and mitral stenosis in the prosthetic valve during the follow-up. **E:** Electrocardiographs before and after pacemaker implantation.

Fifteen months after the prosthetic mitral valve replacement, he started to complain about a chronic cough and intermittent dyspnea. Echocardiographic evaluation showed aortic and mitral valve stenosis and pulmonary venous hypertension. An average 15 mmHg gradient was measured at the level of the aortic valve; and an average 16 mmHg gradient was measured at the level of the mitral valve with continuous wave Doppler. The mean pulmonary pressure was calculated as 35 mmHg based on mild pulmonary insufficiency flow. No significant aortic valve insufficiency was observed. Surgery and other transcatheter treatment options were postponed due to a high risk of complications and family concerns. Currently, the patient is stable and has no dyspnea with intensive oral diuretic treatment, including oral furosemide, chlorothiazide and aldactone combinations.

Informed consent was obtained from the patient and his family to share patient clinical information and imaging photographs anonymously for scientific purposes.

## Discussion

Gaucher disease type IIIC is a rare variant of Gaucher disease that is determined by a distinctive phenotype with homozygous D409H mutation. The presence of this mutation has been associated with the development of oculomotor apraxia and cardiac calcification.[2,9] Our patient with Gaucher disease type IIIC had extensive calcifications on the aortic valve, ascending aorta and aortic arch resulting in a porcelain aorta. It has been reported that in patients with Gaucher disease type IIIC, calcifications may involve intracardiac structures like mitral and aortic valves, and may also extend into the aorta.[2-5,10,11] Our patient had both AS and a porcelain aorta due to calcifications, but did not have a mitral valve involvement, however mitral valve was also involved due to IE.

It has been reported that the existence of calcific aortopathy may increase the possibility of developing ischemic heart disease over an extended period of time.[12] Our patient did not present with findings of ischemic cardiomyopathy. This may be due to the fact that he is still in the pediatric age group and does not have additional comorbidities which are usually present in adults. Porcelain aorta is typically associated with atherosclerosis and systemic inflammation in older adults. However, the condition can also occur at a young age due to infectious and genetic factors, such as Gaucher disease, aortitis, and Singleton Merten syndrome, in children.[13]

In patients with porcelain aorta, difficulties in aortic cross-clamping and embolization risk during the cardiac surgery have led to the consideration of the TAVI procedure as an alternative and appropriate choice.[14-17] Porcelain aorta may be considered a relative contraindication for surgical valve replacement when there is severe AS.[17] However, in children, the best course of action is to perform surgery as the initial treatment due to the patients' longer lifespan and the lack of cumulative experience with TAVI in children. Therefore, our initial decision was to perform surgery in our patient.

The presence of a porcelain aorta is a separate factor that can predict an increased risk of pacemaker requirement after TAVI.[18] Our patient also required a pacemaker implantation due to the development of a complete AV block, 18 months after the TAVI procedure. Therefore, in patients with porcelain aorta, especially after the TAVI procedure, close monitoring of the symptoms and performing periodic ECG and Holter examinations is essential.

In patients with a porcelain aorta who needs cardiac surgery (aortic and/or mitral valve replacement), it is usually difficult or impossible to perform aortic cross-clamping due to extensive calcifications or significant deposits of plaque throughout the ascending aorta, requiring the use of innovative approaches.[19,20]

In patients with severe AS and a porcelain aorta, TAVI has become a crucial therapeutic method. It provides a less intrusive option compared

to conventional surgical methods. However, during the patient follow-up, complications may occur that are predictable, such as pacemaker requirement, or unpredictable, such as mitral cord rupture as in our case.

In conclusion, cardiac involvement may be an important cause of mortality and morbidity in patients with Gaucher disease. Therefore, close cardiac follow-up should be performed. We presented the challenges in the treatment of cardiovascular complications in patients with Gaucher disease type IIIC and demonstrated the importance of individualized treatment approaches, as well as the potential advantages and complications of TAVI. To the best of our knowledge, our patient is the first Gaucher disease type IIIC patient who receieved TAVI procedure for severe AS and porcelain aorta due to calcifications.

### Ethical approval

Informed consent was obtained from the patient and his family to share patient clinical information and imaging photographs anonymously for scientific purposes.

### Author contribution

The authors confirm their contribution to the paper as follows: Study conception and design: MÖ, EA, HD, HHG, İE, MG, EBK; data collection: MÖ, EA, İE; analysis and interpretation of results: MÖ, EA; draft manuscript preparation: MÖ. All authors reviewed the results and approved the final version of the article.

### Source of funding

The authors declares the study received no funding.

### Conflict of interest

The authors declares that there is no conflict of interest.

## REFERENCES

1. Burrow TA, Barnes S, Grabowski GA. Prevalence and management of Gaucher disease. Pediatric Health Med Ther. 2011; 2: 59-73. https://doi.org/10.2147/PHMT.S12499
2. Abrahamov A, Elstein D, Gross-Tsur V, et al. Gaucher's disease variant characterised by progressive calcification of heart valves and unique genotype. Lancet 1995; 346: 1000-1003. https://doi.org/10.1016/s0140-6736(95)91688-1
3. Chabás A, Cormand B, Grinberg D, et al. Unusual expression of Gaucher's disease: cardiovascular calcifications in three sibs homozygous for the D409H mutation. J Med Genet 1995; 32: 740-742. https://doi.org/10.1136/jmg.32.9.740
4. George R, McMahon J, Lytle B, Clark B, Lichtin A. Severe valvular and aortic arch calcification in a patient with Gaucher's disease homozygous for the D409H mutation. Clin Genet 2001; 59: 360-363. https://doi.org/10.1034/j.1399-0004.2001.590511.x
5. Kör Y, Keskin M, Başpınar O. Severe cardiac involvement in Gaucher type IIIC: a case report and review of the literature. Cardiol Young 2017; 27: 1426-1429. https://doi.org/10.1017/S1047951117000579
6. Gumus E, Karhan AN, Hizarcioglu-Gulsen H, et al. Clinical-genetic characteristics and treatment outcomes of Turkish children with Gaucher disease type 1 and type 3: a sixteen year single-center experience. Eur J Med Genet 2021; 64: 104339. https://doi.org/10.1016/j.ejmg.2021.104339
7. Snow T, Semple T, Duncan A, et al. 'Porcelain aorta': a proposed definition and classification of ascending aortic calcification. Open Heart 2018; 5: e000703. https://doi.org/10.1136/openhrt-2017-000703
8. Abramowitz Y, Jilaihawi H, Chakravarty T, Mack MJ, Makkar RR. Porcelain aorta: a comprehensive review. Circulation 2015; 131: 827-836. https://doi.org/10.1161/CIRCULATIONAHA.114.011867
9. Ida H, Rennert OM, Kawame H, Ito T, Maekawa K, Eto Y. Mutation screening of 17 Japanese patients with neuropathic Gaucher disease. Hum Genet 1996; 98: 167-171. https://doi.org/10.1007/s004390050182
10. Spear GS, Beutler E, Hungs M. Congenital Gaucher disease with nonimmune hydrops/erythroblastosis, infantile arterial calcification, and neonatal hepatitis/fibrosis. Clinicopathologic report with enzymatic and genetic analysis. Fetal Pediatr Pathol 2007; 26: 153-168. https://doi.org/10.1080/15513810701563751
11. Altunbas G, Ercan S, Inanç IH, Ozer O, Kervancıoğlu S, Davutoğlu V. Extensive vascular and valvular involvement in Gaucher disease. Asian Cardiovasc Thorac Ann 2015; 23: 446-448. https://doi.org/10.1177/0218492313513598

12. Hata Y, Mochizuki J, Okamoto S, Matsumi H, Hashimoto K. Aortic calcification is associated with coronary artery calcification and is a potential surrogate marker for ischemic heart disease risk: a cross-sectional study. Medicine (Baltimore) 2022; 101: e29875. https://doi.org/10.1097/MD.0000000000029875

13. Nitschke Y, Rutsch F. Inherited Arterial Calcification Syndromes: etiologies and treatment concepts. Curr Osteoporos Rep 2017; 15: 255-270. https://doi.org/10.1007/s11914-017-0370-3

14. Bapat VN, Attia RQ, Thomas M. Distribution of calcium in the ascending aorta in patients undergoing transcatheter aortic valve implantation and its relevance to the transaortic approach. JACC Cardiovasc Interv 2012; 5: 470-476. https://doi.org/10.1016/j.jcin.2012.03.006

15. Matsumoto K, Hisashi Y, Imoto Y. Replacement of the heavily calcified ascending aorta in aortic valve replacement. Asian Cardiovasc Thorac Ann 2015; 23: 349-352. https://doi.org/10.1177/0218492314539949

16. Faggiano P, Frattini S, Zilioli V, et al. Prevalence of comorbidities and associated cardiac diseases in patients with valve aortic stenosis. Potential implications for the decision-making process. Int J Cardiol 2012; 159: 94-99. https://doi.org/10.1016/j.ijcard.2011.02.026

17. Kappetein AP, Head SJ, Généreux P, et al. Updated standardized endpoint definitions for transcatheter aortic valve implantation: the Valve Academic Research Consortium-2 consensus document. J Thorac Cardiovasc Surg 2013; 145: 6-23. https://doi.org/10.1016/j.jtcvs.2012.09.002

18. Pereira E, Ferreira N, Caeiro D, et al. Transcatheter aortic valve implantation and requirements of pacing over time. Pacing Clin Electrophysiol 2013; 36: 559-569. https://doi.org/10.1111/pace.12104

19. Osaka S, Tanaka M. Strategy for porcelain ascending aorta in cardiac surgery. Ann Thorac Cardiovasc Surg 2018; 24: 57-64. https://doi.org/10.5761/atcs.ra.17-00181

20. Isoda S, Osako M, Kimura T, et al. A stepwise aortic clamp procedure to treat porcelain aorta associated with aortic valve stenosis and hemodialysis. Ann Thorac Cardiovasc Surg 2014; 20(Suppl): 725-729. https://doi.org/10.5761/atcs.cr.13-00018

The Turkish Journal of Pediatrics 2024; 66: 649-657
https://doi.org/10.24953/turkjpediatr.2024.4556

Case Report

# Dent's disease: case series from a single center

Hilal Yaşar[1], Emre Leventoğlu[2], Bahar Büyükkaragöz[2], Kibriya Fidan[2], Sevcan A. Bakkaloğlu[2], Oğuz Söylemezoğlu[2]

*[1]Faculty of Medicine, Gazi University, Ankara; [2]Department of Pediatric Nephrology, Gazi University, Ankara, Türkiye.*

## ABSTRACT

**Background.** Dent's disease (DD) is a rare X-linked recessive tubulopathy characterized by low molecular weight proteinuria, hypercalciuria, nephrocalcinosis/nephrolithiasis and chronic kidney disease. With this manuscript, we reported three patients diagnosed as DD in our department in the last 10 years and thereby described the genetics, pathophysiology, clinical presentation, course and management of the disease.

**Cases.** The first case was a male newborn who was consulted to our department after medullary nephrocalcinosis was detected. The second case was a 4-year-old boy who was treated with a diagnosis of urinary tract infection but was found to have proteinuria. Our last case was an 11-month-old male infant who was being followed up for recurrent urinary tract infection and who had millimetric crystalloids in the renal collecting system. Proteinuria and hypercalciuria were present in all cases. Variants were observed in the *CLCN5* gene for the first two cases (c.1852G>A and c.1557+1G>T, respectively) and *OCRL* gene (c.952C>T) for the last case. All patients were recommended oral hydration and a low-salt diet, and hydrochlorothiazide and enalapril were started. No deterioration in kidney function was observed in any patient.

**Conclusion.** DD is a disease that shows different phenotypes even among individuals with mutations in the same gene. Therefore, it should be considered in all patients with hypercalciuria, proteinuria, nephrolithiasis or nephrocalcinosis with/without proximal tubular dysfunction especially in the early childhood period. Classical treatments for hypercalciuria should be utilized, and a patient-based treatment plan should be drawn especially for proteinuria.

**Key words:** chronic kidney disease, hypercalciuria, nephrocalcinosis, nephrolithiasis, proteinuria.

Dent's disease (DD) is a rare X-linked recessive tubulopathy characterized by low molecular weight proteinuria (LMWP), hypercalciuria, nephrocalcinosis/nephrolithiasis and chronic kidney disease.[1] In some cases, microscopic/macroscopic hematuria, hypokalemia, hypophosphatemia and metabolic acidosis may accompany it.[2] The disease may also be accompanied by rickets or osteomalacia and growth retardation.[3] These features are usually detected in males up to 10 years of age. By the age of 50 years, about 80% of males develop kidney failure while females may have a milder phenotype due to X-chromosome inactivation.[4] Nephrolithiasis is rare, although hypercalciuria is seen in some females. Also, LMWP is of moderate severity and CKD rarely develops.[3] Mutations in the *CLCN5* gene are responsible for 60% of the disease and mutations in the *OCRL* gene for 15%, but no mutation has been identified in remaining patients so far.[5]

In this manuscript we report three patients diagnosed as DD in our department in the last 10 years and thereby describe the genetics, pathophysiology clinical presentation and course, laboratory evaluation and management of the disease.

✉ Emre Leventoğlu ▪ dremrelevent@gmail.com

Received 30th Mar 2024, revised 8th Aug 2024, 12th Sep 2024, 2nd Oct 2024, accepted 21st Oct 2024.

**Case Presentations**

***Case 1***

A male infant born to healthy parents after a full-term uneventful pregnancy with a birth weight of 3,750 grams was followed and treated in the neonatal intensive care unit due to central hypoventilation and was consulted to our department as nephrocalcinosis was detected on abdominal ultrasonography (US). There was no parental consanguinity or known history of kidney disease in family members.

On physical examination, his body weight was 3,690 grams (50-75$^{th}$ percentile) and height was 50 cm (50-75$^{th}$ percentile). Blood pressure was normal (90/60 mm Hg, <90$^{th}$ percentile) and he had no peripheral edema. He was receiving respiratory support due to central hypoventilation. Abdominal US revealed no pathology except nephrocalcinosis. Laboratory results showed normal serum creatinine, albumin, and electrolytes. Serum phosphorus was 6 mg/dL (normal range 4.3-9.3 mg/dL), 25-OH-vitamin D and parathyroid hormone (PTH) levels were also within normal ranges. Blood gases revealed a pH of 7.55 and a $HCO_3$ level of 30.4 mmol/L. Urine output was normal. There was no hematuria, and no evidence for hyperoxaluria or hypocitraturia. However, the patient had glucosuria, hypercalciuria, albuminuria and proteinuria. Spot urine albumin/creatinine ratio was 292 mg/g, protein/creatinine 3.2 mg/mg and calcium/creatinine 1.7 mg/mg. β2 microglobulin level in spot urine was high at 24.4 mg/L (normal range 0.04-0.22 mg/L). Tubular reabsorption of phosphate (TRP) was low at 74.2%, and fractional sodium excretion (FeNa) was slightly increased at 3.7%.

Whole exome sequencing analysis revealed a hemizygous c.1852G>A (p.Val618Met) variant classified as likely benign in the *CLCN5* gene. In addition to promoting oral hydration, hydrochlorothiazide and an angiotensin converting enzyme inhibitor (ACEi), enalapril treatments were started due to hypercalciuria and proteinuria, respectively. The patient was then followed up every three months for 2.5 years. During this period, a significant decrease in proteinuria and calciuria was observed, medullary nephrocalcinosis persisted, and no deterioration in kidney functions was observed. However, as the patient had recurrent febrile urinary tract infections (UTIs), prophylactic dosage of nitrofurantoin was commenced. A dimercaptosuccinic acid (DMSA) scan performed at the age of 2 years due to recurrent UTI revealed no renal scar formation and relative cortical functions were normal.

***Case 2***

A 4-year-old boy was admitted to another center due to symptoms compatible with UTI. He was referred to our department for detection of proteinuria on different occasions after treatment. He had a personal medical history of frequent UTIs with normal DMSA scintigraphy.

He was born from non-relative parents at the 31$^{st}$ week of gestation with a birth weight of 2,020 grams (75-90$^{th}$ percentile). His uncle, the mother's brother, had nephrolithiasis with unknown etiology. On admission to our hospital, his body weight was 17 kg (25-50$^{th}$ percentile) and height was 104 cm (25-50$^{th}$ percentile). He was normotensive. The physical examination was unremarkable. The laboratory results revealed normal levels of serum creatinine, albumin and electrolytes. Serum phosphorus was 4.8 mg/dL (normal range 3.8-6.9 mg/dL), 25-OH-vitamin D and PTH were also normal. There was no evidence of polyuria/polydipsia. Urinalysis showed glucose and protein 1+ without hematuria. Urinary protein and calcium excretions were 18.4 mg/m$^2$/hour and 10 mg/kg/day, respectively. Urine albumin level was 220 mg per day (microalbuminuria as 30-300 mg/day). TRP and FeNa were in normal ranges at 93% and 0.5%, respectively. β2 microglobulin level in spot urine was high at 60.2 mg/L. Urinary US was normal, neither nephrolithiasis nor nephrocalcinosis was present.

A hemizygous c.1557+1G>T pathogenic variant was detected in the *CLCN5* gene by

targeted next generation sequencing. Aside from low sodium diet and a high daily water intake, hydrochlorothiazide (1 mg/kg/day) and enalapril (0.1 mg/kg/day) were started. The patient has been followed up in our hospital for 3 years with normal kidney functions. Urinary protein and calcium excretions decreased to 14.2 mg/m$^2$/hour and 1.6 mg/kg/day, respectively. No nephrolithiasis or nephrocalcinosis developed during this period.

***Case 3***

A 3,600 g male infant born at term to a healthy, non-related family was hospitalized for a UTI at postnatal 15$^{th}$ day with complaints of decreased feeding and malaise. He was subsequently followed up for recurrent UTI. At 11 months of age, he was referred to our department due to the presence of a few millimeters of crystalloids in the bilateral renal collecting systems on US.

At the time of admission, growth and development were appropriate for his age and physical examination was unremarkable. In laboratory analysis, serum creatinine, electrolytes, and albumin levels were in normal range. Serum phosphorus, 25-OH-vitamin D and PTH were also normal. There was no acidosis or alkalosis. Urinalysis showed pH 7, specific gravity 1010, no glucosuria, hematuria or pyuria. However, proteinuria and calciuria were present (32 mg/m$^2$/h and 11 mg/kg/day, respectively). Urine albumin level was 421 mg per day (macroalbuminuria >300 mg/day). Spot urine β2 microglobulin level was also markedly elevated (116 mg/L). The DMSA scintigraphy due to the history of UTI was normal.

Genetic analysis by targeted next generation sequencing revealed a c.952C>T (p.Arg318Cys) hemizygous likely pathogenic variant in the *OCRL* gene. Hydrochlorothiazide was started at 1 mg/kg per day due to persistence of hypercalciuria despite dietary recommendations and adequate hydration. Also, enalapril was started due to a mild increase in proteinuria at the age of 2 years. Urinary calcium excretion decreased to 4.6 mg/kg/day when the dose of hydrochlorothiazide was increased to 2 mg/kg/day. However, proteinuria increased to 96 mg/m$^2$/h despite enalapril, and it decreased to 60 mg/m$^2$/h after addition of angiotensin receptor blocker (ARB) as losartan (0.7 mg/kg/day) to the treatment.

During 8 years of follow-up, no significant deterioration was observed in kidney function tests. Kidney dimensions and parenchymal echogenicity were normal on the last urinary US. In the right kidney, there were several crystalloids, the largest of which was 3 mm in diameter in the lower pole. In addition, ophthalmic examination was normal. There were no behavioral problems, nor intellectual deficits. His serum creatine kinase level was slightly high (252.3 U/L, normal range 0-190 U/L), however there was no finding compatible with myopathy.

A summary of clinical information regarding the cases is shown in Table I.

Written informed consent was obtained from parents for the use of clinical and laboratory data for publication.

## Discussion

In this study, we report three pediatric patients with DD who were being followed up in a tertiary pediatric nephrology center. Although variations were found in two different genes, the phenotypic features of all patients were quite different. Despite the presence of proteinuria and hypercalciuria in all patients, their levels varied. Two of the cases had either nephrolithiasis or nephrocalcinosis on urinary US, but remarkably, one patient had no ultrasonographic findings. In this manuscript, we tried to draw attention to this rare disease and its different clinical manifestations. Furthermore, although the variant detected in Case-1 was previously classified as "likely benign", the clinical features of this patient were consistent with DD and no major variant was detected in the whole exome sequencing that could explain the clinical condition. We

**Table I.** Summary of clinical information of the cases.

| Patients | Sex | Variants | At admission | | | | | Drugs | Follow-up durations (years) | At last control | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Age | GFR (mL/min/1.73m$^2$) | Urinary protein excretion | Urinary calcium excretion | Nephrolithiasis / Nephrocalcinosis | | | GFR (mL/min/1.73m$^2$) | Urinary protein excretion | Urinary calcium excretion | Nephrolithiasis / Nephrocalcinosis |
| Case-1 | Male | c.1852G>A in *CLCN5* | 22-days-old | 85.3 | 3.2a | 1.7a | - / + | Hydrochlorothiazide/ Enalapril | 2.5 | 127.3 | 1.8a | 1.1a | - / + |
| Case-2 | Male | c.1557+1G>T in *CLCN5* | 4-years-old | 127.6 | 18.4b | 10.0c | - / - | Hydrochlorothiazide/ Enalapril | 3.0 | 129.7 | 14.2b | 1.6c | - / - |
| Case-3 | Male | c.952C>T in *OCRL* | 11-months-old | 121.7 | 32.0 b | 11.0 c | + / - | Hydrochlorothiazide/ Enalapril/Losartan | 8.0 | 114.3 | 60.0b | 4.6c | + / - |

GFR, glomerular filtration rate.
[a]Spot urine sample; mg/mg creatinine, [b]24-hour urine sample; mg/m$^2$/h, [c]24-hour urine sample; mg/kg/d

believe that this manuscript may pave the way for functional studies to clearly assess the pathogenicity of this variant.

Dent's disease is a rare genetic disorder characterized by proximal tubular dysfunction, described by Dent and Friedman in 1964 as hypercalciuric rickets.[6] It has a highly heterogeneous phenotype. Some cases are diagnosed with asymptomatic proteinuria, while others have nephrocalcinosis/nephrolithiasis and chronic kidney disease. Although the symptoms begin in childhood, asymptomatic patients may be diagnosed in adulthood.[7] Although US had not been performed on the parents of our patients, they had no history of hematuria/nephrolithiasis/urinary tract infection and no symptoms compatible with nephrolithiasis/nephrocalcinosis. However, considering that asymptomatic patients may also be present, we believe family members of every patient with DD, especially the parents of our patients, should be screened for nephrolithiasis/nephrocalcinosis by US.

The most common mutation, the *CLCN5* mutation, causes DD type 1 (DD1), while the rarer *OCRL* mutation causes DD type 2 (DD2). Although *CLCN5* and *OCRL* mutations cause the same disease, they are generally not sufficient to explain phenotypic differences in patients. There is a growing number of patients carrying *CLCN5* or *OCRL* mutations but with incomplete phenotypic features. This phenotypic diversity can lead to unnecessary kidney biopsies, undiagnosed or misdiagnosed DD and, unfortunately, inappropriate treatment.[8] For example, in cases where DD is not considered, patients undergoing kidney biopsy for nephrotic range proteinuria may have focal segmental glomerulosclerosis (FSGS), which may lead to immunosuppressive therapy such as corticosteroids and calcineurin inhibitors.[9] Therefore, even if nephrotic level proteinuria is present, DD should be considered in the differential diagnosis if a patient has accompanying hypercalciuria, nephrolithiasis/ nephrocalcinosis. Some patients with DD2 are likely to have mild extrarenal features such as

muscle weakness, ocular abnormalities and mild intellectual disability.[4] In addition, hidradenitis suppurativa can be seen very rarely in DD2.[10] In these patients, it may be more easily recognized that there is an underlying diagnosis such as DD other than primary FSGS.

The *CLCN5* gene is involved in the production of the electrogenic chloride channel Cl-/H+ antiporter ClC-5 protein. The ClC-5 protein has important roles in the acidification in the endosomes of proximal tubule cells. In the absence of functional CLC-5, the endocytosis is inhibited. Therefore, the reabsorption of LMWPs is disrupted by being located at the brushy edge of the plasma membrane in proximal tubule cells (Fig. 1).[4,11] Furthermore, the endocytosis defect in proximal tubule cells leads to increased PTH concentration in urine and thus to increased 1,25-$(OH)_2$-vitamin D3 levels. Consequently, intestinal absorption of calcium increases and hypercalciuria and nephrocalcinosis/nephrolithiasis may develop.[12] In some in vivo models, despite the same mutation in the *CLCN5* gene, LMWP and hyperphosphaturia have developed in all models, while hypercalciuria and nephrolithiasis have not been observed in some.[13,14]

The *OCRL* gene encodes a member of the inositol polyphosphate-5-phosphatase enzyme family. It is expressed in the trans-Golgi network in glomeruli and all tubule segments and acts as a second messenger in vesicular transport.[15] It has also been shown that *OCRL* mutation disrupts early endosome functions due to defects in F-actin filaments.[16] Furthermore, *OCRL* regulates TRPV6 trafficking in the intestinal epithelium. In the case of *OCRL* mutation, TRPV6 cannot be inhibited, which increases intestinal absorption of $Ca^{2+}$ leading to hypercalciuria (Fig. 2).[16] Both DD2 or Lowe syndrome (which is an X-linked multisystemic disorder characterized by the triad of congenital cataracts, cognitive and behavioral impairment and a renal proximal tubulopathy) can develop due to *OCRL* mutation. However, it seems impossible to determine a genotype-phenotype correlation for this gene, as mutation in the same gene can cause both diseases.[7] However,

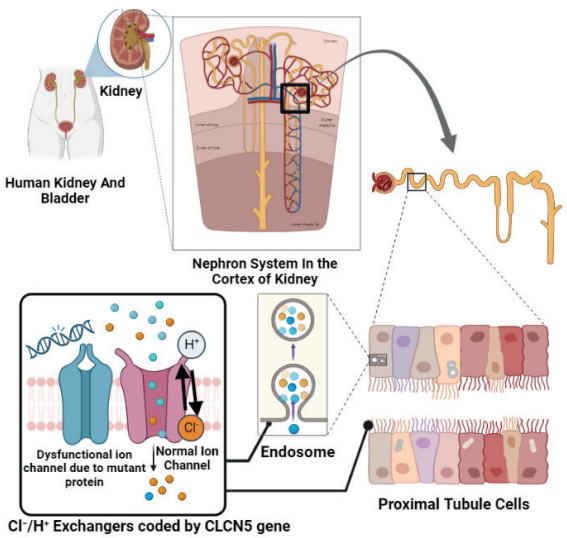



**Fig. 1.** Functions of the CLC-5 protein encoded by the *CLCN5* gene.

The *CLCN5* gene is involved in the production of the electrogenic chloride channel Cl-/H+ antiporter ClC-5 protein. The ClC-5 protein has an important role in the acidification of the endosomes of proximal tubule cells. In the absence of functional CLC-5, endocytosis is inhibited. Created in BioRender. https://BioRender.com/z35o223

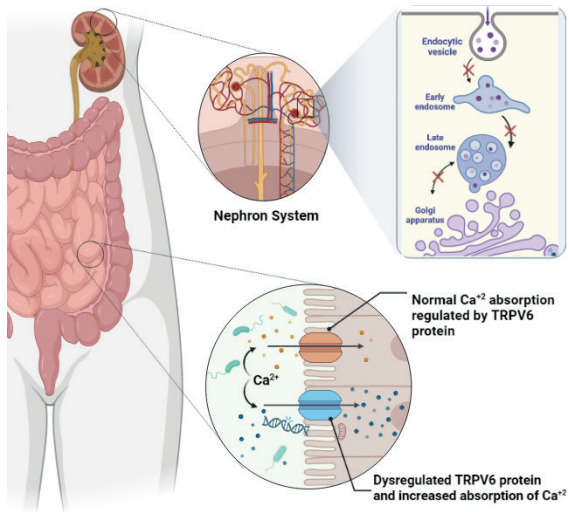



**Fig. 2.** Functions of *OCRL* gene products.

The *OCRL* gene encodes a member of the inositol polyphosphate-5-phosphatase enzyme family. It is expressed in the trans-Golgi network in glomeruli and all tubule segments and acts as a second messenger in vesicular transport. Also, *OCRL* regulates TRPV6 trafficking in the intestinal epithelium. Created in BioRender. https://BioRender.com/u24d663

a review by Gianesello et al. reported that the *OCRL* mutation site and mutation type largely determine the DD2 phenotype. In our study, we cannot comment on the genotype-phenotype relationship due to the limited number of cases. Considering the rarity of the disease, it is thought that a multicenter evaluation with large case series would be appropriate.[17]

Two of our patients had a variant in the *CLCN5* gene, and one had a variant in *OCRL* gene. The c.1852G>A variant in *CLCN5* in Case-1 was previously classified as likely benign.[18] However, despite whole exome sequencing, no other variant was found that could explain LMWP, hypercalciuria and nephrocalcinosis in our patient. Also, in silico analyses, the Combined Annotation Dependent Depletion (CAAD) score was 25.9, Rare Exome Variant Ensemble Learner (REVEL) score was 0.941, phylogenetic P-values (phyloP) were 7.44 and Polymorphism Phenotyping (PolyPhen) score was 0.996.[19] The CADD score is a score that assesses the likelihood of a variant being associated with genetic harm. A value of 15 and above is considered noteworthy for pathogenicity. A score of 25.9 in our patient indicates that the variant is likely to cause loss of function. REVEL is a score that estimates the pathogenicity of rare variants. It ranges from 0 to 1. A high score of 0.941 indicates that the variant is probably harmful. PhyloP is the evolutionary conservation score. A high score of 7.44 indicates that this region is highly evolutionarily conserved and that changes here are likely to be harmful. PolyPhen is a tool that predicts the effect of an amino acid change on protein function. It produces a score between 0 and 1. A high score of 0.996 indicates that the variant is likely deleterious.[20] However, it should be noted that while the detected variant is likely to have a high deleterious effect, in silico assays may not always fully reflect reality as they only provide a prediction of pathogenicity. Therefore, we think that it is very important to identify individuals with the same variant and to re-evaluate the pathogenicity of this variant which was previously classified as likely benign. In particular, functional analyses are needed to definitively classify the detected variant as disease causing/pathologic. The c.1557+1G>T variant in the *CLCN5* gene, which was found in Case-2, was previously described by Tosetto et al.[21] In a patient with DD, a G to T transversion was detected in intron 8 of *CLCN5*, resulting in a splice site mutation and the formation of an mRNA transcript lacking part of exon 8, which was confirmed by real time-polymerase chain reaction (RT-PCR) analysis. The variant was classified as pathogenic as a result of evaluation with tools that predict the pathogenicity of a variant.[21] However, since the phenotypic characteristics of the patient are not detailed in that manuscript, a comparison with our patient cannot be made. The c.952C>T variant in the *OCRL* gene found in Case-3 is a mutation that has been previously detected in patients with DD. In a multicenter study presented by Sekine et al.[22] the same variant was demonstrated in three patients. The age of the patients was between 9.5-15.3 years. It was reported that one patient did not have cataract or intellectual-behavioral impairments, however no evaluation of the other patients was mentioned.[22] In our patient, no extrarenal findings were observed during the 8-year follow-up period, as previously mentioned.

The main goal of DD treatment is to preserve kidney function by reducing hypercalciuria and related complications. Low sodium diet, adequate hydration and thiazide diuretics are classically used for hypercalciuria. In case of hypokalemia, metabolic acidosis and hypophosphatemia, supportive treatments are implemented.[23] In all of our patients, adequate oral fluid intake, low sodium diet and hydrochlorothiazide diuretics were used.

Since Dent's disease is mainly a nonglomerular disease and patients have tubular proteinuria, the rationale for the use of ACEi/ARB therapy is unclear.[23] In a review, some patients treated with ACEi/ARBs had a reduction in proteinuria, some had no effect, and some had a reduction in glomerular filtration rate (GFR).[24] In another

study, it was reported that pre-existing tubular proteinuria may be accompanied by glomerular proteinuria during the follow-up period and enalapril use may be necessary. However, in that article, it was pointed out that GFR may decrease with enalapril.[25] Specifically in the *CLCN5* mutation, FSGS can be seen on kidney biopsy. This supports the hypothesis that *CLCN5* is involved in protein trafficking in podocytes also, and its mutation may cause glomerular proteinuria due to disrupting normal cell physiology and filtration barrier.[26] In a study evaluating the phenotypic spectrum of Dent's Disease and ACEi/ARB response to proteinuria, it was shown that albuminuria could be controlled with ACEi and/or ARB treatment in half of the children. However, genotypes, glomerular lesions, age at treatment initiation and duration of treatment could not explain the difference in antialbuminuric response.[27] All of our patients were prescribed ACEi and/or ARB due to proteinuria and a decrease in proteinuria was shown in each, most significant decline in Case-1. We think that more studies are needed in this field to explain why Case-1 benefited more from antiproteinuric treatment. Fortunately, no patient had a decrease in GFR. In addition, since no biopsy was performed in any of our patients, no definite information about renal histopathology can be given. However, when urine protein analysis was performed in our patients, the significantly higher proportion of tubular proteins in the urine may suggest that glomerular damage was not severe.

In DD, GFR decreases by 1.0 to 1.6 mL/1.73 $m^2$/min per year.[1] Approximately 30% to 80% of male patients with DD develop kidney failure between the ages of 30 and 50 years. The age at which kidney failure develops can be as late as the $7^{th}$ decade. Even within the same family, there are individuals with different ages of onset of kidney failure.[23] The follow-up period in our patients ranged from 2.5 to 8 years. Since all of our patients were in the pediatric age group, we did not observe any patient with kidney failure development. Fortunately, none of our patients had a decrease in GFR.

It is noteworthy that all three patients in our study had a history of recurrent UTIs. The relationship between nephrolithiasis and UTIs is complex. To date, it has been generally emphasized that certain bacteria pose a risk for kidney stone formation.[28] However, a study by Cetin et al. evaluated risk factors for UTI in children with nephrolithiasis and found that recurrent UTI was significantly more common in patients with risk factors for stone formation, especially in patients with hypercalciuria.[29] The presence of marked hypercalciuria in all our patients may have been the reason for the development of recurrent UTI.

In conclusion, DD is a disease that shows different phenotypes even among individuals with the same mutation. It has the potential of leading to impaired kidney function in the absence of appropriate follow-up and treatment. Therefore, DD should be considered in all patients with hypercalciuria, proteinuria, nephrolithiasis or nephrocalcinosis with/without proximal tubular dysfunction especially in early childhood. Classical treatments for hypercalciuria should be utilized, and a patient-based treatment plan should be drawn especially for proteinuria. Since there is a genetic basis, genetic counseling should not be ignored.

## Ethical approval

Informed consent was obtained from the parents of each 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: HY, EL, BB; data collection: HY, EL, BB, KF, SAB and OS; analysis and interpretation of results: HY, EL, BB; draft manuscript preparation: HY, EL, BB. All authors reviewed the results and approved the final version of the manuscript.

**Source of funding**

The authors declares the study received no funding.

**Conflict of interest**

The authors declares that there is no conflict of interest.

## REFERENCES

1. Blanchard A, Curis E, Guyon-Roger T, et al. Observations of a large Dent disease cohort. Kidney Int 2016; 90: 430-439. https://doi.org/10.1016/j.kint.2016.04.022
2. Leggatt G, Gast C, Gilbert RD, Veighey K, Rahman T, Ennis S. Hemizygous loss of function mutations in CLCN5 causing end-stage kidney disease without Dent disease phenotype. Clin Kidney J 2022; 16: 192-194. https://doi.org/10.1093/ckj/sfac127
3. Lieske JC, Milliner DS, Beara-Lasic L, Harris P, Cogal A, Abrash E. Dent disease. In: Adam MP, Mirzaa GM, Pagon RA, et al., editors. Gene Reviews. Seattle, WA: University of Washington; 2012: 1993-2023.
4. Jin YY, Huang LM, Quan XF, Mao JH. Dent disease: classification, heterogeneity and diagnosis. World J Pediatr 2021; 17: 52-57. https://doi.org/10.1007/s12519-020-00357-1
5. Zaniew M, Mizerska-Wasiak M, Załuska-Leśniewska I, et al. Dent disease in Poland: what we have learned so far? Int Urol Nephrol 2017; 49: 2005-2017. https://doi.org/10.1007/s11255-017-1676-x
6. Dent CE, Friedman M. Hypercalcuric rickets associated with renal tubular damage. Arch Dis Child 1964; 39: 240-249. https://doi.org/10.1136/adc.39.205.240
7. Zaniew M, Bökenkamp A, Kolbuc M, et al. Long-term renal outcome in children with OCRL mutations: retrospective analysis of a large international cohort. Nephrol Dial Transplant 2018; 33: 85-94. https://doi.org/10.1093/ndt/gfw350
8. Gianesello L, Del Prete D, Anglani F, Calò LA. Genetics and phenotypic heterogeneity of Dent disease: the dark side of the moon. Hum Genet 2021; 140: 401-421. https://doi.org/10.1007/s00439-020-02219-2
9. Fervenza FC. A patient with nephrotic-range proteinuria and focal global glomerulosclerosis. Clin J Am Soc Nephrol 2013; 8: 1979-1987. https://doi.org/10.2215/CJN.03400313
10. Marzuillo P, Piccolo V, Mascolo M, et al. Patients affected by dent disease 2 could be predisposed to hidradenitis suppurativa. J Eur Acad Dermatol Venereol 2018; 32: e309-e311. https://doi.org/10.1111/jdv.14860
11. Devuyst O, Christie PT, Courtoy PJ, Beauwens R, Thakker RV. Intra-renal and subcellular distribution of the human chloride channel, CLC-5, reveals a pathophysiological basis for Dent's disease. Hum Mol Genet 1999; 8: 247-257. https://doi.org/10.1093/hmg/8.2.247
12. Günther W, Piwon N, Jentsch TJ. The ClC-5 chloride channel knock-out mouse - an animal model for Dent's disease. Pflugers Arch 2003; 445: 456-462. https://doi.org/10.1007/s00424-002-0950-6
13. Piwon N, Günther W, Schwake M, Bösl MR, Jentsch TJ. ClC-5 Cl- -channel disruption impairs endocytosis in a mouse model for Dent's disease. Nature 2000; 408: 369-373. https://doi.org/10.1038/35042597
14. De Matteis MA, Staiano L, Emma F, Devuyst O. The 5-phosphatase OCRL in Lowe syndrome and Dent disease 2. Nat Rev Nephrol 2017; 13: 455-470. https://doi.org/10.1038/nrneph.2017.83
15. Vicinanza M, Di Campli A, Polishchuk E, et al. OCRL controls trafficking through early endosomes via PtdIns4,5P2-dependent regulation of endosomal actin. EMBO J 2011; 30: 4970-4985. https://doi.org/10.1038/emboj.2011.354
16. Wu G, Zhang W, Na T, Jing H, Wu H, Peng JB. Suppression of intestinal calcium entry channel TRPV6 by OCRL, a lipid phosphatase associated with Lowe syndrome and Dent disease. Am J Physiol Cell Physiol 2012; 302: C1479-C1491. https://doi.org/10.1152/ajpcell.00277.2011
17. Gianesello L, Arroyo J, Del Prete D, et al. Genotype phenotype correlation in Dent disease 2 and review of the literature: OCRL gene pleiotropism or extreme phenotypic variability of lowe syndrome? Genes (Basel) 2021; 12: 1597. https://doi.org/10.3390/genes12101597
18. National Library of Medicine. ClinVar-Genomic variation as it relates to human health. Available at: https://www.ncbi.nlm.nih.gov/clinvar/variation/758735/ (Accessed on August 10, 2024).
19. gnomAD browser. SNVX-50090223-G-A(GRCh38), in silico predictors. Available at: https://gnomad.broadinstitute.org/variant/X-50090223-G-A?dataset=gnomad_r4 (Accessed on September 28, 2024).
20. Katsonis P, Wilhelm K, Williams A, Lichtarge O. Genome interpretation using in silico predictors of variant impact. Hum Genet 2022; 141: 1549-1577. https://doi.org/10.1007/s00439-022-02457-6

21. Tosetto E, Ceol M, Mezzabotta F, et al. Novel mutations of the CLCN5 gene including a complex allele and A 5′ UTR mutation in Dent disease 1. Clin Genet 2009; 76: 413-416. https://doi.org/10.1111/j.1399-0004.2009.01212.x

22. Sekine T, Komoda F, Miura K, et al. Japanese Dent disease has a wider clinical spectrum than Dent disease in Europe/USA: genetic and clinical studies of 86 unrelated patients with low-molecular-weight proteinuria. Nephrol Dial Transplant 2014; 29: 376-384. https://doi.org/10.1093/ndt/gft394

23. Ehlayel AM, Copelovitch L. Update on Dent disease. Pediatr Clin North Am 2019; 66: 169-178. https://doi.org/10.1016/j.pcl.2018.09.003

24. van Berkel Y, Ludwig M, van Wijk JAE, Bökenkamp A. Proteinuria in Dent disease: a review of the literature. Pediatr Nephrol 2017; 32: 1851-1859. https://doi.org/10.1007/s00467-016-3499-x

25. Vaisbich MH, Henriques L dos S, Igarashi T, Sekine T, Seki G, Koch VH. The long-term use of enalapril and hydrochlorothiazide in two novel mutations patients with Dent's disease type 1. J Bras Nefrol 2012; 34: 78-81.

26. Solanki AK, Arif E, Morinelli T, et al. A Novel CLCN5 mutation associated with focal segmental glomerulosclerosis and podocyte injury. Kidney Int Rep 2018; 3: 1443-1453. https://doi.org/10.1016/j.ekir.2018.06.003

27. Deng H, Zhang Y, Xiao H, et al. Phenotypic spectrum and antialbuminuric response to angiotensin converting enzyme inhibitor and angiotensin receptor blocker therapy in pediatric Dent disease. Mol Genet Genomic Med 2020; 8: e1306. https://doi.org/10.1002/mgg3.1306

28. Borghi L, Nouvenne A, Meschi T. Nephrolithiasis and urinary tract infections: 'the chicken or the egg' dilemma? Nephrol Dial Transplant 2012; 27: 3982-3984. https://doi.org/10.1093/ndt/gfs395

29. Cetin N, Gencler A, Kavaz Tufan A. Risk factors for development of urinary tract infection in children with nephrolithiasis. J Paediatr Child Health 2020; 56: 76-80. https://doi.org/10.1111/jpc.14495

The Turkish Journal of Pediatrics 2024; 66: 658-665
https://doi.org/10.24953/turkjpediatr.2024.4522

Case Report

# Single-center experience of four cases with iron-refractory iron deficiency anemia (IRIDA)

Gülin Parlak[1], Muhammed Doğukan Aksu[2,3], Fatma Gümrük[4], Şule Ünal[4]

*[1]Department of Pediatrics, Faculty of Medicine, Hacettepe University, Ankara; [2]Faculty of Medicine, Hacettepe University, Ankara; [3]Department of Basic Oncology, Cancer Institute, Hacettepe University, Ankara; [4]Department of Pediatric Hematology, Faculty of Medicine, Hacettepe University, Ankara, Türkiye.*

## ABSTRACT

**Background.** Iron refractory iron deficiency anemia (IRIDA) is a rare autosomal recessive type of anemia characterized by unresponsiveness to oral iron therapy and partial response to parenteral iron therapy. In this article, we report the clinical presentation of four patients with IRIDA admitted to our clinic, including their laboratory values at admission and after oral and parenteral iron treatment, and the analysis of their mutation(s) in *TMPRSS6* gene.

**Case.** Four patients from different families, aged between 3 and 14 years, two girls and two boys, two of whom were from consanguineous marriages, who were diagnosed with iron deficiency anemia in primary health care institutions and referred to our clinic because of inadequate response to oral iron treatment were included. Patients were evaluated for the differential diagnosis of microcytic, hypochromic anemia and investigated for the etiology of IDA. Homozygous or compound heterozygous mutations causing defective matriptase-2 protein expression were detected in the *TMPRSS6* gene; these mutations included four frameshift mutations-two of which were the same in two cases and causing premature terminal stop codons-and a nonsense mutation, all of which were previously demonstrated in the literature. The response to parental iron therapy ranged from complete non-response to mild to good response in hemoglobin levels, but none of the patients showed improvement in iron parameters.

**Conclusions.** Increased awareness of IRIDA and keeping it in mind in the differential diagnosis in the presence of hypochromic microcytic anemia that does not respond to iron treatment will be crucial in improving the diagnosis and treatment of the disease and ultimately enhancing the quality of care for affected individuals.

**Key words:** iron refractory iron deficiency anemia, IRIDA, *TMPRSS6*, parenteral iron.

Iron deficiency anemia (IDA) is the most common type of anemia worldwide, especially in children and adolescents, due to increased physiological iron demand, inadequate intake, chronic blood loss or underlying malabsorptive pathologies.[1,2] The clinical distribution of signs and symptoms of the disease largely depends on the degree of anemia, and oral iron medications are the first-line treatment that should be initiated after laboratory diagnosis of IDA.[1,3] However, there are genetic/hereditary forms of iron-related anemias due to inherited defects in different stages of iron utilization, which complicate the treatment and management of iron deficiency anemia.[4-6]

Iron-resistant iron deficiency anemia (IRIDA) is a rare form of autosomal recessive anemia caused by various mutations in the *TMPRSS6* (MIM #609862) gene, which encodes matriptase-2 (MT2).[7] MT2 is a type II transmembrane serine

✉ Şule Ünal ▪ suleunal@hacettepe.edu.tr

Received 12th Dec 2023, revised 30th Mar 2024, 18th Sep 2024, accepted 30th Sep 2024.

protease responsible for the downregulation of hepcidin expression; it induces the degradation of hemojuvelin and reduces the activity of the BMP/SMAD pathway.[8-11]

This clinical condition is manifested with microcytic, hypochromic anemia, very low mean corpuscular volume (MCV), low serum iron and transferrin saturation index (TSI), normal or high serum ferritin levels and high urinary hepcidin levels; in addition, patients with IRIDA respond poorly to oral iron and only partially to intravenous iron therapy.[6,12,13]

Patients with IRIDA have mild to moderate anemia with a degree of hypochromic microcytosis, and growth and development are usually normal.[13,14] Because of this inconsistency of clinical and laboratory findings, in the absence of routine laboratory screening for anemia, many cases may go unnoticed clinically due to the normal growth and development of affected individuals , hence the estimate of IRIDA frequency in the community.[10]

In this article, the clinical picture, laboratory values at presentation and after iron therapy, and analysis of *TMPRSS6* mutations in four patients referred to our clinic with a diagnosis of iron deficiency unresponsive to oral iron therapy are presented.

## Case presentations

Four patients from different families, aged between 3 and 14 years, two girls and two boys, two of whom were from consanguineous marriages (Case-1 and Case-4) were diagnosed with IDA in primary health care institutions and referred to our clinic because of an inadequate response to oral iron treatment.

Medical history revealed that none of the patients had undergone bone marrow examination and only Case-2 had received an erythrocyte transfusion once. When the family history was questioned, it was noted that only the cousins of the father of Case-2 received intravenous iron treatment regularly; however, no further details could be obtained.

Firstly, it was confirmed that all patients had a varied iron-rich diet, and avoided foods that could lead to iron malabsorption. None of the patients had any recent infections and C-reactive protein values were in the normal range. Except for Case-4 (who presented with dizziness), the other patients had no significant complaints other than mild pallor and fatigue; no abnormal physical examination findings were found in any patient. Furthermore, blood loss was not suspected in patients whose medical histories were taken and fecal occult blood tests were performed. Liver, kidney and thyroid function tests were normal in all patients and no pathological findings were found in urinalyses. IgA and IgG antibodies against tissue transglutaminase were negative and serum IgA levels were within the normal range, making celiac disease unlikely.

Since there was microcytic, hypochromic anemia with low serum iron and low TSI, and no pathological findings were found in the peripheral smear except for hypochromia and poikilocytosis, oral iron (II)-glycine sulfate treatment of 4-6 mg/kg/day for 2 to 3 months was initiated in all patients except Case-4. Since it was confirmed that Case-4 had received oral iron treatment at the appropriate dose and duration in the previous center and the patient was still symptomatic; therefore, intravenous iron treatment was preferred instead of oral iron treatment.

In follow-up examinations of the patients in whom oral iron treatment was initiated, it was observed that there was no adequate response to the treatment, and no improvement in their fatigue. Similar results were observed in the follow-up blood tests of Case-4, in whom parenteral iron therapy was initiated. Hemoglobin electrophoresis was performed for the differential diagnosis of hypochromic microcytic anemia due to a lack of response to iron treatment, and thalassemia was ruled out with laboratory results shown in Table I.

**Table I.** Results of hemoglobin electrophoresis of all cases.

| | Case-1 | Case-2 | Case-3 | Case-4 |
|---|---|---|---|---|
| Hb A (%) | 98.6 | 97.7 | 98.4 | 97.8 |
| Hb A2 (%) | 1.4 | 2.3 | 1.6 | 2.1 |
| Hb F (%) | 0 | 0 | 0 | 0.1 |

Hb, hemoglobin.

**Table II.** Detected mutations in *TMPRSS6* gene.

| Case | *TMPRSS6* Variation | Amino acid Change | Region | Reference Sequence | rsID | Genotype |
|---|---|---|---|---|---|---|
| 1 | c.[1877_1878dupGC] | p.[Lys627fs] | Exon 16 | NM_001374504.1 | rs869320724 | Homozygous |
| 2 | c.[188del] | p.[Leu63fs] | Exon 2 | NM_153609.3 | rs1438085143 | Heterozygous |
| | c.[580G>T] | p.[Glu194*] | Exon 5 | NM_153609.3 | rs761779631 | Heterozygous |
| 3 | c.[1904_1905dupGC] | p.[Lys636Alafs*17] | Exon 16 | NM_153609.3 | N/A | Homozygous |
| 4 | c.[1904_1905dupGC] | p.[Lys636Alafs*17] | Exon 16 | NM_153609.3 | N/A | Homozygous |

rsID, reference single nuclear polymorphism identification.

Therefore, IRIDA was suspected for iron deficiency anemia resistant to oral iron therapy (Case-1 and Case-3) and unresponsive to parenteral iron therapy (Case-4), and the *TMPRSS6* gene was investigated.

For mutation analyses, peripheral blood samples from each patient were collected in tubes containing ethylenediaminetetraacetic acid (EDTA) and genomic DNA was extracted. All 18 exons and exon-intron boundaries of the *TMPRSS6* gene were amplified by polymerase chain reaction (PCR). GenBank accession numbers NM_001374504.1 (for Case-1) and NM_153609.3 (for Case-2 and Case-4) were used as reference sequences. Direct sequencing was performed and the results were analyzed using NextGENe (Version 2.4.2.3/SoftGenetics LLC-USA) and Geneticist Assistant (Version 1.8.1.0/SoftGenetics LLC-USA).

*TMPRSS6* gene sequence analysis revealed homozygous frameshift in exon 16 in Case-1, compound heterozygous frameshift in exon 2 and heterozygous nonsense mutation in exon 5 in Case-2, and a homozygous frameshift mutation causing an early stop codon in exon 16 in Case-3 and Case-4 (Table II).

Case-1and Case-3 were started on intravenous ferric hydroxide sucrose complex, and it was observed that they benefited from parenteral iron therapy compared to oral iron therapy. Case-4 did not respond to intravenous iron treatment and 6 mg/kg/day oral iron (II)-glycine sulfate treatment with vitamin C supplementation was initiated; the patient did not respond to this treatment either and the patient did not attend the follow-up visits in our center and continued his follow-up in his hometown.

Complete blood count, serum iron, ferritin and TSI values of the patients at admission, after oral iron treatment and parenteral iron treatment are shown in Table III.

All the studies were performed in accordance with the Declaration of Helsinki and guidelines for good clinical practice. All legal representatives of the patients were informed, and their informed consent was obtained.

## Discussion

Iron deficiency anemia is a global health problem and is the leading cause of anemia manifested by microcytic and hypochromic erythrocytes.[15]

Other common causes of microcytic anemia that should be excluded when IDA is suspected include anemia of chronic disease, hemoglobinopathies, thalassemia syndromes

**Table III.** Demographic features, complete blood count and serum iron parameters of four patients at the time of presentation and after oral and parenteral iron therapy.

| | | Hb (g/dL) | Hct (%) | MCV (fL) | MCH (pg) | MCHC (g/dL) | RDW-CV (%) | RBC (x10$^6$/μL) | WBC (x10$^3$/μL) | Platelets (x10$^3$/μL) | Serum iron (μg/dL) | TSI (%) | Ferritin (μg/L)[a] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Case-1 | Admission | 8.1 | 27.4 | 55.8 | 16.4 | 29.4 | 19.4 | 4.91 | 7.6 | 582 | 10 | 3 | 62.9 |
| (3 years, M) | After 2 months of oral iron treatment | 8.8 | 31.6 | 62.2 | 17.3 | 27.8 | 19 | 5.08 | 7.72 | 521 | 14 | 5 | 81.2 |
| | After 2 months of parenteral iron treatment | 10.4 | 35.3 | 65.5 | 19.3 | 29.5 | 21.0 | 5.39 | 7.57 | 388 | 13 | 5 | 165.7 |
| Case-2 | Admission | 8.8 | 31.1 | 57.2 | 16.2 | 28.3 | 18.6 | 5.44 | 5.8 | 465 | 8 | 2 | 44.1 |
| (5 years, M) | After 3 months of oral iron treatment | 9.8 | 34.5 | 61.8 | 17.6 | 28.6 | 18.3 | 5.58 | 6.7 | 401 | 11 | 3 | 108.3 |
| | After 2 months of parenteral iron treatment | 10.3 | 34.2 | 64.1 | 19.3 | 30.2 | 19.3 | 5.34 | 7.3 | 382 | 15 | 4 | 286.7 |
| Case-3 | Admission | 7.9 | 26.5 | 52.1 | 15.5 | 29.8 | 20.0 | 5.05 | 6.6 | 653 | 9 | 2 | 63.2 |
| (6 years, F) | After 10 weeks of oral iron treatment | 9.3 | 30.4 | 55.3 | 16.9 | 30.5 | 22.4 | 5.49 | 6.5 | 617 | 12 | 3 | 44.8 |
| | After 3 months of parenteral iron treatment | 11.6 | 36.2 | 69.9 | 22.4 | 32.0 | 23.7 | 5.18 | 7.4 | 438 | 21 | 6 | 205 |
| | After 6 months of parenteral iron treatment | 13 | 42.4 | 79.41 | 24.34 | 30.65 | 16.3 | 5.33 | 8.56 | 407 | 23 | 9.6 | 524.98 |
| Case-4 | Admission | 9.8 | 32.4 | 59.4 | 18 | 30.4 | 20.3 | 5.45 | 6.5 | 467 | 14 | 4 | 107.3 |
| (14 years, F) | 5 weeks after parenteral iron treatment | 9.2 | 30.4 | 59.9 | 18.2 | 30.3 | 21.9 | 5.08 | 9.9 | 383 | 14 | 5 | 152.9 |
| | 6 months after parenteral iron treatment | 9.6 | 32.3 | 60.8 | 18 | 29.6 | 21.5 | 5.32 | 7.1 | 419 | 10 | 4 | 107.1 |
| | 4 months after oral iron and vitamin C treatment | 9.4 | 32.1 | 61.7 | 18 | 29.2 | 19.9 | 5.21 | 9.5 | 400 | NA | NA | NA |

Hb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW-CV, red cell distribution width – coefficient of variation; RBC, red blood cell; WBC, white blood cell; TSI, transferrin saturation index.
[a]Normal range: 11-307 μg/L; NA, Not available; M, male; F, female.

and sideroblastic anemias, especially in congenital cases.

Following the diagnosis of IDA, acquired causes of iron deficiency, such as inadequate dietary iron intake and persistent hemorrhage, should be addressed for optimal treatment.[16] The well-established treatment strategy for IDA is oral ferrous sulphate; in addition, vitamin C has been shown to improve iron absorption.[17] However, inadequate treatment response can occur and in most cases is associated with poor treatment adherence, improper use of oral iron medication (e.g. not taking pills on an empty stomach), dosage errors and inadequate duration of treatment.[16]

Malabsorptive defects should be primarily suspected as the underlying cause of IDA if the patient is compliant with oral iron therapy at the appropriate dose and duration. Infections and inflammatory diseases that upregulate hepcidin, achlorhydria (e.g. chronic proton pump inhibitors, histamine H2 receptor antagonists or anti-acid medication, post-gastrectomy) and celiac disease are among the common causes limiting the absorption of dietary iron.[1,18]

IRIDA should be considered in the differential diagnosis of IDA patients with onset in infancy or childhood whose hematological parameters do not improve with oral iron therapy and/or abnormalities are found in oral iron absorption tests.[6] These patients clearly have hypochromic and microcytic anemia (hemoglobin 6-9 g/dL) with very low MCV (45-65 fL) and TSI (<5%).[10]

In the cases presented above, the IRIDA assessment was based on specific clinical and laboratory findings. Strikingly, the patients showed hypochromic microcytic anemia with significantly reduced serum iron levels accompanied by a low transferrin saturation index and normal serum ferritin levels. The diet of the patients investigated for iron deficiency and its possible causes was questioned and iron deficiency in the daily diet was ruled out. No abnormal findings were found in terms of chronic blood loss and malabsorption syndromes, especially celiac sprue. In addition, all patients had normal C-reactive protein levels, which excluded infectious or inflammatory conditions as acquired factors. Patients who did not respond to iron therapy underwent hemoglobin electrophoresis for the differential diagnosis of hypochromic microcytic anemia, especially thalassemia, and decreased HbA2 levels supporting IDA were noted (Table III).

Finally, an important common characteristic among these cases was the patients' history of minimal to no improvement upon oral iron supplementation, indicating the iron-refractory nature of their anemia. Consequently, the patients were selected to perform a genetic analysis in order to pinpoint the mutation resulting in the expression of the defective matriptase-2 protein.

Since the first identification of IRIDA, more than 45 different mutations, including nonsense, missense and frameshift, have been reported.[13] Of these, the presence of two nonsense mutations was noted to cause a more severe form of the disease compared to two missense mutations or one missense and one nonsense mutation, which is presumably due to the formation of a truncated version of the protein with nonsense mutations.[12] Among the pathogenic mutations identified and extracted from the dbSNP database, missense (rs770897887, s1438085143, rs1373272804, rs199474805, rs855791 and rs1449962575) and frameshift variants (rs1438085143, rs786205060, rs869320724, rs767094129, rs1384933966, rs869320724 and rs137853123) were the most frequently reported, followed by stop-gain variants (CM1411671, rs137853123 and rs137853122) and synonymous variants (rs4820268). These variants have been shown to disrupt iron homeostasis by impairing matriptase-2 autoproteolytic activation and contributing to the development of IRIDA.[13] Furthermore, several single nucleotide polymorphisms (SNPs) associated with IRIDA have been identified in the *TMPRSS6* gene, with rs1373272804, rs1430692214 and rs855791 being the most frequently recorded SNPs associated

with functional outcomes. Exons 5, 7, 13 and 15 are important hotspots for SNPs and are some of the exons that play a critical role in shaping the genetic picture by showing a high frequency of genetic variation.[11]

In our cases, four frameshift mutations—two of which were the same in two cases and caused early terminal stop codons—and a nonsense mutation were detected. Mutations in Case-1, -3, and -4 were located in exon 16, which is responsible for coding the C-terminal trypsin-like serine protease domain of MT-2.[19] Specifically, in Case-3 and Case-4, a premature stop codon was introduced and thought to result in termination of translation before the catalytic serine, as described in a previous case.[20] In Case-2, there was a frameshift mutation in exon 2, which codes for the transmembrane region, and a nonsense mutation in exon 5, which codes for the membrane-proximal SEA domain.[19] Since all the mutations were either frameshift or nonsense and there was no missense mutation, we would expect to see more severe disease in Case-2 because the mutations were in the more proximal exons of the catalytic region of the enzyme compared to Case-1, -3, and -4. Correspondingly, only Case-2 required an erythrocyte suspension transfusion prior to his referral to our clinic.

It has been known that in patients with IRIDA, the causative mutations in *TMPRSS6* occur mostly in exon 15 and exon 16.[21] Interestingly, previous reports have shown that Turkish patients with IRIDA often exhibit a duplication mutation in exon 16, resulting in a frameshift mutation with a premature stop codon (c.1904_1905dupGC).[6,22-24] Supporting this observation, we found that two of our cases (Cases-3 and Case-4) also had the same mutation. This finding might suggest the possibility of a founder effect for this mutation among patients of Turkish origin, particularly in regions like the northern part of Türkiye, where consanguineous marriages are common, as was the case in our study.

In the literature, there is no consensus on the treatment of patients with IRIDA. The patients benefit from parenteral iron treatment often only partially and temporarily. In our management of the cases, proper dosage and duration of oral iron treatment were initially ensured, which was later replaced with parenteral iron. Response to parenteral iron treatment ranged from total unresponsiveness (Case-4) to mild (Case-1) to good response (Case-2 and Case-3) in Hb levels. However, iron parameters did not improve in any of the patients.

In conclusion, IRIDA remains a rare syndrome in established literature and clinical practice, despite being believed to be one of the most common congenital anemias.[10] This discrepancy may, in part, stem from a lack of awareness of the disease and the misattribution of symptoms to non-adherence to oral iron treatment by hematologists. The steps towards diagnosing IRIDA begin with a suspicion of the disease during the differential diagnosis process, which we aim for in the present report. In future studies, we anticipate the discovery of new mutations in *TMPRSS6* and a more precise description of IRIDA's frequency. These advances in understanding and awareness will be crucial in improving the diagnosis and management of IRIDA, ultimately enhancing the quality of care for affected individuals.

## Ethical approval

All the studies were performed in accordance with the Declaration of Helsinki and guidelines for good clinical practice. All legal representatives of the patients were informed, and their informed consent was obtained.

## Author contribution

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

**Source of funding**

The authors declares the study received no funding.

**Conflict of interest**

The authors declares that there is no conflict of interest.

## REFERENCES

1. Miller JL. Iron deficiency anemia: a common and curable disease. Cold Spring Harb Perspect Med 2013; 3: a011866. https://doi.org/10.1101/cshperspect.a011866
2. Camaschella C. Iron deficiency. Blood 2019; 133: 30-39. https://doi.org/10.1182/blood-2018-05-815944
3. Mattiello V, Schmugge M, Hengartner H, von der Weid N, Renella R, SPOG Pediatric Hematology Working Group. Diagnosis and management of iron deficiency in children with or without anemia: consensus recommendations of the SPOG Pediatric Hematology Working Group. Eur J Pediatr 2020; 179: 527-545. https://doi.org/10.1007/s00431-020-03597-5
4. Beutler E, Gelbart T, Lee P, Trevino R, Fernandez MA, Fairbanks VF. Molecular characterization of a case of atransferrinemia. Blood 2000; 96: 4071-4074.
5. Cotter PD, Rucknagel DL, Bishop DF. X-linked sideroblastic anemia: identification of the mutation in the erythroid-specific delta-aminolevulinate synthase gene (ALAS2) in the original family described by Cooley. Blood 1994; 84: 3915-3924.
6. Finberg KE, Heeney MM, Campagna DR, et al. Mutations in TMPRSS6 cause iron-refractory iron deficiency anemia (IRIDA). Nat Genet 2008; 40: 569-571. https://doi.org/10.1038/ng.130
7. De Falco L, Totaro F, Nai A, et al. Novel TMPRSS6 mutations associated with iron-refractory iron deficiency anemia (IRIDA). Hum Mutat 2010; 31: E1390-E1405. https://doi.org/10.1002/humu.21243
8. Folgueras AR, de Lara FM, Pendás AM, et al. Membrane-bound serine protease matriptase-2 (Tmprss6) is an essential regulator of iron homeostasis. Blood 2008; 112: 2539-2545. https://doi.org/10.1182/blood-2008-04-149773
9. Silvestri L, Guillem F, Pagani A, et al. Molecular mechanisms of the defective hepcidin inhibition in TMPRSS6 mutations associated with iron-refractory iron deficiency anemia. Blood 2009; 113: 5605-5608. https://doi.org/10.1182/blood-2008-12-195594
10. Heeney MM, Finberg KE. Iron-refractory iron deficiency anemia (IRIDA). Hematol Oncol Clin North Am 2014; 28: 637-52, v. https://doi.org/10.1016/j.hoc.2014.04.009
11. Ganz T, Nemeth E, Rivella S, et al. TMPRSS6 as a therapeutic target for disorders of erythropoiesis and iron homeostasis. Adv Ther 2023; 40: 1317-1333. https://doi.org/10.1007/s12325-022-02421-w
12. De Falco L, Sanchez M, Silvestri L, et al. Iron refractory iron deficiency anemia. Haematologica 2013; 98: 845-853. https://doi.org/10.3324/haematol.2012.075515
13. Sharma A, Kumar A, Saha PK, Saha L. The role of TMPRSS6 gene polymorphism in iron resistance iron deficiency anaemia (IRIDA): a systematic review. Ann Hematol 2024; 103: 1085-1102. https://doi.org/10.1007/s00277-023-05576-w
14. Camaschella C. How I manage patients with atypical microcytic anaemia. Br J Haematol 2013; 160: 12-24. https://doi.org/10.1111/bjh.12081
15. Camaschella C. Iron-deficiency anemia. N Engl J Med 2015; 372: 1832-1843. https://doi.org/10.1056/NEJMra1401038
16. Yılmaz Keskin E, Yenicesu İ. Iron-refractory iron deficiency anemia. Turk J Haematol 2015; 32: 1-14. https://doi.org/10.4274/tjh.2014.0288
17. DeLoughery TG. Iron deficiency anemia. Med Clin North Am 2017; 101: 319-332. https://doi.org/10.1016/j.mcna.2016.09.004
18. Çakmaklı S, Kaplan Ç, Uzunoğlu M, et al. A novel homozygous nonsense mutation (p.Y78*) in TMPRSS6 gene causing iron-refractory iron deficiency anemia (IRIDA) in two siblings. Turk J Pediatr 2020; 62: 103-108. https://doi.org/10.24953/turkjped.2020.01.014
19. Wang CY, Meynard D, Lin HY. The role of TMPRSS6/matriptase-2 in iron regulation and anemia. Front Pharmacol 2014; 5: 114. https://doi.org/10.3389/fphar.2014.00114
20. Du X, She E, Gelbart T, et al. The serine protease TMPRSS6 is required to sense iron deficiency. Science 2008; 320: 1088-1092. https://doi.org/10.1126/science.1157121
21. De Falco L, Silvestri L, Kannengiesser C, et al. Functional and clinical impact of novel TMPRSS6 variants in iron-refractory iron-deficiency anemia patients and genotype-phenotype studies. Hum Mutat 2014; 35: 1321-1329. https://doi.org/10.1002/humu.22632
22. Lehmberg K, Grosse R, Muckenthaler MU, et al. Administration of recombinant erythropoietin alone does not improve the phenotype in iron refractory iron deficiency anemia patients. Ann Hematol 2013; 92: 387-394. https://doi.org/10.1007/s00277-012-1618-8

23. Sal E, Keskin EY, Yenicesu I, Bruno M, De Falco L. Iron-refractory iron deficiency anemia (IRIDA) cases with 2 novel TMPRSS6 mutations. Pediatr Hematol Oncol 2016; 33: 226-232. https://doi.org/10.3109/08880018.2016.1157229

24. Donker AE, Schaap CC, Novotny VM, et al. Iron refractory iron deficiency anemia: a heterogeneous disease that is not always iron refractory. Am J Hematol 2016; 91: E482-E490. https://doi.org/10.1002/ajh.24561

The Turkish Journal of Pediatrics 2024; 66: 666-671
https://doi.org/10.24953/turkjpediatr.2024.5090

Case Report

# Interpretation of pseudothrombocytopenia using platelet histograms and flags in a hematology autoanalyzer in a healthy child: a case report

Emine Dönmez[1], Zühre Kaya[1]

[1]*Department of Pediatric Hematology, Faculty of Medicine, Gazi University, Ankara, Türkiye.*

## ABSTRACT

**Background.** Pseudothrombocytopenia is a spurious thrombocytopenia caused mostly by ethylenediaminetetraacetic acid (EDTA) use, and if detected early, unnecessary testing and treatment can be avoided. We present pseudothrombocytopenia caused by EDTA and citrate in an asymptomatic healthy child, as well as the value of using peripheral blood smear, platelet histogram, and flag data.

**Case.** A previously healthy 13-year-old girl with thrombocytopenia who developed tonsillitis 12 days previously was referred to our hematology department. Laboratory tests revealed severe thrombocytopenia ($17x10^3$/μL) in EDTA samples. A peripheral blood smear revealed numerous platelet clumping. We hypothesized EDTA-dependent pseudothrombocytopenia and ordered a platelet count by citrate tube. A citrate tube revealed thrombocytopenia with a platelet count of $55x10^3$/μL. The platelet count ($175x10^3$/μL) returned to normal with heparin tubing. All blood samples had a similar platelet histogram and flags in the autoanalyzer. The platelet histogram indicated a serrated/sawtooth curve containing the largest platelet aggregates. Platelet flags alert messages about platelet clumping.

**Conclusions.** Peripheral blood smear is the most reliable test for pseudothrombocytopenia. If the physician has no experience with smear examination, both laboratory technician and physician should be aware of abnormal platelet histograms and platelet clumping messages in platelet flags, which indicate pseudothrombocytopenia.

**Key words:** pseudothrombocytopenia, child, platelet histogram, platelet flags.

Pseudothrombocytopenia is a spurious thrombocytopenia caused by blood tube-dependent antibodies. The majority of antibodies target glycoprotein IIb/IIIa on platelet surfaces. Pseudothrombocytopenia should be considered in the differential diagnosis of thrombocytopenia. In the literature, pseudothrombocytopenia is often misinterpreted as immune thrombocytopenic purpura, resulting in overtreatment, splenectomy, and unnecessary laboratory tests.[1,2]

Although ethylenediaminetetraacetic acid (EDTA) is the most common cause of pseudothrombocytopenia, citrate-induced pseudothrombocytopenia has been documented on a few occasions.[2] When physicians see platelet clusters on a peripheral blood smear, they should consider pseudothrombocytopenia; the diagnosis is confirmed by a repeat blood count using citrate or heparin tubes other than EDTA. If the medical professional has no experience evaluating peripheral blood smears, the diagnosis is commonly missed. As a

✉ Zühre Kaya ▪ zuhrekaya@gmail.com

Received 15th Jul 2024, revised 27th Aug 2024, 3rd Oct 2024, accepted 29th Oct 2024.

result, the technician must be able to accurately interpret platelet histograms and flags before reporting to the physician in the laboratory.[3-5] Furthermore, clinicians need to know how to evaluate platelet histograms and flags.

We present a healthy child who was diagnosed with both EDTA and citrate-induced pseudothrombocytopenia, as well as the value of platelet histogram and flags analysis.

## Case Presentation

A 13-year-old girl was admitted for cancer screening due to a positive family and at the request of her parents, and her complete blood count revealed thrombocytopenia. She was referred to the hematology department for further examination into the cause of thrombocytopenia, such as immune thrombocytopenic purpura. She was asymptomatic upon admission to the hospital, however, she had tonsillitis at least 12 days before. She was administered antibiotics due to a suspected bacterial infection. Her personal history included no thrombocytopenia or bleeding symptoms, and she underwent a successful adenoidectomy when she was 10 years old. Her family history included her father's thyroid cancer diagnosis, her aunt's lung cancer diagnosis, and her sibling's Wilms tumor diagnosis. Upon admission, an automated hematology analyzer (DxH400, Beckman-Coulter Inc., Nyon, Switzerland) revealed isolated thrombocytopenia (platelet count: $17x10^3/\mu L$) with mean platelet volume (MPV): 8.4 fL, platelet distribution width (PDW): 16 fL and typical platelet histogram patterns (Table I and Figure 1). When we examined the peripheral blood smear, we observed numerous platelet clumping. We suspected pseudothrombocytopenia and performed a citrate tube platelet count. Citrate samples again indicated thrombocytopenia (platelet count: $55x10^3/\mu L$, MPV: 9.1 fL, PDW: 17.4 fL), as well as platelet clumping similar to EDTA. The platelet count in the heparin tube sample was normal ($175x10^3/\mu L$, MPV: 10.1 fL, PDW: 18.1 fL). However, platelet clumping was also seen in the peripheral blood smear, and abnormal platelet histogram and flags with heparin. We consulted the referring physician regarding the possibility of reviewing the peripheral blood smear. However, he indicated a lack of experience in interpreting peripheral blood smear assessments of this nature. Concurrently, we requested the laboratory technician to examine the platelet histogram and check for any indications of platelet clumping. He reported an inability to routinely assess the platelet

**Table I.** Simultaneous complete blood count in three distinct blood tube samples.

| Variables | EDTA Samples | Citrate Samples | Heparin Samples |
|---|---|---|---|
| Erythroid series | | | |
| Red blood cell ($x10^6/\mu L$) | 4.68 | 4.05 | 4.51 |
| Hemoglobin (g/dL) | 11 | 9.8 | 10.9 |
| Hematoctrit (%) | 34.1 | 30.1 | 32.9 |
| MCV (fL) | 74.3 | 72.9 | 73.1 |
| RDW (%) | 19.1 | 19.2 | 18.4 |
| White Blood cell | | | |
| Leukocyte count ($x10^3/\mu L$) | 12.1 | 5.5 | 7.4 |
| Neutrophil count ($x10^3/\mu L$) | 6.2 | 2.2 | 4.1 |
| Lymphocyte count ($x10^3/\mu L$) | 4.3 | 2.3 | 2.7 |
| Platelet series | | | |
| Platelet count ($x10^3/\mu L$) | 17 | 55 | 175 |
| Mean platelet volume (fL) | 8.4 | 9 | 10.1 |
| PDW (fL) | 16 | 17.4 | 18.1 |

MCV, mean corpuscular volume; PDW, platelet distribution width; RDW, red cell distribution width.

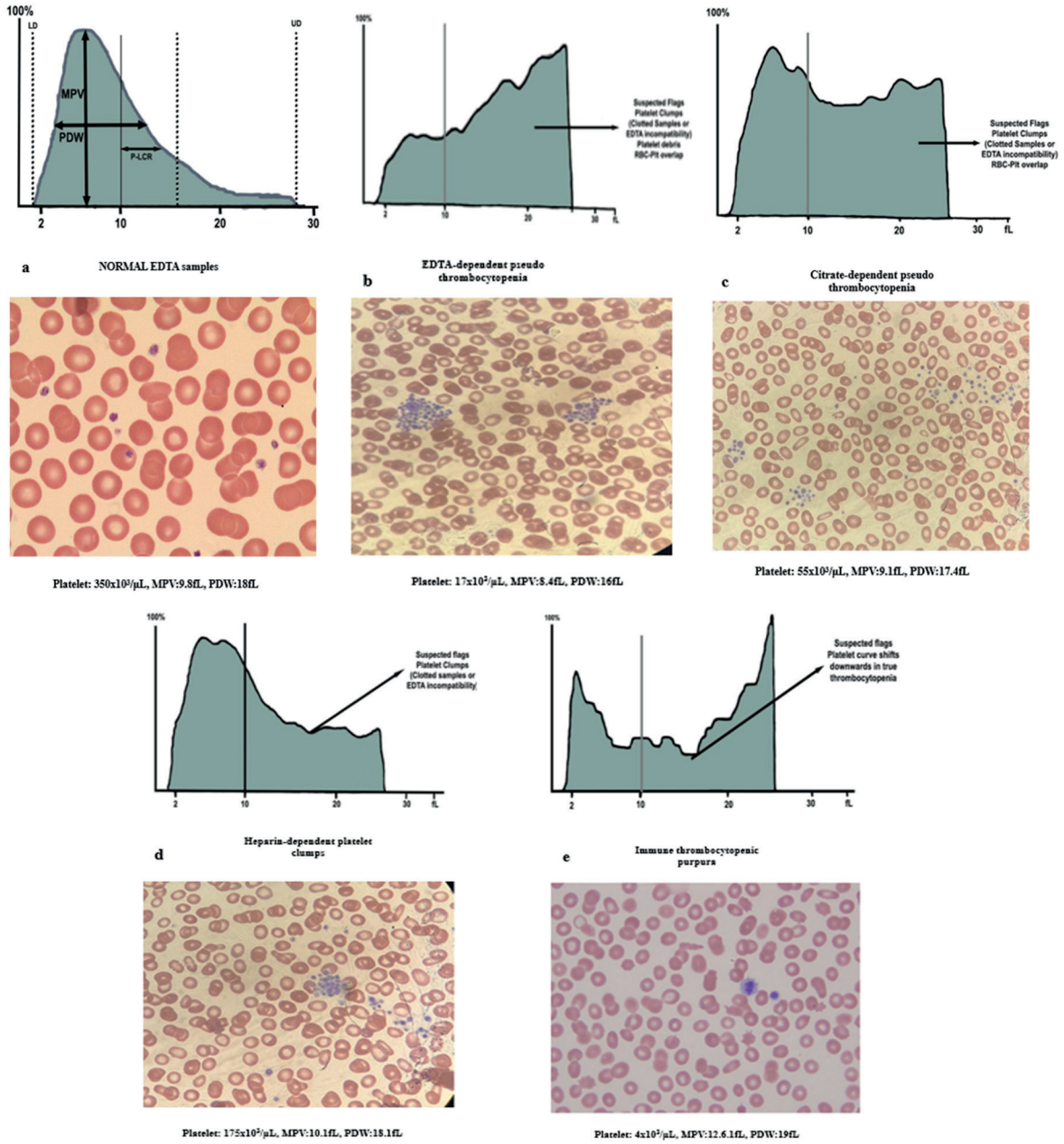



**Fig. 1.** Normal and abnormal platelet histogram, suspected flags, and peripheral blood smear of EDTA **(a,b)**, Citrate **(c)**, and Heparin **(d)** samples for the diagnosis of pseudothrombocytopenia in a child as well as EDTA sample **(e)** in a patient with immune thrombocytopenic purpura.

histogram and associated flags. The platelet histogram displayed a serrated/sawtooth curve, with the largest platelet aggregates appearing as a distinct peak on the platelet curve's rightward shift in EDTA and citrate samples. Furthermore, the platelet flag represents warning signs, such as platelet clumping or large platelets. Although the platelet count returned to normal with the heparin tube, a peripheral blood smear, platelet histogram, and flags suggested some platelet clumping message in the heparin tube, similar to the EDTA and citrate samples (Figure 1). Informed consent was obtained from the parents for the publication of this case report.

## Discussion

In this case study, we present the platelet histogram, flags, and peripheral blood smear results for pseudothrombocytopenia in EDTA and citrate blood samples from a previously healthy child. Figure 1 also shows platelet histograms, flags, and smear results from individuals with normal and immune thrombocytopenic purpura samples collected at the hematology laboratory for comparison.

EDTA anticoagulants are widely used in hospitals for complete blood count (CBC) analysis.[3] The frequency of EDTA-dependent pseudothrombocytopenia ranges from 0.1-2% in hospitalized patients to 15-17% in outpatients tested for thrombocytopenia.[6] Furthermore, 83% of individuals have EDTA-induced pseudothrombocytopenia, and 17% have citrate-dependent pseudothrombocytopenia.[7-10] Multiple anticoagulant-dependent pseudothrombocytopenias have been reported in a variety of tubes, including heparin. Heating the whole blood specimen at 37 °C, in vitro amikacin supplementation or rapid sample analysis are further laboratory procedures for correcting low platelets on the laboratory bench.[10] These methods are time, temperature, and drug-dependent procedures. However, the use of alternate anticoagulant sample tubes is a quick and practical approach for clinicians.[10] In our case, we observed both EDTA and citrate-dependent pseudothrombocytopenia, but the platelet count was corrected using the heparin tube platelet count.

Peripheral blood smears remain the gold standard test for diagnosing pseudothrombocytopenia. However, not every physician has experience assessing peripheral blood smears.[11,12] As a result, most individuals are misdiagnosed as immune thrombocytopenic purpura.[1,2] According to the literature, the majority of individuals with pseudothrombocytopenia are treated as immune thrombocytopenic purpura with steroids, immunoglobulins, or splenectomy, which is unnecessary. In this case, he was referred to our hematology department due to immune thrombocytopenic purpura suspicions. We were aware that the referral physician had not examined the peripheral blood smear. We believe that digital imaging technology will be able to detect pseudothrombocytopenia in routine peripheral blood smears, minimizing the need for physicians to evaluate smear testing.

A modern technology-based automated hematological analyzer, as well as suitable blood sample tubes, are required for reliable complete blood count results.[11-13] Most laboratories continue to report hemogram results obtained using impedance-based automated hematological analyzers. An advanced automated hematology analyzer that uses a fluorescent or optic-based platelet count, is effective for the correction of pseudothrombocytopenia. However, pseudothrombocytopenia is difficult to identify with an impedance platelet count since platelet histograms and flag warning messages are frequently ignored by technicians or physicians. As a result, if the physician has no experience assessing peripheral blood smears, it may be critical for the technician to notice pseudothrombocytopenia on the laboratory bench before reporting it to the physician. Furthermore, physicians should be qualified to interpret platelet histograms and flags in clinical settings.

The platelet histogram measures platelet size, MPV, PDW, and platelet large cell ratio (P-LCR).[10-13] MPV, PDW, and P-LCR values are artificially increased in the presence of platelet clumping or large platelets. However, MPV and PDW values in our patient were similar to those of a healthy sample (Figure 1). The platelet histograms have two flexible discriminators that help distinguish platelet curves from others: the lower and upper discriminators range from 2 to 20 fL. The platelet discriminator has an optimum value of 12 fL. The normal platelet histogram begins with a sharp climb to a peak and then progressively

declines as platelet size increases (Figure 1). This suggests that the majority of platelets are small, with fewer larger ones. The red blood cell peak can be seen starting to the right of the upper discriminator bar. When platelet size approaches 20 fL, it interacts with red blood cells, causing an inaccurate platelet count due to large platelets and platelet aggregates. Additionally, typical normal platelet curves are left-skewed. In cases where the platelet curve exhibits a rightward shift extending beyond the upper platelet discriminator, as observed in our patient, combined with an upper discriminator height of 40% and a serrated or sawtooth pattern, the resulting platelet count may yield an inaccurately low value. However, the platelet curve shifts downward in true thrombocytopenia, such as immune thrombocytopenic purpura (Fig. 1). In addition to the platelet histogram, platelet clumping and large platelet signals obtained from platelet flags should also be considered. Our platelet histogram and flag analysis results revealed platelet clumping in the EDTA and citrate samples. The heparin-based platelet histogram and peripheral blood smear show rare platelet clumping but appear to have a nearly normal platelet count. Our findings show that if EDTA and citrate cause pseudothrombocytopenia, the physician should consider using the heparin tube.

The main limitation of this report is that it is based on a single case. Additional research with a large cohort is required to confirm pseudothrombocytopenia in such individuals.

Finally, if the platelet histogram and flags suggest a platelet cluster warning, the laboratory technician and physician should be aware of pseudothrombocytopenia, one on the laboratory bench and the other in the clinical setting.

**Acknowledgment**

We would like to thank Dr. Onur Özdemir for designing the images presented in Figure 1.

**Ethical approval**

Informed consent was obtained from parents.

**Author contribution**

The authors confirm their contribution to the paper as follows: Study conception and design: ED; data collection: ZK; analysis and interpretation of results: ED, ZK; draft manuscript preparation: ED, ZK. 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. Sharma A, Pinto Pereira LM, Capildeo K, Charles K, Teelucksingh S. Steroid-induced iatrogenic disease after treating for pseudothrombocytopenia. Clin Appl Thromb Hemost 2011; 17: 100-102. https://doi.org/10.1177/1076029609347902
2. Salama A. Autoimmune thrombocytopenia complicated by EDTA- and/or citrate-dependent pseudothrombocytopenia. Transfus Med Hemother 2015; 42: 345-348. https://doi.org/10.1159/000437220
3. Abal CC, Calviño LR, Manso LR, et al. Pseudothrombocytopenia by ethylenediaminetetraacetic acid can jeopardize patient safety - report. EJIFCC 2020; 31: 65-69.
4. Bartels PC, Schoorl M, Lombarts AJ. Screening for EDTA-dependent deviations in platelet counts and abnormalities in platelet distribution histograms in pseudothrombocytopenia. Scand J Clin Lab Invest 1997; 57: 629-636. https://doi.org/10.3109/00365519709055287
5. Yilmaz S, Dağ M, Kizilarslanoğlu MC, Baştürk A. Can pseudothrombocytopenia be recognised at first look? Medicine (Baltimore) 2023; 102: e35395. https://doi.org/10.1097/MD.0000000000035395

6. Nagler M, Keller P, Siegrist D, Alberio L. A case of EDTA-dependent pseudothrombocytopenia: simple recognition of an underdiagnosed and misleading phenomenon. BMC Clin Pathol 2014; 14: 19. https://doi.org/10.1186/1472-6890-14-19

7. Cohen AM, Cycowitz Z, Mittelman M, Lewinski UH, Gardyn J. The incidence of pseudothrombocytopenia in automatic blood analyzers. Haematologia (Budap) 2000; 30: 117-121. https://doi.org/10.1163/15685590051130137

8. Froom P, Barak M. Prevalence and course of pseudothrombocytopenia in outpatients. Clin Chem Lab Med 2011; 49: 111-114. https://doi.org/10.1515/CCLM.2011.013

9. Kovacs F, Varga M, Pataki Z, Rigo E. Pseudothrombocytopenia with multiple anticoagulant sample collection tubes. Interv Med Appl Sci 2016; 8: 181-183. https://doi.org/10.1556/1646.8.2016.4.4

10. Lardinois B, Favresse J, Chatelain B, Lippi G, Mullier F. Pseudothrombocytopenia-a review on causes, occurrence and clinical implications. J Clin Med 2021; 10: 594. https://doi.org/10.3390/jcm10040594

11. Kaya Z. Interpretation of automated blood cell counts. Dicle Medical Journal 2013; 40: 521-528.

12. Dixit S, Jha T, Gupta R, Shah D, Dayal N, Kotru M. Practical approach to the interpretation of complete blood count reports and histograms. Indian Pediatr 2022; 59: 485-491.

13. Thomas ETA, Bhagya S, Majeed A. Clinical utility of blood cell histogram interpretation. J Clin Diagn Res 2017; 11: OE01-OE04. https://doi.org/10.7860/JCDR/2017/28508.10620

Printed by METEKSAN in Ankara, Turkey