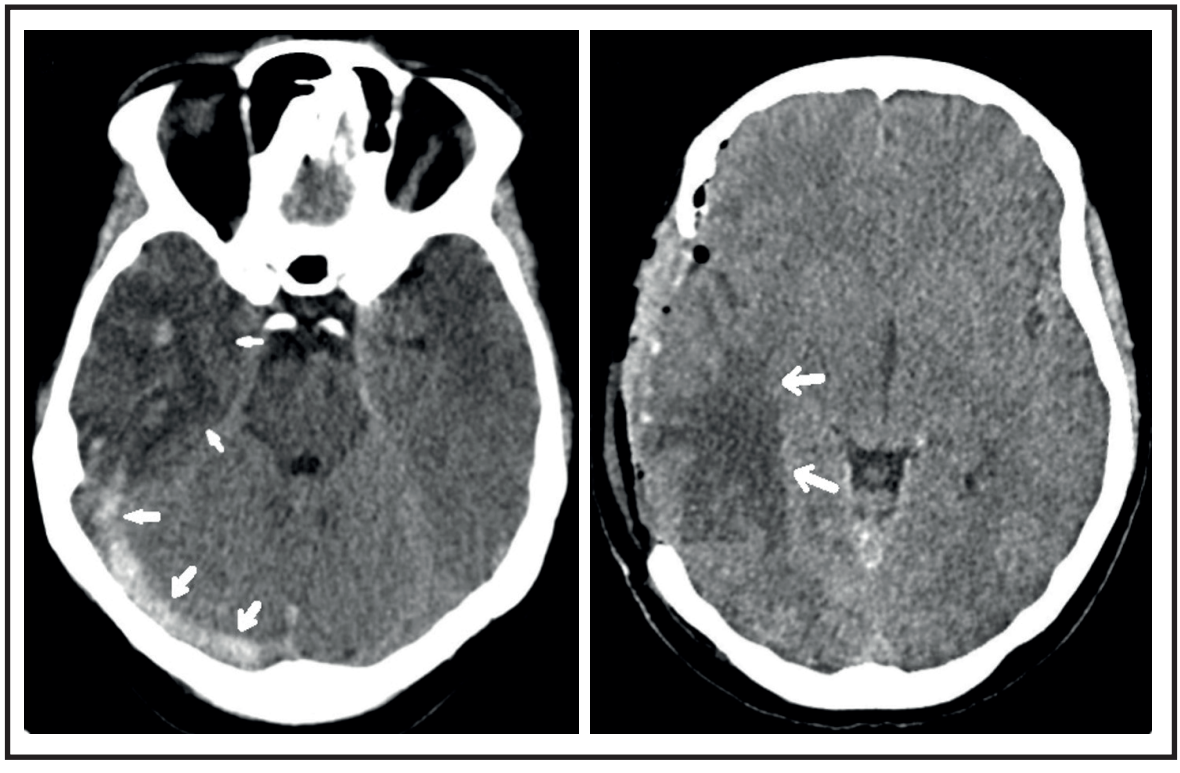
Online ISSN: 2792-0429

Trends in Pediatrics

Volume: **6** Issue: **4** **December 2025**



www.trendspediatrics.com

Trends in Pediatrics

www.trendspediatrics.com

Volume: 6 Issue: 4 December 2025

Online ISSN: 2792-0429

Trends in Pediatrics

Trends in Pediatrics

ISSN (Online): 2792-0429

DOI Prefix: 10.59213

Owner
Owner on behalf of the Aydın Pediatric Society
Prof. Dr. Pınar Uysal, MD *(President)*

Publication Type
International peer-reviewed journal

Publication Frequency and Language
Quarterly (March, June, September, December), English

Abstracting and Indexing
Trends in Pediatrics is abstracted and indexed in Web of Science Emerging Sources Citation Index, Scopus, TR Index, DOAJ (Directory of Open Access Journals), Türkiye Atıf Dizini, J-Gate, Türk Medline, Gale.

Editor in Chief
Prof. Ahmet Anık, MD
Department of Pediatrics, Division of Pediatric Endocrinology, Aydın Adnan Menderes University, Medical Faculty, Aydın, Türkiye
ahmet.anik@adu.edu.tr - https://orcid.org/0000-0002-7729-7872

Publisher
Aydın Pediatric Society

Publisher Address
Aydın, Türkiye
Phone: +90 123 456 78 90
E-mail: info@trendspediatrics.com
Web: www.aydinpediatridernegi.com

Publishing Services
Akdema Informatics and Publishing
Address: Kızılay Mah. Gazi Mustafa Kemal Bulvarı No: 23/8 06420 Çankaya/Ankara
Certificate number: 52576
E-mail: bilgi@akdema.com
Tel: +90 533 166 80 80
Web: www.akdema.com

Trends in Pediatrics

TP Trends in Pediatrics

Advisory Board

Trends in Pediatrics

Advisory Board

Trends in Pediatrics

Advisory Board

Trends in Pediatrics

Research Articles

Case Reports

Trends in Pediatrics

Letters to the Editor

Trends in Pediatrics 2025;6(4):225-232
DOI: 10.59213/TP.2025.255

Original Article

Efficacy and immunologic effects of a synbiotic in children with functional abdominal pain

Hanife Ayşegül Arsoy[1], Tanju Başarır Özkan[1], Taner Özgür[1], Nilüfer Ülkü Şahin[1], Ferah Budak[2]

[1]Department of Pediatric Gastroenterology, Faculty of Medicine, Uludağ University, Bursa, Türkiye
[2]Department of Immunology, Faculty of Medicine, Uludağ University, Bursa, Türkiye

Cite this article as: Arsoy HA, Özkan TB, Özgür T, Şahin NÜ, Budak F. Efficacy and immunologic effects of a synbiotic in children with functional abdominal pain. Trends in Pediatrics 2025;6(4):225-232.

ABSTRACT

Background: The objective of this study was to examine the efficacy of a synbiotic in addressing recurrent abdominal pain in children, including functional abdominal pain, and to assess its impact on serum levels of pro-inflammatory and anti-inflammatory cytokines.

Methods: We included 80 patients diagnosed with Functional Abdominal Pain Not Otherwise Specified according to the Rome IV criteria and divided the sample into two groups: the synbiotic group (Lactobacillus helveticus, Lactobacillus casei, Bifidobacterium lactis, chicory inulin) (Group 1) and the placebo group (Group 2). We inquired about pre-intervention and post-intervention symptoms in both groups and measured their blood cytokine levels. All statistical analyses were performed using SPSS 23 for Windows.

Results: The 80 patients with functional abdominal pain had a mean age of 11.48 ± 3.86 years. The groups were compared for the severity of symptoms before and after the intervention, and no statistical difference was found ($p>0.05$). There was no significant difference between the synbiotic group and the placebo group in terms of pre-intervention serum pro-inflammatory or anti-inflammatory cytokine levels (TNF α, IFN γ, IL-10, TGF β, IL-13), and no statistically significant difference was determined after 8 weeks of synbiotic or placebo administration ($p>0.05$). A comparison was made of pre-treatment and post-treatment cytokine levels in each group. The most significant finding was the substantial increase in IL-13 levels post-treatment in the synbiotic group ($p < 0.001$).

Conclusion: In the present study, no differences were found between the symbiotic and placebo groups with regard to functional abdominal pain symptoms or serum cytokine levels. However, a significant increase in IL-13 levels was detected after treatment in the symbiotic group. There is a need for further research on the optimal dosage and duration of synbiotic application, the type of probiotic that should be administered, and its effect on cytokine levels in functional gastrointestinal diseases.

Keywords: functional gastrointestinal diseases, synbiotics, cytokines

INTRODUCTION

Functional abdominal pain (FAP) is a common condition in children that is diagnosed after a thorough medical assessment when the symptoms cannot be attributed to any other medical condition. The pathology of functional gastrointestinal diseases (FGID) associated with abdominal pain in children is yet to be clarified.[1] The subtypes of FAP, defined by the Rome IV criteria, include conditions such as irritable bowel syndrome (IBS), functional dyspepsia (FD), abdominal migraine, and functional abdominal pain not-otherwise specified (FAPNOS), each of which requires a customized diagnostic and therapeutic strategy.[2] Previous research has reported various causes to explain the symptoms of FGIDs associated with abdominal pain, like altered gut motility, visceral hypersensitivity, abnormal gut-

✉ Hanife Ayşegül Arsoy ▪ draysegulgastro@gmail.com

Received: 27.02.2025 **Accepted:** 30.07.2025

brain axis interactions, gut flora, psychosocial discomfort, and immune system activation.[3-5]

Following a study that revealed bacterial overgrowth in some patients with IBS, the idea that altered bacterial flora influences the development of IBS symptoms has gained attention.[6] Another research indicated a higher prevalence of abnormal bacterial fermentation among children with IBS or functional abdominal pain compared to healthy controls.[7]

Given the effects of gut microbiota on the maturation of the gastrointestinal epithelium, providing a mucosal barrier, visceral hypersensitivity, intestinal immune response, and gut motility, it is believed to play a key role in the pathogenesis of FGIDs. In this regard, probiotics, prebiotics, and synbiotics play a crucial role in gut microbiota and dysbiosis. There are numerous studies on the effects of probiotics, prebiotics, and synbiotics on gut microbiota and their use in gastrointestinal and non-gastrointestinal diseases.[6,8,9] Examining the studies on the effect of probiotics on FGIDs, we see that the body of research is limited and mostly focuses on adults with IBS, and there is no clear consensus on which microorganism should be administered in what dose and for how long, which suggests further research.[10-13]

The present trial aimed to observe the effects of treatments that support the microbiota on symptoms and serum pro-inflammatory and anti-inflammatory cytokine levels in patients with functional abdominal pain.

MATERIALS AND METHODS

The study population comprised patients aged 6 to 18 years who presented to the outpatient clinic for Paediatric Gastroenterology, Hepatology and Nutrition at a tertiary care centre with complaints of recurrent abdominal pain. Using the Rome IV criteria, the patients were diagnosed with FAPNOS, and patients with alarm symptoms of recurrent abdominal pain were excluded.[2]

Exclusion criteria:

1. Presence of another organic cause that could lead to abdominal pain
2. Antibiotic use in the last two months
3. Having another chronic disease involving other systems, including the gastrointestinal tract

The study was designed as a randomized, double-blind, placebo-controlled trial. Informed consent was obtained from study participants and their families. Synbiotic or placebo products, in exactly the same shape and size, and coded by the manufacturer, were randomly distributed to the patients by the head nurse, who was also blinded to the products' contents. The patients received either a synbiotic-containing capsule or a placebo orally once a day for 8 weeks. At the end of eight weeks, we checked the product codes and formed two groups: Group 1, the synbiotic group (n=39), and Group 2, the placebo group (n=41).

Each capsule of the synbiotic product was a Mamsel Maflor® plus capsule containing *Lactobacillus helveticus (L.helveticus), Lactobacillus casei (L.casei), Bifidobacterium lactis (B.lactis), chicory inulin* 100 mg: in total 7x 10^9 CFU of active probiotics. The placebo capsules had no active ingredient, with the same package and form as provided by the manufacturer.

We contacted the patients in both groups by phone weekly to discuss the continuity of their treatment, their clinical status, and any symptoms they were experiencing. We asked the patients to show the location of the abdominal pain, and these locations were recorded. We also asked the patients to keep records regarding their pain each week of treatment for 8 weeks. These records included pain frequency (more than twice a week, twice a week, once a week, or no pain), pain severity (0 = absent, 1=mild, 2=moderate, 3=severe), school absenteeism due to pain, the number of days of absence, and limitations in daily activities due to pain. Pre-treatment data were obtained from records kept for one month before admission, including pain frequency (weekly), pain severity (0=absent, 1=mild, 2=moderate, 3=severe), school absenteeism due to pain, number of days of absence, and limitations in daily activities due to pain. At the end of the eighth week, the patients were examined again in the pediatric gastroenterology outpatient clinic, and they were asked to evaluate the success of the treatment with a score from 1 to 100, and these scores were recorded with the joint decision of the parents.

We took blood samples to measure serum levels of pro-inflammatory cytokines (TNF-α, IFN-γ) and anti-inflammatory cytokines (IL-10, TGF β, IL-13) at the beginning and end of the study. TNF α, IFN γ, IL-10, TGF β, and IL-13 plasma levels were measured in the Immunology Laboratory of our faculty; TNF α, IFN γ, IL-10, TGF β levels were measured using a *Boster Human ELISA* kit, and IL-13

levels using a *Bioscience Human ELISA* kit, all separately and according to the manufacturer's instructions. Optical density values were measured at *450 nm* wavelength, *620 nm* reference wavelength, using an *ELISA microplate* reader *(Sunrise Remote/ Touch Screen, Tecan, Austria)*. We created linear correlations with the results and the standard concentration values, and evaluated the results with separate calibration curves for each cytokine.

The study was approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki of 1975. Approval number was 2014-16/6.

Statistical analysis

We investigated the correlations between the variables using the SPSS 23 statistics software for Windows. We examined the differences between the frequencies of categorical variables using the Chi-square test. We tested the data for normal distribution using the Shapiro-Wilk test. For continuous variables, the differences between the two groups were evaluated using Student's t-test. Differences between the two groups were evaluated using Student's t-test for continuous variables and the Mann-Whitney U test for data that did not fit a normal distribution. G*Power 3.1 statistical program was used for power analysis. The analysis of continuous variables was conducted employing the Wilcoxon signed-rank test for paired data. For all analyses, the significance level was set at $p<0.05$.

RESULTS

Eighty patients with functional abdominal pain were included in the study. The patients had a mean age of 11.48±3.86 years, and 57.5% (n=46) of the patients were female and 42.5% (n=34) were male. The most common localization of abdominal pain was periumbilical, occurring in 78.8% of cases (n=63). We also analyzed factors that affect patients' daily lives, including pain frequency, pain severity, absenteeism from school, the number of days of absence, and limitations in daily activities. Pain frequency was more than twice a week in 61.2% (n=49) of patients, twice a week in 17.5% (n=14) of patients, and once a week in 21.2% (n=17) of patients. Pain severity was moderate for 65% (n=52) of the patients, mild for 20% (n=16), and severe for 15% (n=12).

Thirty-nine (48.8%) of the patients were in the synbiotic group (Group 1) and 41 (51.2%) in the placebo group (Group 2). We found no difference between the groups in terms of the distribution of sex, age, or weight-for-height z-scores, and pain location did not differ significantly between the groups ($p>0.05$) (Table 1). Considering the factors affecting daily life before treatment, such as pain frequency, pain severity, absenteeism from school, the number of days of absence, and limitations in daily activities, we observed no significant difference between the groups ($p > 0.05$) (Table 2). Pre-treatment IL-13, IL-10, IFN-γ, TGF-β, and TNF-α serum levels again did not differ significantly between the groups ($p>0.05$) (Table 2).

After 8 weeks of treatment, complete recovery (100%) was achieved in 64.1% (n=25) of patients in Group 1 and 43.9% (n=18) of patients in Group 2, with no statistically significant difference ($p>0.05$). The reduction in complaints was higher in Group 1 compared to Group 2, with a borderline statistically significant difference ($p=0.05$) (Table 3). Unresponsiveness to treatment was observed in 12.5% (n=5) of patients in Group 1 and 13.8% (n=8) in Group 2, though without a statistically significant difference ($p>0.05$) (Table 3).

Considering the factors affecting daily life after treatment, such as pain frequency, pain severity, absenteeism from school, the number of days of absence, and limitations in daily activities, we observed no significant difference between the groups ($p > 0.05$) (Table 4). Finally, there was no significant difference between the groups in terms of post-treatment IL-13, IL-10, IFN-γ, TGF-β, and TNF-α serum levels ($p>0.05$) (Table 4). A comparison was made of pre-treatment and post-treatment cytokine levels in each group (Table 5). The most significant finding was the substantial increase in IL-13 levels post-treatment in Group 1 ($p < 0.001$). Although IFN γ levels decreased after treatment in both Group 1 and Group 2, this decrease was much more pronounced in Group 1 than in Group 2, respectively, ($p<0.001$),($p=0.010$).

Table 1. Sex, age, weight, height, characteristics and location of abdominal pain of the groups

	Group 1 (n=39)	Group 2 (n=41)	p
Female*	53.8 (21)	61 (25)	0.519#
Age (years)	11.90±3.92	11.09±3.80	0.352€
Weight Z score	0.11±1.20	-0.13±0.96	0.301€
Hight Z score	-0.34±1.44	-0.17±1.21	0.593€
Location of abdominal pain*			
Periumlical	74.4 (29)	82.9 (34)	0.595#
Epigastric	20.5 (8)	12.2 (5)	
Hypogastric	5.1 (2)	4.9 (2)	

*% (n)
#ki-kare test, €student-t test

Table 2. Characteristics of pre-treatment abdominal pain and serum cytokine levels by groups			
Before treatment	**Group 1, n=39**	**Group 2, n=41**	**p**
Pain frequency*			
Once a week	12.8 (5)	29.3 (12)	0.127#
Twice a week	15.4 (6)	19.5 (8)	
More than twice a week	71.8 (28)	51.2 (21)	
Intensity of pain*			
Mild	15.4 (6)	24.4 (10)	0.226#
Moderate	74.3 (29)	56.1 (23)	
Severe	10.3 (4)	19.5 (8)	
School absenteeism*	30.8 (12)	41.5 (17)	0.320#
Days of absence from school in the last 1 month	0 (0-1)	0 (0-1)	0.505±
Restriction in daily activity*	71.8 (28)	75.6 (31)	0.698#
Cytokine levels (pg/ml)			
IL-13	0.42 (0.31-0.96)	0.61 (0.36-1.23)	0.179±
IL-10	5.4 (4.7-7.7)	6.1 (5.3-8.7)	0.112±
IFN-γ	0.9 (0.1-2.8)	0.3 (0.1-2.3)	0.542±
TGF-β	648 (584-738)	651 (586-738)	0.900±
TNF-α	0.1 (0.1-2.3)	0.1 (0.1-0.15)	0.360±

The data are presented as median (25%-75%)
*% (n) # ki-kare test ±Mann-Whitney U test

Table 3. Comparison of treatment results by groups			
Treatment response	**Group 1, n=39**	**Group 2, n=41**	**p**
Complete improvement in symptoms*	64.1 (25)	43.9 (18)	0.070#
Rate of reduction in symptoms (%)	100 (70-100)	80 (40-100)	0.054±
Non-response to treatment*	12.8 (5)	19.5 (8)	0.417#

The data are presented as median (25%-75%)
*% (n) # ki-kare test ±Mann-Whitney U test

DISCUSSION

This prospective, randomized, double-blind, placebo-controlled trial investigated the clinical and immunological effects of a synbiotic (*Lactobacillus helveticus, Lactobacillus casei, Bifidobacterium lactis, and chicory inulin)* in patients with functional abdominal pain. Considering the microbiota and their effects on gastrointestinal immunity, probiotics and synbiotics are still being investigated for the treatment of various gastrointestinal diseases.[14] In our trial, we observed similarities between the study group and placebo group in terms of age, sex, anthropometric measurements, cytokine levels at admission, and complaints.

There are a handful of randomized, double-blind, placebo-controlled trials examining the effects of probiotics and synbiotics on the symptoms of children with functional gastrointestinal diseases. When the Rome IV criteria are applied to children diagnosed with recurrent abdominal pain, IBS constitutes the most common diagnosis, with a rate of up to 45%.[15] The majority of research on probiotics and synbiotics has been focused on IBS, with a paucity of studies conducted on functional abdominal pain. A meta-analysis was conducted in order to evaluate the efficacy of probiotics in treating IBS in children. The analysis revealed that probiotics, particularly mixtures of *L. rhamnosus GG*, *VSL#3,* and three *bifidobacteria* strains, were associated with improvement in abdominal pain seen in IBS.[16]

In their recent position statement on the utilisation of probiotics for the treatment of paediatric gastrointestinal disorders, the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN, 2023) includes a weak recommendation for *L. rhamnosus*

Table 4. Characteristics of post-treatment abdominal pain and serum cytokine levels by groups			
After treatment	**Group 1, n=39**	**Group 2, n=41**	**p**
Pain frequency*			
No pain	68.4 (26)	55 (22)	0.246#
Once a week	15.8 (6)	12.5 (5)	
Twice a week	7.9 (4)	7.5 (4)	
More than twice a week	7.9 (3)	25 (10)	
Intensity of pain*			
No pain	68.4 (26)	55 (22)	0.538#
Mild	10.5 (4)	10 (4)	
Moderate	15.8 (7)	22.5 (10)	
Severe	5.3 (2)	12.5 (5)	
School absenteeism*	8.1 (3)	12.5 (5)	0.528#
Days of absence from school in the last 1 month	0 (0-0)	0 (0-0)	0.276±
Restriction in daily activity*	13.2 (5)	22.5 (9)	0.283#
Cytokine levels (pg/ml)			
IL-13	0.77 (0.43-1.62)	0.7 (0.27-1.33)	0.519±
IL-10	5.1 (4.2-6.2)	5 (4.5-6.3)	0.363±
IFN-γ	0.2 (0.1-0.7)	0.2 (0.1-1.3)	0.700±
TGF-β	949 (863-1058)	894 (807-1014.5)	0.164±
TNF-α	0.1 (0.1-3.9)	0.1 (0.1-5.8)	0.755±

The data are presented as median (25%-75%)
*% (n) # ki-kare test ±Mann-Whitney U test

Table 5. Comparison of cytokine levels of the groups before and after treatment				
		Before treatment	**After treatment**	**p***
Group 1	IL-13	0.42 (0.31-0.96)	0.77 (0.43-1.62)	<0.001
	IL-10	5.4 (4.7-7.7)	5.1 (4.2-6.2)	<0.001
	IFN-γ	0.9 (0.1-2.8)	0.2 (0.1-0.7)	<0.001
	TGF-β	648 (584-738)	949 (863-1058)	<0.001
	TNF-α	0.1 (0.1-2.3)	0.1 (0.1-3.9)	0.527
Group 2	IL-13	0.61 (0.36-1.23)	0.7 (0.27-1.33)	0.246
	IL-10	6.1 (5.3-8.7)	5 (4.5-6.3)	<0.001
	IFN-γ	0.3 (0.1-2.3)	0.2 (0.1-1.3)	0.010
	TGF-β	651 (586-738)	894 (807-1014.5)	<0.001
	TNF-α	0.1 (0.1-0.15)	0.1 (0.1-5.8)	0.103

The data are presented as median (25%-75%)
*Wilcoxon Signed Ranks test

GG (1-3 × 10^9 CFU twice daily) to reduce pain in children diagnosed with IBS.[17] A study of 29 paediatric patients with FD treated with *L. reuteri* found a significant reduction in pain.[18] In one randomized, double-blind, placebo-controlled trial in Turkey, children with functional constipation were given a synbiotic containing *L. casei*, *L. rhamnosus*, *L. plantarum*, and *B. lactis*, inulin, and a significant improvement was found in the synbiotic group in terms of abdominal pain, painful defecation, and defecation frequency.[19] A study of 101 children with chronic abdominal pain found that treatment with *L. reuteri DSM 17938* reduced pain days and intensity.[20] However, a contradictory study has been conducted on the efficacy of probiotics in functional abdominal pain. Eftekhari et al.[21] conducted a randomized,

double-blind, placebo-controlled trial on 80 children aged 4-16 years with functional abdominal pain, and by dividing them into two groups, they gave them *L. reuteri* and a placebo for 4 weeks. In contrast to the positive effects of probiotics reported in the preceding studies, this study found that there was no significant difference in complaints between the probiotics and placebo groups at weeks 4.[21] In the present study, no significant difference was observed in functional abdominal pain symptoms between the synbiotic group, which received a treatment containing *L. helveticus, L. casei, B. lactis, chicory inulin,* and the placebo group. This finding indicates that distinct probiotic strains may exert varied effects on functional abdominal pain, and that the dosage and duration of treatment with probiotic or synbiotic preparations may also result in different outcomes. Furthermore, the pathophysiology of different subgroups of functional abdominal pain disorders has not yet been fully elucidated, so the potential for different responses to different treatments with different treatment durations remains unclear.

Numerous studies have shown mucosal inflammation and elevated pro-inflammatory cytokine levels in functional gastrointestinal disorders, mostly in IBS.[22-25] There is limited research that compares intestinal mucosal damage, microbiota changes, and serum levels of pro-inflammatory cytokines in childhood. Zambruni et al.[26] found impaired gut microbiota and elevated serum levels of pro-inflammatory cytokines in children with growth retardation. One trial from China compared fecal flora and serum cytokine levels among infants with and without bronchopulmonary dysplasia (BPD). The authors highlighted a significant increase in proteobacteria and a significant decrease in firmicutes in the fecal flora of infants with BPD compared to controls. These infants also had significantly higher pro-inflammatory cytokine levels (IL1β, IL-6, TNFα) and significantly lower anti-inflammatory cytokine levels (IL-10).[27]

There are some animal trials on this subject. Liu HY et al.[28] used L. reuteri to treat colitis induced by 3% dextran sulfate sodium in mice. The authors demonstrated that this treatment decreased pro-inflammatory cytokine levels (TNF-α, IL-1β, INFγ) in the colon. Wang et al.[29] administered L. casei Zhang to mice with acute liver failure orally and showed decreased production of IL-1β and TNF-α in serum, as well as decreased hepatic inflammation. Considering the literature, studies in this regard are most often concentrated on experimental animals, and human trials have mostly investigated the correlations between changes in gut microbiota and systemic inflammation. In the current trial, we compared serum levels of pro-inflammatory cytokines (TNFα, INFγ), anti-inflammatory cytokines (IL-10, IL-13), and an immunomodulatory cytokine (TGF-β) among children with functional abdominal pain at the time of diagnosis and after treatment, and we revealed no significant difference compared to the placebo group. The synbiotic we applied, containing *Lactobacillus helveticus, Lactobacillus casei, Bifidobacterium lactis, and chicory inulin,* caused no significant change in cytokine levels compared to the placebo group. This could be due to the strain of probiotics or the administered dose.

On the other hand, when comparing the cytokine levels of the probiotic group before and after treatment, we observed a significant increase in the anti-inflammatory cytokine levels of IL-13. Furthermore, it was demonstrated that pro-inflammatory cytokine IFN-γ levels decreased in both the synbiotic and placebo groups; however, this decrease was significantly greater in the synbiotic group. In a rat model of ulcerative colitis, a combination therapy consisting of *L. acidophilus* and Chinese medicine Huan Kui Le suspension was used, and it was demonstrated that the combined intervention resulted in upregulation of IL-13 and TGF-β and downregulation of IFN-γ in colon protein expression levels, as well as enrichment of the microbiota composition toward beneficial bacteria.[30] A recent study using a mouse model of intestinal dysfunction induced by sodium dextran sulfate and broad-spectrum antibiotics has shown that intestinal dysfunction causes muscle and bone loss in conjunction with microbial imbalances. This study shows *Bifidobacterium animalis subsp. lactis A6* can reduce muscle and bone loss by tweaking the gut microbiome and increasing butyrate-producing bacteria. This, in turn, decreases pro-inflammatory cytokines (e.g., TNF-α, IL-6, IL-1β, IL-17) in the blood.[31] The *Lactobacillus* and *Bifidobacterium* strains used in our study are probiotics that have been extensively studied and are known to exhibit promising properties. The changes in the gut microbiota caused by probiotics, prebiotics, synbiotics, and postbiotics, as well as their effects on dysbiosis and various systems, particularly the immune system, continue to be an exciting and promising area of research.

In conclusion, functional abdominal pain is a complex group of diseases with multiple factors, including genetic, environmental, familial, psychosocial, intestinal motility, impaired gut-brain axis, dysregulation of the mucosal immune system, dysbiosis, mucus secretion, and barrier dysfunction. The present study was unable to demonstrate

that the synbiotic preparation used in this study caused any difference in symptoms or serum cytokine levels in comparison with a placebo in functional abdominal pain, a complex disorder. However, the significant increase in IL-13 serum levels in the symbiotic group after treatment is a noteworthy finding, and further research is required to explore its implications for intestinal microbiota and the clinical manifestations of functional abdominal pain.

CONCLUSION

In the present study, no discrepancy was observed between the synbiotic and placebo groups with regard to functional abdominal pain symptoms or serum cytokine levels. Nevertheless, a notable increase in anti-inflammatory cytokine, IL-13 levels was observed in the synbiotic group following treatment when the groups were compared within themselves. Our study leaves an open door for the efficacy of synbiotics in the treatment of functional abdominal pain in children. Therefore, there is a need for more studies on the use of synbiotics with different strains, different doses, and different durations.

Ethical approval

This study has been approved by the Uludag University Faculty of Medicine Clinical Research Ethics Committee (approval date 02.09.2014, number 2014-16/6). Written informed consent was obtained from the participants.

Author contribution

The authors declare contribution to the paper as follows: Study conception and design: HAA, TBO, TO, FB, NUS; data collection: HAA, NUS, FB; analysis and interpretation of results: HAA, TO; draft manuscript preparation: HAA, TO, TBO. All authors reviewed the results and approved the final version of the article.

Source of funding

This study was supported by the Scientific Research Fund of Uludag University (Project No: KUAP(T)-2015/5).

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Korterink JJ, Ockeloen L, Benninga MA, Tabbers MM, Hilbink M, Deckers-Kocken JM. Probiotics for childhood functional gastrointestinal disorders: a systematic review and meta-analysis. Acta Paediatr. 2014;103:365-72. [Crossref]
2. Hyams JS, Di Lorenzo C, Saps M, Shulman RJ, Staiano A, van Tilburg M. Functional disorders: children and adolescents. Gastroenterology. 2016:S0016-5085(16)00181-5. [Crossref]
3. Crowell MD, Harris L, Jones MP, Chang L. New insights into the pathophysiology of irritable bowel syndrome: implications for future treatments. Curr Gastroenterol Rep. 2005;7:272-9. [Crossref]
4. Simrén M, Barbara G, Flint HJ, et al. Intestinal microbiota in functional bowel disorders: a Rome foundation report. Gut. 2013;62:159-76. [Crossref]
5. Jones MP, Dilley JB, Drossman D, Crowell MD. Brain-gut connections in functional GI disorders: anatomic and physiologic relationships. Neurogastroenterol Motil. 2006;18:91-103. [Crossref]
6. Pimentel M, Chow EJ, Lin HC. Eradication of small intestinal bacterial overgrowth reduces symptoms of irritable bowel syndrome. Am J Gastroenterol. 2000;95:3503-6. [Crossref]
7. Rasquin A, Di Lorenzo C, Forbes D, et al. Childhood functional gastrointestinal disorders: child/adolescent. Gastroenterology. 2006;130:1527-37. [Crossref]
8. O'Mahony L, McCarthy J, Kelly P, et al. Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. Gastroenterology. 2005;128:541-51. [Crossref]
9. Bauserman M, Michail S. The use of Lactobacillus GG in irritable bowel syndrome in children: a double-blind randomized control trial. J Pediatr. 2005;147:197-201. [Crossref]
10. Saps M, Youssef N, Miranda A, et al. Multicenter, randomized, placebo-controlled trial of amitriptyline in children with functional gastrointestinal disorders. Gastroenterology. 2009;137:1261-9. [Crossref]
11. Kaptchuk TJ, Kelley JM, Conboy LA, et al. Components of placebo effect: randomised controlled trial in patients with irritable bowel syndrome. BMJ. 2008;336:999-1003. [Crossref]
12. Ford AC, Talley NJ, Spiegel BMR, et al. Effect of fibre, antispasmodics, and peppermint oil in the treatment of irritable bowel syndrome: systematic review and meta-analysis. BMJ. 2008;337:a2313. [Crossref]
13. Malinen E, Rinttilä T, Kajander K, et al. Analysis of the fecal microbiota of irritable bowel syndrome patients and healthy controls with real-time PCR. Am J Gastroenterol. 2005;100:373-82. [Crossref]
14. Yadav MK, Kumari I, Singh B, Sharma KK, Tiwari SK. Probiotics, prebiotics and synbiotics: safe options for next-generation therapeutics. Appl Microbiol Biotechnol. 2022;106:505-21. [Crossref]

15. Gwee KA, Lee WRW, Chua Q, Chiou FK, Aw MM, Koh YH. The evidence for probiotics in the treatment of digestive disorders in the pediatric population. J Gastroenterol Hepatol. 2025;40:41-7. [Crossref]

16. Xu HL, Zou LL, Chen MB, et al. Efficacy of probiotic adjuvant therapy for irritable bowel syndrome in children: a systematic review and meta-analysis. PLoS One. 2021;16:e0255160. [Crossref]

17. Szajewska H, Berni Canani R, Domellöf M, et al. Probiotics for the management of pediatric gastrointestinal disorders: position paper of the ESPGHAN special interest group on gut microbiota and modifications. J Pediatr Gastroenterol Nutr. 2023;76:232-47. [Crossref]

18. Rahmani P, Ghouran-Orimi A, Motamed F, Moradzadeh A. Evaluating the effects of probiotics in pediatrics with recurrent abdominal pain. Clin Exp Pediatr. 2020;63:485-90. [Crossref]

19. Baştürk A, Artan R, Atalay A, Yılmaz A. Investigation of the efficacy of synbiotics in the treatment of functional constipation in children: a randomized double-blind placebo-controlled study. Turk J Gastroenterol. 2017;28:388-93. [Crossref]

20. Weizman Z, Abu-Abed J, Binsztok M. Lactobacillus reuteri DSM 17938 for the management of functional abdominal pain in childhood: a randomized, double-blind, placebo-controlled trial. J Pediatr. 2016;174:160-4.e1. [Crossref]

21. Eftekhari K, Vahedi Z, Kamali Aghdam M, Noemi Diaz D. A randomized double-blind placebo-controlled trial of lactobacillus reuteri for chronic functional abdominal pain in children. Iran J Pediatr. 2015;25:e2616. [Crossref]

22. Ford AC, Talley NJ. Mucosal inflammation as a potential etiological factor in irritable bowel syndrome: a systematic review. J Gastroenterol. 2011;46:421-31. [Crossref]

23. Holtmann G, Shah A, Morrison M. Pathophysiology of functional gastrointestinal disorders: a holistic overview. Dig Dis. 2017;35(Suppl 1):5-13. [Crossref]

24. Singh M, Singh V, Schurman JV, Colombo JM, Friesen CA. The relationship between mucosal inflammatory cells, specific symptoms, and psychological functioning in youth with irritable bowel syndrome. Sci Rep. 2020;10:11988. [Crossref]

25. Doyle LA, Sepehr GJ, Hamilton MJ, Akin C, Castells MC, Hornick JL. A clinicopathologic study of 24 cases of systemic mastocytosis involving the gastrointestinal tract and assessment of mucosal mast cell density in irritable bowel syndrome and asymptomatic patients. Am J Surg Pathol. 2014;38:832-43. [Crossref]

26. Zambruni M, Ochoa TJ, Somasunderam A, et al. Stunting is preceded by intestinal mucosal damage and microbiome changes and is associated with systemic inflammation in a cohort of peruvian infants. Am J Trop Med Hyg. 2019;101:1009-17. [Crossref]

27. Zhang Z, Jiang J, Li Z, Wan W. The change of cytokines and gut microbiome in preterm infants for bronchopulmonary dysplasia. Front Microbiol. 2022;13:804887. [Crossref]

28. Liu HY, Gu F, Zhu C, et al. Epithelial heat shock proteins mediate the protective effects of limosilactobacillus reuteri in dextran sulfate sodium-induced colitis. Front Immunol. 2022;13:865982. [Crossref]

29. Wang Y, Xie J, Li Y, et al. Probiotic Lactobacillus casei Zhang reduces pro-inflammatory cytokine production and hepatic inflammation in a rat model of acute liver failure. Eur J Nutr. 2016;55:821-1. [Crossref]

30. Aximujiang K, Kaheman K, Wushouer X, Wu G, Ahemaiti A, Yunusi K. Lactobacillus acidophilus and HKL suspension alleviates ulcerative colitis in rats by regulating gut microbiota, suppressing TLR9, and promoting metabolism. Front Pharmacol. 2022;13:859628. [Crossref]

31. Chen M, Li Y, Zhai Z, et al. Bifidobacterium animalis subsp. lactis A6 ameliorates bone and muscle loss via modulating gut microbiota composition and enhancing butyrate production. Bone Res. 2025;13:28. [Crossref]

Trends in Pediatrics 2025;6(4):233-238
DOI: 10.59213/TP.2025.261

Original Article

Midline brain abnormalities and associated endocrine dysfunctions: a clinical and MRI-based study

Yavuz Özer[1], Hande Turan[2], Gürkan Tarçın[2], Dilek Bingöl Aydın[2], Büşra Demirtaş[2], Olcay Evliyaoğlu[2], Sema Saltık[3], Oya Ercan[2]

[1]Department of Pediatric Endocrinology, Zeynep Kamil Women and Children's Diseases Training and Research Hospital, İstanbul, Türkiye
[2]Department of Pediatric Endocrinology, Cerrahpaşa Faculty of Medicine, İstanbul University-Cerrahpaşa, İstanbul, Türkiye
[3]Department of Pediatric Neurology, Cerrahpaşa Faculty of Medicine, İstanbul University-Cerrahpaşa, İstanbul, Türkiye

* This study was previously presented as an abstract titled "Konjenital Beyin Malformasyonlarının Endokrin Bozukluklar ile İlişkisinin Değerlendirilmesi: Bir Ön Çalışma" at the XXV. National Pediatric Endocrinology and Diabetes Congress, held on October 6-10, 2021.

Cite this article as: Özer Y, Turan H, Tarçın G, et al. Midline brain abnormalities and associated endocrine dysfunctions: a clinical and MRI-based study. Trends in Pediatrics 2025;6(4):233-238.

ABSTRACT

Background: This study aimed to assess the spectrum and prevalence of endocrine disorders in pediatric patients with midline brain abnormalities (MBA).

Methods: This retrospective observational study was conducted at a tertiary pediatric endocrinology center and included patients younger than 18 years of age with MBA. Clinical data were obtained from medical records.

Results: The study included 17 patients (52.9% male) with a median age of 11.1 (8.9–15.7) years. The median age at first admission was 5.3 (1.5–9.9) years, and the median follow-up period was 6.8 (2.1–7.9) years. The most common clinical finding at admission was short stature (29.5%). Brain magnetic resonance imaging most frequently revealed corpus callosum abnormalities (52.9%), followed by septo-optic dysplasia (17.6%). Endocrine disorders were present in 82.3% of patients with MBA. The most frequently observed endocrine disorder was multiple pituitary hormone deficiency (41.2%). In addition, isolated endocrine disorders such as central hypothyroidism (17.6%), growth hormone deficiency (11.8%), diabetes insipidus (5.9%), and hypogonadotropic hypogonadism (5.9%) were observed. When each endocrine disorder was evaluated individually, central hypothyroidism emerged as the most frequently identified condition (58.8%). Three patients had no detectable endocrine dysfunction.

Conclusions: Endocrine disorders were observed in 82.3% of patients with MBA, with central hypothyroidism being the most common when considered individually. The high prevalence of endocrine disorders in children with MBA underscores the importance of routine endocrine screening in this population.

Keywords: children, endocrine disorders, hypopituitarism, midline brain abnormalities

INTRODUCTION

The hypothalamus, pituitary gland, and other endocrine organs play a crucial role in regulating growth, reproduction, metabolism, and fluid balance.[1] The pituitary gland originates from the Rathke's pouch and the neuroectoderm of the diencephalon, with its development occurring around the sixth to seventh week of embryogenesis. Congenital and developmental abnormalities of the hypothalamic-pituitary axis include pituitary hypoplasia or agenesis, ectopic posterior pituitary, absence of the pituitary bright spot, duplication of the pituitary gland or stalk, empty

✉ Yavuz Özer ▪ yavuzcp@yahoo.com

Received: 21.03.2025 **Accepted:** 14.06.2025

sella syndrome, and various midline congenital anomalies. Additionally, midline structural defects such as optic nerve hypoplasia, absence of the septum pellucidum, and corpus callosum abnormalities may coexist with these conditions.[2]

Children with midline brain abnormalities (MBA) often present with neurological impairments, including developmental delay and hydrocephalus.[3] Given the potential risk of morbidity and mortality associated with undiagnosed endocrinopathies, recognizing the clinical manifestations of hormone deficiencies in these patients is essential. Hypopituitarism, if left undiagnosed, may lead to severe complications such as hypoglycemia, adrenal crisis, and increased mortality.[4] Reports in the literature suggest that the prevalence of endocrinopathies in children with neuroanatomical anomalies can be as high as 82%.[4-8]

Pediatric endocrinologists frequently evaluate children and adolescents with MBA to assess potential endocrine dysfunction. In addition to clinical symptoms, anthropometric measurements and laboratory investigations aid in diagnosing various pituitary endocrinopathies.[9] Furthermore, brain magnetic resonance imaging (MRI) of the hypothalamic-pituitary region is a key diagnostic tool for identifying structural abnormalities associated with endocrine dysfunction.[9,10]

This study aimed to evaluate the prevalence and spectrum of endocrine disorders in pediatric patients with MBA.

MATERIAL AND METHODS

This retrospective study included 17 patients diagnosed with MBA. Patients younger than 18 years of age who were followed at a tertiary pediatric endocrinology center for endocrine evaluation due to MBA between January 2011 and January 2021 were enrolled. In some cases, MBA was incidentally detected during neuroimaging performed for non-endocrine indications, while in others, it was identified during evaluation for suspected endocrine dysfunction. Patients diagnosed with an endocrinopathy after the detection of MBA underwent additional hormonal assessments as part of routine endocrine follow-up. Data on patient demographics, including age, sex, age at presentation, auxological measurements, endocrine laboratory findings, and MRI results, were retrieved from medical records. Anthropometric percentiles and standard deviation scores (SDS) were calculated using the Child Metrics online calculator (http://www.childmetrics.com), based on the national child growth reference data for Turkey.[11]

Exclusion criteria

Patients with acquired brain abnormalities due to trauma, infection, or malignancy were excluded. Additionally, cases with septum pellucidum variation and empty sella, considered normal anatomical variants due to their high prevalence in the general population, were not included in the study.[12,13]

Endocrine assessments

Clinical findings, baseline laboratory tests, and dynamic endocrine function tests were evaluated by a pediatric endocrinologist. The diagnosis of hypopituitarism was established according to current clinical guidelines.[14-18] Growth hormone deficiency (GHD) was defined as an insufficient response to two different stimulation tests, after excluding chronic diseases, in patients with a mean height of less than −2 SDS for sex or a low growth velocity.[14] Central hypothyroidism was diagnosed in cases with low or normal thyrotropin (TSH) levels and reduced free thyroxine (FT4) concentrations.[15] Central adrenal insufficiency was identified based on morning cortisol levels and confirmed with adrenocorticotropic hormone (ACTH) stimulation tests.[16] Central diabetes insipidus was diagnosed using serum and urine osmolality measurements, supplemented by a water deprivation test when necessary.[17] Hypogonadism was assessed through luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels, as well as responses to a gonadotropin-releasing hormone (GnRH) stimulation test.[18] Multiple pituitary hormone deficiencies (MPHD) were defined as the presence of at least two deficiencies among growth hormone (GH), TSH, ACTH, and gonadotropins.[4] MRI scans were reviewed by an experienced neuroradiologist to assess structural abnormalities. All clinical evaluations, endocrine laboratory tests, and imaging assessments were performed as part of routine patient care.

Ethical approval

This retrospective study involving human participants was in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This study was approved by the Clinical Research Ethical Committee of Istanbul University-Cerrahpasa Cerrahpasa Medical Faculty (Approval number: 83045809-604.01.01-370476). Due to the retrospective nature of the study, informed consent was not required.

Statistical analysis

All statistical analyses were conducted using SPSS 21.0 (IBM Corp., Armonk, NY, USA). Categorical variables were expressed as numbers and percentages, while continuous variables were presented as means and standard deviations for normally distributed data. For non-normally distributed continuous variables, the median and interquartile range (25th–75th percentiles) were reported.

RESULTS

The study included 17 patients, of whom 52.9% (n=9) were male, with a median age of 11.1 (8.9–15.7) years. The median age at admission was 5.3 (1.5–9.9) years, and the median follow-up duration was 6.8 (2.1–7.9) years. Demographic, clinical, and laboratory findings of the patients are presented in Table 1. The median SDS for weight, height, and body mass index were -1.51 [(-2.10)–0.3], -2.19 (-3.0–0.66), and -0.15 (-1.85–1.5), respectively. The height SDS values among all patients ranged from -7.26 to +2.75. Tall stature (height SDS: +2.75) was observed in one patient, which is atypical for pituitary hormone deficiencies. The patient was diagnosed with Sotos syndrome, a condition associated with excessive childhood growth. No endocrine abnormalities were detected, and cranial MRI demonstrated corpus callosum hypoplasia.

The most frequently observed clinical finding at admission was short stature (29.5%). Additional presenting features included cleft lip/palate, neuromotor delay, optic nerve hypoplasia, electrolyte imbalances, autism, undescended testis, and tall stature (Table 1).

Brain MRI revealed corpus callosum abnormalities as the most common MBA (52.9%). Other malformations included septo-optic dysplasia (SOD), polymicrogyria with pituitary hypoplasia, pituitary stalk interruption syndrome, ectopic neurohypophysis, holoprosencephaly, and Chiari malformation type 1 (Table 2).

Endocrine disorders were identified in 14 patients (82.3%), while no endocrine pathology was detected in three cases. MPHD were observed in 41.2% of the cases, as detailed in Table 3. Additionally, isolated endocrine disorders—including central hypothyroidism, GHD, diabetes insipidus, and hypogonadotropic hypogonadism—were also observed. When each endocrine disorder was evaluated individually, central hypothyroidism was the most frequently identified condition (58.8%, n=10). Among the three cases without endocrine abnormalities, all had corpus callosum abnormalities. One patient presented with hydrocephalus and GHD, along with cavum septum pellucidum et vergae variation, which is considered a normal anatomical variant (Table 3).

DISCUSSION

This study investigated the relationship between neuroanatomical abnormalities and endocrine dysfunction in patients with MBA. Endocrine disorders were detected in 82.3% of children referred to our tertiary pediatric endocrinology center with MBA. Previous studies have reported the prevalence of endocrinopathies in similar populations to range between 37% and 82%.[4-8] Variability in reported prevalence rates may stem from differences in study design and patient selection criteria. Since endocrine dysfunction may develop progressively over time, an initially normal pituitary function does not exclude the possibility of future endocrinopathy. In particular, patients with SOD and hypothalamic-pituitary dysfunction are frequently diagnosed within the first two years of life.[19,20] Furthermore, Cerbone et al.[6] demonstrated that individuals with SOD may continue to develop hormone deficiencies

Table 1. Admission findings of cases

Clinical Finding	n	%
Short stature	5	29.5
Neuromotor retardation	3	17.6
Cleft lip/palate	2	11.8
Optic nerve hypoplasia	1	5.9
Optic nerve hypoplasia + short stature	1	5.9
Electrolyte imbalance	1	5.9
Electrolyte imbalance + cleft lip/palate	1	5.9
Autism	1	5.9
Cryptorchidism	1	5.9
Tall stature	1	5.9

Table 2. Brain abnormalities of cases on MRI

Brain Malformation	n	%
Corpus callosum abnormality (agenesis, dysgenesis, hypoplasia)	9	52.9
Septo-optic dysplasia	3	17.6
Polymicrogyria and pituitary hypoplasia	1	5.9
Pituitary stalk interruption syndrome	1	5.9
Ectopic posterior pituitary	1	5.9
Holoprosencephaly	1	5.9
Chiari malformation type 1	1	5.9

Table 3. Endocrine status of cases		
Endocrine Disorder	n	%
Multiple pituitary hormone deficiency	7	41.2
Central hypothyroidism + growth hormone deficiency + adrenal insufficiency	2	
Central hypothyroidism + adrenal insufficiency	1	
Central hypothyroidism + diabetes insipidus	1	
Central hypothyroidism + hypogonadotropic hypogonadism	1	
Central hypothyroidism + growth hormone deficiency	1	
Adrenal insufficiency + diabetes insipidus	1	
Central Hypothyroidism	3	17.6
No detected endocrine disorder	3	17.6
Growth hormone deficiency	2	11.8
Diabetes insipidus	1	5.9
Hypogonadotropic hypogonadism	1	5.9

throughout adolescence. Delayed or missed diagnoses can lead to irreversible complications, increasing both morbidity and mortality risks.[5,7,9] Therefore, children with midline cerebral and intracranial malformations require thorough endocrine evaluation and long-term follow-up to monitor potential hormonal dysfunction.[5,21]

Not all congenital MBA are necessarily associated with hypopituitarism.[5,6,22] In our study, corpus callosum anomalies were identified in the three patients without any endocrine dysfunction. Corpus callosum abnormalities are among the most frequently detected MBA during routine prenatal ultrasonography and are associated with more than 200 syndromes.[22,23] However, their relationship with endocrine dysfunction remains unclear. While some cases remain asymptomatic, others present with neuromotor impairment, intellectual disability, seizures, and endocrine disorders.[3,24] Additionally, associated structural abnormalities, such as gyrus dysplasia and cortical heterotopia, may influence prognosis when combined with corpus callosum anomalies.[25] Endocrine dysfunction is particularly common in holoprosencephaly and SOD, as these conditions significantly impact hypothalamic and pituitary development.[19,26] In such cases, hormonal deficiencies are more likely to result from hypothalamic dysfunction rather than direct pituitary anomalies.[21]

The diagnostic utility of brain MRI for detecting endocrinopathies has been debated. Sensitivity and specificity have been reported as 67.9% and 83.3%, respectively, indicating that abnormal MRI findings alone may not reliably predict endocrine dysfunction, nor does a normal MRI exclude the possibility of hormonal abnormalities.[7] Nonetheless, MRI remains a valuable tool for identifying structural lesions. Given the lack of standardized guidelines for the endocrinological management of patients with MBA, follow-up and treatment should be individualized.[9] Specific MRI abnormalities have been linked to an increased risk of early-onset hypopituitarism, emphasizing the need for lifelong endocrine monitoring in these patients.[5,6] Furthermore, the severity of MBA does not always correlate with the degree of endocrine dysfunction.[5]

The prevalence and distribution of endocrine disorders in children with neuroanatomical abnormalities vary widely. While growth hormone deficiency is frequently cited as the most common endocrinopathy in this population, followed by hypothyroidism, ACTH insufficiency, and diabetes insipidus,[8,21] our study identified central hypothyroidism as the most prevalent disorder (52.9%). Qian et al.[7] reported that ACTH insufficiency was the most frequently observed endocrine disorder in these cases. Previous research has indicated that patients with MPHD often present with GH and TSH deficiencies as the most common hormonal deficits.[27] Pituitary hypoplasia can cause endocrine deficiencies in the spectrum ranging from isolated GHD to panhypopituitarism in patients with SOD. The most common endocrine findings reported in patients with SOD are central hypothyroidism, GHD, and adrenal insufficiency.[19,20,28] In our study, patients with SOD exhibited a range of endocrine abnormalities, including hypothyroidism, GHD, and adrenal insufficiency. In holoprosencephaly, endocrine dysfunction—particularly adrenal insufficiency, diabetes insipidus, GHD, and hypogonadism—is frequently observed due to severe midline defects affecting hypothalamic and

pituitary development.[26] In our study, the patient with holoprosencephaly had both adrenal insufficiency and diabetes insipidus.

Additionally, hypothyroidism has been linked to developmental delay in patients with corpus callosum abnormalities.[25] In a study by Qian et al.,[7] 20.9% of children with developmental delay were diagnosed with hypothyroidism. Congenital central hypothyroidism, characterized by low thyroxine and inappropriately low or normal TSH levels, is often missed in neonatal screening programs that rely solely on TSH measurements. In countries where neonatal screening does not include direct T4 assessment, early detection and treatment may be delayed. Given the high prevalence of endocrine disorders in children with MBA, early diagnosis and timely intervention for central hypothyroidism are critical to minimizing neurological complications and improving long-term outcomes.[29]

A key limitation of this study is the small sample size, which may limit the generalizability of the findings. Additionally, the retrospective study design did not allow for long-term follow-up, restricting our ability to assess the progression of endocrinopathies over time. Future prospective studies with larger cohorts are needed to better characterize the natural history of endocrine dysfunction in children with MBA.

CONCLUSION

Endocrine disorders were identified in 82.3% of children with MBA, with central hypothyroidism being the most frequently observed endocrinopathy. Given the high prevalence of hormonal dysfunction in this population, routine endocrine assessment should be conducted regardless of the presence or absence of clinical symptoms. Early diagnosis and appropriate management are essential to mitigating morbidity and preventing further neurological impairment. A multidisciplinary approach involving pediatric endocrinologists, neurologists, and radiologists is crucial for optimizing the care of these patients.

Ethical approval

This study has been approved by the Ethics Committee of Istanbul University-Cerrahpaşa (approval date 26.04.2022, number 83045809-604.01.01-370476). Due to the retrospective nature of the study, informed consent was not required.

Author contribution

The authors declare contribution to the paper as follows: Study conception and design: YÖ, OE; data collection: YÖ, BD, HT, DT, DAB, OE, SS, OE; analysis and interpretation of results: YÖ, OE; draft manuscript preparation: YÖ, OE. 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. Hong GK, Payne SC, Jane JA. Anatomy, physiology, and laboratory evaluation of the pituitary gland. Otolaryngol Clin North Am. 2016;49:21-32. [Crossref]
2. Chaudhary V, Bano S. Imaging of pediatric pituitary endocrinopathies. Indian J Endocrinol Metab. 2012;16:682-91. [Crossref]
3. Shankar P, Zamora C, Castillo M. Congenital malformations of the brain and spine. Handb Clin Neurol. 2016;136:1121-37. [Crossref]
4. Birkebaek NH, Patel L, Wright NB, et al. Endocrine status in patients with optic nerve hypoplasia: relationship to midline central nervous system abnormalities and appearance of the hypothalamic-pituitary axis on magnetic resonance imaging. J Clin Endocrinol Metab. 2003;88:5281-6. [Crossref]
5. Traggiai C, Stanhope R. Endocrinopathies associated with midline cerebral and cranial malformations. J Pediatr. 2002;140:252-5. [Crossref]
6. Cerbone M, Güemes M, Wade A, Improda N, Dattani M. Endocrine morbidity in midline brain defects: differences between septo-optic dysplasia and related disorders. EClinicalMedicine. 2020;19:100224. [Crossref]
7. Qian X, Fouzdar Jain S, Morgan LA, Kruse T, Cabrera M, Suh DW. Neuroimaging and endocrine disorders in paediatric optic nerve hypoplasia. Br J Ophthalmol. 2018;102:906-10. [Crossref]
8. Haddad NG, Eugster EA. Hypopituitarism and neurodevelopmental abnormalities in relation to central nervous system structural defects in children with optic nerve hypoplasia. J Pediatr Endocrinol Metab. 2005;18:853-8. [Crossref]
9. Brener A, Kozyrev DA, Shiran SI, et al. Incidental findings on brain Magnetic Resonance Imaging (MRI) in pediatric endocrine patients. Endocr Pract. 2020;26:1105-14. [Crossref]
10. Chapman PR, Singhal A, Gaddamanugu S, Prattipati V. Neuroimaging of the pituitary gland: practical anatomy and pathology. Radiol Clin North Am. 2020;58:1115-33. [Crossref]

11. Demir K, Özen S, Konakçı E, Aydın M, Darendeliler F. A Comprehensive online calculator for pediatric endocrinologists: ÇEDD çözüm/TPEDS metrics. J Clin Res Pediatr Endocrinol. 2017;9:182-4. **[Crossref]**

12. Scoffings DJ, Kurian KM. Congenital and acquired lesions of the septum pellucidum. Clin Radiol. 2008;63:210-9. **[Crossref]**

13. Chiloiro S, Giampietro A, Bianchi A, et al. Diagnosis of endocrine disease: primary empty sella: a comprehensive review. Eur J Endocrinol. 2017;177:R275-85. **[Crossref]**

14. Collett-Solberg PF, Ambler G, Backeljauw PF, et al. Diagnosis, genetics, and therapy of short stature in children: a growth hormone research society international perspective. Horm Res Paediatr. 2019;92:1-14. **[Crossref]**

15. Wassner AJ. Pediatric hypothyroidism: diagnosis and treatment. Paediatr Drugs. 2017;19:291-301. **[Crossref]**

16. Patti G, Guzzeti C, Di Iorgi N, et al. Central adrenal insufficiency in children and adolescents. Best Pract Res Clin Endocrinol Metab. 2018;32:425-44. **[Crossref]**

17. Baldeweg SE, Ball S, Brooke A, et al. Society for endocrinology clinical guidance: inpatient management of cranial diabetes insipidus. Endocr Connect. 2018;7:G8-11. **[Crossref]**

18. Zhu J, Feldman HA, Eugster EA, et al. Practice variation in the management of girls and boys with delayed puberty. Endocr Pract. 2020;26:267-84. **[Crossref]**

19. Cemeroglu AP, Coulas T, Kleis L. Spectrum of clinical presentations and endocrinological findings of patients with septo-optic dysplasia: a retrospective study. J Pediatr Endocrinol Metab. 2015;28:1057-63. **[Crossref]**

20. Alt C, Shevell MI, Poulin C, Rosenblatt B, Saint-Martin C, Srour M. Clinical and radiologic spectrum of septo-optic dysplasia: review of 17 cases. J Child Neurol. 2017;32:797-803. **[Crossref]**

21. Garcia-Filion P, Borchert M. Optic nerve hypoplasia syndrome: a review of the epidemiology and clinical associations. Curr Treat Options Neurol. 2013;15:78-89. **[Crossref]**

22. des Portes V, Rolland A, Velazquez-Dominguez J, et al. Outcome of isolated agenesis of the corpus callosum: a population-based prospective study. Eur J Paediatr Neurol. 2018;22:82-92. **[Crossref]**

23. Sotiriadis A, Makrydimas G. Neurodevelopment after prenatal diagnosis of isolated agenesis of the corpus callosum: an integrative review. Am J Obstet Gynecol. 2012;206:337.e1-5. **[Crossref]**

24. D'Antonio F, Pagani G, Familiari A, et al. Outcomes associated with isolated agenesis of the corpus callosum: a meta-analysis. Pediatrics. 2016;138:e20160445. **[Crossref]**

25. Garcia-Filion P, Epport K, Nelson M, et al. Neuroradiographic, endocrinologic, and ophthalmic correlates of adverse developmental outcomes in children with optic nerve hypoplasia: a prospective study. Pediatrics. 2008;121:e653-9. **[Crossref]**

26. Dubourg C, Bendavid C, Pasquier L, Henry C, Odent S, David V. Holoprosencephaly. Orphanet J Rare Dis. 2007;2:8. **[Crossref]**

27. Blum WF, Deal C, Zimmermann AG, et al. Development of additional pituitary hormone deficiencies in pediatric patients originally diagnosed with idiopathic isolated GH deficiency. Eur J Endocrinol. 2013;170:13-21. **[Crossref]**

28. Murray A, Schwartz T, Hornung L, Lawson S. Pituitary deficiencies related to optic nerve hypoplasia and visual acuity. Clin Endocrinol (Oxf). 2024;101:507-15. **[Crossref]**

29. Fink C, Vedin AM, Garcia-Filion P, Ma NS, Geffner ME, Borchert M. Newborn thyroid-stimulating hormone in children with optic nerve hypoplasia: associations with hypothyroidism and vision. J AAPOS. 2012;16:418-23. **[Crossref]**

Trends in Pediatrics 2025;6(4):239-244
DOI: 10.59213/TP.2025.265

Original Article

Impact of the COVID-19 pandemic on central precocious puberty: a retrospective cohort study from Türkiye

Gülcan Seymen[1], Müge Atar[2]

[1]Department of Pediatric Endocrinology, Ümraniye Training and Research Hospital, University of Health Sciences, İstanbul, Türkiye
[2]Department of Pediatric Endocrinology and Diabetes, Faculty of Medicine, Süleyman Demirel University, Isparta, Türkiye

Cite this article as: Seymen G, Atar M. Impact of the COVID-19 pandemic on central precocious puberty: a retrospective cohort study from Türkiye. Trends in Pediatrics 2025;6(4):239-244.

ABSTRACT

Objective: This study aimed to investigate the clinical and demographic characteristics of girls diagnosed with central precocious puberty (CPP) before and during the COVID-19 pandemic to identify potential changes in incidence and contributing factors.

Methods: A retrospective cohort study was conducted in two pediatric endocrinology centers in Turkiye. Girls treated with GnRH Gonadotropin-Releasing Hormone (GnRH) analogs between March 2018 and March 2022 were categorized into two groups: the pre-pandemic group (2018–2020) and the pandemic group 2020–2022). Clinical, anthropometric, and hormonal data were analyzed. Patients with organic lesions, genetic disorders, or medications affecting puberty were excluded.

Results: CPP diagnoses increased significantly during the pandemic, rising from 0.5% (32/6,446) in the pre-pandemic period to 2.1% (160/7,436) during the pandemic. Basal, peak LH, and peak LH/peak FSH levels were significantly higher in the pandemic group ($p < 0.01$), while BMI and Tanner stages showed no significant differences.

Conclusion: The study highlights a marked increase in CPP diagnoses during the pandemic. Elevated basal and peak LH levels suggest a more pronounced hormonal activation, potentially influenced by pandemic-related factors. Further research is necessary to elucidate the underlying mechanisms, including environmental and psychological contributors.

Keywords: central precocious puberty, COVID-19 pandemic, GnRH analogs, pediatric endocrinology, lifestyle changes

INTRODUCTION

Various measures have been implemented to mitigate the impact of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) pandemic, which has affected the entire world. In line with these measures, stay-at-home orders and school closures have had both physiological and psychological effects on children.[1] Moreover, obesity rates have increased due to reduced physical activity, disrupted sleep patterns, and altered eating habits.[2] As the pandemic's impact lessens over time, our understanding of how it affected puberty is improving.

Puberty is a critical period that leads to complex endocrinological changes and enables the attainment of reproductive capacity. Some mechanisms that initiate the pubertal period have not yet been elucidated. However, it is known that changes in energy balance, neurotransmitters, and neuropeptide expression, and genetic and epigenetic factors, contribute to the beginning of pubertal signals.[3]

✉ Gülcan Seymen ▪ gulcansk@gmail.com

Received: 25.03.2025 **Accepted:** 29.08.2025

Early pubertal maturation could lead to rapid skeletal development and premature epiphyseal closure, which may be associated with a psychological burden due to potential short stature.[4]

Several studies have shown that admissions due to early pubertal changes have increased in different countries during the COVID-19 pandemic.[5,6] Although some studies from Turkiye have shown that the age of onset of pubertal changes started earlier, no differences were found in others.[7,8] Several mechanisms, such as the direct effect of SARS-CoV-2 infection, electronic device use, sedentary lifestyles, obesity, and sleep disturbances, have been hypothesized to explain increased precocious puberty, but the entire mechanism needs clarification.[6,8] This study aimed to compare the clinical and demographic properties of patients with precocious puberty admitted before and after the pandemic.

METHODS

The study included patients admitted to the Pediatric Endocrinology Departments of Ümraniye Training and Research Hospital and Süleyman Demirel University, located in two different cities in Turkiye. These patients were diagnosed with precocious puberty and treated with GnRH analogs before and after the COVID-19 pandemic. The patients were categorized into two groups: the pre-pandemic group (admitted and started GnRH analog treatment between March 2018 and March 2020) and the pandemic group (admitted and started GnRH analog treatment between March 2020 and March 2022), covering 24 months before and after the start of the pandemic lockdown. The participants' medical records were reviewed retrospectively.

Clinical data, including Tanner staging, anthropometric measurements, and hormonal profiles, were collected. Hormonal profiles included luteinizing hormone (LH), follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), estradiol (E2), and, if available, other hormones such as dehydroepiandrosterone sulfate (DHEAS), 17-hydroxyprogesterone (17-OHP), prolactin (PRL), and free thyroxine (FT4). Pediatric endocrinologists assessed all patients for pubertal staging according to the Marshall and Tanner criteria.[9] Bone age was determined using left-hand X-rays analyzed with the Greulich-Pyle method.[10] Height, weight, and body mass index data were taken retrospectively from patient files. Body mass index (BMI), height SDS, and weight SDS were calculated using an online calculator based on Turkish children's growth standards.[11]

Overweight and obesity were defined as BMI values between the 85th and 95th percentiles and ≥95th percentile for age and gender, respectively.

Central precocious puberty (CPP) in girls was defined as the onset of secondary sexual characteristics before 8 years of age, accompanied by advanced bone age (≥1 year above chronological age) and a pubertal response to GnRH stimulation (peak LH >5 IU/L, peak LH/FSH ratio >1, or basal LH >0.3 IU/L).[6,8,12]

If pubertal changes began after eight years of age but progressed faster than six months between two pubertal stages, these cases were classified as accelerated pubertal development. Only patients diagnosed with CPP and who were initiated on GnRH analogue therapy were included in the study. Cases with slow progression that did not require immediate treatment were excluded.

Patients with organic lesions (e.g., hypothalamo-pituitary tumors), congenital malformations, oncological diseases, neurosurgical or genetic disorders, or medications affecting puberty were excluded from the study.

Serum levels of LH (mIU/mL), FSH (mIU/mL), and estradiol (pg/mL) were measured using the immunochemiluminescence method (ICMA, ADVIA Centaur XPT, Siemens, USA). Samples for FSH and LH during GnRH stimulation tests were obtained at 20, 40, and 60 minutes following intravenous administration of 100 µg/m² (maximum 100 µg) LHRH (LHRH Ferring ampule) if basal FSH and LH levels were inconclusive.

Ethical approval

This study was approved by the Clinical Research Ethics Committee of the Ministry of Health, University of Health Sciences, Ümraniye Training and Research Hospital on August 25, 2022 (Ethics Committee No: 19659). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical analysis

The statistical analysis was performed using the IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., Armonk, NY, USA). The distribution of the data was assessed using the Kolmogorov-Smirnov test. As the data followed a normal distribution, they were expressed as mean ± standard deviation. For the comparison of normally distributed data

between two groups, the Independent Samples t-test was used. Categorical variables were analyzed using the Chi-square test. A p-value of <0.05 was considered statistically significant for all tests.

RESULTS

During the pre-COVID-19 period, 6,446 patients presented various complaints, compared to 7,436 patients during the COVID-19 period. Of these, CPP diagnoses accounted for 32 cases pre-pandemic (0.5%) and 160 cases during the pandemic (2.1%). Notably, the incidence of new CPP cases requiring GnRH analog therapy nearly quadrupled during the pandemic compared to the pre-pandemic period.

The demographic, anthropometric, and clinical characteristics of patients were compared based on their admission periods (pre-pandemic vs. pandemic) (Table 1, Table 2).

Among Tanner stages, stage IV was the most frequent in the pandemic group (26.3%), whereas stage III was the most common in the pre-pandemic group (62.5%). Menarche was reported in 20 (12.5%) patients in the pandemic group and 4 (12.5%) in the pre-pandemic group. Although no statistically significant difference was found in BMI category distribution, the proportion of normal-weight patients was higher in the pandemic group (15.0% vs. 6.3%).

No significant differences were observed between the groups in terms of bone age (10.0 ± 1.7 vs. 10.08 ± 1.4 years, p = 0.33) or BA/CA ratio (1.22 ± 0.13 vs. 1.20 ± 0.12, p = 0.26). Additionally, estradiol, basal and peak FSH, prolactin, DHEAS, and TSH levels were similar between groups. However, basal LH (2.08 ± 1.89 vs. 1.37 ± 1.03 IU/L), peak LH (20.5 ± 9.1 vs. 10.7 ± 6.5 IU/L), and peak LH/FSH ratio (1.45 ± 0.35 vs. 1.10 ± 0.30) were significantly higher in the pandemic group (all p < 0.01), indicating a more pronounced hormonal activation.

DISCUSSION

This study observed an increase in the number of admissions, in the diagnosis of CPP, and in the requirement for GnRH therapy during the pandemic compared to the pre-pandemic period. Consistent with our findings, several studies from Italy confirmed the association between the pandemic and the increased incidence of precocious puberty.[8,13,14] Stagi et al. compared cases of precocious puberty during the pandemic period with the same timeframe over the preceding five years and reported a significantly higher rate of newly diagnosed cases during the pandemic (37 cases per year versus 17.8 ± 1.3 cases per year pre-pandemic).[6] Similarly, Verzani et al. found an increase in suspected precocious puberty cases during the pandemic in a retrospective review of consultation reports (215 vs. 87 patients).[13] Studies from Turkiye also support this trend, with Yüksek Acinikli et al. reporting a threefold increase in CPP and rapidly progressive early puberty diagnoses after the pandemic[7], and Acar et al. documenting that CPP cases more than doubled after the pandemic compared to the previous three years.[15] As supported by this study, a multicenter study by Yeşiltepe Mutlu et al. also reported that the onset of puberty occurred earlier and the need for pubertal suppression therapy increased during the pandemic period compared to the previous year in Turkish children.[8] These findings suggest that the global pandemic, while primarily affecting respiratory health, also had significant effects on the endocrine system.[16]

Table 1. Demographic and anthropometric characteristics of CPP patients, comparing pre-pandemic and pandemic groups

Variable	Pre-pandemic (n=32)	Pandemic (n=160)	p-value
Age (years)	8.35 ± 1.21	8.16 ± 0.98	0.27
Weight SDS	1.04 ± 0.70	1.07 ± 0.90	0.34
Height SDS	0.79 ± 0.74	1.12 ± 0.94	0.11
BMI SDS	0.87 ± 0.78	0.77 ± 0.98	0.15
Obesity (%)	62.5	58.8	0.27
Overweight (%)	31.3	26.3	0.23
Normal Weight (%)	6.3	15.0	0.16
Bone Age (years)	10.0 ± 1.7	10.08 ± 1.4	0.33
BA/CA Ratio	1.22 ± 0.13	1.20 ± 0.12	0.26

BA: Bone age, CA: Chronologic age
Pre-pandemic group: patients diagnosed between March 2018 and March 2020 (n = 32).
Pandemic group: patients diagnosed between March 2020 and March 2022 (n = 160).

Table 2. Clinical and hormonal features of CPP patients in the pre-pandemic and pandemic groups

Variable	Pre-pandemic (n=32)	Pandemic (n=160)	p-value
Tanner Stage II n (%)	4(12.5)	34(21.3)	0.27
Tanner Stage III n (%)	20(62.5)	64(40.0)	0.31
Tanner Stage IV n (%)	4(12.5)	42(26.3)	0.21
Tanner Stage V n (%)	4(12.5)	20(12.5)	0.47
Menarche n (%)	4(12.5)	20(12.5)	0.45
Basal LH (mIU/mL)	1.37 ± 1.03	2.08 ± 1.89	<0.01*
Peak LH (mIU/mL)	10.7 ± 6.5	20.5 ± 9.1	<0.01*
Estradiol (pg/mL)	28.5 ± 23.2	26.8 ± 20.5	0.33
Peak FSH (mIU/mL)	10.06 ± 6.2	14.3 ± 5.2	0.27
Basal FSH (mIU/mL)	3.83 ± 2.02	4.43 ± 4.74	0.36
Peak LH/ peak FSH	1.10 ± 0.30	1.45± 0.35	<0.01*
17-OHP (ng/mL)	**1.2 ± 0.4**	**1.4 ± 0.5**	0.07
DHEAS (µg/dL)	112 ± 26	116 ± 29	0.32
Prolactin (ng/mL)	14.0 ± 3.5	13.2 ± 3.0	0.22
FT4 (ng/dL)	1.15 ± 0.18	1.18 ± 0.20	0.45
TSH (mIU/L)	2.1 ± 0.6	2.3 ± 0.7	

*p<0.05 (Independent samples T test)
The values corresponding to Tanner stages and menarche represent the number of patients (n) and the percentage (%) within each group.
Abbreviations: LH = Luteinizing Hormone; FSH = Follicle-Stimulating Hormone; 17-OHP = 17-Hydroxyprogesterone; DHEAS = Dehydroepiandrosterone sulfate; FT4 = Free Thyroxine; TSH = Thyroid-Stimulating Hormone.

Pandemic-related restrictions, including school closures, changes in eating habits, reduced physical activity, increased screen time, poor sleep quality, and elevated stress levels, are potential factors influencing pubertal timing and progression.[14] Several studies have linked the rise in precocious puberty cases during the pandemic to an increase in BMI, potentially driven by higher food consumption and decreased physical activity.[8] Interestingly, our study found that while BMI in the pandemic group was comparable to the pre-pandemic group, the need for GnRH therapy increased. Stagi et al. observed a notable increase in Δ BMI-SDS in the pubertal progression group, suggesting a possible link between weight changes and pubertal progression.[6] Accelerated weight gain during the pandemic may explain the significantly higher number of precocious puberty cases observed in our study. On the contrary, there was no significant difference in weight and BMI among groups in our study. However, we were unable to investigate the body composition, which could impact puberty onset, due to a lack of lean mass and fat mass measurements. Certain studies from Turkiye also showed that BMI-SDS was similar in patients with CPP diagnosed before and after the pandemic,[6,14] indicating that increased admissions for precocious puberty cannot be solely attributed to BMI changes. This may be explained by reduced physical activity, which can lead to increased body fat without altering BMI.[5] Our study found that the admission age of the two groups was similar, aligning with findings from Turkiye and other countries.[7,17] However, Yeşiltepe Mutlu et al. reported that patients with precocious puberty were younger during the pandemic compared to the pre-pandemic period.[8] These conflicting results may reflect differences in the timing of enrollment after the onset of clinical signs, as fear of illness during the pandemic likely led to delayed admissions.[18] In our study, the relatively higher rate of normal-weight cases in the pandemic group, though not statistically significant, may reflect increased parental vigilance and altered referral patterns during lockdown periods, rather than a true shift in the weight–puberty relationship.

Similar to our findings, Stagi et al. observed elevated basal and peak LH levels in the pandemic group[6], although some studies did not find significant differences in LH levels.[5,19] Reduced melatonin secretion, potentially linked to increased screen and electromagnetic field exposure, may lead to disinhibition of GnRH secretion and contribute to earlier onset of puberty.[20,21] It is well established that SARS-CoV-2 infections can impact the central nervous system (CNS) through ACE-2 receptor binding, using olfactory or hematogenous pathways, as well as via the cytokine

storm.[22] Given that ACE-2 receptors are also expressed in the ovaries and testes, it is plausible that SARS-CoV-2 may play a role in triggering early puberty through direct viral effects and additional influencing factors.

Lastly, the presence of endometrial rhyme is a highly specific marker of estrogenic secretion and significant pubertal activation.[19] It is plausible that the estrogenic surge was more pronounced in patients who experienced pubertal onset during the lockdown period. This heightened estrogenic activity may have contributed to the development of endometrial rhyme, even in the presence of similar laboratory findings.

Limitations

This study recognizes several limitations that may have influenced the findings and interpretations. One notable limitation is the lack of data regarding participants' exposure to electronic devices, which has been hypothesized to play a significant role in the increase of precocious puberty cases during the pandemic. Without quantifying screen time or electromagnetic field exposure, it remains challenging to fully assess their impact on pubertal development.

Additionally, stress—an established trigger for hormonal changes and pubertal onset—was not directly measured in this study. Pandemic-related factors such as fear of illness, prolonged social isolation, and school closures likely heightened stress levels in children, which may have contributed to the observed rise in precocious puberty cases. However, cortisol levels or other biomarkers of stress were not evaluated, limiting the study's ability to establish a clear connection.

Finally, the retrospective nature of the study and reliance on existing medical records might have introduced selection biases or inconsistencies in data collection. Future prospective studies that account for these variables, including stress and electronic device usage, are essential to better understand the mechanisms driving the increase in precocious puberty cases during the pandemic.

CONCLUSION

This study highlights a significant rise in central precocious puberty (CPP) cases during the COVID-19 pandemic, indicating a potential interplay between environmental, psychological, and biological factors. While lifestyle changes such as reduced physical activity, increased screen time, and altered eating habits have been widely implicated, our findings suggest that hormonal activation may have played a crucial role beyond these behavioral shifts. The observed increase in basal, peak LH, and peak LH/peak FSH levels reinforces the need to explore potential neuroendocrine triggers, including stress-related and viral mechanisms. Given the long-term implications of early puberty on metabolic and psychosocial health, further studies are essential to unravel the precise mechanisms and develop effective preventive and therapeutic strategies.

Ethical approval

This study has been approved by the Clinical Research Ethics Committee of the Ministry of Health, University of Health Sciences, Ümraniye Training and Research Hospital (approval date 25.08.2022, number 19659). Written informed consent was obtained from the participants.

Author contribution

The authors declare contribution to the paper as follows: Study conception and design: GS, MA; data collection: GS, MA; analysis and interpretation of results: GS, MA; draft manuscript preparation: GS. 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. Procentese F, Esposito C, Gonzalez Leone F, et al. Psychological lockdown experiences: downtime or an unexpected time for being? Front Psychol. 2021;12:577089. [Crossref]
2. Hauerslev M, Narang T, Gray N, Samuels TA, Bhutta ZA. Childhood obesity on the rise during COVID-19: a request for global leaders to change the trajectory. Obesity (Silver Spring). 2022;30:288-91. [Crossref]
3. Livadas S, Chrousos GP. Molecular and environmental mechanisms regulating puberty initiation: an integrated approach. Front Endocrinol (Lausanne). 2019;10:828. [Crossref]
4. Aguirre RS, Eugster EA. Central precocious puberty: from genetics to treatment. Best Pract Res Clin Endocrinol Metab. 2018;32:343-34. [Crossref]

5. Chioma L, Bizzarri C, Verzani M, et al. Sedentary lifestyle and precocious puberty in girls during the COVID-19 pandemic: an Italian experience. Endocr Connect. 2022;11:e210650. [Crossref]

6. Stagi S, De Masi S, Bencini E, et al. Increased incidence of precocious and accelerated puberty in females during and after the Italian lockdown for the coronavirus 2019 (COVID-19) pandemic. Ital J Pediatr. 2020;46:165. [Crossref]

7. Acinikli KY, Erbaş İM, Besci Ö, Demir K, Abacı A, Böber E. Has the frequency of precocious puberty and rapidly progressive early puberty increased in girls during the COVID-19 pandemic? J Clin Res Pediatr Endocrinol. 2022;14:302-7. [Crossref]

8. Yesiltepe Mutlu G, Eviz E, Haliloglu B, et al. The effects of the Covid-19 pandemic on puberty: a cross-sectional, multicenter study from Turkey. Ital J Pediatr. 2022;48:144. [Crossref]

9. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child. 1969;44:291-303. [Crossref]

10. Greulich WW, Pyle SI. Radiographic atlas of skeletal development of hand and wrist. 2nd ed. Stanford: Stanford University Press; 1971.

11. Neyzi O, Bundak R, Gökçay G, et al. Reference values for weight, height, head circumference, and body mass index in Turkish children. J Clin Res Pediatr Endocrinol. 2015;7:280-93. [Crossref]

12. Carel JC, Léger J. Clinical practice. Precocious puberty. N Engl J Med. 2008;358:2366-77. [Crossref]

13. Verzani M, Bizzarri C, Chioma L, Bottaro G, Pedicelli S, Cappa M. Impact of COVID-19 pandemic lockdown on early onset of puberty: experience of an Italian tertiary center. Ital J Pediatr. 2021;47:52. [Crossref]

14. Street ME, Sartori C, Catellani C, Righi B. Precocious puberty and Covid-19 into perspective: potential increased frequency, possible causes, and a potential emergency to be addressed. Front Pediatr. 2021;9:734899. [Crossref]

15. Acar S, Özkan B. Increased frequency of idiopathic central precocious puberty in girls during the COVID-19 pandemic: preliminary results of a tertiary center study. J Pediatr Endocrinol Metab. 2021;35:249-51. [Crossref]

16. Gnocchi M, D'Alvano T, Lattanzi C, et al. Current evidence on the impact of the COVID-19 pandemic on paediatric endocrine conditions. Front Endocrinol (Lausanne). 2022;13:913334. [Crossref]

17. Trujillo MV, Rungvivatjarus T, Klein KO. Incidence of central precocious puberty more than doubled during COVID-19 pandemic: single-center retrospective review in the United States. Front Pediatr. 2022;10:1007730. [Crossref]

18. Kim JY, van de Wall E, Laplante M, et al. Obesity-associated improvements in metabolic profile through expansion of adipose tissue. J Clin Invest. 2007;117:2621-37. [Crossref]

19. Barberi C, Di Natale V, Assirelli V, Bernardini L, Candela E, Cassio A. Implicating factors in the increase in cases of central precocious puberty (CPP) during the COVID-19 pandemic: experience of a tertiary centre of pediatric endocrinology and review of the literature. Front Endocrinol (Lausanne). 2022;13:1032914. [Crossref]

20. Sangün Ö, Dündar B, Çömlekçi S, Büyükgebiz A. The effects of electromagnetic field on the endocrine system in children and adolescents. Pediatr Endocrinol Rev. 2015;13:531-45.

21. Waldhauser F, Waldhauser M, Lieberman HR, Deng MH, Lynch HJ, Wurtman RJ. Bioavailability of oral melatonin in humans. Neuroendocrinology. 1984;39:307-13. [Crossref]

22. Nagu P, Parashar A, Behl T, Mehta V. CNS implications of COVID-19: a comprehensive review. Rev Neurosci. 2020;32:219-34. [Crossref]

Trends in Pediatrics 2025;6(4):245-252
DOI: 10.59213/TP.2025.279

Original Article

Endocrinological evaluation of children and adolescents with hereditary spherocytosis: a cross-sectional retrospective multicenter study

Şeyma Türkmen[1], Sebla Güneş[2], Yusuf Ziya Aral[3], Yeşim Oymak[4], Sultan Okur Acar[4], Behzat Özkan[5], Ahmet Anık[2]

[1]Department of Pediatrics, Faculty of Medicine, Aydın Adnan Menderes University, Aydın, Türkiye
[2]Department of Pediatric Endocrinology, Faculty of Medicine, Aydın Adnan Menderes University, Aydın, Türkiye
[3]Department of Pediatric Hematology and Oncology, Faculty of Medicine, Aydın Adnan Menderes University, Aydın, Türkiye
[4]Department of Pediatric Hematology and Oncology, Dr. Behçet Uz Children's Hospital, İzmir, Türkiye
[5]Department of Pediatric Endocrinology, Dr. Behçet Uz Children's Hospital, İzmir, Türkiye

Cite this article as: Türkmen Ş, Güneş S, Aral YZ, et al. Endocrinological evaluation of children and adolescents with hereditary spherocytosis: a cross-sectional retrospective multicenter study. Trends in Pediatrics 2025;6(4):245-252.

ABSTRACT

Background: Hereditary spherocytosis (HS) is one of the most common causes of hereditary hemolytic anemia among erythrocyte membrane disorders. Endocrine complications are commonly seen in chronic anemias, such as β thalassemia major and sickle cell anemia; these include growth retardation, thyroid dysfunction, hypoparathyroidism, carbohydrate metabolism disorders, bone metabolism disorders, vitamin D3 deficiency/ insufficiency, delayed puberty, and adrenal insufficiency. However, studies on endocrine problems in HS are limited.

Methods: This study evaluated 40 children with HS. Data included clinical features, anthropometric parameters, pubertal stages, and laboratory evaluation for growth, thyroid, parathyroid, carbohydrate metabolism, bone metabolism, gonadal, and adrenal functions.

Results: The cohort comprised 24 females and 16 males, with a median age of 10.0 years. Two patients (5%) had severe HS requiring regular transfusions and chelation therapy. Short stature was observed in 5%, low body mass index in 10%, obesity in 5%, and subclinical hypothyroidism in 5% of patients. Vitamin D insufficiency or deficiency was noted in 55% of the cohort. Patients with vitamin D insufficiency or deficiency were significantly older than those with sufficient levels ($p = 0.043$). Impaired fasting glucose was found in 32.5%. Neither diabetes mellitus nor adrenal insufficiency was detected. Ferritin levels were elevated in 62.5% of patients, but showed no significant association with anthropometric parameters. None of the patients had signs of delayed puberty.

Conclusions: Endocrine complications, such as vitamin D insufficiency/ deficiency, short stature, and impaired fasting glucose, necessitate regular monitoring and early intervention in HS. Older age was associated with vitamin D insufficiency and deficiency in this population, underscoring the importance of age-specific surveillance. Further research with larger cohorts is required to validate these findings and optimize monitoring strategies for pediatric HS patients.

Keywords: hereditary spherocytosis, pediatric, endocrine, hemolytic anemia, complication

INTRODUCTION

Hereditary spherocytosis (HS) is the most common cause of hereditary hemolytic anemia among erythrocyte membrane disorders, with an incidence of approximately 1:2000. It typically follows an autosomal dominant inheritance, although recessive and de novo cases have also been reported.[1-3] The majority of HS cases are due

✉ Şeyma Türkmen ▪ drpieni@hotmail.com

Received: 29.04.2025 **Accepted:** 13.08.2025

to defects in erythrocyte membrane proteins such as spectrin or ankyrin, although defects in band three and protein 4.2 can also occur.[4] Clinical severity is variable, ranging from asymptomatic cases to severe hemolytic anemia, and common findings include anemia, jaundice, and splenomegaly.[5,6] Diagnosis is based on clinical and laboratory findings such as spherocytes in peripheral blood smear, elevated mean corpuscular hemoglobin concentration (MCHC), and a positive osmotic fragility (OF) test.[7] Quantitative examination of erythrocyte membrane proteins may be performed in atypical cases or when the diagnosis is uncertain.[8] Management of HS includes supportive care and, when necessary, splenectomy.[9] In addition to hematologic manifestations, chronic hemolysis in HS may result in complications such as bilirubin gallstones, aplastic crises, and, rarely, iron overload due to repeated transfusions or increased iron absorption.[9] Severe cases may present with hemosiderosis due to repeated transfusions, skeletal abnormalities from bone marrow expansion, growth retardation, and secondary endocrinopathies.[10] However, most patients compensate for hemolysis and remain asymptomatic, apart from fatigue and pallor.[11]

Endocrine complications are commonly seen in other chronic anemias like β-Thalassemia Major and Sickle Cell Anemia, which include growth retardation, thyroid dysfunction, hypoparathyroidism, carbohydrate metabolism disorders, bone metabolism disorders, vitamin D3 deficiency/insufficiency, delayed puberty, and adrenal insufficiency. However, studies on endocrine problems in HS are limited.[12,13]

Transfusion-related iron overload is a major concern in HS and other chronic anemias, affecting various organs, including the liver, heart, and endocrine system.[14] In addition to transfusional iron overload, non-transfusional iron overload due to hemochromatosis gene heterozygosity has also been documented in HS patients.[15,16] Lifelong chronic hemolysis and erythropoietic activity may increase iron absorption in patients with mild HS, suggesting that even mild HS is not entirely benign, and patients with mild HS should be monitored for iron overload. In addition, chronic hemolysis and ineffective erythropoiesis lead to increased tissue oxygen demand and chronic hypoxia, which, together with the persistent inflammatory state induced by ongoing hemolysis, may contribute to dysfunction of endocrine organs and dysregulation of the hypothalamic–pituitary axis.[10,11,16]

Although endocrine dysfunctions are expected to be relatively rare in HS patients, they can cause significant problems. To prevent these complications, regular monitoring of anemia severity, growth, and development is essential. Ferritin levels should be checked periodically, and iron chelation therapy should be initiated when necessary.[9]

We hypothesized that children and adolescents with HS may experience endocrine complications, and this study aimed to perform a comprehensive endocrine evaluation to better characterize their frequency and nature, highlighting the importance of early recognition and follow-up.

MATERIAL AND METHODS

This cross-sectional, retrospective study included 40 patients diagnosed with HS between 2004 and 2020 at the Departments of Pediatric Hematology in two tertiary referral centers: Aydın Adnan Menderes University Faculty of Medicine and Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital. The study was approved by the Non-Interventional Clinical Research Ethics Committee of Aydın Adnan Menderes University Faculty of Medicine (date: 21.03.2023, number: 2024-63)

HS diagnosis was based on a positive family history, MCHC > 35 g/dL, spherocytes in peripheral blood smears, reticulocytosis, elevated serum total and indirect bilirubin levels, a negative direct Coombs test, and a positive OF test. Other potential causes of hemolytic anemia, such as pyruvate kinase (PK) and glucose-6-phosphate dehydrogenase (G6PD) deficiencies, were excluded. Data collected from the hospital records included birth dates, age at diagnosis, presenting complaints, height, weight, body mass index (BMI), pubertal stages, number of erythrocyte transfusions, and chelation therapy. Puberty was defined by testicular volume ≥4 ml in boys and breast development at Tanner stage 2 in girls. Pubertal delay was defined as the absence of pubertal signs after age 14 in boys and 13 in girls.[17] Anthropometric parameters (height, weight, and BMI) were compared between prepubertal and pubertal patients to evaluate whether pubertal status could influence the presentation or detection of endocrine abnormalities. Laboratory tests performed after an eight-hour fast included glucose, ferritin, cortisol, parathormone (PTH), adrenocorticotropic hormone (ACTH), calcium (Ca), phosphorus (P), alkaline phosphatase (ALP), free thyroxine (fT4), thyroid-stimulating hormone (TSH), glycosylated hemoglobin (HbA1c), and insulin-like growth factor-1

(IGF-1). Pubertal patients were also tested for luteinizing hormone (LH), estradiol (E2)/testosterone (T), and follicle-stimulating hormone (FSH). Glucose, Ca, P, and ALP were measured using spectrophotometry, ferritin, cortisol, ACTH, PTH, fT4, TSH, IGF-1, FSH, LH, E2, and T were measured using Electrochemiluminescence Immunoassay (ECLIA), and 25-OH vitamin D3 and HbA1c were measured using High-Performance Liquid Chromatography (HPLC).

Definitions

Growth[18,19]

- Short stature: Height <-2 SDS for age and gender,
- Obesity: BMI >2 SDS for age and gender

Carbohydrate metabolism[20]

- Impaired fasting glucose: Fasting glucose 100-125 mg/dL,
- Diabetes mellitus: Fasting glucose ≥126 mg/dL, HbA1c ≥6.5%,

Bone metabolism[21]

- Vitamin D insufficiency: Serum 25-OH vitamin D3 12-20 ng/mL,
- Vitamin D deficiency: Serum 25-OH vitamin D3 <12 ng/mL,
- Asymptomatic elevated parathormone: PTH >65 pg/mL with normal Ca, P, and ALP levels

Thyroid[22]

- Overt hypothyroidism: Free T4 <0.8 ng/dL (Reference range for free T4: 0.8–1.48 ng/dL),
- Subclinical hypothyroidism: TSH> 5 mIU/mL with normal free T4,

Adrenal[23]

- Primary adrenal insufficiency: Basal cortisol <3 µg/dL and ACTH >100 pg/mL.

Statistical analysis

Statistical analyses were performed using IBM SPSS Version 21 (Statistical Package for the Social Sciences for Windows, Armonk, NY, IBM Corp.). The behavior of the quantitative variables was specified using measures of centralization and variance: Mean ± SD. Fisher's Exact (where sample size was low) and Chi-square test were used to determine differences in proportions or relationships between categorical variables. For a categorical variable, percentages and population proportions were compared using a One-Sample chi-square test. Non-parametric methods were used to compare group means when the assumptions of normality and homogeneity of variance were not met. Specifically, the Mann–Whitney U test was applied for comparisons between two independent groups (e.g., ferritin levels, vitamin D status, and impaired fasting glucose) with respect to anthropometric and laboratory parameters. For each case, the statistical significance was established at $p<0.05$.

RESULTS

Of the 40 patients included in the study, 24 (60%) were female and 16 (40%) were male. The median age of the patients was 10 years (1.47-17.66 years). Two patients (5%) had severe HS requiring regular blood transfusions, and one of these patients had undergone splenectomy. Both patients were receiving oral chelation therapy (deferasirox). Despite the splenectomy performed 13 years ago, the need for regular monthly transfusions continued. The average height, weight, and BMI SDS of the patients were -0.15 ± 0.67, -0.26 ± 0.4, and -0.34 ± 0.91, respectively. Seventeen patients (42.5%) were pubertal. Four patients (10%) had a BMI below -2 SDS, and two patients (5%) had short stature and obesity. Those with severe HS growth retardation were not observed in one, while the other, who had undergone splenectomy, later developed persistent short stature and required growth hormone treatment six years after the procedure, with subsequent improvement in height SDS. None of the patients showed signs of delayed puberty. There were no significant differences in height, weight, and BMI SDS between prepubertal and pubertal patients (p=1, p=0.565, and p=0.624, respectively) (Table 1).

Ferritin levels were elevated in 62.5% of the patients with a mean level of 129.88 ± 160.5 ng/mL. High ferritin levels were observed in one of two patients with short stature, two of three with low weight, and in three of four with low BMI. Ferritin levels were not significantly associated with short height, low weight, or low BMI (p=0.44, p=0.23, and p=0.31, respectively). No significant difference in ferritin levels was found between prepubertal and pubertal patients (p=0.652) (Table 1).

IGF-1 levels were normal for age in all patients. The average basal cortisol level was 9.10 ± 3.93 µg/dL, and the average ACTH level was 20.57 ± 10.1 pg/mL. No patient had a basal cortisol level <5 µg/dL. The average 25 (OH)

Table 1. Mean height, weight, and BMI SDS values of the patients

	All patients	Prepubertal	Pubertal	p*
n	40	23	17	
Height SDS	-0.15 ± 0.67	-0.04 ± 0	-0.31 ± 0.71	1
Weight SDS	-0.26 ± 0.4	-0.23 ± 0.62	-0.33 ± 0.4	0.565
BMI SDS	-0.34 ± 0.91	-0.38 ± 1.075	-0.21 ± 0.759	0.624
Ferritin (ng/mL)	129.88 ± 160.5	144.4 ± 198.59	110.24 ± 88.6	0.652

BMI, body mass index; SDS, standard deviation score.
* Comparison between prepubertal and pubertal patients.

vitamin D3 level was 19.91 ± 10.32 ng/mL. Eighteen (45%) patients had normal 25 (OH) vitamin D3 levels, 13 (32.5%) had insufficiency, and 9 (22.5%) had deficiency. All patients had normal Ca, P, and ALP values. Seven patients (17.5%) had elevated parathormone levels, and 42.9% of these had low 25 (OH) vitamin D3 levels. No patients had symptoms of rickets. Subclinical hypothyroidism was found in two patients (5.0%), but no cases with goiter or overt hypothyroidism were detected. Thirteen (32.5%) patients had impaired fasting glucose, whereas 27 (67.5%) patients had normal fasting blood glucose levels. None of the patients had hypoglycemia or diabetes mellitus (DM). The mean hemoglobin A1c was 4.2 ± 1.02%. In two (5%) patients, the HbA1c level was found to be <2%. The fasting blood glucose levels of these individuals were within normal ranges, and they did not belong to the transfusion-dependent severe HS patient group. No patient had an HbA1c level > 6.5% (Table 2).

No significant differences were found in subclinical hypothyroidism rates, fasting blood glucose levels, or 25(OH) vitamin D3 levels (insufficiency/deficiency) between prepubertal and pubertal patients (p=0.388, p=0.301, p=0.499, p=0.986, respectively) (Table 3).

In post hoc analyses, additional comparisons were performed to assess whether vitamin D3 insufficiency or deficiency and impaired fasting glucose were associated with clinical parameters. These two conditions were selected for subgroup analysis due to adequate sample sizes. Patients with vitamin D3 insufficiency or deficiency were older than those with sufficient vitamin D3 levels (121.5 vs. 68.5 months, p = 0.043). Hemoglobin levels at diagnosis did not differ significantly between these groups (8.10 vs. 9.65 g/dL, p = 0.34). Similarly, patients with impaired fasting glucose did not show significant differences in age (111 vs. 121 months, p = 0.644) or diagnostic hemoglobin levels (8.2 vs. 8.9 g/dL, p = 0.84) compared to those without impaired fasting glucose (Table 4).

Table 2. Laboratory characteristics of children with hereditary spherocytosis

	All patients, n=40	Prepubertal, n=23	Pubertal, n=17	p
Glucose (mg/dL)	91.9 ± 11.55	91.48 ± 13.85	92.47 ± 7.78	0.775
HbA1c (%)	4.2 ± 1.02	4.21 ± 1.05	4.19 ± 1.0	0.923
Calcium (mg/dL)	9.61 ± 0.39	9.63 ± 0.39	9.58 ± 0.4	0.697
Phosphorus (mg/dL)	4.9 ± 0.8	4.99 ± 0.9	4.79 ± 0.63	0.691
Alkaline phosphatase (U/L)	167.1 ± 50.16	169.87 ± 48.72	163.35 ± 53.33	0.69
Parathyroid hormone (pg/mL)	41.52 ± 25.57	37.49 ± 19.32	46.98 ± 32.02	0.292
25(OH) vitamin D3 (ng/mL)	19.91 ± 10.32	21.77 ± 9.71	17.39 ± 10.87	0.151
fT4 (ng/dL)	1.04 ± 0.1	1.06 ± 0.09	1.03 ± 0.12	0.428
TSH (mIU/mL)	2.14 ± 1.14	2.32 ± 1.27	1.9 ± 0.91	0.389
Cortisol (µg/dL)	9.10 ± 3.93	9.49 ± 4.19	8.16 ± 3.52	0.298
Adrenocorticotropic Hormone (ACTH) (pg/mL)	20.57 ± 10.1	21.24 ± 10.97	19.67 ± 9.03	0.805

mg/dL, milligrams per deciliter; HbA1c, glycated hemoglobin; U/L, units per liter; pg/mL, picograms per milliliter; 25(OH) vitamin D3, 25-hydroxyvitamin D3; ng/mL, nanograms per milliliter; fT4, free thyroxine; ng/dL, nanograms per deciliter; TSH, thyroid-stimulating hormone; mIU/mL, milli-international units per milliliter; µg/dL, micrograms per deciliter.

Table 3. Frequency of endocrinological problems in hereditary spherocytosis

	Patient (n)	%
Short stature	2	5
Low BMI	4	10
Obesity	2	5
Overt hypothyroidism	0	0
Subclinical hypothyroidism	2	5
Vitamin D insufficiency	13	32.5
Vitamin D deficiency	9	22.5
Asymptomatic elevated parathyroid hormone levels	7	17.5
Impaired fasting glucose	13	32.5
Diabetes mellitus	0	0
Adrenal insufficiency	0	0
Hypoparathyroidism	0	0

BMI: Body mass index

DISCUSSION

Despite being less frequent than other complications, endocrine problems in HS can lead to significant health issues. The strength of our study lies in being the first to conduct a comprehensive endocrinological evaluation of HS in childhood; however, the absence of a healthy control group limits our ability to determine the extent to which these findings differ from the general pediatric population. Another limitation of our study is the relatively small sample size, which may have reduced the statistical power of subgroup analyses. In addition, fructosamine levels, which may provide a more accurate assessment of glucose metabolism in hemolytic anemias, were not measured. This study focused on endocrinological problems, which revealed short stature (5%), low BMI (10%), obesity (5%), subclinical hypothyroidism (5%), vitamin D deficiency and insufficiency (55%), asymptomatic elevated parathyroid hormone levels (17.5%), and impaired fasting glucose (32.5%) (Table 3).

In our cohort, patients with vitamin D insufficiency or deficiency were significantly older than those with sufficient levels. This finding may suggest that vitamin D deficiency becomes more apparent over time due to the cumulative effects of chronic hemolysis, reduced sunlight exposure, or nutritional factors. Alternatively, older age itself may act as an independent risk factor, emphasizing the need for age-specific surveillance in pediatric patients with HS. However, no significant difference was observed in hemoglobin levels at diagnosis — a parameter commonly used to reflect disease severity in HS — between groups with and without vitamin D deficiency.

Similarly, patients with impaired fasting glucose did not show significant differences in age or diagnostic hemoglobin levels compared to those without impaired fasting glucose. These results suggest that the development of certain endocrine abnormalities in HS may not be directly related to disease severity or age alone. Further studies with larger patient populations are warranted to better elucidate the underlying risk factors and mechanisms contributing to endocrine dysfunction in this setting.

Research has not shown a significant gender difference for HS; in our study, girls were more common.[24,25] Severe cases of HS with life-threatening anemia requiring frequent transfusions constitute about 3–5% of cases.[11] Consistent with previous research, 5% of the patients in our study had severe HS requiring frequent blood transfusions.

Among the 40 individuals diagnosed with HS in our study, 5% were underweight, 5% were short, and 10% had a BMI <-2 SDS. None of these patients with growth retardation had undergone splenectomy. On the other hand, among severe patients, growth retardation was not observed in one, while the other, who had undergone splenectomy, later developed it. The patient in the severe HS group who had a splenectomy 13 years prior was started on growth hormone treatment seven years ago due to short stature. After a five-year follow-up, the height SDS value was -0.74 SDS. Growth and developmental delay have been reported

Table 4. Comparison of clinical parameters according to 25(OH)D3 status and fasting glucose levels

Characteristic	25(OH)D3 Insufficiency/ Deficiency (n=22)	Sufficient 25(OH) D3 (n=18)	p value	Impaired Fasting Glucose (n=13)	Normal Fasting Glucose (n=27)	p value
Median age (months, IQR)	121.5 (107–179)	68.5 (68–191)	0.043	111 (78–191)	121 (83–133)	0.644
Median hemoglobin at diagnosis (g/dL, IQR)	8.1 (6.5–10.1)	9.65 (7.2–10.6)	0.34	8.2 (7.2–10.6)	8.9 (6.8–10.4)	0.84

IQR, interquartile range; 25(OH)D3, 25-hydroxyvitamin D3; g/dL, grams per deciliter

in severe HS cases, but there is no clear information on its prevalence.[26] Chronic anemia and long-standing tissue hypoxia are the most significant underlying mechanisms. The relatively mild symptoms in HS patients compared to other chronic hemolytic anemias and the resulting delay in seeking healthcare services contribute to these mechanisms and exacerbate growth retardation.[27] Hemoglobin levels, in particular, increase significantly after splenectomy, positively affecting growth.[6] Therefore, suboptimal growth in children with HS is often considered a relative indication for splenectomy.[28] The growth retardation observed in our subjects did not involve those with severe HS, as the mild or moderate HS group likely sought medical attention later, undergoing a chronic anemic process before experiencing symptoms. Additionally, there were only two patients with severe HS. One of these two patients had a splenectomy, but required growth hormone treatment six years later due to short stature. Several previous investigations present differing findings: In a 2017 study by Das et al.[29] involving 82 HS patients, 32% of the children were underweight, and 26% were short. They reported that while splenectomy did not reverse growth retardation, anemia significantly improved over an average follow-up of 4.5 years post-splenectomy. Another study by Bader-Meunier et al.[30] reported that five prepubertal adolescents with HS showed a height increase of approximately two SDS along growth charts after surgery.

Pubertal delay may result from hemosiderosis due to repeated transfusions in individuals with severe HS who do not undergo splenectomy.[31] Of the patients in our study, 42.5% had reached puberty, and none of the patients experienced delayed puberty.

Extramedullary hematopoiesis (EMH), the abnormal formation of hematopoietic tissue outside of the bone marrow, has been reported in HS. Calhoun et al.[32] reported the first case of an EMH-related tumor in the right adrenal gland of a nine-year-old male with HS in 2001. Demir et al.[33] reported a 13-year-old girl with HS who had an adrenal gland ganglioneuroma in 2012. Although EMH is a rare cause of adrenal mass, it should be considered in patients with congenital hemolytic anemia such as HS.[32] We did not find any evidence of adrenal insufficiency in any of the patients in our study.

While numerous studies have examined bone metabolism in hemolytic anemias, few have focused on HS.[34] Schündeln et al.[35] conducted a study with 45 hemolytic anemia patients and found that the average 25-OH vitamin D level was 19.1 ± 5.7 (12.8–30.2) ng/mL in HS patient and 9.3 ± 7.4 (1-25.2) ng/mL in sickle cell anemia patients. The 25-OH vitamin D level was below 20 ng/mL in 86.7% of patients with sickle cell anemia and 61.5% of patients with HS. In a different study, the average 25-OH vitamin D level was significantly lower in children with HS than that of healthy controls (17.74 ± 7.76 ng/mL and 24.04 ± 11.70 ng/mL, respectively).[34] In our study, the average 25 (OH) vitamin D3 level was 19.91 ± 10.32 ng/mL, and 55% suboptimal vitamin D levels. These findings indicate the importance of monitoring the vitamin D status of patients with HS.

Excessive iron load can lead to thyroid dysfunction, particularly in the first decade of life, along with growth retardation. Most data on transfusional iron overload relates to thalassemia major. Studies indicate that thyroid dysfunction often presents as overt hypothyroidism without goiter and with negative autoantibodies.[36] Masuno et al. reported a case of primary hypothyroidism in HS in 1982.[37] In our study, overt hypothyroidism was not observed, but subclinical hypothyroidism was present in 5% of the patients. Although treatment indications for subclinical hypothyroidism are unclear, we believe HS patients should be regularly monitored for thyroid dysfunction.

Hypoparathyroidism, a late complication of iron overload, is frequently seen in the second decade of life. It results from the suppression of parathyroid secretion caused by bone resorption due to increased erythropoiesis secondary to chronic anemia or from iron accumulation in the parathyroid glands.[36] Secondary hyperparathyroidism can also occur due to low 25-OH vitamin D levels. In a study conducted by Schündeln et al.[35] in 2014, comparing 17 homozygous sickle cell anemia patients with 14 HS patients, they reported that approximately half of the patients with severe vitamin D deficiency developed secondary hyperparathyroidism. In our study, while none of the patients were found to have hypoparathyroidism, 17.5% had hyperparathyroidism. In parallel with the study by Schündeln et al.,[35] vitamin D deficiency was observed in 42.9% of the patients with hyperparathyroidism.

In hereditary hemolytic anemias, causing iron overload, impaired glucose tolerance, and diabetes mellitus are mostly seen in the second decade of life. Iron accumulation in the pancreas leads to defective microcirculation, resulting in impaired oxygen supply and, consequently, insulin deficiency. Most studies on this subject are related to thalassemia major.[36] In hemolytic anemias, metabolic control of glucose should be done through plasma or serum fructosamine levels. Hemolysis reduces overall glucose uptake and HbA1c, leading to falsely low values. Therefore,

HbA1c should be interpreted with caution in the presence of hemolysis.[38] In our study, 32.5% of the patients had impaired fasting glucose. No patient had diabetes mellitus.

CONCLUSION

We believe that the results of this study, which investigated endocrine complications in children diagnosed with hereditary spherocytosis and reported low vitamin D status, short stature, subclinical hypothyroidism, and impaired fasting glucose, need to be validated by studies conducted with larger patient populations and appropriate control groups. Notably, vitamin D insufficiency/deficiency was found to be associated with older age, which may suggest a potential age-related vulnerability in this group. Additionally, similar to other hemolytic anemias, HS patients should be closely monitored for endocrine problems.

Ethical approval

This study has been approved by the Aydın Adnan Menderes University Ethics Committee (approval date 21.03.2023, number 2024-63). Written informed consent was obtained from the participants.

Author contribution

The authors declare contribution to the paper as follows: Study conception and design: AA, YZA, ŞT; data collection: ŞT, YO, SOA, BÖ; analysis and interpretation of results: ŞT, AA, SG; draft manuscript preparation: ŞT, SG. 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. Farias MG. Advances in laboratory diagnosis of hereditary spherocytosis. Clin Chem Lab Med. 2017;55:944-8. [Crossref]
2. Iolascon A, Avvisati RA, Piscopo C. Hereditary spherocytosis. Transfus Clin Biol. 2010;17:138-42. [Crossref]
3. Ciepiela O. Old and new insights into the diagnosis of hereditary spherocytosis. Ann Transl Med. 2018;6:339. [Crossref]
4. Güngör A, Yaralı N, Fettah A, Ok-Bozkaya İ, Özbek N, Kara A. Hereditary spherocytosis: retrospective evaluation of 65 children. Turk J Pediatr. 2018;60:264-9. [Crossref]
5. Liao L, Deng ZF, Qiu YL, Chen P, Chen WQ, Lin FQ. Values of mean cell volume and mean sphered cell volume can differentiate hereditary spherocytosis and thalassemia. Hematology. 2014;19:393-6. [Crossref]
6. Bolton-Maggs PHB, Langer JC, Iolascon A, Tittensor P, King MJ, General haematology task force of the British committee for standards in haematology. Guidelines for the diagnosis and management of hereditary spherocytosis-2011 update. Br J Haematol. 2012;156:37-49. [Crossref]
7. Da Costa L, Galimand J, Fenneteau O, Mohandas N. Hereditary spherocytosis, elliptocytosis, and other red cell membrane disorders. Blood Rev. 2013;27:167-78. [Crossref]
8. Risinger M, Kalfa TA. Red cell membrane disorders: structure meets function. Blood. 2020;136:1250-61. [Crossref]
9. Gallagher PG. Abnormalities of the erythrocyte membrane. Pediatr Clin North Am. 2013;60:1349-62. [Crossref]
10. Ayhan AC, Yildiz I, Yüzbaşıoğlu S, et al. Erythrocyte membrane protein defects in hereditary spherocytosis patients in Turkish population. Hematology. 2012;17:232-6. [Crossref]
11. Kiliç MA, Özdemir GN, Tahtakesen TN, et al. Clinical features and outcome of children with hereditary spherocytosis. J Pediatr Hematol Oncol. 2022;44:e306-9. [Crossref]
12. Haliloglu B, Tuysuz G, Tayfun F. Endocrinopathies in Turkish children with thalassemia major. J Pediatr Res. 2017;4:216-9. [Crossref]
13. Mandese V, Bigi E, Bruzzi P, et al. Endocrine and metabolic complications in children and adolescents with Sickle Cell Disease: an Italian cohort study. BMC Pediatr. 2019;19:56. [Crossref]
14. Ozment CP, Turi JL. Iron overload following red blood cell transfusion and its impact on disease severity. Biochim Biophys Acta. 2009;1790:694-701. [Crossref]
15. Fargion S, Cappellini MD, Piperno A, Panajotopoulos N, Ronchi G, Fiorelli G. Association of hereditary spherocytosis and idiopathic hemochromatosis. A synergistic effect in determining iron overload. Am J Clin Pathol. 1986;86:645-9. [Crossref]
16. Tole S, Amid A, Baker J, Kuo K, Pugi J, Carcao M. Mild Hereditary spherocytosis without aHereditary haemochromatosis: an unrecognised cause of iron overload. Acta Haematol. 2019;141:256-60. [Crossref]
17. Gohil A, Eugster EA. Delayed and precocious puberty: genetic underpinnings and treatments. Endocrinol Metab Clin North Am. 2020;49:741-57. [Crossref]
18. Collett-Solberg PF, Ambler G, Backeljauw PF, et al. Diagnosis, genetics, and therapy of short stature in children: a growth hormone research society international perspective. Horm Res Paediatr. 2019;92:1-14. [Crossref]

19. Styne DM, Arslanian SA, Connor EL, et al. Pediatric obesity-assessment, treatment, and prevention: an endocrine society clinical practice guideline. J Clin Endocrinol Metab. 2017;102:709-57. [Crossref]

20. American Diabetes Association. 13. children and adolescents: standards of medical care in diabetes-2021. Diabetes Care. 2021;44:S180-99. [Crossref]

21. Munns CF, Shaw N, Kiely M, et al. Global consensus recommendations on prevention and management of nutritional rickets. J Clin Endocrinol Metab. 2016;101:394-415. [Crossref]

22. Hanley P, Lord K, Bauer AJ. Thyroid disorders in children and adolescents: a review. JAMA Pediatr. 2016;170:1008-19. [Crossref]

23. Kirkgoz T, Guran T. Primary adrenal insufficiency in children: diagnosis and management. Best Pract Res Clin Endocrinol Metab. 2018;32:397-424. [Crossref]

24. Inati A, Noun P, Kabbara N, et al. A multicenter study on the Lebanese experience with hereditary spherocytosis. Pediatr Blood Cancer. 2014;61:1895-6. [Crossref]

25. Oliveira MCLA, Fernandes RAF, Rodrigues CL, Ribeiro DA, Giovanardi MF, Viana MB. Clinical course of 63 children with hereditary spherocytosis: a retrospective study. Rev Bras Hematol Hemoter. 2012;34:9-13. [Crossref]

26. Gallagher PG, Glader B. Hereditary spherocytosis, hereditary elliptocytosis, and other disorders associated with abnormalities of the erythrocyte membrane. In: Greer JP, Arber DA, Rodgers GM, editors. Wintrobe's clinical hematology. 12th ed. Philadelphia, : Lippincott Williams & Wilkins; 2009:912-930.

27. Das A, Bansal D, Das R, Trehan A, Marwaha RK. Hereditary spherocytosis in children: profile and post-splenectomy outcome. Indian Pediatr. 2014;51:139-41. [Crossref]

28. Englum BR, Rothman J, Leonard S, et al. Hematologic outcomes after total splenectomy and partial splenectomy for congenital hemolytic anemia. J Pediatr Surg. 2016;51:122-7. [Crossref]

29. Das A, Bansal D, Trehan A, Das R. Growth failure in hereditary spherocytosis and the effect of splenectomy. Indian Pediatr. 2017;54:563-6. [Crossref]

30. Bader-Meunier B, Gauthier F, Archambaud F, et al. Long-term evaluation of the beneficial effect of subtotal splenectomy for management of hereditary spherocytosis. Blood. 2001;97:399-403. [Crossref]

31. Eber S, Lux SE. Hereditary spherocytosis-defects in proteins that connect the membrane skeleton to the lipid bilayer. Semin Hematol. 2004;41:118-41. [Crossref]

32. Calhoun SK, Murphy RC, Shariati N, Jacir N, Bergman K. Extramedullary hematopoiesis in a child with hereditary spherocytosis: an uncommon cause of an adrenal mass. Pediatr Radiol. 2001;31:879-81. [Crossref]

33. Demir HA, Ozdel S, Kaçar A, Senel E, Emir S, Tunç B. Ganglioneuroma in a child with hereditary spherocytosis. Turk J Pediatr. 2012;54:187-90.

34. Cesur M, Temiz F, Acıpayam C, Kılınc M, Seringec Akkececi N. Disordered bone metabolism in hereditary spherocytosis patients. Hematology. 2019;24:276-81. [Crossref]

35. Schündeln MM, Goretzki SC, Hauffa PK, et al. Impairment of bone health in pediatric patients with hemolytic anemia. PLoS One. 2014;9:e108400. [Crossref]

36. Wonke B, De Sanctis V. Clinical aspects of transfusional iron overload. Rev Clin Exp Hematol. 2000;4:322-36. [Crossref]

37. Masuno M, Origasa T, Takai T, et al. A case of primary hypothyroidism with hereditary spherocytosis. Nihon Naika Gakkai Zasshi. 1982;71:985-9.

38. Kutter D, Thoma J. Hereditary spherocytosis and other hemolytic anomalies distort diabetic control by glycated hemoglobin. Clin Lab. 2006;52:477-81.

Trends in Pediatrics 2025;6(4):253-259
DOI: 10.59213/TP.2025.289

Original Article

Comparative analysis of clonidine and L-DOPA stimulation tests in diagnosing growth hormone deficiency in children and adolescents

Fatih Kilci[1], Emre Sarıkaya[1]

[1]Department of Pediatric Endocrinology, Kocaeli City Hospital, Kocaeli, Türkiye

Cite this article as: Kilci F, Sarıkaya E. Comparative analysis of clonidine and L-DOPA stimulation tests in diagnosing growth hormone deficiency in children and adolescents. Trends in Pediatrics 2025;6(4):253-259.

ABSTRACT

Background: Growth hormone deficiency (GHD) is a significant cause of growth failure in children and is diagnosed through growth hormone (GH) stimulation tests. However, the sensitivity and specificity of these tests vary, leading to different protocols across centers. Clonidine and Levodopa (L-DOPA) are two commonly used GH-stimulating agents in pediatric endocrinology, yet data on their diagnostic performance and comparative effectiveness remain limited. This study aimed to evaluate the results of L-DOPA and clonidine stimulation tests in patients undergoing evaluation for suspected GHD at our clinic.

Methods: A retrospective analysis was conducted on patients who underwent both L-DOPA and clonidine stimulation tests between January 2020 and January 2025. Demographic, anthropometric, and biochemical parameters, including Insulin-like growth factor-1 (IGF-1) and Insulin-like growth factor-binding protein-3 (IGFBP-3) levels, were recorded. Tests were performed after an overnight fast, with oral administration of L-DOPA (10 mg/kg) and clonidine (150 mg/m²) at 08:00–09:00 AM, followed by GH measurements at 0, 30, 60, 90, and 120 minutes. A peak GH level <7 ng/mL in both tests was used to define GHD.

Results: A total of 133 patients (median age: 10 years, range: 1.3–16.1; 62.4% male) were included. There were 67 patients diagnosed with GHD and 66 patients without GHD. The median peak GH response was significantly higher with clonidine (6.9 ng/mL) than with L-DOPA (3.2 ng/mL) ($p<0.001$). In 95.5% of cases, the L-DOPA test yielded lower peak GH responses than the clonidine test. There were no significant differences between the GHD and non-GHD groups in terms of age, sex, height standard deviations (SD), body mass index (BMI) SD, or IGF-1 SD. However, the GHD group had a significantly higher proportion of pubertal cases, along with significantly lower IGFBP-3 SD levels and peak GH responses on both the L-DOPA and clonidine tests compared to the non-GHD group.

IGFBP-3 SD showed a weak positive correlation with peak GH responses in both the L-DOPA ($r=0.261$, $p=0.044$) and clonidine ($r=0.294$, $p=0.033$) tests in the GHD group. Additionally, in the GHD group, a weak negative correlation was observed between BMI SD and peak GH responses in the clonidine test ($r = -0.279$, $p = 0.032$), whereas no correlation was observed between BMI SD and peak GH responses in the L-DOPA test ($p = 0.358$).

Conclusions: Our findings indicate that clonidine stimulation results in significantly higher GH peaks compared to L-DOPA and demonstrates greater specificity. Using clonidine as the first-line stimulation test may reduce the number of unnecessary tests and associated costs in the diagnostic process. Furthermore, IGFBP-3 levels appeared to be more closely associated with GHD than IGF-1 levels, suggesting that IGFBP-3 could serve as an additional diagnostic marker. Larger-scale studies are warranted to validate these findings and optimize GHD screening strategies.

Keywords: body mass index, clonidine, growth hormone deficiency, insulin-like growth factor 1, insulin-like growth factor binding protein 3, levodopa

✉ Fatih Kilci ▪ fatihkilci@gmail.com

Received: 21.05.2025 **Accepted:** 19.10.2025

INTRODUCTION

Growth hormone deficiency (GHD) is a significant cause of short stature and impaired growth in children.[1] The diagnosis of GHD relies on growth hormone (GH) stimulation tests, which assess the ability of the pituitary gland to secrete GH in response to pharmacological stimuli.[2] However, the sensitivity and specificity of these tests vary depending on the agents used, the test protocols, and the diagnostic thresholds applied.[3,4] Due to these variations, there is ongoing debate regarding the most reliable and accurate GH stimulation test for diagnosing GHD in pediatric patients.

Among the various GH stimulation agents, clonidine and Levodopa (L-DOPA) are two of the most commonly used in pediatric endocrinology.[5,6] Clonidine, an α2-adrenergic agonist, stimulates GH release by reducing hypothalamic somatostatin tone, whereas L-DOPA, a dopamine precursor, exerts its effect by increasing dopaminergic stimulation of GH secretion.[7] Despite their widespread use, the diagnostic efficacy and relative superiority of these agents remain uncertain. Previous studies have reported conflicting findings regarding GH peak responses to clonidine and L-DOPA, and the optimal cut-off values for GHD diagnosis remain a topic of debate.[8-10]

Given the variability in GH responses and the limited data comparing the diagnostic utility of these two agents, further investigation is warranted to determine their effectiveness in clinical practice. Understanding the performance of these tests in different populations and settings is essential for refining diagnostic algorithms and improving the accuracy of GHD diagnosis.

In this study, we aimed to evaluate the results of GH stimulation tests with clonidine and L-DOPA in pediatric patients with suspected GHD evaluated at our clinic. By analyzing the GH peak responses to these agents, we sought to compare their diagnostic efficacy and assess their clinical utility in distinguishing children with GHD from those with normal GH secretion.

METHODS

Study design and setting

This retrospective study reviewed patients' medical records who underwent both L-DOPA and clonidine GH stimulation tests for suspected GHD at our clinic between January 2020 and January 2025. Patients with systemic or endocrine disorders affecting GH secretion, or with syndromic short stature, were excluded. Growth hormone stimulation tests were performed in children with a height below −2 standard deviations (SD) for chronological age and/or a subnormal height velocity (below the 25th percentile). In addition, other clinical indications such as delayed bone age, height more than 1.5 SD below the mid-parental target height, physical features suggestive of growth hormone deficiency, and low serum insulin-like growth factor-1 (IGF-1) and insulin-like growth factor-binding protein-3 (IGFBP-3) levels were also considered in accordance with current clinical guidelines.[11] The study protocol received approval from the local institutional review board (approval number: 2024/127).

Data collection and diagnostic procedures

Anthropometric data, including height, weight, and body mass index (BMI), were collected at the time of testing by a single experienced nurse. Measurements were taken without shoes and in light clothing, using a device with an accuracy of ±0.1 cm for height and ±0.05 kg for weight. The anthropometric measurements of the patients were evaluated according to the national auxological references.[12] Participants' pubertal stages were determined according to the Tanner classification system.[13] For analytical purposes, patients were classified as prepubertal if at Tanner stage 1 and as pubertal if at Tanner stage 2 or above, in line with the physiological onset of puberty.

All tests were conducted in the morning between 08:00 and 09:00 following an overnight fast of at least eight hours, while patients were in a euthyroid and eucortisolemic state. An intravenous catheter was placed before the test initiation. As per departmental protocol, the L-DOPA test was conducted first, with a second test using clonidine for those who did not pass the L-DOPA test, and sex-steroid priming was not performed in peripubertal children.

For the L-DOPA stimulation test, L-DOPA was administered orally at a dose of 10 mg/kg, with a maximum dose not exceeding 500 mg. For the clonidine stimulation test, clonidine was administered orally at a dose of 150 mcg/m² of body surface area, with a maximum dose not exceeding 250 mcg.

Blood samples for GH measurement were collected at baseline and at 30, 60, 90, and 120 minutes for both tests. The highest GH value obtained during either test was recorded as the peak GH level. A peak GH concentration below 7 ng/mL in both tests was considered diagnostic of GHD.[14] Those with a peak GH response below 7 ng/mL in both tests formed the GHD group, while those with a peak

GH response greater than or equal 7 ng/mL in the second test formed the non-GHD group.

Serum IGF-1 and IGFBP-3 levels were also measured at baseline, and their SD's were calculated according to national standards based on chronological age and sex.[15] GH levels were measured using the electrochemiluminescence immunoassay method with the Roche Diagnostics Cobas® e801 system (Roche Diagnostics, Mannheim, Germany). Serum IGF-1 and IGFBP-3 concentrations were determined using the chemiluminescence immunoassay technique with the Maglumi SNIBE X3® analyzer (Snibe Diagnostics, Shenzhen, China).

Statistical analysis

Statistical analysis was performed using SPSS, version 23.0 (IBM Inc., Armonk, NY, USA). Categorical variables were presented as frequencies and percentages, while normality was assessed using the Kolmogorov-Smirnov test. Depending on the data distribution, parametric data were described as mean ± standard deviation, and nonparametric data were presented as median and range (minimum-maximum). The chi-square test or Fisher's exact test was used to compare the differences of categorical variables presented as counts (percentages). The Student's t-test was used to compare parametric variables between the two groups, while the Mann-Whitney U test was applied for nonparametric data. Correlation analyses between GH peak responses (to L-DOPA and clonidine separately) and auxological/laboratory parameters were performed using the Spearman correlation test, as the data did not show normal distribution. A value of $p < 0.05$ was considered statistically significant.

RESULTS

A total of 133 patients, with a median age of 10 years (range: 1.3–16.1), were included in the study, of which 62.4% were male. There were 67 patients diagnosed with GHD and 66 patients without GHD. Forty-five patients (33.8%) were in the pubertal stage. The median peak GH response to the clonidine test for all cases was 6.9 ng/mL (range: 0.3–18), which was significantly higher than the median peak GH response to the L-DOPA test, with 3.2 ng/mL (range: 0.1–6.7) ($p<0.001$). Except for six patients, all others (95.5%) exhibited lower peak GH responses on the L-DOPA test compared to the clonidine test.

There were no significant differences between the two groups in bone age, chronological age, gender, height SD, BMI-SD, or IGF-1 SD. However, the GHD group had a significantly higher proportion of pubertal cases, along with significantly lower IGFBP-3 SD levels and peak GH responses on both the L-DOPA and clonidine tests compared to the non-GHD group (Table 1).

When analyzed by pubertal status, no significant difference in L-DOPA-stimulated peak GH responses was observed between prepubertal and pubertal patients within the GHD

Table 1. Clinical and Laboratory Characteristics of the Study Population

	Total (n=133)	GHD Group (n=67)	Non-GHD Group (n=66)	P-Value
CA, years, median (range)	10 (1.3 to 16.1)	9.95 (1.3 to 15)	8.85 (1.6 to 16.1)	0.117
BA, years median (range)	7.8 (0.5 to 14)	7.8 (0.5 to 13)	6.75 (0.75 to 14)	0.272
Male, n (%)	83 (62.4)	45 (67.2)	38 (57.6)	0.286
Weight SD, median (range)	-1.77 (-4.6 to 0.75)	-1,7 (-4.6 to 0.75)	-1,8 (-3,05 to 0,52)	0.333
Height SD, median (range)	-2.47 (-4.7 to -1.45)	-2.54 (-4.7 to -2)	-2.44 (-4 to -1.45)	0.204
BMI SD, median (range)	-0.4 (-2.5 to 1.6)	-0.39 (-2.5 to 1.6)	-0.42 (-2.24 to 1.3)	0.208
IGF1 SD, median (range)	-1.69 (-4.29 to 0.45)	-2.09 (-3.7 to -0.23)	-1.88 (-4.29 to 0.45)	0.164
IGFBP3 SD, median (range)	-0.89 (-3.84 to 1.7)	-1.25 (-3.84 to 1.3)	-0.62 (-2.34 to 1.7)	0.008
Proportion of Pubertal Patients (%)	33.8	43.3	24.2	0.028
Peak GH Response to L-DOPA (ng/mL), median (range)	3.2 (0.1 to 6.7)	2.55 (0.17 to 5.9)	4.34 (0.17 to 6.7)	<0.001
Peak GH Response to Clonidine (ng/mL), median (range)	6.9 (0.3 to 18)	4.2 (0.39 to 6.9)	11.1 (7.24 to 18)	<0.001

CA: Chronological age, BA: Bone age, BMI: Body mass index, IGF1: Insulin-like growth factor-1, IGFBP3: Insulin-like growth factor-binding protein-3, GH: Growth hormone, GHD: Growth hormone deficiency, SD: Standard deviation.

group. However, a statistically significant difference was observed in clonidine-stimulated peak GH responses, with higher responses in prepubertal patients than in pubertal patients (Table 2). In contrast, in the non-GHD group, no significant differences were observed between prepubertal and pubertal participants in either the L-DOPA or the clonidine stimulation tests (Table 3).

In the GHD group, no significant correlations were observed between GH peak responses (to both L-DOPA and clonidine) and age, weight, height, or IGF-1 SD. However, a weak positive correlation was found between IGFBP-3 SD and GH peak responses to the L-DOPA test (r = 0.261, p = 0.044). Similarly, a weak positive correlation was also identified between IGFBP-3 SD and GH peak responses to the clonidine test (r=0.294, p=0.033). Additionally, a weak negative correlation was observed between BMI SD and GH peak responses to the clonidine test (r = –0.279, p = 0.032) in the GHD group. These findings are summarized in Table 4.

DISCUSSION

In patients with suspected GHD, random serum GH measurements are not useful for diagnosis. Therefore, two different GH stimulation tests are required, and both tests must yield abnormal results to confirm GHD.[11,16] In the diagnosis of GHD in children, various stimulation agents such as the insulin tolerance test (ITT), clonidine, glucagon, L-DOPA, arginine, and GH-releasing peptide-2 are used.[5] Although ITT is considered the gold standard, its use in pediatric GHD diagnosis is limited due to the need for close medical supervision, poor reproducibility, and the unpleasant symptoms associated with hypoglycemia. [7] Additionally, the low specificity and sensitivity of traditional GH stimulation tests significantly reduce their diagnostic reliability, as no single test is sufficiently sensitive and specific to confirm the diagnosis.[3,17] Moreover, these tests have poor reproducibility, and their results can be influenced by factors such as age, sex, puberty, nutritional status, and body weight.[6]

In this study, we demonstrated that the clonidine test elicited significantly higher peak GH responses compared to the L-DOPA test in children and adolescents with suspected GHD. Notably, while the majority of patients showed this pattern, a small subset exhibited discordant responses between the two tests, emphasizing the variability inherent in GH stimulation testing and the need for careful interpretation of results in clinical practice. In addition, we observed a negative correlation between BMI-SD and GH peak levels in the clonidine test.

Some studies suggest that L-DOPA is a more sensitive test for stimulating GH secretion, particularly in pediatric populations.[8] In contrast, others have shown that this test has lower sensitivity than the clonidine test but similar specificity and has been shown to increase accuracy when combined with arginine.[18-20] The findings of the present investigation, in which all subjects first underwent the L-DOPA stimulation test, and those who failed to meet the 7 ng/mL GH threshold were subsequently evaluated using the clonidine test, suggest that clonidine is a more robust and efficacious GH secretagogue than L-DOPA. Based on

Table 2. Comparison of Peak GH Responses to L-DOPA and Clonidine Tests According to Pubertal Status in the GHD Group

Stimulation Test	Pubertal Status (n)	Peak GH (ng/mL), median (range)	P-Value
L-DOPA	Pubertal (29)	2.8 (0.1 to 5.9)	0.728
	Prepubertal (38)	2.4 (0.18 to 5.1)	
Clonidine	Pubertal (29)	3.7 (0.4 to 6.9)	0.035
	Prepubertal (38)	5.54 (0.3 to 6.9)	

GH: Growth hormone.

Table 3. Comparison of Peak GH Responses to L-DOPA and Clonidine Tests According to Pubertal Status in the Non-GHD Group

Stimulation Test	Pubertal Status (n)	Peak GH (ng/mL), median (range)	P-Value
L-DOPA	Pubertal (16)	3.45 (0.17 to 6.4)	0.151
	Prepubertal (50)	4.45 (1.32 to 6.76)	
Clonidine	Pubertal (16)	10.2 (7.6 to 18)	0.976
	Prepubertal (50)	11.1 (7.2 to 18)	

GH: Growth hormone.

Table 4. Correlation of Clinical and Biochemical Parameters with Peak GH Responses in L-DOPA and Clonidine Stimulation Tests

Variable	L-DOPA– GHD Group	L-DOPA – Non-GHD Group	Clonidine– GHD Group	Clonidine– Non-GHD Group
Age (years)				
r	0.034	-0.108	-0.209	-0.024
p	0.783	0.387	0.089	0.109
Height-SD				
r	0.056	0.031	0.145	0.190
p	0.650	0.804	0.240	0.127
Weight-SD				
r	-0.0069	-0.0021	-0.104	-0.053
p	0.578	0.976	0.402	0.672
BMI-SD				
r	-0.114	-0.005	-0.279	-0.107
p	0.358	0.978	0.032	0.391
IGF-1 SD				
r	0.043	0.020	0.025	0.031
p	0.733	0.875	0.844	0.804
IGFBP-3 SD				
r	0.261	0.070	0.294	0.047
p	0.044	0.595	0.033	0.720

BMI: Body mass index, IGF1: Insulin-like growth factor-1, IGFBP3: Insulin-like growth factor-binding protein-3, GHD: Growth hormone deficiency, r: Spearman Rho correlation coefficient, SD: Standard deviation.

our findings, we recommend using the clonidine test as the primary diagnostic approach for GHD, as this strategy could reduce unnecessary tests and improve diagnostic efficiency. Additionally, our study suggests that adopting lower cut-off values for diagnosing GHD on the L-DOPA test may be warranted, which could further refine diagnostic criteria and facilitate earlier detection of the condition.

It is well known that malnutrition and chronic undernutrition can blunt GH responses during stimulation tests, despite normal or even elevated endogenous GH secretion.[6] In our study, although some patients had low weight and BMI SD values, these parameters did not differ significantly between the GHD and non-GHD groups. Therefore, we believe the comparison of GH responses between the groups remained valid. Nevertheless, the potential influence of nutritional status should be kept in mind, particularly when interpreting borderline test results.

Body mass index significantly affects GH secretion, leading to reduced spontaneous and stimulated GH release in obese individuals. Furthermore, the GH response to various stimuli, including clonidine, shows a negative correlation with BMI.[21-23] In line with the literature, our study revealed a weak negative correlation between BMI SD and GH peak responses to the clonidine stimulation test in patients diagnosed with GHD. However, no such correlation was found with the L-DOPA stimulation test. This discrepancy may be due to the differing mechanisms of action of the two agents and the generally lower GH peaks elicited by L-DOPA, which could limit the detection of BMI-related effects. Therefore, the clonidine test appears to be a more reliable and superior diagnostic tool than L-DOPA when evaluating GH response in relation to BMI.

During puberty, sex steroids influence the GH/IGF-I axis, resulting in enhanced GH secretion and responsiveness to diverse stimuli, including the administration of testosterone or estrogen.[24,25] However, there is no consensus regarding the role of sex steroids in priming for GH stimulation tests. Some studies suggest that prepubertal children may fail GH stimulation tests due to the influence of sex steroids[26,27], while other studies have found that puberty itself does not significantly impact the GH response to stimuli.[17,28] A recent study by Ibba et al.[9] evaluating the reliability of clonidine in diagnosing GHD in children and adolescents, as well as the effect of puberty on the GH response to clonidine, suggested that clonidine is effective and reliable in both prepubertal and pubertal children, without the need for sex steroid priming. Interestingly, in our study, GH responses to the

clonidine test were lower in pubertal than in prepubertal cases within the GHD group. This finding suggests that, despite hormonal fluctuations during puberty, clonidine remains a reliable diagnostic tool for GHD across different pubertal stages, reinforcing the argument that sex steroid priming may not be necessary for the accurate assessment of GH response in this population.

Serum IGF-1 and IGFBP-3 levels indicate endogenous GH secretion in healthy children, making them potential screening tools for GHD.[29] However, the diagnostic utility of IGF-1 and IGFBP-3 in distinguishing GHD from non-GHD remains a matter of debate. Previous studies have reported conflicting results regarding their predictive value. For instance, Jensen et al. found that the sensitivity of IGF-1 and IGFBP-3 for diagnosing GHD was 90% and 81%, respectively.[30] In contrast, Boquete et al.[31] reported that IGF-1 was superior to IGFBP-3 in identifying GHD. On the other hand, Iwayama et al. demonstrated that IGF-1 had poor diagnostic accuracy as a screening test for GHD and concluded that IGF-1 alone should not be used for screening purposes.[32] In our cohort, IGF-1 SD did not differ significantly between GHD and non-GHD groups, whereas IGFBP-3 SD was significantly lower in the GHD group, consistent with its closer association with GH status. These findings suggest that IGFBP-3 may provide additional insight into GH deficiency, but, due to modest differences, it cannot reliably serve as a standalone diagnostic marker, underscoring the continued necessity of GH stimulation testing for accurate diagnosis.

Limitations

Our study has several limitations. First, its retrospective design may introduce selection bias and limit the generalizability of our findings. Second, the diagnosis of GHD was based on GH stimulation tests, which are known to have variability in reproducibility and specificity. Third, the lack of ITT data, which is considered the gold standard for GHD diagnosis, may limit the diagnostic accuracy of our findings. Additionally, we did not assess other potential biomarkers or dynamic testing methods that could further refine GHD diagnosis. Future prospective studies with larger cohorts and alternative biomarkers are needed to validate our findings.

Conclusion

The current study confirms that clonidine induces a higher GH response than L-DOPA and that BMI SD negatively correlates with GH levels during the clonidine test. Additionally, IGFBP-3 SD was significantly lower in the GHD group, indicating an association with GH deficiency, whereas IGF-1 SD did not differ between groups. Given its superior GH response, using clonidine as the initial GH stimulation test may reduce the number of unnecessary tests and lower diagnostic costs. These findings underscore the need for novel biomarkers to enhance the accuracy of GHD diagnosis and screening.

Ethical approval

This study has been approved by the Kocaeli City Hospital Ethics Committee (approval date 31.10.2024, number 2024-127). Written informed consent was obtained from the participants.

Author contribution

The authors declare contribution to the paper as follows: Study conception and design: FK, ES; data collection: FK, ES; analysis and interpretation of results: FK; draft manuscript preparation: FK. 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. Deodati A, Cianfarani S. The rationale for growth hormone therapy in children with short stature. J Clin Res Pediatr Endocrinol. 2017;9:23-32. [Crossref]
2. Richmond EJ, Rogol AD. Growth hormone deficiency in children. Pituitary. 2008;11:115-20. [Crossref]
3. Binder G, Reinehr T, Ibáñez L, et al. GHD diagnostics in Europe and the US: an audit of national guidelines and practice. Horm Res Paediatr. 2019;92:150-6. [Crossref]
4. Stanley T. Diagnosis of growth hormone deficiency in childhood. Curr Opin Endocrinol Diabetes Obes. 2012;19:47-52. [Crossref]
5. Kamoun C, Hawkes CP, Grimberg A. Provocative growth hormone testing in children: how did we get here and where do we go now? J Pediatr Endocrinol Metab. 2021;34:679-96. [Crossref]
6. Yau M, Rapaport R. Growth hormone stimulation testing: to test or not to test? that is one of the questions. Front Endocrinol (Lausanne). 2022;13:902364. [Crossref]

7. Yuen KCJ, Johannsson G, Ho KKY, Miller BS, Bergada I, Rogol AD. Diagnosis and testing for growth hormone deficiency across the ages: a global view of the accuracy, caveats, and cut-offs for diagnosis. Endocr Connect. 2023;12:e220504. [Crossref]
8. Petriczko E, Szmit-Domagalska J, Horodnicka-Józwa A, Walczak M. Prognostic value of the L-Dopa and clonidine GH stimulation tests in patients with endogenous growth hormone deficiency - retrospective analysis after discontinuation of treatment - own experience. Pediatr Endocrinol Diabetes Metab. 2009;15:13-9.
9. Ibba A, Guzzetti C, Casula L, et al. Reliability of clonidine testing for the diagnosis of growth hormone deficiency in children and adolescents. Clin Endocrinol (Oxf). 2018;89:765-70. [Crossref]
10. Castelli B, De Santis R, Carrera S, Malanima MA, De Masi S, Stagi S. L-DOPA test in the diagnosis of childhood short stature: evaluation of growth hormone peaks over time. Endocrinol Diabetes Metab. 2024;7:e70000. [Crossref]
11. Growth Hormone Research Society. Consensus guidelines for the diagnosis and treatment of growth hormone (GH) deficiency in childhood and adolescence: summary statement of the GH Research Society. GH Research Society. J Clin Endocrinol Metab. 2000;85:3990-3. [Crossref]
12. Neyzi O, Bundak R, Gökçay G, et al. Reference values for weight, height, head circumference, and body mass index in Turkish children. J Clin Res Pediatr Endocrinol. 2015;7:280-93. [Crossref]
13. Marshall WA, Tanner JM. Variations in pattern of pubertal changes in girls. Arch Dis Child. 1969;44:291-303. [Crossref]
14. Collett-Solberg PF, Ambler G, Backeljauw PF, et al. Diagnosis, genetics, and therapy of short stature in children: a growth hormone research society international perspective. Horm Res Paediatr. 2019;92:1-14. [Crossref]
15. Guven B, Can M, Mungan G, Acikgoz S. Reference values for serum levels of insulin-like growth factor 1 (IGF-1) and IGF-binding protein 3 (IGFBP-3) in the West Black Sea region of Turkey. Scand J Clin Lab Invest. 2013;73:135-40. [Crossref]
16. Poyrazoğlu Ş, Akçay T, Arslanoğlu İ, et al. Current practice in diagnosis and treatment of growth hormone deficiency in childhood: a survey from Turkey. J Clin Res Pediatr Endocrinol. 2015;7:37-44. [Crossref]
17. Ghigo E, Bellone J, Aimaretti G, et al. Reliability of provocative tests to assess growth hormone secretory status. Study in 472 normally growing children. J Clin Endocrinol Metab. 1996;81:3323-7. [Crossref]
18. Guzzetti C, Ibba A, Pilia S, et al. Cut-off limits of the peak GH response to stimulation tests for the diagnosis of GH deficiency in children and adolescents: study in patients with organic GHD. Eur J Endocrinol. 2016;175:41-7. [Crossref]
19. Weldon VV, Gupta SK, Klingensmith G, et al. Evaluation of growth hormone release in children using arginine and L-dopa in combination. J Pediatr. 1975;87:540-4. [Crossref]
20. Sizonenko PC, Clayton PE, Cohen P, Hintz RL, Tanaka T, Laron Z. Diagnosis and management of growth hormone deficiency in childhood and adolescence. Part 1: diagnosis of growth hormone deficiency. Growth Horm IGF Res. 2001;11:137-65. [Crossref]
21. Lee J, Yoon J, Kang MJ, et al. Influence of body mass index on the growth hormone response to provocative testing in short children without growth hormone deficiency. J Korean Med Sci. 2013;28:1351-5. [Crossref]
22. Loche S, Guzzetti C, Pilia S, et al. Effect of body mass index on the growth hormone response to clonidine stimulation testing in children with short stature. Clin Endocrinol (Oxf). 2011;74:726-31. [Crossref]
23. Stanley TL, Levitsky LL, Grinspoon SK, Misra M. Effect of body mass index on peak growth hormone response to provocative testing in children with short stature. J Clin Endocrinol Metab. 2009;94:4875-81. [Crossref]
24. Mauras N, Rogol AD, Haymond MW, Veldhuis JD. Sex steroids, growth hormone, insulin-like growth factor-1: neuroendocrine and metabolic regulation in puberty. Horm Res. 1996;45:74-80. [Crossref]
25. Keenan BS, Richards GE, Ponder SW, Dallas JS, Nagamani M, Smith ER. Androgen-stimulated pubertal growth: the effects of testosterone and dihydrotestosterone on growth hormone and insulin-like growth factor-I in the treatment of short stature and delayed puberty. J Clin Endocrinol Metab. 1993;76:996-1001. [Crossref]
26. Marin G, Domené HM, Barnes KM, Blackwell BJ, Cassorla FG, Cutler GB. The effects of estrogen priming and puberty on the growth hormone response to standardized treadmill exercise and arginine-insulin in normal girls and boys. J Clin Endocrinol Metab. 1994;79:537-41. [Crossref]
27. Martínez AS, Domené HM, Ropelato MG, et al. Estrogen priming effect on growth hormone (GH) provocative test: a useful tool for the diagnosis of GH deficiency. J Clin Endocrinol Metab. 2000;85:4168-72. [Crossref]
28. Cavallo L, Acquafredda A, Liuzzi S, et al. Growth hormone release during insulin tolerance, clonidine, arginine and growth hormone releasing hormone tests in short normal children and adolescents. J Endocrinol Invest. 1992;15:131-5. [Crossref]
29. Juul A, Skakkebaek NE. Prediction of the outcome of growth hormone provocative testing in short children by measurement of serum levels of insulin-like growth factor I and insulin-like growth factor binding protein 3. J Pediatr. 1997;130:197-204. [Crossref]
30. Jensen RB, Jeppesen KA, Vielwerth S, et al. Insulin-like growth factor I (IGF-I) and IGF-binding protein 3 as diagnostic markers of growth hormone deficiency in infancy. Horm Res. 2005;63:15-21. [Crossref]
31. Boquete HR, Sobrado PGV, Fideleff HL, et al. Evaluation of diagnostic accuracy of insulin-like growth factor (IGF)-I and IGF-binding protein-3 in growth hormone-deficient children and adults using ROC plot analysis. J Clin Endocrinol Metab. 2003;88:4702-8. [Crossref]
32. Iwayama H, Kitagawa S, Sada J, et al. Insulin-like growth factor-1 level is a poor diagnostic indicator of growth hormone deficiency. Sci Rep. 2021;11:16159. [Crossref]

Trends in Pediatrics 2025;6(4):260-267
DOI: 10.59213/TP.2025.304

Original Article

The impact of colchicine therapy and disease characteristics on vitamin B12 status in pediatric patients with familial Mediterranean fever

Sıla Atamyıldız Uçar[1], Mahmut Seyfeddin Öz[2], Eray Tunce[1], Betül Sözeri[1]

[1]Department of Pediatric Rheumatology, Ümraniye Training and Research Hospital, University of Health Sciences, İstanbul, Türkiye
[2]Department of Pediatrics, Ümraniye Training and Research Hospital, University of Health Sciences, İstanbul, Türkiye

Cite this article as: Atamyıldız Uçar S, Öz MS, Tunce E, Sözeri B. The impact of colchicine therapy and disease characteristics on vitamin B12 status in pediatric patients with familial mediterranean fever. Trends in Pediatrics 2025;6(4):260-267.

ABSTRACT

Objective: To determine whether prolonged colchicine therapy and higher colchicine doses are independently associated with vitamin B12 deficiency in pediatric familial Mediterranean fever (FMF) patients, after adjusting for age as a confounding factor.

Methods: This retrospective study included pediatric FMF patients with biallelic exon 10 *MEFV* mutations, followed between August 2016 and September 2024. Patients receiving colchicine treatment for at least 12 months and with available serum vitamin B12 measurements were included. Vitamin B12 levels were categorized as deficient (≤200 pg/mL) or normal (>200 pg/mL). Demographic data, colchicine treatment duration and dosage, clinical features, and Pras disease severity scores were recorded.

Results: Among 339 patients, 193 (56.9%) were female. The median age at FMF diagnosis was 5 years (interquartile range [IQR], 3-9), and at vitamin B12 testing was 13 years (IQR, 9-16). The median of vitamin B12 levels was 317 pg/mL (236.7-447), and 12.1% of patients had vitamin B12 deficiency. Patients with vitamin B12 deficiency had significantly longer colchicine duration (72 months [48-144] vs. 60 months [24-96], p=0.034) and higher daily colchicine doses (1.33±0.4 vs. 1.12±0.4, p=0.004) compared to those with normal vitamin B12 levels. Patients with a colchicine duration of more than 96 months had the lowest vitamin B12 status (p=0.008) and the highest frequency of vitamin B12 deficiency (p=0.014). Receiver operating characteristic (ROC) system analysis identified an age threshold of 12.2 years as predictive for vitamin B12 deficiency (area under the curve=0.708; sensitivity=85.4%; specificity=53.4%). At the time of vitamin B12 measurement, 28 (8.3%) were colchicine-resistant FMF patients, and vitamin B12 deficiency was significantly more common in colchicine-resistant FMF patients compared to colchicine-responsive FMF patients (n=21, 7%) (p=0.03). No significant association was observed between *MEFV* mutation subtypes (p=0.35), nor between PRAS severity categories (p=0.71) with vitamin B12 status.

Conclusion: Vitamin B12 deficiency appears to be associated with age in pediatric FMF patients. Although prolonged colchicine treatment and higher daily doses were associated with lower B12 levels, these associations were not independent of age. Routine monitoring may be considered in adolescents and with long-standing disease. Further prospective studies are needed to clarify the long-term impact of colchicine on vitamin B12 levels.

Keywords: familial Mediterranean fever, vitamin B12, colchicine, pediatrics, *MEFV* gene

✉ Betül Sözeri ▪ drbetulsozeri@gmail.com

Received: 22.06.2025 **Accepted:** 28.10.2025

INTRODUCTION

Familial Mediterranean fever (FMF) is the most common monogenic autoinflammatory disorder of childhood, characterized by recurrent episodes of fever and serositis.[1] The disease is caused by autosomal recessive mutations in the Mediterranean fever (*MEFV)* gene, particularly pathogenic variants located in exon 10, including M694V, M680I, and V726A, which are strongly associated with an earlier disease onset and a more severe disease phenotype.[2,3] Colchicine has remained the cornerstone of FMF treatment since its introduction in 1972, effectively preventing inflammatory attacks and significantly reducing the risk of secondary amyloidosis.[4] Although generally well tolerated, colchicine could cause gastrointestinal side effects, including abdominal pain, decreased appetite, nausea, vomiting, and diarrhea.[5,6] Due to its anti-mitotic properties, colchicine may also interfere with intestinal absorption, including vitamin B12.[5,6]

Vitamin B12 (cobalamin) is a water-soluble vitamin essential for hematological function and neurologic development, particularly in pediatric populations.[7,8] Experimental data suggest that colchicine may impair vitamin B12 absorption by downregulating intrinsic factor receptors in the terminal ileum and accelerating gastrointestinal transit time.[9,10] In addition, disease-related factors, such as *MEFV* genotype and disease activity, may further influence vitamin B12 status. Patients with more severe clinical phenotypes, particularly those with biallelic exon 10 mutations, often require prolonged or higher-dose colchicine therapy[11], which could increase the risk of subtle nutritional deficiencies, including vitamin B12 deficiency. [12]

Despite these mechanisms, the relationship between colchicine treatment, FMF genotype, disease severity, and serum vitamin B12 levels remains underexplored, especially in pediatric populations.

In this study, we aimed to determine whether prolonged colchicine therapy and higher colchicine doses are independently associated with vitamin B12 deficiency in pediatric FMF patients with biallelic exon 10 mutations, after adjusting for age as a potential confounder.

MATERIALS AND METHODS

This retrospective, single-center study included pediatric patients diagnosed with FMF who carried biallelic *MEFV* exon 10 mutations and were followed at our referral pediatric rheumatology clinic between August 2016 and September 2024. Eligible patients had a recorded serum vitamin B12 measurement in their medical files and had been receiving colchicine therapy for at least 12 months at the time of assessment.

The diagnosis of FMF was established according to the Eurofever/PRINTO classification criteria[13] and/or the Turkish pediatric FMF criteria.[14] Based on EULAR (the European Alliance of Associations for Rheumatology) recommendations, the standard dose of colchicine is 1.2 mg/m^2/day, with age-adjusted daily dosing defined as follows: ≤0.5 mg/day for children under 5 years of age, 0.5–1.0 mg/day for children aged 5–10 years, and 1.0–1.5 mg/day for children over 10 years of age.[15] Colchicine resistance was defined as experiencing more than one FMF attack per month for at least six months despite receiving the maximum age-appropriate colchicine dosage.[16]

Exclusion criteria included current or recent usage of vitamin B12 supplementation or proton pump inhibitors; presence of known gastrointestinal malabsorption syndromes (e.g., inflammatory bowel syndrome, celiac disease), or endocrinological diseases; patients on a vegan or vegetarian diet, those with incomplete medical records, or those older than 18 years at the time of vitamin B12 testing.

Demographic and clinical data were collected retrospectively from patient records, including age, sex, disease duration, colchicine dosage, and treatment duration, clinical manifestations, and *MEFV* gene results. Laboratory parameters included serum vitamin B12 concentrations. Vitamin B12 concentrations were measured using a chemiluminescent immunoassay (ARCHITECT i2000SR, Abbott Diagnostics, Germany), with a reference range of 183–883 pg/mL. All samples were obtained in the fasting state and processed in the same institutional laboratory under standardized protocols. Hemolyzed or lipemic samples were excluded from the analysis. Vitamin B12 deficiency was defined as ≤200 pg/mL and normal as >200 pg/mL, according to international pediatric standards.[7] Genetic testing of the *MEFV* gene was performed using conventional Sanger sequencing and recorded from archived results.

Colchicine treatment duration at the time of vitamin B12 testing was categorized into four groups based on the distribution of treatment lengths: Group 1, 12-24 months; Group 2, 25-60 months; Group 3, 61-96 months; and Group 4, >96 months. Disease severity at the time of B12 testing was assessed retrospectively using the Pras

severity score.[17,18] Ethical approval was obtained from the local ethics committee (approval date: 10 April 2025, approval number: B.10.1.TKH.4.34.H.GP.0.01/125) and was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical analysis

All statistical analyses were performed using IBM SPSS (Statistical Package for Social Sciences) Software version 30.0 (IBM Co., Armonk, NY, USA). The distribution of numerical variables was assessed using visual methods (histograms) and the Kolmogorov-Smirnov/Shapiro-Wilk tests to assess normality. Descriptive statistics were presented as medians and interquartile ranges (IQR, 25th-75th percentiles) or as means and standard deviations for continuous variables. Categorical variables were presented as numbers and frequencies and compared with the chi-square test or Fisher's exact test, as appropriate. Prevalence estimates with 95% confidence intervals (CI) were calculated using the bootstrap method. Non-normally distributed continuous numerical data were analyzed using the Mann-Whitney U test or Kruskal-Wallis test, as appropriate. Receiver operating characteristic (ROC) curve analysis was performed to determine the optimal age cut-off value for predicting vitamin B12 deficiency. The area under the curve (AUC) was calculated to evaluate diagnostic performance, and the optimal threshold was determined using the Youden index, which maximizes the sum of sensitivity and specificity. The Hosmer-Lemeshow test was used as a model-fitting test for binary logistic regression analysis. A two-sided p-value less than 0.05 was considered statistically significant.

RESULTS

A total of 339 pediatric FMF patients with biallelic *MEFV* exon 10 mutations and documented serum vitamin B12 levels were included in the study. Of these, 193 patients (56.9%) were female. The median age at symptom onset was 4 years (IQR, 2-7), and at diagnosis was 5 years (IQR, 3-9) (Table 1). The most common genotype was M694V/M694V, followed by M680I/M694V and M694V/V726A.

At the time of vitamin B12 measurement, the median age was 13 years (IQR, 9-16), and 41 patients (12.1% [95% CI: 8.6–15.6]) had B12 deficiency. The median duration of colchicine treatment prior to vitamin B12 measurement was 60 months (IQR 24-96), and the mean colchicine dose at the time of testing was 1.15±0.4 mg/day (Table 1).

Patients with vitamin B12 deficiency had both significantly longer duration and higher daily dosage of colchicine exposure compared to those with normal B12 levels (p=0.034 and p=0.004, respectively) (Table 2). Patients in the longer colchicine treatment duration groups had higher frequencies of vitamin B12 deficiencies (p=0.014) and lower median vitamin B12 levels (p=0.008), suggesting a potential inverse relationship between colchicine exposure and vitamin B12 levels (Table 3). Regarding serum vitamin B12 levels, Group 4 had significantly lower values compared to Group 1 (p=0.004) and Group 2 (p=0.006).

Vitamin B12 deficiency was significantly more common among patients receiving more than 1 mg/day compared to those receiving 1 mg/day or less (19.1% vs. 8.7%)

Table 1. Characteristics of pediatric patients with FMF

	FMF n = 339 (100%)
Sex, female, n (%)	193 (56.9)
Age at symptom onset, years [a]	4 (2-7)
Age at FMF diagnosis, years [a]	5 (3-9)
Age at vitamin B12 measurement, years [a]	13 (9-16)
Colchicine treatment duration, months [a]	60 (24-96)
Colchicine dose, mg/day [b]	1.15 ± 0.4
Colchicine resistance, n (%)	28 (8.3)
Vitamin B12 status, n (%)	
Deficient (≤200 pg/mL)	41 (12.1)
Normal (>200 pg/mL)	298 (87.9)
Serum vitamin B12 level, pg/mL [a]	317 (236.7-447)
Pras severity score [a]	8 (7-10)
Pras severity category, n (%)	
Mild	47 (13.9)
Moderate	201 (59.3)
Severe	91 (26.8)
MEFV mutations	
Homozygous M694V	188 (55.5)
Compound heterozygous M694V	115 (33.9)
Non-M694V compound heterozygote	36 (10.6)

[a] Values represented as median (interquartile range Q1–Q3)
[b] Values represented as mean ± standard deviation
FMF, familial Mediterranean fever; n, number

(p=0.006). Patients receiving more than 1 mg/day (n=229, 67.6%, 331 pg/mL [IQR, 258-452.5]) had significantly lower serum vitamin B12 levels compared to those receiving 1 mg/day or less (n=110, 32.4%, 278 pg/mL [IQR, 217-396.5]) (p<0.001).

Neither median vitamin B12 levels nor the frequency of vitamin B12 deficiency differed significantly by M694V mutation status (p=0.179 and p=0.35, respectively) (Table 2). The median serum B12 levels did not differ significantly among Pras severity groups (p=0.97), and no association was found between Pras severity and vitamin B12 status (p=0.71) (Table 2).

Twenty-eight patients (8.3%) were classified as colchicine-resistant at the time of vitamin B12 measurement, and were receiving anti-interleukin-1 therapy, with a median biologic treatment duration of 22.5 months (IQR, 9.3-31.7) (Table 4). Colchicine-resistant patients were significantly older at the time of vitamin B12 measurement (p<0.001), had significantly longer colchicine exposure (p<0.001), and received a higher daily dose (p<0.001). Vitamin B12 deficiency was more frequent among colchicine-resistant

Table 2. Comparison of FMF patients with vitamin B12 deficiency and normal vitamin B12 levels

	Deficient vitamin B12 levels n = 41 (12.1%)	Normal vitamin B12 levels n = 298 (87.9%)	p-value
Sex, female, n (%)	24 (58.5)	169 (56.7)	0.83
Age at symptom onset, years [a]	4.9 (3.5-8.0)	3.5 (2-6.9)	0.064
Age at FMF diagnosis, years [a]	8 (5-11.5)	5 (3-9)	<0.001
Age at vitamin B12 measurement, years [a]	15 (13-17)	12 (8.8-15)	<0.001
Colchicine treatment duration, months [a]	72 (48-114)	60 (24-96)	0.034
Colchicine dose, mg/day [b]	1.33 ± 0.4	1.12 ± 0.4	0.004
Colchicine resistance, n (%)	7 (17.1)	21 (7)	0.03
Pras severity score [a]	8 (7-9)	8 (7-9)	0.3
Pras severity category, n (%)			
Mild	4 (9.8)	43 (14.4)	0.71
Moderate	25 (61)	176 (59.1)	
Severe	12 (29.2)	79 (26.5)	
MEFV mutations			
Homozygous M694V	19 (46.3)	169 (56.7)	0.35
Compound heterozygous M694V	18 (43.9)	97 (32.6)	
Non-M694V compound heterozygote	4 (9.8)	32 (10.7)	

[a] Values represented as median (interquartile range Q1–Q3)
[b] Values represented as mean ± standard deviation
FMF, familial Mediterranean fever; n, number

Table 3. Vitamin B12 status and levels according to duration of colchicine therapy in pediatric FMF patients

	Group 1 12-24 months n=87, 25.7%	Group 2 25-60 months n=70, 20.6%	Group 3 61-96 months n=87, 25.7%	Group 4 >96 months n=90, 28%	p-value
Age at vitamin B12 measurement, years [a]	9 (5-14)	11 (7.8-14)	12 (10-15)	15 (13-17)	<0.001*
Vitamin B12 deficiency, n (%)	5 (5.7)	7 (10)	13 (14.9)	16 (16.8)	0.014
Serum Vitamin B12 level, pg/mL [a]	332 (252-494)	350.5 (256.5-491)	307 (231-448)	295 (226-390)	0.008**

[a] Values represented as median (interquartile range Q1–Q3)
* Post hoc pairwise comparisons using Mann–Whitney U tests with Bonferroni correction (adjusted significance threshold p < 0.0083) showed statistically significant differences in age between Group 4 and Groups 1–3 (all p < 0.001) and between Group 1 and Group 3 (p < 0.001).
**Post hoc pairwise comparisons using Mann–Whitney U tests with Bonferroni correction (adjusted significance threshold p < 0.0083) showed statistically significant differences in serum vitamin B12 levels between Group 4 and Group 1 (p = 0.004) and between Group 4 and Group 2 (p = 0.006).
FMF, familial Mediterranean fever; IQR, interquartile range; n, number

Table 4. Comparison of demographic characteristics and vitamin B12 levels between colchicine-responsive and colchicine-resistant pediatric FMF patients

	Colchicine-responsive n=311 (91.7%)	Colchicine-resistant n=28 (8.3%)	p-value
Sex, female, n (%)	174 (55.9)	19 (67.9)	0.22
Age at symptom onset, years [a]	4 (2-7)	2.6 (1.5-7)	0.23
Age at FMF diagnosis, years [a]	5 (3-9)	5 (3-8.7)	0.54
Age at vitamin B12 measurement, years [a]	12 (9-15)	15 (13-18)	<0.001
Colchicine treatment duration, months [a]	60 (24-96)	114 (72-132)	<0.001
Colchicine dose, mg/day [b]	1.12 ± 0.4	1.5 ± 0.5	<0.001
Anti-IL-1 therapy duration, months [a]	-	22.5 (9.3-31.7)	-
Vitamin B12 deficiency, n (%)	34 (10.9)	7 (25)	0.03
Serum vitamin B12 level, pg/mL [a]	321 (239-449)	286.5 (189-357.5)	0.053

[a] Values represented as median (interquartile range Q1–Q3)
[b] Values represented as mean ± standard deviation
FMF, familial Mediterranean fever; n, number

Table 5. Univariate and multivariate logistic regression analysis for predictors of vitamin B12 deficiency in pediatric FMF patients

	Univariate Analysis		Multivariate Analysis	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Age	1.22 (1.11-1.35)	<0.001	1.22 (1.08-1.37)	<0.001
Colchicine Dose	2.98 (1.40-6.33)	0.005	1.47 (0.59-3.66)	0.41
Colchicine Duration	1.01 (0.99-1.01)	0.096	0.997 (0.99-1.01)	0.47
Colchicine Resistance	2.72 (1.08-6.86)	0.035	1.80 (0.66-4.88)	0.25

Hosmer-Lemeshow test chi-square = 5.54, p = 0.69
CI, confidence interval; FMF, familial Mediterranean fever; OR, odds ratio

patients compared to the colchicine-responsive group (p=0.03). Although the median serum vitamin B12 level appeared lower in the colchicine-resistant group, this difference did not reach statistical significance (p=0.053) (Table 4).

A significant inverse correlation was observed between age and vitamin B12 levels (Spearman's ρ = –0.366, p < 0.001), indicating that older patients tended to have lower B12 concentrations. Age also correlated positively with the colchicine treatment duration (Spearman's ρ=0.514, p<0.001), suggesting that age-related decline in B12 levels may be partially mediated by longer cumulative colchicine exposure over time.

In univariate logistic regression analysis, age (p<0.001), colchicine dose (p=0.005), and colchicine resistance (p=0.035) were significantly associated with vitamin B12 deficiency. However, in multivariate analysis, only age remained a significant predictor (p<0.001), while the associations with colchicine dose (p=0.41), colchicine duration (p=0.47), and colchicine resistance (p=0.25) were no longer significant (Table 5). Model fit was assessed using the Hosmer-Lemeshow goodness-of-fit test, which indicated an adequate fit (χ^2=5.54, p=0.69). ROC analysis revealed that an age threshold of 12.2 years best predicted vitamin B12 deficiency, with an AUC of 0.708 (standard error: 0.037; 95% CI: 0.635-0.780; p<0.001), yielding a sensitivity of 85.4% and specificity of 53.4% (Figure 1).

DISCUSSION

In this retrospective study, we evaluated serum vitamin B12 concentrations in pediatric patients with FMF carrying biallelic exon 10 mutations, along with clinical and genetic variables, including colchicine treatment duration, disease severity, and *MEFV* variants. Our findings indicate that vitamin B12 deficiency is relatively common, 12.1%, among children with FMF, and that older age (>12.2 years)

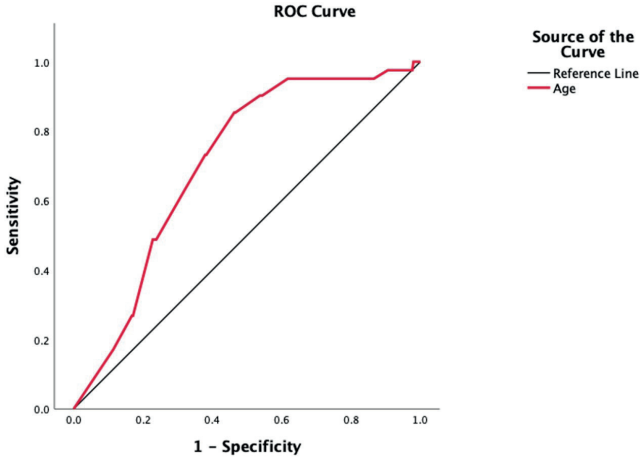


Figure 1. Receiver operating characteristics (ROC) curve for age in predicting vitamin B12 deficiency among pediatric FMF patients. The optimal cut-off point was 12.2 years (AUC = 0.708 [SE: 0.037; 95% CI: 0.635–0.780], sensitivity = 85.4%, specificity = 53.4%).

AUC, area under the curve; CI, confidence interval

emerged as an independent risk factor for reduced vitamin B12 levels.

Colchicine remains the mainstay of FMF treatment. However, its side effect profile primarily involves gastrointestinal symptoms, including diarrhea, abdominal pain, and vomiting.[5,6] In literature, it has been suggested that long-term and high-dose colchicine therapy may impair vitamin B12 absorption by altering intestinal motility and inhibiting the function of intrinsic factor-B12 receptors in the terminal ileum.[5-7] Our findings are consistent with these proposed mechanisms, as we observed a statistically significant decrease in vitamin B12 levels among patients with longer colchicine treatment duration (>96 months). In a study by Yılmaz et al.[19], a significant reduction in mean serum vitamin B12 levels, from 418 pg/ml to 240 pg/ml, was observed after the initiation of colchicine therapy within 16.5±9.8 months, which concluded that administration of colchicine may lead to a reduction in vitamin B12 levels.

In our FMF cohort, the frequency of vitamin B12 deficiency was 12.1% (95% Cl: 8.6–15.6), which appears higher than the reported prevalence of 5.5% in a large cohort of 3,163 healthy children from the same country.[20] Previous studies have reported conflicting results regarding the impact of colchicine on vitamin B12 levels. Gemici et al.[21] reported significantly lower vitamin B12 levels in FMF patients compared with the healthy control group, suggesting a colchicine-related effect. Conversely, Başaran et al.[22] found no relationship between colchicine duration and vitamin B12 levels but observed lower vitamin B12 levels in patients receiving higher colchicine doses (>1 mg/day). In our study, we demonstrated that both longer duration (>96 months) and higher daily colchicine doses (>1 mg/day) were independently associated with lower serum vitamin B12 levels, supporting a dose-dependent impact on vitamin B12 metabolism or absorption. However, these associations did not remain significant after adjusting for age. Multivariate analysis models suggest that the effects of colchicine-related variables may be confounded by age, as older patients are more likely to receive longer treatment durations.

While the majority of our cohort carried at least one M694V mutation, no statistically significant differences in vitamin B12 levels were observed when stratified by mutation status. These results suggest that M694V mutation status alone does not appear to affect serum B12 concentrations. This finding supports that colchicine-related factors, such as dose and duration, rather than genotype itself, may play a more prominent role in reducing vitamin B12 status.

Despite our hypothesis that greater disease severity would correlate with lower vitamin B12 levels due to chronic inflammation, no significant relationship was found between the Pras severity score and vitamin B12 concentrations. This may, in part, reflect the limitations of cross-sectional severity indices in reflecting cumulative disease burden. Similarly, in a study by Tetik Dincer et al.[23], vitamin B12 levels were not affected by attack frequency, suggesting that attack frequency alone may be insufficient to reflect disease activity.

Notably, colchicine-resistant FMF patients in our study had a significantly higher prevalence of vitamin B12 deficiency than colchicine-responsive patients. However, this group also had older age and longer colchicine treatment duration, which may confound the observed association. These findings highlight the multifactorial nature of vitamin B12 status in FMF and suggest that disease severity score or colchicine response alone may not adequately reflect risk for subclinical micronutrient deficiencies. Identifying and correcting vitamin B12 deficiency in this subgroup could help prevent additive complications such as anemia, fatigue, or impaired growth, and may improve overall disease management and quality of life.

Our findings suggest that FMF patients older than 12.2 years of age and those under long-term colchicine therapy (>96 months) may be at increased risk of vitamin B12

deficiency, regardless of specific *MEFV* variants. The observed association between prolonged colchicine therapy and lower vitamin B12 levels may, in part, reflect increasing patient age as both variables are closely linked. Furthermore, our multivariate logistic regression analysis confirmed that age was the main independent predictor of vitamin B12 deficiency. In addition, vitamin B12 deficiency appeared to be more frequent in adolescents with FMF than in healthy pediatric populations.[20,23] The finding that age emerged as the only independent predictor of vitamin B12 deficiency may be explained by biological plausibility. During adolescence, rapid growth, pubertal changes, and dietary shifts increase the metabolic demand for vitamin B12 and may alter its absorption[20], potentially confounding the association with treatment-related factors. In addition, in FMF patients, prolonged colchicine exposure during these critical developmental years may further contribute to the risk of deficiency, highlighting the combined effect of age and long-term therapy. Based on the analyses, our findings indicate that the prevalence of vitamin B12 deficiency may warrant routine screening in FMF patients during adolescence, particularly among those receiving long-term colchicine therapy. Such an approach could facilitate early recognition and timely management of deficiency in this vulnerable subgroup.

Our study has several strengths, including a large cohort, exclusion of potential confounders such as vitamin B12 supplementation and comorbid malabsorption conditions, and detailed stratification by genotype and disease severity. However, this study has several limitations. Due to its retrospective design, data on patients' nutritional status, dietary vitamin B12 intake, and pubertal stage were lacking, and baseline vitamin B12 measurements before colchicine initiation were not available. Moreover, the lack of a healthy control group limits direct comparison of vitamin B12 levels between FMF patients and the healthy pediatric population. Further prospective studies comparing vitamin B12 levels with other vitamin levels and dietary intake of vitamins could be more important. Longitudinal assessments of vitamin B12 levels before initiation and after long-term colchicine treatment would provide further understanding of the specific impact of treatment over time.

In conclusion, this study underscores the potential for vitamin B12 deficiency in pediatric FMF patients, particularly in adolescent patients. While those receiving long-term (>96 months), higher dose (>1 mg/day) colchicine therapy, or colchicine resistance appeared to be associated with lower vitamin B12 levels, age was the independent predictor of vitamin B12 deficiency. Further prospective studies are warranted to better elucidate the long-term impact of colchicine on vitamin B12 metabolism and to determine whether routine screening should be recommended for all pediatric FMF patients undergoing colchicine treatment.

Ethical approval

This study has been approved by the Umraniye Traning and Research Hospital, University of Health Sciences Ethics Committee (approval date 10.04.2025, number B.10.1.TKH.4.34.H.GP.0.01/125).

Author contribution

The authors declare contribution to the paper as follows: Study conception and design: SAU, BS; data collection: SAU, MSÖ, ET; analysis and interpretation of results: SAU, ET, BS; draft manuscript preparation: SAU, BS. 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. Lancieri M, Bustaffa M, Palmeri S, et al. An update on familial mediterranean fever. Int J Mol Sci. 2023;24:9584. [Crossref]
2. Ayaz NA, Tanatar A, Karadağ ŞG, Çakan M, Keskindemirci G, Sönmez HE. Comorbidities and phenotype-genotype correlation in children with familial Mediterranean fever. Rheumatol Int. 2021;41:113-20. [Crossref]
3. Tunce E, Atamyıldız Uçar S, Polat MC, et al. Predicting homozygous M694V genotype in paediatric FMF: multicentre analysis and scoring system proposal. Rheumatology (Oxford). 2025;64:4816-24. [Crossref]
4. Batu ED, Basaran O, Bilginer Y, Ozen S. Familial mediterranean fever: how to interpret genetic results? How to treat? A quarter of a century after the association with the mefv gene. Curr Rheumatol Rep. 2022;24:206-12. [Crossref]
5. Stamp LK, Horsley C, Te Karu L, Dalbeth N, Barclay M. Colchicine: the good, the bad, the ugly and how to minimize the risks. Rheumatology (Oxford). 2024;63:936-44. [Crossref]

6. Webb DI, Chodos RB, Mahar CQ, Faloon WW. Mechanism of vitamin B12 malabsorption in patients receiving colchicine. N Engl J Med. 1968;279:845-50. [Crossref]

7. Green R, Miller JW. Vitamin B12 deficiency. Vitam Horm. 2022;119:405-39. [Crossref]

8. Rasmussen SA, Fernhoff PM, Scanlon KS. Vitamin B12 deficiency in children and adolescents. J Pediatr. 2001;138:10-7. [Crossref]

9. Demir A, Akyüz F, Göktürk S, et al. Small bowel mucosal damage in familial Mediterranean fever: results of capsule endoscopy screening. Scand J Gastroenterol. 2014;49:1414-8. [Crossref]

10. Mansueto P, Seidita A, Chiavetta M, et al. Familial mediterranean fever and diet: a narrative review of the scientific literature. Nutrients. 2022;14:3216. [Crossref]

11. Tunce E, Uçar SA, Coşkuner T, et al. Preliminary evaluation for the development of a scoring system to predict homozygous M694V genotype in familial mediterranean fever patients: a single-center study. J Clin Rheumatol. 2025;31:7-11. [Crossref]

12. Ekinci RMK, Balci S, Serbes M, Dogruel D, Altintas DU, Yilmaz M. Decreased serum vitamin B12 and vitamin D levels affect sleep quality in children with familial Mediterranean fever. Rheumatol Int. 2018;38:83-7. [Crossref]

13. Gattorno M, Hofer M, Federici S, et al. Classification criteria for autoinflammatory recurrent fevers. Ann Rheum Dis. 2019;78:1025-32. [Crossref]

14. Yalçinkaya F, Ozen S, Ozçakar ZB, et al. A new set of criteria for the diagnosis of familial Mediterranean fever in childhood. Rheumatology (Oxford). 2009;48:395-8. [Crossref]

15. Ozen S, Demirkaya E, Erer B, et al. EULAR recommendations for the management of familial Mediterranean fever. Ann Rheum Dis. 2016;75:644-51. [Crossref]

16. Ozen S, Kone-Paut I, Gül A. Colchicine resistance and intolerance in familial mediterranean fever: Definition, causes, and alternative treatments. Semin Arthritis Rheum. 2017;47:115-20. [Crossref]

17. Pras E, Livneh A, Balow JE, et al. Clinical differences between North African and Iraqi Jews with familial Mediterranean fever. Am J Med Genet. 1998;75:216-9. [Crossref]

18. Ozen S, Aktay N, Lainka E, Duzova A, Bakkaloglu A, Kallinich T. Disease severity in children and adolescents with familial Mediterranean fever: a comparative study to explore environmental effects on a monogenic disease. Ann Rheum Dis. 2009;68:246-8. [Crossref]

19. Yilmaz R, Ozer S, Ozyurt H, Erkorkmaz U. Serum vitamin B12 status in children with familial mediterranean fever receiving colchicine treatment. HK J Paediatr. 2011;16:3-8.

20. Kara İS, Peker NA, Dolğun İ, Mertoğlu C. Vitamin B12 level in children. J Curr Pediatr. 2023;21:127-34. [Crossref]

21. Gemici AI, Sevindik ÖG, Akar S, Tunca M. Vitamin B12 levels in familial Mediterranean fever patients treated with colchicine. Clin Exp Rheumatol. 2013;31:57-9.

22. Başaran Ö, Uncu N. Kolşisin tedavisi altındaki ailevi akdeniz ateşi tanısı ile takip edilen çocuk hastalarda vitamin B12 düzeylerinin değerlendirilmesi. Güncel Pediatri. 2018;16:86-92. [Crossref]

23. Tetik Dincer B, Ozcelik G, Urganci N. Comparison of vitamin D, B12, and folic acid levels according to attack frequency in familial mediterranean fever cases. Sisli Etfal Hastan Tip Bul. 2024;58:359-62. [Crossref]

Trends in Pediatrics 2025;6(4):268-274
DOI: 10.59213/TP.2025.332

Original Article

Evaluation of the clinical characteristics of patients with PFAPA syndrome according to attack triggers

Lütfiye Koru[1], Elif Selcen Yabancı Erten[2], Feray Kaya[1], Elif Küçük[1], Zelal Aydın[1], Eda Nur Dizman[1], Hatice Kübra Dursun[1], Merve Özen Balcı[1], Ufuk Furkan Özdemir[1], Kübra Öztürk[1], Fatih Haşlak[1]

[1]Department of Pediatric Rheumatology, Faculty of Medicine, İstanbul Medeniyet University, İstanbul, Türkiye
[2]Department of Pediatrics, Faculty of Medicine, İstanbul Medeniyet University, İstanbul, Türkiye

Cite this article as: Koru L, Yabancı Erten ES, Kaya F, et al. Evaluation of the clinical characteristics of patients with PFAPA syndrome according to attack triggers. Trends in Pediatrics 2025;6(4):268-274.

ABSTRACT

Objective: Based on our clinical observations, this study aimed to evaluate the spectrum of triggers for febrile attacks in children with periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) and to investigate whether clinical characteristics differ by trigger presence or type, providing insights relevant for clinicians managing PFAPA.

Methods: The study enrolled children diagnosed with PFAPA by a pediatric rheumatologist according to the European Registry of Autoinflammatory Diseases (EUROFEVER), developed by the Paediatric Rheumatology International Trials Organisation (PRINTO), who had been followed at our tertiary care center for at least six months and were not on prophylaxis. Patients were stratified by the presence of attack triggers. Those with triggers were classified into infection/vaccination, physical/emotional stress, or food intake categories. A comparative analysis of demographic, clinical, and laboratory characteristics was performed between groups during attacks.

Results: Triggers were identified in 31.4% of patients (n = 53), most commonly infection/vaccination (16.0%), followed by physical/emotional stress (8.9%) and food intake-related factors (7.1%). Median breastfeeding duration was significantly shorter in the trigger group (17 vs. 24 months, p=0.032). No significant differences were found in the cardinal PFAPA features (oral aphthae, cervical lymphadenitis, and tonsillitis) by trigger status or type. However, constipation during attacks was more frequent in the group with physical/emotional stress (p=0.020).

Conclusion: The clinical phenotype of PFAPA appears largely independent of trigger type. However, shorter breastfeeding duration among patients with triggers suggests early-life factors may influence trigger susceptibility. Additionally, stress-related triggers may link to other symptoms, such as constipation. Recognizing these patterns may help tailor supportive strategies for PFAPA management.

Keywords: fever, stomatitis, aphthous, triggers

INTRODUCTION

Periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) represents one of the most common forms of autoinflammatory diseases (AIDs) in the pediatric age group. The syndrome generally presents in early childhood, characterized by recurrent febrile episodes lasting 3 to 7 days and recurring every 2 to 8 weeks. These flares are typically accompanied by pharyngitis, cervical lymphadenopathy, and/or aphthous stomatitis. Between attacks, affected children mostly remain asymptomatic, demonstrating normal growth and development.[1-4]

Although the pathogenesis of PFAPA has not been fully elucidated, it is thought that innate immune system dysregulations and abnormal cytokine responses play

✉ Fatih Haşlak ▪ drfatihhaslak@gmail.com

Received: 14.08.2025 **Accepted:** 16.11.2025

a role in the development of attacks.[5-7] Clinicians who follow PFAPA are aware that, as in other AIDs such as Familial Mediterranean Fever (FMF), there may be various factors that can trigger attacks in patients. This topic is better defined in FMF, where pyrin activation associated with *MEFV* gene variations has been shown to lower the inflammatory threshold, potentially initiating attacks in response to various environmental or internal triggers.[8-10] In contrast, data on attack triggers in PFAPA are limited. Studies in the literature have reported that emotional stress and changes in the microbial environment may be associated with PFAPA attacks.[11,12]

We hypothesized that specific environmental or physiological triggers may influence the occurrence and clinical characteristics of PFAPA attacks. Based on our biological knowledge and clinical observations, this study aimed to evaluate the factors that trigger attacks in PFAPA patients. We also explored whether clinical characteristics differ by trigger type. Our study aims to make a unique contribution to the literature by examining a wide range of triggers and their clinical effects.

MATERIALS AND METHODS

Patients and data collection

The study enrolled children diagnosed with PFAPA by a pediatric rheumatologist according to the European Registry of Autoinflammatory Diseases (EUROFEVER), developed by the Paediatric Rheumatology International Trials Organisation (PRINTO) who had been followed at our tertiary care center for at least 6 months and were not on prophylaxis.[13] Prophylactic treatment refers to the continuous use of colchicine for attack prevention. Patients who were receiving colchicine prophylaxis at the time of evaluation were excluded from the study. Demographic characteristics and clinical parameters observed during febrile episodes were retrospectively retrieved from patient medical records.

This study was conducted in compliance with the Helsinki Declaration and local laws and regulations. Informed consent was obtained from the patients and their legal caregivers. The ethics committee of a tertiary center approved our study (18/07/2024/725).

Grouping of patients and classification of triggers

Patients were interviewed face-to-face during outpatient clinic visits regarding triggers and were asked to respond based on attacks experienced within the last three attacks. They were then stratified into two groups: those with and those without triggers preceding febrile episodes. Triggers were classified into three main categories: (1) infection/vaccination, (2) physical/emotional stress, and (3) food intake. Some patients experienced more than one trigger type during different attacks. Infection was defined as the presence of physician-confirmed or microbiologically proven infection, or at least two infection-related symptoms (e.g., cough, rhinorrhea) in the patient or household members within 7 days prior to the attack; all other episodes were classified as PFAPA flares. In our study, infection-triggered PFAPA attacks were defined as episodes meeting PFAPA criteria but preceded by mild upper respiratory or other infection-related symptoms occurring within seven days before fever onset, either in the patient or household members. These symptoms resolved in parallel with the PFAPA flare, without a pathogen-specific treatment response. In contrast, episodes with clear evidence of active infection requiring antimicrobial therapy or showing pathogen-specific findings were classified as infectious episodes rather than PFAPA attacks. Infection and vaccine-related triggers were grouped due to their shared potential to activate the innate immune response in genetically susceptible individuals.[14] Physical stress (cold exposure, intense exercise) and emotional stress were combined under a single category, as stress-induced neuroimmune alterations may activate similar inflammatory pathways.[15,16] The food intake category consisted of potential triggers, including milk, yogurt, cocoa, and processed packaged foods.

Statistical analysis

We performed the statistical analysis using SPSS for Windows, version 25.0 (SPSS Inc., Chicago, IL). The Kolmogorov–Smirnov test was used to assess the distribution of continuous variables. Variables with a normal distribution were presented as mean ± standard deviation, whereas those with a non-normal distribution were presented as median (minimum–maximum). Categorical variables, expressed as numbers (percentages), were compared using the chi-square test or Fisher's exact test,

as appropriate. Continuous variables were compared using the Student's t-test, Mann–Whitney U test, or Kruskal–Wallis test, depending on the distribution and number of groups. A p-value < 0.05 was considered statistically significant.

RESULTS

Characteristics and comparative analysis of patients with and without triggers

The investigation encompassed 169 children, of whom 53 patients (31.4%) had triggers and 116 (68.6%) had no triggers. The gender distribution was 65.7% (n=111) male and 34.3% (n=58) female. The median age at diagnosis was 4.3 (1.0–9.4) years in patients with triggers and 4.3 (1.1–9.7) years in those without triggers. Breastfeeding duration was significantly shorter in patients with triggers compared to those without triggers (17 (0–48) months *vs*. 24 (0–54) months, $p = 0.032$). No statistically significant differences were observed in other demographic, clinical, or laboratory characteristics between the two groups (Table 1).

Comparative analysis of clinical characteristics and laboratory findings by trigger types

In the overall cohort, the most common trigger type was infection/vaccination (n=27, 16.0%), followed by physical/emotional stress (n=15, 8.9%) and food intake-related triggers (n=12, 7.1%). Among patients with infection- or vaccination-triggered episodes, one patient had an attack following vaccination. Mevalonate kinase deficiency (MKD) was excluded based on clinical evaluation and genetic testing showing no pathogenic MVK variants. Comparative analysis according to trigger types revealed that constipation was significantly more frequent in the physical/emotional stress group ($p = 0.020$). No other statistically significant differences were observed in demographic, clinical, or laboratory characteristics among the trigger type groups (Table 2).

DISCUSSION

In this study, we evaluated triggers for PFAPA flares, including their distribution and association with clinical, demographic, and laboratory characteristics. These parameters were compared by trigger presence/absence and, among patients with triggers, by trigger type. The most frequent trigger was infection/vaccination, followed by physical/emotional stress and food intake. The sole significant difference between the groups was a shorter breastfeeding duration in patients with triggers. When stratified by trigger type, constipation was more frequently observed in the physical/emotional stress group. These findings suggest that the presence or type of trigger in PFAPA may be associated with certain clinical features, although the overall clinical profile remains largely consistent.

To our knowledge, no previous study has systematically evaluated such a broad spectrum of triggers in PFAPA; in this respect, our work represents one of the most comprehensive analyses to date. Previous reports have generally focused on a single trigger, most often infectious exposures or stress, and have not described their relative frequencies.[11,12] In our study, the most frequent trigger was infection or vaccination, followed by physical/emotional stress, and, less commonly, food-related factors. This trigger profile is not unique to PFAPA but shares common features with infectious triggering mechanisms described in certain AIDs.[17,18] In these disorders, the primary pathology involves excessive or inappropriate activation of the innate immune system.[18] Pattern-recognition receptors (e.g., Toll-like receptors, NOD-like receptors) detect viral or bacterial components and activate the inflammatory cascade in a regulated manner. In AIDs, however, the activation threshold of this system is lowered.[17,18] Although a genetic mutation is rarely identified in PFAPA, it is likely that defects in regulatory mechanisms and epigenetic alterations lead to similarly low-threshold activation of the innate immune system, with infection or vaccination serving as potent stimuli for flare initiation. Vaccination, in particular, is a temporally distinct and easily recognizable event for caregivers, increasing the likelihood that it will be recalled and linked to the onset of an episode. Additionally, although infrequently reported, food intake-related triggers are noteworthy. Dietary antigens may modulate inflammatory responses through the mucosal immune system, and alterations in gut microbiota composition can influence the severity of IL-1–mediated inflammation.[19,20] These biological mechanisms, together with the high detectability of such events by caregivers, may account for the prominence of these triggers among patients.

When patients with triggers were compared with those without, the only statistically significant difference was a shorter breastfeeding duration in the trigger group. Although the clinical relevance of this finding is not entirely clear, early cessation of breastfeeding may lead to alterations in gut microbiota composition, inadequate maturation of the mucosal immune system, and reduced oral tolerance.[21,22] Human milk oligosaccharides in breast

Table 1. Comparison of clinical and demographic characteristics of patients with and without attack triggers

	Patients with triggers (n=53)	Patients without triggers (n=116)	*p* value
Male gender (n, %)	38 (71.7%)	73 (62.9%)	0.265
*Age at diagnosis (Year) Median	4.3 (1.0-9.4)	4.3 (1.1-9.7)	0.819
*Number of monthly attacks	1 (0-5)	1.5 (0-4)	0.628
*Duration of attack (Day)	4 (2-10)	4.5 (1-10)	0.215
*Highest fever value in the attack (^{0}C)	40 (38-41)	40 (38-41)	0.594
*Time between attacks (Day)	15 (7-90)	25 (7-90)	0.688
Clinical findings during the attack (n, %)			
Sore throat	53 (100%)	113 (97.4%)	0.553
Oral aphthae	29 (54.7%)	59 (50.9%)	0.642
Lymphadenitis	44 (83%)	83 (71.6%)	0.110
Abdominal pain	34 (64.2%)	67 (57.8%)	0.432
Headache	15 (28.3%)	29 (25%)	0.650
Diarrhea	9 (17%)	24 (20.7%)	0.573
Constipation	4 (7.5%)	4 (3.4%)	0.260
Family history (n, %)			
Periodic fever syndrome	33 (62.3%)	75 (64.7%)	0.764
Tonsillectomy	27 (50.9%)	53 (45.7%)	0.526
*Breastfeeding duration (months)	17 (0-48)	24 (0-54)	0.032
Vaccination status (n, %)	45 (84.9%)	109 (94.0%)	0.078
Genetic and laboratory findings			
MEFV variation (n, %)	17 (51.5%)	36 (50%)	0.885
M694V variation (n, %)	5 (15.2%)	13 (18.1%)	0.714
Exon 10 variation (n, %)	11 (33.3%)	19 (26.8%)	0.491
*,**CRP (mg/L)*	44 (8.7-156)	60.6 (7.9-338)	0.091
*,**ESR (mm/h)*	25.5 (4-56)	25 (10-89)	0.945
*,**Neutrophil count (×10^9/L)*	8.9 (2.2-27.1)	9 (1.2-23.9)	0.750
*,**Lymphocyte count*	2.5 (1.1-9.8)	3.1 (0.9-7.7)	0.086
*,**Platelet count (×10^9/L)*	295 (212-526)	301 (171-603)	0.506

*: Median (Min-Max)
**: During the attack
CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; MEFV: Mediterranean fever gene.

milk support the colonization of beneficial gut bacteria, while bioactive components such as immunoglobulin A, lactoferrin, and antimicrobial peptides play a critical role in protecting against infections and modulating inflammatory responses.[23,24] The absence of these protective mechanisms may increase susceptibility to infectious or other environmental stimuli, thereby lowering the threshold for disease flares. Indeed, in a study of 150 PFAPA patients, Rigante *et al.*[25] demonstrated that breastfeeding for ≥6 months was associated with lower attack frequency and severity, higher rates of spontaneous remission, and that prolonged breastfeeding was an independent protective factor against disease activity in PFAPA. In our study, the shorter breastfeeding duration observed in patients with triggers suggests that the diminished protective effect of breastfeeding might increase the likelihood of trigger-related attacks. Moreover, the association between shorter breastfeeding duration and the development of allergic and autoimmune diseases has been demonstrated in multiple epidemiological studies, potentially predisposing the immune system toward a more reactive phenotype in response to environmental stimuli.[26-28] As an early-life factor, inadequate breast milk intake may shape gut microbiota development, immune tolerance, and

Table 2. Comparison of clinical and genetic features according to trigger types in PFAPA patients

	Attack Trigger Types (n, %)			
	Infection/Vaccination (27, 16%)	**Physical/Emotional Stress (15, 8.9%)**	**Food intake (12, 7.1%)**	***p***
Male gender (n, %)	20 (76.9%)	10 (66.7%)	8 (66.7%)	0.582
*Age at diagnosis (Year)	5 (1.1-8.2)	3.5 (1.1-9.4)	3.8 (1-6.7)	0.889
*Number of monthly attacks	1 (1-5)	1.5 (0-3)	1 (1-2)	0.958
*Duration of attack (Day)	4 (2-10)	4 (2-10)	4 (3-7)	0.541
*Highest fever value in the attack (^{0}C)	40 (38.6-42)	40 (38.5-41)	40 (38-41)	0.924
*Time between attacks (Day)	15 (7-60)	20 (10-90)	20 (15-30)	0.809
Clinical findings during the attack (n, %)				
Sore throat	26 (100%)	15 (100%)	12 (100%)	0.707
Oral aphthae	15 (57.7%)	7 (46.7%)	7 (58.3%)	0.859
Lymphadenitis	23 (88.5%)	12 (80%)	9 (75%)	0.326
Abdominal pain	19 (73.1%)	10 (66.7%)	5 (41.7%)	0.257
Headache	8 (30.8%)	6 (40%)	1 (8.3%)	0.279
Diarrhea	4 (15.4%)	3 (20%)	2 (16.7%)	0.930
Constipation	0 (0%)	3 (20%)	1 (8.3%)	0.020
Family history (n, %)				
Periodic fever syndrome	17 (65.4%)	10 (66.7%)	6 (50%)	0.775
Tonsillectomy	17 (65.4%)	6 (40%)	4 (33.3%)	0.188
*Breastfeeding duration (months)	17 (0-42)	15 (1-30)	22 (0-48)	0.149
Vaccination status (n, %)	21 (80.8%)	13 (86.7%)	11 (91.7%)	0.173
Genetic and laboratory findings				
MEFV variation (n, %)	7 (26.9%)	9 (60%)	1 (8.3%)	0.532
*, **CRP (mg/L)*	54.7 (8.7-156)	42 (13.3-119.6)	37.8 (18.7-98)	0.267
*, **ESR (mm/h)*	16.5 (4-38)	28 (21-51)	34 (17-56)	0.414
*, **Neutrophil count (×10^9/L)*	8.3 (2.2-27.1)	6.6 (2.5-14.3)	10.9 (3.6-21.2)	0.262
*, **Lymphocyte count (×10^9/L)*	2.1 (1.1-5.0)	2.7 (1.2-4.4)	2.6 (1.7-9.8)	0.164
*, **Platelet count (×10^9/L)*	304 (227-525)	280 (212-526)	370 (219-433)	0.312

*: Median (Min-Max)

**: During the attack

CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; MEFV: Mediterranean fever gene.

resistance to infections, thereby providing a biological basis for increased trigger sensitivity in PFAPA patients. Moreover, alterations in gut microbiota composition have been suggested to play a role in PFAPA pathogenesis by influencing mucosal immune responses and oral tolerance. In this context, approaches targeting the gut microbiota, including probiotic supplementation, may potentially help in modulating disease activity.[29]

When evaluated by trigger presence/absence or trigger type, no significant differences were observed in the cardinal PFAPA manifestations during attacks (oral aphthae, cervical lymphadenitis, tonsillitis). PFAPA shares some clinical features with FMF, such as recurrent fever, aphthous stomatitis, and abdominal pain, which may sometimes complicate the differential diagnosis. However, PFAPA attacks are usually shorter and exhibit more regular periodicity. MEFV gene variants may also contribute to overlapping inflammatory phenotypes between PFAPA and FMF.[30,31] In our previous study, these findings were also similar regardless of *MEFV* variation status or early response to colchicine.[32] These parallel results suggest that the core clinical phenotype of PFAPA may be largely independent of genetic factors, trigger history, and early treatment response. However, the significantly higher frequency of constipation during attacks in patients reporting

stress as a trigger suggests that stress may influence the gastrointestinal system through alterations in gut motility and neuroimmune interactions.[33] Therefore, although the fundamental clinical phenotype may remain unchanged, supportive interventions targeting stress-related triggers, particularly those potentially associated with additional symptoms such as constipation, should be considered in PFAPA management.

The main limitation of our study is its retrospective design, which risks recall bias, as trigger presence and type were based on patient or caregiver reports. For infectious triggers, mild symptoms could have been overlooked or misremembered. The absence of standardized trigger definitions may have introduced subjectivity in classification, and microbiological confirmation was not systematic in all patients. The relatively small number of patients in each trigger subgroup may also have limited the statistical power, and the observed difference in constipation should therefore be interpreted with caution. Despite these limitations, the study benefits from a large, well-characterized cohort and a systematic comparison of clinical features by trigger presence and type, thereby strengthening the reliability of our observations. The main strength of our study lies in being the first to examine attack triggers in children diagnosed with PFAPA syndrome.

In conclusion, our findings show that PFAPA's core clinical phenotype is largely independent of trigger type. However, higher constipation frequency in patients with stress-related triggers suggests additional symptoms may emerge in this subgroup. The shorter duration of breastfeeding in patients with triggers implies that early-life factors may influence trigger development. Infections, vaccinations, and certain foods were identified as triggers, highlighting the importance of evaluating trigger history in PFAPA's clinical management.

Ethical approval

This study has been approved by the Istanbul Medeniyet University Göztepe Training and Research Hospital (approval date 18/07/2024, number 725). Written informed consent was obtained from the participants.

Author contribution

The authors declare contribution to the paper as follows: Study conception and design: LK, FK, EK; data collection: ESYE, ZA, END, HKD; analysis and interpretation of results: FH, MÖB, UFÖ; draft manuscript preparation: LK, KÖ. 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. Hofer M, Pillet P, Cochard MM, et al. International periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis syndrome cohort: description of distinct phenotypes in 301 patients. Rheumatology (Oxford). 2014;53:1125-9. [Crossref]
2. Marshall GS, Edwards KM, Butler J, Lawton AR. Syndrome of periodic fever, pharyngitis, and aphthous stomatitis. J Pediatr. 1987;110:43-6. [Crossref]
3. Thomas KT, Feder HM, Lawton AR, Edwards KM. Periodic fever syndrome in children. J Pediatr. 1999;135:15-21. [Crossref]
4. Adrovic A, Yıldız M, Kanber M, et al. Performance of recently proposed periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis (PFAPA) syndrome criteria in a region endemic for familial Mediterranean fever. Rheumatol Int. 2020;40:91-6. [Crossref]
5. Brown KL, Wekell P, Osla V, et al. Profile of blood cells and inflammatory mediators in periodic fever, aphthous stomatitis, pharyngitis and adenitis (PFAPA) syndrome. BMC Pediatr. 2010;10:65. [Crossref]
6. Stojanov S, Lapidus S, Chitkara P, et al. Periodic fever, aphthous stomatitis, pharyngitis, and adenitis (PFAPA) is a disorder of innate immunity and Th1 activation responsive to IL-1 blockade. Proc Natl Acad Sci U S A. 2011;108:7148-53. [Crossref]
7. Kolly L, Busso N, von Scheven-Gete A, et al. Periodic fever, aphthous stomatitis, pharyngitis, cervical adenitis syndrome is linked to dysregulated monocyte IL-1β production. J Allergy Clin Immunol. 2013;131:1635-43. [Crossref]
8. Kucuk E, Senpolat S, Kaya F, et al. Reported triggers and self-reported management strategies in children with familial mediterranean fever. J Clin Rheumatol. 2026;32:1-7. [Crossref]
9. Parlar K, Ates MB, Onal ME, Bostancı E, Azman FN, Uğurlu S. Factors triggering familial mediterranean fever attacks, do they really exist? Intern Emerg Med. 2024;19:1007-13. [Crossref]
10. Karadag O, Tufan A, Yazisiz V, et al. The factors considered as trigger for the attacks in patients with familial Mediterranean fever. Rheumatol Int. 2013;33:893-7. [Crossref]
11. Levinsky Y, Butbul Aviel Y, Ahmad SA, et al. PFAPA flares observed during COVID outbreak: can emotional stress trigger PFAPA attacks? A multicenter cohort study. Pediatr Rheumatol Online J. 2022;20:46. [Crossref]

12. Galili T, Shaikh N, Khokhar A. et al. Microbes of the tonsils in PFAPA (Periodic Fever, Aphthous stomatitis, Pharyngitis and Adenitis) syndrome - a possible trigger of febrile episodes. Pediatr Infect Dis J. 2016;35:579-84.

13. Gattorno M, Hofer M, Federici S, et al. Classification criteria for autoinflammatory recurrent fevers. Ann Rheum Dis. 2019;78:1025-32. [Crossref]

14. Jeyaratnam J, Ter Haar NM, Lachmann H. et al. Genetic and phenotypic characteristics of 114 patients with mevalonate kinase deficiency. Pediatr Rheumatol. 2015;13:P25. [Crossref]

15. Stojanovich L, Marisavljevich D. Stress as a trigger of autoimmune disease. Autoimmun Rev. 2008;7:209-13. [Crossref]

16. Ploesser M, Silverman S, Diaz JDL, Zincke MT, Taylor MB. The link between traumatic stress and autoimmune rheumatic diseases: a systematic scoping review. Semin Arthritis Rheum. 2024;69:152558. [Crossref]

17. Manthiram K, Zhou Q, Aksentijevich I, Kastner DL. The monogenic autoinflammatory diseases define new pathways in human innate immunity and inflammation. Nat Immunol. 2017;18:832-42. [Crossref]

18. Masters SL, Simon A, Aksentijevich I, Kastner DL. Horror autoinflammaticus: the molecular pathophysiology of autoinflammatory disease. Annu Rev Immunol. 2009;27:621-68. [Crossref]

19. Petersen C, Round JL. Defining dysbiosis and its influence on host immunity and disease. Cell Microbiol. 2014;16:1024-33. [Crossref]

20. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. Cell. 2014;157:121-41. [Crossref]

21. Walker WA, Iyengar RS. Breast milk, microbiota, and intestinal immune homeostasis. Pediatr Res. 2015;77:220-8. [Crossref]

22. Ballard O, Morrow AL. Human milk composition: nutrients and bioactive factors. Pediatr Clin North Am. 2013;60:49-74. [Crossref]

23. Bode L. Human milk oligosaccharides: every baby needs a sugar mama. Glycobiology. 2012;22:1147-62. [Crossref]

24. Donovan SM, Comstock SS. Human milk oligosaccharides influence neonatal mucosal and systemic immunity. Ann Nutr Metab. 2016;69:42-51. [Crossref]

25. Rigante D, Candelli M. A potential link between outcome of Periodic Fever, Aphthous Stomatitis, Pharyngitis, Cervical Adenitis (PFAPA) syndrome in children and breastfeeding: a retrospective single-center cohort snapshot. Children (Basel). 2024;11:1559. [Crossref]

26. Oddy WH. Breastfeeding, childhood asthma, and allergic disease. Ann Nutr Metab. 2017;70:26-36. [Crossref]

27. Friedman NJ, Zeiger RS. The role of breast-feeding in the development of allergies and asthma. J Allergy Clin Immunol. 2005;115:1238-48. [Crossref]

28. Li WJ, Gao YC, Hu X, et al. Association between breastfeeding and the risk of autoimmune diseases: a systematic review and meta-analysis. Autoimmun Rev. 2025;24:103801. [Crossref]

29. Batu ED, Kaya Akca U, Basaran O, Bilginer Y, Özen S. Probiotic use in the prophylaxis of periodic fever, aphthous stomatitis, pharyngitis, and adenitis (PFAPA) syndrome: a retrospective cohort study. Rheumatol Int. 2022;42:1207-11. [Crossref]

30. Adrovic A, Sahin S, Barut K, Kasapcopur O. Familial Mediterranean fever and periodic fever, aphthous stomatitis, pharyngitis, and adenitis (PFAPA) syndrome: shared features and main differences. Rheumatol Int. 2019;39:29-36. [Crossref]

31. Yildiz M, Adrovic A, Ulkersoy I, et al. The role of Mediterranean fever gene variants in patients with periodic fever, aphthous stomatitis, pharyngitis, and adenitis syndrome. Eur J Pediatr. 2021;180:1051-8. [Crossref]

32. Koru L, Erten ESY, Dursun HK, et al. Colchicine treatment in PFAPA: how long should we wait for the clinical response? Eur J Pediatr. 2025;184:322. [Crossref]

33. Qin HY, Cheng CW, Tang XD, Bian ZX. Impact of psychological stress on irritable bowel syndrome. World J Gastroenterol. 2014;20:14126-31. [Crossref]

Trends in Pediatrics 2025;6(4):275-278
DOI: 10.59213/TP.2025.226

Case Report

Sinus vein thrombosis requiring cranial decompression after oral contraceptive use in an adolescent girl

Emine Yılmaz Orulluoğlu[1], Esra Serdaroğlu[2], Merve Yazol[3], Pelin Kuzucu[4], Zühre Kaya[1]

[1]Department of Pediatric Hematology, Faculty of Medicine, Gazi University, Ankara, Türkiye
[2]Department of Pediatric Neurology, Faculty of Medicine, Gazi University, Ankara, Türkiye
[3]Department of Pediatric Radiology, Faculty of Medicine, Gazi University, Ankara, Türkiye
[4]Department of Neurosurgery, Faculty of Medicine, Gazi University, Ankara, Türkiye

Cite this article as: Yılmaz Orulluoğlu E, Serdaroğlu E, Yazol M, Kuzucu P, Kaya Z. Sinus vein thrombosis requiring cranial decompression after oral contraceptive use in an adolescent girl. Trends in Pediatrics 2025;6(4):275-278.

ABSTRACT

Although sinus vein thrombosis is rare in childhood, drugs play an important role in the etiology. Sinus vein thrombosis should be excluded with urgent imaging in the presence of headache, weakness, and papilledema lasting longer than 1 week, especially in adolescent girls who require hormonal medication due to oligomenorrhoea. If thrombosis is detected in these cases, urgent surgical intervention will be a life-saving approach if the thrombosis and infarct area in the brain are widespread and lead to herniation. We discussed the recovery of a 17-year-old obese girl after the use of oral contraceptives due to menstrual irregularity, the place of surgical approach in the treatment of thrombosis, and the recovery of shifting and herniation due to diffuse sinus vein thrombosis after emergency decompressive surgery. Consent was obtained from the patient's parents beforehand.

Keywords: adolescent, cranial decompression, hormone, sinus vein thrombosis

INTRODUCTION

Cerebral sinus vein thrombosis (SVT) is an uncommon complication in children, with a prevalence of 0.4-0.7 per 100,000, but it can cause morbidity and mortality if not diagnosed and treated early.[1] While infections are the most common cause of SVT, cardiac diseases, renal diseases, malignancies, and drugs are other causes.[1,2]

It is difficult to recognize SVT because of the diversity of clinical symptoms and findings. The superior sagittal sinus, transverse sinus, and sigmoid sinus are most commonly affected.[3] Patients may present with different clinical pictures, including headache, confusion, seizure, focal neurological findings, encephalopathy, and seizure depending on the localization of the affected brain. Anticoagulant treatment may not be adequate, especially in the presence of rapidly changing findings such as clouding of consciousness and a decrease in coma scale, depending on the extent of thrombus and infarction; emergency surgical intervention may be planned in cases to prevent an increase in intracranial pressure, shifting, and herniation.[1]

We report the successful management of life-threatening SVT after oral contraceptive (OCS) use in an adolescent girl with emergency surgical decompression and anticoagulant therapy.

✉ Emine Yılmaz Orulluoğlu ▪ emine07@windowslive.com

Received: 14.12.2024 **Accepted:** 26.07.2025

CASE REPORT

A 17-year-old female patient presented to the emergency department with complaints of severe headache for 1 week and slowed movements and left extremity weakness that started 1 day ago. Physical examination revealed a body mass index of 26 kg/m^2, an obese appearance, and signs of hirsutism. In vital signs, blood pressure was 120/75 mmHg, pulse rate was 70 beats/minute, and temperature was 36.7°C. Neurological examination revealed consciousness and central facial paralysis on the right, muscle strength was 2/5 in the left lower and upper extremities, and other system examinations were normal. Glasgow Coma Scale was 10. Papilledema was detected. Laboratory findings included hemoglobin: 10.2 g/dL, MCV: 83.5 fL, platelet count: 13,000/mm^3, WBC: 3350/mm^3, and absolute neutrophil count: 1750/mm^3, sodium: 135, potassium: 3.8, creatinine: 0.68. Prothrombin time: 13 seconds, D-Dimer: 3.3 was high. Brain tomography performed at an external center revealed no pathology. Magnetic resonance imaging (MRI) of the brain performed in our center at his presentation to us with complaints of increased headache and weakness revealed changes compatible with diffuse thrombus in the right transverse sinus, sigmoid sinus and jugular vein (Figure 1), signal increase and oedema compatible with venous hemorrhagic ischemia in the supratentorial right temporal, right basal ganglia and thalamic region (Figure 2), and changes suggestive of right to left shifting and uncal herniation. When her history was questioned, it was learnt that she had no previous thrombosis and no known systemic disease, and she had been using OCS containing 0.02 mg ethinylestradiol and 3 mg drospirenone for 1 year due to oligomenorrhoea. The patient underwent anterior temporal lobectomy and thrombectomy with urgent surgical decompression due to diffuse thrombus, shifting, and herniation findings, and altered consciousness on brain MRI (Figure 2)

Anti-nuclear antibody, perinuclear anti-cytoplasmic antibody, anti-cytoplasmic antibody, anticardiolipin antibody IgM and IgG, and Lupus anticoagulant were found to be negative. Antithrombin III, Protein S, and Protein C

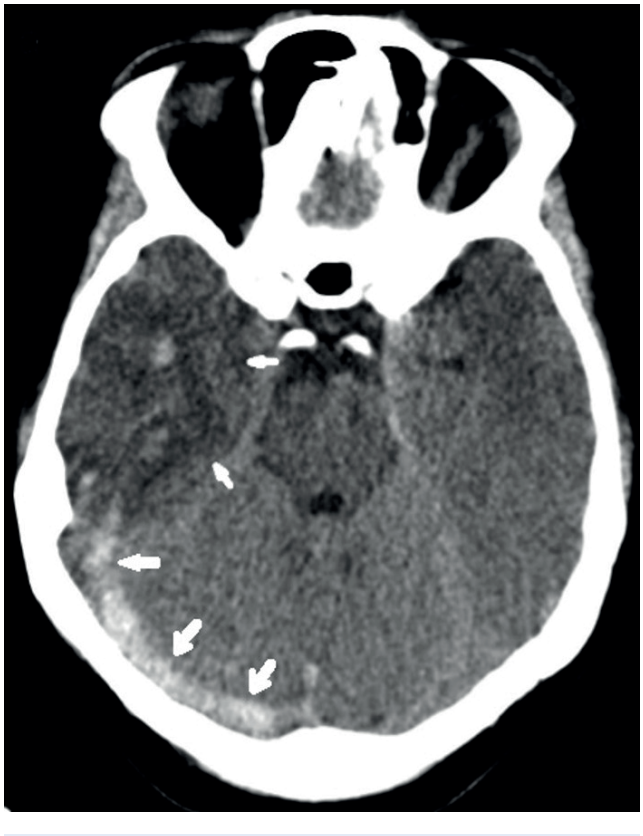


Figure 1. Diffuse thrombus in the right transverse sinus, sigmoid sinus, and jugular vein

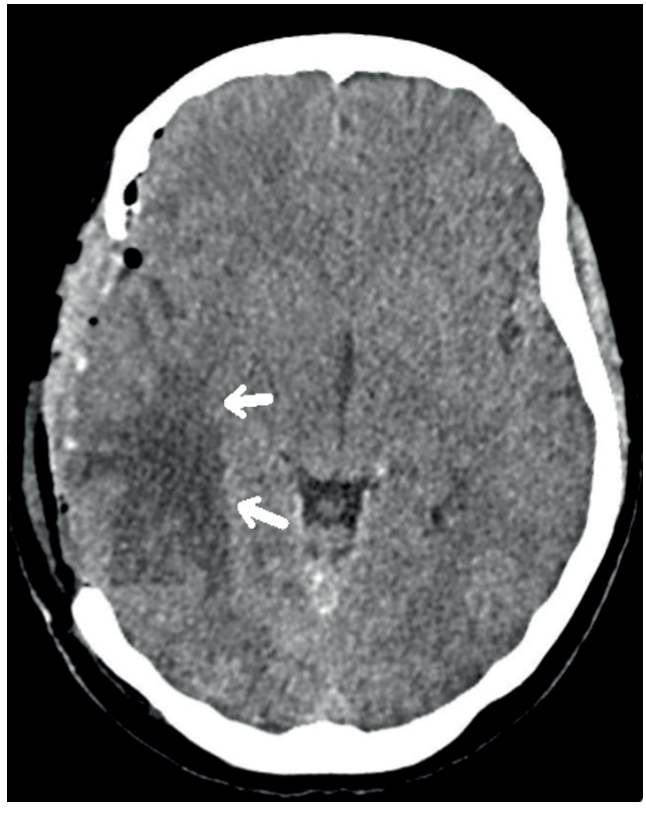


Figure 2. Postoperative imaging and edema areas of the patient who underwent anterior temporal lobectomy and thrombectomy

levels were normal. Factor V Leiden and prothrombin 20210A mutations were not detected. Low molecular weight heparin (LMWH) was started with the diagnosis of right lateral SVT. The patient was intubated for 24 hours after the operation and was conscious after extubation. Left upper extremity muscle strength was 1/5. On the fifth postoperative day, it was observed that the upper extremity muscle strength of the patient who was taken to physical rehabilitation increased significantly (4/5). On the 10th day of anticoagulant treatment, the patient's complaints improved almost completely, and the patient was discharged with an outpatient follow-up planned. No recurrence of SVT was observed during prophylactic anticoagulant treatment for six months.

DISCUSSION

Although SVT may be observed at all ages, it is most commonly observed in the neonatal and young adult age group. It is 3 times more common in women.[4] It is observed secondary to prothrombotic conditions, including pregnancy, puerperium, and OCS use.[5-8] In patients with hereditary thrombophilia risk factors, triggering factors, including systemic diseases, trauma, drug use (including hormones and steroids), infection, and malignancies predispose to thrombosis formation.[9] OCSs have been reported to increase the risk of thromboembolism. In this article, the development of life-threatening SVT in an obese adolescent girl after OCS use and its correction with decompressive surgery is presented because it is a rare case.

When focal neurological symptoms and signs develop in the presence of new-onset severe headache and seizures in children, SVT should be considered, especially if risk factors are present, and urgent investigations and treatments should be planned for the diagnosis. In a study by Gosk-Bierska et al. the most common symptom was headache, with a rate of 87%, and the most common examination finding was papilledema, with a rate of 55%.[10] The most commonly used radiological examinations for the diagnosis of cerebral thrombosis are brain MRI and MR venography.[4] Brain computed tomography (CT) is an easily accessible imaging modality, but its role in the diagnosis of SVT is limited. In 25% of patients, neurological examinations may be completely normal, and CT may be normal.[11] In the present case, although no pathology was detected initially on brain tomography performed after presentation with severe headache and weakness lasting 1 week, the presence of papilledema indicated that MRI was more useful in detecting thrombus.

Although SVT is rare, it may result in high mortality and morbidity in the absence of early diagnosis and treatment. The presence of increased intracranial pressure and venous infarcts accompanying diffuse thrombus is a dangerous picture, and the patient may die within hours due to herniation. The presence of impaired consciousness and cerebral hemorrhage are considered to be markers of poor prognosis. The priority is to stabilize the patient and prevent or reverse cerebral herniation.[12,13] For this purpose, head elevation, sedation, administration of mannitol or 3% NaCl, and hyperventilation are emergency approaches.[14] If herniation cannot be prevented despite these, surgical removal of hemorrhagic infarction or decompressive hemicraniectomy may be required in patients with unilateral hemispheric lesions with the expectation of a better functional recovery.[15] In this case, emergency decompressive surgery and thrombectomy were preferred in the first stage because the cause of increased intracranial pressure was diffuse thrombosis, and shifting and herniation were found on brain MRI. In addition, anticoagulant and thrombolytic therapies are used in the medical treatment of cerebral vein thrombosis.[16] In clinical practice, the use of anticoagulants such as intravenous heparin or LMWH may contribute to the control of intracranial pressure. In different studies, LMWH treatment monitored with anti-factor Xa levels has been shown to be safe. LMWH, which was started after surgery in our patient, led to rapid clinical improvement and discharge in a short time. No recurrence of SVT was observed during prophylactic anticoagulant treatment for six months.

The importance of imaging modalities such as MR and MR Venography at the time of clinical suspicion, even if brain tomography is normal in patients with headache, is seen in this case. There is a need for studies with more case series in the recognition and follow-up of cerebral thrombosis cases.

In conclusion, in the presence of clinical findings such as severe headache lasting more than 1 week, altered consciousness, and papilledema in obese adolescent girls requiring hormonal drug use, urgent imaging should be planned for early diagnosis of SVT, and the decision for medical or urgent surgical treatment should not be delayed.

Ethical approval

No ethics committee was obtained for the case report, and informed consent was obtained from the patient and her family.

Author contribution

The authors declare contribution to the paper as follows: Study conception and design: EYO, ZK; data collection: ES, MY; analysis and interpretation of results: PK, EY, ZK; draft manuscript preparation: MY. 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. Kaya Z. Cerebral venous sinus thrombosis. In: Orhaner B, editor. Thrombosis in Childhood. 1st ed. Ankara: Türkiye Clinics; 2020. p. 79-85.
2. Guzelkucuk Z, Karapınar DY, Gelen SA, et al. Central nervous system thrombosis in pediatric acute lymphoblastic leukemia in Turkey: A multicenter study. Pediatr Blood Cancer. 2023;70:e30425. [Crossref]
3. Renowden S. Cerebral venous sinus thrombosis. Eur Radiol. 2004;14:215-26. [Crossref]
4. Ehtisham A, Stern BJ. Cerebral venous thrombosis: a review. Neurologist. 2006;12:32-8. [Crossref]
5. Bousser MG, Chiras J, Bories J, Castaigne P. Cerebral venous thrombosis-a review of 38 cases. Stroke. 1985;16:199-213. [Crossref]
6. Dzialo AF, Black-Schaffer RM. Cerebral venous thrombosis in young adults: 2 case reports. Arch Phys Med Rehabil. 2001;82:683-8. [Crossref]
7. Allroggen H, Abbott RJ. Cerebral venous sinus thrombosis. Postgrad Med J. 2000;76:12-5. [Crossref]
8. Lanska DJ, Kryscio RJ. Stroke and intracranial venous thrombosis during pregnancy and puerperium. Neurology. 1998;51:1622-8. [Crossref]
9. Saposnik G, Barinagarrementeria F, Brown RD, et al. Diagnosis and management of cerebral venous thrombosis: a statement for healthcare professionals from the American Heart Association/ American Stroke Association. Stroke. 2011;42:1158-92. [Crossref]
10. Gosk-Bierska I, Wysokinski W, Brown RD, et al. Cerebral venous sinus thrombosis: Incidence of venous thrombosis recurrence and survival. Neurology. 2006;67:814-9. [Crossref]
11. Kaya D. Diagnosis and treatment of cerebral venous sinus thrombosis. Turk J Neurol. 2017;23:94-104. [Crossref]
12. Kimber J. Cerebral venous sinus thrombosis. QJM. 2002;95:137-42. [Crossref]
13. Bushnell C, Saposnik G. Evaluation and management of cerebral venous thrombosis. Continuum (Minneap Minn). 2014;20:335-51. [Crossref]
14. Fischer C, Goldstein J, Edlow J. Cerebral venous sinus thrombosis in the emergency department: retrospective analysis of 17 cases and review of the literature. J Emerg Med. 2010;38:140-7. [Crossref]
15. Filippidis A, Kapsalaki E, Patramani G, Fountas KN. Cerebral venous sinus thrombosis: review of the demographics, pathophysiology, current diagnosis, and treatment. Neurosurg Focus. 2009;27:E3. [Crossref]
16. Duman T, Uluduz D, Midi I, et al. A multicenter study of 1144 patients with cerebral venous thrombosis: the VENOST study. J Stroke Cerebrovasc Dis. 2017;26:1848-57. [Crossref]

Trends in Pediatrics 2025;6(4):279-283
DOI: 10.59213/TP.2025.229

Case Report

COVID-19 related mucormycosis and hemophagocytic lymphohistiocytosis in a child with juvenile myelomonocytic leukemia

Kübra Aslan[1], Hatice Beyza Akkan[2], Fırat Özçelik[3], Kerem Kökoğlu[4], Alper Özcan[2], Can Acıpayam[5], Ayşenur Paç Kısaarslan[6], Benhur Şirvan Çetin[7], Özlem Canöz[8], Munis Dündar[3], Halit Canatan[1], Ekrem Ünal[2,9,10]

[1]Department of Medical Biology, Betül-Ziya Eren Genome and Stem Cell Center, Erciyes University, Kayseri, Türkiye
[2]Division of Pediatric Hematology, Department of Pediatrics, School of Medicine, Erciyes University, Kayseri, Türkiye
[3]Department of Medical Genetics, School of Medicine, Erciyes University, Kayseri, Türkiye
[4]Department of Otorhinolaryngology, School of Medicine, Erciyes University, Kayseri, Türkiye
[5]Division of Pediatric Hematology, Department of Pediatrics, School of Medicine, Sütçü İmam University, Kahramanmaraş, Türkiye
[6]Division of Pediatric Rheumatology, Department of Pediatrics, School of Medicine, Erciyes University, Kayseri, Türkiye
[7]Division of Pediatric Infectious Disease, Department of Pediatrics, School of Medicine, Erciyes University, Kayseri, Türkiye
[8]Department of Pathology, School of Medicine, Erciyes University, Kayseri, Türkiye
[9]Medical Point Hospital Pediatric Hematology and Oncology Clinic, Gaziantep, Türkiye
[10]School of Health Sciences, Hasan Kalyoncu University, Gaziantep, Türkiye

Cite this article as: Aslan K, Akkan HB, Özçelik F, et al. COVID-19 related mucormycosis and hemophagocytic lymphohistiocytosis in a child with juvenile myelomonocytic leukemia. Trends in Pediatrics 2025;6(4):279-283.

ABSTRACT

Juvenile myelomonocytic leukemia (JMML) is a childhood hematological cancer that often results from mutations in the PTPN11 gene. This study aims to report an original proband with JMML, COVID-19, HLH, and mucormycosis phenotypes.

In JMML patients, secondary HLH associated with SARS-CoV-2 and mucor infection has not been reported in the literature. We identified a missense variant c.226G>C (p. Glu76Gln) (NM_001330437) in *PTPN11*.

The patient suffered from SARS-CoV-2-related secondary Hemophagocytic lymphohistiocytosis (HLH) and mucormycosis in the right eye and the synoidal ethmoidal sinuses, which eventually led to the patient's death due to cardiopulmonary arrest.

We report a child with JMML complicated with COVID-19-related secondary HLH and mucormycosis, which underlines the need for effective and urgent treatment to reduce further mortalities.

Keywords: JMML, secondary HLH, mucor, zygomycosis, COVID-19, *PTPN11* mutation

INTRODUCTION

Juvenile myelomonocytic leukemia (JMML) is a rare pediatric hematopoietic malignancy frequently associated with *PTPN11* gene mutations, which dysregulate the RAS/MAP-K signaling pathway and drive clonal proliferation. Splenomegaly, hepatomegaly with lymphadenopathy, pallor and skin rash, thrombocytopenia, and anemia are common symptoms of JMML. Allogeneic hematopoietic

✉ Ekrem Ünal ▪ ekremunal@erciyes.edu.tr

Received: 23.12.2024 **Accepted:** 17.09.2025

stem cell transplantation remains the primary curative therapy.[1]

Mutations in the *RAS* signaling pathway genes may lead to JMML.[2] Mutations in *PTPN11* are also frequently found in 35% of JMML patients. The *PTPN11* gene is located on chromosome 12q24.13 and spans 91.568 bp.[3] The *PTPN11* gene encodes a phosphatase named Src Homology 2 domain–containing protein tyrosine Phosphatase-2 (SHP-2). SHP-2 functions in the signal transduction of several cytokine and growth factor receptors, such as GM-CSF and IL-3, and positively regulates the RAS/MAPK signaling pathway. Somatic *PTPN11* mutations render myeloid progenitor cells to become more sensitive to GMCSF, which leads to clonal expansion of monocytic and macrophagic cells in blood and bone marrow.[4]

Hemophagocytic lymphohistiocytosis (HLH), a severe inflammatory syndrome, can be primary (genetic) or secondary, often triggered by infections, malignancies, or autoimmune conditions.[5]

Given their compromised immune status, patients with underlying hematologic conditions like JMML are particularly susceptible to developing secondary HLH, especially when faced with infections such as SARS-CoV-2. Concurrent opportunistic infections, like mucormycosis, further complicate these cases.[6] This report details a unique case of a child with JMML and a somatic *PTPN11* mutation who developed severe COVID-19-related secondary HLH complicated by mucormycosis, ultimately leading to a fatal outcome. This case highlights the complex and often lethal interplay between these conditions, underscoring the critical need for prompt and aggressive management.

CASE REPORT

Necessary information was given to the patient and her family for the study, and a consent form was obtained. A 4-year-old Syrian immigrant girl was admitted to the hospital due to complaints of fever, cough, and runny nose. Depending on the thrombocytopenia and low hemoglobin level, she was referred to the pediatric hematology and oncology clinic. At physical examination, she had fever, abdominal distension, and the liver was 3-4 cm palpable. Complete blood count revealed Hemoglobin:7,8 gr/dl, Platelet:14000/mm^3 Leukocyte:6450/mm^3 Lymphocyte:3170/mm^3, Absolute Neutrophil Count: 650/mm^3. Other Laboratory test results showed elevated CRP (45 mg/L) and procalcitonin (3.9 µg/mL), reduced fibrinogen (95 mg/dL). Other parameters were as follows: INR: 1,29, d-dimer:1570, Antithrombin 3: 20 T/D, bilirubin:1,5/0,8, Albumin: 1,9, and Reticulocyte:1%. Coombs's test was negative. CD panel cells was within normal range: CD3: 2044 cells/cm^2, CD4: 912 cells/cm^2, CD8:1091 cells/cm^2, CD16/56:240.9 cells/cm^2, CD19:1335 cells/cm^2. CD4/CD8 ratio was decreased (CD4/CD8: 40/57). Antibody isotypes were within normal range. IgA:107 mg/dl, IgM:101 mg/dl, IgG:1452 mg/dl, IgM: 46.5 mg/dl (Table 1). She was treated with frozen plasma, erythrocyte suspension, and thrombocyte suspension, and was given Cefepime because of high fever. Because of persistent thrombocytopenia and anemia, Bone marrow aspiration was carried out twice. To assess the cytotoxicity of natural killer cells and rule out primary HLH, a cytotoxicity assay using a cell-tracking dye was performed.

Because of persistent fever, a SARS-CoV-2 PCR test was performed, and the result returned positive. The patient

Table 1. Criteria at Diagnosis of HLH

Criteria	At Diagnosis	Reference Value
1. Fever	Yes	
2. Splenomegaly	Yes	
3. Cytopenia (>2)		
Hemoglobin	8,3 g/dl	12-16 g/dl
Platelets	7x10^3 ul	130-400x10^3 ul
4. Hypertriglyceridemia and/or hypofibrinogenemia		
Tryglicerides	133 mg/dl	40-130 mg/dl
Fibrinogen	161 mg/dl	180-350 mg/dl
5 Hemophagocytosis	Yes	
6. Ferritin	140 ng/ml	4-67 ng/ml

was referred to the pediatric infectious disease clinic. The patient was suspected of having hemophagocytosis, and intravenous immunoglobulin (IVIG) was administered at a dose of 2 g/kg. Additionally, methylprednisolone was given at a dose of 30 mg/kg/day (for 3 days) and continued as 2 mg/kg.

COVID-19-related pneumonic infiltration was observed in her lungs. Skin petechial rash and hepatosplenomegaly appeared in the patient. Bacterial reproduction was not observed in the blood and urine. Despite the replacement therapy, thrombocytopenia and anemia continued. Therefore, bone marrow aspiration was examined once again. Hemophagocytosis was observed in the bone marrow aspirate (Figure 1a).

In vitro NK cytotoxicity test results were comparable between the patient and the healthy control (Figure 1b).

To identify the underlying genetic defect, clinical-exome sequencing was performed at the Erciyes University School of Medicine Department of Medical Genetics.

Molecular analyses revealed a missense variant c.226G>C (p. Glu76Gln) (NM_001330437) in *PTPN11.* The variant fraction was 17%. Given the patient's clinical presentation with JMML, the use of a bone marrow sample, the low variant fraction, and the known pathogenicity of the variant, this alteration was considered a somatic variant contributing to the patient's phenotype (Figure 1c). According to clinical exome sequencing results, the case was diagnosed as JMML.

In follow-up, pain in the right eye, redness, and edema around the eye developed. Necrotic areas were observed on intranasal evaluation, and the patient was operated on urgently for invasive fungal rhinosinusitis (Figure 1d) (Video). The patient was re-operated on three days later again due to the progression of necrosis to the facial skin and nasal septum (Figure 1e). The pathological examination of the patient's nasal tissues revealed fungal hypha growth (Figure 1f). Based on the clinical findings, treatment with liposomal amphotericin B was initiated empirically. Mucorales were isolated from the samples obtained. Despite two surgeries, the infection could not be

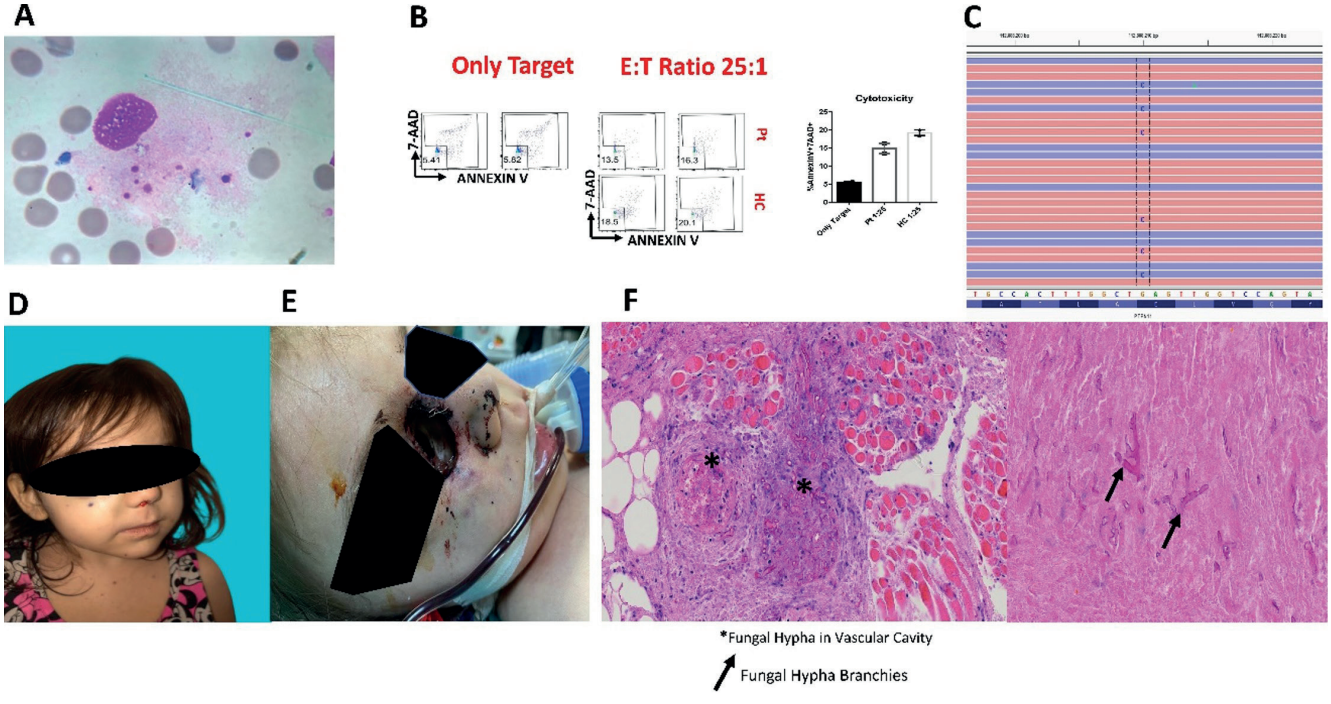


Figure 1. Patient presents with JMML phenotype, also with HLH and mucormycosis. **(a)** HLH was observed on bone marrow aspiration **(b)** Peripheral blood mononuclear cells isolated from the patients and healthy control showed comparable cytotoxicity to the target cells. (HC: Healthy Control, Pt: Patient) **(c)** Whole Exome Sequencing revealed a somatic missense mutation in the *PTPN11* gene **(d)** Patient's facial appearance before the ethmoidectomy, septectomy, and periorbital debridement operation **(e)** Patient's facial appearance after the operation **(f)** Fungal hypha in her nasal tissue; * symbol represents fungal hypha in vascular cavity and hypha branches indicated with ↗ symbol.

controlled and worsened, so posaconazole was added to the treatment. The general condition deteriorated during follow-up, and the patient died eight days after the onset of ocular findings and seven days after the first operation.

DISCUSSION

JMML is a rare but lethal disease, especially in early childhood, with an annual incidence of 1.2 per million children. The majority of JMML patients harbor mutations in *PTPN11, NF1, KRAS,* or *NRAS*. While somatic *PTPN11* mutations may cause JMML, the germline mutations may lead to Noonan syndrome. The symptoms and phenotype of Noonan syndrome are quite different than those of JMML.[6] Noonan syndrome is characterized by small stature, pulmonary valve stenosis, hypertelorism, mild intellectual disability, ptosis, and skeletal malformations. JMML usually presents in early childhood (2-4 years) with a male predominance. JMML occurs due to clonal proliferation of transformed hematopoietic progenitors and is marked with expansion of granulocytic and monocytic lineages.[1] In affected children with JMML, skin rash, pallor, cough, hepatomegaly, or splenomegaly are observed.[6] Our patient did not present with Noonan syndrome symptoms. Accordingly, clinical exome sequencing data revealed a somatic variant in the *PTPN11* gene, c.226G>C (p. Glu76Gln). The variant fraction was 17%. By the time the result was reported, the patient had died; therefore, no confirmatory study could be performed. Consistent with the *PTPN11* defect, she has thrombocytopenia, anemia, and hepatosplenomegaly.

HLH is a life-threatening disorder in children and adults. The primary or familial (F)HLH results from genetic defects. Mutations in *PRF1, UNC13, STX11, STXBP2* genes may cause F-HLH.[7] Molecular genetic analyses revealed no pathogenic variant in the genes associated with familial HLH. Secondary HLH may be observed due to malignancy, hematologic disease, or viral infections.[8] Prevalence of SARS-CoV-2 among pediatric cancer patients has been predicted as 1.3% which is higher than other pediatric populations.[9] Having a hematologic malignancy, such as leukemia, lymphoma, or having had hematopoietic stem cell transplantation, may increase the susceptibility of pediatric patients to COVID-19 infection.[9] Our patient showed secondary HLH related to SARS-CoV-2 infection. The observation that *in vitro* NK cell cytotoxicity was comparable between the patient and the healthy control supports this hypothesis. To treat HLH, IVIG and methylprednisolone were given. However, due to JMML, the treatment was ineffective.

Zygomycosis (Mucormycosis) infection has increased over the last decade and is particularly common among patients with hematologic malignancies or who have undergone HSCT.[10] Invasive fungi were known to be opportunistic pathogens causing sinusitis, rhinonasal infections.[11] The patient had swelling and a rash on the right eye, consistent with mucormycosis related to COVID-19. Her ethmoidal and sphenoidal sinuses were affected by mucor, and she also had necrotic lesions in her skin. Treatment approaches of mucormycosis depend on antifungal agents such as liposomal amphotericin B, Posaconazole, or other azole compounds.[12] She was treated with liposomal amphotericin B and posaconazole, but her clinical course did not improve. Our patient, to the best of our knowledge, is the first patient in the literature with mucor infection and COVID-19-related secondary HLH in a JMML case.

CONCLUSION

In conclusion, the coexistence of secondary HLH and mucor infection may be fatal for children with JMML. In our patient, SARS-CoV-2 infection contributed to a complicated clinical course with mucormycosis, HLH, and JMML. Secondary HLH due to SARS-CoV-2 infection, combined with the underlying somatic genetic defect in *PTPN11* and the concurrent presence of other causative infectious agents such as Mucormycosis became lethal.

Acknowledgements

The authors thank to Dr. Ahmet Eken for critical review of the manuscript.

Ethical approval

This study has been approved by the Ethics Committee of Erciyes University (approval date 06.01.2021, number 2021/17). Written informed consent was obtained from parents of the patient.

Author contribution

The authors declare contribution to the paper as follows: Study conception and design: EU, KA; data collection: KA, FO, KK, AO, CA, AK, BSC, OC; analysis and interpretation of results: KA, HBA, EU, MD, HC; draft manuscript preparation: KA, HBA. All authors reviewed the results and approved the final version of the article.

Source of funding

This work was supported partly by the Erciyes University BAP grant, TCD-2019-8751 to EU.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

1. Locatelli F, Algeri M, Merli P, Strocchio L. Novel approaches to diagnosis and treatment of Juvenile Myelomonocytic Leukemia. Expert Rev Hematol. 2018;11:129-43. [Crossref]
2. Stieglitz E, Taylor-Weiner AN, Chang TY, et al. The genomic landscape of juvenile myelomonocytic leukemia. Nat Genet. 2015;47:1326-33. [Crossref]
3. PTPN11 Gene - GeneCards | PTN11 Protein | PTN11 Antibody. Available at: https://www.genecards.org/cgi-bin/carddisp.pl?gene=PTPN11 (Accessed on Jan 10, 2022).
4. Lachenaud J, Strullu M, Baruchel A, Cavé H. Juvenile myelomonocytic leukemias. Bull Cancer. 2014;101:302-13. [Crossref]
5. Cansever M, Zietara N, Chiang SCC, et al. A rare case of Activated Phosphoinositide 3-Kinase Delta Syndrome (APDS) presenting with hemophagocytosis complicated with hodgkin lymphoma. J Pediatr Hematol Oncol. 2020;42:156-9. [Crossref]
6. Alfayez M, Issa GC, Patel KP, et al. The clinical impact of PTPN11 mutations in adults with acute myeloid leukemia. Leukemia. 2021;35:691-700. [Crossref]
7. Akyol S, Ozcan A, Sekine T, et al. Different clinical presentation of 3 children with familial hemophagocytic lymphohistiocytosis with 2 novel mutations. J Pediatr Hematol Oncol. 2020;42:e627-9. [Crossref]
8. Abuzaid O, Akyol S, Alcalı SC, Unal E. Hemophagocytic lymphohistiocytosis: pouring gasoline on the cytokine storm. Kastamonu Med J. 2022;2:30-4. [Crossref]
9. Kebudi R, Kurucu N, Tuğcu D, et al. COVID-19 infection in children with cancer and stem cell transplant recipients in Turkey: A nationwide study. Pediatr Blood Cancer. 2021;68:e28915. [Crossref]
10. Celkan T, Kizilocak H, Evim M, et al. Hepatosplenic fungal infections in children with leukemia-risk factors and outcome: a multicentric study. J Pediatr Hematol Oncol. 2019;41:256-60. [Crossref]
11. Böhme A, Ruhnke M, Buchheidt D, et al. Treatment of fungal infections in hematology and oncology-guidelines of the Infectious Diseases Working Party (AGIHO) of the German Society of Hematology and Oncology (DGHO). Ann Hematol. 2003;82(Suppl 2):S133-40. [Crossref]
12. Greenberg RN, Scott LJ, Vaughn HH, Ribes JA. Zygomycosis (mucormycosis): emerging clinical importance and new treatments. Curr Opin Infect Dis. 2004;17:517-25. [Crossref]

Trends in Pediatrics 2025;6(4):284-288
DOI: 10.59213/TP.2025.252

Case Report

Next generation sequencing identifies a novel variant in the *NR5A1* gene in a 46,XY female with complete gonadal dysgenesis

Ömer Günbey[1], İhsan Esen[1], Firdevs D. Paksoy[1], Deniz Ökdemir[1]

[1]Division of Endocrinology, Department of Pediatrics, School of Medicine, Fırat University, Elazığ, Türkiye

Cite this article as: Günbey Ö, Esen İ, Paksoy FD, Ökdemir D. Next generation sequencing identifies a novel variant in the *NR5A1* gene in a 46, XY female with complete gonadal dysgenesis. Trends in Pediatrics 2025;6(4):284-288.

ABSTRACT

To report a novel variant in the *NR5A1* gene as a cause of 46,XY complete gonadal dysgenesis (Swyer syndrome). A 12.5-year-old prepubertal girl presented with the complaint of short stature and was evaluated in our clinic after pelvic ultrasonography revealed an absent uterus and ovaries. In pubertal examination, she was at Tanner stage I, had a fully female phenotype, and no clitoromegaly. Gonads were not palpable on examination. Laboratory investigations, including hemogram and biochemical tests, were normal. Celiac antibodies were negative, and thyroid function tests were within normal limits. Patient's serum LH level was 11.7 mIU/mL, FSH 81.3 mIU/mL, estradiol <15 pg/mL, total testosterone <7.0 ng/dL, DHEA-S 64.4 µg/dL, ACTH 18.1 pg/mL, and cortisol 13.4 µg/dL. Magnetic resonance imaging revealed a hypoplastic uterus in a band-like shape, with no visible ovaries. No appearance consistent with testicular tissue was identified. Karyotype analysis revealed a 46,XY pattern. Estrogen therapy was initiated for the patient. No pathogenic variant was detected in the *SRY* gene analysis. As part of the molecular analysis using the next-generation sequencing (NGS) method, a heterozygous p.V15L (c.43G>T) variant was detected in the *NR5A1* gene (NM_004959.5). A total of 26 genes were screened as part of this panel, including *DHX37, SRY, WT1, DHH, ZFPM2, PPP1R12A, NR5A1, GTF2H5, MAP3K1, HSD17B4, SOX9, DMRT3, NR0B1, and GATA4*. In summary, this case report describes a novel heterozygous *NR5A1* variant identified in an adolescent with 46,XY complete gonadal dysgenesis (Swyer syndrome).

Keywords: *NR5A1* gene, 46,XY complete gonadal dysgenesis, Swyer syndrome

INTRODUCTION

First described in 1955, 46,XY complete gonadal dysgenesis (Swyer Syndrome) is a rare disorder of sex development (DSD).[1,2] Its estimated prevalence is approximately 1 in 80,000 live births.[1] The etiology of 46,XY complete gonadal dysgenesis has been associated with variants in several genes, including *DHH, DHX37, DMRT1, LHX9, MAP3K1, NR5A1, SOX8, SOX9, SRY, and ZFPM2*.[3] The *nuclear receptor subfamily 5 group A member 1 (NR5A1)* gene, also known as *steroidogenic factor-1 (SF-1)*, encodes a transcription factor belonging to the nuclear receptor superfamily.[4,5] It is highly expressed in tissues where steroid synthesis occurs, particularly in the gonads and adrenal glands.[5]

During bipotential gonadal development, NR5A1 plays a critical role in promoting regression of Müllerian structures in 46,XY individuals by activating anti-müllerian hormone (AMH) secretion.[5,6] Additionally, it induces testosterone production in Leydig cells, facilitating the virilization of external genitalia and testicular descent.[5,7] Variants in *NR5A1* have been reported in individuals with disorders of sex development (DSD) affecting both 46,XY and 46,XX individuals during the neonatal or pubertal periods.[5] The first *NR5A1* variant was identified in 1999 in a 46,XY individual with adrenal insufficiency and a female phenotype.[8] Subsequent studies have demonstrated that *NR5A1* variants can lead to 46,XY DSD without adrenal insufficiency.[9]

✉ Ömer Günbey ▪ omergunbey50@gmail.com

Received: 21.02.2025 **Accepted:** 22.07.2025

Here, we present a previously unreported variant in the *NR5A1* gene in a patient diagnosed with 46,XY complete gonadal dysgenesis without adrenal insufficiency.

CASE REPORT

A 12.5-year-old prepubertal girl presented with the complaint of short stature and was evaluated in our clinic after pelvic ultrasonography revealed an absent uterus and ovaries. Her medical history revealed that she was born at full term via normal spontaneous vaginal delivery with a birth weight of 3300 grams, and her mental and motor development was appropriate for her age. There was a consanguinity between her parents, who are first cousins. She had a healthy younger brother who was three years younger.

On physical examination, vital signs were normal. Her height was 143.7 cm (-1.80 SDS), weight 33.8 kg (-1.88 SDS), and body mass index (BMI) 16.4 kg/m² (-1.29 SDS). Systemic examination findings were normal. On pubertal examination, Tanner stage was I, and the patient's External Masculinization Score (EMS) was 0; the external genitalia had a completely female appearance. Gonads could not be palpated on examination.

Laboratory investigations, including hemogram and biochemical tests, were normal. Celiac antibodies were negative, and thyroid function tests were within normal limits. Patient's serum LH level was 11.7 mIU/mL, FSH 81.3 mIU/mL, estradiol <15 pg/mL, total testosterone <7.0 ng/dL, DHEA-S 64.4 µg/dL, ACTH 18.1 pg/mL, and cortisol 13.4 µg/dL (Table 1). Because the patient's basal cortisol level was above 10 µg/dL, we did not perform an ACTH stimulation test.

Bone age was 10.5 years using the Greulich-Pyle method on hand and wrist X-rays. Magnetic resonance imaging revealed a hypoplastic uterus in a band-like shape, with no visible ovaries. No appearance consistent with testicular tissue was identified. Karyotype analysis revealed a 46,XY pattern. Estrogen therapy was initiated for the patient. No pathogenic variant was detected in the *SRY* gene analysis. As part of the molecular analysis using the next-generation sequencing (NGS) method, a heterozygous p.V15L (c.43G>T) variant was detected in the *NR5A1* gene (NM_004959.5) (Figure 1). A total of 26 genes were screened as part of this panel, including *DHX37, SRY, WT1, DHH, ZFPM2, PPP1R12A, NR5A1, GTF2H5, MAP3K1, HSD17B4, SOX9, DMRT3, NR0B1, and GATA4*. Segregation analysis revealed no detectable variant in the other family members, including the mother, father, and sibling. The variant identified in our patient was considered to be de novo. Due to the risk of a gonadal tumor, gonadectomy was initially planned. However, the procedure could not be performed because informed consent for gonadectomy was not obtained from the patient and their family. The family is advised to have gonadectomy at each follow-up appointment. If consent is obtained during follow-up, gonadectomy will be reconsidered.

Table 1. Laboratory data of the case

Test	Result	Reference range
Alpha-fetoprotein (AFP) (ng/mL)	< 0.1	0-8.2
Beta Human Chorionic Gonadotropin (ß-hCG) (mIU/mL)	< 0.1	0-4
Dihydrotestosterone (DHT) (µg/L)	0.19	0.016 - 0.07
Free Testosterone (ng/L)	0.78	<8.4
1,4 Delta Androstenedione (µg/L)	< 0.30	0.77-2.25
17-Hydroxyprogesterone (17OHP) (µg/L)	0.53	0.18-2.3
Follicle Stimulating Hormone (FSH) (IU/L)	81.3	0.6-4.1
Luteinizing Hormone (LH) (IU/L)	11.7	<0.02-0.3
Estradiol (pg/mL)	<15	<20
Testosterone (ng/dL)	<7	<20
Anti-mullerian Hormone (AMH) (ng/mL)	0.01	<8.6
Adrenocorticotropic Hormone (ACTH) (pg/mL)	18.1	0-46
Cortisol (µg/dL)	13.4	3-21
Fasting Blood Sugar (mg/dL)	84	70-100
Sodium (Na) (mmol/L)	140	135-145
Potassium (K) (mEq/L)	4.6	3.5-5.1

DISCUSSION

Heterozygous *NR5A1* variants are known causes of gonadal dysgenesis.[3] To date, more than 180 different mutations in the *NR5A1* gene have been identified.[4] The loss of function of the *NR5A1* gene can cause complete or partial gonadal dysgenesis in 46,XY individuals. Different clinical phenotypes may occur in patients carrying the same variant.[5] The frequency of *NR5A1* variants in cases of complete gonadal dysgenesis is reported to be 4-10%.[4,10] *NR5A1* variants are commonly encountered in cases of 46,XY Disorders of Sex Development (DSD).[5] In this case report, we present a novel variant in the *NR5A1* gene as the cause of 46,XY complete gonadal dysgenesis (Swyer syndrome).

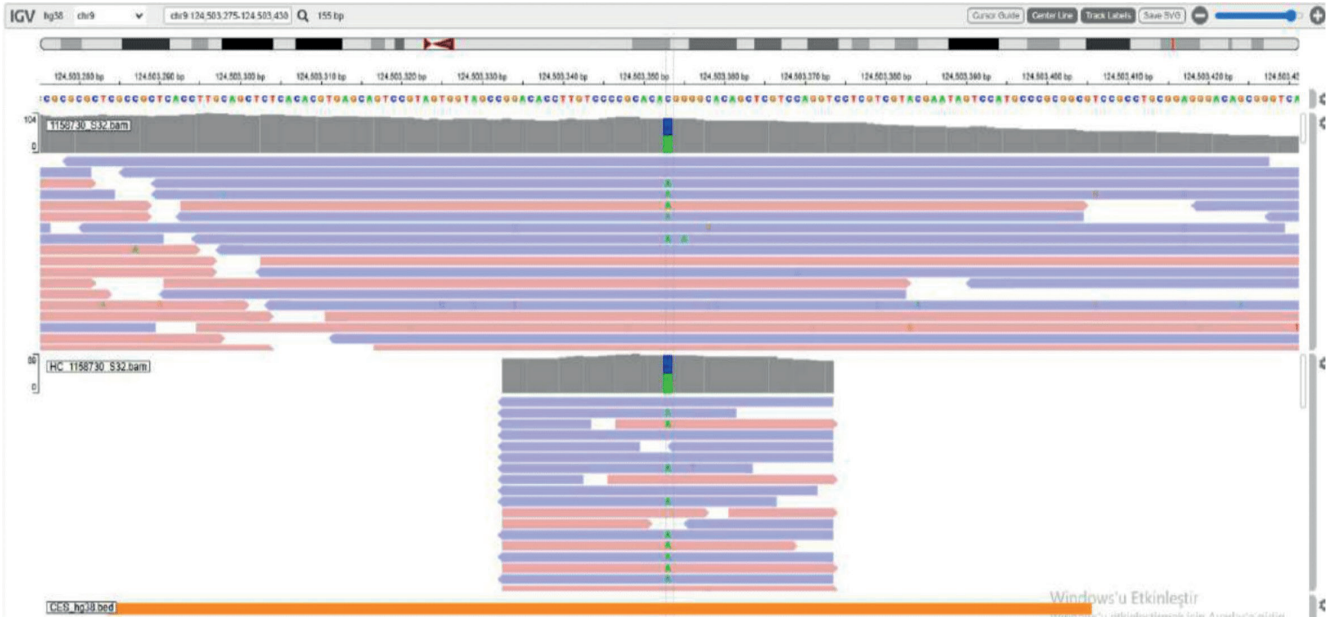


Figure 1. Heterozygous p.V15L (c.43G>T) variant detected in exon 9 (NM_004959.5) in the *NR5A1* gene

In a cohort of 289 patients with 46,XY DSD, 143 patients (49.5%) were diagnosed with 46,XY DSD. Among the 45 patients with a genetic etiology identified for 46,XY DSD, a variant of the *NR5A1* gene, p.Tyr138Ter (c.414C>G/wt), was found in one patient (2.2%).[11] In another cohort of 400 individuals with sex development disorders, the frequency of *NR5A1* mutations was 4% in patients with complete gonadal dysgenesis, while it was 20% in those with partial gonadal dysgenesis and partial androgen insensitivity syndrome.[10] Complete androgen insensitivity syndrome should be considered in the differential diagnosis of karyotype 46,XY, phenotype female cases. Complete androgen insensitivity syndrome was excluded in this case because she was 12 years old, had no pubertal findings, and had no findings in favor of testicular tissue in laboratory and imaging tests. The majority of *NR5A1* variants affect DNA binding activity, and phenotypic differences can be observed depending on the characteristics of the encoded protein.[9] A study reviewed 81 cases of 46,XY CGB with *NR5A1* gene variants published until 2014.[9] In that study, the appearance of external genitalia varied, with ambiguous genitalia in 31%, clitoromegaly in females in 25%, complete female phenotype in 16%, complete male phenotype in 17%, and hypospadias in males in 11%.[9] The clinical spectrum is variable, with 46,XY individuals displaying a female phenotype or ambiguous genitalia, while milder cases may result in infertility.[5] Our adolescent case, who was born with a female phenotype and raised as a girl, exhibited a completely female phenotype, despite having a 46,XY karyotype.

Based on characteristics observed in *NR5A1* knockout mice, initial human studies focused on 46,XY individuals with primary adrenal insufficiency, complete gonadal dysgenesis, and Müllerian structures.[4] Subsequent studies have identified individuals with *NR5A1* variants who have normal adrenal function.[9] In studies, adrenal insufficiency was seen more frequently in homozygous variations in the *NR5A1* gene.[4,9] The variant in our case is heterozygous, and adrenal insufficiency was not detected. The most important laboratory finding in cases of complete gonadal dysgenesis, as observed in our case, is elevated FSH and LH levels, indicating hypergonadotropic hypogonadism, along with low estradiol levels.[2]

In a case of a 46,XY individual with complete gonadal dysgenesis associated with an *NR5A1* variant, the mother, who also carried the same variant, was diagnosed with 46,XX primary ovarian insufficiency.[7] This suggests that the same variant can cause complete gonadal dysgenesis in 46,XY individuals and ovarian insufficiency in 46,XX individuals. The heterozygous variant detected in our case is consistent with dominant inheritance or a de novo mutation. Genetic analysis of the *NR5A1* gene performed on our patient's mother, father, and sibling revealed NO variant. The variant detected in our patient is thought to be de novo.

Molecular analysis of the DNA sample obtained from our patient using next-generation sequencing revealed a change in the first zinc finger of the *NR5A1* gene's DNA-binding domain, where valine was replaced by leucine (V15L heterozygous). To the best of our knowledge, this variant has not yet been associated with any clinical condition and is reported to be extremely rare in population databases.[12] In silico analyses suggest that the variant may be deleterious.[12] Previously, the V15M heterozygous variant at the same position in the *NR5A1* gene has been associated with gonadal dysgenesis and ovarian insufficiency.[13-15] Three cases involving this variant have been reported: the first being a 4-month-old infant with 46,XY karyotype (*SRY* positive) and partial gonadal dysgenesis; the second being a 28-year-old female with 46,XX karyotype and premature ovarian insufficiency; and the third being a case of primary ovarian insufficiency in a study that evaluated 269 patients for 18 genes.[13-15] A comparison of these cases with ours is provided in Table 2.

In Swyer syndrome, estrogen replacement therapy is required to develop secondary sexual characteristics.[1] This is typically followed by cyclical estrogen and progesterone replacement therapy until around the age of 50.[1] Early initiation of estrogen therapy is crucial to ensure adequate bone mineral density during adolescence.[16] Delayed treatment can lead to a decrease in bone mineral density.[16] Estrogen therapy was initiated at the time of diagnosis in our case.

In Swyer syndrome, early diagnosis is important due to the risk of gonadal malignancy.[16] The risk of gonadoblastoma and dysgerminoma is estimated to be between 15-35%.[16] Bilateral gonadectomy is recommended as soon as the diagnosis is made, as there is no role for gonadal biopsy.[17] In our case, laparoscopy with gonadectomy was recommended to the family, but they declined. The patient will be followed up with imaging and laboratory tests for the potential development of gonadal tumors.

This case report describes a novel heterozygous p.V15L (c.43G>T) variant in the *NR5A1* gene identified in a 12.5-year-old girl diagnosed with Swyer syndrome, characterized by short stature and delayed puberty. It adds to the growing body of literature on the genetic basis of Swyer syndrome. The management of 46,XY complete gonadal dysgenesis requires a multidisciplinary approach, emphasizing the importance of early identification for effective counseling. This includes guidance on hormone replacement therapy, bone health, sexual function, fertility options, and the potential risk of gonadal malignancy. The heterozygous p.V15L variant in the *NR5A1* gene should be investigated as a cause of Swyer syndrome in girls with 46,XY karyotype and no adrenal insufficiency who present with short stature and delayed puberty. A novel *NR5A1* variant associated with complete gonadal dysgenesis may not only aid clinicians in interpreting genetic screening results but also inform the development of future therapeutic approaches. More studies are needed to elucidate the phenotype-genotype relationship of the *NR5A1* gene.

Table 2. Comparison of *NR5A1* gene V15M variant cases with our case.

Case	V15M [13]	V15M [14]	V15M [15]	V15L*
Age	4 months	28 years		12.5 years
Clinical	Partial gonadal dysgenesis	Secondary amenorrhea	Primary ovarian insufficiency	Complete gonadal dysgenesis
Phenotype	Female	Female	Female	Female
FSH (IU/L)	9.5	29		81.3
LH (IU/L)	2.3	10.7		11.7
Estradiol (pg/mL)	-	26		<15
Testosterone (ng/dL)	<3	-		<7
AMH (ng/mL)	7.3	0.4		0.01 (2.6–80)
ACTH (pg/mL)	19	-		18.1
Cortisol (µg/dL)	12	-		13.4
Karyotype	46,XY	46,XX		46,XY
Laparoscopy	Bilateral labial testes, epididymis, and vas deferens, no Müllerian structures	-		-

* The case reported in this article

Ethical approval

Informed consent was obtained for the case report.

Author contribution

The authors declare contribution to the paper as follows: Study conception and design: ÖG, İE, DÖ; data collection: FDP, ÖG; analysis and interpretation of results: ÖG, İE; draft manus preparation: ÖG. 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. King TFJ, Conway GS. Swyer syndrome. Curr Opin Endocrinol Diabetes Obes. 2014;21:504-10. **[Crossref]**
2. Mutlu GY, Kırmızıbekmez H, Aydın H, Çetiner H, Moralıoğlu S, Celayir AC. Pure gonadal dysgenesis (Swyer syndrome) due to microdeletion in the SRY gene: a case report. J Pediatr Endocrinol Metab. 2015;28:207-10. **[Crossref]**
3. Elzaiat M, McElreavey K, Bashamboo A. Genetics of 46,XY gonadal dysgenesis. Best Pract Res Clin Endocrinol Metab. 2022;36:101633. **[Crossref]**
4. Fabbri-Scallet H, de Sousa LM, Maciel-Guerra AT, Guerra-Júnior G, de Mello MP. Mutation update for the NR5A1 gene involved in DSD and infertility. Hum Mutat. 2020;41:58-68. **[Crossref]**
5. Luppino G, Wasniewska M, Coco R, et al. Role of NR5A1 gene mutations in disorders of sex development: molecular and clinical features. Curr Issues Mol Biol. 2024;46:4519-32. **[Crossref]**
6. Tuhan H, Anik A, Catli G, et al. A novel mutation in steroidogenic factor (SF1/NR5A1) gene in a patient with 46 XY DSD without adrenal insufficiency. Andrologia. 2017;49:10.1111/and.12589. **[Crossref]**
7. Lourenço D, Brauner R, Lin L, et al. Mutations in NR5A1 associated with ovarian insufficiency. N Engl J Med. 2009;360:1200-10. **[Crossref]**
8. Achermann JC, Ito M, Ito M, Hindmarsh PC, Jameson JL. A mutation in the gene encoding steroidogenic factor-1 causes XY sex reversal and adrenal failure in humans. Nat Genet. 1999;22:125-6. **[Crossref]**
9. Pedace L, Laino L, Preziosi N, et al. Longitudinal hormonal evaluation in a patient with disorder of sexual development, 46,XY karyotype and one NR5A1 mutation. Am J Med Genet A. 2014;164A:2938-46. **[Crossref]**
10. Buonocore F, Clifford-Mobley O, King TFJ, et al. Next-generation sequencing reveals novel genetic variants (SRY, DMRT1, NR5A1, DHH, DHX37) in adults with 46,XY DSD. J Endocr Soc. 2019;3:2341-60. **[Crossref]**
11. Ata A, Özen S, Onay H, et al. A large cohort of disorders of sex development and their genetic characteristics: 6 novel mutations in known genes. Eur J Med Genet. 2021;64:104154. **[Crossref]**
12. Franklin. Available at: https://franklin.genoox.com/clinical-db/variant/snp/chr9-127265632-C-A?app=acmg-classification (Accessed on Jan 11, 2025).
13. Lin L, Philibert P, Ferraz-de-Souza B, et al. Heterozygous missense mutations in steroidogenic factor 1 (SF1/Ad4BP, NR5A1) are associated with 46,XY disorders of sex development with normal adrenal function. J Clin Endocrinol Metab. 2007;92:991-9. **[Crossref]**
14. Jaillard S, Sreenivasan R, Beaumont M, et al. Analysis of NR5A1 in 142 patients with premature ovarian insufficiency, diminished ovarian reserve, or unexplained infertility. Maturitas. 2020;131:78-86. **[Crossref]**
15. Eskenazi S, Bachelot A, Hugon-Rodin J, et al. Next generation sequencing should be proposed to every woman with “idiopathic” primary ovarian insufficiency. J Endocr Soc. 2021;5:bvab032. **[Crossref]**
16. Michala L, Goswami D, Creighton SM, Conway GS. Swyer syndrome: presentation and outcomes. BJOG. 2008;115:737-41. **[Crossref]**
17. McCann-Crosby B, Mansouri R, Dietrich JE, et al. State of the art review in gonadal dysgenesis: challenges in diagnosis and management. Int J Pediatr Endocrinol. 2014;2014:4. **[Crossref]**

Trends in Pediatrics 2025;6(4):289-296
DOI: 10.59213/TP.2025.348

Case Report

An enigmatic diagnosis of abdominal tuberculosis in children: Two case reports

Arya Krisna Manggala[1], Ni Putu Siadi Purniti[2], Ida Bagus Subanada[2], Ayu Setyorini Mestika Mayangsari[2], I Putu Gede Karyana[3], I Gusti Ngurah Sanjaya Putra[3], Ni Nyoman Metriani Nesa[3], I Gusti Ayu Sri Mahendra Dewi[4], Pande Putu Yuli Anandasari[5], Ni Made Adi Tarini[6]

[1]Department of Child Health, Faculty of Medicine, Udayana University, Ngoerah Hospital, Denpasar, Bali, Indonesia
[2]Division of Respirology, Department of Child Health, Faculty of Medicine, Udayana University, Ngoerah Hospital, Denpasar, Bali, Indonesia
[3]Division of Gastrohepatology, Department of Child Health, Faculty of Medicine, Udayana University, Ngoerah Hospital, Denpasar, Bali, Indonesia
[4]Department of Anatomical Pathology, Faculty of Medicine, Udayana University, Ngoerah Hospital, Denpasar, Bali, Indonesia
[5]Department of Radiology, Faculty of Medicine, Udayana University, Ngoerah Hospital, Denpasar, Bali, Indonesia
[6]Department of Clinical Microbiology, Faculty of Medicine, Udayana University, Ngoerah Hospital, Denpasar, Bali, Indonesia

This abstract was presented as a poster at the 10th Indonesia Pediatric Respirology Meeting, held on July 20-21, 2024.

Cite this article as: Manggala AK, Purniti NPS, Subanada IB, et al. An enigmatic diagnosis of abdominal tuberculosis in children: Two case reports. Trends in Pediatrics 2025;6(4):289-296.

ABSTRACT

Diagnosing abdominal tuberculosis (TB) is challenging due to its uncommon symptoms and limited diagnostic tools, which can delay treatment and increase mortality. We present two cases of abdominal TB diagnosed using different approaches. In case 1, a 6-year-old girl with a month-long history of ascites, whose father had treatment-resistant TB, was evaluated. The tuberculin test was positive, but the Xpert MTB/RIF Ultra assay was negative. Laparoscopy revealed miliary nodules, and histopathology confirmed the presence of granulomas with Langhans giant cells. In case 2, a 1-year-old girl presented with seven months of ascites and no known TB contact. The tuberculin test was negative, but an abdominal CT scan showed hepatic TB. The in-house polymerase chain reaction (PCR) for TB targeting the IS3-like element IS987 family transposase in our laboratory was positive. Both patients were diagnosed with abdominal TB, treated with the extrapulmonary (EPTB) regimen, and showed significant improvement. Diagnosing abdominal TB is complex, requiring a thorough history and adequate examination support. This might prevent delays in treatment that potentially increase complications.

Keywords: abdominal tuberculosis, children, diagnosis, histopathology, PCR

INTRODUCTION

Tuberculosis (TB) is a chronic infectious disease that remains a worldwide health issue.[1] It affects more than 70 million children aged 0 to 14 years, resulting in an estimated 230,000 deaths, which positions it among the top 10 leading causes of death. In Indonesia, pediatric TB accounts for about 12% of all TB cases, which translates to over 80,000 instances.[1,2]

Abdominal TB, as an extrapulmonary manifestation, is rare but serious and can affect various abdominal structures, including the gastrointestinal tract, peritoneum, abdominal solid organs, and lymphatic drainage.[3] In China, 14.55% of 2,130 children with EPTB had abdominal TB, making it the fourth most common type of EPTB after meningitis, pleurisy, and lymphatic TB.[4] Symptoms like abdominal discomfort, distention, ascites, diarrhea, vomiting, and

✉ Arya Krisna Manggala ▪ aryakrisnamanggala@gmail.com

Received: 07.09.2025 **Accepted:** 12.11.2025

pain are common, but these do not always lead directly to a diagnosis of TB without strong clinical evidence.[3,5] The nonspecific presentation can also mimic other conditions, such as inflammatory diseases, malignancies, or intestinal perforation, leading to a delay in diagnosis or could evoke unnecessary surgery.[5] Moreover, the absence of clear recommendations for diagnostic tools for abdominal TB complicates the diagnosis, particularly in resource-limited settings.[6]

In this report, we highlight two cases of pediatric abdominal TB diagnosed at our tertiary hospital. These cases illustrate the complexities of diagnosing, underscoring the importance of a comprehensive diagnostic approach and thorough clinical examination. They also emphasize the significance of timely and efficient treatment to prevent severe complications linked to abdominal TB.

CASE PRESENTATION

Case 1

A 6-year-old girl was admitted with abdominal distension and discomfort lasting for one month. She had no history of fever, cough, night sweats, or weight loss. Her father, diagnosed with bacteriologically confirmed pulmonary TB (PTB) eight months before her symptoms began, was undergoing treatment for TB, which had been deemed a failure. The girl had not received the *Bacillus Calmette–Guérin* (BCG) vaccine.

Her current clinical status was within normal limits; however, anthropometry indicated undernutrition, with mid-upper arm circumference (MUAC) at 76% of the standard reference value. The abdomen was distended (abdominal circumference: 62 cm), with positive bowel sounds, undulation, and shifting dullness. The laboratory findings indicated mild anemia, with a hemoglobin (HGB) level of 10.70 g/dL and a mean corpuscular volume (MCV) of 75.50 fL. Other laboratory tests, including liver function tests, were within normal limits.

The abdominal X-ray showed distension and a sentinel loop in the left upper quadrant (Figure 1A), while the ultrasound revealed ascites (Figure 1B). The MTB/RIF Ultra (Cepheid, Sunnyvale, California, USA) test on sputum was negative, but a positive tuberculin test showed a 20 mm induration. A chest X-ray revealed hilar lymphadenopathy (Figure 2), giving a TB score of eight. Subsequently, to assess for extrapulmonary TB (EPTB), an ascitic fluid sample was obtained via laparoscopic examination. During the procedure, multiple whitish tubercles were observed (Figure 3A). Although the MTB/RIF Ultra test of the fluid was negative, the adenosine deaminase (ADA) level

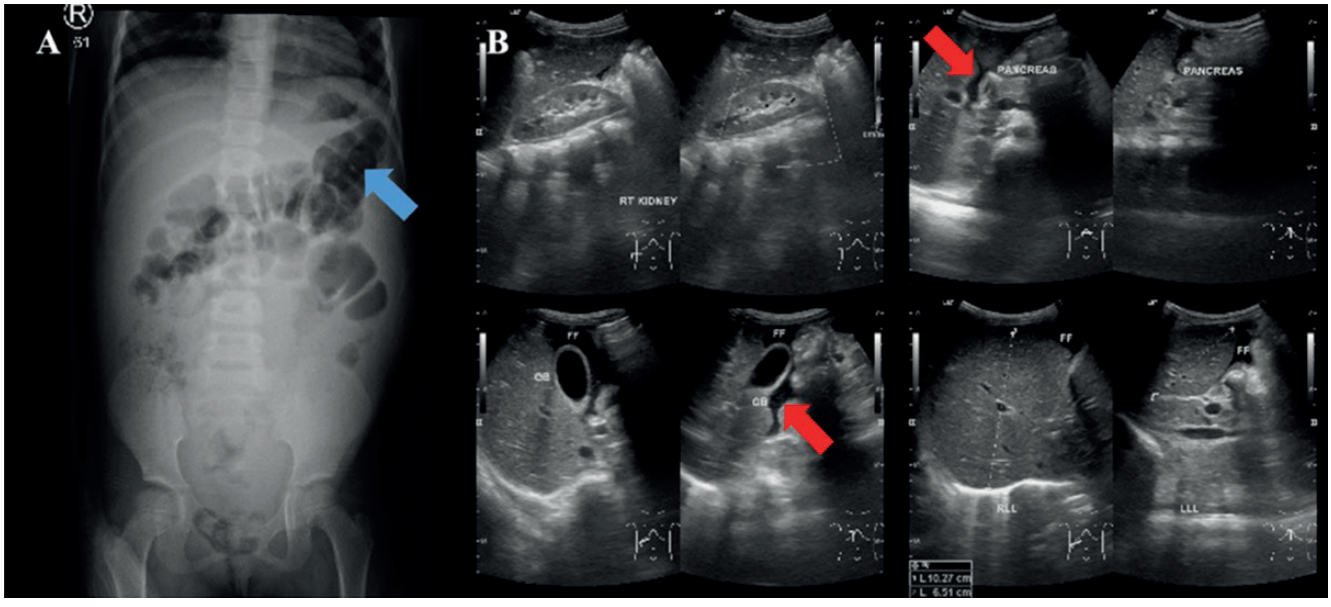


Figure 1. The abdominal radiograph and ultrasound examination

A) A sentinel loop observed in the upper left quadrant on abdominal radiograph (blue arrow) **B)** Abdominal ultrasound examination showed free fluid in the abdominal and pelvic cavities (red arrow), confirming the presence of ascites.

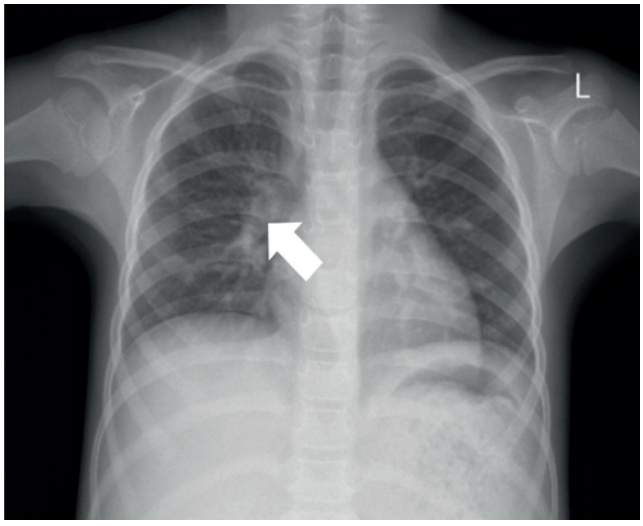


Figure 2. Chest radiograph in Case 1 showed hilar lymphadenopathy (white arrow)

was elevated at 58 U/L, and histopathology confirmed abdominal TB with epithelioid granulomas and Langhans giant cells (Figure 3B). The HIV test was nonreactive.

She started the intensive phase of anti-tuberculosis therapy (ATT) with four tablets of a pediatric fixed-dose combination of Rifampicin (R), Isoniazid (H), Pyrazinamide (Z) (75/50/150), and Ethambutol (20 mg/kg/day). She followed the 6-month therapy following national guidelines, and showed clinical improvement, including a 20% reduction in abdominal circumference (to 50 cm) and an increase in MUAC (from 76% to 79%), with no gastrointestinal or respiratory issues.

Case 2

A 1-year-old girl was referred to our tertiary hospital from X Province Hospital with a primary complaint of ascites that had persisted for seven months. She also had a persistent fever and cough for three months. Significant weight loss was noted three months before admission to X Hospital. The patient's family and close contacts had no known history of tuberculosis exposure. Her immunization was completed.

She was previously diagnosed with sepsis caused by *Gamella morbillorum*, massive ascites, and clinically suspected PTB. She had been treated at the previous hospital for about one month with the following regimen: vancomycin for 12 days, a single-drug ATT for 19 days, and prednisone for 18 days. However, the ascites did not improve.

Her vital signs were within normal limits, but the anthropometric assessment indicated undernourishment (MUAC 76%). The abdominal examination showed distention (60 cm) with distant bowel sounds. The ascites drainage inserted at the previous hospital was well-placed,

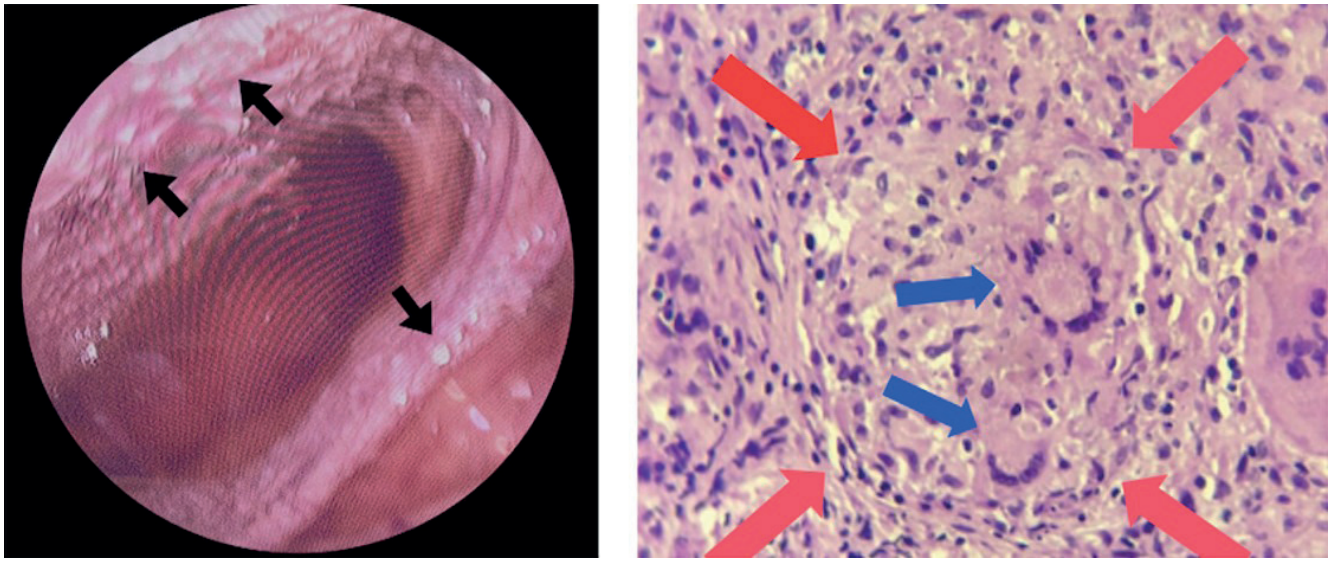


Figure 3. A) During laparoscopic examination for ascitic fluid retrieval, a whitish tubercle was identified in the peritoneum (black arrow). **B)** Histopathological analysis showed the presence of epithelioid histiocytes forming granulomatous (red arrow) and Langhans giant cells (blue arrow).

aiding in the removal of excess fluid. The tuberculin skin test showed an 8 mm induration, and the chest radiograph from the previous hospital showed hilar right lymphadenopathy (Figure 4). The MTB/RIF Ultra from gastric lavage was negative.

The laboratory results showed microcytic hypochromic anemia with an HGB of 10.00 g/dL and MCV of 65.20 fL. The liver function, bilirubin, and septic markers were normal. An earlier abdominal ultrasound revealed massive ascites. An abdominal CT with contrast revealed ascites in the abdominal and pelvic cavities, dilatation of the inferior vena cava (IVC) due to thrombus formation, a hypodense lesion with peripheral enhancement in the hepatic parenchyma, minimal nodular peritoneal thickening, and reactive lymph nodes in the inguinal regions, suggesting abdominal (hepatic) TB (Figure 5A).

Ascitic fluid analysis revealed a serum-ascites albumin gradient (SAAG) of 1.5 g/dL (transudate). Subsequently, the MTB/RIF Ultra was negative, and the ADA was 20 U/L. Given the inconclusive findings, we opted for in-house polymerase chain reaction (PCR) testing of the ascitic fluid rather than laparoscopy due to the significant ascites. The primers and target gene used for the PCR were provided by our Clinical Microbiology Laboratory, as follows: PCR TB E1 (5'-CCTGCGAGCGTAGGCGTC) and PCR TB E2 (5'-CCGTCCAGCGCCGCTGTCGG), targeting the IS3-like element IS987 family transposase. DNA extraction was performed using the QIAamp DNA Mini Kit, and amplification was carried out with a T100 Thermal Cycler (Bio-Rad) using GoTaq Green Master Mix. The ascitic fluid sample tested positive for TB by PCR, thereby confirming the diagnosis of abdominal TB. The patient completed the intensive phase of ATT with two pediatric fixed-dose combination RHZE tablets once daily for 2 months and entered the continuation phase. (Figure 5B).

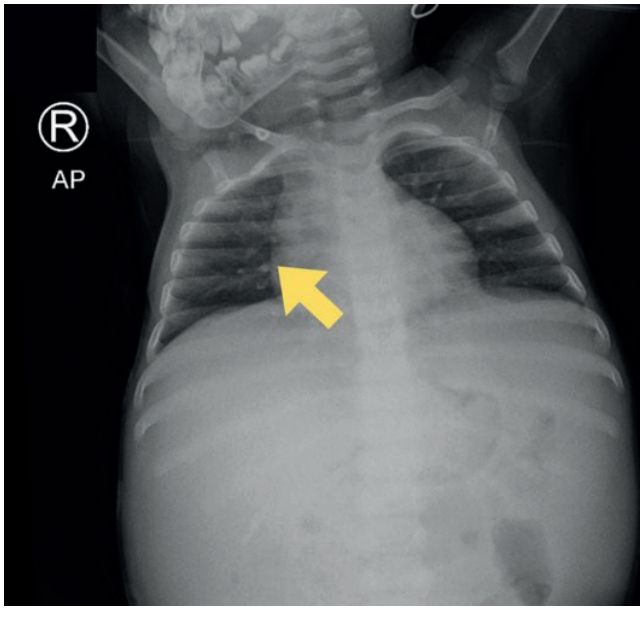


Figure 4. Chest radiograph in Case 2 showed right hilar lymphadenopathy (yellow arrow)

The antibiotic treatment was stopped upon admission to our hospital due to minimal signs of bacterial infection. Abdominal circumference reduced by 11.6% and MUAC increased by 78.8% within 2 weeks of treatment.

The patient's parents gave their consent for these cases to be published for academic purposes, with the assurance that their identities would remain confidential. Ethical approval was granted by our institution's ethics committee.

DISCUSSION

Abdominal TB in children is a rare but significant form of EPTB, presenting with diagnostic and management challenges due to its atypical presentation and potential for severe complications.[7] Based on anamnesis and physical examination, abdominal TB was suspected in Case 1 due to distention and a history of contact with PTB, while in Case 2, it was suspected because of distention, fever, cough, and weight loss. In both cases, malnutrition and anemia may also support a chronic infection.

Contacts of TB patients are at increased risk of acquiring either PTB or EPTB.[8] A study in Pakistan found a family history of TB significantly associated with EPTB, especially in children aged 0-4 years.[9] Additionally, a previous study in Bali stated that an incomplete BCG history is associated with a threefold higher risk of developing EPTB.[10] Although no studies have explored the association between failed TB treatment in adults and EPTB in children, it is likely that the abdominal TB in Case 1 was related to her father's PTB as the index case, along with an incomplete BCG vaccination history.

Following initial suspicion, TST usually shows increased induration, indicating TB infection. A study in Turkey found a 69.2% TST positivity rate among children with abdominal TB.[11] In Case 1, a 20 mm induration was observed, which typically indicates TB infection. In contrast, in Case 2, an 8 mm induration was noted, which was considered doubtful given the patient's negative immunosuppressive status.

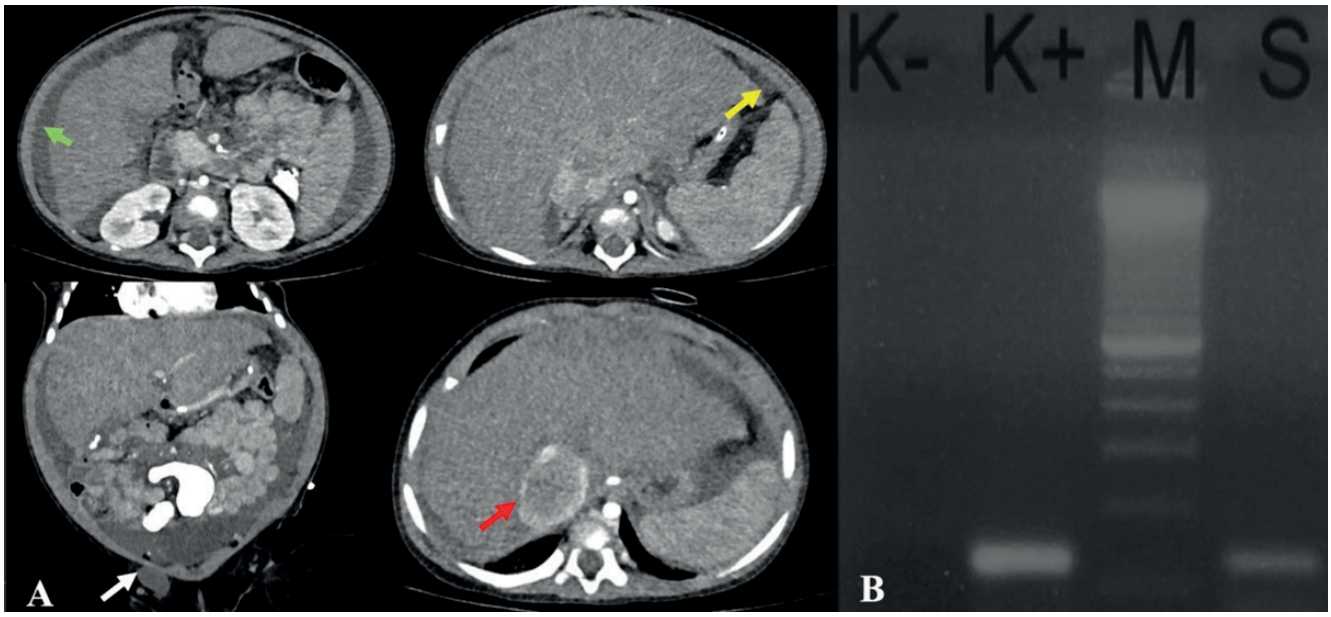


Figure 5. A) Contrast-enhanced abdominal CT scan revealed ascites in the abdominal cavity (green arrow), nodular peritoneal thickening (yellow arrow), reactive lymph nodes in the inguinal region (white arrow), and a hypodense lesion with peripheral enhancement in the hepatic parenchyma (red arrow). **B)** The results of a PCR test to detect specific DNA sequences related to *M. tuberculosis*. The lanes on the gel include a negative control (K-), a positive control (K+), a molecular weight marker (M), and the sample lane (S). The presence of a band in the sample lane, aligning with the positive control, indicates a positive result for TB DNA in the patient's specimen.

Therefore, while the TST can raise suspicion, it must be interpreted in conjunction with other diagnostic tools for a more accurate diagnosis.[12]

Recent national guidelines for TB in children recommend ruling out PTB if EPTB is suspected.[13] This might indicate an infectious source of initial involvement in the development of abdominal TB. Both cases showed negative MTB/RIF Ultra results from sputum or gastric aspirate. However, chest X-rays revealed right hilar lymphadenopathy, which is a common radiological hallmark of primary TB in children, seen in 50-70% of cases.[14]

The abdominal CT is preferred for detailed visualization, revealing inflammation in abdominal structures and differentiating between TB-related ascites and cancer.[15] Abdominal CT can also show peritoneal or intestinal wall thickening, a key feature of abdominal TB.[5,16] In a case series in Turkey, the most common findings in abdominal CT were lymphadenopathy, peritoneal thickening, ascites, hepatosplenomegaly, and multiple nodules.[11] In Case 2, there was a hypodense lesion with peripheral enhancement in the hepatic parenchyma and minimal nodular peritoneal thickening, which aligns with hepatic TB features.

Molecular testing, including ascitic fluid examination, acid-fast bacilli (AFB) staining, MTB/RIF, and PCR, is crucial in diagnosing abdominal TB. In peritoneal TB, ascitic fluid typically has SAAG < 1.1 g/dL, while SAAG > 1.1 g/dL is commonly seen in patients with cirrhosis or inferior vena cava (IVC) obstruction.[17] In Case 2, ascitic fluid analysis revealed a SAAG > 1.1 g/dL, correlating with abdominal CT findings that suggested IVC obstruction as the cause of the transudate pattern. The association between IVC obstruction and abdominal TB is rarely reported, but it may result from acquired IVC thrombosis caused by external compression from swollen retroperitoneal lymph nodes, which distort the IVC and promote thrombus formation.[18,19] Consequently, we closely monitored homeostatic function (INR at 1.2) and signs of obstruction.

The MTB/RIF Ultra, introduced by the WHO in 2017, is claimed to have higher sensitivity than the previous version, as it includes two distinct multi-copy amplification targets (IS6110 and IS1081) and has a larger DNA reaction chamber compared to Xpert MTB/RIF.[20] In a study conducted by Slail *et al.*,[21] the sensitivity and specificity of Xpert MTB/RIF Ultra from ascitic fluid were 75% and 93%, respectively, with a positive predictive value (PPV) of 60% and a negative

predictive value (NPV) of 96%.[21] However, in these cases, the MTB/RIF Ultra results from ascitic fluid were negative. The recent WHO guidelines conditionally recommend Xpert MTB/RIF and Xpert MTB/RIF Ultra as initial diagnostic tests, due to low certainty in peritoneal fluid specimens.[22]

The ADA test has been reported to be highly effective in diagnosing abdominal TB.[23] A recent meta-analysis by Mahajan *et al*.[24] in 2023 reported a pooled sensitivity of 90% and specificity of 94% for the ADA test compared with bacteriologically confirmed *M. tuberculosis* or histopathology. The study also found that a cut-off value above 30 U/L resulted in an area under the curve (AUC) of 0.95.[24] Both patients underwent the ADA test, with Case 1 showing a higher level (58 U/L). In contrast, Case 2 had an ADA level of 20 U/L, which was not indicative of abdominal TB. This could be due to early-stage abdominal TB or the patient's immunocompromised state from sepsis.[23]

The PCR testing for *M. tuberculosis* DNA is a highly sensitive and specific method, with a specificity of 96-99%, making it valuable in diagnosing TB when traditional methods fall short.[25] In Case 2, the positive PCR result was crucial in confirming the abdominal TB diagnosis, despite the weak Mantoux test reaction. Histopathology is another key diagnostic tool, often serving as the reference standard when culture results are limited.[26] Caseating granulomas, characterized by central necrosis surrounded by epithelioid cells, Langhans giant cells, and infiltrating lymphocytes, are typically present in TB.[27] In Case 1, histopathological examination confirmed these granulomas and Langhans giant cells, reinforcing the diagnosis of abdominal TB. However, microbiological confirmation could not be established in this case, as AFB staining and mycobacterial culture were not performed.

The AFB staining was not performed in our diagnostic work-up because, according to the current national tuberculosis guidelines, this method has low sensitivity.[13] Therefore, AFB staining is no longer recommended as the primary bacteriological diagnostic tool for tuberculosis.[13] Mycobacterial culture, although still considered the gold standard, was also not performed in these cases due to its lengthy turnaround time and its frequent negative results, particularly in paucibacillary forms of the disease.[5] Because of these limitations, we combined several diagnostic modalities—including imaging and molecular tests (Xpert MTB/RIF, PCR), ADA testing, and histopathological examination—to achieve a more comprehensive and reliable diagnosis.

Finally, the diagnosis of abdominal TB in both patients was confirmed through several examinations (Table 1). Following national guidelines for EPTB treatment, anti-tuberculosis therapy (ATT) was promptly initiated. Both patients demonstrated a satisfactory treatment response, including a reduction in abdominal circumference and weight gain, confirming that the diagnosis was consistent with the positive treatment outcomes.

Conclusion

Abdominal TB in children is complex and challenging to diagnose, requiring a high index of suspicion and a thorough, repeated diagnostic approach. Accurate diagnosis relies on a combination of history, physical examination, laboratory tests, imaging, molecular testing, and histopathology. While proper management can improve prognosis, the risk of long-term complications remains, highlighting the need for continuous monitoring to ensure full recovery and prevent recurrence.

Acknowledgements

The authors would like to express their sincere gratitude to the Department of Microbiology, Department of Anatomical Pathology, Department of Clinical Pathology, and Department of Radiology of Ngoerah Hospital, Bali, for their valuable support in sample processing and interpretation of diagnostic findings. Their technical expertise and collaboration were essential in completing this case report.

Ethical approval

This study has been approved by the Ethics Committee of the Faculty of Medicine, Udayana University (approval number 1755/UN14.2.2.VII.14/LT/2025). Written informed consent was obtained from the participants.

Author contribution

The authors declare contribution to the paper as follows: Study conception and design: AKM, NPSP, IBS, ASMM, IPGK, IGNSP, NNMN; data collection: AKM, NPSP, IBS, IPGK, IGNSP, IGASMD, PPYA, NMAT; analysis and interpretation of results: AKM, NPSP, IBS, IPGK, IGNSP, IGASMD, PPYA, NMAT; draft manuscript preparation: AKM, NPSP, ASMM, NNMN, IGASMD, PPYA, NMAT. All authors reviewed the results and approved the final version of the article.

Table 1. The different diagnostic approaches in both cases

	Case 1	**Case 2**
Gender	Girl	Girl
Age (years)	6	1
Body weight	20 kg	9.5 kg
Signs and symptoms	Abdominal distention	Abdominal distention, fever > 2 weeks, cough > 2 weeks, decrease in body weight
Contact history	Father with PTB on treatment	Not known
BCG vaccination	No	Yes, scar (+)
TST	Positive (≥ 10 mm)	<10 mm
HIV examination	Negative	Negative
Sputum and/or Gastric lavage MTB/RIF Ultra	*M. tuberculosis* not detected (Sputum)	*M. tuberculosis* not detected (Gastric lavage)
Chest radiograph examination	Hilar lymphadenopathy	Hilar lymphadenopathy
Abdominal plain radiograph	Sentinel loop in the upper left quadrant	N/A
Abdominal ultrasound	Ascites	Ascites
Abdominal CT	N/A	Ascites in the abdominal and pelvic cavities, dilatation of the inferior vena cava (IVC) due to thrombus formation, a hypodense lesion with peripheral enhancement in the hepatic parenchyma, minimal nodular peritoneal thickening, and reactive lymph nodes in the inguinal regions
Ascitic MTB/RIF Ultra	*M. tuberculosis* not detected	*M. tuberculosis* not detected
Acid-fast bacilli	N/A	Negative
Ascitic fluid analysis	N/A	Mono 94.3% Poly 5.7% Albumin ascitic 1.4 g/dL Albumin serum 2.9 g/dL Protein total 2.3 g/dL LDH 63 SAAG: 1.5 g/dL
ADA test	58 U/L	20 U/L
PCR TB ascites	N/A	Positive
Histopathology	Epithelioid histiocytes forming granulomatous structures and Langhans giant cells	N/A
Treatment	2RHZE/4RH [FDC (75/50/150) 4 tablets + Ethambutol 20 mg/kg/day ~ 400 mg OD]	2RHZE/4RH [FDC (75/50/150) 2 tablets + Ethambutol 20 mg/kg/day ~ 200 mg OD]

PTB, pulmonary tuberculosis; TST, tuberculin skin test; BCG, bacilli Calmette–Guérin; LDH, lactate dehydrogenase; CT, computed tomography; SAAG, serum-ascites-albumin-gradient; ADA, adenosine deaminase; PCR, polymerase chain reaction; TB, tuberculosis; RHZE, rifampicin, isoniazid, pyrazinamide, and ethambutol; RH, rifampicin and isoniazid; FDC, fixed dose combination; OD, once daily; N/A, not available.

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. World Health Organization (WHO). Global tuberculosis report 2023. Geneva: WHO; 2023.
2. UNICEF. Desk review: pediatric tuberculosis with a focus on Indonesia. Indonesia: UNICEF; 2022.
3. Sartoris G, Seddon JA, Rabie H, Nel ED, Schaaf HS. Abdominal tuberculosis in children: challenges, uncertainty, and confusion. J Pediatric Infect Dis Soc. 2020;9:218-27. **[Crossref]**
4. Chu P, Chang Y, Zhang X, et al. Epidemiology of extrapulmonary tuberculosis among pediatric inpatients in mainland China: a descriptive, multicenter study. Emerg Microbes Infect. 2022;11:1090-102. **[Crossref]**
5. Lancella L, Abate L, Cursi L, et al. Abdominal tuberculosis in children: a case series of five p. Microorganisms. 2023;11:730. **[Crossref]**
6. Lukosiute-Urboniene A, Dekeryte I, Donielaite-Anise K, Kilda A, Barauskas V. Challenging diagnosis of abdominal tuberculosis in children: case report. Int J Infect Dis. 2022;116:130-2. **[Crossref]**
7. Cho JK, Choi YM, Lee SS, et al. Clinical features and outcomes of abdominal tuberculosis in southeastern Korea: 12-years of experience. BMC Infect Dis. 2018;18:699. **[Crossref]**
8. Laghari M, Sulaiman SAS, Khan AH, Talpur BA, Bhatti Z, Memon N. Contact screening and risk factors for TB among the household contact of children with active TB: a way to find source case and new TB cases. BMC Public Health. 2019;19:1274. **[Crossref]**
9. Dubois MM, Brooks MB, Malik AA, et al. Age-specific clinical presentation and risk factors for extrapulmonary tuberculosis disease in children. Pediatr Infect Dis J. 2022;41:620-5. **[Crossref]**
10. Utami MDA, Purniti NPS, Subanada I, Mm AS. Faktor risiko infeksi tuberkulosis milier dan ekstraparu pada anak penderita tuberkulosis. Sari Pediatri. 2021;22:290-6. **[Crossref]**
11. Çay Ü, Gündeşlioğlu ÖÖ, Alabaz D. Evaluation of cases with abdominal tuberculosis ın children: 10 years of experience from a single center in Turkey. South Clin Ist Euras. 2022;33:298-303. **[Crossref]**
12. Rana S, Farooqui MR, Rana S, Anees A, Ahmad Z, Jairajpuri ZS. The role of laboratory investigations in evaluating abdominal tuberculosis. J Family Community Med. 2015;22:152-7. **[Crossref]**
13. Kementerian Keshatan Republik Indonesia. Petunjuk Teknis Tata laksana Tuberkulosis Anak dan Remaja Indonesia 2023. Indonesia: Kementerian Kesehatan Republik; 2023.
14. Concepcion NDP, Laya BF, Andronikou S, Abdul Manaf Z, Atienza MIM, Sodhi KS. Imaging recommendations and algorithms for pediatric tuberculosis: part 1-thoracic tuberculosis. Pediatr Radiol. 2023;53:1773-81. **[Crossref]**
15. Sinan T, Sheikh M, Ramadan S, Sahwney S, Behbehani A. CT features in abdominal tuberculosis: 20 years experience. BMC Med Imaging. 2002;2:3. **[Crossref]**
16. World Gastroenterology Organisation Global Guidelines. Digestive tract tuberculosis. World Gastroenterology Organisation; 2021.
17. Seo YS. 2017 Korean Association for the Study of the Liver (KASL) clinical practice guidelines for ascites and related complications: what has been changed from the 2011 KASL clinical practice guidelines? Korean J Gastroenterol. 2018;72:179. **[Crossref]**
18. Abid R, Oueslati I, Bousetta N, et al. Inferior vena cava thrombosis complicating tuberculosis. Ann Clin Case Rep. 2018;3:1534.
19. Khaladkar DSM, Choure DAA, Jain DS. Abdominal ganglionic tuberculosis with inferior vena cava and common iliac vein thrombosis- A case report. Glob J Med Res.2019;29:11-6.
20. World Health Organization (WHO). WHO meeting report of a technical expert consultation: non-inferiority analysis of xpert MTB/RIF ultra compared to xpert MTB/RIF. Geneva: WHO; 2017.
21. Slail MJ, Booq RY, Al-Ahmad IH, et al. Evaluation of Xpert MTB/RIF Ultra for the diagnosis of extrapulmonary tuberculosis: a retrospective analysis in Saudi Arabia. J Epidemiol Glob Health. 2023;13:782-93. **[Crossref]**
22. World Health Organization (WHO). WHO consolidated guidelines on tuberculosis: rapid diagnostics for tuberculosis detection; module 3: diagnosis. 3rd ed. Geneva: WHO; 2024.
23. Dahale AS, Dalal A. Evidence-based commentary: Ascitic adenosine deaminase in the diagnosis of peritoneal tuberculosis. J Gastrointest Infect. 2022;12:57-60. **[Crossref]**
24. Mahajan M, Prasad ML, Kumar P, et al. An updated systematic review and meta-analysis for the diagnostic test accuracy of ascitic fluid adenosine deaminase in tuberculous peritonitis. Infect Chemother. 2023;55:264-77. **[Crossref]**
25. Shen Y, Fang L, Ye B, Yu G. Meta-analysis of diagnostic accuracy of nucleic acid amplification tests for abdominal tuberculosis. PLoS One. 2023;18:e0289336. **[Crossref]**
26. Ahmad R, Changeez M, Khan JS, et al. Diagnostic accuracy of peritoneal fluid geneXpert in the diagnosis of intestinal tuberculosis, keeping histopathology as the gold standard. Cureus. 2018;10:e3451. **[Crossref]**
27. Maulahela H, Simadibrata M, Nelwan EJ, et al. Recent advances in the diagnosis of intestinal tuberculosis. BMC Gastroenterol. 2022;22:89. **[Crossref]**

Trends in Pediatrics 2025;6(4):297-298
DOI: 10.59213/TP.2025.380

Letter to the Editor

Comment on: "Low bone mineral density in rare metabolic disorders: data from a Turkish cohort of patients with glycogen storage disorders and organic acidemias"

Mahmood Dhahir Al-Mendalawi[1]

[1]Department of Paediatrics, Al-Kindy College of Medicine, University of Baghdad, Baghdad, Iraq

Cite this article as: Al-Mendalawi MD. Comment on: Low bone mineral density in rare metabolic disorders: data from a Turkish cohort of patients with glycogen storage disorders and organic acidemias. Trends in Pediatrics 2025;6(4):297-298.

Dear Editor,

In the most recent issue of Trends in Pediatrics, Akin and Goksoy[1] used dual-energy X-ray absorptiometry (DEXA) to assess bone mineral density (BMD) in Turkish pediatric patients with rare metabolic disorders of organic acidemias (OA) and glycogen storage diseases (GSD). They found low BMD in the studied OA and GSD populations and observed that these populations were affected by certain modifiable determinants, such as vitamin D status and dietary calcium intake. Akin and Goksoy[1] thankfully highlighted numerous valuable limitations of the study. We herein introduce another valued one. Notwithstanding, monitoring bone health status using BMD by DEXA requires reference to BMD reference values (BMDRVs). Age, sex, weight, and ethnicity are among the many determinants that control BMDRVs[2], and BMDRVs have been introduced for certain pediatric populations based on these determinants.[3-5] Türkiye is among forerunner countries that formulated local pediatric BMDRVs in 2006 to help practicing pediatricians and endocrinologists monitor bone health integrity in pediatric Turkish population, especially among those with chronic illnesses.[6] Akin and Goksoy[1] in the study methodology unexpectedly referred to a foreign standard (2019 ISCD Official Position)[7] rather than a local one[6] in evaluating BMD in the study population. As a result, the study's findings might be halted, and consequently, their clinical applicability could be additionally jeopardized by the aforementioned limitation.

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. Kumru Akin B, Goksoy E. Low bone mineral density in rare metabolic disorders: data from a Turkish cohort of patients with glycogen storage disorders and organic acidemias. Trends in Pediatr. 2025;6:194-201. [Crossref]
2. Guss CE, McAllister A, Gordon CM. DXA in children and adolescents. J Clin Densitom. 2021;24:28-35. [Crossref]
3. Lopez-Gonzalez D, Wells JC, Cortina-Borja M, Fewtrell M, Partida-Gaytán A, Clark P. Reference values for bone mineral density in healthy Mexican children and adolescents. Bone. 2021;142:115734. [Crossref]

✉ Mahmood Dhahir Al-Mendalawi ▪ mdalmendalawi@yahoo.com

Received: 11.11.2025 **Accepted:** 16.11.2025

4. Prajantawanich K, Manpayak T, Pooliam J, Nakavachara P. Updated reference values for BMD and lean mass measured by DXA in Thai children. J Bone Miner Metab. 2024;42:728-40. **[Crossref]**

5. Khadilkar A, Oza C, Sanwalka N, et al. Reference data for Lunar iDXA for the assessment of bone health in Indian children and youth: a cross-sectional study. Indian Pediatr. 2025;62:578-85. **[Crossref]**

6. Goksen D, Darcan S, Coker M, Kose T. Bone mineral density of healthy Turkish children and adolescents. J Clin Densitom. 2006;9:84-90. **[Crossref]**

7. ISCD. 2019 ISCD official positions pediatric. Available at: https://iscd.org/learn/official-positions/pediatric-positions/. (Accessed on Nov 8, 2025).

Trends in Pediatrics 2025;6(4):299-300
DOI: 10.59213/TP.2025.388

Letter to the Editor

Authors' reply to the letter: "Low bone mineral density in rare metabolic disorders: data from a Turkish cohort of patients with glycogen storage disorders and organic acidemias"

Burcu Kumru Akın[1], Emine Göksoy[2]

[1]Division of Nutrition and Diet, Department of Pediatrics, Gaziantep Cengiz Gökçek Maternity and Children's Hospital, Gaziantep, Türkiye
[2]Division of Pediatric Metabolism, Department of Pediatrics, Faculty of Medicine, Adnan Menderes University, Aydın, Türkiye

Cite this article as: Kumru Akın B, Göksoy E. Authors' reply to the letter: "Low bone mineral density in rare metabolic disorders: data from a Turkish cohort of patients with glycogen storage disorders and organic acidemias". Trends in Pediatrics 2025;6(4):299-300.

We sincerely thank the authors of the Letter to the Editor for their interest in our study and for the opportunity to clarify the methodological basis of our bone mineral density (BMD) assessments. In our study, all DXA results were interpreted in accordance with the 2019 Pediatric Official Positions of the International Society for Clinical Densitometry (ISCD), which define the current global standard for pediatric densitometry.[1] An explicit and essential statement from the ISCD 2019 pediatric positions is the following: "Z-scores should be generated from reference data matched to age, sex, and the manufacturer's reference database used by the DXA system." ISCD does not state or imply that country-specific or locally developed pediatric reference data should be used.

Although the 2006 reference curves were generated using a Hologic device, these curves are not part of Hologic's embedded pediatric reference database. Therefore, despite being a valuable national dataset, the 2006 Turkish pediatric BMD reference values[2] cannot be technically applied within an ISCD-compliant DXA workflow and were not used in our study, as doing so could result in inaccurate or non-standardized Z-score interpretation. Moreover, the 2006 Turkish reference curves were developed exclusively from healthy, normally growing children. ISCD emphasizes that growth patterns and body composition in children with chronic illnesses differ substantially from those of healthy peers, and therefore normative datasets derived from healthy populations may lead to misleading Z-score interpretation in chronically ill groups.

A review of current Turkish pediatric DXA practices also supports the methodological approach used in our work. Several recent theses evaluating BMD in chronic pediatric conditions have consistently relied on DXA-derived Z-scores generated from the manufacturer's reference database and have interpreted them using ISCD definitions and cut-off values. In recent theses[3-6] conducted on pediatric patients in Türkiye, the assessment of bone mineral density has been based on ISCD guidelines. In these studies, the ISCD-recommended Z-score threshold (≤ –2 SD) has been used to define low BMD, and measurements have been interpreted using device-specific reference databases provided by the DXA manufacturer. Moreover, many of these works explicitly highlight adherence to ISCD standards as a methodological strength. This demonstrates that contemporary academic research in Türkiye consistently employs ISCD- and manufacturer-based DXA interpretation in line with international standards, rather than utilizing the 2006 national reference curves. In addition, it is evident

✉ Burcu Kumru Akın ▪ burcu-kumru@hotmail.com

Received: 20.11.2025 **Accepted:** 24.11.2025

that the ISCD criteria have also been used in an academic study conducted in Türkiye.[7] International practices further support this approach. For example, Brazil has a national pediatric BMD reference dataset.[8] Nevertheless, Brazilian clinical studies evaluating BMD in hepatic glycogen storage disease and published in Nutrients (high-impact journal) in 2021 calculated Z-scores exclusively using ISCD 2019 recommendations and manufacturer-specific reference data rather than their national curves.[9] This demonstrates that even countries with strong population-specific datasets prioritize ISCD-compliant Z-score generation to ensure device compatibility and international comparability. In rare metabolic disorders such as glycogen storage diseases and organic acidemias, where study populations are small and global harmonization of data is essential, adherence to ISCD methodology is particularly critical.

In light of these considerations, our use of the ISCD 2019 pediatric positions and Hologic's manufacturer-calibrated pediatric reference database reflects current international best practice, aligns with ISCD expectations for device-specific DXA interpretation, and is consistent with contemporary Turkish and global pediatric literature. For these reasons, although scientifically valuable, the 2006 national pediatric BMD reference curves could not be applied methodologically or technically within an ISCD-compliant framework. We hope that this explanation clarifies the rationale underlying our methodological choices and contributes to constructive scientific dialogue.

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. ISCD (International Society for Clinical Densitometry). 2019 ISCD pediatric official positions. Available at: https://iscd.org
2. Goksen D, Darcan S, Coker M, Kose T. Bone mineral density of healthy Turkish children and adolescents. J Clin Densitom. 2006;9:84-90. **[Crossref]**
3. Kılıç MN. Evaluation of nutritional status and micronutrients in patients with urea cycle disorder [Specialization thesis]. İstanbul: Sağlık Bilimleri Üniversitesi; 2025.
4. Kardaş F. Retrospective and prospective evaluation of clinical and laboratuary parameters of the patients with phenylketonuriia and hyperphenylalalinemia followed by the department of the Pediatric Nutrition and Metabolism in the Erciyes University Faculty of Medicine [Specialization thesis]. Kayseri: Erciyes Üniversitesi; 2019.
5. Kocamaz H. Comparison of bone mineral density, tissue transglutaminase and marsh staging in adolescents and children with celiac disease [Specialization thesis]. Elazığ: Fırat Üniversitesi; 2012.
6. Salar HB. Evaluation of bone mineral density and related factors in children with inflammatory bowel disease [Specialization thesis]. İstanbul: Sağlık Bilimleri Üniversitesi; 2022.
7. Hacıhamdioğlu DÖ, Kalman S, Gök F. Long-term results of children diagnosed with idiopathic nephrotic syndrome; single center experience. Turk Pediatri Ars. 2015;50:37-44. **[Crossref]**
8. Fonseca AS, Szejnfeld VL, Terreri MT, Goldenberg J, Ferraz MB, Hilário MO. Bone mineral density of the lumbar spine of Brazilian children and adolescents aged 6 to 14 years. Braz J Med Biol Res. 2001;34:347-52. **[Crossref]**
9. Jacoby JT, Bento Dos Santos B, Nalin T, et al. Bone mineral density in patients with hepatic glycogen storage diseases. Nutrients. 2021;13:2987. **[Crossref]**