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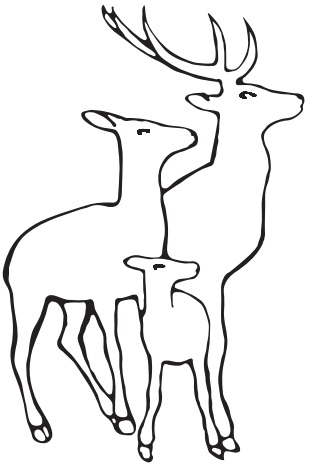

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Original Article

Effectiveness of a blood culture bundle in reducing contamination rates in a neonatal intensive care unit

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ABSTRACT

Background. Isolating microorganisms from blood cultures is the gold standard for identifying the cause of sepsis. However, contamination of the blood culture is a significant barrier to the blood culture's utility. In this study, we aimed to evaluate the impact of blood culture bundles on the incidence of contamination in the neonatal intensive care unit (NICU).

Methods. A prospective research to compare pre-bundle and bundle periods was created. During the bundle period, a bundle for blood culture sampling was implemented. The numbers of unnecessary antibiotic days and hospital stay following a false positive blood culture were used to calculate costs.

Results. A total of 320 neonatal blood culture procedures were included. The rate of blood culture contamination was 3.8% in the bundle and 12.5% in the pre-bundle period, this was significantly higher in the pre-bundle period ($p<0.001$). The implementation of the blood culture bundle reduced blood culture contamination by 69.6%. The average number of hospital days attributed to blood culture contamination was 3.8 days. The average cost of a hospital stay due to contamination of one blood culture was $883.12. During the study, 14 blood culture contaminations, 54 unnecessary NICU stay days were avoided and $12549.6 were saved.

Conclusions. We found that the blood culture bundle program was successful at decreasing the blood culture contamination, preventing additional hospital stay and treatment costs in the NICU.

Key words: blood culture bundle, blood culture contamination, cost-effectiveness; neonatal intensive care unit, neonates.

Infections are never as frequent and life-threatening as in the neonatal period. Neonatal sepsis is a clinical syndrome in which clinical signs and laboratory results of infection are present in the first month of life and a specific agent is produced in blood culture. Despite the advances in neonatology, it is still an important cause of morbidity and mortality. The incidence of neonatal sepsis is reported to be between 1-8.1 per 1000 live births and lower in developed countries.[1]The lack of sepsis-specific findings in the neonatal period, the narrow repertoire of clinical findings in newborns, and the fact that non-infectious clinical conditions frequently encountered in this period have similar findings pose a serious problem for early diagnosis and

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treatment of neonatal sepsis. Another important problem is that early empirical antibiotic treatment without diagnostic confirmation has to be resorted to very frequently in order to avoid morbidity and more importantly mortality with early intervention. This leads to unnecessary and prolonged treatment of newborns with potentially dangerous broad-spectrum antibiotics as well as prolonged hospitalizations and significant costs.[2,3]

The diagnosis of sepsis in the newborn is made by evaluating clinical and laboratory findings together. The gold standard is the detection of growth in blood culture. However, detection of growth in blood cultures in newborns does not always give very accurate results due to the difficulties of growth due to insufficient blood sample collection and high contamination rates.[4,5] A blood culture contamination is defined as a microorganism that is supposed to be introduced into the culture during either specimen collection or processing and that is not pathogenic for the patient.[6] The rate of contaminated blood culture in the neonatal period is 2.6-18%.[7] There are numerous studies on practices to reduce blood culture contamination in children and neonates, including dedicated blood collection teams, application of different skin disinfection solutions, commercially produced blood culture collection packs, staff training programs, and improvements in hand hygiene.[8-11]

In this pre and post intervention study we aimed to evaluate the impact of blood culture bundles on the incidence of blood culture contamination rates in the neonatal intensive care unit (NICU).

Materials and Methods

This observational prospective pre-post intervention study was conducted in the NICU of the University of Health Sciences, İzmir Faculty of Medicine, Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital between May 2022 and May 2023. The hospital is a 400-bed pediatric teaching hospital and the NICU is a 58-bed level 4 unit with approximately 1300 patients admitted annually, which is an advanced referral center for a very large patient population including neonatal cardiac surgery and neonatal surgery.

The study covered two periods: a pre-bundle phase (May–December 2022) and a post-bundle phase (January–May 2023), each lasting six months. The checklist for the blood culture contamination precaution package (Supplementary Table S1) was prepared using guidelines from the Turkish Society of Clinical Microbiology.[12]

Sample size: A priori power analysis was conducted to determine the adequacy of the number of cases by using Medcalc v.12.3.0 (MedCalc Software bvba, Broekstraat 52, 9030 Mariakerke, Belgium). As false positive blood culture rates were not known in our unit prior to the study, false positive culture rates were estimated at 5% based on published literature. With a power of 80% and $\alpha = 0.05$, we aimed to include 160 infants before and after initiation of the intervention to demonstrate that the false positive blood culture rate decreased from 5% to 0.5% with the use of the blood culture bundle.[13]

The patients included in the study included a total of 320 (160 study, 160 control group) neonates up to 28 days postnatal and/or 44 weeks postconceptional who were hospitalized in the NICU and required diagnostic blood cultures at any time during hospitalization. Patients with antibiotic use within 48 hours before blood culture, patients with underlying skin problems and patients whose results could not be obtained were excluded from the study. Among the patients hospitalized in the NICU before the start of the study, patients with a similar gestational week with the newborns included in the study group were selected retrospectively and taken as the control group.

During both periods, blood culture collection adhered to an aseptic technique. For this purpose, hands were cleaned with antibacterial liquid soap (Klorhexin scrub antibacterial liquid

soap 4% - Ekin Medical), the patient's skin was cleaned with povidone iodine 10% solution (Betadix 10% solution- Natural Medical Pharma) using sterile gloves, waited at least 30 seconds and then povidone iodine was removed from the skin with 70% alcohol. Then, 1 mL of blood collected with a 22 gauge or larger needle was contained in a pediatric blood culture bottle (BACTEC PedPlus, BD Diagnostics, Maryland, USA) and sent to the laboratory. Each blood culture bottle was placed in the BACTEC FX automated system (BD Diagnostics, Maryland, USA). Identification and antimicrobial susceptibility testing of the bacteria was performed using the automated VITEK-2 COMPACT (bioMérieux, Marcy l'Etoile, France) system. Gram-positive bacteria identification and antimicrobial susceptibility testing were performed using the VITEK-2 AST-P664 card. Gram-negative bacteria identification and antimicrobial susceptibility testing were done with VITEK-2 cards AST-N420, AST-N423, and AST-N326. Yeast identification was performed using a VITEK-2 AST-YST card. Antifungal susceptibility of the yeasts was determined by broth microdilution method with Sensititre YeastOne plate (Thermo Fisher Diagnostics BV, Landsmeer, The Netherlands).

At the beginning of the blood culture bundle implementation period, all doctors and nurses in charge of blood culture collection were informed about all items related to the checklist (Supplementary Table S1) with the blood culture contamination precaution package and its necessity. During blood culture collection using the checklist, one health care worker (HCW) performed the blood culture collection and the second HCW guided and filled the checklist form. Every procedure was observed by a supervisor nurse.

The patients' gender, gestational week, birth weight, postnatal age (in days), presence of sepsis clinic, C-reactive protein (CRP) results, and results of bacteria grown in blood cultures, if any, were obtained from file notes and electronic medical record database. The data were evaluated and recorded by three investigators and the investigators were blinded to patient information to protect patient confidentiality.

Statistics were performed with SPSS statistical software (version 22; SPSS, Chicago, IL, USA). Average age, gestational age, and age at the culture collection were expressed as median, interquartile range (IQR, Q1-Q3) and the number of hospital stays were expressed as mean±SD. Student's t-test for dependent and independent groups was used for comparisons of normally distributed numerical data, and Mann-Whitney U tests were used for non-normally distributed numerical data. Chi-square test was used to analyze categorical data. A p-value < 0.05 was considered statistically significant.

Economical evaluation

For patients misclassified as having bloodstream infections, the number of unnecessary antibiotic days was recorded according to unit protocol. Direct medical care cost items were calculated from the hospital perspective using a combination of micro-costing technique (resource-based accounting method) and hospital list data. Attributable length of stay for hospital admission was considered as the span of days for the treatment of central line associated bloodstream infection (CLABSI). Based on the number of patients affected due to blood culture contamination and the number of unnecessary antibiotic days, the extra hospitalization fees of the patients were calculated as Social Security Institution (SSI) payment. The investigators recorded the costs first in Turkish Lira (TL) and converted them to USD ($), using the average exchange rate between TL to USD currency between 01 May 2022 and 01 May 2023 ($1 = 17.69 TL, 1 TL = $0.056).

The local ethical committee of the Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital gave its approval to this investigation with the date and registration number 12.01.2023/797, and informed consent forms were obtained from each participant prior to enrollment.

Results

A total of 320 neonatal blood culture procedures were analyzed, with 160 in the pre-bundle (control) and 160 in the bundle (intervention) period.

Comparison of the study and control group

There were 112 (70%) male and 48 (30%) female patients in the study group and 89 (55.6%) male and 71 (44.4%) female patients in the control group, and the males were significantly higher in the study group (p=0.008). The average gestational age and birth weight were not different between study and control group (p>0.05). The average age at blood culture collection was 28.5 days in the study group and 6.7 days in the control group, and significantly longer in the study group (p<0.001). The demographic features are given in Table I.

CRP levels were <0.5 mg/dL in 120 (75%) patients in the study group, and 131 (81.9%) in the control group, and no significant difference was present between the groups (p>0.05). Fifty-one (31.9%) patients in the study group and 37 (23.1%) patients in the control group had clinical signs of sepsis, showing no significant difference (p>0.05).

Isolated microorganisms and blood culture results

When the patients were evaluated according to their clinical conditions, CRP results and blood culture growth results; 102 (63.8%) patients in the study group and 101 (63.1%) patients in the control group were evaluated as true negative. Among the 26 patients with confirmed bloodstream infections, the most common isolated microorganisms were *Klebsiella pneumoniae* (n=8; 30.8%), coagulase negative staphylococci (CoNS) (n=4, 15.4%), *Serratia marcescens* (n=4, 15.4%), *Candida albicans* (n=3, 11.5%), *Candida tropicalis* (n=2, 7.7%), *Escherichia coli* (n=2, 7.7%), *Staphylococcus aureus* (n=2, 7.7%), and *Acinetobacter baumannii* (n=1, 3.8%).

All the detected blood culture contaminants were CoNS. The rate of blood culture contamination in the study group was 3.8% (n=6) and 12.5% (n=20) in the control group, and significantly higher in the control group (p<0.001). The implementation of the blood culture bundle decreased the blood culture contamination by 69.6%. Blood culture results and the number of unnecessary hospitalization days are given in Table II.

Economical and clinical evaluation of additional burden of blood culture contamination

The number of hospital stay day attributable to blood culture contamination was 3.8±1.5 (range from 2 to 6) days. In Türkiye, NICU payment by the social security system is based on a fixed payment per day and was 411152 TL ($232.4) per patient day. Therefore the average cost of hospital stay attributable to contamination of

Table I. Demographic characteristics and results of the pre and post-bundle groups.

	Post-bundle Group	Pre-bundle Group	p
Gender, n (%)			
Male	112 (70%)	89 (55.6%)	0.008
Female	48 (30%)	71 (44.4%)	
Gestational age (day), mean ±SD (min-max)	32.7±4.6 (23-41)	33.0±4.2 (23-40)	>0.05
Birth weight (gram), mean ±SD (min-max)	2014.8 ± 932.4 (680-4070)	2079.8 ± 872.4 (520- 4440)	>0.05
Age at culture collection (day), median (Q1-Q3)	20.5 (8- 42)	4 (2-10)	<0.001
CRP positivity, n (%)	40 (25%)	29 (18.1%)	>0.05
Clinical appearance of sepsis, n (%)	51 (31.9%)	37 (23.1%)	>0.05

CRP, C-reactive protein; SD, standard deviation.

Table II. Blood culture results and number of unnecessary hospitalization days.

	Post-bundle Group	Pre-bundle Group	p
Blood culture growth, n (%)	22 (13.8%)	33 (20.6%)	>0.05
True negative, n (%)	102 (63.8%)	101 (63.1%)	>0.05
True positive, n (%)	16 (10%)	10 (6.3%)	>0.05
Suspected, n (%)	36 (22.5%)	29 (18.1%)	>0.05
Contamination, n (%)	6 (3.8%)	20 (12.5%)	<0.01

one blood culture was calculated as $883.12 (ranging from minimum $464.8 to maximum $1394.4).

Within the implementation of the blood culture bundle, 14 blood culture contaminations were prevented during the study, preventing 54 unnecessary NICU stay days and saving $12549.6 in a 6-month period. In a model of NICU with an annual number of 1000 blood culture sampling, within the implementation of the blood culture bundle, a total of 87 per 1000 blood culture samples will be prevented. In this way, a minimum of 174 days, a maximum of 522 days, and an average of 330 days of unnecessary hospitalization due to blood culture contamination per 1000 blood cultures will be prevented. The total money saved within the implementation of the blood culture bundle will range from $40437.6-$12131.28 with an average of $76692 per 1000 blood culture sample. The cost savings that can be reduced by preventing blood culture contamination as a result of package application are given in Table III.

Discussion

The neonatal period is the most common period in which sepsis occurs during infancy. Neonatal sepsis is a clinical condition whose definitive diagnosis is based on growth in blood culture and which requires hospitalization for diagnosis and treatment.[14] In this pre and post-intervention study we aimed to evaluate the impact of the blood culture bundles on the incidence of blood culture contamination rates in the NICU, and with the implementation of a blood culture bundle the rate of blood culture contamination decreased significantly by 69.6%. The number of hospital stay attributable to blood culture contamination was 3.8±1.5 days and the average cost of hospital stay attributable to the contamination of one blood culture was calculated as $883.12. The Clinical and Laboratory Standards Institute recommends

Table III. Cost-savings that can be reduced by preventing contamination with the bundle implementation.

Definition	
NICU pay per day by the social security system ($)	232.4
Total additional cost per contaminated blood culture ($), mean ±SD (min-max)	883.12±268.35 (464.8-1394.4)
Savings with the implementation of the bundle ($)	12549.6
Number of days of hospitalization saved by prevention 87 contamination /1000 blood cultures, mean ±SD (min-max)	330±100.5 days (174-522)
1000 blood cultures / 87 contamination prevention Total Savings ($), mean ±SD (min-max)	76692 ±23358.6 (40437.6-121312.8)

NICU, Neonatal Intensive Care Unit; SD, standard deviation.

a blood culture contamination target of 3% or less.[15] However, this rate is reported in a wide range of 2.85-9.1% in the literature.[16,17] In neonates, due to the difficulties in obtaining blood cultures, these rates can be in a wide range of 2.6-18% and much higher.[7,18-20]

The collecting blood culture procedure consists of many steps such as hand hygiene, skin disinfection, blood culture bottle preparation, blood collection, and handling of samples to the laboratory. Improper practices that cause contaminated blood culture can occur in each of these steps. In order to reduce the contamination rates, many applications have been made that affect the various stages of the blood culture procedure. Bundle applications ensure that the correct practices are carried out at every stage of blood culture collection, while at the same time ensuring regular training repetition and awareness raising of personnel. This leads to an exponential increase in the power of these practices.[20]Hand disinfection and hygiene before blood culture collection is one of them.

As a result of increasing compliance with hand hygiene in a tertiary NICU, the contamination rate for CoNS was reduced from 4.2±2.4 to 1.9±1.8 per 1000 patient days.[21]Routine wearing of sterile gloves prior to blood culture collection has been shown to reduce contamination rates by 50% compared to wearing optional sterile gloves.[22]Staff training and awareness are also effective in reducing blood culture contamination rates. In a study in which a staff training intervention program was implemented in an intensive care unit (ICU), it was reported that monthly ICU blood culture contaminant rates during the intervention period were reduced to an average of 3.7% compared to 9.5% in the baseline period.[10]In another study, contamination rates were found to be statistically significant at 14 versus 5.6 per 100 blood cultures before and after planned training on what to look out for during the blood culture collection procedure in the ICU.[23]

Besides these efforts, a dedicated blood culture sampling bundle and checklist had an exponential effect on lowering the blood culture contamination, and our experience also supported the effectiveness of the blood culture bundle. In addition to all these single applications, the idea that the application of two or three combinations of these applications together theoretically reduces blood culture contamination rates more has brought blood culture contamination prevention package applications to the agenda. In a non-randomized study in which only sterile collection packs and hand hygiene and skin cleaning were standardized in the NICU, the false positive rate, which was 4.6% before the application, was reduced to 0.6% after this application.[20] A 12-month quality improvement (QI) program that implemented a package of transfer, inoculation, skin antisepsis, aseptic pack and blood volume optimization to reduce the blood culture contamination rate by 50% in a neonatal unit resulted in a blood culture contamination rate reduction from 2% to 1%.[24]

Blood culture contamination results in unnecessary laboratory tests, inappropriate antimicrobial therapy, long-term antimicrobial resistance, as well as prolonged hospital stay and increased cost.[25,26] The number of hospital stay attributable to blood culture contamination was 3.8 days. In addition to the negative effects of hospitalization on mother-infant bonding and maintenance of breastfeeding, the mandatory empirical use of antibiotics in treatment has undesirable consequences in terms of its negative effects on all systems of the newborn, whose maturation and reserves are extremely limited, and the resulting dysbiosis.[27] In a study comparing nurses taking blood cultures without a standard protocol with nurses taking blood cultures with sterile kits using a special sterile collection kit and laboratory-trained phlebotomy teams taking blood cultures, contamination rates associated with usual care, sterile kits and phlebotomy teams were found to be 4.34%, 1.68% and 1.10%, respectively, and the annual net savings using sterile kit and phlebotomy team strategies compared

to the first group were $483.219 and $288.980, respectively.[28]

In our study, with the implementation of the blood culture bundle, $12549.6 in a 6-month period was saved. In addition, when the blood culture number increases, the money saved would increase up to $12131.28 per 1000 blood culture sample. A previous study estimated annual cost saving of approximately £250.100 with implantation of blood culture bundle. In a study evaluating hospital costs for patients with negative, false positive and true positive blood culture results, an average additional cost of $8.720 per contamination event was reported, supporting our study.[29]

This study has certain limitations. First of all, despite being a prospective, pre and post-intervention study, it lacks the advantages of a randomized control study. The patients in the two periods were not homogenous regarding age. In addition, we measured the cost of blood culture contamination as a direct cost of hospital stay, however, we did not account for indirect costs, such as additional laboratory investigations.

In conclusion, we found that the blood culture bundle program was successful at decreasing the blood culture contamination, preventing additional hospital stay and treatment costs in the NICU.

Supplementary materials

Supplementary materials for this article are available online at https://doi.org/10.24953/turkjpediatr.2025.5613

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Ethical approval

The study was approved by Ethical Committee of the Dr. Behçet Uz Pediatric Diseases and Surgery Training and Research Hospital (date: 12.01.2023, number: 797).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: ŞÇ, NK, MYÇ; data collection, analysis and interpretation: OHK, TK, AB, İD; draft manuscript preperation: ŞÇ, OHK. All authors reviewed the results, critically reviewed the manuscript and approved the final version for publication.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Association of miR-149 gene rs2292832 polymorphism with necrotizing enterocolitis in preterm infants

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ABSTRACT

Background. Necrotizing enterocolitis (NEC) is a prevalent and challenging intestinal disease in premature infants, lacking a specific pathogen consistently associated with its occurrence. Effectively preventing and treating NEC to reduce mortality rates remains a significant contemporary challenge. The present study aimed to explore the correlation between microRNA-149 gene polymorphism and NEC in premature infants in a Chinese Han population.

Methods. The expression levels of serum miR-149 were determined using reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Polymorphism detection of the miR-149 gene rs2292832 polymorphism was performed by polymerase chain reaction. Multivariate logistic regression analysis was employed to investigate the association between the rs2292832 polymorphism and risk factors for NEC in preterm infants.

Results. General clinical data were compared between 102 preterm infants diagnosed with NEC and 263 preterm infants without NEC. Significant differences were observed in gestational age and birth weight. However, no significant differences were found in antenatal steroid use, sex, or feeding patterns between the two groups. The expression level of serum miR-149 was significantly reduced in premature infants with NEC, and there were differences in the allele frequency of the miR-149 rs2292832 polymorphism between the NEC group and control group. Specifically, the T allele and TT genotype of rs2292832 were associated with an increased susceptibility to NEC. Furthermore, both gestational age and the rs2292832 polymorphism showed a significant association with NEC risk, with the rs2292832 polymorphism of miR-149 being identified as the most prominent risk factor for NEC development in preterm infants.

Conclusions. The rs2292832 gene polymorphism of miR-149 may potentially exert an influence on susceptibility to NEC.

Key words: Necrotizing enterocolitis (NEC), Gene polymorphism, MiR-149, rs2292832.

Necrotizing enterocolitis (NEC) is a prevalent and challenging-to-predict inflammatory intestinal disease in newborns, resulting from the complex interplay of multiple factors. These factors encompass preterm birth, formula feeding, abnormal colonization of intestinal microbiota, intestinal mucosal ischemia, infection, and dysbiosis.[1,2] Relevant statistical data indicate that NEC primarily affects premature and low-birth-weight infants. The incidence rate among preterm infants ranges from 5% to 7%, while it reaches between 4% and 13% in premature newborns weighing less than 1500 grams. Furthermore, NEC remains a leading cause of mortality.[3-5] As a leading cause of mortality and morbidity in neonatal intensive care units, NEC exhibits a high mortality rate ranging from 15% to

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30%.[1,6] Additionally, children afflicted by this disease often encounter adverse prognostic factors such as neurodevelopmental delay, growth disorders, and intestinal dysfunction. These complications not only significantly escalate healthcare costs but also profoundly impact the long-term quality of life for these children.[7] However, despite extensive research on clinical risk factors associated with NEC occurrence thus far no specific pathogen has been consistently linked to enterocolitis development. Our understanding regarding the pathogenesis of NEC remains limited. Relying solely on clinical risk factors fails to elucidate individual variations in susceptibility to or severity of NEC among preterm infants. This indicates the involvement of genetic risk factors in disease pathogenesis.[8] The potential genetic predisposition for NEC is gradually gaining recognition with expectations that genetic investigations will provide novel insights into its pathogenesis.

MicroRNAs (miRNAs) are a class of small, highly conserved non-coding RNAs that play a crucial role in regulating various cellular and metabolic pathways by binding to target mRNAs and modulating the expression of candidate proteins. Single nucleotide polymorphisms (SNPs) within miRNA sequences or their binding sites can potentially disrupt the interaction between miRNAs and mRNA targets, leading to dysregulation of gene expression.[9] Consequently, SNPs in miRNAs have been implicated in the pathogenesis of numerous diseases. Previous studies have demonstrated significant downregulation of miR-149 in preeclampsia placenta[10], with lower levels correlating with disease severity and potential diagnostic utility for preeclampsia.[11] MiR-149 is also involved in trophoblast cell proliferation, migration, and invasion[12], while its reduced expression level in preeclampsia placenta regulates trophoblast cell behavior.[13] Moreover, decreased levels of miR-149-5p contribute to impaired angiogenesis in HUVEC cells.[14] Additionally, overexpression of miR-149-5p can alleviate brain ischemia/reperfusion (I/R) injury by targeting Notch2. Gene polymorphisms play a pivotal role in the pathogenesis of various diseases.[15]

The investigation of candidate genes associated with the occurrence and progression of NEC can provide insights into the molecular mechanisms underlying NEC. In the Korean population, it has been observed that the TT genotype (T>C, rs2292832) of miR-149 is linked to an increased risk of spontaneous abortion.[16] Conversely, the C allele of rs2292832 in miR-149 may confer protection against gastric mucosal atrophy.[17] Furthermore, in an Iranian population using a recessive inheritance model (TT/(TC+CC)), a significant association between the rs2292832 variant in miR-149 and colorectal cancer susceptibility has been identified.[18] Additionally, studies have demonstrated decreased expression levels of serum miR-149-5p in patients with inflammatory bowel disease.[19] However, the impact of the rs2292832 polymorphism site in miR-149 on preterm infants' susceptibility to NEC remains unclear.

This study aimed to further investigate the impact of miR-149 rs2292832 polymorphism on NEC by detecting differences in genotype and allele frequencies between preterm infants with and without NEC, providing novel insights for early diagnosis and treatment of NEC.

Materials and Methods

Study participants

In this study, 102 preterm infants diagnosed with NEC and admitted to the neonatal intensive care unit (NICU) of Women and Children's Hospital of Ningbo University were selected as the NEC group based on inclusion and exclusion criteria. Simultaneously, a total of 263 preterm infants admitted to our hospital's NICU who did not develop NEC were selected as the control group. The NEC group included infants with gestational ages ranging from 28 to 36 weeks, while the control group included infants with gestational ages ranging from 28.3 to 36.6 weeks. General patient data including sex, gestational age, antenatal steroid use,

birth weight, and feeding characteristics were recorded. The inclusion criteria specifically required: (1) Neonates younger than 28 days; (2) Clear diagnosis of NEC according to Bell staging diagnostic standards with a stage greater than II. Exclusion criteria mainly included: (1) Presence of congenital intestinal malformations; (2) Gastroenteritis; (3) Inherited metabolic disorders; (4) History of hypoxia or asphyxia; (5) Pneumonia, intestinal atresia, Hirschsprung's disease or other conditions; (6) Incomplete medical records or death within a few hours after admission; (7) Sepsis. To exclude infants with sepsis, we identified cases based on clinical symptoms, signs, and laboratory findings. Specifically, if an infant exhibited fever or hypothermia, lethargy, tachypnea, tachycardia, and other clinical manifestations, along with laboratory evidence such as abnormal white blood cell counts (either increased or decreased), elevated C-reactive protein levels, and positive blood cultures, the case was classified as sepsis. Infants diagnosed with sepsis were excluded from the study because sepsis can significantly impact intestinal function and systemic inflammatory response, potentially confounding the accurate assessment of the relationship between miR-149 gene polymorphisms and NEC. To ensure a more precise investigation of the association between the target gene polymorphism and NEC, we opted to exclude these cases.

This study has been approved by the Women and Children's Hospital of Ningbo University Medical Ethics Committee (Ethics No. 2019-ky-042, Ethics Approval Date: 2019-6-22) and informed consent forms have been signed by all newborn guardians. All procedures adhere to the principles outlined in the Helsinki Declaration.

Isolation of DNA and genotyping

After the diagnosis of NEC, peripheral blood samples (0.5 ml) were collected from infants in both the control group and NEC group within 24 hours of admission, and placed in EDTA anticoagulation tubes. Subsequently, the blood samples were stored in epoxy resin tubes and kept at -80°C. The control group underwent blood sampling during the 42-day routine physical examination. Blood samples from both groups were uniformly analyzed within one week following the blood draw. Total DNA extraction and PCR amplification were performed using the QIA amp DNA Blood Mini Kit 51104 (cat No. 51304) (Qiagen, Germany), while Primer 5.0 software was utilized for primer design. This study focused on a specific SNP of the miR-149 gene, namely rs2292832. The PCR reaction conditions were as follows: pre-denaturation was performed at 95°C for 3 minutes; denaturation was carried out at 94°C for 30 seconds; annealing occurred at 55°C for 30 seconds, with a total of 35 cycles; subsequently, extension took place at 72°C for 30 seconds, followed by a final extension step at the same temperature lasting for 10 minutes. The amplified products were identified through agarose gel electrophoresis. Sanger sequencing was employed to determine the sequence of the amplified products. Finally, SNP typing analysis was conducted using Seq-Man software.

Quantitative real-time PCR (RT-qPCR)

The total RNA of miR-149 was extracted individually, followed by reverse transcription into cDNA using the cDNA as a template for RT-qPCR amplification. Subsequently, the resulting cDNA was amplified using the Roche Light Cycler® 96 instrument and reverse transcription reagents. The expression level of miR-149 was analyzed by RT-qPCR, and the relative gene expression level was calculated using the $2^{-\Delta\Delta Ct}$ formula to reflect the multiplex change of miR-149 gene expression. U6 served as an internal control for miR-149.

Statistical analysis

Data processing was performed using SPSS 16.0 statistical software in this study. The effect size analysis (input: effect size w = 0.3, alpha error probability = 0.05, power (1-beta error probability) = 0.95, df = 5) indicated that the required sample size for calculation was 220.

Quantitative data were presented as mean ± standard deviation, while qualitative data were expressed as percentages (%). Student's t-test was employed for comparing two groups of quantitative data, and the χ^2 test was used for comparing two groups of qualitative data. Additionally, unconditional logistic regression analysis was conducted to investigate the association between gene polymorphisms and the susceptibility and severity of NEC. Statistical significance was defined as $P < 0.050$.

Results

The general clinical characteristics of NEC individuals

This study enrolled a total of 102 preterm infants diagnosed with NEC and 263 preterm infants admitted to the NICU without NEC. Our actual sample size was 365, resulting in a power of 99.8%, which provides high confidence in our results. In terms of gestational age, the NEC group included infants with gestational ages ranging from 28 to 36 weeks, while the control group included infants with gestational ages ranging from 28.3 to 36.6 weeks. The NEC group had an average gestational age of 32.5 ± 2.9 weeks, while the control group had an average gestational age of 33.4 ± 2.3 weeks, demonstrating a significant difference between the two groups (P = 0.003). Regarding birth weight, the NEC group had an average birth weight of 2040 ± 206 g, whereas the control group had an average birth weight of 2120 ± 213 g; these values also exhibited a significant difference (P = 0.001). The antenatal steroid use was similar in both groups (P = 0.702). There was no substantial disparity in the sex ratio between the two groups (P = 0.529). Furthermore, there was no notable variation in feeding characteristics, whether breastfeeding, formula feeding, or both—between the two groups ($P > 0.050$, Table I). The onset of NEC in preterm infants was about two weeks after birth.

The genotype and allele frequencies of miR-149 rs2292832 gene polymorphisms

The distribution of allele and genotype frequencies of the miR-149 rs2292832 polymorphism in NEC patients is summarized in Table II. Analysis of the rs2292832 genotype revealed a significant association between the T allele and NEC onset, with a high occurrence frequency of 77.45% (χ^2 = 10.527, 95% CI = 1.848 [1.271-2.686], P = 0.001). Conversely, the C allele showed a relatively lower association, occurring at a frequency of only 22.55%. Within the NEC group, there was a relatively higher frequency of homozygous TT genotype (62.75%) than the control group (45.25%), while heterozygous mutant genotype CT and recessive homozygous CC genotypes had lower frequencies in this group (29.41% and 7.84%, respectively) than the control group (39.54% and 15.21%, respectively). In summary, the T allele of rs2292832 genotype was associated with an increased risk for NEC susceptibility compared to the relatively low genetic predisposition observed with the C allele (Table II).

The rs2292832 locus constituted a significant risk factor for the development of NEC in premature infants

The association between miR-149 rs2292832 and the risk of NEC in premature infants was further investigated using multivariate logistic regression analysis. In this analysis, gestational age showed a significant association with NEC risk (odds ratio [OR] = 0.574, 95% CI 0.348-0.947, P = 0.030. However, birth weight, antenatal steroid use, sex, and feeding did not show a significant association with NEC risk ($P > 0.050$). On the other hand, the genotype rs2292832 polymorphism exhibited a significantly positive association with NEC risk (OR = 4.009, 95% CI 1.517-10.597, P = 0.005, Table III). The forest plot visually demonstrates that the miR-149 rs2292832 polymorphism was the most prominent risk factor for NEC development in preterm infants (Fig. 1).

Table I. The clinical information of two study groups.

Characteristics	NEC (n=102)	Control (n=263)	P values
Gestational age, weeks, mean±SD	32.5 ± 2.9	33.4±2.3	0.003
Birth weight, grams, mean±SD	2040 ±206	2120±213	0.001
Antenatal steroid use, n (%)			0.702
Yes	84 (82.4)	212 (80.6)	
No	18 (17.7)	51(19.4)	
Sex, n (%)			0.529
Male	53 (51.96)	127 (48.29)	
Female	49 (48.04)	136 (51.71)	
Feeding type, n (%)			0.943
Breastmilk	39 (38.24)	96 (36.50)	
Formula	31 (30.39)	84 (31.94)	
Both	32 (31.37)	83 (31.56)	
Time of diagnosis, week, mean±SD	1.83±0.42		

NEC, necrotizing enterocolitis; SD, standard deviation.

Table II. The genotype and allele frequencies of miR-149 rs2292832 gene polymorphisms.

Genotype / Allele	NEC (n=102), n (%)	Control (n=263), n (%)	$\chi 2$	OR (95%CI), median (Q1-Q3)	P
Genotype					
CC	8 (7.84)	40 (15.21)	-	1	-
CT	30 (29.41)	104 (39.54)	0.700	1.442 (0.610-3.412)	0.403
TT	64 (62.75)	119 (45.25)	5.940	2.689 (1.187-6.091)	0.015
Allele					
C	46 (22.55)	184 (34.98)	-	1	-
T	158 (77.45)	342 (65.02)	10.527	1.848 (1.271-2.686)	0.001

CI, confidence interval; NEC, necrotizing enterocolitis; OR, odds ratio.

Table III. Multivariate logistic regression analysis of risk factors for NEC in preterm infants.

Variables	Multivariate analysis		
	OR	95% CI	P value
Gestational age	0.574	0.348-0.947	0.030
Birth weight	1.186	0.717-1.964	0.507
Antenatal steroid use	0.834	0.454-1.532	0.559
Sex	1.208	0.756-1.930	0.430
Feeding	0.965	0.547-1.701	0.902
Rs2292832	4.009	1.517-10.597	0.005

CI, confidence interval; NEC, necrotizing enterocolitis; OR, odds ratio.

The expression level of miR-149 was downregulated in individuals with NEC

RT-qPCR analysis was employed to assess miR-149 expression levels in individuals from both groups and revealed that serum miR-149 expression level was significantly reduced in NEC compared to controls (Fig. 2A). To investigate the association between miR-

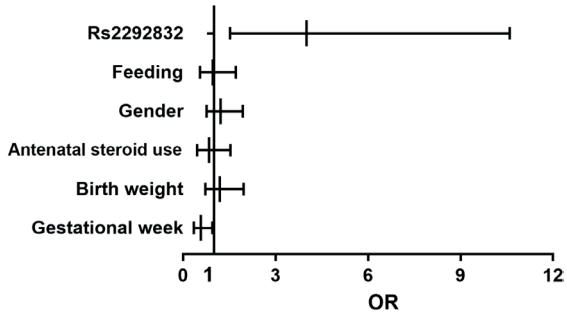

Fig. 1. Forest map analysis of risk factors for necrotizing enterocolitis (NEC) in preterminfants.
OR, odds ratio.

149 gene polymorphism locus rs2292832 and preterm infants with NEC, we further examined expression levels across different genotypes within both the NEC and control groups. The results demonstrated that within both groups, the homozygous CC genotype exhibited significantly higher miR-149 expression levels than the mutant TT + TC genotype did; moreover, this gap in miR-149 expression level between CC genotype and mutant TT + TC genotype appeared more pronounced within the NEC group compared to controls (Fig. 2B). These findings suggested the potential association between miR-149 gene polymorphism locus rs2292832 and NEC might be related to its mediation in miR-149 levels.

Discussion

In this study, the expression level of serum miR-149 was significantly lower in preterm infants with NEC, and the allele frequency of the miR-149 rs2292832 polymorphism differed between the NEC and control groups. Specifically, the T allele and TT genotype of rs2292832 were associated with an increased susceptibility to NEC. NEC is a severe gastrointestinal disorder with significant implications in the neonatal period, often necessitating urgent surgical intervention and posing a grave threat to life.[20] Khasawneh et al.[21] have highlighted that preterm infants constitute the primary high-risk group for developing NEC. Despite extensive research efforts, the pathogenesis of NEC remains elusive; however, given its variable severity among newborns, genetic factors may play a pivotal role in its onset. Notably, *CPS1* T1405N polymorphism may be associated with the risk of NEC in preterm infants[22], and functional variants of the *CPS1* gene may be associated with NEC susceptibility.[23] Our study aimed to investigate polymorphisms associated with NEC and elucidate potential mechanisms underlying this condition to facilitate the development of personalized treatment strategies.

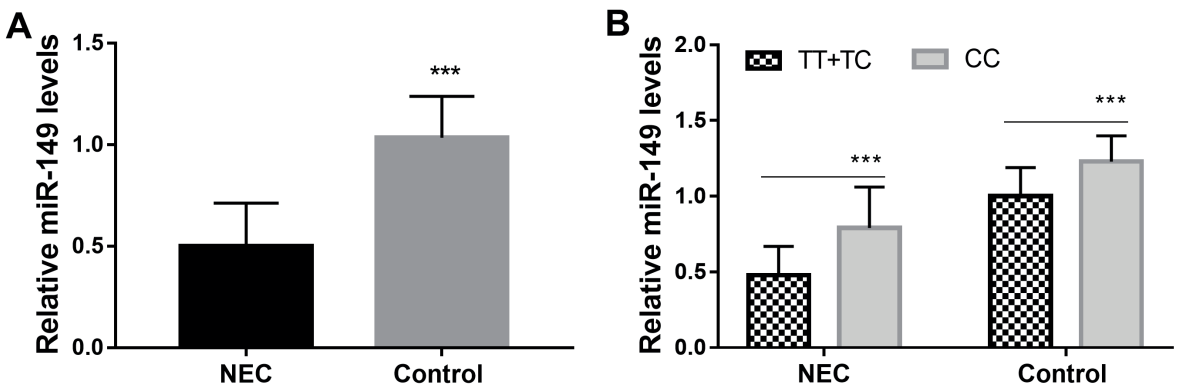

Fig. 2. Expression level of miR-149. **A.** Expression of miR-149 was reduced in the necrotizing enterocolitis group. **B.** Expression levels of different genotypes of rs2292832.
***$P < 0.001$. NEC, necrotizing enterocolitis.

Investigating candidate genes associated with the occurrence and progression of NEC can provide insights into the molecular mechanisms underlying NEC. This study aimed to explore the relationship between a genotype polymorphism (rs2292832) in the miR-149 gene and NEC. The study included 102 preterm infants with NEC and 263 infants without NEC hospitalized during the same period. The analysis of clinical data revealed a significant association between gestational age and birth weight in both the NEC group and the control group, as indicated by our findings. The results revealed significant associations between gestational age, rs2292832 polymorphism, and NEC risk. Furthermore, the miR-149 rs2292832 polymorphism emerged as a prominent risk factor for NEC development in preterm infants. Additionally, serum levels of miR-149 were significantly reduced in children with NEC, consistent with previous research findings, indicating decreased expression of serum miR-149-5p in patients with inflammatory bowel disease.[19] In both the NEC group and control group, the homozygous CC genotype exhibited significantly higher expression levels of miR-149 compared to mutant TT + TC genotypes; moreover, this difference was more pronounced within the NEC group than in controls. Previous studies have linked miR-149 TT (T > C, rs2292832) to an increased risk of spontaneous abortion[16], while suggesting that the C allele of miR-149 rs2292832 may confer protection against gastric mucosal atrophy.[17] In the Chinese Han population, carrying the TT genotype or T allele for rs2292832 polymorphism in the miR-149 gene has been found to elevate gastric cancer risk.[24] Similarly, the TT genotype has been associated with the clinical stage of nasopharyngeal carcinoma.[25] Moreover, Iranian population studies have shown an association between the TT genotype of rs2292832 in miR-149 and coronary artery disease.[26] Additionally, they have found a significant correlation between the recessive genetic model TT / (TC + CC) for mir-RS299832 and cancer susceptibility.[18] After analyzing the rs2292832 genotype, it was observed that the allele T exhibits a significant association with the onset of NEC, while the proportion of heterozygous mutant genotype CT and recessive homozygous CC genotype in the NEC group was relatively small. Therefore, it can be inferred that the T allele of rs2292832 genotype was linked to an increased susceptibility to NEC, whereas the C allele demonstrates a comparatively lower association. These findings suggest that rs2292832 genotype polymorphism may be implicated in NEC risk, with individuals carrying the T allele or TT genotype being more susceptible to NEC.

Gene polymorphism can influence gene expression and function. In individuals with the rs2292832 genotype, those carrying the TT genotype demonstrate an increased susceptibility to NEC. This association may be attributed to the downregulation of miR-149 expression mediated by the TT genotype. Additionally, studies have demonstrated decreased expression levels of serum miR-149-5p in patients with inflammatory bowel disease.[19] This also coincided with our research findings regarding the lower expression level of miR-149 in NEC individuals. MiR-149 has been extensively reported in the literature for its involvement in various biological processes such as cell proliferation, apoptosis, and inflammatory response.[27] Dysregulated miR-149 expression could potentially compromise intestinal mucosal barrier function and exacerbate inflammatory responses, thereby heightening the risk of NEC. These studies provided compelling evidence supporting our conclusions while further emphasizing the intricate relationship between genotype, miR-149, and NEC. It is proposed that miR-149 may play a significant role in the pathogenesis of NEC.

However, this study has several limitations. Despite previous research indicating that breastfeeding confers a protective effect against NEC[28], our study did not observe a significant association between feeding methods and the risk of NEC. This discrepancy may be attributed to the relatively smaller sample size, which lacked sufficient statistical power to detect

the subtle influence of feeding methods on NEC risk. Although we endeavored to match relevant factors between the control and NEC groups, unexplained variables may still have influenced the results. Additionally, fetuses in the control group were generally more mature than those in the patient group, possibly due to the samples primarily originating from a single hospital with limited capacity for preterm infants. The relatively small sample size of the included standard samples may also contribute significantly to this deviation. This difference underscores the higher likelihood of NEC development in preterm infants with smaller gestational ages. Moreover, this study focused on the relationship between the miR-149 gene rs2292832 polymorphism and NEC susceptibility but did not analyze genetic variations associated with different stages of NEC or provide data on other inflammatory issues, intestinal perforation, or patient prognosis. Future studies should expand the sample size to explore genetic differences in these areas and provide more evidence for accurate diagnosis and treatment of NEC in preterm infants.

In conclusion, miR-149 rs2292832 polymorphism may be implicated in NEC. Specifically, the T allele of rs2292832 exhibited a significant correlation with NEC morbidity, and within NEC patients, there was a relatively high prevalence of individuals carrying the T allele and TT genotype.

Ethical approval

This study has been approved by the Women and Children's Hospital of Ningbo University Medical Ethics Committee. All procedures adhere to the principles outlined in the Helsinki Declaration. Informed consent forms have been signed by all newborn guardians.

Author contribution

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

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Turkish pediatricians' knowledge, attitudes, and awareness of respiratory syncytial virus (RSV) infection and immunization strategies: a cross-sectional study

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ABSTRACT

Background. This study aims to assess Turkish pediatricians' knowledge and attitudes regarding respiratory syncytial virus (RSV) infection and its current immunization strategies.

Methods. From May 10 to June 4, 2024, we invited 1603 pediatricians who subscribed to the website of The Turkish Pediatrics Atelier via e-mail to respond to an online questionnaire. A total of 401 pediatricians responded.

Results. Of pediatricians, 11% stated that they routinely use chest X-ray (CXR) for diagnosing RSV illness. When managing RSV-positive patients, while 44.4% of pediatricians indicated that they need a CXR if there are lung auscultation findings, the rate of routine CXR usage was 22.7%. While most pediatricians (74.8%) stated that they prefer nebulized salbutamol and/or corticosteroid; 43.4% used hypertonic saline; and 22.7% used nebulized epinephrine as a treatment option. While 60.3% of pediatricians had no information about the maternal RSV vaccine; 58.1% stated that they would recommend it to only willing women; 16% stated that they would not recommend it; and 25.9% indicated that they would recommend it to every pregnant individual. While most pediatricians (79.8%) had knowledge about nirsevimab; 14% indicated that it was not approved in children worldwide; 49.1% stated that it is more effective than palivizumab; and 37.9% indicated that they would start administrating it immediately after its approval and availability in Türkiye.

Conclusions. The use of CXR and administration of non-evidence-based therapies in diagnosing and managing RSV illness were relatively high. Additionally, there is a notable gap in knowledge and awareness regarding the maternal RSV vaccine and nirsevimab.

Key words: respiratory syncytial virus, immunization, nirsevimab, pediatrician.

Respiratory syncytial virus (RSV) is a predominant cause of acute lower respiratory tract infection (ALRTI), hospital admissions, and hospitalization in children under two years of age throughout the world regardless of income status, resulting in a considerable global burden on healthcare systems.[1] While healthy infants born at term are mostly hospitalized due to RSV, premature infants and those with underlying heart and lung disease are at the highest risk for serious illness.[2-4] It was estimated that globally in 2019, there were 33

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million RSV-associated ALRTI incidents, 3.6 million RSV-associated ALRTI hospitalizations, and over 100,000 deaths in children aged under 60 months.[5]

Many patients continue to be treated with non-evidence based therapies, although the mainstay of therapy for RSV infection remains supportive care, which includes respiratory support and maintaining hydration and nutrition status.[6] Prevention of RSV infection approaches include preventive measures to avoid RSV exposure and immunization.[7] Palivizumab, which was first recommended in 1998, is the first humanized monoclonal antibody used for immunoprophylaxis during the bronchiolitis season in infants at risk of severe ALRTI.[8,9] With the approval of the first bivalent RSV prefusion F protein–based vaccine in pregnancy to prevent RSV illness during the infancy period and a new RSV-neutralizing antibody (nirsevimab), 2023 has been considered a game-changing year in preventing morbidity and mortality of RSV infections in infants.[10,11]

This study aimed to evaluate the knowledge, attitudes, and practices of Turkish pediatricians regarding RSV infection and current immunization strategies

Materials and Methods

Study design

We performed a cross-sectional study using an online questionnaire form and distributed it to pediatricians through the involvement of the Turkish Pediatrics Atelier. The Turkish Pediatrics Atelier group was first established on the Telegram application on October 31, 2018. It has also had a website since 2023 (https://www.pediatriatolyesi.com). The group consists mainly of pediatricians, pediatric sub-specialists, pediatric surgeons, child psychiatry specialists, and physicians serving the pediatric population including pediatric dermatologists, pediatric ear, nose, and throat (ENT) specialists, pediatric radiologists, and pediatric urologists. Group members were included by invitation and memberships were confirmed by the Telegram group directors. The group functions as a professional forum where members can seek expert opinions on various pediatric topics, including radiological images, dermatological conditions, laboratory results, and drug dosages.[12] Its Telegram channel and website have approximately 2600 and 1600 registered members, respectively.

An online questionnaire, prepared using Google Forms, was sent via email to 1,603 members of the Turkish Pediatrics Atelier's website between May 10 and June 4, 2024. The questionnaire consisted of questions regarding the pediatricians' basic demographic features, knowledge, attitudes and behaviors toward RSV illness and its current immunization strategies. The pediatricians provided consent for participation in the online survey. This study was approved by the Ataşehir Memorial Hospital Ethics Committee.

Data analysis

Data were presented as mean ± standard deviation or median (25th-75th percentile) according to their distribution characteristics. Chi-square or Fisher's exact tests were used to compare categorical variables. Significance was set at $p < 0.05$. All these analyses were carried out using Jamovi 1.6 software.

Results

A total number of 401 (25%) pediatricians from 60 cities of Türkiye participated in this study. The median age of the pediatricians was 42 years (35-50), with 53.6% were female. The demographic features of the pediatricians are presented in Table I.

RSV general knowledge

Overall, 85.5% of pediatricians had managed at least one RSV case in their practice, while 81.5% had diagnosed at least one RSV case in the last year. Rapid antigen test and polymerase chain reaction (PCR) from nasopharynx swab

Table I. The demographic characteristics of the pediatricians.

Variables		n	%
Gender	Male	186	46.4%
	Female	215	53.6%
Specialty	Pediatrician	349	87%
	Pediatric subspecialist	52	13%
Professional experience in pediatrics	<5 years	46	11.5%
	5-10 years	92	22.9%
	10-20 years	122	30.4%
	>20 years	141	35.2%
Working setting	Private hospitals	137	34.2%
	Training and research / university hospitals	127	31.7%
	State hospitals	88	21.9%
	Private practice	43	10.7%
	Outpatient clinics	6	1.5%

preference among the pediatricians were 55.6% and 33.4%, respectively. Of the pediatricians 11% stated that they routinely use chest X-ray (CXR) for diagnosing RSV illness.

According to the pediatricians in this study, indications of ordering a CXR for children diagnosed with RSV illness were as follows; lung auscultation findings (44.4%), respiratory distress (26.2%), and high fever (1.5%). Among the pediatricians, 22.7% reported routinely ordering a chest X-ray (CXR) after diagnosing RSV illness. It was observed that pediatricians working in private practice had lower rates of ordering CXR compared with pediatricians working in hospitals ($p < 0.01$). Additionally, there was a statistically significant difference ($p = 0.015$), with pediatrician having >10 years of experience indicating lower CXR necessity (19% vs 29.7%).

While most pediatricians (75.8%) stated that they hospitalized at least one patient with RSV infection in the last year, 6.2% of pediatricians reported that they had hospitalized every RSV-positive patient. In the past year, at least one patient of 171 pediatricians (42.6%) required admission to the pediatric intensive care unit (PICU), while at least one patient of 219 pediatricians (54.6%) required high-flow nasal cannula (HFNC) oxygen therapy after being diagnosed with RSV illness. There were 13 (3.2%) pediatricians who stated that they lost at least one patient secondary to RSV infection.

91.3% of pediatricians reported that RSV is a seasonal virus that causes epidemics between November and March. The majority of pediatricians (84.4%) stated that RSV is the most common cause of LRTI in children under 2 years of age. The thoughts of the pediatricians regarding the hospitalization requirement and mortality of RSV, influenza, and SARS-CoV-2 infection in children are shown in the Table II.

99.8% of pediatricians considered congenital heart disease as a risk factor for RSV infection, followed by prematurity (99.5%), neuromuscular diseases (97.8%), HIV infection (94.5%), trisomy 21 (81.8%), and frequent infections (26.7%).

According to the survey, the most common indication for hospitalization due to RSV illness was severe respiratory distress (99%) and it was followed by decreased food or fluid intake (98.2%), toxic appearance or lethargy (97.7%), low (<92%) peripheral oxygen saturation (96.7%), apnea (93.7%), being younger than 3 months of age (88.2%), infiltration on the CXR imaging (46.6%), auscultation findings (28.6%), high fever (10.2%), and coughing (2.7%). The treatment preferences for RSV illness are presented in the Table III.

Table II. The thoughts of the pediatricians regarding the hospitalization requirement and mortality of RSV, influenza, and SARS-CoV-2 infection in childhood.

Statement	True		False	
	n	%	n	%
The hospitalization requirement of RSV illness is lower than SARS-CoV-2 infection	144	36.7	248	63.3
The mortality rate of RSV illness is higher than SARS-CoV-2 infection	338	86.2	54	13.8
The mortality rate of RSV illness is higher than influenza infection	354	90.3	38	9.7
The hospitalization requirement of RSV illness is higher than influenza infection	340	86.7	52	13.3

RSV, respiratory syncytial virus; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Table III. The treatment preferences of the pediatricians and pediatric sub-specialists for RSV illness.

Treatment Option	n	%
Nebulized salbutamol and/or corticosteroids	300	74.8
Nasal oxygen support	255	63.6
Hydration and oxygen	189	47.1
Nebulized 3% hypertonic saline	174	43.4
Cool mist	109	27.2
Nebulized epinephrine	91	22.7
Antibiotics	46	11.5
Cough syrups	4	1

Knowledge and attitudes towards immunization strategies

Among the pediatricians, 26.4%, 14.5%, and 6.7% believed that the administration of a single dose nirsevimab in the first RSV season in healthy infants under 8 months of age, the RSV vaccine for pregnant individuals, and immunoprophylaxis with palivizumab during the RSV season in infants born at ≤ 32 weeks of gestational age are not a prevention method for RSV illness, respectively. According to the responses of pediatricians, the most effective immunization strategies for preventing complications of RSV infection were; maternal RSV vaccination as an active immunization, passive immunization with monoclonal antibodies, and being naturally infected with RSV with a rate of 59.4%, 33.4%, and 7.2%, respectively.

39.7% of pediatricians who participated in the survey had information regarding the bivalent RSV vaccine during pregnancy to prevent RSV illness in the infancy period. There was no statistically significant relationship between the knowledge of this vaccine and professional experience of the pediatricians (>10 years vs <10 years, p = 0.061). It was seen that the pediatricians working in the private sector were more knowledgeable about maternal vaccination against RSV during pregnancy than the pediatricians working in the public sector (p = 0.007). Only 20.9% of pediatricians stated that they were aware of the maternal vaccination schedule.

58.1% of the pediatricians stated that they would recommend the maternal RSV vaccination to pregnant women who have willingness to be vaccinated, 25.9% stated that they would recommend the vaccine to every pregnant individual, and 16% stated that they would not recommend the vaccine during the pregnancy period. There was no statistically significant relationship between the recommendation of the maternal vaccination and the professional experience of the pediatricians (>10 years vs <10 years, p = 0.084) or the type of affiliated

institutions of the pediatricians (private or public, p = 0.719).

Most of the pediatricians (61.8%) who participated the study stated that they have administrated palivizumab to at least one child in their practice. While 79.8% of pediatricians have knowledge about nirsevimab as a monoclonal antibody recently approved against RSV, 14% stated that it was not approved in children worldwide. A significant proportion of pediatricians (49.1%) thought that it is more effective than palivizumab as passive immunization. While the rate of the correct answers about the recommended nirsevimab administration timing (younger than 8 months of age who were born during or are entering their first RSV season) and route (intramuscularly as a single dose regardless of infants' age) was 61%, the rate of the correct answers about the recommended dosage of nirsevimab was 30.7%.

The most important determinants for the pediatricians who participated in the survey for recommending nirsevimab were as follows: the studies and literature data (51.6%), the recommendations of experts and trustworthy organizations (41.6%), the product's cost (4%), families' requests (2.2%), and their physician colleagues (0.5%). While a significant proportion of pediatricians (37.9%) stated that they would immediately start administrating nirsevimab after its approval by the Ministry of Health and availability in the market in their country, 30.2% indicated that they would wait for the literature on local experiences before adopting its use.

Discussion

According to our study, it was observed that most pediatricians lacked sufficient knowledge of the RSV vaccine and its vaccination schedule. In addition, while the majority of pediatricians were aware of nirsevimab, a monoclonal antibody against RSV, their knowledge of the correct dosing regimen was insufficient.

In our study, intriguingly, 11% of physicians routinely ordered CXR's to diagnose RSV infection. While 44.4% of pediatricians stated that they ordered a CXR if there were lung auscultation findings, the use of routine CXR rate was 22.7% in RSV-positive patients. It was observed that physicians working in private practice and with more professional experience had statistically significant lower rates of CXR requirement ($p < 0.01$ and $p = 0.015$, respectively). According to the guidelines, CXR should be performed only in patients with severe bronchiolitis with signs of pulmonary complications (i.e., pneumothorax) and leading to PICU admission.[13,14] It is important to consider that CXR in children with RSV illness may be completely normal or have non-specific findings such as perihilar opacities, atelectasis, hyperinflation, and rarely consolidation or air-leak which are also encountered in other viral infections.[15] Instead of routine CXR in cases of RSV bronchiolitis, it is more appropriate to perform CXR in children with respiratory distress or suspected pulmonary complications.

Our findings indicate that 81.5% of pediatricians diagnosed at least one case of RSV illness, and 75.8% reported hospitalizing at least one patient with RSV. Additionally, 42.6% of physicians had at least one RSV-positive patient requiring PICU admission, while 54.6% had at least one patient requiring HFNC oxygen therapy in the past year. Of pediatricians, 3.2% stated that they had lost at least one patient secondary to RSV illness during their whole career. In a survey from Italy applied to pediatricians from February to May 2023[16], nearly 97.5% of pediatricians stated that they had managed RSV-positive bronchiolitis cases. Of these, 53.1% managed more than 10 patients in the past season. In that study, 93.8% of family pediatric practitioners had hospitalized patients secondary to RSV bronchiolitis. The pediatricians in that study mentioned RSV as the leading cause of hospitalization for respiratory infections between November and March in infants <1 year old in 90% of cases.[16]

According to a recent cohort study of 7998 hospitalized adults before the RSV vaccine was introduced in the United States, it was concluded that RSV illness was at least as severe

as influenza or COVID-19 among unvaccinated patients and considerably more severe than influenza or COVID-19 among vaccinated patients hospitalized with those diseases.[17] Among 847 hospitalized pediatric patients, it was revealed that RSV illness was the leading cause and required higher oxygen support and non-invasive ventilation compared with children with COVID-19 and influenza in a season with respiratory pathogen co-circulation between October 1, 2021, and April 30, 2022.[18] Our results were compatible with these recent studies as the most of pediatricians stated that the hospitalization requirement and mortality of RSV was substantially higher than influenza or COVID-19 (Table II).

According to the current evidence-based guidelines[13,19], there is high-level evidence indicating no benefit of nebulized beta-2 agonists, epinephrine, and corticosteroids in infants with bronchiolitis for the outcomes of admission to hospital, oxygenation, hospitalization, or length of stay. When we compare our results with these up-to-date guidelines[13,19], we found excessive use of nebulized salbutamol and/or corticosteroid (74.8%) and also relatively overuse of nebulized epinephrine (22.7%) and antibiotics (11.5%) in our study. Although the recommendations in the bronchiolitis guidelines do not recommend routine use of nebulized hypertonic saline[13,19], it was revealed that 43.4% of physicians in our study preferred it as a treatment option. Intriguingly, 27.2% of pediatricians stated that they use cool mist for the treatment of RSV illness in spite of the fact that it is not advised in the recent bronchiolitis guidelines.[13,19]

Another key objective of our study was to assess the knowledge and attitudes of pediatricians in our country regarding the recently approved maternal RSV vaccine and the monoclonal antibody nirsevimab. Our results showed that there is a gap in knowledge concerning the bivalent RSV vaccine in pregnancy. A majority of pediatricians (60.3%) had no information about this vaccine with no statistically significant relationship between the knowledge and professional experience of the pediatricians. It was also found that awareness of the vaccine among pediatricians working in the private sector was statistically higher than among those working in the public sector ($p = 0.007$). According to recent literature[20-22], the maternal RSV vaccine has the potential to reduce both all-cause LRTIs and RSV-related illnesses, while also having long-term benefits for lung health. Although the approval of the maternal RSV vaccine is groundbreaking, considerable additional effort to guide decision-making and implementation is paramount.[20] Healthcare professionals are the most trusted consultants and influencers in vaccination decisions according to the current literature.[23,24] The majority of pediatricians (58.1%) in the present study stated that they would recommend the maternal RSV vaccine to only willing women, while 16% stated that they would not recommend this vaccine to pregnant individuals. Only about one-quarter of pediatricians (25.9%) indicated that they would recommend the vaccine to every pregnant individual. In a survey study[16]from Italy in which pediatricians participated, 25% of pediatricians thought that, at the time, the RSV vaccine was available for active immunization; 69.6% thought that the vaccine was not yet available and 5.5% had no opinion. Nevertheless, this study was conducted between February and May 2023, before the approval of the vaccine by the Food and Drug Administration (FDA).[16] The lack of knowledge regarding RSV vaccination restricts its widespread use, resulting in infants losing the protective benefits against RSV infection. Furthermore, when infants contract RSV, they face an increased risk of morbidity and potentially mortality.

While a substantial majority of pediatricians (79.8%) had knowledge about nirsevimab, 14% indicated that it was not approved in children worldwide. Nearly one-half of pediatricians (49.1%) stated that it is more effective than palivizumab as a passive immunization. Of pediatricians, 61% correctly answered the recommended timing and route of

administration of nirsevimab. A considerable majority of pediatricians (69.3%) did not know the recommended dosage of nirsevimab. Only 37.9% of pediatricians indicated that they would start using nirsevimab immediately after its approval and availability in Türkiye. In the study mentioned above[16], while 69.6% of physicians stated that nirsevimab is designed to prevent RSV infection in all infants and children in their first season, 25.5% had no thought. While 76.2% of pediatricians indicated that nirsevimab has demonstrated significant efficacy in reducing hospitalizations and outpatient healthcare visits for RSV illness, 22.3% remained neutral in the same study.[16]As mentioned above, the study was conducted between February and May 2023, before the approval of nirsevimab by the FDA.[16] To the best of our knowledge, there is no published English study, conducted after the approval of maternal RSV vaccine and nirsevimab, regarding the knowledge and awareness towards this vaccine and nirsevimab among pediatricians, obstetricians, or gynecologists who have a crucial impact on parents and pregnant individuals' decision-making. It is essential to enhance the level of knowledge regarding the vaccine and monoclonal antibody, which are effective in protecting against RSV, as well as to ensure their accessibility. Therefore, organizing training programs for healthcare professionals working with infants will be of paramount importance.

Although the present study provides information regarding the knowledge and attitudes towards RSV infection and awareness of its current immunization strategies among Turkish pediatricians, it does not include all pediatricians in our country and the demographic data could not be completely homogenized as the major limitation of our study.

In conclusion, this study revealed that pediatricians lack sufficient knowledge and awareness about the RSV vaccine and nirsevimab. Furthermore, CXR usage and administration of non-evidence-based therapies in the diagnosis and management of RSV illness were at a relatively high rate among the pediatricians who participated in the present study. There is a gap in knowledge and awareness regarding the maternal RSV vaccine and nirsevimab, which may pose a significant obstacle to reducing RSV-related hospitalizations and outpatient healthcare utilization. Improving the knowledge towards RSV infection and the awareness of its current immunization strategies among parents, family practitioners, pediatricians, and obstetricians are suggested to reduce RSV morbidity and mortality in children especially under 2 years of age in Türkiye and throughout the world. To address the existing knowledge gaps regarding RSV immunization, it is essential to design and implement targeted educational programs for pediatricians and healthcare professionals. These programs should focus on the latest scientific evidence, provide detailed information on vaccination, and cover the correct timing, administration route, and dosing regimen for nirsevimab.

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Ethical approval

This study was approved by Ataşehir Memorial Hospital Ethics Committee (date: 15.02.2024, number: 2024/8).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: AS, İY, EG; data collection: AS, İY, EG, EZ, SY, OY; analysis and interpretation of the results: AS, EG, İY, EZ, SY; draft manuscript preparation: İY, OY, EG, AS All authors reviewed the results and approved the final version of the article.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Enhancing clinical decision-making: a scenario-based patient simulation study using QR code-based algorithms for the management of acute intoxication-type inborn errors of metabolism

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ABSTRACT

Background. Acute intoxication-type inborn errors of metabolism (IEMs) present significant challenges in pediatric care. Prompt recognition and appropriate management are essential to prevent serious complications and reduce mortality. Recent studies increasingly highlight the use of quick response (QR) code-based tools to facilitate rapid intervention, particularly in emergency departments and primary healthcare settings. In this study, the effectiveness of a newly developed QR code-based algorithm, designed to support the accurate recognition and effective management of acute intoxication-type IEMs and, indirectly, to reduce sequelae and mortality, was evaluated for the first time.

Methods. This study included 113 pediatric residents from two centers, one with (Group 1, n=77) and one without (Group 2, n=36) a mandatory pediatric metabolism rotation. All participants completed a scenario-based simulation with 10 clinical questions on a standardized patient case of acute intoxication-type inborn errors of metabolism, both before and after using the QR code-based algorithm. The algorithm, developed in accordance with international guidelines, was accessed via mobile devices. Pre- and post-intervention responses were compared using appropriate statistical tests. The effectiveness of the QR code in guiding the management of a simulated patient was analyzed.

Results. Of the participants, 73 (64.6%) were female and 40 (35.4%) male; the median age was 28.0 years. Forty-two residents (37.2%) had previous experience in a pediatric metabolism unit. Correct identification of urgent treatment increased from 77.9% to 97.3% ($p<0.001$). Preliminary diagnosis improved from 79.6% to 88.5% ($p=0.050$). Only 0.9% initially selected the correct treatment sequence versus 81.4% post-intervention ($p<0.001$). Hemodialysis decisions improved from 81.4% to 95.6% ($p<0.001$). Satisfaction was high, with 92.0% assigning an average score of 93.3/100.

Conclusion. Considering the limited knowledge of rare diseases among physicians, scenario-based simulation training and the widespread use of QR code-accessed algorithms in emergency departments appear essential to improve outcomes in patients at risk of severe complications.

Key words: acute intoxication-type inborn errors of metabolism, QR code algorithms, scenario-based training.

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Healthcare services are continuously evolving in line with technological advances, and digitalization is at the forefront of this transformation. Quick Response (QR) codes have become a vital tool facilitating the storage, sharing, and accessing of information, supporting numerous applications in the healthcare sector.[1-3]

QR codes have a broad range of applications in the provision of healthcare education and services, and have been employed in numerous areas to date, such as medical school student training[4], resident physician training in emergency departments[5], the provision of evidence-based care to newborns and mothers in emergency departments[6], the expediting of consent processes in emergency departments[7], the management of emergencies such as anaphylaxis[8,9], in family medicine residency clinics[1], facilitating pharmacy service operations[10], COVID-19 pandemic management[11] and health profile card creation, while also providing access to healthcare and educational services for those with disabilities.[8,12] The advantages of QR code systems in these areas have been reported, along with their efficacy in enhancing various healthcare processes.

Inborn errors of metabolism (IEMs), although individually rare, collectively represent a significant group of disorders. Awareness-raising activities to highlight inborn errors of metabolism are organized worldwide, and physicians receive both pre-graduate and post-graduate training to ensure the proper management of patients.[13] In acute intoxication type IEMs, such as organic acidaemia, maple syrup urine disease, urea cycle disorders, and fatty acid oxidation defects, sequelae or death may occur if acute attacks are not managed correctly.[14,15] The rapid initiation of disease-specific treatment, which these patients require, can be lifesaving.[16] Therefore, the widespread adoption of technologies such as QR codes, which have the potential to facilitate patient management and expedite treatments in emergency departments, may play an important role.

The current literature includes only a limited number of studies examining the use of QR code-based algorithms for the management of patients with IEM, A study in the United Kingdom has put forward recommendations for both patients and healthcare professionals related to the management of metabolic emergencies such as hyperammonaemia. These are based on Royal College of Paediatrics and Child Health[17] and British Inherited Metabolic Disease Group[18,19] guidelines, in which the emphasis is on acting swiftly to save lives.

This study evaluates the effectiveness of a QR code-based algorithm developed in Pediatric Metabolism Department, designed to aid in the accurate recognition, management, and reduction of sequelae and mortality risks associated with acute intoxication-type IEMs—such as urea cycle disorders, organic acidaemias, maple syrup urine disease, and fatty acid oxidation defects—across all levels of care, from primary to tertiary. To achieve this, pediatric residents from two centers, one with and one without a pediatric metabolism department, were included. Responses to patient scenario questions were analyzed both before and after their exposure to the QR code algorithm, considering whether they had completed a rotation in a pediatric metabolism department. This approach allowed us to assess both the general applicability of the algorithm and its utility in different training environments.

Materials and Methods

Included in the study were pediatric residents who were assigned to two groups, with Group 1 comprised of residents from the Ankara University Faculty of Medicine Department of Pediatrics, in which a metabolism rotation is included as part of pediatric training, and Group 2 comprising residents from the Gazi Yaşargil Training and Research Hospital Department of Pediatrics, in which the training does not include a metabolism rotation. Pediatric residents in Group 1 are required to undergo mandatory foundational pediatric metabolism

training, as stipulated by university regulations, and this training is assessed through an annual examination. The basic characteristics of the respondent residents, including age, gender and details of their pediatric training, were also recorded.

A total of 113 residents participated in the study, including 77 residents from the Ankara University Faculty of Medicine Department of Pediatrics (Group 1) and 36 residents from the Gazi Yaşargil Training and Research Hospital Department of Pediatrics (Group 2). All residents participated in a Scenario-Based Patient Simulation in which they were presented with 10 questions (Supplementary Materials) related to acute intoxication-type IEM. The baseline information for this scenario included the neonatal history, consanguinity status, sibling history, presenting complaints, clinical progression, and physical examination findings of each case. Prior to applying the QR code-based algorithm, all residents answered the same 10 questions related to each case based on their prior knowledge, and subsequently answered the same 10 questions after accessing the QR code-based algorithm (Fig. 1). Of the 10 questions, eight were multiple choice questions, while two were open-ended. The responses to the open-ended questions were categorised as correct, incorrect, incomplete, or unanswered. Pediatric residents utilized the hospital internet or their personal mobile internet for access to the QR code. During the time each participant answered the scenario questions, one of authors was present alongside them at both centers, and the questions were presented to the participants one at a time. This ensured that returning to and modifying previous questions were prevented.

This algorithm was devised taking into account the most common acute intoxication-type IEMs, such as urea cycle disorders, organic acidaemia, maple syrup urine disease and fatty acid oxidation defects. The algorithm steps (Fig. 1) were developed by the Ankara University Faculty of Medicine Department of Pediatric Metabolism based on international guidelines, reviews and clinical experience.[16,20-25]

For the creation of the QR codes, a web-based "QR-Code Generator"[26] was utilized. The pediatric residents participating in the study accessed the disease management protocol in one step by scanning the QR code using their mobile phones, tablets, or computer cameras.

In the descriptive analysis of the data, mean±standard deviation was used for continuous variables with a normal distribution, median (25^{th} and 75^{th} percentiles, Q1-Q3) for variables with a non-normal distribution, and percentages for categorical variables. For comparisons of two groups, between-group differences in the means of the groups were analysed with a t-test, and differences in median values were analysed with a Mann-Whitney U test. When comparing percentages, Pearson's chi-square or Fisher's exact tests were used for independent variables, while the McNemar test was employed for dependent variables. A p-value less than 0.05 was considered statistically significant for all analyses. IBM SPSS Statistics for Macintosh (Version 22.0. Armonk, NY: IBM Corp.) was used for the analysis of data.

This study was approved by the Institutional Review Board (IRB) of Ankara University Faculty of Medicine Ethics Committee (Date: 15 February 2024, Number: İ02-117-24)

Results

Of the residents, 73 (64.6%) were female, and 40 (35.4%) were male, and the median age of the residents was 28.0 years (26.0–31.0) and the median duration of residency was 1.5 years (1.0–3.0). Among the residents, 42 (37.2%) had previously worked in the pediatric metabolism department of their institution, while three had worked in a metabolism department during external rotations, due to the lack of a metabolism department in their institution. Furthermore, 83 residents (73.5%) had worked in a pediatric emergency department, 83 (73.5%) in a pediatric intensive care and 82 (72.6%) in a neonatal intensive care department (Table I).

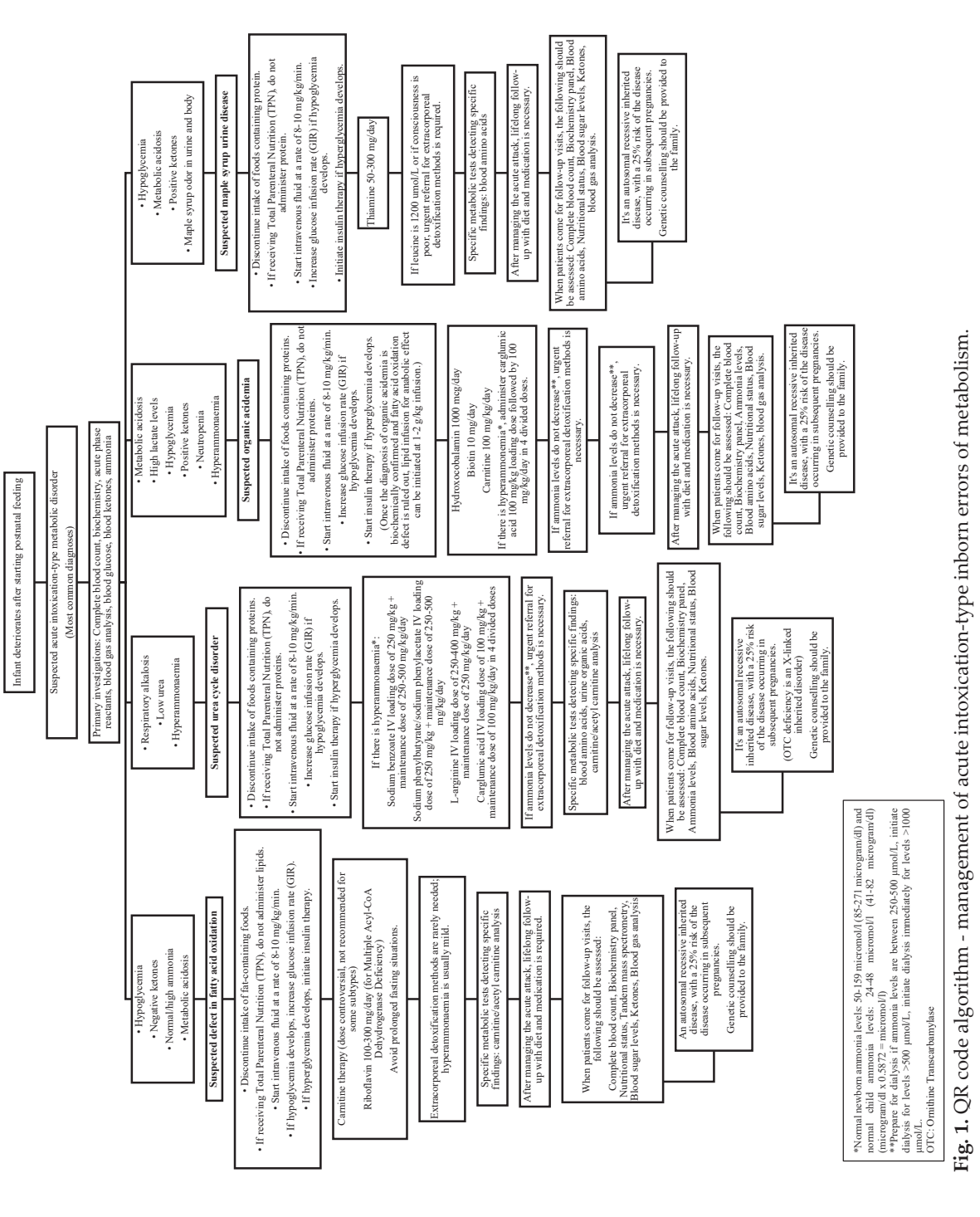

Fig. 1. QR code algorithm - management of acute intoxication-type inborn errors of metabolism.

Table I. Baseline characteristics of participants.

	Total (n=113)	Group 1 (n=77)	Group 2 (n=36)
Resident Age, years			
Median	28.0	27.0	31.0
(min-max)	24.0-46.0	24.0-41.0	24.0-46.0
[25th-75th]	26.0-31.0	26.0-29.0	27.0-35.0
Mean (Standard deviation)	29.1 (4.2)	27.8 (2.9)	31.8 (5.2)
Gender, n (%)			
Female	73 (64.6)	63 (81.8)	10 (27.8)
Male	40 (35.4)	14 (18.2)	26 (72.2)
Duration of Residency, years			
Median	1.5	1.5	1.9
(min-max)	0.1-4.5	0.1-4.5	0.1-3.5
[25th-75th]	1.0-3.0	1.0-3.0	0.5-2.5
Mean (Standard deviation)	1.7 (1.2)	1.8 (1.2)	1.7 (1.1)
Previously Worked in a Metabolism Department? n (%)			
Yes	42 (37.2)	39 (50.6)	3 (8.3)
No	71 (62.8)	38 (49.4)	33 (91.7)
Previously Worked in a Pediatric Emergency Department?			
Yes	83 (73.5)	61 (79.2)	22 (61.1)
No	30 (26.5)	16 (20.8)	14 (38.9)
Previously Worked in a Pediatric Intensive Care Department? n (%)			
Yes	83 (73.5)	57 (74.0)	26 (72.2)
No	30 (26.5)	20 (26.0)	10 (27.8)
Previously Worked in a Neonatology Department? n (%)			
Yes	82 (72.6)	55 (71.4)	27 (75.0)
No	31 (27.4)	22 (28.6)	9 (25.0)

Of the total, 108 (95.6%) of the residents accurately identified the initial tests to be performed upon the patient's first presentation, and the QR code directive did not support any significant changes in the initially requested tests. Information on respiratory alkalosis, hyperammonaemia and hypoglycaemia was provided alongside the patient's laboratory results, and the QR code-based algorithm presented differential flowcharts to be applied for different acute intoxication-type IEMs, including fatty acid oxidation defects, urea cycle disorders, organic acidaemia and maple syrup urine disease (Fig. 1). One of the scenario questions focused on the identification of the priority initial treatment to be administered before proceeding with a differential diagnosis based on the provided laboratory information. Of the total, 88 residents (77.9%) before access to the QR code-based algorithm and 110 residents (97.3%) after access gave correct responses to the question ($p<0.001$), and a comparable difference was noted in both groups (respectively, Group 1 after accessing QR code vs. before accessing QR code: 80.5% vs. 97.4%, $p<0.001$; Group 2 after accessing QR code vs. before accessing QR code: 72.2% vs. 97.2%, $p=0.004$). The residents were then asked about the appropriate actions to take upon the development of hyperglycaemia in the patient (blood sugar 340 mg/dl), with some incorrect options available for selection, such as reducing the rate of glucose infusion

or reducing the rate of dextrose infusion, both of which can lead to increased catabolism. Of the total, 64 residents (56.6%) answered the question correctly without using the QR code-based algorithm, whereas 104 residents (92.0%) answered correctly after accessing the QR code-based algorithm flowchart recommendations (p<0.001). The correct answer was to initiate insulin – an important step in increasing anabolism, and the contribution of the QR code-based algorithm to the correct identification of this crucial treatment step was observed in both Group 1 and Group 2 (respectively, Group 1 after accessing QR code vs. before accessing QR code: 58.4% vs. 93.5%, p<0.001; Group 2 after accessing QR code vs. before accessing QR code: 52.8% vs. 88.9%, p=0.002).

Of the total, 90 residents (79.6%) were able to determine a preliminary diagnosis for the scenario patient based on their existing knowledge, and this rate increased to 88.5% with QR code-based algorithm assistance (p=0.050). The residents not only considered the possibility of urea cycle disorders in the patient, but also took action to prepare for emergency treatments aimed at reducing ammonia levels, such as sodium benzoate and sodium phenylacetate, considering the advice received from the QR code-based algorithm. After being instructed to place treatment orders for the patient, only one resident (0.9%) provided the correct order without the QR code-based algorithm, while the use of the QR code-based algorithm resulted in 92 residents (81.4%) providing complete and accurate treatment orders with correct dosages (p<0.001).

When the hyperammonaemia persisted (ammonia: 680 μmol/L), despite the administration of ammonia-lowering treatments, and emergency haemodialysis was deemed necessary, the residents were asked for treatment recommendations. Of the total, 92 residents (81.4%) made this critical decision based on existing knowledge, and the number increased to 108 (95.6%) after accessing the QR code-based data, demonstrating a significant improvement (p <0.001). The benefit of the QR code-based algorithm in critical haemodialysis decision-making was found to be greater among the residents of the centre with a metabolism department (respectively, Group 1 after accessing QR code vs. before accessing QR code: 81.8% vs. 97.4%, p <0.001).

When asked about the specific metabolic tests to be requested to support a diagnosis, Group 1 residents answered correctly at a rate of 80.5% based on their existing knowledge, whereas Group 2 residents answered correctly at a rate of 69.4%. The QR code-based algorithm increased the correct response rate for the tests to be requested among all residents in both groups, from 77% to 90.3% (p=0.001). It was noted that the QR code-based algorithm was beneficial to both groups in terms of identifying the routine tests to be requested during subsequent follow-up visits (respectively, Group 1 after accessing QR code vs. before accessing QR code: 79.2% vs. 97.2%, p<0.001; Group 2 after accessing QR code vs. before accessing QR code: 72.2% vs. 97.2%, p=0.004). The final question regarding the scenario patient, who was born to a consanguineous marriage, concerned recommendations for future pregnancies. While 83% of the residents provided the correct information for genetic counselling, this number increased to 91.2% following the QR code-based algorithm recommendations (p<0.001).

When asked about their satisfaction with the QR code-based algorithm application, 104 of the residents (92.0%) reported being satisfied, assigning it an average score of 93.3 out of 100 (Table II).

Discussion

The QR code-based algorithm applied in the present study was found to be successful in correctly identifying life-saving recommendations for the management of acute intoxication-type inborn errors of metabolism. In metabolic emergencies such as hyperammonaemia, which are rare but pose a high risk of complications within

Table II. Responses to patient scenarios with and without QR code algorithm.
Scenario:

- A male infant who had no complaints after birth begins to deteriorate after starting to feed.
- He was brought to the emergency department at 3 days old due to vomiting, feeding intolerance and altered consciousness.
- There is a history of consanguinity between the parents.
- He was born at term, weighing 3300 grams.
- The family's first child had treatment at a hospital for vomiting, weakness and seizures at 14 days old, and passed away.
- On examination, he appeared weak and prone to sleepiness. There are no other pathological findings in the examination.

Patient Scenario Questions, Correct Answer Rates, n (%)	Total Before accessing to QR code	Total After accessing to QR code	p	Group 1 Before accessing to QR code	Group 1 After accessing to QR code	p	Group 2 Before accessing to QR code	Group 2 After accessing to QR code	p
Question 1: What tests should be performed initially for the patient?	108 (95.6)	112 (99.1)	1.000	74 (96.1)	77 (100.0)	-	34 (94.4)	35 (97.2)	1.000
Question 2: Respiratory alkalosis and hyperammonaemia (ammonia: 392 μmol/L), hypoglycaemia were detected in the patient. Acute phase reactants are negative. What should be the initial treatment approach in the emergency department?	88 (77.9)	110 (97.3)	<0.001	62 (80.5)	75 (97.4)	<0.001	26 (72.2)	35 (97.2)	0.004
Question 3: During follow-up, the patient's blood sugar is measured as 340 mg/dl, what would you do?	64 (56.6)	104 (92.0)	<0.001	45 (58.4)	72 (93.5)	<0.001	19 (52.8)	32 (88.9)	0.002
Question 4: What is the possible preliminary diagnosis for the patient and what should be the second-line treatments to be reached?	90 (79.6)	100 (88.5)	0.050	62 (80.5)	71 (92.2)	0.035	28 (77.8)	29 (80.6)	1.000
Question 5: Please provide medication orders for the patient. (Open-ended question)			<0.001			<0.001			-
- Correct answer	1 (0.9)	92 (81.4)		1 (1.3)	64 (83.1)		0 (0.0)	28 (77.8)	
- Incorrect answer	4 (3.5)	3 (2.7)		2 (2.6)	1 (1.3)		2 (5.6)	2 (5.6)	
- Incomplete answer	10 (8.8)	0 (0.0)		3 (3.9)	0 (0.0)		7 (19.4)	0 (0.0)	
- Unanswered	98 (86.7)	18 (15.9)		71 (92.2)	12 (15.6)		27 (75.0)	6 (16.7)	

Table II. Continued.

Patient Scenario Questions, Correct Answer Rates, n (%)	Total Before accessing to QR code	Total After accessing to QR code	p	Group 1 Before accessing to QR code	Group 1 After accessing to QR code	p	Group 2 Before accessing to QR code	Group 2 After accessing to QR code	p
Question 6: Despite initiating ammonia-lowering treatments, the patient's ammonia level continues to rise (ammonia: 680 μmol/L). What treatment should be applied at this stage?	92 (81.4)	108 (95.6)	<0.001	63 (81.8)	75 (97.4)	<0.001	29 (80.6)	33 (91.7)	0.289
Question 7: What are the priority metabolic tests to be requested for diagnosis?	87 (77.0)	102 (90.3)	0.001	62 (80.5)	71 (92.2)	0.022	25 (69.4)	31 (86.1)	0.070
Question 8: After the patient's general condition has improved and the diagnosis has been clarified, what are your recommendations for treatment follow-up?	100 (88.5)	107 (94.7)	0.092	72 (93.5)	74 (96.1)	0.687	28 (77.8)	33 (91.7)	0.125
Question 9: Which of the following tests should be scheduled when patients come for follow-up?	87 (77.0)	110 (97.3)	<0.001	61 (79.2)	75 (97.4)	<0.001	26 (72.2)	35 (97.2)	0.004
Question 10: The patient's mother mentions a desire to become pregnant again in the future, how would you inform the family? (Open-ended question)			<0.001			0.001			0.070
- Correct answer	83 (73.5)	103 (91.2)		57 (74.0)	71 (92.2)		26 (72.2)	32 (88.9)	
- Incorrect answer	5 (4.4)	0 (0.0)		2 (2.6)	0 (0.0)		3 (8.3)	0 (0.0)	
- Unanswered	25 (22.1)	10 (8.8)		18 (23.4)	6 (7.8)		7 (19.4)	4 (11.1)	
Were you satisfied with the QR code? Yes	-	104 (92.0)		-	73 (94.8)		-	31 (86.1)	
QR code satisfaction score (out of 100)	-	93.3		-	97.8		-	83.9	

hours, physicians easy access resources in the absence of sufficient experience or knowledge contributed significantly to effective patient management and addressed the urgent time-sensitive nature of treatment.

Medical training focuses on common diagnoses and treatments, but this study explored acute intoxication-type inborn errors of metabolism, a rare condition. Many physicians lack knowledge of rare diseases, leaving them unprepared to manage such cases.[27] Studies show over 90% of physicians lack knowledge of rare conditions, with less than 5% confident in managing them. Over 75% believe curricula should include more training on diagnosing and managing rare diseases.[27] Delays in diagnosing rare conditions significantly affect survival, with research showing that insufficient physician knowledge increases complications and mortality.[28] These findings suggest that there is a need to disseminate educational materials on rare conditions, both during medical education and in postgraduate training.[27,29-31]

Providing access to digital databases through such tools as QR code applications, and thus providing easy access to such data as educational materials, algorithms and guidelines, could contribute to a transformative strategy for the more effective management of emergencies related to rare conditions.[27,32]

Diagnosing rare conditions is challenging for non-specialist physicians, as they often require distinguishing overlapping symptoms and specialized tests.[33] While numerous guidelines exist in literature for the diagnosis and treatment of rare diseases, physician awareness and easy access to these guidelines are crucial for the effective management of these conditions.[8,34] For physicians outside the field of rare diseases, quickly accessing resources and managing emergencies can be challenging. This study used a urea cycle disorder scenario, asking residents to diagnose based on findings like respiratory alkalosis, hyperammonaemia, and low urea using a QR code-based algorithm. The algorithm significantly improved pediatric residents' diagnostic abilities and their ordering of crucial metabolic tests for higher-level referrals.

In urea cycle disorders, the priority is to stop catabolism, which raises ammonia levels. This is achieved by halting protein intake and initiating high-glucose intravenous hydration.[16,35] Approximately three-quarters of the pediatric residents participating in our study were able to initiate this critical treatment based on their existing knowledge, while nearly all residents were able to do so after accessing the QR-code application. In response to the open-ended question, "Please provide medication orders for the patient," only one resident physician was able to respond correctly before accessing the QR-code application but was a senior resident with previous experience in a centre with a metabolism department who completed rotations in the neonatal and intensive care units, and who had been involved in the management of numerous metabolic patients. Following the use of the QR code application, more than three-quarters of the residents correctly and comprehensively answered the medication order question, highlighting the contribution of the QR code algorithm to both the diagnosis and initial treatment processes.

Another crucial life-saving recommendation in our scenario relates to extracorporeal detoxification decisions (such as haemodialysis). Authors generally agree on the importance of the early initiation of haemodialysis.[36,37] In the sixth step of our scenario, despite the initiation of ammonia-lowering treatments, the patient's ammonia level was measured at 680 μmol/L. After accessing the QR code-based algorithm, the respondent physicians were able to make a prompt hemodialysis decision. It was observed that pediatric residents trained in centres with pediatric metabolism departments were able to make more effective decisions related to dialysis after accessing the QR code-based algorithms. In contrast, in the centre without a pediatric metabolism department, although there was an increase in the rate of dialysis decisions after accessing the QR code-based algorithms,

the difference was not statistically significant. The small number of participants in Group 2 is a significant limitation of the present study and increasing the sample size may yield more accurate evidence-based results.

After stabilizing and managing the acute episode in patients, it is essential to ensure they are referred to specialized centers with third-level pediatric metabolism departments for comprehensive long-term care. These centers are equipped to provide the multidisciplinary expertise required for the ongoing management of metabolic disorders, including tailored treatment plans, dietary adjustments, and monitoring for potential complications. By including these referral steps in the algorithm, the goal is not only to guide resident physicians through the immediate management process but also to emphasize the importance of continuity of care. This approach aims to enhance the residents' understanding of the broader, long-term needs of metabolic patients and foster awareness of the critical role that specialized care plays in improving patient outcomes. Our observations indicate that the QR code-based algorithm enhanced the education and awareness of resident physicians of the required tests during follow-up, as well as recommendations for future pregnancies. Genetic counselling based on detailed family information should be provided by an expert, as a crucial intervention.[38] In Türkiye, with a consanguinity rate of 21%, they should consider that rare diseases inherited in an autosomal recessive manner may be more common than expected. Sharing scientific information with families at every stage in the prevision of healthcare services can play a crucial role in fostering healthier future generations.[39]

The digitalization of medical education and hybrid learning models have rapidly advanced worldwide, accelerated by the COVID-19 pandemic, familiarizing healthcare students and professionals with these methods.[40] Studies show that new-generation physicians quickly adapt to digital materials, preferring softcopy resources on devices for their portability and information accessibility over hardcopy materials[40-42] In the present study, the satisfaction levels of the resident physicians regarding the QR code-based algorithm were investigated, and nearly all reported being satisfied, giving very high ratings. This pioneering study in the field of rare conditions suggests that raising awareness among physicians and assisting them in the management of patients with life-saving critical interventions through this method can be considered a highly effective approach.

This preliminary study evaluates the use of a QR code and algorithm-based material for simulating patient management by pediatric residents, though with limitations. The design does not compare the QR code to a printed algorithm, and success depends on the combined material rather than the QR code alone. The authors suggest such algorithms could aid pediatric emergency management of IEM patients, given the difficulty of quickly accessing guidelines and literature. While simulation questions align with the algorithm and are multiple-choice, real-life cases are more complex. This study, while requiring further refinement for real-world application, offers a foundation for developing new digital tools.

The reason for dividing the residents into groups in our study is that participation was from two different centers, and the most significant distinction between these two centers is the presence or absence of a metabolism department. Working as a resident in a center with a metabolism department allows for certain experience with metabolic diseases through multidisciplinary patient management in other departments, even if the residents have not yet completed their metabolism rotation. We believe that the residents from these two centers have different levels of awareness and background knowledge regarding metabolic diseases. For this reason, we aimed to compare the two centers. However, the number of metabolic patients encountered by each resident is not the same. Therefore, as a subgroup analysis, we created subgroups of residents who had completed and not completed a

rotation in the metabolism department. Another limitation of our study is that not all pediatric residents working in centers with a pediatric metabolism department have completed their metabolism rotation. However, due to frequent consultations that occur when they rotate through pediatric emergency, pediatric intensive care, neonatal intensive care, or other departments, they are generally well-versed in the approach to metabolic patients. The percentages for rotations in these departments are high as shown in Table I. Moreover, residents are required to undergo mandatory foundational metabolism training, assessed through an annual examination. Although they have not completed the rotation, the infrastructure of the center means that residents are accustomed to managing metabolic patients. Nevertheless, it can be discussed as a limitation that not all residents in Group 1, where the center has a metabolism department, have completed their metabolism rotation. Another significant limitation of our study is that the difficulty and quality levels of each question vary, and a discrimination index could not be calculated. For this reason, rather than creating a total score for all questions, each question was assessed based on its individual accuracy percentages. Nevertheless, future studies that can evaluate question quality and discrimination indices would be invaluable for assessing this algorithm.

Our algorithm, based on seven international guidelines, reviews, and clinical experience, requires further development and study. As a preliminary example, it tested a QR code-integrated homemade algorithm. Since pediatric residents mostly encounter common diseases, rare disease algorithms may mislead without proper training. Therefore, QR codes must be paired with detailed training before use on real patients. This study provides only preliminary findings.

In conclusion, the digitalization of our world is transforming the nature of educational materials accessed by resident physicians. There is a global preference for resources that are easily transportable and quickly accessible, and that provide the right amount of information needed. Considering the inadequate knowledge of physicians of rare conditions, we believe that scenario-based patient simulation training and the widespread adoption of QR code-based algorithms in emergency departments are necessary for improving the outcomes of patients who may otherwise face mortality or significant sequelae. Although the contribution to education was not measured during the data collection phase due to the design of this study, it is believed that further studies of this nature could make significant contributions to postgraduate and in-service training.

Supplementary materials

Supplementary materials for this article are available online at https://doi.org/10.24953/turkjpediatr.2025.5208

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Ethical approval

This study was approved by the Institutional Review Board (IRB) of Ankara University Faculty of Medicine (Date: 15 February 2024 Number: İ02-117-24).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: MKY, FTE; data collection: MKY, GBY, MT, NO; Analysis and interpretation of results: MKY, FTE; draft manuscript preparation: MKY, GBY, MT, NO; draft manuscript preparation: MKY, GBY, MT, NO, FTE. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Comprehensive analysis of 1103 infants referred to a single center due to positive newborn screening test for phenylketonuria

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ABSTRACT

Objective. Phenylketonuria (PKU) is a prevalent inherited metabolic disorder, resulting from biallelic pathogenic variants in the *PAH* gene. This study aimed to assess the clinical characteristics of 1103 infants referred to a single center due to positive newborn screening (NBS) tests for PKU, providing insights into screening and diagnosis.

Methods. The health records of infants who were referred with suspicion of PKU through the Turkish national NBS program to a single referral center between January 2016 and January 2023 were retrospectively reviewed. The study analyzed demographic data, clinical findings, and diagnostic results from hospital records. Logistic regression analysis identified significant predictors of age at admission.

Results. This study highlights significant regional differences within Türkiye regarding DBS collection, result reporting, and age at admission. Significant delays in age at admission (expressed as median, [Q1-Q3]) were noted in the Eastern Anatolia (34 days [27-42]), Southeastern Anatolia [34 days (25-42)], and Black Sea regions [26 days (19-33)]. Out of the referred infants, 5.1% and 2.4% had transient tyrosinemia and transient hyperphenylalaninemia, respectively, and these transient conditions were more prevalent among neonates with a history of jaundice. Phenylalanine level was normal in 38.1% of the patients and was considered false positive. Among the 26 (2.36%) patients admitted after 90 days (late admissions), there were 2 PKU patients with untreated Phe levels >20 mg/dL (n=2). Among the 140 infants requiring treatment, 1.43% (n=2) were late admissions (>90 days). A history of PKU in the family and higher initial Phe levels were associated with earlier admissions.

Conclusion. This comprehensive analysis underscores the need to enhance NBS programs, particularly in regions with identified delays. Improving healthcare infrastructure, increasing awareness, and implementing targeted health policies are crucial for timely diagnosis and treatment. Future research should address regional disparities and optimize screening protocols to improve outcomes for affected infants.

Key words: hyperphenylalaninemia, phenylketonuria, newborn screening, geographic disparities, delayed diagnosis.

Phenylketonuria (PKU) is one of the most common inherited metabolic disorders, caused by pathogenic variants in the *PAH* gene, which encodes the enzyme phenylalanine hydroxylase. This enzyme, which requires tetrahydrobiopterin (BH4) as a cofactor, converts phenylalanine (Phe) into tyrosine. If left untreated, the accumulation of Phe leads to irreversible damage in the brain, resulting in global developmental delay, intellectual

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disability, and various other neuropsychiatric problems.[1]

The frequency of elevated Phe (hyperphenylalaninemia, HPA) varies depending on country, region, and ethnicity. The incidence is estimated to be 1:10,000 in Europe[2], 1:25.000 in the United States, and 1:15,924 in China.[3] In recent studies published worldwide, it is noted that Türkiye has the highest incidence of PKU.[4,5] Özalp et al.[6] reported, the incidence of PKU in Türkiye as 1:4,172 in 1986. Based on the latest report by the Ministry of Health, the incidence of phenylketonuria in Türkiye is 1 in 4,500 live births.[7]

In the 1960s, Robert Guthrie developed a microbiological test, leading to the initiation of the first newborn screening (NBS) program for PKU in Massachusetts, USA, in 1963.[8] Later, more sensitive methods, such as fluorometric and spectrometric techniques, were developed. Türkiye started a pilot program in 1983, expanding it nationwide in 2006.[9] Ideally, newborns with PKU should be diagnosed and start treatment within the first fifteen days of life. Current guidelines classify patients who begin treatment after three months as "late-diagnosed".[2]

This study represents the clinical characteristics of 1103 patients identified through an NBS program. By examining demographic data, clinical findings, and diagnostic results, we aimed to provide valuable insights that can enhance the workings of the NBS program.

Materials and Methods

Study design and participants

In this retrospective study, the health records of infants who were referred with suspicion of PKU through the national NBS program to the Division of Pediatric Metabolism at Hacettepe University İhsan Doğramacı Children's Hospital between January 2016 and January 2023 were reviewed. Patient information was obtained from the parents. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki and was approved by the Hacettepe University Ethics Committee for Non-Interventional Clinical Studies.

Sample collection and screening protocol

According to the protocol of the national NBS program, capillary blood samples collected from the newborns using a Guthrie card at healthcare institutions are sent as dried blood spots (DBS) to designated screening laboratories by the Ministry of Health. For samples with a Phe level of 2.1 mg/dL or higher, additional diagnostic and clinical follow-up actions are initiated. The blood test results, conducted by the screening laboratory and transmitted to the provinces through the NBS Program Web Application, lead to infants suspected of having PKU being referred to pediatric nutrition and metabolism clinics by their registered family physicians. The Ministry's screening algorithm does not include a third DBS; however, the results of patients who had a third DBS collected were also documented (Fig. 1), which may have been collected due to previous samples being improperly collected or shipped. The "time of DBS result" was defined as the interval between the sample collection date and the result finalization date. The "age at admission " was defined as the age of the patient (in days) when they first applied to our center. "Late admission" was defined as age at admission older than 90 days.

There are various classifications for PKU.[2,10-12] We classified patients as follows: patients who do not require treatment (Group 1; untreated Phe level 2–6 mg/dL), and patients who require treatment (Group 2; untreated Phe level 6–20 mg/dL, and Group 3; untreated Phe level >20 mg/dL). Transient HPA and transient tyrosinemia are temporary conditions, characterized by elevated blood phenylalanine (≥2.1 mg/dL) or tyrosine (>3 mg/dL) levels, usually detected during routine NBS, normalizing spontaneously at follow-up. In our center, phenylalanine and tyrosine levels were measured using quantitative amino acid analysis by high performance liquid chromatography.

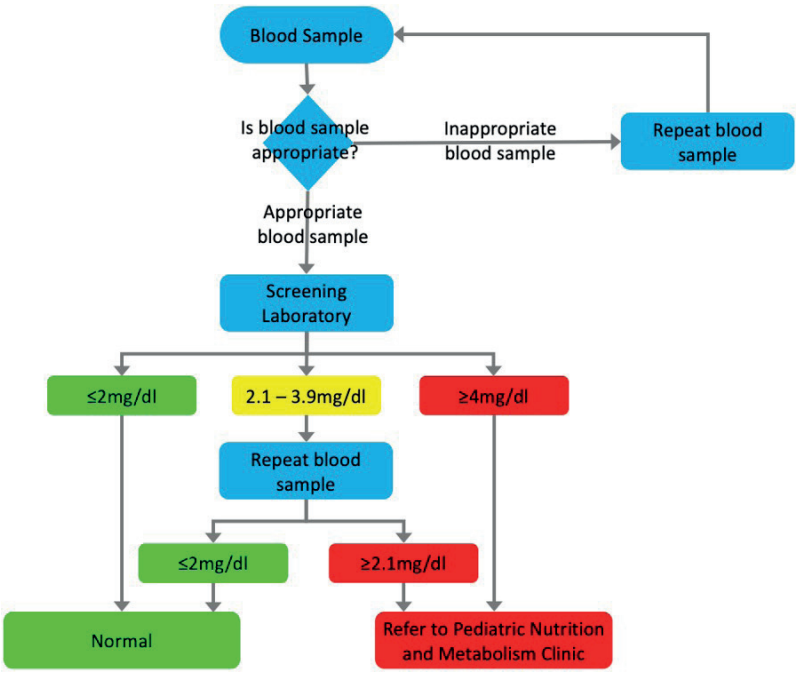

Fig. 1. Implementation of the phenylketonuria screening and referral algorithm in Türkiye.

Statistical analyses

Data analysis was performed using SPSS v26.0 (SPSS Inc., Chicago, USA). Distribution of the variables was assessed using both visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov and Shapiro-Wilk tests). Descriptive statistics were reported as mean ± standard deviation (SD) for normally distributing quantitative data, as median, range (minimum–maximum), and interquartile range (IQR, Q1-Q3) for non-normally distributing quantitative data, and as frequencies and percentages for qualitative data. The Mann-Whitney U test was utilized to assess the differences between two independent groups with non-parametric data. Depending on the test conditions, either chi-square or Fisher's exact tests were employed to compare categorical variables. As DBS test features, age at admission, plasma Phe level were not normally distributed the Kruskal-Wallis tests were conducted to compare these parameters and the ordinal variables among the geographical regions (7 groups). The Mann-Whitney U test was performed to test the significance of pairwise differences using Bonferroni correction (p=0.0023) to adjust for multiple comparisons when investigating the associations between non-normally disturbed variables. The correlation coefficients and their significance were calculated using the Spearman test. The univariate analyses to identify variables associated with age at admission were investigated using Mann-Whitney U test. In univariate analyses, variables were selected for inclusion in multivariate analysis based on statistically significant factors, typically with a p-value <0.05. In addition, parameters considered to be clinically relevant or potentially influential were included. Thus, the following variables were included in the model: family history of PKU, geographical region, and 1st DBS result. For the multivariate analysis, the possible factors identified with univariate analyses were further entered into the logistic regression analysis to determine independent predictors of patient "age at admission" and "late admission". Logistic regression model was constructed by enter method, using variables associated with "age at admission" and "late

admission". Hosmer-Lemeshow goodness of fit statistics were used to assess the fitness of the logistic regression model. To preserve the integrity of the model in the presence of multicollinearity, only one variable from each highly correlated set was incorporated. The cut-off value of the first DBS result in predicting the age at admission was analyzed using receiver operating characteristics (ROC) curve analysis. While evaluating the area under the curve, a 5% type-I error level was used to accept a statistically significant predictive value of the test variables.

Results

Overview of patient characteristics

1103 patients referred from the national NBS program for elevated Phe level between January 2016 and January 2023 were included. The demographic and neonatal clinical characteristics of the patients, and the initiated treatments, and the final diagnoses are shown in Table I, and the laboratory findings are shown in Table II. The majority of referred infants were either normal (38.1%) or had mild HPA not requiring treatment (38.3%), and the rest had transient or hereditary disorders of phenylalanine or tyrosine metabolism, or a variety of diagnoses unrelated to this pathway, most commonly galactosemia. In total, there were 140 infants who required treatment.

The timing of DBS collections and DBS results within the NBS program are summarized in Table II. 26 infants (2.36%) had late admissions (after 90 days). 18 of these infants had normal Phe levels, 5 had HPA, 2 had PKU with untreated Phe level >20 mg/dL, and 1 had transient tyrosinemia.

Comparison of geographical regions

Significant differences were observed among the geographical regions in Türkiye, regarding the age at the 1st, 2nd, and 3rd DBS samples, and the time it took for the 1st and 2nd DBS tests to finalize as well as the age at admission to the center. In the Black Sea region, the time for sample collection and result finalization was later compared to the Central Anatolia region. In the Southeastern and Eastern Anatolia regions, the collection time for the 3rd DBS, the time of 1st DBS result, and the age at admission were later compared to the Central Anatolia region. In the Black Sea region, the age of 1st DBS collection was later when compared to the Southeastern and Eastern Anatolia regions, but the age at admission to our center was found to be earlier. In the Aegean region, the time of 1st DBS result and time of 2nd DBS result was earlier when compared to the Eastern Anatolia region. Patients from the Central Anatolia and Marmara regions were younger at the age of admission (Supplementary Table S1 and S2).

Comparison of clinical and laboratory characteristics across patient groups

The median age at admission was 20.5 days (Q1-Q3: 17.5-25.5, min-max: 12-62 days) for patients with first DBS Phe levels higher than 20 mg/dL (n=12), and 22.5 days (Q1-Q3: 13-36.5, min-max:12-42 days) for patients with second DBS results higher than 20 mg/dL (n=4).

Supplementary Table S3 shows differences in the 1st, 2nd, and 3rd DBS results and the initial plasma Phe levels among groups categorized by neonatal hospitalization status, low birth weight status, history of antibiotic use, preterm and term births, presence of neonatal jaundice, and phototherapy treatment. No significant differences in Phe levels were observed between the Transient HPA (n=26) and Transient Tyrosinemia (n=56) groups regarding neonatal hospitalization or antibiotic use (p=1, and p=0.86, respectively). However, a history of neonatal jaundice was 4.5 times more common in the Transient Tyrosinemia group compared to the Transient HPA group, and this difference was statistically significant (p=0.04). No significant differences were observed in gestational age, birth weight, 1st DBS, 2nd DBS, and 3rd DBS results (Supplementary Table S3).

Table I. General characteristics of infants referred through newborn screening for phenylketonuria (N=1103).

Characteristics	
Female sex, n (%)	539 (48.9%)
Consanguinity, n (%)	272 (24.7%)
Maternal age, yr, mean ± SD	29 ± 5.4
Paternal age, yr, mean ± SD	32.7 ± 6
Family history of PKU, n (%)	85 (7.7%)
Gestational age, wk, mean ± SD	38.3 ± 2.1
Birth weight, gr, mean ± SD	3166 ± 572.2
History of neonatal hospitalization, n (%)	182 (16.5%)
Neonatal jaundice	71 (6.4%)
Neonatal respiratory distress	21 (1.9%)
Premature birth	39 (3.5%)
Congenital pneumonia	13 (1.1%)
Urinary tract infection	6 (0.5%)
Sepsis	12 (1%)
Others*	20 (11.8%)
History of neonatal jaundice, n (%)	167 (15.1%)
Phototherapy, n (% among jaundice)	81 (48.5%)
No treatment, n (% among jaundice)	86 (51.5%)
Age at admission, days, median (Q1-Q3), min-max	25 (18-34), 3-410
1st plasma Phe level (at our center), mg/dL (n=1103), median (Q1-Q3), min-max	2.1 (1.26-3.3), 0.1-55
1st plasma Tyr level (at our center), mg/dL (n=1103), median (Q1-Q3), min-max	2 (1.51-2.51), 0.49-11
Diagnosis, n (%)	
Normal	421 (38.1%)
Disorders of Phe or Tyr metabolism	
Group 1; untreated Phe level 2–6 mg/dL	422 (38.3%)
Group 2 PKU; untreated Phe level 6–20 mg/dL	63 (5.7%)
Group 3 PKU; untreated Phe level >20 mg/dL	70 (6.3%)
Defects of BH4 metabolism or DNAJC12	5 (0.5%)
Transient tyrosinemia	56 (5.1%)
Transient HPA	26 (2.4%)
HPA + transient tyrosinemia	16 (1.5%)
Transient HPA + transient tyrosinemia	7 (0.6%)
Transient tyrosinemia + Group 2 PKU	2 (0.2%)
Maternal PKU syndrome	5 (0.5%)

*Others: Arrhythmia, early rupture of membranes, hypoglycemia, hypoxic-ischemic encephalopathy, necrotizing enterocolitis, intrauterine growth restriction, polycythemia, respiratory distress syndrome
** Group 1: untreated Phe level 2–6 mg/dL (patients not requiring treatment); Group 2: untreated Phe level 6–20 mg/dL (patients requiring treatment); Group 3: untreated Phe level >20 mg/dL (patients requiring treatment).
HPA, hyperphenylalaninemia; Phe, phenylalanine; PKU, phenylketonuria; Tyr, tyrosine.

Table I. Continued.

Characteristics	
Other inherited metabolic disorders	10 (0.9%)
Galactosemia	7 (0.6%)
Citrin deficiency	1 (0.1%)
I-cell disease	1 (0.1%)
Lipoprotein lipase deficiency	1 (0.1%)
Follow up / Treatment, n (%)	
No follow-up required	510 (46.3%)
Follow-up required	593 (53.7%)
Lost to follow-up	188 (17.0%)
Followed without treatment	265 (24%)
Followed with treatment	140 (12.7%)
Diet	70 (6.4%)
Sapropterin monotherapy for PKU	65 (5.9%)
Neurotransmitter precursors and sapropterin	5 (0.5%)

*Others: Arrhythmia, early rupture of membranes, hypoglycemia, hypoxic-ischemic encephalopathy, necrotizing enterocolitis, intrauterine growth restriction, polycythemia, respiratory distress syndrome
** Group 1: untreated Phe level 2–6 mg/dL (patients not requiring treatment); Group 2: untreated Phe level 6–20 mg/dL (patients requiring treatment); Group 3: untreated Phe level >20 mg/dL (patients requiring treatment).
HPA, hyperphenylalaninemia; Phe, phenylalanine; PKU, phenylketonuria; Tyr, tyrosine.

Table II. DBS timing and phenylalanine levels in infants referred via newborn screening (N=1103).

	1st DBS	2nd DBS	3rd DBS
Age at collection (days)	1 (1-4), 0-734 (n=1103)	7 (5-13), 3-178 (n=898)	16 (13-21), 16-219 (n=492)
Time it took for the result (days)	6 (4-8), 1-62 (n=1035)	7 (5-9), 1-42 (n=886)	7 (5-9), 1-41 (n=492)
Phe level (mg/dL)	2.5 (1.7-3.7), 0.2-40 (n=1035)	2.5 (2.2-3.2), 0.4-32 (n=886)	2.4 (2.2-2.7), 0.6-6.6 (n=492)

Values given as median (Q1-Q3), min-max, followed by the number of infants (n).
DBS, dried blood spot, Phe, phenylalanine.

There were no significant differences in gestational age and birth weight parameters between the HPA (n=422) and normal (n=422) groups (p=0.07-0.655). However, there were significant differences in the 1st, 2nd, and 3rd DBS Phe levels (p<0.001) between normal and HPA groups. The DBS Phe levels were higher in the HPA group (Supplementary Table S3).

Patients with transient HPA, transient tyrosinemia, and transient HPA combined with transient tyrosinemia were recorded as a single "transient" group and compared with the normal group (Supplementary Table S3). There were no differences in gestational age, birth weight, 2nd DBS, and 3rd DBS values. The risk of neonatal hospitalization in the transient group was 1.6 times higher compared to the normal group (p=0.04). The history of jaundice in the transient group was 2.7 times more common compared to the normal group (p=0.001).

Determinants of age at admission

There was a statistically significant relationship between having a family history of PKU and age at admission: The median day of admission was

19 (3-86) days for patients with a family history of PKU and 26 (4-410) days for those without (p<0.001). For patients with Phe levels measured at our center below 4 mg/dL (n=504), the median age at admission was 26 days (Q1-Q3: 21-34, min-max: 4-410), while for patients with Phe levels above 4 mg/dL (n=209), the median age at admission was 14 days (Q1-Q3: 10-20, min-max: 4-138), and this difference was statistically significant (p<0.001). Spearman correlation analysis between the 1st DBS Phe level and age at admission to our center showed a moderate, negative correlation (r= -0.41, p<0.001). ROC curve analysis for predicting early admission based on the 1st DBS Phe level yielded a cut-off value of 4.05 mg/dL (area under the curve: 0.816, 95% confidence interval: 0.777–0.854, p<0.001, sensitivity: 90.2%, specificity: 68%; Supplementary Fig. S1). Logistic regression analysis showed that positive family history of PKU, certain geographical regions, higher 1st DBS Phe level were associated with earlier age at admission (Table III). In Eastern Anatolia, Southeastern Anatolia, Mediterranean and the Black Sea regions, the age of admission was found to be significantly later (Supplementary Table S1 and S2).

Discussion

The results of our study highlight several critical findings related to the clinical characteristics of patients identified through the national NBS program for disorders of Phe metabolism, and to the workings of the NBS program itself. Our comprehensive analysis of 1103 patients offers valuable insights into the demographics, clinical features, and final diagnosis.

Regional disparities in newborn screening and healthcare access

One of the significant findings of our study is significant geographical differences in the timing of DBS sample collection, result reporting and admission time, which could reflect variations in healthcare infrastructure, accessibility, and geographical challenges. These disparities can be attributed to factors such as healthcare infrastructure, availability of adequate healthcare workers, and health policies. Additionally, family-related factors such as beliefs, education level, and awareness may contribute to the delays in collecting and transporting DBS samples to the center. Efforts should be made to speed up sample

Table III. Significant predictors of age at admission and late admission by logistic regression.

Variables in model	β coefficient	Standard error	p value	Odds ratio	95% CI
Significant predictors of "age at admission"					
Constant	-2.589	0.722	<0.001	0.075	-
Family History of PKU	0.762	0.364	0.037	2.143	1.049-4.377
Region	0.362	0.081	<0.001	1.436	1.226-1.682
1st DBS Phe level	3.01	0.23	<0.001	20.279	12.910-31.854
Significant predictors of "late admission"					
Constant	-4.139	1.149	0.000	0.016	-
Region	0.265	0.087	0.002	1.303	1.1-1.545

The model is statistically significant with p <0.001; Hosmer- Lemeshow: 0.446; Nagelkerke R^2: 0.442; overall accuracy: 84.9%. In the second logistic regression model examining factors influencing late admission, the region was found to be a significant factor with p=0.002; Hosmer-Lemeshow: 0.233; Nagelkerke R^2: 0.050; overall accuracy: 97.8%. All regions were included in the model separately. Descriptive and comparative characteristics of the differences between regions are given in Supplementary Table S1 and S2.
CI, confidence interval; DBS, dried blood spot; Phe, phenylalanine; PKU, phenylketonuria.

transport and analysis, either by establishing a new screening center closer to these regions or by optimizing logistical algorithms for more efficient sample processing and transfer.

Detection of transient and secondary metabolic conditions

Transient tyrosinemia and transient HPA result from the immaturity of hydroxylation enzymes or hepatocyte damage. Transient tyrosinemia is also associated with ascorbic acid deficiency and prematurity.[13] Transient conditions can also occur in other metabolic diseases causing liver damage, such as galactosemia and citrin deficiency. The incidence of transient tyrosinemia of the newborn varies between 0.2-10%.[14] We observed an incidence of 5.1% among the referred infants, consistent with existing data. Studies have shown that maternal/neonatal complications are more frequent in the Transient Tyrosinemia group, but long-term cognitive outcomes do not differ from those of normal infants.[15,16] We demonstrated that jaundice was more prevalent in the Transient Tyrosinemia group compared to the Transient HPA group. Furthermore, the comparison of Transient HPA and/or Transient Tyrosinemia with normal patients showed higher rates of neonatal jaundice and hospitalization in the transient group. To our knowledge, no previous studies have compared these groups. Our results suggest that mild Phe or tyrosine elevations detected by NBS are more likely to be transient if the newborn has a history of jaundice or hospitalization, which can guide counseling.

In our study, we identified patients with various other inherited metabolic disorders, diagnosed incidentally. These included galactosemia (n=7), citrin deficiency (n=1), I-cell disease (n=1) and lipoprotein lipase deficiency (n=1). Studies from our country have reported cases of citrin deficiency, as documented by Zeybek et al.[17]; while Yekeduz et al.[18] and Unal et al.[19] reported cases of maple syrup urine disease, galactosemia, and tyrosinemia. According to the literature, Shakespeare et al.[20] reported cases of galactosemia (n=8), biopterin defects (n=2), and tyrosinemia type 1 (n=1). In our study, we identified patients with DNAJC12 deficiency (n=1) and disorders of BH4 metabolism, which include 6-pyruvoyltetrahydropterin synthase deficiency (n=3) and dihydropteridine reductase deficiency (n=1), which are among the secondary aims of NBS for PKU. However, there have been no reported cases of I-cell disease, and lipoprotein lipase deficiency. The patient is the first to be diagnosed with lipoprotein lipase deficiency based on findings from the NBS, where inaccurately elevated measurement of the patient's blood Phe levels due to the lipemic nature of the sample ultimately led to the diagnosis. Additionally, one patient was diagnosed with congenital leukemia presenting with massive hepatomegaly. This patient has been reported previously.[21] Careful physical examination to detect signs such as cholestasis, dysmorphism, leukocoria, neurological findings, and hepatomegaly is crucial, especially in patients identified through screening, as it helps identify other disorders, including other inherited metabolic disorders.

We identified five cases of maternal PKU syndrome presenting with severe congenital anomalies, consistent with previous studies.[22,23] Timely DBS collection is crucial, as delayed sampling may lead to the normalization of the newborn's Phe levels, potentially missing the diagnosis of maternal PKU. NBS programs offer secondary benefits, including the detection of maternal metabolic disorders.[10,24,25]

Impact of family history and referral threshold on early diagnosis

Our study revealed that there is substantial variation in the age at admission based on a family history of PKU. Patients with a positive family history presented earlier compared to those without such a history. It is essential to consider that these families might seek medical attention proactively without waiting for a referral. In line with the Ministry algorithm, patients with an initial Phe level above 4 mg/dL are referred immediately without waiting

for a second sample. Our analysis confirms that a first DBS Phe level above 4.05 mg/dL is associated with an earlier clinical presentation. This finding aligns with the Ministry algorithm, suggesting that the current referral threshold effectively facilitates earlier medical consultation and diagnosis.

Variability in false positive rates due to screening methods and cutoff level variations

In our study, 38% of individuals with elevated phenylalanine levels in NBS were determined to have Phe within normal limits. The false positive rate for PKU screening in Türkiye ranges from 44% to 76.2%.[18,26,27] Studies conducted in different regions of the world have reported false positive rates ranging from 81% to 94%.[4,28] While direct comparisons are complicated by disparities in measurement techniques and threshold values, the false positive rate identified at our center seems to reside within an acceptable spectrum. Systematic annual dissemination of data regarding false positive and false negative rates, in addition to the positive and negative predictive values by the Ministry of Health, would be instrumental in enhancing and refining the newborn screening initiative.

Considerations for optimizing the Turkish PKU NBS Program

Considering our findings, it is imperative to enhance the NBS programs and follow-up protocols. The early detection and treatment of PKU is crucial in preventing severe long-term complications. Based on our findings, several measures can be taken to prevent delayed diagnoses, both across the entire population and in specific regions. Firstly, increasing the accessibility and efficiency of healthcare services in regions with identified delays, such as Eastern Anatolia, Southeastern Anatolia, Mediterranean and the Black Sea regions, is crucial. This can be achieved by improving healthcare infrastructure, ensuring the availability of adequate healthcare workers, and implementing targeted health policies. It is necessary to educate families about the importance of timely screening and follow-up, particularly in regions with lower levels of awareness.

This study is one of the largest single-center studies conducted examining NBS. It offers a comprehensive analysis of demographic data, clinical findings, and diagnostic results, contributing valuable insights into the workflow of NBS programs. The study highlights regional disparities in healthcare access, guiding policy decisions to improve healthcare delivery in underserved areas. The occurrence of two delayed diagnoses in patients with a classical PKU is regrettable, and similar situations are likely to have occurred in other centers. To prevent delayed diagnoses, it is crucial to implement strategies that ensure timely and accurate screening and follow-up.

Our study has some limitations. The study's retrospective design relies on the accuracy and completeness of historical medical records, which may introduce biases or data inaccuracies. As a single-center study, the findings may not be generalizable to other regions or countries with different healthcare infrastructures and practices. Given that the national NBS program is centrally managed by the Ministry of Health, open access to screening processes and outcome data would facilitate further improvements. Transparent data sharing could help refine screening protocols, address regional disparities and optimize the overall effectiveness of the NBS

In conclusion, our study underscores the importance of timely and accurate NBS for phenylalanine metabolism disorders. By improving early diagnostic and follow-up practices, we can significantly impact the clinical outcomes and quality of life of affected individuals. Future research should focus on addressing regional disparities and optimizing NBS protocols to ensure that all newborns receive the best possible start in life.

Supplementary materials

Supplementary materials for this article are available online at https://doi.org/10.24953/turkjpediatr.2025.5263

Ethical approval

The study was approved by Hacettepe University Ethics Committee (date: 21.09.2021, number: 15/44, 2021/09).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: ABK, KÇ, YY , AD, SS, AT; data collection: ABK, KÇ, BP, MG; analysis and interpretation of results: ABK, KÇ YY; draft manuscript preparation: ABK, KÇ, YY, AD, SS, AT. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Increased serum YKL-40 levels in children with sickle cell disease

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ABSTRACT

Background: YKL-40 is a glycoprotein secreted by various cell lines during inflammation and vascular dysfunction. Sickle cell disease (SCD) also involves inflammation and endothelial dysfunction processes. Thus, we aimed to assess the levels of YKL-40 in pediatric SCD patients.

Methods: We evaluated serum levels of YKL-40 in children with steady state SCD and those with vaso-occlusive crisis (VOC) episodes and compared them with healthy subjects.

Results: Overall, 33 children with SCD and 33 healthy controls participated in this study. Serum YKL-40 concentrations of children with steady state SCD were significantly higher than the concentrations found in the healthy controls (median [Q1-Q3]: 71.0 [53.3-133.3] vs. 43.6 [37.9-69.9] ng/mL, p=0.001). Seventeen of the 33 children with SCD (51.5%) had a VOC during the one-year follow-up period. Steady state and VOC episode YKL-40 did not significantly differ in children who were experiencing VOC during the one-year follow-up (77.6 [55.2-126.8] vs. 69.7 [49.3-100.0] ng/mL, p=0.381). During VOC episodes, children with SCD had significantly higher YKL-40 levels than the healthy controls (69.7 [49.3-100.0] vs. 43.6 [37.9-69.9] ng/mL, p=0.005). YKL-40 levels at steady state and during VOC episodes did not show significant correlation (p=0.955).

Conclusions: YKL-40 may have a potential role in the inflammation component of SCD. Circulating YKL-40 levels may be used to monitor chronic inflammation in SCD patients.

Key words: endothelial dysfunction, inflammation, sickle cell disease, YKL-40, chitinase-3-like protein-1, human cartilage glycoprotein 39.

Sickle cell disease (SCD) is an autosomal recessive genetic disease and is one of the most common hemoglobinopathies in the world. The term *SCD* covers mutations in the gene that encodes the beta globin subunit of hemoglobin, leading to a group of diseases (e.g., sickle cell anemia [SCA], hemoglobin SC disease, and hemoglobin S-beta-thalassemia). Substituting glutamic acid with valine at the sixth position of the beta-globin chain leads to the formation of abnormal hemoglobin, known as hemoglobin S (HbS).[1] HbS is the most common abnormal hemoglobin in Türkiye.[2]

The complex pathophysiology of SCD involves HbS polymerization, the sickling of red blood cells, vaso-occlusion, increased blood viscosity, oxidative stress and inflammation, endothelial dysfunction, and ischemia-reperfusion injury. The symptoms mainly occur as a result of

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the abnormal sickling of red blood cells and various subsequent complications.[3] Pain crisis, anemia, infections, acute chest syndrome, delayed growth and development, stroke, and long-term organ damage are common symptoms of SCD.[1,3] Pain crises or vaso-occlusive crises (VOC) are hallmarks of SCD. Polymerization of deoxygenated HbS leads to decreased deformability of red blood cells, and the characteristic "sickle" shape occurs. Rigid sickled red blood cells lead to tissue ischemia by adhering to the vascular endothelium and obstructing the vasculature. Ultimately, severe pain episodes arise due to the ischemic tissue damage and inflammation.[4]

In the pathophysiology of vaso-occlusion, interactions between sickled red blood cells, activated endothelial cells, leukocytes, and platelets play a crucial role. Multicellular aggregation, increased oxidative stress, and a pro-inflammatory environment created by these cells underlie VOCs.[4] Previously, higher neutrophil and platelet counts and high levels of platelet factors 3 and 4, extracellular hemoglobin, and lactate dehydrogenase have been reported during VOCs.[5-8] Chronic hemolysis in SCD induces oxidative stress, and increased oxidative stress triggers inflammatory cytokine release.[9] Furthermore, ischemia-reperfusion injury secondary to microvascular occlusions promotes chronic inflammation in SCD.[10] Both acute and chronic inflammatory responses have been associated with VOCs.[3,4] The inflammatory mediators C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), interleukin 8 (IL-8), transforming growth factor-beta (TGF-β), and interleukin 17 (IL-17) have been shown to be significantly increased in patients with SCD and VOCs when compared to healthy controls.[11,12]

YKL-40 (chitinase-3-like protein-1, human cartilage glycoprotein 39) is a mammalian glycoprotein related in sequence to family 18 of bacterial and fungal chitinases. However, it does not exhibit enzymatic activities. While YKL-40 is expressed in various cell types, its exact biological function is currently unknown. Increased levels of YKL-40 have been linked to inflammation, tissue remodeling, angiogenesis, and cancer.[13,14] Previous studies have investigated the role of YKL-40 in various cardiovascular, gastrointestinal, endocrinological, immunological, musculoskeletal, neurological, respiratory, urinary, and infectious diseases and found contradictory results. YKL-40 has been found to be an important proinflammatory protein in certain diseases. However, in some other diseases, it has not been found to be significantly different from healthy individuals.[15] YKL-40 is identified to be a potential biomarker of inflammation and vascular dysfunction.[16] SCD and VOCs also are also characterized by inflammation and endothelial dysfunction. A meeting abstract reported higher serum YKL-40 and cytokine levels in children with SCD in comparison with their healthy counterparts, and weak correlations between serum YKL-40 levels and salivary cytokine levels.[17] To the best of our knowledge, original research comparing serum YKL-40 levels between pediatric SCD patients and healthy children has not previously been published. The primary objective of this study was to evaluate serum levels of YKL-40 in children with steady state SCD and those with VOC episodes and compare them with healthy subjects. The secondary objective was to investigate correlations between YKL-40 and the inflammatory biomarkers CRP, IL-6, and TNF-α in children with SCD.

Materials and Methods

Study design and study sample

This comparative cross-sectional study was conducted at Mersin University Hospital and investigated the inflammatory biomarkers of children with SCD. Children with SCD and age- and sex-matched healthy controls were the subjects. Children with SCD followed in the Pediatric Hematology department and healthy children admitted to the well-child outpatient clinic were included in the study. Inclusion criteria for the patient group were as follows:

having been diagnosed with SCD based on hemoglobin electrophoresis and genetic testing; age 2 to 17 years; and having consent from his/her parents or legal guardians to participate in the study. For the patient group, exclusion criteria were the presence of VOC at last one month prior to the study, chronic simple or blood exchange transfusions, drug use except hydroxyurea, sickle hepatopathy, renal dysfunction, hypertension, endocrine disorder, overweight and obesity, serious infections and inflammatory diseases, and smoking. Inclusion criteria for the control group were as follows: being healthy and age 2-17 years; having normal weight; not smoking; having no medical or family history of congenital hematologic disease, blood disorder, chronic systemic disease, genetic disorder or malignancy; and having consent from his/her parents or legal guardians to participate in the study. Child assent and parent informed consent were obtained for participation. This study was approved by Mersin University Clinical Research Ethics Committee (2016-04-14/108).

Data collection

All children with SCA (n=23) and hemoglobin S-beta-thalassemia (n=10) who were admitted to the Pediatric Hematology clinic between May 1, 2016 and May 1, 2017 and fulfilled the appropriate criteria constituted the study sample. For each SCD patient, an age- and gender-matched healthy child with normal growth and development was also included in the study. In total, data were collected from 33 children with SCD and 33 healthy children.

Baseline evaluations and one-year follow-ups were conducted for the patients. Laboratory data were collected from 33 patients with steady state SCD and from 33 healthy controls at the baseline evaluation. In 17 patients who had VOCs during the one-year follow-up period, a second evaluation was performed, and laboratory data were recollected at the VOC episode. Serum samples of the SCD and control groups were obtained on the day of admission to the hospital.

Laboratory measurements

Serum YKL-40, IL-6, TNF-α, and CRP levels, as well as complete blood count values in venous blood samples, were obtained. YKL-40, IL-6, and TNF-α concentrations were determined by an enzyme-linked immunosorbent assay (ELISA). The measurements were carried out using the YKL-40 human ELISA kit (201-12-2034 SunredBio), IL-6 ELISA kit (KAP 1261 Diasource) and TNF-α-ELISA kit (KAP 1751 Diasource). The manufacturer's protocols for each ELISA kit were followed. The DSXTM Four-Plate Automated ELISA Processing System MikroELISA device was utilized for analysis. For each sample, the concentrations of YKL-40, IL-6, and TNF-α were determined by calculating them from the curves and equations obtained from plotting the optical density values corresponding to the known concentrations of standards provided in each analysis kit. IL-6 and TNF-α levels were only measured in the patient group. CRP levels were analyzed using the immunoturbidimetric method and a CRP value less than 5 mg/L was considered normal.

Statistical analysis

Statistical Package for the Social Sciences version 21.0 (IBM SPSS Corp.; Armonk, NY, USA) was used for statistical analysis. The Shapiro-Wilk test and histograms were used to test for normality. For continuous variables, mean ± standard deviation or median (with first and third quartiles, Q1-Q3) values are provided; for categorical variables, numbers (n) and percentages (%) are provided as descriptive statistics. The two independent groups (patient and healthy control groups) were compared with the Student t-test or Mann-Whitney U test, and the two dependent groups (steady state and VOC episode groups) were compared with the Wilcoxon test. The three independent groups (steady state, VOC episode, and healthy control groups) were compared with the Kruskal-Wallis test. Relationships between continuous variables were examined with the Spearman correlation coefficient. Categorical variables were compared with the chi-square test. The statistical significance level was set at $p<0.05$.

Results

Overall, 33 children with SCD and 33 healthy controls participated in this study. The mean age of the children with SCD was 12.4 ± 3.7 years, and the mean age of the healthy controls was 11.7 ± 4.2 years (p=0.460) Twenty of the children with SCD (60.6%) and 21 of the healthy controls (63.6%) were male (p=0.800). All children with SCD were using hydroxyurea.

Serum YKL-40 concentrations of children with steady state SCD were significantly higher than the concentrations found in the healthy controls (71.0 [53.3-133.3] vs. 43.6 [37.9-69.9] ng/mL, p=0.001). CRP concentrations in the serum of children with steady state SCD was significantly increased compared to the healthy controls (p=0.001). White blood cell (WBC) count did not differ between the children with steady state SCD and the healthy controls (p=0.380, Table I).

Seventeen children with SCD (51.5%) had a VOC during the one-year follow-up period. The median duration between the baseline evaluation at steady state and the second evaluation at VOC episode was 5.8 ± 2.7 months (min-max: 1.5-10 months). Steady state and VOC episode YKL-40, CRP, IL-6, and TNF-α levels did not significantly differ in children with SCD who were experiencing VOC during the one-year follow-up (p>0.05). WBC count significantly increased during VOC compared with the WBC count in steady state SCD (14.30 [10.27-19.83] vs. 10.50 [8.70-13.37] x10^3/μL, p=0.017). During VOC episodes, children with SCD had significantly higher YKL-40 and CRP levels than the healthy controls (p=0.005 and p<0.001, respectively). Table II shows a comparison of inflammatory biomarkers at steady state SCD and VOC episodes, as well as a comparison of VOC episode biomarker levels with healthy controls.

When grouped according to the occurrence of VOCs during the one-year follow-up, there were no significant differences in the steady-state concentrations of YKL-40, CRP, IL-6, TNF-α, or in the steady-state WBC counts between children with SCD who experienced VOCs and those who did not (p > 0.05). There was no significant difference in baseline WBC counts among children with SCD who experienced VOCs during the one-year follow-up, those who did not experience VOCs, and healthy controls (Table III).

Steady state YKL-40 levels were moderately correlated with steady state CRP levels in children with SCD (rho=0.40, p=0.022). There was no significant correlation between steady state YKL-40 levels and steady state IL-6, TNF-α, or WBC values (p>0.05). VOC episode biomarker levels did not show significant correlations (p>0.05). Steady state YKL-40 levels and VOC episode YKL-40 levels did not show significant correlation (rho= -0.015, n=17, p=0.955). In healthy subjects, YKL-40 levels, CRP levels, and WBC counts did not show significant correlations (p>0.05).

Discussion

This study showed that serum YKL-40 and CRP levels increased in children and adolescents with steady state SCD and with VOC episodes, and steady state YKL-40 was correlated with

Table I. Comparison of YKL-40, CRP, and WBC values between patients with steady state SCD and healthy controls.

Biomarkers	Steady state SCD (n=33)	Healthy controls (n=33)	**p**
YKL-40, ng/mL	71.00 (53.3-133.3)	43.60 (37.9-69.9)	0.001
CRP, mg/L	4.32 (2.71-10.24)	0.98 (0.40-3.65)	0.001
WBC, x10^3/μL	12.00 (9.20-15.00)	10.62 (8.65-13.44)	0.380

Mann-Whitney U test, data are presented as median and interquartile range (Q1-Q3).
CRP, C-reactive protein; SCD, sickle cell disease; WBC, white blood cell.

steady state CRP, while steady state and VOC episode YKL-40 levels did not significantly differ.

The expression pattern of YKL-40 points to its potential involvement in sterile inflammation and endothelial dysfunction, even though its precise function is still unknown.[18] A comprehensive review indicated that YKL-40 can be considered a marker for systemic inflammatory and autoimmune disorder diagnosis, prognosis, and disease activity.[13] Higher serum concentrations of YKL-40 are known to be associated with severe forms of

Table II. Comparison of inflammatory biomarkers at steady state and VOC episode, and comparison of VOC episode biomarker levels with healthy controls.

Biomarkers	Steady state (n=17)	VOC episode (n=17)	p*	Healthy controls (n=33)	p**
YKL-40, ng/mL	77.56	69.74	0.381	43.60	0.005
	(55.22-126.76)	(49.31-100.02)		(37.9-69.9)	
CRP, mg/L	7.10	6.57	0.149	0.98	<0.001
	(3.91-13.15)	(3.25-25.0)		(0.40-3.65)	
WBC, x10^3/μL	10.50	14.30	0.017	10.62	0.053
	(8.70-13.37)	(10.27-19.83)		(8.65-13.44)	
IL-6, pg/mL	6.57	35.27	0.266	-	-
	(4.02-26.22)	(10.77-174.15)			
TNF-α, pg/mL	21.05	15.25	0.831	-	-
	(15.27-42.42)	(11.07-37.42)			

*Wilcoxon test, data are presented as median and interquartile range (Q1-Q3), comparison of steady state and VOC episode biomarker levels in patient group experiencing VOC during the one-year follow-up.
**Mann-Whitney U test, data are median and interquartile range (IQR 25-75%), comparison of patients' vaso-occlusive crisis episode biomarker levels with healthy controls' biomarker levels.
CRP, C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor alpha; VOC, vaso-occlusive crisis; WBC, white blood cell.

Table III. Comparison of inflammatory biomarkers between patients experiencing vaso-occlusive crisis and those who were not during the one-year follow-up, and healthy controls.

Baseline bio-markers	SCD without VOC (n=16)	SCD with VOC (n=17)	p*	Healthy controls (n=33)	p**
YKL-40, ng/mL	65.00	77.56	0.692	43.60	0.003
	(51.2-135.2)[a]	(55.22-126.76)[a]		(37.9-69.9)[b]	
CRP, mg/L	2.85	7.10	0.061	0.98	0.001
	(1.25-8.32)[a]	(3.91-13.15)[a]		(0.40-3.65)[b]	
WBC, x10^3/μL	14.25	10.50	0.072	10.62	0.175
	(9.95-16.50)	(8.70-13.37)		(8.65-13.44)	
IL-6, pg/mL	10.94	6.57	0.746	-	-
	(3.72-187.56)	(4.02-26.22)			
TNF-α, pg/mL	37.45	21.05	0.296	-	-
	(15.73-159.06)	(15.27-42.42)			

*Mann-Whitney U test, data are presented as median and interquartile range (Q1-Q3), comparison of SCD patients course with and without VOC.
**Kruskal-Wallis test, data are presented as median and interquartile range (Q1-Q3), comparison of the three groups; different letters represent significant differences at $p < 0.05$ probability level.
CRP, C-reactive protein; IL-6, interleukin-6; SCD, sickle cell disease; TNF-α, tumor necrosis factor alpha; VOC, vaso-occlusive crisis; WBC, white blood cell.

inflammatory diseases and cardiovascular inflammatory conditions.[15,19-21] Higher levels of YKL-40 were expected in patients with beta-thalassemia major. However, the level of YKL-40 was found to be within the normal range in patients between the ages of 15–69 and only a slight correlation was found with the liver status.[22] Previous studies have explained their findings with the involvement of YKL-40 in the inhibition of vascular endothelial cell apoptosis, inducing the loss of endothelial barrier function and endothelial-mesenchymal transition.[15,19] Endothelial dysfunction and inflammation have also been demonstrated in children with SCD and are present in both VOCs and in steady state SCD.[23] Therefore, YKL-40 is also likely to be elevated in children with SCD. For the first time in the literature, the current study demonstrates increased serum YKL-40 levels in children with SCD both in VOCs and in steady state compared to healthy controls.

The main inflammatory events in SCD involve increases in leukocyte numbers and their activation, expression of adhesion molecules in leukocytes, proinflammatory cytokines, neutrophilic extracellular traps, secretory phospholipase A2 enzyme, placental growth factor, and leukotriene E4, as well as decreases in anti-inflammatory cytokines.[24] The inflammatory state in SCD involves the production and secretion of numerous pro-inflammatory mediators. The cytokines TNF-α, IL-1-alpha, IL-6, IL-17, interferon-gamma, platelet-derived CD40 ligand and herpesvirus entry mediator ligand (TNSF14), IL-1-beta and IL-18; and the chemokines IL-8, monocyte chemoattractant protein-1, regulated on activation, normal T cell expressed and secreted (RANTES), platelet factor 4, macrophage inflammatory protein 1-alpha, eotaxin-1 and fractalkine; the growth factors granulocyte-macrophage colony-stimulating factor, macrophage colony-stimulating factor, TGF-β and pro-angiogenic molecules; and the acute phase proteins CRP and pentraxin-3 are reported to be elevated in SCD patients when compared to controls.[25,26] Our findings add to the existing literature on elevated YKL-40 in both steady-state SCD and VOCs. Our findings also support the existing literature on upregulated acute phase protein CRP both in steady state SCD and in VOCs.

IL-6 is an activated cytokine that is significantly increased in the peripheral blood of patients with SCD. IL-6 has been found to be increased in steady state SCD and further increased during vaso-occlusion.[27,28] We also demonstrated an increase in serum IL-6 levels during VOC compared with steady state SCD, although it was statistically insignificant. Expression of TNF-α was reported to be normal or increased in both steady state SCD and VOC episodes.[28] We found consistently statistically similar TNF-α levels in steady state SCD and VOC episodes. Due to a limited research budget, we did not measure IL-6 and TNF-α concentrations in the healthy control group.

Previous studies investigating predictor biomarkers of VOC provide evidence of the association between high-adhesive phenotypes and the occurrence of VOCs. Higher adhesion biomarkers during steady state SCD were found to predict the frequent occurrence of VOCs.[29,30] Steady state TNF-α, IL-6, and WBC levels, taken together, were found to discriminate between low and high VOC risk groups in children with SCD.[31] A previous study of risk prediction modeing for VOCs in children with SCD reported that YKL-40 failed to distinguish the patients with VOC and those with steady-state disease, and patients with a high risk of VOC could not be detected via YKL-40.[31] Aforementioned feasibility study evaluated YKL-40 to predict VOC risk[31], the current study, on the other hand, evaluated YKL-40 levels in children with SCD in comparison with healthy controls. While the current study did not aim to assess the predictive value of inflammatory biomarkers during steady-state SCD and VOC, a comparison of children experiencing VOC and those who were not during the one-year

follow-up revealed a non-significant difference in steady state YKL-40, CRP, WBC, IL-6, and TNF-α levels. This finding may contribute to research in the prediction of VOCs among individuals with SCD.

Rees and Gibson reviewed more than 100 different blood and urine biomarkers described in SCD and concluded that these biomarkers are mostly closely intercorrelated.[32] Our findings add to the existing literature on a positive correlation between serum YKL-40 and CRP in steady-state SCD.

Chronic transfusions and sickle organopathies may alter biomarker profiles of SCD patients, and altered biomarkers of hemolysis, anemia, and hypoxemia, as well as damage to specific organs, may alter biomarkers of inflammation.[32] For this reason, we did not include children with SCD receiving chronic simple or blood exchange transfusions, with sickle hepatopathy or nephropathy, or with other potential inflammatory conditions. Hydroxyurea is linked to decreased inflammatory cytokines in children with SCD.[33] Therefore, the use of hydroxyurea by all of our patients contributed to ensuring group homogeneity.

This study has several limitations. First, various patient factors and environmental factors (eg, hypoxia, dehydration, stress, low humidity, exposure to cold or weather changes) may trigger and exacerbate VOCs, and inflammatory profiles may vary depending on the triggers.[34,35] However, we did not assess the potential trigger(s) of VOC in our patients. Second, some genotypes are associated with the severity of SCD and VOC[36], but we did not consider the inflammation-related gene polymorphisms or SCD haplotypes of our patients. Third, VOC has previously been suggested to consist of sequential phases and inflammatory markers that increase from the beginning of a pain event and become significant in the severe, constant pain phase.[37] We cannot know for certain that we took blood samples from all patients in the same phase of VOC, but we can say that serum samples were obtained from patients with severe, debilitating pain within 24 hours of hospital admission.

In conclusion, serum YKL-40 and CRP levels in children and adolescents with SCD were higher both in steady state SCD and during VOCs, and steady state YKL-40 was correlated with steady state CRP. Our findings suggest a potential role of YKL-40 in the inflammation component of SCD. Circulating YKL-40 levels may be used to monitor chronic inflammation in SCD patients. Further studies utilizing serial measurements are warranted to clarify the peak timing and decline dynamics of YKL-40, and to determine whether it may serve as an earlier indicator of VOCs or demonstrate superiority over other serologic inflammatory markers.

Ethical approval

The study was approved by Mersin University Clinical Research Ethics Committee (date: 14.04.2016, number: 2016-108).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: VA, SÜ, ÖT, BT, ŞBF, FK; data collection: VA, ŞBF, FK; analysis and interpretation of results: ÖT, BT; draft manuscript preparation: VA, SÜ, ÖT. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Exploring quality of life and related clinical factors in children with tree nut allergies

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ABSTRACT

Background. In Türkiye, tree nut allergy (TNA) is the most common form of food allergy, characterized by persistence and the potential for life-threatening reactions. This study aimed to evaluate the quality of life (QoL) of Turkish children aged 0-12 years with IgE-mediated TNA and explore influential factors, including parental anxiety.

Materials and **Methods.** Primary caregiver-parents of children diagnosed with TNA completed the Food Allergy Quality of Life Questionnaire-Parent Form (FAQLQ-PF) and State-Trait Anxiety Inventory (STAI) to assess QoL and parental anxiety, respectively.

Results. Of 120 eligible patients diagnosed with TNA, 88 were included in the study. Questionnaires were completed by mothers in 79 cases (90%) and fathers in 9 cases (10%). Parents reported significantly higher FAQLQ-PF scores for children with hazelnut allergy, a history of anaphylaxis, and those who had to use an adrenaline auto-injector. There was significant but weak correlations between FAQLQ-PF and anxiety (STAI) domains. The multivariate linear regression analysis revealed that having a hazelnut allergy, a history of anaphylaxis, and higher parental state anxiety were all associated with higher FAQLQ-PF scores, but fathers tended to report better level of QoL.

Conclusion. QoL for children with TNA is influenced by several factors such as adverse life experiences, local and situational factors, and parental anxiety. Understanding these diverse factors is crucial for enhancing the well-being of children with TNA.

Key words: anxiety, child, nuts, parent, quality of life.

IgE-mediated food allergy affects up to 6% of children, with varying etiologies across different geographies and cultures.[1,2] Peanuts are common allergens in Western countries, while tree nut allergy (TNA) is most prevalent and the leading cause of anaphylaxis among Turkish children.[3-5] Strict avoidance of index allergens and immediate access to rescue medications remain the most commonly used management strategies.[1] However, oral food immunotherapy and biological agents have recently been introduced to mitigate the severity of reactions to inadvertent exposures.[2] Although, with appropriate management, morbidity in children with food allergies is generally low and mortality is rare, food allergies significantly impact the quality of life (QoL) of both children and their families.[1] Several factors,including gender, age,

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disease severity, and concurrent allergies, are associated with lower QoL in children, and these associations vary by population. For instance, a Turkish study linked lower QoL with older age, anaphylaxis, asthma, maternal age over 30, and higher maternal education, while a Greek study found that anaphylactic reactions, epinephrine auto-injector usage, and multiple food allergies decreased QoL.[6,7] Understanding these factors is crucial for improving the overall well-being of these children and their families.

Food allergies (FA) can substantially affect the QoL of children, not only through dietary limitations but also by impacting social, emotional, and psychological well-being. Parental anxiety further exacerbates these challenges, disrupting daily routines and fostering avoidance behaviors. Despite this, data on the influence of parental anxiety on QoL in children with TNA are scarce. Therefore, our study aims to evaluate the food allergy-related QoL (FAQL) in children with TNA, as perceived by their parents, and to explore the factors—including parental anxiety—that influence this assessment.

Materials and Methods

Study population

This study was carried out with primary caregivers (mothers/fathers) of patients aged 12 years and younger who were diagnosed with TNA at Hacettepe University Hospital, Department of Pediatric Allergy between January 1, 2018 and December 31, 2020. During outpatient clinic visits, we requested parents to complete online surveys about their children's TNAs. For those who consented, we subsequently sent a survey link via text message. For the designation of the primary caregiver, parents were given the autonomy to determine who is most responsible for the child's care and upbringing. In cases of uncertainty, the decision was made based on the understanding that the parent who prioritizes the child's health—especially in the context of TNAs—should serve as the primary caregiver. All patients were required to have sensitization to the relevant tree nut by skin prick test, extract-specific IgE and allergen molecule specific IgE (Ana o 3/Pis v 1 for cashew/pistachio, Cor a 14/Cor a 9/Cor a 11 for hazelnut, Jug r 1/Jug r 2 for walnut).[8] The diagnosis of TNA was required to have been made at least 6 months prior by the presence of a consistent history or positive outcome at the oral food challenge (OFC) or high level of sensitization as described previously.[3,9] Parents who could not be contacted, who did not give informed consent, and whose child was older than 12 years of age were not included into the study. This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Hacettepe University Ethics Committee for Non-Interventional Clinical Studies (GO 21/745). All parents gave written informed consent before the study.

Questionnaires

With the questionnaire, information such as sociodemographic characteristics, comorbid diseases, adrenaline auto-injector (AAI) usage, tree nut and other FAs, healthcare utilizations such as unscheduled healthcare use/emergency and hospital admissions due to TNA were collected. The data obtained from the questionnaires were cross-checked with the information in the hospital electronic database and inconsistencies were resolved through interviews. Internationally recognized instruments renowned for their validity and reliability—the Food Allergy Quality of Life Questionnaire-Parent Form (FAQLQ-PF) and the State-Trait Anxiety Inventory (STAI)—were used to assess children's food allergy-related quality of life from the parents' perspective and parental anxiety, respectively.[10-12]

Food Allergy Quality of Life Questionnaires-Parent Form: To summarize briefly, the FAQLQ-PF is designed to gauge the quality of life related to FAs in children from the parental perspective. It has a scale of 30 questions, and its Turkish translation has previously been shown to be

valid and reliable.[6,10] Each question in the scale is answered with a 6-point Likert type variable (0-none to 6-extreme). The scale has a total of 3 sub-dimensions: emotional impact, food anxiety, social and nutritional restrictions. The total score of the scale ranges from 0 to 6, and a low score indicates a high quality of life.[10]

State-Trait Anxiety Scale: The State-Trait Anxiety Inventory (STAI) is a recognized tool for measuring situational and long-term anxiety. It has a total of 40 statements in the scale and its Turkish translation and adaptation has previously been shown to be valid and reliable.[11] The first twenty items measure the level of anxiety related to the situation (STAI-S), and items 21 to 40 measure the trait anxiety (STAI-T).[11,12] Accordingly, obtaining 0-19 points from the scale are not significant levels of anxiety, 20-39 points mean mild, 40-59 points mean moderate, 60-79 points mean severe anxiety, and individuals with a score of 60 and above need professional support.[11]

Statistical analyses

Descriptive analysis were presented with frequency and percentage for categorical variables, and mean±standard deviation, or median (Q1-Q3) for continuous variables. Conformity of continuous variables to normal distribution was examined by visual (histogram and probability graphs) and analytical methods (Kolmogorov-Smirnov / Shapiro-Wilk tests). Independent group comparisons for categorical variables were made with chi-square ($\chi 2$) or Fisher tests. Student's t-test was used in comparison of two independent groups for continuous variables, and one-way analysis of variance (ANOVA) method was used in comparisons of three or more groups. The relationship between STAI and FAQLQ-PF scores was investigated by correlation analysis including Pearson test in parametric and Spearman test in non-parametric correlation analyses. The independent factors predicting the FAQLQ-PF total score were analysed by multivariate linear regression model using possible factors detected in univariate analyses. Statistical significance, the type-1 error level was determined as 5%. G*Power version 3.1.9.7 statistical software was used to calculate the required sample size and statistical power. SPSS for Windows (version 23.0, IBM SPSS Statistics, Chicago, IL) were used for the remaining statistical evaluations.

Results

Study group characteristics

There were 120 eligible patients diagnosed with TNA during the study period and 88 patients were included in the study, resulting in an inclusion rate of 73.3% . As the primary caregivers, 79 (89.8%) of the questionnaires were completed by mothers and 9 (10.2%) by fathers. From the children, 31 (35.2%) were female and 57 (64.8%) were male. The median age at diagnosis was 12 months (Q1-Q3: 7.25-19.5) and median months of follow-up was 40 months (Q1-Q3: 10.25–65.75). The most common TNA was hazelnut (72.7%), followed by cashew, pistachio, walnut and almond. In the context of their life time, 42 (47.7%) and 5 (5.7%) patients had been admitted to the emergency department, and required hospitalization due to TNA, respectively. A total of 42 (47.7%) patients had been exposed to the food they were allergic to in the last year. A total of 49 (55.7%) and 16 (18.2%) patients had a lifetime and current history of anaphylaxis.

Although all patients were prescribed AAIs, only 84.1% of the patients had AAIs. A total of 13 (14.8%) patients had to use AAI at least once in their lives due to a tree nut allergy (Table I). When comparing clinical features across age groups, both having an AAI (p=0.007) and the occurrence of anaphylaxis (p=0.019) were statistically significant. Post-hoc analysis with Bonferroni correction revealed that the 0–3 age group had significantly lower rates of AAI possession (p=0.004) and a lower incidence of anaphylaxis (p=0.008) compared to the 4–6 age group (Table I).

Table I. Clinical characteristics of patients according to age groups.

	Entire group	Age groups			p
	(n:88)	0-3 years (n:29)	4-6 years (n:37)	7-12 years (n:22)	
Female gender	31 (35.2%)	12 (41.4%)	10 (27%)	9 (40.9%)	NS
Age of diagnosis (mo) †	14.51±12.63	16.03±13.09	12.51±8.78	15.86±16.9	NS
Current TN allergy					
Hazelnut	64 (72.7%)	20 (69%)	27 (73%)	17 (77.3%)	NS
Cashew	56 (63.6%)	18 (62.1%)	24 (64.9%)	14 (63.6%)	NS
Pistachio	51 (58%)	17 (58.6%)	22 (59.5%)	12 (54.5%)	NS
Walnut	49 (55.7%)	19 (65.5%)	19 (51.4%)	11 (50%)	NS
Almond	25 (28.4%)	12 (41.4%)	7 (18.9%)	6 (27.3%)	NS
Multiple TN allergy	68 (77.3%)	22 (75.9%)	30 (81.1%)	16 (72.7%)	NS
Concomitant all. disease					NS
Allergic rhinitis	15 (17%)	1 (3.4%)	8 (21.6%)	6 (27.3%)	
Atopic dermatitis	12 (13.6%)	5 (17.2%)	5 (13.5%)	2 (9.1%)	
Other food allergy	3 (3.4%)	0	2 (5.4%)	1 (4.5%)	
Asthma	15 (17%)	7 (24.1%)	2 (5.4%)	6 (27.3%)	
Having an AAI*	74 (84.1%)	20 (69%)	36 (97.3%)	18 (81.8%)	0.007
Having ever used an AAI	13 (14.8%)	2 (9.1%)	8 (21.6%)	3 (16.7%)	NS
Exposure to TN (last year)	42 (47.7%)	12 (41.4%)	18 (48.6%)	12 (54.5%)	NS
Anaphylaxis due TN					
Ever**	49 (55.7%)	10 (34.5%)	25 (67.6%)	14 (63.6%)	0.019
Current	16 (18.2%)	4 (36.4%)	6 (24%)	6 (42.9%)	NS
Ever emergency dept. use	42 (47.7%)	11 (37.9%)	19 (51.4%)	12 (54.5%)	NS
Ever hosp. due to TN allergy	5 (5.7%)	1 (3.4%)	2 (5.4%)	2 (9.1%)	NS

Values are presented as number (%); †: mean±standard deviation; AAI, Adrenaline auto-injector; NS, non-significant; TN, Tree nut.

* Post-hoc analysis performed with Bonferroni correction, shows statistically significant difference between 0-3 and 4-6 age group (p = 0.004).

** Post-hoc analysis performed with Bonferroni correction, shows statistically significant difference between 0-3 age and 4-6 age group (p = 0.008).

Food allergy related quality of life

The mean FAQLQ-PF score of the study group was 3.55±1.34, and there was no statistically significant difference between age groups (0-3 years: 3.15±1.28, 4-6 years: 3.76±1.42, 7-12 years: 3.73±1.19; Table II) and parents' gender (3.61±1.33 for mothers vs 3.23±1.42 for fathers; p=0.32).

The scores for children with a hazelnut allergy (p= 0.049), with a previous history of tree nut-induced anaphylaxis (p=0.008), with a history of allergic rhinitis (p=0.008), and those who had to use an AAI (p=0.005) were significantly higher than those without these conditions (Table II).

State-trait anxiety inventory

The STAI scores, categorized by the child's age groups, are presented in the Table III. Specifically, 55.6% reported mild, 38.6% moderate, and 5.6% severe state anxiety. In addition, 38.6% reported mild, 57.9% moderate, and 3.4% reported severe trait anxiety. The mean STAI-S and STAI-T scores in fathers (36.40±11.54 and 40.07±8.43, respectively) were numerically lower than those in mothers (38.66±13.37 and 43.15±10.31, respectively), but the differences were not

Table II. Total and subscale scores of FAQLQ-PF according to study variables.

	Total score (mean ± SD)	p	Emotional impact (mean ± SD)	p	Food anxiety (mean ± SD)	p	Social & dietary limitation (mean ± SD)	p
Sex		0.153		0.057		0.58		0.169
Male	3.70±1.39		2.78±1.12		3.97±1.33		3.06±1.74	
Female	3.27±1.20		3.37±1.47		4.15±1.59		3.59±1.65	
Age groups		0.148*		0.056*		0.001*		0.700*
0-3 yr	3.15±1.28		2.67±1.26		3.22±1.66		3.56±1.61	
4-6 yr	3.76±1.42		3.34±1.48		4.50±1.27		3.42±1.86	
7-12 yr	3.37±1.19		3.51±1.21		4.52±1.16		3.16±1.54	
TN allergy		0.833		0.686		0.557		0.728
Multiple	3.57±1.37		3.19±1.41		4.14±1.53		3.37±1.75	
Single	3.49±1.24		3.05±1.28		3.91±1.42		3.52±1.52	
TN allergy								
Hazelnut (+ / -)	3.72±1.32 / 3.09±1.031	0.049	3.34±1.38 / 2.68±1.28	0.043	4.25±1.48 / 3.63±1.47	0.084	3.57±1.67 / 2.96±1.71	0.140
Cashew (+ / -)	3.37±1.30 / 3.85±1.36	0.107	3.01±1.33 / 3.43±1.44	0.175	3.95±1.55 / 4.32±1.39	0.265	3.17±1.75 / 3.82±1.54	0.085
Pistachio (+ / -)	3.35±1.31 / 3.83±1.35	0.099	3.01±1.36 / 3.37±1.39	0.220	3.96±1.49 / 4.26±1.50	0.361	3.08±1.79 / 3.85±1.46	0.035
Walnut (+ / -)	3.74±1.39 / 3.31±1.24	0.138	3.35±1.43 / 2.92±1.30	0.151	4.15±.1.57 / 4.00±1.41	0.627	3.71±1.75 / 3.01±1.56	0.051
Almond (+ / -)	3.19±1.17 / 3.69±1.38	0.119	2.75±1.08 / 3.32±1.46	0.079	3.62±1.57 / 4.27±1.44	0.081	3.22±1.75 / 3.48±1.68	0.530
Ever anaphylaxis		0.008		0.050		0.008		0.114
Yes	3.88±1.34		3.53±1.36		4.46±1.45		3.66±1.71	
No	3.13±1.23		2.70±1.27		3.61±1.44		3.08±1.64	
Having AAI		0.633		0.923		0.285		0.911
Yes	3.58±1.39		3.17±1.44		4.16±1.55		3.41±1.69	
No	3.39±1.04		3.13±1.05		3.69±1.13		3.36±1.77	
Use AAI		0.005		0.023		0.025		0.023
Yes	4.30±0.83		3.72±0.77		4.99±0.86		4.21±1.18	
No	3.41±1.41		3.04±1.50		3.95±1.58		3.25±1.73	

AAI, Adrenaline auto-injector; Ed, Emergency department; SD, standard deviation; TN, Tree nut.
†ANOVA test; *In the post-hoc pairwise comparisons performed with Bonferroni correction, 0-3 age group vs. the 4-6 age group=0.001, 0-3 age group vs. the 7-12 age group=0.004, 4-6 age group vs. 7-12 age group=1.000.

Table II. Continued.

	Total score (mean ± SD)	p	Emotional impact (mean ± SD)	p	Food anxiety (mean ± SD)	p	Social & dietary limitation (mean ± SD)	p
ED admition		0.087		0.308		0.433		0.012
Yes	3.29±1.39		3.00±1.32		3.95±1.64		2.93±1.74	
No	3.78±1.25		3.31±1.43		4.21±1.36		3.84±1.54	
Hospit. due TN		0.362		0.413		0.230		0.626
Yes	4.08±1.44		3.66±1.39		4.75±1.09		3.84±1.95	
No	3.52±1.33		3.13±1.38		4.04±1.51		3.38±1.69	
Asthma		0.297		0.449		0.111		0.654
Yes	3.22±1.41		2.91±1.48		3.52±1.75		3.22±1.41	
No	3.62±1.32		3.21±1.36		4.20±1.43		3.44±1.75	
Allergic rhinitis		0.008		0.030		0.003		0.055
Yes	4.38±0.90		3.86±0.91		5.10±0.79		4.17±1.28	
No	3.38±1.35		3.02±1.42		3.88±1.53		3.24±1.73	
Atopic dermatitis		0.345		0.288		0.818		0.243
Yes	3.21±1.09		2.76±1.28		3.99±1.31		2.87±1.88	
No	3.60±1.37		3.22±1.39		4.10±1.53		3.49±1.66	
Sibling allergy		0.065		0.032		0.211		0.129
Yes	4.07±1.00		3.78±1.13		4.48±1.40		3.95±0.97	
No	3.42±1.38		3.00±1.40		3.98±1.51		3.26±1.81	
Mother's educ.		0.480		0.687		0.332		0.624
<University	3.71±1.20		3.26±1.27		4.33±1.17		3.54±1.63	
≥University	3.49±1.39		3.12±1.43		3.99±1.61		3.35±1.73	

AAI, Adrenaline auto-injector; Ed, Emergency department; SD, standard deviation; TN, Tree nut.
†ANOVA test; *In the post-hoc pairwise comparisons performed with Bonferroni correction, 0-3 age group vs. the 4-6 age group=0.001, 0-3 age group vs. the 7-12 age group=0.004, 4-6 age group vs. 7-12 age group=1.000.

statistically significant (p=0.545 and p=0.281 for STAI-S and STAI-T, respectively).

When the relationships between anxiety scores and baseline demographic and clinical characteristics of the patients were investigated using bivariate tables, there were higher STAI-S scores for the parents who needed to use AAI (p<0.001) and higher STAI-T scores in parents whose child was hospitalized due to TNA (p=0.031, Table III). There was also a strong positive correlation between STAI-S and STAI-T scores (r=0.584; p<0.001).

The relationship between food allergy related quality of life and parental anxiety

When we analyzed the association between STAI scores and the total/subscales of FAQLQ-PF scores using correlations, we found overall significant but weak positive correlations between parental anxiety and QoL, as well as its subscales (Table IV). These findings suggest that a higher level of anxiety in parents is weakly associated with a decrease in the child's QoL from the parents' perspective. Furthermore, when the association between STAI and total FAQLQ scores was analysed using univariate linear regression analyses, the increase in STAI-S (B=0.032; 95% confidence interval [CI]: 0.011-0.053; p=0.003) and STAI-T scores (B=0.037; 95% CI: 0.001 - 0.065; p=0.009) was associated with an increase in total FAQLQ-PF scores (a decrease in QoL). When we look at the correlation between parental anxiety levels and FAQLQ-PF scores, there was a weak positive correlation between mother's STAI-S and FAQLQ-PF scores (r=0.306; p=0.009). Similarly, it turned out to be a weak positive correlation between mother's STAI-T and FAQLQ-PF scores (r=0.241; p=0.040). On the other hand, there was no statistically significant correlation either between father's STAI-S and FAQLQ-PF scores (r=0.310; p=0.261) or STAI-T and FAQLQ-PF scores (r=0.447; p=0.095).

Multivariate linear regression analysis was performed to model the relationship between FAQLQ-PF (dependent variable) and independent variables by assuming a linear relationship between the variables. In the model where STAI-S was included, fathers as primary caregiver (B= -1.035; 95% CI: -1.761 / -0.310; p=0.006), having hazelnut allergy (B =0.717; 95% CI: 0.058 / 1.376; p=0.033), having a history of anaphylaxis (B=0.707; 95% CI: 0.171 / 1.244; p=0.010), and STAI-S scores (B=0.024; 95% CI: 0.004 / 0.044; p=0.019) were significant predictors (Table V). When analysis was repeated by including STAI-T but not STAI-S, similar predictors were depicted except borderline non-significance achieved for STAI-T (Table V). Our findings indicate that considering fathers' viewpoints may yield more favorable assessments of children's QoL. Parents of children with hazelnut allergies and with a history of anaphylaxis tend to report lower QoL for their children. Notably, higher parental state anxiety, as opposed to trait anxiety, is significantly linked to a lower perceived QoL in the child.

Discussion

This study, one of the few to assess QoL in children with TNA[13,14], differs from previous research by incorporating parental anxiety in two domains—state and trait anxiety. While our findings align with earlier studies demonstrating the detrimental impact of negative experiences on QoL[13], they also provide novel insights. First, our data underscore the potential impact of parental state anxiety on reported QoL. Second, the findings suggest that local factors, such as specific nut allergies, may further influence QoL. Additionally, our study indicates that assessing QoL from the father's perspective may yield distinct results, though this issue requires further clarification.

Limited data exist regarding food allergy-related quality of life in children under 12 years old. When comparing our group's total scores with previous studies using the same scale, our FAQLQ-PF scores fall on the lower (negative) side (0–3 years: 3.15±1.28; 4–6 years: 3.76±1.42; 7–12 years: 3.73±1.19).[15,16] These results are consistent with the Turkish validation study

Table III. STAI-S and STAI-T scores according to study variables.

	STAI-S scores	p	STAI-T scores	p
Sex		0.149		0.622
Male	36.79±12.51		43.02±10.67	
Female	41.00±13.75		41.90±8.89	
Prim.Caregivers		0.545		0.281
Mother	38.66±13.37		43.15±10.31	
Father	36.40±11.54		40.07±8.43	
Age groups		0.186*		0.877*
0-3 yr	36.52±10.30		41.86±10.02	
4-6 yr	41.24±15.33		43.14±10.45	
7-12 yr	35.59±11.52		42.77±9.77	
TN allergy		0.801		0.241
Multiple	38.81±12.77		43.31±10.56	
Single	36.45±14.09		40.30±7.83	
TN allergy				
Hazelnut (+/-)	38.36±11.61 / 38.04±16.55	0.920	42.53±10.32 / 42.88±9.46	0.887
Cashew (+/-)	37.89±13.10 / 38.94±13.11	0.720	41.98±10.20 / 43.75±9.81	0.430
Pistachio (+/-)	38.35±13.22 / 38.16±12.96	0.946	42.12±10.49 / 43.32±9.49	0.581
Walnut (+/-)	38.12±11.95 / 38.46±14.44	0.904	43.49±10.74 / 41.54±9.11	0.368
Almond (+/-)	34.08±10.84 / 39.94±13.53	0.057	40.64±10.40 / 43.41±9.87	0.245
Ever anaphylaxis		0.399		0.761
Yes	39.33±14.20		42.92±10.73	
No	36.95±11.45		42.26±9.22	
Having AAI		0.414		0.485
Yes	38.77±13.33		42.30±10.10	
No	35.64±11.42		44.36±9.91	
Use AAI		<0.001		0.318
Yes	51.77±14.76		44.69±11.91	
No	35.59±11.22		41.64±9.57	
ED admition		0.967		0.904
Yes	38.33±12.76		42.76±10.15	
No	38.22±13.43		42.50±10.05	
Hospitalization		0.560		0.031
Yes	41.60±10.99		52.00±5.91	
No	38.07±13.18		42.06±9.98	
Asthma		0.545		0.687
Yes	36.40±14.10		41.67±9.61	
No	38.66±12.88		42.82±10.18	

*ANOVA test; AAI, Adrenaline autoinjector; ED, Emergency department ; STAI, State-Trait anxiety inventory; TN, Tree nut.

Table III. Continued.

	STAI-S scores	p	STAI-T scores	p
Allergic rhinitis		0.899		0.281
Yes	38.67±15.64		40.07±8.86	
No	38.19±12.56		43.15±10.24	
Atopic dermatitis		0.359		0.915
Yes	41.50±11.77		42.33±7.78	
No	37.76±13.23		42.67±10.39	
Sibling allergy		0.375		0.089
Yes	40.72±10.05		46.22±9.29	
No	37.64±13.69		41.70±10.08	
Mother's educ.		0.289		0.446
<University	35.92±10.44		41.32±9.72	
≥University	39.21±13.90		43.14±10.20	

*ANOVA test; AAI, Adrenaline autoinjector; ED, Emergency department ; STAI, State-Trait anxiety inventory; TN, Tree nut.

Table IV. Results of correlations between STAI-State/Trait scores and FAQLQ-PF total/subdimension scores.

	FAQLQ-Total Score		FAQLQ-Emotional Impact		FAQLQ-Food Anxiety		FAQLQ-Social and Dietary Limitations	
	r	p value	r	p value	r	p value	r	p value
STAI-State	0.310	0.003	0.286	0.007	0.238	0.026	0.291	0.006
STAI-Trait	0.278	0.009	0.341	0.001	0.134	0.214	0.264	0.013

FAQLQ, Food Allergy Quality of Life Questionnaire; r, Pearson correlation coefficient; STAI, State-Trait Anxiety Inventory

Table V. Results of multivariate linear regression analysis including FAQLQ total score and STAI scores.

Independent variables	STAI-S included			STAI-T included		
	B	95% CI	p	B	95% CI	p
Primary caregiver (Father/mother)	-1.035	-1.761/-0.310	0.006	-1.035	-1.774 / -0.296	0.007
Age groups	0.240	-0.103/0.582	0.167	0.200	-0.146 / 0.547	0.253
Current TN allergy						
Hazelnut (+/-)	0.717	0.058 /1.376	0.033	0.796	0.132 / 1.460	0.019
Cashew (+/-)	-0.246	-1.287 / 0.796	0.640	-0.332	-1.381 / 0.718	0.531
Pistachio (+/-)	-0.438	-1.462 / 0.586	0.397	-0.286	-1.312 / 0.740	0.580
Walnut (+/-)	0.357	-0.261 / 0.975	0.253	0.275	-0.357 / 0.907	0.389
Almond (+/-)	-0.501	-1.207 / 0.206	0.162	-0.616	-1.315 / 0.083	0.083
Anaphylaxis (+/-)	0.707	0.171 / 1.244	0.010	0.755	0.214 / 1.295	0.007
STAI scores	0.024	0.004 – 0.044	0.019	0.024	-0.001 / 0.050	0.062

CI, Confidental interval; FAQLQ, Food Allergy Quality of Life Questionnaires; STAI, State-Trait Anxiety Inventory; TN, Tree nut.

of the FAQLQ-PF and a recent study from another region in Türkiye[6,17], yet they align with findings from studies conducted in Ireland, the United States, and Thailand, suggesting consistency across diverse populations.[10,18-20] This discrepancy may be attributed to the persistent and severe nature of tree nut allergies and the particular characteristics of our study group.

Our age subgroup analysis revealed that the 0–3 age group had significantly lower rates of adrenaline auto-injector possession (p=0.004) and a lower incidence of anaphylaxis (p=0.008) compared to the 4–6 age group. These findings were anticipated, as adrenaline auto-injectors (0.1 mg) are not available for children under 1 year in Türkiye, and younger children are under strict maternal supervision, reducing the need for auto-injector prescriptions. Notably, most anaphylaxis events in this age group occurred during the first exposure, indicating the onset of an allergy.

Bivariate comparisons demonstrated that FAQLQ-PF scores were lower in children who experienced anaphylaxis and in those who used adrenaline auto-injectors compared to those who did not. This effect was evident across all three components—emotional impact, food anxiety, and social limitations—in children who used adrenaline auto-injectors, whereas in children who experienced anaphylaxis, significant differences were observed only in the emotional impact and food anxiety. We have previously shown that parents are often hesitant to use adrenaline auto-injectors[21]; thus, the observed differences likely reflect not only the experience of anaphylaxis but also the decision-making process regarding auto-injector use. Furthermore, patients with allergic rhinitis exhibited lower FAQLQ-PF scores than those without, which we attribute to their older age and cumulative negative experiences.

Research on parental anxiety in the context of children's food allergies has yielded mixed results.[1,22-26] Some studies report that mothers of food-allergic children experience higher stress and anxiety levels compared to mothers of non-allergic children, while others find no significant differences in anxiety or depression levels between these groups.[27,28] Given that parents play a crucial role in shaping their children's emotional development—through mechanisms such as emotional contagion—understanding parental anxiety is essential.

To our knowledge, only two previous studies have examined the connection between food allergy-related quality of life in children and parental anxiety.[29,30] DunnGalvin et al.'s[29] study, using the GAD-7 questionnaire, reported a significant link between parental general anxiety and FAQLQ in Russian children and adolescents, while Acaster et al.'s[30] study, using the Hospital Anxiety and Depression Scale (HADS), found that parental anxiety significantly predicted a higher burden for peanut-allergic children. Although our study reaffirms the association between parental anxiety and QoL, our findings offer a nuanced perspective by focusing specifically on state anxiety. While state and trait anxiety scores were correlated in our study, the greater predictive capacity of state anxiety provides a deeper understanding of how to enhance the accuracy of QoL assessments in the context of food allergies. Different assessment tools (e.g., STAI, GAD-7, and HADS) measure various dimensions of anxiety; thus, observed disparities may stem from these inherent differences.

We would like to emphasize that it could be argued that using trait anxiety scores in this study provides greater clinical relevance than state anxiety. Nonetheless, in our study, trait and state anxiety scores were correlated, with significant overlap observed in the results of the multivariate analyses. We speculate that the state anxiety experienced by parents may be influenced by the recall of past TNA-related experiences during the completion of the questionnaire.

We also observed that assessing QoL from the father's perspective may yield better insights at multivariate analysis. Whether parental anxiety

differs between mothers and fathers is complex, with studies yielding mixed results.[28,31] Some evidence suggests that mothers experience greater anxiety, particularly regarding their children's health and safety, while fathers may exhibit concerns about other aspects of their children's well-being.[32] Given the low number and limited representation of fathers in our study, we recognize the need for gender-specific studies among parents to validate these findings.

Our study also demonstrated that the presence of a hazelnut allergy negatively impacts FAQLQ, implying that local factors contribute to QoL. In Türkiye, where hazelnuts are a major agricultural product and a staple in the diet, hazelnut allergy is a primary cause of IgE-mediated food allergies and anaphylaxis, heightening parental concerns about accidental exposure.[3-5,33]

Limitations include the lack of a prospective design to document the evolving effect of state anxiety on FAQLQ-PF scores over time and a modest sample size, which may increase the potential for type II errors. Specifically, the limited number of fathers in the study restricts the generalizability of our conclusions to that group. Additionally, participants with lower socioeconomic or cultural backgrounds or those inattentive while completing the questionnaire may have influenced the results—a common issue in questionnaire-based studies. Moreover, it can be placed among limitations that we couldn't include parents who could not be contacted or who did not give informed consent to this study. Nevertheless, to our knowledge this study is the only one exclusively dedicated to TNA in the pediatric population in the Eastern Mediterranean region and represents the first effort to explore various domains of anxiety within this context.

In conclusion, the QoL of children with TNA, as perceived by their parents, is influenced by universal factors such as adverse life experiences, local factors like culinary culture, situational factors such as state anxiety, and potentially parent-specific factors such as gender. Understanding these multifaceted influences is crucial for identifying the predictors of food allergy related quality of life in children and ultimately enhancing their well-being.

Ethical approval

The study protocol was reviewed and approved by the Institutional Review Board of Hacettepe University (date: 15.06.2021, number: 21/745) and those who gave informed consent for the study completed the questionnaire.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: ZGO, BES; data collection: AA, UMS, OUS; analysis and interpretation of results: ZGO, BES; draft manuscript preparation: ZGO, BES. All authors reviewed the results and approved the final version of the article.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Diagnostic persistence, autistic traits, and resilience in youth and adolescents with attention deficit hyperactivity disorder

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ABSTRACT

Objective: This study aimed to determine whether children diagnosed with attention deficit hyperactivity disorder (ADHD) continue to receive this diagnosis during adolescence and young adulthood, and to examine the relationships between autistic traits, psychological resilience, emotion regulation levels, and the continuity of diagnosis.

Methods: In the initial evaluations conducted between 2012 and 2013, 121 children diagnosed with ADHD began medication treatment. From this group, 20 participants aged 13 to 25 who agreed to participate in the second evaluation, conducted between 2020 and 2022, were included in this study. The presence of ADHD in their second evaluation was determined using the DSM-5 criteria. Psychiatric comorbidities in adolescents were screened using the Schedule for Affective Disorders and Schizophrenia for School-Age Children - Present and Lifetime Version, 2016 Turkish Adaptation of the DSM-5, while for adults, the DSM-5 criteria were utilized. Parents completed the Social Responsiveness Scale and the Family Assessment Device, while the young participants completed the Child and Youth Resilience Measure and the Difficulties in Emotion Regulation Scale.

Results: Among the participants, 10 individuals (50%) continued to have an ADHD diagnosis, exhibiting lower psychological resilience and significantly more autistic traits. No difference in emotional dysregulation was observed between those with and without an ADHD diagnosis, and a negative relationship between autistic traits and psychological resilience was identified.

Conclusion: The continuity of an ADHD diagnosis during adolescence and young adulthood may be associated with psychological resilience and autistic traits. However, the limited number of participants and the cross-sectional design highlight the need for larger longitudinal studies to further explore the cause-and-effect relationships.

Key words: adolescence, attention deficit hyperactivity disorder, autism spectrum disorder, emotion regulation, psychological resilience.

Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder that begins in childhood and is characterized by symptoms of inattention, hyperactivity, and impulsivity.[1] It is estimated that ADHD affects 5.9% of adolescents and 2.5% of adults.[2,3] Studies indicate that 60-85% of children diagnosed with ADHD continue to exhibit symptoms during adolescence.[4] Additionally, research on the prevalence of ADHD in adulthood has

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produced varying findings, with a reported persistence rate ranging from 5% to 75%.[5]

Factors associated with the continuity of an ADHD diagnosis during adolescence and young adulthood include familiarity with ADHD, psychosocial adversity, comorbidity with conduct, mood, and anxiety disorders, parental conflict, childhood sexual assault, lower educational attainment, income loss, severity of ADHD, family history of psychopathology, family and school functioning, behavioral impairment, lower intelligence quotient (IQ), and cyclothymic, irritable, or anxious temperament.[6-12]

Autistic traits, which encompass social deficits, communication challenges, and repetitive behaviors that do not meet the criteria for a diagnosis of autism spectrum disorder (ASD), are present in approximately 30% of children diagnosed with ADHD.[13,14] These children often exhibit a more severe form of ADHD compared to those without autistic traits. Furthermore, they experience greater impairments in functional areas such as academics, activities, and social interactions compared to their peers without autistic traits.[15-18]

Emotion regulation refers to an individual's ability to adjust their emotional state in an adaptive and goal-directed manner.[19] Recent studies emphasize that difficulties in emotion regulation are a fundamental component of the ADHD diagnosis.[20] In cases where ADHD is accompanied by deficient emotional self-regulation from childhood through adolescence, higher rates of psychiatric comorbidities, particularly oppositional defiant disorder (ODD), persistence of ADHD, and social problems, along with functional impairments, have been observed during follow-up.[21]

Psychological resilience is the capacity to effectively cope with, adapt to, or manage stress and challenging circumstances.[22] Research has shown that psychological resilience tends to be weaker in adolescents and young adults diagnosed with ADHD compared to those without the diagnosis.[23,24] Emerging adults with ADHD identify several important resilience factors, including strategies for managing ADHD, supportive relationships, acceptance, a positive perception of their ADHD, tailored non-stigmatizing support, and engagement in meaningful activities.[25] Better psychological resilience among adolescents with ADHD is correlated with improved psychosocial functioning and a lower incidence of depression and anxiety disorders in young adulthood.[26]

Upon reviewing the literature, while various studies indicate that difficulties in emotion regulation are associated with the persistence of ADHD diagnoses during adolescence[21,27], other research has shown that autistic traits exacerbate the clinical presentation of ADHD, increase comorbidities, and lead to poorer functioning.[15-17] A longitudinal study conducted with a population sample indicated that autistic traits and ADHD traits are reciprocal, often decreasing, persisting, or emerging together over time.[28] However, to the best of our knowledge, no research has specifically examined the impact of autistic traits on the continuity of ADHD diagnoses, nor have studies investigated the effect of psychological resilience on ADHD diagnosis continuity.

Considering the negative impact of autistic traits and emotion dysregulation on ADHD's clinical presentation, we aimed to investigate the relationship between the continuity of the ADHD diagnosis in adolescents and adults diagnosed in childhood and these variables. Additionally, we sought to explore the relationship between psychological resilience and the persistence of the ADHD diagnosis, emphasizing the need to assess both negative and positive traits in psychiatric evaluations. This study, utilizing a cross-sectional design due to the limited sample size, focuses on the continuation of the ADHD diagnosis as individuals transition from childhood to adolescence and young adulthood, particularly regarding autistic traits, psychological resilience, and emotion regulation.

We hypothesized that psychological resilience will be weaker, problems with emotional regulations will be more pronounced, autistic traits and clinical deterioration will be more common in the group with an ongoing ADHD diagnosis.

Materials and Methods

Participants

The participants in this study consisted of individuals who were included in the research conducted by Ünal et al. between 2012 and 2013 , during which they were diagnosed with ADHD based on assessments performed at that time and began medication treatment while aged 6 to 18 years.[29] These individuals were contacted again by phone between January 2020 and December 2022, invited to the Department of Child and Adolescent Psychiatry at Hacettepe University, and those who volunteered to participate were included in the study after it was confirmed that they met the inclusion criteria during psychiatric evaluations.

Participants with intellectual disabilities, anxiety disorders, mood disorders, ASD, psychotic disorders, substance use disorders, chronic illnesses, and neurological conditions were excluded from the study during the initial assessments in childhood. However, children with specific learning disorders, ODD, and conduct disorder (CD) comorbidities were included in the study. In our current study, participants with chronic and neurological conditions, ASD, intellectual disabilities, psychotic disorders, and substance use disorders were excluded.

Out of the 121 individuals targeted for participation, 35 could not be reached, 2 were diagnosed with ASD, and 64 chose not to participate for various reasons (such as work commitments, residing in another city, pandemic conditions, or unwillingness to volunteer). As a result, 20 participants (3 aged 18, 1 aged 25, and the remaining participants under 18) with ages ranging from 13 to 25 years (7 females and 13 males) were included in our study. The flow of the study sample formation is illustrated in Fig. 1.

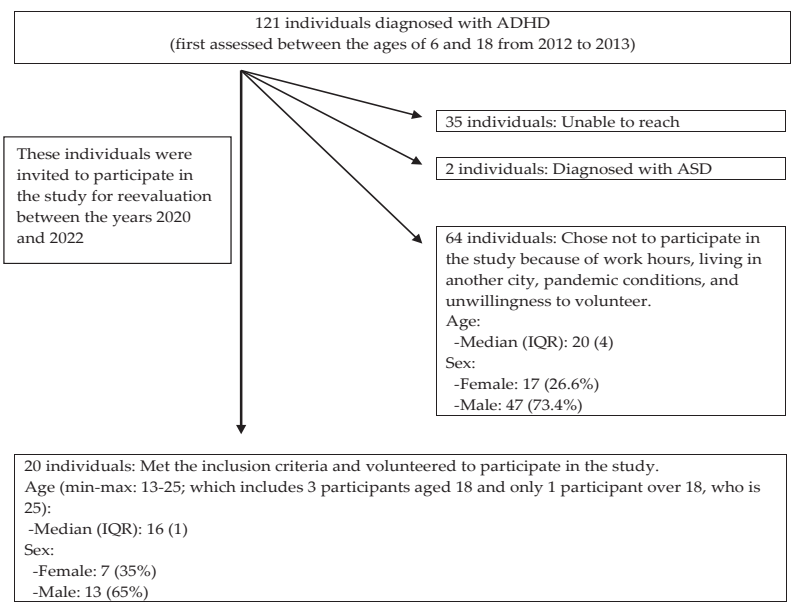

Fig. 1. Flowchart of the study sample.

ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; IQR, interquartile range.

If the participants were under 18 years of age, written consent was obtained from both themselves and their parents; if they were over 18, consent was obtained from the participants themselves.

Ethics approval

The ethical approval for this study was granted by the Hacettepe University Non-Interventional Clinical Research Ethics Committee on November 5, 2019, with decision number 2019/26-36.

Materials

In our study, the ongoing presence of an ADHD diagnosis among participants was determined according to DSM-5 criteria.[1] For participants under the age of 18, the presence of additional psychiatric comorbidities and an ASD diagnosis was assessed using the Schedule for Affective Disorders and Schizophrenia for School-Age Children - Present and Lifetime Version, 2016 Turkish Adaptation of the DSM-5 (K-SADS-PL-DSM-5-T), administered by a child and adolescent psychiatrist. For participants aged 18 and over, all diagnoses available in the K-SADS were screened during the psychiatric evaluation based on DSM-5 criteria.

The sociodemographic information form, prepared by the researchers, and the clinical information form, which inquired about ADHD-related clinical information and psychosocial stressors, were completed by the parents. All of the scales described below were completed by the participants during their second evaluations in adolescence and young adulthood. The scales used in the study are described below:

Schedule for Affective Disorders and Schizophrenia for School-Age Children - Present and Lifetime Version, DSM-5 - Turkish Adaptation (K-SADS-PL-DSM-5-T): This semi-structured interview, developed by Kaufman et al.[30] and adapted in Turkish by Ünal et al.[31], assesses ADHD and comorbid disorders in children and adolescents. It was utilized to evaluate the continuity of the ADHD diagnosis. This interview tool was used with participants under the age of 18.

Social Responsiveness Scale (SRS): This 65-item scale evaluates autism-like symptoms in children aged 4 to 18. Developed by Constantino et al.[32] and adapted by Ünal et al.[33], it was used to compare autistic traits between participants with and without an ADHD diagnosis. This scale was completed by the parents of all participants.

Child and Youth Resilience Measure (CYRM-12): Developed by Liebenberg et al.[34], this 12-item scale measures resilience in individuals over 10 years old. The Turkish validity and reliability study was conducted by Arslan.[35] This scale assessed the relationship between ADHD diagnosis and resilience levels and was completed based on self-report by all participants in our study.

Difficulties in Emotion Regulation Scale (DERS): A 36-item scale designed for individuals aged 18 and older, developed to measure difficulties in emotion regulation.[36] The Turkish validation was completed by Rugancı and Gençöz.[37] This scale was used to investigate emotion regulation difficulties in participants with and without a persistent ADHD diagnosis and was completed based on self-reports by all participants in our study. Due to the majority of our sample being comprised of participants under the age of 18, the necessity of using this scale with this age group has been noted as a limitation.

Family Assessment Device (FAD): This scale, utilizing the McMaster Family Functioning Model, assesses family structure and functionality.[38] The Turkish validity and reliability study was conducted by Bulut.[39] It was utilized to examine the relationship between the continuity of the ADHD diagnosis and family functioning. The scale was completed by all participants.

Hollingshead-Redlich Scale: This scale measures family socioeconomic and sociocultural status.[40] It was completed based on information obtained from the family by the researcher.

Clinical Global Impression Scale (CGI): Developed to assess psychiatric disorders across all ages, this scale includes sections for illness severity, global improvement, and efficacy index.[41] It was used to determine the severity of ADHD-related problems and was completed based on the clinical assessment conducted by the researcher.

Global Assessment Scale (GAS): A clinician-administered scale assessing functionality, with scores ranging from 0 to 100, used to determine the participants' levels of functioning over the past week.[42] This scale was completed following the clinical assessment conducted by the researcher.

Statistical analysis

The Statistical Program for Social Sciences (SPSS) version 23.0 was used for data analysis. The normality of numerical variables was assessed using the Shapiro-Wilk goodness-of-fit test, while the homogeneity of variance was examined with Levene's test. The independent two-sample t-test was employed for normally distributed variables, and the Mann-Whitney U test was used for those that were not normally distributed. Categorical variables are presented as numbers and percentages, and Pearson's chi-square test and Fisher's exact test were utilized for analysis based on expected frequencies. Spearman's correlation test was employed to assess relationships between two quantitative variables. The significance level was set at $p < 0.05$, and effect sizes were computed for each statistical test to determine clinical relevance.

Results

Sociodemographic and clinical characteristics of participants

The sociodemographic characteristics of the participants, the status of the ongoing ADHD diagnosis, and other clinical variables are presented in Table I. Information regarding psychiatric comorbidities are included in Table II.

Table I. Sociodemographic and clinical characteristics of participants.

Variable	N=20
	Median (IQR)
Age	16 (1)
Socio-economic level	3 (0)
CGI disease severity score	3 (2)
CGI improvement score	2 (1.75)
	Mean ± SD
GAS score	73.60 ± 13.50
	N (%)
Sex	
Female	7 (35%)
Male	13 (65%)
Mother's education level	
Below high school	9 (45%)
High school and above	11 (55%)
Father's education level	
Below high school	4 (20%)
High school and above	16 (80%)
ADHD diagnosis	
Yes	10 (50%)
No	10 (50%)
ADHD treatment	
Continues treatment	4 (20%)
Stopped treatment	16 (80%)
Psychiatric treatment in the last six months	
Yes	5 (25%)
No	15 (75%)
Psychosocial stressor	
Yes	8 (44.4%)
No	10 (55.6%)
Self-harming behavior	
Yes	4 (25%)
No	12 (75%)
Suicide plan/attempt	
Yes	3 (19%)
No	13 (81%)
Forensic case history	
Yes	4 (20%)
No	16 (80%)

For those to which the t-test was applied, the Mean ± Standard Deviation notation was used. For those to which the Mann-Whitney U test was applied, the Median (Interquartile Range) notation was used. The percentages given in the table are column percentages.
ADHD, attention deficit hyperactivity disorder; CGI, Clinical Global Impression Scale; GAS, Global Assessment Scale.

Table II. Psychiatric comorbidities of participants, N (%).

Variable	N=20
Depression	
None	16 (80%)
Threshold/subthreshold	4 (20%)
Social anxiety disorder	
None	14 (70%)
Threshold/subthreshold	6 (30%)
Generalized anxiety disorder	
None	17 (85%)
Threshold/subthreshold	3 (15%)
Specific phobia	
None	14 (70%)
Threshold/subthreshold	6 (30%)
Tic disorder	
None	18 (90%)
Threshold/subthreshold	2 (10%)
Obsessive-compulsive disorder	
None	15 (75%)
Threshold/subthreshold	5 (25%)
Panic disorder	
None	19 (95%)
Threshold/subthreshold	1 (5%)

The percentages given in the table are column percentages.

Comparison of groups with and without ongoing ADHD diagnosis

In the group still diagnosed with ADHD, the GAS score was significantly lower, indicating poorer functionality. In this group, CGI scores for disease severity and improvement were significantly higher, suggesting greater current severity and less improvement compared to the past. The Social Responsiveness Scale (SRS) score was also significantly higher, reflecting a greater presence of autistic traits, while the CYRM-12 score was significantly lower, indicating reduced resilience. There were no significant differences between the two groups in terms of the DERS and FAD scores (Table III).

A negative correlation was found across the entire group between psychological resilience and autistic traits ($r = -0.530$, $p < 0.05$), and psychological resilience also negatively related to lack of emotional clarity and difficulty in goal-directed behavior (DERS subscales) ($r = -0.669$, $p < 0.05$; $r = -0.583$, $p < 0.05$; Table IV). Additionally, there was no significant difference between the two groups regarding comorbid psychiatric disorders (Supplementary Table S1).

There were no significant differences between the groups continuing to receive an ADHD diagnosis and those who did not, regarding the following variables: sociodemographic characteristics, prenatal/perinatal problems, developmental features, psychiatric treatment history, and familial and psychosocial stressors (Supplementary Table S2).

Discussion

This study aimed to determine whether adolescents and young adults who were initially diagnosed with ADHD during childhood and started on medication continue to meet the ADHD diagnosis, and to compare current adolescents and young adults with and without an ADHD diagnosis in terms of autism-like symptoms, psychological resilience, and emotion regulation levels. However, the inability of a significant portion of individuals over the age of 18 to participate in the study, the limited number of participants, and the likelihood that these individuals are still seeking treatment, as indicated by the median age of participants being 16, suggest that the findings should primarily be considered in the context of adolescent characteristics.

Sixty to eighty-five percent of children diagnosed with ADHD continue to demonstrate symptoms during adolescence.[4] We found that the rate of retaining an ADHD diagnosis from childhood into adolescence is 50%. This rate is consistent with findings reported by other studies in the literature.[43,44] However, the limited number of participants and the heterogeneous distribution in terms of age in our study prevent us from interpreting this finding as the continuation rate of the ADHD diagnosis during adolescence.

Table III. Comparison of the mean ranks of the scale scores of groups with ongoing ADHD diagnosis and those no longer diagnosed with ADHD.

Variable	ADHD (+) (N=10)	ADHD (-) (N=10)	Test statistic	p-value	Effect size
GAS	6.85	14.15	-2.788	0.005*	-0.623
CGI-disease severity	14.33	6.10	-3.402	0.001*	-0.780
CGI-improvement	11.93	8.83	-2.652	0.008*	-0.663
CYRM-12	7.65	13.35	-2.165	0.030*	-0.484
SRS	11.17	5.89	-2.243	0.025*	-0.579
DERS-lack of emotional awareness	9.81	8.28	-0.628	0.530	-0.152
DERS-lack of emotional clarity	11.00	7.22	-1.550	0.121	-0.375
DERS-nonacceptance of emotional responses	10.75	7.44	-1.359	0.174	-0.329
DERS-limited access to emotion regulation strategies	9.69	7.31	-1.001	0.317	-0.250
DERS-impulse control difficulties	10.44	7.72	-1.108	0.268	-0.268
DERS-difficulty engaging in goal-directed behavior	10.43	7.00	-1.432	0.152	-0.358
FAD-problem solving	7.81	10.06	-0.922	0.356	-0.223
FAD-communication	9.75	8.33	-0.583	0.560	-0.141
FAD-role	9.25	8.78	-0.193	0.847	-0.046
FAD-affective responsive	9.38	8.67	-0.296	0.767	-0.071
FAD-affective involvement	9.50	8.56	-0.389	0.697	-0.094
FAD-behavioral control	10.06	8.06	-0.831	0.406	-0.201
FAD-general functionality	7.75	10.11	-0.975	0.329	-0.236

p-value was obtained using the Mann-Whitney U test.
*p<0,05
ADHD: attention deficit hyperactivity disorder, CGI: Clinical Global Impression Scale, CYRM-12: Child and Youth Resilience Measure-12, DERS: Difficulties in Emotion Regulation Scale, FAD: Family Assessment Device, GAS: Global Assessment Scale, SRS: Social Responsiveness Scale

Since the participants consisted of individuals who were diagnosed and began treatment during childhood (even though we found the treatment continuation rate to be 20%), and given that ADHD treatment is known to reduce symptom burden in adolescence and young adulthood[45,46], this rate might have been higher if examined in a population that had never received treatment.

In our study, the most common threshold and subthreshold psychiatric comorbidities in young people diagnosed with ADHD were depression, anxiety disorders, and obsessive-compulsive disorder. This finding aligns with previous studies suggesting that these conditions are frequent among youth and adults with ADHD.[47] Those diagnosed with depression and anxiety disorders were excluded during the initial evaluation; however, these comorbidities were still detected at high rates in our sample. Surprisingly, no cases of conduct or substance use disorders were found. This may be due to the study sample not living in a risky social environment and beginning ADHD treatment at an early age. Adolescents who participated in the study may have been more willing to complete time-consuming activities, such as scales and psychiatric interviews, while seeking help, which could explain the higher prevalence of internalizing disorders compared to disruptive disorders in our sample. If those who declined or were unreachable had been included, conduct disorder, substance use disorder, or academic and social issues might have been more apparent. Nonetheless, 10% of participants failed a class, 20% experienced peer bullying, 20% were involved in criminal

Table IV. Correlation test results between participants' SRS, CYRM-12, and DERS scale scores.

	SRS	CYRM-12	DERS- lack of emotional clarity	DERS- lack of emotional awareness	DERS- nonacceptance of emotional responses	DERS- limited access to emotion regulation strategies	DERS- impulse control difficulties	DERS- difficulty engaging in goal-directed behavior
SRS								
CYRM-12	-0.530*							
DERS- lack of emotional clarity	0.205	-0.669*						
DERS- lack of emotional awareness	0.185	-0.229	0.532*					
DERS- nonacceptance of emotional responses	0.050	-0.116	0.242	-0.245				
DERS- limited access to emotion regulation strategies	0.187	-0.164	0.282	-0.013	0.790*			
DERS- impulse control difficulties	0.111	-0.290	0.443	0.073	0.870*	0.732*		
DERS- difficulty engaging in goal-directed behavior	0.369	-0.583*	0.522*	0.026	0.584*	0.465	0.620*	

Spearman correlation test was performed.
*Correlation is significant at the p<0.05 level.
CYRM-12: Child and Youth Resilience Measure-12, DERS: Difficulties in Emotion Regulation Scale, SRS: Social Responsiveness Scale

cases, 25% had a history of self-harm, and 19% reported suicide plans or attempts. This indicates that some participants faced impairments in their individual, relational, educational, and social functioning despite the absence of conduct or substance abuse disorders. The attention and impulse control deficits in individuals diagnosed with ADHD can lead to deteriorating social skills, academic failure, accidents, antisocial behavior, and occupational and relational problems, even without comorbid diagnoses.[48,49]

In our study, we found that the group still meeting the ADHD diagnosis during adolescence exhibited significantly higher levels of autistic traits. Previous studies have shown that features of autism are associated with a worse clinical course in patients diagnosed with ADHD, and children diagnosed with both ADHD and autistic traits continued to exhibit these traits in their ten-year follow-up. They experienced more problems in interpersonal relationships as well as in educational and neurocognitive areas, and they had a higher burden of early-onset psychopathology.[16,17] To the best of our knowledge, no study has specifically investigated the direct effect of autistic traits on the continuation of an ADHD diagnosis. While the limitations of our study's methodology and its cross-sectional nature prevent us from making definitive interpretations, examining this issue in larger samples and through longitudinal designs could help fill the existing gap in the literature.

We found that the level of psychological resilience was significantly higher in the group that no longer met the ADHD diagnosis during adolescence compared to the group that still had the diagnosis. While some studies examining resilience in children diagnosed with ADHD found no significant differences in resilience between children diagnosed with ADHD and controls[50,51], others suggested that resilience is lower in youth diagnosed with ADHD compared to controls[23,26]. The differing results may be attributed to the multidimensional nature of resilience, which includes individual, familial, and social-environmental factors. Resilience involves having multiple skills to cope with challenges.[52] In our study, the lower

resilience observed in the ADHD group may be related to the negative impact of ADHD on cognitive factors, self-regulation skills, peer relationships, and family functioning. Studies with larger samples and investigations of these multidimensional resilience factors are needed to shed light on this issue.

We found a significant negative relationship between resilience and autistic traits in all participants. To the best of our knowledge, there is currently no study examining the relationship between autistic traits and resilience. However, it has been shown that young people with autistic traits experience significant impairments in their social functioning and quality of life.[53,54] Considering the multidimensional nature of resilience, it can be anticipated that young people with autistic traits may be more vulnerable in terms of resilience due to their psychosocial challenges. In our study, the lower resilience observed in the group with a continuing diagnosis of ADHD may be associated with the higher prevalence of autistic traits in this group. In the correlation analysis conducted with all participants, we found a negative relationship between resilience and autistic traits. However, further analytical methods are needed to determine whether the lower psychological resilience in individuals with ADHD is related to the core symptoms of ADHD itself or is influenced by autistic traits. Unfortunately, we could not perform this analysis due to the limited sample size per group for examining the relationship between autistic traits and resilience in both groups with and without a diagnosis. It is recommended that this gap in the literature regarding the relationship between autistic traits and resilience be investigated in studies with larger samples.

We also found a significant negative relationship between resilience and both lack of emotional clarity and difficulty in engaging in goal-directed behavior. However, we did not find a significant relationship between resilience and variables such as lack of emotional awareness, non-acceptance of emotional responses, limited access to emotion regulation strategies, and impulse control difficulties, which are other sub-dimensions of emotional regulation difficulties. Based on this finding, it can be concluded that resilience and emotion regulation are related but distinct concepts, with resilience encompassing a broader scope that includes emotion regulation.[55]

We did not find any statistically significant differences in emotion regulation skills between the groups that met the ADHD diagnosis during adolescence and those that did not. Emotional dysregulation in children with ADHD is linked to a higher incidence of psychiatric comorbidities, poorer social functioning, and persistence of ADHD.[21] Our inability to find differences in emotion regulation skills may be due to improved emotional control from childhood treatment and ongoing neurodevelopment in adolescence.[56,57] Studies have shown that psychostimulants reduce emotional lability and irritability in children[58,59], and similar beneficial effects are observed in adults.[60,61] However, it is important to note this limitation, as the scale measuring emotion regulation difficulties is primarily intended for adults. Additionally, the limited sample size may have contributed to our inability to detect differences between the groups with and without a diagnosis.

Our study has methodological limitations. We did not anticipate a low number of participants over the age of 18, which led to applying the DERS, typically for adults, to adolescents. Additionally, the study coincided with the pandemic, resulting in heterogeneous findings as some participants were evaluated before and others after the pandemic. Finally, the small sample size intended for a longitudinal study resulted in a cross-sectional design, limiting our ability to use advanced analytical techniques and necessitating caution in interpreting both significant and non-significant results.

Despite this limitation, the thorough evaluations of participants in both initial and follow-up assessments, the use of a semi-structured interview to identify comorbidities,

and the investigation of possible psychosocial challenges are key strengths of our study. While the number of participants was small, they were comprehensively examined regarding various psychosocial and clinical variables. Furthermore, although this study followed a cross-sectional design, the lack of existing research on the relationship between autistic traits, psychological resilience, and the continuity of the ADHD diagnosis suggests that our study lays a foundation for establishing cause-and-effect relationships in future larger-sample longitudinal studies.

This study found that half of the adolescents diagnosed with ADHD in childhood and treated still met the criteria for an ADHD diagnosis. These adolescents exhibited more autistic traits and lower psychological resilience compared to those who no longer met the diagnosis. No significant differences were found in emotion regulation skills. Additionally, significant negative relationships were observed between autistic traits and psychological resilience, as well as between resilience and certain emotion regulation difficulties. These results should be interpreted with caution due to the limited sample size and cross-sectional nature of the study. Future follow-up studies with larger samples will enhance our understanding of the factors affecting the persistence of ADHD diagnosis.

Supplementary materials

Supplementary materials for this article are available online at https://doi.org/10.24953/turkjpediatr.2025.5460

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Ethical approval

The study was approved by Non-Interventional Clinical Studies Ethics Committee of Hacettepe University (date: 05.11.2019, number: 2019/26-36).

Author contribution

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

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Cornell Assessment of Pediatric Delirium: Turkish translation and validation

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ABSTRACT

Background: Hypoactive delirium may go unrecognized unless routinely screened. At present, there is no valid screening tool for delirium in the Turkish language. This study was conducted to translate the Cornell Assessment of Pediatric Delirium (CAPD) into Turkish and to evaluate its validity and reliability.

Methods: In this is validation study, CAPD assessments were conducted by pediatric intensive care unit nurses and compared with assessments by a child psychiatrist.

Results: A total of 76 patients were included, 37 participants (48.6%) were younger than 24 months, and 22 participants (28.9%) had developmental disabilities. Prevalence of delirium was 25.0% (n=19). Inter-rater agreement for the identification of delirium by psychiatrists was strong and reliable, with a Cohen's kappa value of 0.86 (95% confidence interval [CI]: 0.72-0.99). Inter-rater reliability for nurses was also significant, with a Cohen's kappa of 0.74 (95% CI, 0.57-0.91). Inter-rater reliability ranged from 0.64 to 0.84 for each CAPD item except item 6, indicating reliable scoring. Sensitivity and specificity improved when the CAPD cut-off score was increased from 9 (100% and 95%, respectively) to 11 (100% and 98.02%, respectively). Subgroup analyses showed high sensitivity and specificity in patients with developmental delay (96%) and in patients under 2 years of age (96%) when the CAPD cut-off score was 9. However, specificity decreased slightly to 93% in patients under 6 months of age.

Conclusion: The Turkish CAPD, the first delirium screening scale translated into Turkish, has demonstrated validity and reliability in screening for delirium in children of all ages, including those with developmental disabilities.

Key words: delirium, critical care, child, infant, language.

According to DSM-V criteria, delirium refers to an acute onset and fluctuating neurological disturbance involving consciousness and cognition over a brief period of time, and occurs in the presence of an underlying medical condition.[1] The gold standard diagnosis of delirium is psychiatric evaluation. However, psychiatric evaluation is not feasible for

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routine monitoring of delirium in the pediatric intensive care unit (PICU) setting. Therefore, standardized and validated screening tools that enable rapid and reliable screening of patients in real-time are of the utmost importance.

Delirium occurs frequently in critically ill children. Although the prevalence of delirium in PICUs varies with regional and disease variations, an international large-sample study reported that one in four critically ill children had delirium.[2] Studies have shown that pediatric delirium is associated with longer intensive care unit (ICU) stay[3-5], increase in costs, prolonged mechanical ventilation and higher odds of death.[2-9] Therefore, rapid recognition of delirium is important to allow for effective intervention. In recent years, several screening tools have been proposed for children admitted to the PICU, including the Pediatric Confusion Assessment Method for Intensive Care Unit (pCAM-ICU)[10], the Sophia Observation Withdrawal Symptoms-Pediatric Delirium (SOS-PD) scale[11] and the Cornell Assessment for Pediatric Delirium (CAPD).[12] The CAPD is a quick and easy bedside tool based on the nurses' observational assessments during care.[3,13,14] It is designed to recognize all types of delirium for any pediatric age[2,5,15,16], regardless of the presence of developmental disabilities.[5,12,17] Since 2016, the European Society of Pediatric and Neonatal Intensive Care (ESPNIC) has recommended the CAPD as the assessment tool to diagnose delirium among children and infants (grade of recommendation: A).[6] The CAPD has been internationally translated, adapted, and validated into Chinese, Japanese, Portuguese, Spanish, Italian and Danish.[14,18-23] In a nationwide study, 57.9% of PICUs in Türkiye did not use any delirium screening tool and the number of centers where delirium screening was routinely and regularly performed was only three (15.8%).[24]

Currently, there is no validated Turkish screening tool for the diagnosis of pediatric delirium. The primary objectives of this study were to translate the CAPD from English to Turkish, ensure its cross-cultural adaptation, and to analyze the validity and reliability of the Turkish version. The secondary aim was to test the screening efficacy of the Turkish CAPD in specific subgroups, including developmental status and age categories.

Materials and Methods

Description of the CAPD

The CAPD is a screening tool for identifying symptoms of delirium. It consists of eight questions, each scored on a scale of 0 to 4, with responses ranging from 'never' to 'always'. The total score ranges from 0 to 32, with a CAPD score of 9 or higher indicating the presence of delirium. Patients with a score of 9 or higher are categorized as "delirium present," while those with a score below 9 are categorized as "delirium absent." There are anchor points available to be used as a point of reference when scoring the CAPD in children under two years of age.[15]

Translation

The CAPD was translated following the International Society for Pharmacoeconomics and Outcomes Research's (ISPOR) good practice guidelines[25] for translation and cross-cultural adaptation (Fig. 1). The following steps were followed in the process: 1. *Preparation*: Permission was obtained from the developers of the original scale during the translation process. The authors met with the original developers of the CAPD to understand the background and theory and to ensure the correct use and interpretation of the assessment tool. 2. *Forward translation*: The translation from English to Turkish was conducted by a translator familiar with medical terminology. 3. *Back translation*: The Turkish text was translated into English by a translator who is a native English speaker but had no prior knowledge of the CAPD. 4. *Back translation review and harmonization*: The original scale and back translation were compared by the developers. 5. *Cognitive debriefing*: Clinical applicability was reviewed by pediatric

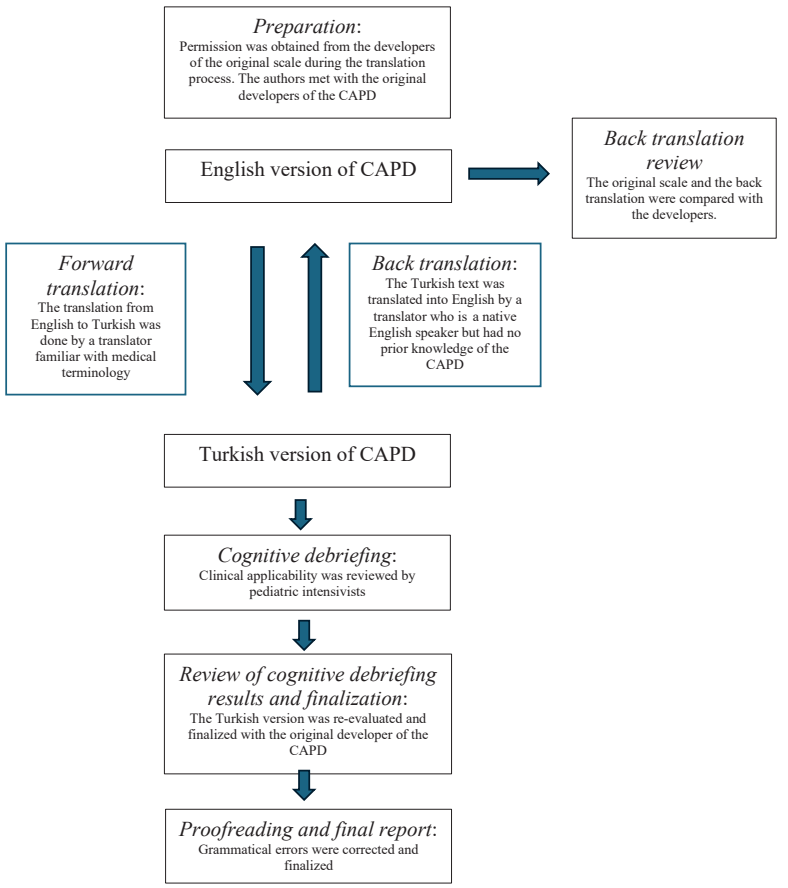

Fig. 1. Translation process.

intensivists. 6. *Review of cognitive debriefing results and finalization*: The Turkish version was re-evaluated with the original author to ensure that it retained its original meaning. 7. *Proofreading and final report*: Grammatical errors were corrected and finalized.

Training

Training was provided by the original author of the CAPD to the PICU specialist leading the study. This specialist then organized training sessions for nurse leaders (a 'train-the-trainers' model). The nurses on the ward were then trained according to the original developer's guidelines, with a maximum of three nurses attending each session. Thereafter, each PICU nurse had to complete three accurate CAPD assessments at the bedside in the presence of the supervisors, in order to be approved for independent delirium screening. Difficulties and questions in the assessment were clarified. Separately, the Child Psychiatry team organized a consensus session. A standardized psychiatric assessment form was created by consensus in accordance with the gold standard DSM-V criteria for pediatric delirium.

Assessing CAPD performance

This validation study was executed in a 32-bed medical-surgical PICU in a major urban academic medical center between November 2021 and August 2022. The study protocol was approved by the Institutional Review Board of Ankara City Hospital (No: E2-21-731, Date: 14.07.2021).

All patients who were admitted to the PICU for any reason and who had been in hospital for more than 48 hours were included in the study. Informed consent forms were obtained from

the families during hospitalization. Patients without informed consent were excluded from the study. Thirty-two beds were numbered and a *'True Random Number Generator'* tool (Randomness and Integrity Services LTD., Dublin, Ireland, www.random.org) was used to assign four bed numbers for each scheduled study day. If the same participant was part of the random selection, the participant had a maximum of four assessments. The definition of "significant clinical developmental delay" is on the basis of clinical evaluation and/or parental report of developmental problems affecting the child's behavior or communication capability. Children with a history of mild or transient developmental problems (e.g., needing occupational therapy or having motor or speech delays) were not included in this category.[12] Demographic and clinical data were recorded on each subject. The patient's level of sedation was assessed with the Richmond Agitation and Sedation Scale (RASS)[26] every four hours. If any of the selected beds had a patient with a RASS score less than -3 for the entire shift (i.e.: unarousable to verbal stimulation), CAPD scoring was not performed. Otherwise, the CAPD scores were assessed twice daily by the bedside nurse (Supplementary Tables S1, S2).

A set of double-blind matched nurse assessments were performed. The bedside nurse completed the CAPD as a paper checklist. Then, the supervisor nurse recorded the CAPD assessment. This was followed by an assessment by 2 blinded psychiatrists. If the child was diagnosed with delirium by either psychiatrist, the healthcare team was notified so that appropriate treatment could be given. Once inter-rater reliability was established, for the remainder of the study each patient had one CAPD and one psychiatric assessment. In our study, the DSM-V criteria were used as the gold standard for confirming the diagnosis of delirium, and the CAPD's validity was evaluated by comparing its results with this diagnostic standard.

Statistics

Patient characteristics including demographics, reasons for admission, and level of sedation were summarized using descriptive statistics. Interrater reliability was quantified using Cohen's κ coefficient.[27,28] The receiver operating characteristic (ROC) analysis was performed to find the optimal CAPD cut-off score; subsequently, sensitivity and specificity were calculated for the overall sample. In addition, in order to explore CAPD performance in subgroups, validity measures were described by age groups and developmental status. All confidence intervals (CI) were adjusted for the possible correlation between observations within subjects using a ratio estimator method.[29,30] Analyses were performed in R version 4.4.3 (R Foundation for Statistical Computing, Vienna, Austria).

Results

A total of 77 patients were enrolled in the study and 120 paired CAPD and psychiatric assessments were completed. One patient did not complete any study assessments. The remaining 76 patients completed at least one paired assessment (CAPD plus psychiatric evaluation). Thirty-eight nurses and three psychiatrists participated in study assessments.

Characteristics of the participants

Among the participants, 42 (55.3%) were male, and 37 (48.6%) were younger than 24 months. The age distribution of the participants is reported in Table I. Additionally, 22 participants (28.9%) had developmental delay. The diagnoses at the time of admission to the PICU and the underlying conditions can also be seen in Table I. Thirty-four participants (44.7%) received noninvasive ventilation, 23 participants (30.3%) received invasive mechanical ventilation, and 4 participants (5.3%) received supplemental oxygen. Fifteen participants (19.7%) were not receiving any

Table I. Biological and clinical characteristics of the study cohort.

Characteristic	n (%)
Gender	
Male	42 (55.4%)
Female	34 (44.7%)
Age	
0-24 mo	37 (48.6%)
2-5 yr	11 (14.5%)
6-12 yr	15 (19.7%)
13-18 yr	13 (17.2%)
Developmental disability[a]	
No	54 (71.1%)
Yes	22 (28.9%)
Respiratory support	
Oxygen	4 (5.3%)
Noninvasive mechanical ventilation	34 (44.7)
Ventilator	23 (30.3)
None	15 (19.7)
Diagnoses[b]	
Cardiac	11 (14.5%)
Genetic disorder	17 (22.4%)
Hematologic/oncologic	11 (14.5%)
Infectious/Pneumonia	24 (31.6%)
Bronchiolitis	14 (18.4%)
Metabolic	6 (7.9%)
Neurologic	14 (18.4%)
Postoperative/other	30 (39.5%)

[a] See text for description of categories. [b] Including all primary and secondary diagnoses

form of respiratory support. In addition, 35 participants (46.1%) received sedation, 18 of whom (51.4%) received dexmedetomidine. The incidence of delirium was 25.0% (n=19 patients) according to the gold standard psychiatric assessment for the diagnosis of delirium. Of these cases, 10 (52.6%) patients had hyperactive delirium, 8 (42.1%) patients had hypoactive delirium, and 1 (5.3%) patient had a mixed type of delirium.

Criterion Standard and CAPD Performance

Inter-rater Reliability

The first 33 psychiatric assessments were performed independently by two psychiatrists, each blinded as to the other's assessment. The concordance between the psychiatrist assessments was excellent, with a strong and reliable agreement (Cohen's Kappa = 0.86, 95% CI: 0.72-0.99). The first 64 CAPD scores were performed independently by two nurses, each blinded as to the other's assessment. The inter-rater reliability of nurses was 0.74 overall (Cohen's Kappa = 0.74, 95% CI, 0.57-0.91). When each CAPD item was assessed individually, the inter-rater reliability ranged from 0.76 to 0.84 (indicating substantial to near-perfect agreement), except for item 6 (Cohen's Kappa = 0.58, 95% CI: 0.39-0.77; Table II).

The sensitivity and specificity for the 120 CAPD assessments were 100% and 95%, respectively, using a cut-off point of 9 or higher, consistent with the original CAPD validation study. In our study, the optimal cut-off that maximizes sensitivity and specificity is a CAPD score of 11. Using this cut-off, sensitivity is 100% and specificity is 98.02% (Supplementary Figure S1).

The gold standard psychiatric diagnosis showed a 96% agreement with the CAPD screening test. Among the participants, there were five false positives (children with a CAPD score of 9 or higher in whom the psychiatrist did not diagnose delirium).

Subgroup analyses of CAPD performance against the gold standard of psychiatric diagnosis showed high sensitivity (100%) and specificity (96%) for patients with developmental delay. CAPD performance showed high sensitivity (100%) and specificity (96%) in participants aged less than 2 years. In infants <6 months of age, sensitivity remained at 100% and specificity decreased slightly (93%).

Discussion

Without routine screening, the diagnosis of delirium – especially in children – is often missed. The absence of a pediatric delirium screening tool in the native language complicates the recognition, prevention, and appropriate treatment of pediatric delirium. Therefore, it is imperative to have a linguistically and culturally appropriate delirium screening tool available for use in Turkish PICUs.

The CAPD is the first screening tool for delirium to be translated into Turkish. This study shows that the Turkish version of the CAPD is both valid and reliable, and results are consistent with the gold-standard psychiatric assessment for delirium. Implementing routine screening for delirium in Turkish PICUs will increase awareness of pediatric delirium, and may reduce morbidity and improve care nationwide.

In many prospective studies, a CAPD score of 9 or above has been used as a definition for delirium.[2,6,12,14,17,18,20,22,31-33] Our data were in agreement with these previous reports, with a sensitivity of 100% and specificity of 95% when using the same cut-off. In this study, we found that a cut-off score of 11 further increased specificity (98.02%) without sacrificing specificity (still 100%). Further studies in Turkish PICUs will be necessary to replicate this finding before changing the CAPD cut-off score.

In practice, the diagnosis of delirium is challenging in extremely young and/or developmentally delayed children.[15] Similar to the English version of the CAPD[12], we demonstrated good validity (100% sensitivity and 96% specificity) in detecting delirium in children younger than 2 years of age, with decreased specificity of the CAPD (93%) in patients younger than 6 months. However, in contrast with the reported literature, we demonstrated excellent specificity (96%) even in children with developmental disabilities. Kaur et al. showed an increase in specificity and positive diagnostic value from 66% to 97% and from 47% to 89%, respectively, when combining CAPD with RASS fluctuations in children with developmental delay compared to CAPD alone.[17] We were aware of this finding when planning our current study and added a footnote to the final version of the Turkish CAPD: *'Fluctuation in RASS values (during at least six hours of follow-up) is a typical finding for delirium'*, along with a checklist for RASS fluctuation (obvious/occasional/never). During the preparatory training of nurses, we stressed the significance of RASS fluctuations as indicative of changes in the patient's level of consciousness. We believe that attention to RASS fluctuations when scoring the CAPD may have contributed to the nurses' ability to identify delirium with high sensitivity and specificity, even in children with developmental disabilities.

Table II. Inter-rater reliability of individual CAPD items.

Item	Cohen's Kappa* (95% CI)
1. Does the child make eye contact with the caregiver?	0.84 (0.84-0.84).
2. Are the child's actions purposeful?	0.76 (0.76-0.76)
3. Is the child aware of his/her surroundings?	0.84 (0.84-0.84)
4. Does the child communicate needs and wants?	0.84 (0.84-0.84)
5. Is the child restless?	0.76 (0.76-0.76)
6. Is the child inconsolable? / *Is it difficult to calm the child?*	0.58 (0.39-0.77)
7. Is the child underactive -very little movement while awake?	0.76 (0.76-0.76)
8. Does it take the child a long time to respond to interactions?	0.64 (0.48-0.80)

*Note for interpretability purposes that a Cohen's Kappa of 0.41-0.60 denotes moderate agreement, 0.61-0.8 denotes substantial agreement, and 0.81-0.99 denotes near perfect agreement.[34]
CAPD: Cornell Assessment of Pediatric Delirium, CI: confidence interval.

The processes of translation and cross-cultural adaptation are complex and challenging. It is important to retain the characteristics of the original version while taking into account the regional use of expressions. The inter-rater reliability of the CAPD scores among nurses was 0.74 (Cohen's Kappa = 0.74, 95% CI, 0.57-0.91). This indicates substantial agreement among nurses overall. For each individual item in the CAPD, inter-rater reliability ranged from 0.64 to 0.84 (substantial to near-perfect agreement), except for item 6 ("Is the child inconsolable?"), which showed only moderate agreement (Cohen's Kappa = 0.58, 95% CI: 0.39-0.77). This is consistent with Hoshino et al.'s Japanese study which showed that item 6 had the lowest inter-rater agreement (Cohen's Kappa 0.67).[22] Japanese and Turkish belong to the same language family (Altaic language family) and the Altaic language family has a different sentence structure than the Euro-Indian language family. As a result, this relationship may be indicative of a translation problem. It is also notable that 4 out of the 5 patients who were falsely identified as delirious by the CAPD were of Syrian origin and did not speak Turkish. This language barrier made it difficult to calm the children. This may have led to decreased interrater reliability of item 6, as only some nurses spoke Arabic. To improve agreement for item 6, a discussion was held with the evaluators and it was suggested that wording for this item should be clarified. In our view, the translation of question 6 should be changed to "*Çocuğu sakinleştirmek zor mu? (Is it difficult to calm the child?)*". This suggested modification may improve agreement between assessors and make communication more effective. We suggest that future studies in Turkish (and other) PICUs should consider evaluating the interrater reliability of item 6 with this alternate wording. Therefore, it is crucial to translate and validate original screening tools into various native languages according to guidelines.

There are some limitations to this study. It was conducted in a single institution. It needs to be replicated in a multi-institutional study. Patients with significant language barrier had a higher false positive rate, reflecting the difficulty in assessing these individuals. Further research is needed to replicate and address the best diagnostic approaches in this population, and the possibility of a higher CAPD cut-off point needs to be investigated in larger studies.

Conclusion

In conclusion, the Turkish version of the CAPD has high sensitivity and specificity compared to the criterion-standard psychiatric assessment. The Turkish CAPD is a valid and reliable screening tool for detecting and monitoring pediatric delirium in children of all ages and developmental stages.

Supplementary materials

Supplementary materials for this article are available online at https://doi.org/10.24953/turkjpediatr.2025.5237

Ethical approval

The study protocol was approved by the Institutional Review Board of Ankara City Hospital (date: 14.07.2021, number: E2-21-731).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: EU, NOK, GD, EA, MO, YM, CT ; data collection: EU, NOK, EA, MO, EK, YM, GD ; analysis and interpretation of results: EU, NOK, EA, MO, EK, YM, GD; draft manuscript preparation: EU, NOK, CT. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Evaluating the reliability and validity of the Turkish version of the Parents' Perceptions of Uncertainty Scale (PPUS-TR)

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ABSTRACT

Background: The disease process can negatively affect both children and their parents, causing them to experience uncertainty. This study aims to determine whether the Turkish version of the Parents' Perceptions of Uncertainty Scale (PPUS) is a valid and reliable instrument for measuring Turkish parents' perceptions of uncertainty.

Methods: Data were collected from 351 parents. Data collection tools included the Descriptive Data Form, PPUS, and the Brief Symptom Inventory (BSI). Language, face, and content validity, descriptive statistics, internal consistency analyses, explanatory and confirmatory factor analyses, and convergent validity analyses were conducted.

Results: The content validity index (CVI) was calculated as 0.96. As a result of the exploratory factor analysis, a four-factor structure with 23 items explaining 57.98% of the total variance was obtained. Confirmatory factor analysis supported the model fit. The Cronbach's alpha coefficient for the final version of the scale was 0.923. Convergent validity showed a significant positive relationship with the BSI (r=0.69).

Conclusions: The Turkish version of PPUS (PPUS-TR) was found to be a valid and reliable measurement tool.

Key words: nursing, parents, pediatrics, uncertainty.

The concept of uncertainty has been defined in healthcare as the inability to determine the meaning of disease-related events, which occurs when the decision-maker cannot assign a precise value to objects or events or predict outcomes accurately.[1] Uncertainty in illness can arise from limited information, unpredictable symptoms, unclear disease progression, inadequate social and healthcare support, and difficulties in understanding or making sense of disease-related developments.[2] Therefore, especially in chronic diseases, the disease is often accompanied by uncertainty, significantly affecting patients' adaptation to the process, quality of life, and disease prognosis.[1,3] So much so that adapting to life during the disease and overcoming its uncertainty is sometimes shown as a more significant source of stress than the disease itself.[4]

The experience of uncertainty negatively affects both child and adult patients, significantly raising anxiety levels and reducing tolerance even in healthy children and adolescents.[5] The uncertainty created by the phenomenon of chronic disease due to its variable nature and unpredictable process also causes similar

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problems in children and adolescents. In two different studies conducted with children with autism[6] and children with attention deficit-hyperactivity disorder[7], it was found that there is a positive relationship between uncertainty and anxiety and that uncertainty intolerance increased the incidence of anxiety disorders. Disease uncertainty has also been associated with a lower quality of life in children diagnosed with cancer.[8] Uncertainty related to chronic illnesses in children and adolescents negatively affects their psychosocial well-being throughout the diagnosis, care, and rehabilitation processes. Similarly, the uncertainty experienced by primary caregivers, particularly parents, also has an adverse impact.

The perception of uncertainty experienced by the parents of pediatric patients was defined by Merle H. Mishel.[9] Mishel states that uncertainty, as a perceptual variable, prevents a precise evaluation of events and limits coping; the ability to resolve uncertainty also affects how well a person can cope with any situation.[1,10] Considering the profound impact of parents' emotional state on their children, particularly during illness, Mishel assumed that parents' ability to navigate the uncertainties of illness could significantly boost their children's confidence and peace of mind.[10] It is stated that parents' perception of uncertainty regarding their child's illness increases the level of uncertainty and stress experienced by the sick child.[11] Even if the treatment outcome is positive, the perception of uncertainty causes families to have difficulty adapting to the process and coping with the current situation.[12] Research has indicated that the perception of uncertainty not only adversely affects parents' mental health but also leads to an increase in their children's levels of uncertainty, resulting in psychological issues.[13-16] In this context, identifying uncertainty in parents of children and adolescents with chronic diseases with valid and reliable methods is essential in terms of protecting the mental health of children and adolescents and their parents, managing the disease process more effectively, and facilitating adaptation to the process.

The Parent Perception of Uncertainty Scale (PPUS)[10] has been widely used for parents' perceptions of uncertainty. The scale has been translated into many languages, such as Arabic, Chinese, and Spanish[17-20], and the versions in these cultures have been found to have high validity and reliability. It has also been determined that the scale has been used in studies conducted with parents of children with many different diagnoses.[21-24] On the other hand, to date there have been no tool that measures the perceptions of uncertainty of parents of children with chronic diseases in Türkiye although the perceptions of uncertainty experienced by this population have been mentioned in some qualitative studies.[25,26] Within the scope of the literature review, a congress abstract was found stating that the psychometric properties of the Turkish version of the PPUS (PPUS-TR) were performed.[27] Still, since the full text of the study was not available, the details of the analyses could not be accessed. Furthermore, no study using this version in Türkiye has been obtained.

The effectiveness of intervention studies relies on identifying specific needs within target populations using valid, reliable, measurable, and repeatable tools. In this context, a valid and reliable measurement is essential for interventions designed to support Turkish parents facing uncertainty about their children's chronic illnesses. Additionally, given the evolution of healthcare systems and cultural shifts since the PPUS was developed, it is necessary to confirm its applicability for assessing parental uncertainty across different cultures in a changing world. Therefore, this study addresses these gaps by evaluating the PPUS's validity and reliability in measuring Turkish parents' perceptions of uncertainty. The aims of our study were to determine the validity and reliability of PPUS-TR and the relationship between PPUS-TR and the Brief Symptom Inventory (BSI) in parents of children with chronic diseases in Türkiye.

Materials and Methods

Study methodology

This study's methodological design was to test the psychometric properties of the Turkish version of the PPUS for parents of children between the ages of 0-18 years with chronic diseases.

Participants

The research population included parents of newborns, children, and adolescents with chronic diseases between the ages of 0-18 years who were receiving inpatient and/or outpatient treatment in Ankara. The research was conducted in the clinics of one university and two state hospitals to ensure a diverse and representative sample. The following clinics were visited in the institutions where approval was obtained to conduct the study: pediatric emergency, pediatric surgery, pediatric intensive care, neonatal intensive care, general pediatrics, hematology, endocrinology, cardiology, cardiovascular surgery, neurology, orthopedics and traumatology, plastic and reconstructive surgery, urology, adolescent ward, and infant ward. Convenience sampling method was chosen to select the sample from the population. The criteria for parents to be included in the study were as follows: 1) having at least one child between the ages of 0-18 years with a chronic disease (such as diabetes, epilepsy, asthma, or cancer), 2) confirming that the child does not have any mental or physical condition that would prevent participation in the study, 3) being able to read and understand Turkish. Parents who did not meet these criteria were excluded from the study.

In scale adaptation studies, the recommendation is to reach at least 8-10 times the number of items[28]; some sources even suggest reaching 200-500 people during the translation and adaptation processes, regardless of the number of items on the scale.[29,30] To align with literature recommendations and achieve ten times the scale items, the target sample size was set at a minimum of 350 participants.

Procedures

The research was conducted per international guidelines on the cross-cultural adaptation of self-report scales.[30,31] First, due to Mishel's death, who had the right to authorize all versions of the scale, the necessary permission was obtained by contacting the institution where Mishel worked when she developed the scale. The scale was translated by two native Turkish speakers proficient in English: one with no clinical background, and the other with an academic specializing in psychiatry/psychology. Both translators received a report addressing complex or ambiguous expressions and word choice justifications. Their translations were synthesized, discrepancies discussed, and merged into a single version. This version was then back-translated by two other translators. All versions were reviewed by 10 psychiatry/psychology experts. A field expert finalized the pre-test version of the scale. After obtaining the necessary permissions from the institutions, the final version of the scale was piloted with 20 parents who met the inclusion criteria. The second researcher regularly visited the institutions from October 2022 to February 2024 until the target number of participants was reached. After explaining the research's purpose and scope, forms were administered face-to-face to parents who verbally and in writing confirmed their willingness to participate. Filling out the scales took approximately 15 minutes. Participants provided feedback on the clarity of scale items and their appearance, but this data was excluded to ensure study rigor. Once face and language validity were confirmed, the scale was administered to 351 parents, completing the research process.

Outcome measures

Descriptive data form: This 13-question form, developed by the researchers based on a literature review[17,32], includes information on participants' age, gender, marital status, education level, income status, knowledge of the diagnosis, and opinion about the severity of the diagnosis, as well as the child's age, gender,

diagnosis, diagnosis time, primary caregivers, and numbers of hospitalizations.

The Parent Perception of Uncertainty Scale (PPUS): Mishel developed PPUS to describe parents' perceptions of uncertainty.[10] PPUS is a 5-point Likert-type scale consisting of 31 items. The scale has four sub-dimensions: "Ambiguity" refers to the lack of clues or uncertainty regarding the planning or execution of the child's care. "Lack of clarity" is related to the lack of clarity regarding receiving or perceiving information about the child's treatment and care system. "Lack of information" refers to the absence of information regarding the diagnosis and severity of the condition. "Unpredictability" includes items related to the inability to make daily or future predictions about symptoms and disease outcomes. While the scale's total Cronbach's alpha value is 0.91, its subscales have Cronbach's alpha values of 0.87, 0.81, 0.73, and 0.72, respectively. The lowest score to be obtained from the scale is 31, the highest score is 155, and an increase in the score indicates that the perception of uncertainty increases.[10]

Brief Symptom Inventory (BSI): The scale, developed by Derogatis[33] as a short form of the 90-item Symptom Checklist (SCL-90-R), allows individuals to evaluate their psychological state across various dimensions and consists of 53 items. The scale has five sub-dimensions: "anxiety", "depression", "negative self", "somatization" and "hostility". BSI is a 5-point Likert-type scale; the total score that can be obtained from the scale varies between 0 and 212. A high total score indicates the frequency of psychological symptoms. The Turkish scale adaptation was conducted in two studies.[34,35] These studies found that the internal consistency coefficient for the entire BSI varied between 0.95 and 0.96, and that of the subscales ranged between 0.55 and 0.86.

Statistical analysis

All validity and reliability analyses were conducted using SPSS 29 and AMOS 29. Participants' characteristics were described with descriptive statistics. Expert opinions were examined with the content validity index (CVI) in the 'language and appearance' validity phase. Correlation coefficients were calculated to assess each item's relationship with its sub-dimension, and corrected item-total correlations were used to minimize random measurement errors.[36] The minimum value for corrected item-total item correlation coefficients was accepted as 0.3. In addition, the anti-image correlation matrix was calculated to determine whether the items were sufficiently related. The coefficients in the diagonal of the anti-image correlation matrix were examined to see whether they were greater than 0.5.[37] The reliability analysis for the sub-dimensions was conducted using Cronbach's alpha coefficient, including item deletion. A half reliability analysis was also performed, by calculating the Spearman-Brown coefficient. The data set's suitability for factor analysis was assessed through the Kaiser-Meyer-Olkin (KMO) value and Bartlett's test. Following these assessments, exploratory factor analysis was performed, and Cronbach's alpha coefficients were recalculated for the new scale structure. The validity of the factor structure was evaluated using confirmatory factor analysis employing the indices given in Table I.

Table I. Criterion ranges of model fit indices.

Fit index	Perfect fit range	Acceptable fit range
$\chi 2/df$	$0 \leq \chi 2/sd \leq 2$	$2 \leq \chi 2/sd \leq 3$
AGFI	$0.90 \leq AGFI \leq 1.00$	$0.85 \leq AGFI \leq 0.90$
GFI	$0.95 \leq GFI \leq 1.00$	$0.90 \leq GFI \leq 0.95$
CFI	$0.95 \leq CFI \leq 1.00$	$0.90 \leq CFI \leq 0.95$
NFI	$0.95 \leq NFI \leq 1.00$	$0.90 \leq NFI \leq 0.95$
RFI	$0.95 \leq RFI \leq 1.00$	$0.90 \leq RFI \leq 0.95$
IFI	$0.95 \leq IFI \leq 1.00$	$0.90 \leq IFI \leq 0.95$
RMSEA	$0.00 \leq RMSEA \leq 0.05$	$0.05 \leq RMSEA \leq 0.08$

AGFI, Adjusted Goodness of Fit Index; CFI, Comparative Fit Index; GFI, Goodness of Fit Index; IFI, Incremental Fit Index; NFI, Normed Fit Index; RFI, Relative Fit Index; RMSEA, Root Mean Square Error of Approximation; $\chi 2/df$, chi-square/degrees of freedom.

Convergent validity was determined through Pearson correlation analysis between the scale's total score and the BSI score, with all analyses conducted at a significance level of 0.05.

Ethical considerations

Ethical approvals were obtained from Hacettepe University Health Sciences Research Ethics Committee, decision number GO22/13-66. Necessary permissions were also obtained from the hospitals, which allowed the research to be conducted.

Results

Sample characteristics

A total of 351 participants were included, with a mean age of 34.1±5.5 years, predominantly consisted of mothers (74.1%). Almost all parents were married (98.9%), and the majority had a high school education or higher (61.6%), with 73.8% reporting a medium income level. A minority (8.0%) were unsure of their child's diagnosis, and nearly half (47.3%) reported caring for the child with their spouse. Parents rated the severity of the child's illness at an average of 8.7±1.7. Approximately 45.9% of the children were under three years old, with a nearly equal gender distribution. Children had various chronic diagnoses, including heart conditions (20.8%), diabetes (15.1%), and epilepsy (14.2%). Most children had been hospitalized at least once (40.2%), and the duration of diagnosis was less than six months (Table II).

Language, face, and content validity

After the translation processes were completed, all versions of the scale (see procedure section) were sent with a draft final form to 10 academics who are experts in the field of psychiatric nursing. Experts were asked to make a face evaluation regarding the understandability and necessity of each statement. In addition, for the first stage of content validity, experts rated the suitability of each statement on a scale from 1 to 4. In line with the experts' opinions, the scale was finalized by a field expert, and the CVI was calculated. According to the Davis technique[38], the CVI value, which is expected to be 0.8 and above, was found to be 0.96.

In the final stage of face and content validity, a pilot study was conducted with 20 parents who met the inclusion criteria. With the suggestion of five parents, a word ("expect") was removed from one item of the scale (item 7) to make it more comprehensible. Thus, the item became clearer and more understandable in its Turkish version.

Internal consistency-1

As a result of the correlation analysis, the relationship between the items and the sub-dimension they belonged to was determined. The corrected correlation coefficient of the 29th item was below 0.3, and the item was deleted (Table III). After this stage, the analyses continued with 30 items. In the correlation analysis conducted to determine the relationship between the scale items and the total item, the correlation coefficient was found to be over 0.3 for all items, and as a result of the anti-image matrix, the diagonal values were over 0.5. The sub-dimension reliability analysis was calculated using the Cronbach alpha coefficient when the item was deleted. Cronbach's alpha coefficient for the reliability of all scale items was found to be 0.945, 0.919 for ambiguity, 0.836 for lack of clarity, 0.796 for unpredictability, and 0.658 for lack of information. No item was excluded from the analysis at these stages. In addition, as a result of the split-half reliability analysis, the Cronbach alpha coefficient of the first part was found to be 0.912, and that of the second part was found to be 0.882. The Spearman-Brown coefficient value was found to be 0.926.

Exploratory factor analysis

The KMO value indicating the suitability of the data set for factor analysis was found to be 0.937, and Bartlett's test was found to be

Table II. Characteristics of the sample.

Characteristics	n (%) or mean±SD
Parents' age, years	34.1 ± 5.5
Parents' gender	
Mother	260 (74.1%)
Father	91 (25.9%)
Parents' marital status	
Married	347 (98.9%)
Single	4 (1.1%)
Parents' educational status	
Only literate	4 (1.1%)
Elementary	131 (37.3%)
Highschool	99 (28.2%)
Bachelor's	100 (28.5%)
Postgraduate	17 (4.9%)
Parents' perceived income status	
Low	70 (19.9%)
Medium	259 (73.8%)
High	22 (6.3%)
Parents' knowledge of child's diagnosis	
Knows the right diagnosis	323 (92.0%)
Misunderstands / does not know the diagnosis	28 (8.0%)
Caregivers of child	
Mother	53 (15.1%)
Mother and father	166 (47.3%)
Mother, father, grandmother/mother-in-law	95 (27.1%)
Mother and grandmother/mother-in-law	25 (7.1%)
Other	12 (3.4%)
Parents' opinion of severity of the diagnosis*	8.7 ± 1.7
Child's age	
0-6 months	35 (10.0%)
7-12 months	51 (14.5%)
1-3 years	75 (21.4%)
4-5 years	86 (24.5%)
6-12 years	66 (18.8%)
13-18 years	38 (10.8%)
Child's gender	
Boy	186 (53.0%)
Girl	165 (47.0%)
Child's diagnosis	
Heart failure	73 (20.8%)
Diabetes	53 (15.1%)
Epilepsy	50 (14.2%)
Chronic renal failure	34 (9.7%)
Thyroid disease	33 (9.4%)
Asthma	32 (9.1%)
Obesity	21 (6.0%)
Other**	55 (15.7%)
Time since child's diagnosis	
0-6 months	141 (40.2%)
7-12 months	83 (23.6%)
1-3 years	56 (16.0%)
4-5 years	27 (7.7%)
5+ years	44 (12.5%)
No. of hospitalizations	
0	80 (22.8%)
1	128 (36.5%)
2	65 (18.5%)
3	32 (9.1%)
4	15 (4.3%)
≥ 5	31 (8.8%)

*Measured on a 10-point scale. SD, standard deviation.
**Other diseases: cystic fibrosis, liver cyst, hearing loss, esophageal atresia, neuroblastoma, soft tissue sarcoma, cerebral palsy, eczema, juvenile idiopathic arthritis.

significant (x^2 [435]:5738.180 , p=0.0001). In the factor analysis, items 1, 2, 12, 21, and 25 were excluded because they had high loadings on different sub-dimensions, and items 17 and 20 were excluded from the analysis because they had low factor loadings. A structure with four sub-dimensions was obtained with the remaining 23 items (Table IV). The first sub-dimension explained 38.677% of the variance, the second sub-dimension explained 8.418% of the variance, the third sub-dimension explained 5.921% of the variance, and the fourth sub-

Table III. Corrected item-total correlation values in each sub-scale.

Ambiguity	Corrected item - Total correlation	Cronbach's alpha if item deleted	Lack of clarity	Corrected item - Total correlation	Cronbach's alpha if item deleted	Unpredictability	Corrected item - Total correlation	Cronbach's alpha if item deleted	Lack of information	Corrected item - Total correlation	Cronbach's alpha if item deleted
A_3	0.696	0.911	LC_2	0.583	0.813	U_11	0.820	0.463	LI_1	0.483	0.669
A_4	0.659	0.913	LC_5	0.719	0.794	U_19	0.704	0.693	LI_12	0.387	0.672
A_8	0.728	0.91	LC_6	0.59	0.813	U_23	0.695	0.709	LI_26	0.441	0.594
A_13	0.695	0.911	LC_7	0.568	0.817	U_27	0.755	0.588	LI_28	0.422	0.600
A_15	0.705	0.911	LC_9	0.549	0.817				LI_30	0.327	0.641
A_16	0.651	0.913	LC_10	0.595	0.809						
A_17	0.583	0.916	LC_14	0.431	0.836						
A_18	0.782	0.907	LC_29	0.197	-						
A_20	0.57	0.916	LC_31	0.484	0.828						
A_21	0.71	0.911									
A_22	0.554	0.917									
A_24	0.711	0.911									
A_25	0.443	0.920									

A, ambiguity; LC, lack of clarity; LI, lack of information; U, unpredictability.

dimension explained 14.966%. The total variance explained by the four sub-dimensional structures was 57.982%. Eigenvalues were determined to be 8.896, 1.936, 1.362, and 1.142 in the sub-dimensions.

At this stage, it was also tested whether a single score could be obtained from the 23 items and four sub-dimensions obtained from the exploratory factor analysis, in other words, the additivity feature. ANOVA with Tukey's test for nonadditivity showed that the 23 items forming the scale were homogeneous and interrelated (p=0.0001), and the items confirmed additivity (p=0.0001).

Table IV. Exploratory factor analysis with Varimax rotation.

Items	Factors			
	1	2	3	4
A_16	0.795			
A_15	0.774			
A_4	0.735			
A_18	0.727			
A_3	0.706			
A_24	0.705			
A_8	0.680			
A_13	0.607			
A_22	0.464			
LC_7		0.706		
LC_5		0.697		
LC_14		0.682		
LC_10		0.530		
LC_6		0.458		
LC_9		0.432		
LC_31		0.418	0.404	
U_19			0.752	
U_23			0.714	
U_27			0.654	
U_11			0.576	
LI_30				0.756
LI_28				0.637
LI_26				0.625

A, ambiguity; LC, lack of clarity; LI, lack of information; U, unpredictability.

Internal consistency-2

As a result of the exploratory factor analysis, the Cronbach alpha value for the entire scale was 0.923, for ambiguity 0.906, for lack of clarity 0.813, for unpredictability 0.801, and for lack of information was 0.860. As a result of the split-half reliability analysis, the Cronbach alpha coefficient of the first part was found to be 0.913, and for the second part, 0.811. On the other hand, Spearman-Brown coefficient value was found to be 0.830.

Confirmatory factor analysis

Fig. 1. gives the path diagram of the confirmatory factor analysis conducted to test whether the factor structure obtained as a result of the exploratory factor analysis was valid. Four of the fit indices showed an "acceptable" level of model fit, while the others showed an "excellent" level of model fit.

Convergent validity

BSI was used to determine the scale's convergent validity. The BSI had a positive, significant relationship with the overall score of the scale (r=0.69) and the subscales (0.64; 0.57; 0.50; 0.41, respectively; Table V).

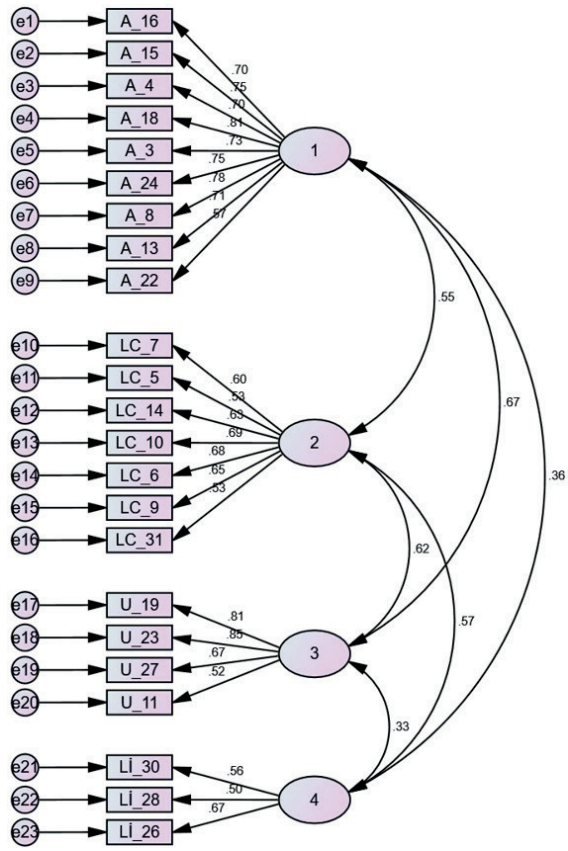

Fig. 1. Diagram from confirmatory factor analysis.

Four of the fit indices showed an "acceptable" level of model fit, while the others showed an "excellent" level of model fit (χ2/sd=2.47; AGFI=0.898; GFI=0,936; CFI=0.956; NFI=0.962; RFI=0.975; IFI=0.957; RMSEA=0.063).

A, ambiguity; AGFI, Adjusted Goodness of Fit Index; CFI, Comparative Fit Index; GFI, Goodness of Fit Index; IFI, Incremental Fit Index; LC, lack of clarity; LI, lack of information NFI, Normed Fit Index; RFI, Relative Fit Index; RMSEA, Root Mean Square Error of Approximation; U, unpredictability; χ2/df, chi-square/degrees of freedom.

Discussion

This study aimed to determine whether PPUS is a valid and reliable tool for measuring Turkish parents' perceptions of uncertainty. Within the scope of the literature review, since the full text of the study was not available in a summary report[27] regarding the psychometric properties

Table V. Correlation between Parental Uncertainty Perception Scale-Turkish Form score and general psychological symptoms of the Brief Symptom Inventory.

Variables (Mean ± SD)	Parents' perception of uncertainty (63.29±17.42)	Ambiguity (27.88±8.98)	Lack of clarity (16.70±5.68)	Unpredictability (12.47±3.94)	Lack of information (6.22±2.48)	Psychological symptoms (43.58±28.64)
Psychological symptoms	0.69*	0.64*	0.57*	0.50*	0.41*	1.00

SD, standard deviation.
*p<0.001

of the Turkish version of the PPUS, the detailed analyses of the scale could not be examined. In addition, to our knowledge no study was found in Türkiye using this scale. In this case, it was thought that due to the lack of details regarding validity and reliability analyses, a sufficient reference was not provided to the literature, and the scale's suitability for Turkish culture could not be fully assessed. Considering the persistent need to assess the uncertainty perceptions of parents of children with chronic diseases in Türkiye, this study addresses this gap by presenting a thorough adaptation process along with comprehensive validity and reliability analyses of the PPUS, thereby making a substantial contribution to the existing literature.

In our study, PPUS-TR provided high face and validity in line with the original scale. The 29th item in the "lack of clarity" sub-dimension of the original scale was deleted because it had a low coefficient in the correlation analysis in which its relationship with the sub-dimension it belonged to was determined. This item refers to the expression of trust by the parents that nurses will be present when needed. While all other items emphasize aspects such as the severity of the child's illness, assuming responsibility for the child's care, and understanding the illness process, this particular item focuses solely on trust in nurses. In this context, item 29 may have shown a low correlation, as it does not fall under any of the subscales due to its distinctiveness from the other items and the fact that its direct relationship with uncertainty is not immediately clear. Similarly, in the Chinese adaptation of the scale, item 29 was removed from the scale due to its low associations with the rest of the scale.[20] As a result of the KMO value (0.937) and Bartlett test (p=0.0001) used to evaluate the suitability and adequacy of the sample size for the analysis for construct validity, it was found that the sample size was sufficient for factor analysis. In the exploratory factor analysis, items 1, 2, 12, 17, 20, 21 and 25 were deleted. The removal of items 1, 2, 12, 21, and 25 due to high loadings on different sub-dimensions indicates that these items lacked specificity to a single construct, potentially introducing conceptual overlap across factors. Additionally, the removal of items 17 and 20 because of low factor loadings highlights that these items may not adequately represent the latent constructs being measured. Despite the deleted items, the factor structure of the scale in our study overlaps with the factor structure of the original scale.[10] The confirmatory factor analysis results show that the model fit indices are sufficient and the model is valid. In addition, the four factors that explain 57.982% of the total variance due to the factor analysis show that the scale has significant structural validity in the Turkish sample. This supports the fact that the scale's factor structure is also suitable for Turkish parents. Similar findings were obtained in other studies where PPUS was adapted to different cultures[17-20], and it was emphasized that PPUS is a tool that can be adapted to different cultures.

When reliability analyses were evaluated, the fact that Cronbach alpha coefficients were found to be relatively high indicates that the scale's internal consistency is strong. Cronbach alpha coefficient was 0.860 in the Spanish version[19], 0.930 in the Arabic version[17], and 0.825 in the cancer-specific adapted Chinese version.[20] In this study, the total Cronbach alpha coefficient of the scale was 0.923, and for the sub-dimensions, it varied between 0.801 and 0.906, confirming that the scale and its sub-dimensions are reliable. At the same time, the high results of the split-half reliability analyses (Cronbach alpha of first part: 0.913, of second part: 0.811) reveal that the scale gives consistent results at a general level. These findings show that the scale is a reliable tool. In addition, the significant positive correlation between PPUS and BSI (r=0.69) supports the convergent validity of the PPUS, suggesting that parents' perceptions of uncertainty may be meaningfully associated with psychological symptoms such as depression and anxiety. In another study where both the PPUS and BSI were used together, a significant positive correlation between these

two scales was found.[39] Additionally, in other studies using the PPUS, consistent with the findings of our study, parents' perception of uncertainty was significantly related to factors such as anxiety, depression, and stress.[20,40,41]

According to the analyses, PPUS-TR has taken its final form with 23 items and a 4-factor structure. However, there are some limitations to this study. First, the predominance of mothers among participants may affect the generalizability of the findings to all parents. Additionally, the study was limited to hospitals in one city, potentially overlooking cultural and socioeconomic differences. In addition, the sample consists of parents of children with many different types of chronic diseases; the results may differ in a sample consisting only of specific patient groups. Therefore, the authors recommend using the scale in future studies in samples where mothers and fathers are equally distributed in different geographical regions and specific disease groups. In addition, since our sample includes parents of children aged 0–18 years, the authors also recommend examining parental perceptions of uncertainty in the context of different age groups.

In conclusion, this study demonstrates that the PPUS-TR is a valid and reliable tool for measuring the perception of uncertainty among Turkish parents. At the same time, further studies with more diverse and extensive samples may help better understand the role of uncertainty perception in the disease process and determine which factors affect this uncertainty perception. Adding the Turkish version of the scale to adaptation studies conducted in different cultures through this study may also pave the way for comparative studies on how parents' uncertainty perception is shaped in various cultural contexts.

Ethical approval

The study was approved by Hacettepe University Health Sciences Research Ethics Committee (date: 06.09.2022, number: GO22/13-66). Necessary permissions were also obtained from the hospitals, which allowed the research to be conducted. Informed consent was obtained from all participants.

Author contribution

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

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Conflict of interest

The authors declare that there is no conflict of interest.

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Short Communication

An *Escherichia coli* pseudo-outbreak in the intensive care units of a university hospital

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ABSTRACT

Background. The term 'pseudo-outbreak' refers to a condition in which a microorganism is found in cultures at a greater rate than expected due to contamination of materials that would normally be sterile. This situation cannot be clinically correlated with the infection suggested by the culture results. This can be confusing depending on the patient's clinical condition, especially in intensive care units (ICU). The pseudo-outbreak with *Escherichia coli* in patients in ICUs will be discussed in this study to emphasize the importance of strict adherence to microbiology policies and procedures.

Methods. In September 2022, growths of *Escherichia coli* were found in the endotracheal aspirate cultures of six children and eighteen adults in the ICU.

Results. The identification of the same microbial agent in 24 patients prompted an investigation into a potential outbreak. The infection control committee compiled a comprehensive patient list to facilitate the assessment. Given that the healthcare personnel and infrastructure of each ICU were distinct and functioned independently, the possibility of cross-contamination within these units was deemed unlikely. Consequently, attention was directed toward the microbiology laboratory as a potential source of the outbreak. A thorough review of culture processing steps and laboratory equipment was conducted. This investigation revealed that the saline solution used for the passage of endotracheal aspiration cultures was contaminated, suggesting a laboratory-associated contamination event as the probable cause.

Conclusions. By strictly adhering to the latest protocols, the disinfection and sterilization chain can ensure the safe use of both invasive and non-invasive medical equipment. This manuscript aims to raise awareness among pediatricians and pediatric infectious disease specialists regarding the occurrence of pseudo-outbreaks. A pseudo-outbreak is indicative of a disruption in the sterilization chain.

Key words: pseudo-outbreak, *Escherichia coli*, contamination.

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An increase in hospital infections, typically from a single species, that is mistakenly interpreted as a true epidemic is known as a pseudo-epidemic of nosocomial infections, and they can be very challenging to identify.[1]

Infectious diseases that spread rapidly within a community are central to most dictionary definitions of an 'outbreak.[2] An outbreak is defined as occurring when the number of reported cases exceeds the expected number for a specific time frame and the cases are connected by epidemiological or microbiological characteristics.[3] However, two or more epidemiologically linked cases with outbreak potential, or even a single case of a newly emerging or previously eradicated disease, can also be considered an outbreak.[4] In some diseases, such as smallpox and poliomyelitis due to wild poliovirus, even a single case is considered an outbreak.[4] The term 'pseudo-outbreak' refers to a situation in which a microorganism is detected in cultures at a higher rate than expected and that cannot be clinical correlated with the infection suggested by the culture results due to contamination of materials that would normally be sterile.[5] ICU's, transplant units, oncology, and endoscopy units are areas that have risks for both outbreaks and pseudo-outbreaks.[6,7]Potential sources of bacterial contamination include bulk diluents, saline bottles, patient beds, ventilators, switches, door handles, sinks, blood collection tubes, liquid hand soaps, soap dispensers, disinfectants, in solutions with nutrients, anesthetics and, laboratory personnel.[8] Contamination and pseudo-outbreaks result in wasted time, and labor and can also invalidate experiments. Early detection of a pseudo-outbreak significantly reduces inappropriate antibiotic use, associated complications, healthcare and laboratory costs, mortality, and hospitalizations.[6-8] Therefore, distinguishing between true outbreaks and pseudo-outbreaks at an early stage is crucial to mitigating these negative consequences.[6]

The first and most important step in combating any infectious disease is prompt identification and recognition of the condition. The importance of a hospital surveillance system that enables rapid and extensive information sharing as well as opinion sharing, and collaborative risk assessment and management, is emphasized. The key elements of a first hospital response include isolation protocols, laboratory capability, and case identification. Sample collection, transportation, and management procedures adhere to protocols established by the World Health Organization and The Centers for Disease Control and Prevention.[3] Healthcare environments need to be an environment where people can report errors without fear of condemnation or punishment.[8] All healthcare personnel involved in patient care should be aware of and receive training in infection control.[8]

Healthcare institutions should actively review processes in high-risk areas to identify potential sources of contamination. It is imperative to designate experts to oversee infection prevention and control at the facility and subsequent administrative levels in order to facilitate a programmatic approach that prioritizes responsibility, supervision, and coordination through ongoing monitoring and assessment. The advent of numerous newly identified or unique infectious agents as well as the reappearance of infectious illnesses that affect the entire world have been the hallmarks of the past few decades. This study aims to provide an overview of the hospital response framework for the early detection and timely treatment of patients with infectious illnesses. The fundamental components of hospital preparedness in the event of a cluster of infectious diseases are discussed. Additionally, the study seeks to determine whether an observed increase in cases represents a true outbreak or a pseudo-outbreak and outlines the necessary steps for appropriate intervention.

Materials and Methods

The list of patients with *Escherichia coli* growth in endotracheal aspirate (ETA) cultures from September 2022 was obtained from the

Infection Control Committee of Marmara University Pendik Training and Research Hospital. *Escherichia coli* growth was observed in the ETA cultures of 18 adult patients in the ICU and 6 pediatric patients in the pediatric ICU (PICU) in September 2022. The adult patients were hospitalized in the Anesthesia and Reanimation ICU, Internal Medicine ICU, General ICU, Cardiovascular Surgery ICU, and Organ Transplantation ICU

Results

Pediatric patients are presented in Table I. A 3.5-year-old boy with pneumonia, a 6-month-old boy with acute respiratory distress syndrome (ARDS), a 6.5-year-old girl diagnosed with a craniocervical mass who had a surgical wound infection, an 8-month-old girl with bronchopulmonary dysplasia and respiratory failure, a 2.5-month-old girl with diarrhea, a 3-year-old boy with aspiration pneumonia, pleural effusion and pneumothorax and a 3.9 month old male with a thorax tube, a Glasgow coma score of seven, fever, and hypotension were admitted to the PICU.

Escherichia coli was isolated from the ETA cultures of 24 patients across all intensive care units (ICUs). The interpretation of these findings was challenging, as some patients exhibited clinical signs suggestive of pneumonia, including increased respiratory secretions, respiratory acidosis, and an elevated requirement for ventilator support. Due to institutional limitations, bacterial sequencing could not be performed; however, antibiogram results were identical across all cases, raising concerns regarding potential contamination or a pseudo-outbreak.. In other words, bacterial susceptibility and resistance were the same in all isolates. This isolate was determined to be sensitive to penicillins, aminoglycosides, and sulfonamides, and moderately sensitive to cephalosporins. Therefore, it was thought to be the same strain. Different staff members and different units were providing treatment for the patients. Additionally, on the day of their ICU admission, three patients had positive cultures (Table I). These results implied that the infection originated outside of the ICUs and at a common place like laboratory.

Upon observing an unusually high frequency of isolations of the same microorganism, with bacteria exhibiting identical susceptibility profiles across multiple ICUs within a single week, the Hospital Infection Control Committee was notified. It was determined that the Hospital Infection Control Committee had also identified the situation, prompting the initiation of an outbreak investigation. A comprehensive patient list was compiled. Given that the affected patients were located in different ICUs throughout the hospital, under the care of distinct medical teams, and not in direct

Table I. Characteristics of patients in the pediatric intensive care unit.

Case	Age (month)	Sex	Primary diagnosis	Timing of bacterial growth in ETA culture (days post-intubation)
1	42	Male	Pneumonia	13*
2	6	Male	ARDS	9
3	78	Female	Craniocervical mass, wound infection	13
4	8	Female	Bronchopulmonary dysplasia and respiratory failure	1
5	2.5	Female	Diarrhea	1
6	35	Male	Aspiration pneumonia, empyema and pneumothorax	1*

*Tracheostomized patients.
ARDS, acute respiratory distress syndrome; ETA, endotracheal aspirate.

proximity to one another, the investigation shifted focus to the microbiology laboratory—the common point connecting these disparate units. It was determined that a 250 mL bag of 0.9% sodium chloride (normal saline), used for the passage of cultures, had been accessed with a syringe multiple times over a period of 5–6 days, leading to contamination of the saline. To confirm this hypothesis, a sample from the saline bag was cultured, and the same bacterial strain was identified. The recognition of the pseudo-outbreak took approximately two weeks.

To address this issue, the saline bag was replaced with single-use 10 mL disposable physiological serum, which is now used for a maximum of 5–6 patients before being discarded. Additionally, disposable syringes are now utilized to prevent contamination.

The laboratory manager, infection prevention specialists, and relevant personnel conducted a comprehensive process assessment, developed improvement action plans, and provided retraining on established policies and procedures. Furthermore, emphasis was placed on reinforcing fundamental infection prevention and control measures, including proper hand hygiene, thorough cleaning and disinfection of workstations, and sanitation of commonly handled objects and surfaces.

Discussion

In critical care settings where rapid clinical decision-making is essential, such as ICUs and transplantation units, or in conditions requiring early intervention, such as sepsis, this situation can be particularly challenging. It may lead to diagnostic confusion, unnecessary distractions, and adverse effects associated with unwarranted treatment. In this study, a pseudo-outbreak of *E. coli* occurred due to contamination of the saline water used for passage of ETA cultures in the laboratory. Similarly, Mumcuoglu et al.[5] isolated *S. marcescens* strains from blood cultures of 22 patients in two different ICUs. The Hospital Infection Control Committee conducted an environmental investigation and identified the surfaces of blood collection tubes as the source of contamination.[5] Since the patients exhibited no clinical signs or symptoms consistent with infection, it was easier to determine that this was a pseudo-outbreak.[5] Eldridge et al.[8] during the investigation of a *B. cepacia* pseudo-outbreak, found that laboratory personnel caused cross-contamination through the use of non-sterile saline diluent. The susceptibility patterns of all isolates were found to be the same as in this study.[8] The laboratory environment can act as potential sources of contamination, highlighting the need for regular surveillance and training in microbiology laboratories.[8]

Pseudo-outbreaks with many different organisms have been reported in the literature. Pseudo-outbreak can occur not only with bacteria but also with viruses. Hellinger et al.[9] published their work about an adenovirus pseudo-outbreak in the bronchoalveolar lavage (BAL) specimens of seven ICUs patients due to adenovirus DNA on the bronchoscope. In the same month, specimens were taken from all patients and healthcare workers with nasopharyngeal swabs in the ICUs. The bronchoscope lumens were scanned. Inactive adenoviral DNA was detected only in the index case and in the bronchoscope. Adenovirus did not grow in the culture. Sterilization of bronchoscopes with ethylene oxide eliminated the contamination. A previous study reported an unusual finding in which 22 healthcare workers tested positive despite wearing masks due to the COVID-19 pandemic.[10] Flipse et al.[10] later described a pseudo-outbreak of *Bordetella parapertussis*, which was traced to swabs contaminated with *B. parapertussis* DNA. The investigation revealed that the contamination resulted from a manufacturing defect in the swabs, highlighting the potential for diagnostic errors due to faulty laboratory supplies. Additional invasive diagnostic procedures, time and cost may be required to confirm the diagnosis.

Stern et al.[11] presented a study about a *Rhizobium* pseudo-outbreak. *Rhizobium* was isolated from six surgical tissue cultures and was determined to be due to improper laboratory tissue handling with contaminated saline. Non-sterile saline has been associated with pseudo-outbreaks of pathogens such as *Burkholderia cepacia, Rhizobium, Legionella*, etc. In the present study, it was related to *E. coli*. Nagano et al.[12] described a pseudo-outbreak of *Mycobacterium lentiflavum* in their study. Sputum and BAL were both contaminated due to the use of tap water, and in environmental research they had found that tap water was passed through the bronchoscope before each procedure.[12] In the latter study, the issue was identified only after three years, leading to the administration of multiple antibiotics to some patients. This resulted in unnecessary antibiotic use, potentially contributing to antimicrobial resistance, adverse effects, and increased healthcare costs. In contrast, in the current study, the issue was detected at an early stage, allowing for timely intervention.

A follow-up period of either one or two months should be carried out, at the very least, to avoid false-positive cultures in lab settings. Continuous communication among clinicians, laboratory technicians, and the Infection Control Committee is essential in interpreting unusual findings resulting from contamination. A pseudo-outbreak of *Bacillaceae* spp. bloodstream infection resulting from improper use of medical cotton wool during blood culture collection was described in the study by Borcan et al.[13]

Over a five-month period, *Bacillaceae* spp.-positive blood cultures were obtained from 60 patients, with two patients receiving treatment. Control measures included the removal of cotton wool contaminated with *Bacillaceae* spp. and the implementation of periodic training on proper blood culture collection techniques.[13]

The study had some limitations. For example, DNA sequencing was not performed to confirm whether the *E. coli* isolates were from the same strain.

In healthcare settings, distinguishing between outbreaks and pseudo-outbreaks can be challenging. Even when utilizing gold-standard diagnostic methods such as cultures, laboratory results should not replace clinical evaluation; physical examination findings must be considered in differentiating a true outbreak from a pseudo-outbreak.

Laboratory processing errors, such as deviating from standard disposable sterile transport media to a larger, contaminated saline distribution source or using non-sterile syringes, have been linked to pseudo-outbreaks. Effective infection control requires timely collaboration and communication, particularly in the prevention of pseudo-outbreaks.

This article highlights potential weaknesses in infection control protocols and provides practical recommendations for improving the standard of care and patient safety in healthcare settings. Identifying the source of an outbreak as early as possible is crucial, along with investigating discrepancies in infection control measures and microbiological processing protocols.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: BP, SÖD, EK; data collection: BP, NP, IK; analysis and interpretation of results: BP, EK, SÖD; draft manuscript preparation: BP, SÖD. All authors reviewed the results and approved the final version of the article.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Short Communication

Inflammatory marker comparison in childhood brucellosis: predicting osteoarticular involvement

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ABSTRACT

Background. Although the use of inflammatory markers in diagnosing *Brucella*-related complications has been the subject of research, studies on osteoarticular disease are insufficient, especially in children. This study aimed to compare inflammatory markers in children diagnosed with brucellosis, distinguishing between those with and without osteoarticular involvement (OI).

Methods. In this retrospective study, patients diagnosed with brucellosis from 1 month to 18 years of age were evaluated. Data collected included age, gender, OI, treatment duration, complete blood count, inflammatory markers including neutrophil-monocyte ratio (NMR), monocyte-lymphocyte ratio, neutrophil-lymphocyte ratio, platelet-lymphocyte ratio, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR) and *Brucella* serum agglutination test (SAT) results. OI was confirmed by MRI in symptomatic patients. The results of patients with and without OI were compared.

Results. The study included 38 patients, 23.7% having OI (8 with sacroiliitis and 1 with spondylitis). The median age of patients with OI was significantly higher than those without (p=0.037). All patients with OI (n = 9, 100%) had an SAT titer ≥ 1/640. Among patients without OI, 62% (n = 18) had an SAT titer ≥1/640. This difference was statistically significant (p = 0.028). Patients with OI had higher CRP levels (p=0.038) but similar ESR levels compared to those without. WBC levels were significantly lower in the group with OI (p=0.015). NMR was significantly higher in those with OI (p=0.012).

Conclusions. Lower WBC counts and higher CRP and NMR levels can predict OI in children with brucellosis at the time of admission. However, our findings should be validated through prospective studies involving larger patient groups.

Key words: brucellosis, osteoarticular brucellosis, neutrophile-to-monocyte ratio.

Brucellosis is one of the most prevalent zoonoses, causing some serious public health consequences.[1] Globally, approximately 500,000 human cases of brucellosis are reported annually.[2] According to European Center for Disease Prevention and Control (ECDC) data, the notification rate in Europe was 0.04 cases per 100,000 population.[3] Brucellosis is also endemic in Türkiye, with widespread disease throughout the country; however, the regions with the highest incidence are the southeast and east parts of the country. The Turkish Ministry of Health reported the incidence of brucellosis as 12.3 per 100,000 in 2019.[4]

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Osteoarticular involvement (OI) is a widely reported manifestation in adults and is also the primary complication of brucellosis in children.[5,6] Brucellosis can lead to prolonged fever, hemophagocytic lymphohistiocytosis, endocarditis, meningitis, as well as peripheral arthritis, osteomyelitis, and, less commonly, severe osteoarticular complications such as spondylitis and sacroiliitis.[7] These complications in children not only cause school failure and economic burden due to hospitalization and long-term antibiotic use but also cause psychological problems along with loss of workforce, especially in adolescents. Therefore, early recognition of complications is essential.

Hematological parameters, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) are easily accessible markers commonly used in the follow-up of infections. Although the use of inflammatory markers in diagnosing brucellosis complications is a subject of research, studies on OI and the use of these markers to manage the disease remain limited, especially in children. This study aims to compare the inflammatory markers between patients with and without osteoarticular involvement.

Materials and Methods

The medical records of 48 patients (age range: 1 month to 18 years) diagnosed with brucellosis at the Department of Pediatrics and Pediatric Infectious Diseases in Konya City Hospital between July 2022 and January 2024 were retrospectively reviewed. After excluding the patients with incomplete demographic information, test results, or those transferred to another hospital for follow-up and treatment, the remaining 38 patients were enrolled. The following data were recorded for the patients: age, gender, presence of OI, presence of fever, treatment duration, complete blood count (including leukocyte, lymphocyte, neutrophil, monocyte, and platelet counts, mean platelet volume), inflammatory markers [neutrophil-to-monocyte ratio (NMR), monocyte-to-lymphocyte ratio (MLR), neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), CRP, and ESR], and *Brucella* serum agglutination test (SAT) results at the time of admission. Serum samples were incubated with *Brucella abortus* antigen (*Brucella abortus* tube antigen supplied by the Public Health Institution of Türkiye, Ankara, Türkiye). *Brucella* antigen was evaluated for agglutinin particles for 24 h at 37°C, and samples with ≥ 1/160 were considered positive.

Definitions

OI was confirmed by magnetic resonance imaging (MRI) in patients with symptoms. Symptoms indicative of sacroiliitis included hip pain, inability to walk, and restriction of movement. Similarly, spinal pain, tenderness along the paravertebral region, and restricted movement were considered as symptoms of spondylitis.

The inflammatory markers of patients with and without OI were compared.

Statistical analysis

Statistical analysis was performed using SPSS statistical software (version 29.0.2.0; SPSS). Categorical variables were analyzed using relative frequencies, whereas numerical variables were analyzed using median or mean values (depending on whether they had a normal distribution). Categorical variables were compared using Pearson χ^2 and Fisher's exact tests. Numerical variables were compared using the t-test or the nonparametric Mann–Whitney *U* test. A $p < 0.05$ was considered to be statistically significant.

Ethical approval

The study was approved by the Necmettin Erbakan University Ethics Committee (date: 05.07.2024, number: 2024/5064)

Results

A total of 38 patients diagnosed with brucellosis were included in the study. Of the patients 65.8% were male and 34.2% female. The median age of the patients was 13 years (IQR: 9-15). Upon admission, 47.4% of the patients (18/38) presented with a fever. OI was proven by MRI in 23.7% (9/38) of the patients (sacroiliitis in 8 patients and spondylitis in 1 patient), while the other patients had no organ involvement. When comparing patients with and without OI, no significant gender difference was found (p=0.95). However, the mean age of patients with OI was significantly higher than those without (p=0.004).

From a diagnostic perspective, SAT titers ranged from 1/160 to 1/5120 among all patients. All patients with OI (n = 9, 100%) had an SAT titer ≥ 1/640. Among patients without OI, 62% (n = 18) had an SAT titer ≥1/640. This difference was statistically significant (p = 0.028). When comparing treatment durations, patients with OI received treatment significantly longer (median 6 weeks vs. 10,5 weeks, p=0.002).

Regarding symptoms and laboratory findings suggestive of an inflammatory process, there was no significant difference in fever between the two groups (p=0.18). The level of CRP was higher in patients with OI (p=0.039), while ESR was similar between the groups. Regarding hematologic parameters, white blood cell (WBC) count was significantly lower in the group with OI (p=0.013). Although the absolute lymphocyte, neutrophil, and monocyte counts were similar, NMR was significantly higher in the group with OI (p=0.012). The other markers, MLR, NLR, PLR, and MPV, were similar between the groups (Table I).

Table I. Demographics and laboratory findings of the patients (N=38).

	Patients with osteoarticular involvement	Patients without osteoarticular involvement	p-value
Number of patients (%)	9 (23.7%)	29 (76.3%)	
Age (year)*	14.6 ±2.2 (10-17)	11.1 ±4.4 (3-17)	0.004
Gender, n (%)			0.950
Male	6 (66.7%)	19 (65.5%)	
Female	3 (33.3%)	10 (34.5%)	
Treatment duration (week)**	11 (9-12)	6 (6-7)	0.002
SAT titer ≥1/640 (%)	100%	62%	0.028
WBC (/μL)**	5960 (5380-7390)	7480 (6485-9660)	0.013
ANC (/μL)**	2770 (2270-3900)	3570(2645-4740)	0.080
ALC (/μL)*	2478±663 (1170-3530)	3056 ±1374 (1040-6440)	0.235
AMC (/μL)**	500 (470-800)	550 (425-735)	0.893
NMR**	0.2 (0.2-0.2)	0.1 (0.1-0.2)	0.010
MLR*	0.2±0.1 (0.2-0.3)	0.2±0.2 (0.1-1)	0.786
NLR*	1.1±0.5 (0.3-1.9)	1.7±1.8 (0.1-8.7)	0.303
PLT (/μL)*	286888±63142 (193000-381000)	282586±92422 (78000-456000)	0.897
PLR**	120.9 (96.6-137.5)	101.4 (68.6-120.6)	0.277
MPV (fL)*	9.2±0.5 (8.5-9.9)	9.9±0.9 (8.3-11.8)	0.064
ESR (mm/h)**	29 (20-45)	17 (8-35)	0.150
CRP (mg/L)**	30.2 (22.2-55)	9.8 ((0.9-32.3)	0.039

*mean ± SD (min-max), ** median (Q1-Q3)

ALC, Absolute lymphocyte count; AMC, Absolute monocyte count; ANC, Absolute neutrophile count; CRP, C-reactive protein; ESR, Erythrocyte sedimentation rate; MLR, Monocyte-to-lymphocyte ratio; MPV, Mean corpuscular volume; NLR, Neutrophile-to-lymphocyte ratio; NMR, Neutrophile-to-monocyte ratio; PLR, Platelet-to-lymphocyte ratio; PLT, Platelet count; SAT, Serum agglutination test; WBC, White blood cell count.

Discussion

This study indicates that the readily accessible and cost-effective inflammatory markers CRP, WBC, and NMR can serve as indicators of OI in brucellosis. In previous studies, peripheral arthritis was predominantly observed in the pediatric population, whereas sacroiliitis and spondylitis were more frequently identified in adolescents and adults.[8,9] Our study also revealed that patients with OI were older than the others, suggesting the potential utility of these markers, particularly in the adolescent age group. Some studies have indicated that OI in childhood brucellosis is more prevalent among males[9,10], while others have suggested a higher incidence in females.[11,12] In our study, no significant gender difference was observed between patients with and without OI. In the group with OI, the SAT titer was ≥1/640 at diagnosis, and it was statistically different from the group without OI. Although the relationship between SAT titers and disease severity or the presence of complications has not been established, our findings were consistent with those in the study by Çiftdoğan et al. regarding the serologic test results.[13]

Moreover, in the presence of osteoarticular complications, the literature indicates a recommendation for a combined antibiotic treatment regimen lasting 3-6 months to achieve a complete cure and prevent relapse.[14,15] In our study, the treatment duration for patients with osteoarticular disease was significantly longer due to delayed radiological and clinical improvement. It is noteworthy that adolescents may be at risk of experiencing antibiotic side effects due to prolonged treatment.

Kayaaslan and colleagues, in their study involving 700 adult patients, found that CRP and ESR values were significantly elevated in those with hepatitis, epididymoorchitis, neurobrucellosis, and OI.[16] A similar multicenter study in China also demonstrated that increased ESR and CRP levels were associated with complicated brucellosis.[17]Research on pediatric patients with osteoarticular complications supports these findings in adults.[13,18] In our study, CRP levels were significantly higher in the group with OI. However, there was no statistically significant difference between the groups concerning ESR elevation. This discrepancy might be because the study was based on blood test results taken at the time of admission, with CRP rising faster than ESR.[19] For more accurate disease monitoring, serial measurements of ESR might be necessary. Upon admission, evaluating CRP levels in patients presenting with joint pain at admission could provide more accurate information regarding OI.

Several mechanisms may explain the hematological changes seen in brucellosis, such as hypersplenism, bone marrow suppression, direct bacterial infection of hematologic cells, and immune-mediated destruction, which can result in leukopenia, anemia, and thrombocytopenia.[20] Our study found that the mean WBC was lower in patients with OI, although the groups had similar thrombocyte levels. Recent research highlights that LMR, NLR, and PLR are valuable markers of systemic inflammation in various diseases.[21-23] Olt et al. reported a significant decrease in NLR in brucellosis patients compared to healthy individuals and a higher median NLR in those with arthritis.[24] Another study observed significantly elevated PLR and NLR in children with *Brucella* arthritis.[25] In our study, only the NMR, which has been shown to be beneficial in sepsis, COVID-19, and non-infectious inflammation, was significantly higher in patients with OI.[26-28] To the best of our knowledge, this is the first study evaluating NMR in brucellosis. MPV has also been identified as an inflammation marker in many diseases, with two studies indicating higher MPV in children with arthritis-positive brucellosis compared to healthy controls and those with arthritis-negative brucellosis.[21,25,29] Sen et al. found lower MPV in brucellosis patients with specific organ involvement versus those with uncomplicated brucellosis. In our study, mean MPV was lower in the OI

group, but this difference was not statistically significant.[30]

Our study has several limitations. First, since our study is retrospective, we could only evaluate the blood tests taken at the time of the patients' initial presentation. Changes in inflammatory markers during the follow-up could not be observed. Second, the design of the study does not include a control group. Third, due to the retrospective nature of the study, the patients' symptoms and the duration of these symptoms could not be analyzed in detail. Consequently, the relationship between the stage of infection (acute, subacute, or chronic) and inflammatory markers could not be evaluated.

In conclusion, lower WBC and higher CRP and NMR levels can serve as indicators for predicting OI at the time of admission. These readily accessible markers could assist clinicians in the early identification of complications, facilitating timely intervention. Prospective studies with larger cohorts and serial marker evaluations are required to validate and expand on our results, improving the accuracy and reliability of inflammatory markers in diagnosing and managing osteoarticular complications in pediatric brucellosis.

Ethical approval

The study was approved by the Necmettin Erbakan University Ethics Committee (date: 05.07.2024, number: 2024/5064).

Author contribution

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

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Case Report

Tension gastrothorax in late-presenting congenital diaphragmatic hernia: a diagnostic dilemma

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ABSTRACT

Background. Tension gastrothorax is a rare life-threatening condition that occurs when the stomach is herniated into the thoracic cavity, most often through the congenital left posterolateral diaphragmatic defect, causing a mediastinal shift when distended with gas and fluid.

Case presentation. A previously healthy 2-year-old boy was admitted with acute abdominal pain, vomiting and dyspnea. Chest X-ray was initially interpreted as hydropneumothorax, but after careful observation the decision was made to insert a nasogastric tube and to perform a computerized tomography scan to confirm the suspicion of tension gastrothorax. Laparotomy was performed the following day, organs were repositioned into the abdomen and reconstruction of the left hemidiaphragm was conducted.

Conclusion. When symptoms of respiratory distress occur in an otherwise healthy child, tension gastrothorax should be on the list of differential diagnosis. It is important to recognize distinct radiographic features of this life-threatening condition in order to promptly manage it. Initial placement of nasogastric tube for decompression should be followed by the reduction of the organs into the abdomen and diaphragmatic repair.

Key words: tension gastrothorax, congenital diaphragmatic hernia, children.

Approximately 90% of congenital diaphragmatic hernias (CDH) are diagnosed with prenatal imaging or within the first 24 hours after birth. The ones that present later have a better prognosis because there is no pulmonary hypoplasia or hypertension.[1] Late-onset CDH has a variety of clinical presentations and one of them is a rare life threatening condition - tension gastrothorax. Horst et al.[2] showed an incidence of 5.1% (5/98) CDH presenting as tension gastrothorax in a 13 year period. It is caused by herniation of the stomach into the thoracic cavity, most often through the congenital left posterolateral diaphragmatic defect. When the stomach is significantly distended with gas and fluid it causes a mediastinal shift which can often be misinterpreted as tension pneumothorax leading to incorrect management with increased morbidity and mortality.[3,4]

The aim of this case report is to present typical clinical and radiographic features of tension gastrothorax and suggest the best treatment options for this rare and potentially fatal condition.

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Case presentation

A previously healthy 2-year-old boy was admitted to our hospital with acute abdominal pain, vomiting and dyspnea which occurred while playing outside the home.

On admission, a board certified pediatrician performed auscultation that revealed diminished breath sounds in the left hemithorax. The patient was sent to the radiology department for imaging evaluation.

Chest radiography demonstrated an air filled structure with fluid in the left hemithorax and a significant mediastinal shift (Fig. 1a). Initially it was interpreted as hydropneumothorax and the child was admitted to the intensive care unit for thoracocentesis. Gastric gas was not seen in the left upper abdomen after careful observation of the chest X-ray, so the decision was made to insert a nasogastric tube (NGT) which led to the resolution of respiratory symptoms. That directed us to the assumption of potential tension gastrothorax (Fig. 1b). In line with this, the patient was further evaluated by a computerized tomography (CT).

A CT scan was done to confirm the diaphragmatic defect and it revealed stomach in the left hemithorax, filled with air and fluid. The spleen with accessory spleen and left colic flexure had an intrathoracic position. The left lung parenchyma was compressed with signs of right mediastinal shift (Fig. 2a and Fig. 2b).

After the imaging evaluation, the patient was refered to a board sertified pediatric surgeon and underwent surgery the following day. A left subcostal incision was made. Intraoperatively, left posterolateral diaphragmatic defect was verified with multiple organs protruding in the thoracic cavity including the stomach, spleen, part of the colon and great omentum. Organs were repositioned into the abdomen and a reconstruction of the left hemidiaphragm

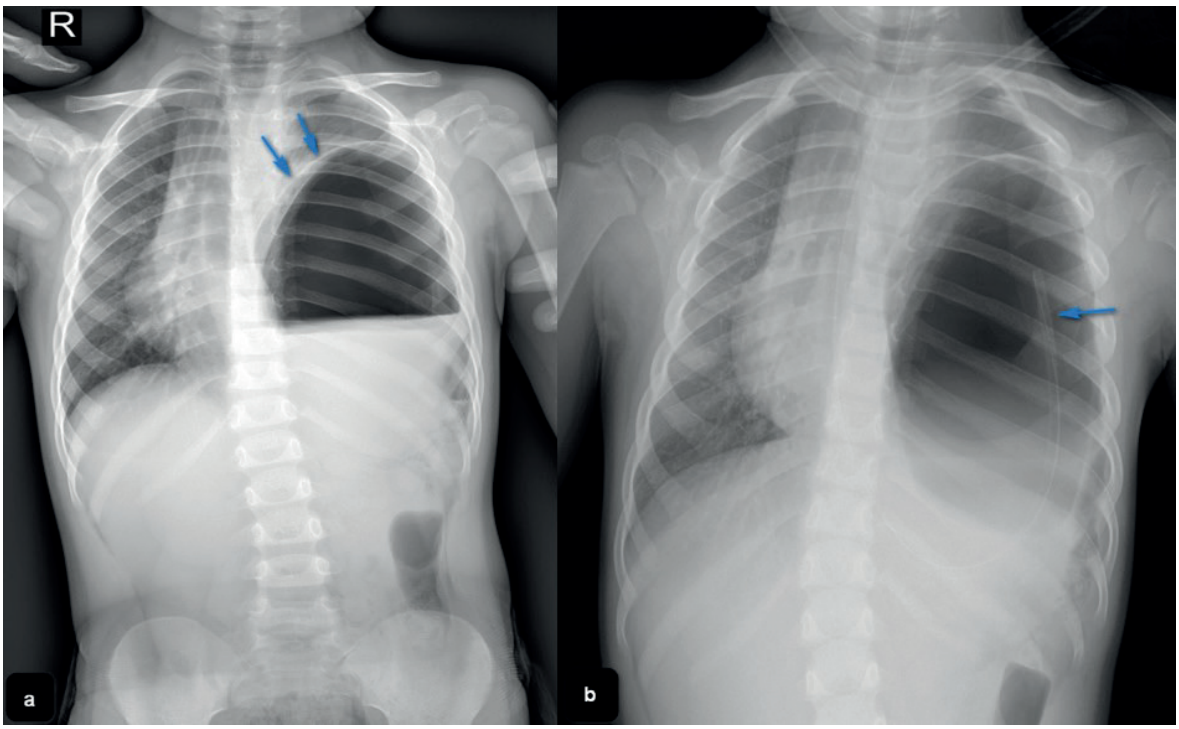

Fig. 1. Initial abdominal radiographs in erect position. **a)** A structure with an air-fluid level is observed in the left hemithorax. Its superior margin is formed by the stomach wall and the compressed lung (arrows), and the mediastinal structures are shifted to the right. **b)** A nasogastric tube (arrow) placed into the stomach is positioned in the left hemithorax.

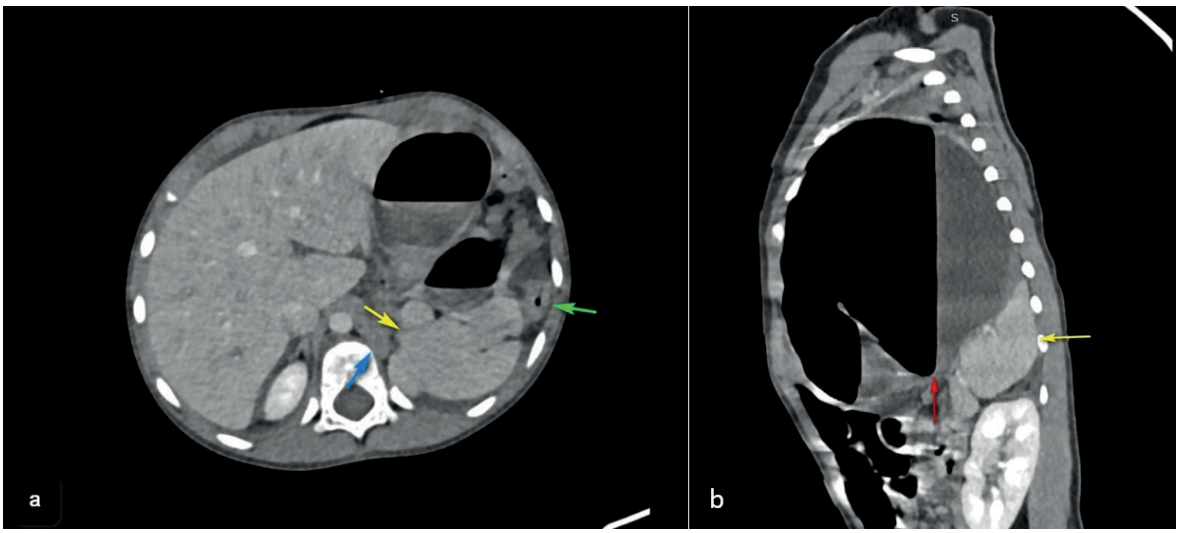

Fig. 2. Computerized tomography (CT) images. **a)** Axial CT image showing the defect in the left diaphragm (blue arrow), herniation of the spleen and accesory speen into left hemithorax (yellow arrow), and the herniation of the colon into the left hemithorax (green arrow). **b)** Sagittal CT image showing the air-fluid level in the herniated stomach (red arrow) and the herniated spleen (yellow arrow).

was conducted with direct sutures, thus repairing the existing defect. The operation was performed without complications.

The postoperative course was uneventful and the patient was discharged from the hospital after 7 days (Fig. 3). Follow up exams were performed immediately after surgery and after one week. Since the patient was healthy and without any difficulties he returned to his daily activities. One month after the surgery he showed no complications during the physical exam. No further follow up exams have been performed since.

A written informed consent was obtained from the parents of the patient for this publication.

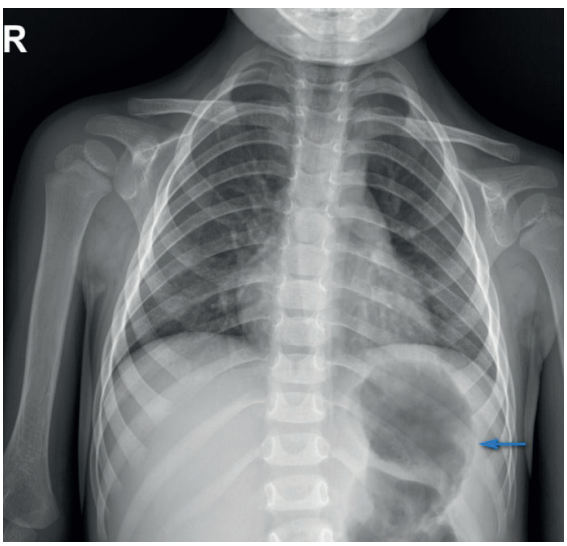

Fig. 3. Postoperative radiograph, showing re-expansion of the left lung with normal position of the mediastinal structures. The gastric bubble (arrow) is positioned below the left hemidiaphragm.

Discussion

Tension gastrothorax represents intrathoracic position of stomach, distended with air or fluid, causing a mediastinal shift. The most common site of herniation in children is pre-existing left posterolateral diaphragmatic defect (Bochdalek).[4] At the level of diaphragmatic defect, increased abdominal pressure causes protrusion of the stomach into the thoracic cavity and twisting of the gastroesophageal junction. This causes a one-way valve mechanism which leads to rapid distension of the stomach with air or fluid.

Initial symptoms can be nonspecific such as abdominal or chest pain, coughing and dyspnea eventually leading to life-threatening conditions

such as severe respiratory distress and cardiac arrest.[5] Approximately 10% of patients with CDH present after the age of 5 months, with a very small number of those developing tension gastrothorax.[6] In the case of severe clinical symptoms in an otherwise healthy child and the need for urgent diagnosis and treatment, mistakes can be made. The presence of an air-filled structure in the left hemithorax with mediastinal shift on the chest X-ray can be misinterpreted as tension pneumothorax.

In the case of tension pneumothorax, the gastric air is seen under the left hemidiaphragm which is well-defined and depressed. The parenchyma of the left lung is medially compressed. On the other hand, the left hemidiaphragm is poorly defined in the case of gastrothorax and a gastric bubble is not seen in the left upper quadrant of the abdomen. Superior margin of the air filled structure is formed by the compressed lung parenchyma and the stomach wall.[7]

Even though prompt management is needed, careful interpretation of the chest X-ray is essential for the correct diagnosis. In order to confirm the suspicion of tension gastrothorax, an NGT can be placed to show the position of the stomach on the X-ray. The final step in radiological diagnosis is a CT scan of the thorax to precisely visualize all herniated structures.[8] Management of tension gastrothorax, when diagnosed, consists of several options. From our experience and upon reviewing the literature, the following are the recommended options:[9,10]

1. Initial treatment should be placing an nasogastric or orogastric tube to decompress the stomach, which may not be easy because of te altered anatomy of esophagogastric junction and can sometimes be done with endoscopic assistance.
2. Endoscopic stomach decompression if the patient has stable vital parameters.
3. Transthoracic needle decompression of stomach if previous ways of decompression fail or are not possible to be performed due to the patient's hemodynamic or respiratory worsening, which should be assesed by an anesthesiologist. This method does not have a high success rate and makes a patient susceptible to contamination and infection.
4. Emergency laparotomy/thoracotomy, stomach reposition and diaphragmatic defect repair, which is also the definitive treatment in all cases. The laparotomy or thoracotomy approach choice is left for the surgeon to decide. Laparotomy gives the surgeon the option to also explore other organs and provides a more prompt reduction of the stomach while a thoracotomy allows an easier repair of the diaphragm.

This case report aims to add to knowledge of the diagnosis and treatment of tension gastrothorax in pediatric patients.

When the symptoms of respiratory distress occur in a previously healthy child, especially if there was an event leading to increased abdominal pressure, timely and adequate diagnostic methods must be performed to confirm the diagnosis of tension gastrothorax. Prompt management is preceded by an X-ray showing distinct signs opposite to tension pneumothorax. Initial placement of NGT is important for the diagnosis as well as to decompress the stomach, followed by the reduction of the organs into the abdomen and diaphragmatic repair.

Ethical approval

A written informed consent was obtained from the parents of the patient for this publication.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: SC, ID, NZ, PP; data collection and literature review: SC, ID, NZ, SS, PP; draft manuscript preparation: SC, ID, DN. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

Blocked D phenomenon implicated in a diagnostic dilemma in RhD-hemolytic disease affecting twins: case report and review of literature

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ABSTRACT

Background. The Rh blood group system is the most common cause of hemolytic disease of the fetus and newborn (HDFN). Rh antigens are fully expressed at birth unlike ABO antigens which are weakly expressed. Sensitization to the D antigen can occur with exposure to < 0.1 mL of fetal blood. In rare cases of HDFN, these passively transferred IgG anti-D antibodies coat the D antigens on the newborn's red blood cells and interfere with the agglutination of D-positive red cells when tested with IgM anti-D typing reagents, resulting in false-negative Rh(D) typing. This "blocked D phenomenon," can pose a diagnostic challenge.

Case presentation. This case report describes twins with HDFN born to a Rh(D) negative mother. Both cord blood and neonatal blood were incorrectly typed as Rh(D) negative using routine typing reagents, creating a diagnostic dilemma. The combination of a positive direct antiglobulin test (DAT), the mother's RhD-negative status, a positive indirect antiglobulin test (IAT), and discordant or unexpected RhD typing in the neonate raised suspicion of blocked D phenomenon. Paired samples from the parents and neonates were analysed. Following gentle heat elution at 45°C for 10 minutes, the neonatal red cells were re-typed as RhD positive using the conventional tube technique with monoclonal IgM anti-D. At the 6-month follow-up, both infants were phenotyped as O RhD positive.

Conclusions. The possibility of the blocking phenomenon should be considered while interpreting blood group results from fetal or neonatal samples in an alloimmunized pregnancy with potent antibodies. All pregnant women, regardless of their RhD type, should be tested for clinically significant unexpected serum antibodies during pregnancy. Elution methods help in identifying correct D antigen when Rh(D) typing gives uncertain results. Antiglobulin testing with anti-IgG should be performed to detect antibodies causing hemolytic disease of the fetus and newborn (HDFN).

Key words: Alloantibody, Anti-D antibody, blocking antibody, hemolysis, hyperbilirubinemia, Rh-typing.

While the incidence of Rhesus hemolytic disease of fetus and newborn (Rh HDFN) has declined significantly with the advent of immune prophylaxis, cases involving other alloantibodies, such as anti-K, have become increasingly common, but still Rh blood group system remains the leading cause of HDFN.[1] It is the most complex blood group system in humans, with individuals classified as Rh-positive or Rh-negative based on the presence or absence of the major D antigen on their red blood cells. Although more than 55 Rh antigens have been identified, the most clinically significant ones are D, C, c, E, and e antigens.[2]

It is well established that no "d antigen" exists, and RhD negativity is characterized by the

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absence of the D antigen. Production of the D antigen begins as early as 5 weeks of gestation and is completed before birth.[3] Pregnant mothers lacking the D antigen can develop anti-D antibodies upon exposure to even a small amount of RhD-positive allogeneic red cells (as little as 0.1 mL).[4] HDFN occurs when maternal IgG antibodies cross the placenta and destroy fetal or neonatal red blood cells.[5] In rare cases, if a neonate's red blood cells are heavily coated with passively transferred maternal IgG anti-D antibodies, the coating may interfere with agglutination of D-positive red cells when tested with commercial IgM anti-D typing reagents. This can result in a false-negative Rh(D) typing. This phenomenon, known as the "blocked D phenomenon," occurs due to the saturation of D antigen sites by the antibodies, preventing detection by routine serological testing.[6,7]

Here, we present twins with RhD hemolytic disease of the newborn, exhibiting the rare blocked D phenomenon, which resulted in false-negative RhD typing in the neonates.

Case presentation

A 35-year-old second gravida mother delivered dichorionic diamniotic (DCDA) twins at 38 weeks gestation via caesarean section at a local hospital. Her blood group was B Rh negative, while her husband's blood group was O Rh positive. She had received anti-D immunoglobulin prophylaxis following the birth of her first child 8 years ago. There was no history of blood transfusions during the past 8 years, and her first child did not experience jaundice after delivery. The antenatal period was uneventful, but antibody screening was not conducted at the primary healthcare unit. Both the babies were vigorous at birth and weighed 2680 and 2690 grams respectively. Cord blood group of both twins conducted at the primary unit were reported as O RhD negative.

The twin neonates were referred to our hospital at 22 hours of life due to hyperbilirubinemia. On examination, both babies were icteric up to their palms and soles and were noted to be pale. Vital signs were normal. Laboratory findings for Twin A were consistent with HDFN, including indirect hyperbilirubinemia (total bilirubin: 19.8 mg/dL, direct bilirubin: 1.1 mg/dL, indirect bilirubin: 18.7 mg/dL), anemia (hemoglobin: 11 g/dL) with corrected reticulocyte count of 9% (Table I).[8,9] Intensive phototherapy was initiated, and preparations were made for a double-volume exchange transfusion (DVET).

Similarly, Twin B also exhibited manifestations of HDFN, presenting with anemia (hemoglobin: 11.3 g/dL) and indirect hyperbilirubinemia (total bilirubin: 19.9 mg/dL, direct bilirubin: 1.2 mg/dL, indirect bilirubin: 18.7 mg/dL) with a corrected reticulocyte count of 11% (Table I).

The indirect antiglobulin test (IAT) performed on maternal blood was positive, with a titer of 1:512 (Anti Human Globulin – Tulip diagnostics). DVET was performed for both twins using O-negative whole blood, following American Academy of Pediatrics (AAP) guidelines. After exchange transfusion, both babies were administered intravenous immunoglobulin (IVIG) due to rising bilirubin level and packed red blood cell transfusions for anemia (Hemoglobin level was 9.1 and 9.3 g/dL for twin A and B respectively).

The peripheral smear of both twins showed polychromasia, anisocytosis, and nucleated red blood cells (RBCs). Both twins were initially typed as O Rh(D) negative using the conventional tube technique with monoclonal IgM anti-D (Biolab Pvt Ltd). The direct antiglobulin test (DAT), conducted using the gel technique (Tulip Matrix Gel system CC 2400), showed a strong positive result (4+).

The combination of a positive DAT, the mother's RhD-negative status, a positive IAT, and discordant or unexpected RhD typing in the neonate raised suspicion of immune-mediated HDFN, with a potential blocked D phenomenon. Paired samples from the parents and twin babies were sent to the immunohematology

Table I. Laboratory results of Twin A and B in the first 3 days of admission.

Parameter	Reference interval	22 HOL		34 HOL*		42 HOL		56 HOL		72 HOL	
		Twin A	Twin B	Twin A	Twin B	Twin A	Twin B	Twin A	Twin B	Twin A	Twin B
Hemoglobin (g/dL)	19.3 + 2.2	11.0	11.3	-	-	9.1**	9.3**	-	-	-	-
Hematocrit (%)	61 + 7	35.8	36.2	-	-	26.3	27	-	-	-	-
Total bilirubin (mg/dL)	< 8	19.8	19.9	14.2	14.6	15.8#	15.6#	11.6	12.1	8.6	9.9
Direct bilirubin (mg/dL)	< 0.6	1.1	1.2	1.4	1.7	2.1	1.9	2.1	2.2	1.4	1.9
Indirect bilirubin (mg/dL)	< 7.4	18.7	18.7	12.8	12.9	13.7	13.7	9.5	9.9	7.2	8.0
Direct Coombs test	Negative	4+	4+	-	-	-	-	-	-	-	-
Corrected reticulocyte count (%)	3.2 + 1.4	9	11	-	-	-	-	-	-	-	-

Values reported as mean ± standard deviation. The reference values are from: Christensen RD: Expected hematologic values for term and preterm neonates. In Christensen RD, editor: Hematologic Problems of the Neonate, Philadelphia, 2000, Saunders, p. 120.[8], and Wu Alan HB. Tietz Guide to Laboratory Tests. 4th ed. Philadelphia: WB Saunders; 2006.[9]
* Values six hours post double volume exchange transfusion, **Values before packed RBC transfusion, #Values before intravenous immunoglobulin
HOL: hours of life

department for analysis. Neonatal red cells underwent gentle heat elution at 45°C for 10 minutes. Following elution, the red cells were re-typed as RhD positive using the conventional tube technique with monoclonal IgM anti-D. (Biolab Pvt Ltd). At the 6-month follow-up, blood group of both infants were phenotyped as O RhD positive. Written parental consent was obtained for the publication of the case report.

Discussion

HDFN is characterized by an increased rate of RBC destruction. Diagnostic clues indicating hemolysis include an elevated reticulocyte count, unconjugated hyperbilirubinemia, and characteristic red cell abnormalities on the peripheral smear. Hemolysis in newborns can be categorized into immune-mediated and non-immune-mediated causes, based on its etiology. Immune-mediated hemolysis, such as HDFN, results from maternal antibodies targeting fetal RBC antigens. In contrast, non-immune-mediated causes include conditions like alpha-thalassemia major, hereditary spherocytosis, and glucose-6-phosphate dehydrogenase (G6PD) deficiency.[1,5,10]

Using the keywords "Alloantibody, anemia, Anti-D antibody, anti-globulin test, blocking antibody, Coombs test, exchange transfusion, hemolysis, hemolytic anemia" we performed a literature search in PubMed (from 1966 until 2024), EMBASE (from 1966 until 2024), and Google Scholar (from 1960 until 2024) and identified 17 case reports of blocked phenomenon involving D, K, and Fya antigens, whose characteristics are shown in Table II. We have identified 12 cases involving a blocking phenomenon caused by D antibodies, 4 cases related to anti-K and anti-C antibodies, and 1 case involving an anti-Fya antibody.

In the present case, non-immune causes of hemolysis were excluded, as the twins were born to an RhD-negative mother with confirmed RhD incompatibility between the parents, and both babies had a positive direct antiglobulin test (DAT). Initial typing of both twins indicated O Rh(D) negative using the conventional tube technique with monoclonal IgM anti-D (Biolab Pvt Ltd). Given the clinical context and laboratory findings, blocked D phenomenon was suspected. To confirm this, neonatal red cells were subjected to gentle

Table II. Laboratory tests and clinical characteristics of Blocked phenomenon cases published in the literature.

Study	Blood group and Rh typing of mother	Obstetric history	Initial blood group and Rh typing of neonate	Maternal blood test for antibodies	Other antibodies in maternal blood	Infant's blood test for antibodies	Elution method	Blood group, Rh typing of neonate after Elution	Management
Hannon J et al., 2007[11]	NA	NA	NA	NA	Anti-K	NA	NA	NA	NA
Sulochana PV et al., 2008 [6]	B RhD negative	G2P2	B RhD negative	IAT 4+ Anti D 1:1024	Anti-C	DAT 3+ Anti -D 1:512	Heat elution	B RhD positive	3 ET
Moiz B et al., 2008[12]	O RhD negative	G2P1	A RhD negative	IAT strong positive	-	DAT strong +	CDP	A RhD positive	Packed cell transfusions for anemia
Lee E et al., 2009[13]	A Rh D+ R(1)R(1) K negative	G3P3	A, R(1)r K negative	DAT 5+	Anti -K 1:256	DAT 5+	CDP	A, R(1)r K positive	Mild jaundice
Verma A et al., 2013[14]	AB RhD negative	G10P8	O RhD negative	IAT 4+ Anti -D 1:256	-	DAT 4+	CDP	A RhD positive	3 Intrauterine transfusions
Lee E et al., 2015[15]	A, R1R1 K- Fy(a-)	NA	A, R1R1 K-	NA	Anti-Fy^a titre 1:256	DAT 3+	CDP, Flow cytometry, anti-Fy^a MIMI-19	A, R1R1 K-Fy(a+b+)	PT
Jain A et al., 2015[16]	AB RhD negative	P2L2	A RhD negative	Anti -D 1:1024	Anti-C, anti-S	DAT 4+	Acid elution, Heat elution	A RhD positive	3 ET
Wang H et al., 2017[17]	O RhD negative	G2P1	O RhD uncertain	IAT 4+ Anti -D 1:2048	-	DAT 4+	Heat elution	O RhD positive	ET, top up transfusion
Manfroi S et al., 2017[18]	O Rh D positive, R1R1, K-, S+s-, Lu (a-b+)	G2P1L1	O RhD positive, R1R1, K-, S+s-, Lu (a-b+)	-	Anti-K: 1:1024, anti-s (titre 1), anti-Lu (titre 1)	DAT 4+ Anti- K 1:256	Acid elution	O RhD + K+	3 Intrauterine transfusions, PT, 2 top up transfusions
Das S et al., 2019[19]	A RhD negative	G2P1	O RhD negative	IAT 4+	Anti-Le	DAT 4+	Heat elution	O RhD variant type III	IVIG, PT, top up transfusion

*Anti-K 3+, **Both twins had similar results and clinical course, #Rh typing was inconsistent

CDP: chloroquine diphosphate, DAT: direct antiglobulin test, ET: exchange transfusion, IAT: indirect antiglobulin test, IVIG: intravenous immunoglobulin, NA: details not available, PT: phototherapy

Table II. Continued.

Study	Blood group and Rh typing of mother	Obstetric history	Initial blood group and Rh typing of neonate	Maternal blood test for antibodies	Other antibodies in maternal blood	Infant's blood test for antibodies	Elution method	Blood group, Rh typing of neonate after Elution	Management
Subramaniyan R. et al., 2019[20]	B RhD negative	G3P2L1A1	B RhD uncertain[#]	IAT 4+ Anti -D 1:1024	-	DAT 4+	Heat elution	B RhD positive	IVIG, PT, top up transfusion
Mani A et al., 2019[21]	A RhD negative	G3P1L1A1	O RhD negative	Anti -D 1:512	Anti-C 1:32	DAT +	Heat elution	O RhD positive	4 Intrauterine transfusions
	B RhD negative	G2P1L1A0	B RhD negative	IAT 1:32	Anti-C, Anti-E	DAT+	Heat elution	B RhD positive	Intensive PT
Naik A et al., 2020[22]	A RhD negative	G2P1	O RhD negative	IAT 4+ Anti -D 1:1024	Anti-C 1:128	DAT 4+	Heat elution, acid elution, CDP	O RhD positive	ET
Moosavi M et al., 2020[23]	K, Jka negative	G4P3	O RhD negative K, Jka negative	-	Anti-K 1:1024 Anti-Jka 1:4	DAT 2+ DAT C3 3+	Modified gentle heat elution	O RhD negative K positive	ET, IVIG, intensive PT
Novoselac J et al., 2020[24]	A Rh D positive, K negative	G5P3A2	AB RhD positive, K negative	Anti-K 3+	Anti-K 1:32	DAT 3+	Acid elution	AB RhD positive, K positive	PT, 3 top up transfusions
Sil S et al., 2022[25]	A RhD negative	G2P1L1	AB RhD negative	Anti-D 1: 128	-	DAT 4+	Acid elution	AB RhD positive	Intensive PT, IVIG
Thakkar GH et al., 2024[26]	B RhD negative	G4P3L2A1	O RhD negative	Anti -D 1:512	-	DAT 4+	Heat elution	O RhD positive	PT, top up transfusion
Present study**, 2025	B RhD negative	G2P2L1	O RhD negative	IAT 4+ Anti -D 1:512	-	DAT 4+	Saline wash, Heat elution	O RhD positive	ET, IVIG, intensive PT, top up transfusion

*Anti-K 3+, **Both twins had similar results and clinical course, #Rh typing was inconsistent
CDP: chloroquine diphosphate, DAT: direct antiglobulin test, ET: exchange transfusion, IAT: indirect antiglobulin test, IVIG: intravenous immunoglobulin, NA: details not available, PT: phototherapy

heat elution and post elution blood group was confirmed as O RhD positive. The blocked D phenomenon, characterized by the saturation of RhD antigens with maternal IgG anti-D antibodies, was first demonstrated in vitro by Wiener in 1944.[7] The false-negative D typing, or blocked D phenomenon, has been attributed to the prozone effect. This occurs when an excess of antibodies saturates the D antigen sites with anti-D antibodies, preventing their detection. Importantly, the anti-D antibodies do not need to be of a high titer to cause this phenomenon.

Hannon et al.[11] first described phenomenon of blocking antibodies due to anti-K in 2007. Sulochana et al.[6] described a case of blocked D due to a maternal IgM anti-D titer of 32, with an IgG titer of 1,024. In the same year, Moiz et al.[12] described a similar case of blocked D phenomenon, using CDP to elute antibodies.

Similarly, Verma et al.[14] reported a case of blocked D in RhD hemolytic disease of the fetus, where at 20 weeks gestation, the maternal anti-D titer was 256, as determined by the conventional tube technique. Wang et al.[17] and Subramaniyan et al.[20] independently reported cases of blocked D in RhD hemolytic disease of the fetus, where the maternal anti-D titer was 1:1,024. Thakkar et al.[26] reported a case of blocked D in RhD hemolytic disease of the fetus with a maternal anti-D titer of 1:512, closely resembling our case. These cases emphasize that high titers of anti-D can result in the blocking phenomenon, potentially leading to misinterpretation of RhD typing.

The blocking phenomenon is not exclusive to anti-D antibodies. Similar false-negative blood typing results have been reported with other antigens. For example, two cases of false-negative K1 typing of fetal cells were attributed to the blocking effect of maternal IgG anti-K antibodies.

Lee et al.[13] reported a case of blocking of fetal K antigen, and demonstrated that antenatal anti-K1 samples with a titer of 256 or higher can inhibit K1 antigens. Additionally, Lee et al.[15] showed evidence of blocking of Fy antigen sites in a simulated experiment, where high-titer human-murine hybridoma anti-Fy (HIMA-19) antibodies interfered with the detection of Fy antigens. Manfroi et al.[18] reported K-antigen blocking phenomenon in a case of HDFN wherein the maternal anti-K titre was 1:1,024. Moosavi et al.[23] reported a case of HDFN due to anti-K antibodies (titre 1: 1,024) which was diagnosed using a modified gentle heat elution. These cases underscore that the blocking phenomenon can occur with various blood group antigens, posing challenges to accurate typing and diagnosis.

Novoselac et al.[24] reported a case involving anti-K antibodies with a titer of 1:32 that effectively masked K antigens on neonatal red blood cells. The presence of additional alloantibodies, including anti-C, anti-S, and anti-E, in maternal serum alongside anti-D was also documented.[16,21,22] Unlike anti-D antibodies, even a low titer of anti-K can block antigens on neonatal red blood cells. Moreover, the antibody titer does not necessarily correlate with the severity of HDFN.[24]

Das et al.[19] reported a variant D phenotype that mimicked blocked D phenomenon. D variants can be classified into two types: weak D and partial D. Partial D antigen variant lacks one or more of the D epitopes whereas weak D antigen variant has all the D epitopes but are expressed weakly. Individuals with partial D antigens may produce anti-D antibodies because they lack certain D epitopes, while individuals with weak D antigens typically do not produce anti-D antibodies, as the full set of D epitopes is still present, albeit weakly expressed. This distinction is important in the context of RhD typing and immunization risk, as weak D antigens may result in misinterpretation of RhD status, while partial D antigens may lead to alloimmunization.[19]

Upon repeating the Rh typing at six months of life, blood groups of both infants were confirmed to be RhD positive, which was consistent with findings from other studies.

However, this result differed from the report by Das et al.[19], who identified the case as a weak D variant. The weak D variant typically shows all the D epitopes but with weak expression, which can lead to potential misinterpretation of RhD status in certain settings.

Heat elution, chloroquine diphosphate (CDP), and glycine-EDTA are commonly used methods for eluting antibodies from red blood cells. Heat elution involves exposing red blood cells to a temperature of 56°C to release antibodies. Sil et al.[25] used acid elution method to elute antibodies to detect blocked D phenomenon.

CDP, widely used in hematology laboratories, is effective for eluting IgG antibodies while preserving the integrity of the red cell membrane, making it a preferred alternative to heat elution.[27] However, Katharia et al.[28] found that the glycine-EDTA method is more effective than CDP in reducing the strength of the direct antiglobulin test (DAT) reaction and offers greater accuracy.

If unexpected antibodies are detected during pregnancy, their blood group specificity should be identified. A limited-reagent RBC panel can help exclude clinically significant antibodies other than D. Tests for fetal anemia include amniocentesis, cordocentesis, ultrasound, and Doppler assessment of cerebral artery peak velocity.[29] There is limited data on critical titers for non-Rh antibodies in pregnancy. A titer of 64 has been recommended for anti-Fya in HDFN. However, in pregnancies affected by anti-K, antibody titers and amniotic fluid analysis do not reliably predict fetal anemia severity. If there is no fetomaternal ABO incompatibility, maternal serum or infant eluate should be tested against paternal RBCs. Rh-HDFN should not be attributed solely to anti-D, as other alloantibodies may also contribute to hemolysis.[30]

Conclusions

The possibility of the blocking phenomenon should be considered while interpreting blood group results from fetal or neonatal samples in an alloimmunized pregnancy with potent antibodies. A false-negative RhD grouping can occur if maternal IgG antibodies saturate all available antigen sites on fetal red blood cells, preventing the anti-D reagent from binding. In such cases, a thorough clinical history, details of any intrauterine transfusions and the results of previous immunohematological investigations during the perinatal period, are crucial for accurate diagnosis. All pregnant women, regardless of their RhD type, should be tested for clinically significant unexpected serum antibodies during each pregnancy, ideally during their first visit to the obstetrician. Anti-globulin testing with anti-IgG should be performed to detect antibodies causing hemolytic disease of the fetus and newborn.

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Ethical approval

This case report does not require approval of Ethics committee. Written informed consent of parents was obtained. The identity of patients was not disclosed in the manuscript.

Author contribution

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

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Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

Duchenne muscular dystrophy with Kocher-Debre-Semelaigne syndrome: a double jeopardy

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ABSTRACT

Background. Duchenne muscular dystrophy (DMD) is a progressive X-linked dystrophinopathy with onset in early childhood. Affected individuals present predominantly with proximal lower limb weakness and pseudohypertrophy of calf musculature being a prominent sign, heralding the onset of contractures in the large joints of lower limbs. Kocher-Debre-Semelaigne syndrome (KDSS) refers to the muscular pseudohypertrophy that develops in children with long-standing hypothyroidism.

Case presentation. We present an 11-year-old boy with progressive walking difficulty for two years and associated decrease in appetite and chronic constipation. Physical examination revealed mild soft goitre, proximal lower limb weakness, areflexia (except for preserved weak ankle reflex), soft hypertrophy of bilateral calf muscles and latissimus dorsi, with bilateral dynamic ankle joint contractures. Investigations showed moderately elevated total serum creatine phosphokinase (CPK) levels, elevated serum thyroid stimulating hormone (TSH), low free T4, normal free T3 and elevated serum anti-thyroid peroxidase and anti-thyroglobulin antibody titers. A diagnosis of hypothyroidism secondary to Hashimoto's thyroiditis with Kocher-Debre-Semelaigne syndrome (KDSS) (thyroid myopathy) was made while multiplex ligation-dependent probe amplification confirmed DMD. He was started on steroids and levothyroxine. On follow up, he had improvement in activity, appetite and motor movements (North Star Ambulatory Assessment score 3 to 7).

Conclusion. As a very rare coincidence, our patient suffered from two different diseases with similar presentation which are DMD and KDSS. Subtle clinical clues of joint contractures and goitre helped us identify these unrelated co-existing diseases. An alternate diagnosis must be thought of when all clinical findings cannot be explained by a single disease.

Key words: Duchenne muscular dystrophy, Kocher-Debre-Semelaigne syndrome, dystrophinopathy, hypothyroidism, pseudohypertrophy.

Duchenne muscular dystrophy (DMD) is a progressive X-linked dystrophinopathy that results mostly from large deletions affecting the dystrophin gene.[1] Affected children present predominantly with proximal lower limb weakness and pseudohypertrophy of calf musculature with weakness progressing gradually and leading to contractures in the large joints of lower limbs. Kocher-Debre-Semelaigne syndrome (KDSS) refers to the muscular pseudohypertrophy that develops in children with long-standing hypothyroidism.[2] Creatine phosphokinase (CPK) levels are elevated in both conditions, making the differentiation of the two difficult. Both these conditions can lead to worsening of ambulation with cardiac dysfunction being evident in both conditions. Muscle weakness usually improves

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to normal within a few weeks to months after initiation of levothyroxine therapy in thyroid myopathy. Hence, the course of illness and treatment response is a good clinical predictor for differentiation between the two conditions. DMD has a more severe course with gradual worsening of ambulation despite treatment with steroids. We would like to emphasize the importance of a thorough clinical examination to pick clues towards an alternative diagnosis when all clinical findings cannot be explained by a single disease. It is important not to miss the diagnosis of a treatable condition with a high index of clinical suspicion and easily performed thyroid function tests.

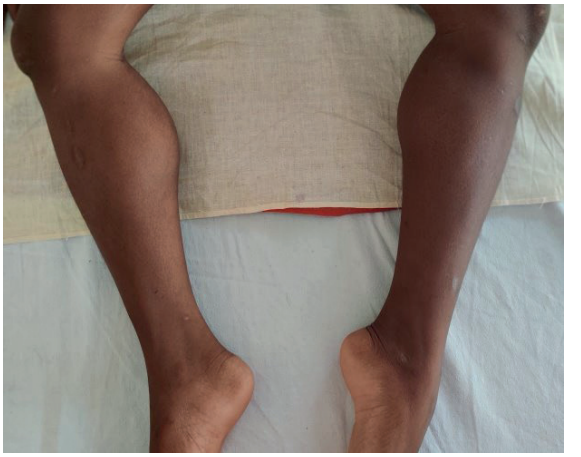

Fig. 1. Bilateral hypertrophy of calf muscles and ankle contractures.

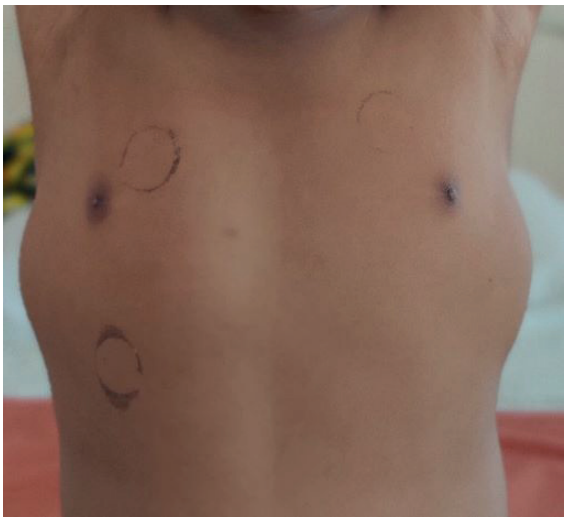

Fig. 2. Bilateral hypertrophy of latissimus dorsi.

Case presentation

An 11-year-old developmentally normal boy with normal intelligence, firstborn to non-consanguineous parents, presented to us with progressive difficulty in walking for two years that gradually progressed to need for support to stand for the past two months. On probing further, a history of frequent falls while walking for the past 5 years was reported by parents, although no medical support was sought then. There was a history of decreased appetite and chronic constipation for 2 years but no muscle pain, dark colored urine, breathlessness, or cold intolerance. There was no relatable family history. On examination, anthropometric assessment was normal for age (weight 37 kg, height 144 cm, body mass index 17.8 kg/m^2). Genital examination revealed Tanner's stage 2 which was normal for age. A soft swelling of the thyroid gland was noted. Lower limb proximal weakness, areflexia (except for preserved weak ankle reflex), hypertrophy of bilateral calf muscles (soft on palpation) (Fig. 1) and latissimus dorsi (Fig. 2) with bilateral dynamic ankle joint contractures were remarkable. Upper limbs were normal. He had a mild truncal weakness. Gower's sign could not be elicited as he could not stand without support. North Star Ambulatory Assessment (NSAA) score at admission was 3. Higher mental functions and intellect were normal on examination. The possibility of muscular dystrophy was considered. Total serum CPK levels were moderately elevated (3979 U/L) with elevated aspartate aminotransferase (AST) levels (205 U/L). Thyroid function tests, performed in view of adolescent age for puberty, goitre or autoimmune thyroiditis, revealed elevated serum thyroid stimulating hormone (TSH, 27.8 μIU/L), low free T4 (0.75 ng/dL) and normal free T3 (3.5 pg/mL). Serum anti-thyroid peroxidase (>1300 units/mL) and anti-thyroglobulin (>500 IU/mL) titers were elevated. A diagnosis of hypothyroidism secondary to Hashimoto's thyroiditis with KDSS (thyroid myopathy) was made. He was started on oral

levothyroxine supplementation at 2 μg/kg/day (50 μg per day). Given the progressive joint contractures, dystrophinopathy was considered the most likely cause of muscular dystrophy . Electromyography revealed slow conduction and low amplitude motor units, a myopathic pattern, which could not differentiate between DMD and KDSS. Multiplex ligation-dependent probe amplification (MLPA) was performed for the Dystrophin gene. It revealed deletion of exons 49 and 50, confirming the diagnosis of DMD. He was started on deflazacort orally at 0.9 mg/kg once a day and regular physiotherapy. Echocardiogram revealed mild left ventricular systolic dysfunction (ejection fraction 50% [normal > 55%]), suggestive of early cardiomyopathy. Though asymptomatic at the time, he was started on enalapril as progression over time was expected. The patient was regularly followed for 12 months. Thyroid function tests at 8 months of follow up revealed normal serum TSH (4.1 μIU/L), normal free T4 (1.94 ng/dL) and normal free T3 (3.3 pg/mL). Serum antibody titres (anti-thyroid peroxidase: 699 units/mL and anti-thyroglobulin 254 IU/mL) had decreased and the thyroid swelling had reduced in size on follow up. The child reported improvement in appetite and in the ability to stand and walk with support, with improvement in NSAA score to 7 at the end of follow up.

Informed consent was obtained from the parents for publication of this report. Genetic counselling was provided to the family regarding the management of the patient and the risk of recurrence in blood relatives.

Discussion

DMD, a relentlessly progressive and life-limiting neuromuscular disease is the most common muscular dystrophy (one in 3500 males worldwide).[1,3] It is an X-linked dystrophinopathy with males most affected in late childhood. The most common pathology is large deletions in the *DMD* gene in about 70 % of patients, and point mutations or partial duplications in few.[1] Affected individuals usually present by five to seven years of age with predominant proximal lower limb weakness which rapidly progresses to truncal weakness. Pseudohypertrophy of muscles seen in our patient is a prominent sign that progresses from soft and flabby to firm and fibrous over time, heralding the onset of contractures in the large joints of lower limbs. The apparent late onset of symptoms of DMD as in our patient may happen when parents do not notice the mild early symptoms. They usually become wheelchair bound by 11-12 years of age and succumb to cardiac or respiratory illness by late teens.

KDSS, on the other hand, is the name given to muscular pseudohypertrophy that develops in children with long-standing hypothyroidism.[2] Its incidence is <10% among patients with thyroid myopathy. It is more common in the age group of 3-10 years. Hashimoto's thyroiditis diagnosed in our patient, is a common cause and has female preponderance, unlike KDSS.[4] Individuals with hypothyroidism may present with lethargy, somnolence, cold intolerance and sometimes depression, or may not have other symptoms of hypothyroidism, making the diagnosis difficult. They can also have symptoms due to muscular involvement like easy fatigability, cramps and stiffness which has been collectively called thyroid myopathy.[5]

Both DMD and hypothyroidism can affect the functioning of the heart. Patients with DMD may develop cardiomyopathy usually by the second decade of life, however, they often are relatively asymptomatic due to limited activity and mobility with disease progression.[6] Hypothyroidism is also associated with cardiac problems, bradycardia, pericardial effusion, reduced cardiac output, reduced ejection fraction (as seen in our patient) and cardiac failure if untreated.[7] Thus, a reduced functioning of the heart does not help to differentiate the two conditions.

CPK levels are usually significantly elevated in DMD. Moderate elevation can be seen later in the course of the disease due to significant

muscle fibrosis, as in our patient. In a previous study, CPK was shown to have a sensitivity and negative predictive value of 100% and specificity and positive predictive values of 91% and 88.8% respectively in DMD.[8]

The CPK levels are also elevated in patients with hypothyroidism, including KDSS, again making it difficult to differentiate the two conditions. Skeletal muscles have type 2 deiodinase enzyme which converts T4 to T3 required for muscle function. Hypothyroidism-induced impaired metabolism causes the accumulation of glycogen, glycosaminoglycans and connective tissue, leading to muscular hypertrophy.[9] Pseudohypertrophy usually occurs in the limbs, tongue and facial muscles and can bring in a diagnostic conundrum as in our case.

According to the American Thyroid Association guidelines, an increased serum CPK or serum lactate dehydrogenase (LDH) persisting for more than 2 weeks, even if asymptomatic, warrants a TSH level to be obtained to rule out thyroid myopathy.[10] Muscle weakness usually improves to normal within a few weeks to months after the initiation of levothyroxine therapy.[2,9] Hence, the best clinical predictor to differentiate between DMD and KDSS is the course of disease and response to treatment.

Our patient represents a rare instance of two distinct conditions with overlapping presentations, DMD and KDSS. Distinction of certain characteristics between the two conditions can aid in establishing a diagnosis (Table I). Both of these conditions can lead to worsening of ambulation. The presence of joint contractures and a just perceptible thyroid gland swelling were the clinical clues that led to a diagnosis of both conditions. DMD has a more severe course with gradual worsening of ambulation despite treatment with steroids. The presenting features of our patient posed a diagnostic challenge, raising the question of whether we were faced with a case of DMD with Hashimoto's thyroiditis or DMD with KDSS. The improvement in our patient's NSAA score from 3 to 7 over 12 months could be due to a combination of steroid therapy, physiotherapy, and appropriate management of the co-existing hypothyroidism. Although the dilemma still prevails, this response represents a more significant improvement than typically expected solely from steroid therapy in DMD, suggesting a multifactorial contribution, including the resolution of hypothyroid-related symptoms. A high index of suspicion and easily performed thyroid function tests help in avoidance of labelling this highly treatable condition as DMD, which carries a poor prognosis until definitive treatments become accessible and affordable. It is important not to miss the diagnosis of a treatable condition that can significantly improve the quality of life of the patient.

Thyroid function tests should be performed in all children with muscle hypertrophy and weakness to diagnose KDSS since it is a treatable cause with a good prognosis. DMD and KDSS can have similar clinical presentations and can rarely co-exist. An alternate diagnosis should be looked for when all clinical findings cannot be explained by a single disease.

Ethical approval

Written informed consent for publication of the child's clinical details was obtained from the concerned parents.

Author contribution

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

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Table I. Features common to and distinguishing between DMD and KDSS.

Features common to DMD and KDSS		
Fatigue and weakness		
Calf muscle pseudohypertrophy		
Proximal muscle weakness		
Elevated CPK levels		
Hyporeflexia/areflexia		
Non specific myopathic pattern on EMG		
Features distinct between DMD and KDSS		
	DMD	KDSS
Progression of symptoms	Progressive difficulty in walking with history of frequent falls points more towards DMD.	Weakness in KDSS is symmetrical between limbs and is usually not progressive, and shows response to treatment in weeks to months
Contractures	More common with DMD	Less likely due to KDSS but can still occur with prolonged muscle weakness.
Truncal weakness	Common in DMD	Not a typical feature of KDSS
Reflexes	Hyporeflexia/ areflexia	Delayed relaxation of reflexes seen
CPK levels	Elevated significantly	Moderate elevation
Cardiac findings	Cardiomyopathy more common in DMD	Unusual in KDSS
Genetic testing	Dystrophin gene deletion	No genetic predilection

Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

A rare association of osteogenesis imperfecta and juvenile idiopathic arthritis: case reports and literature review

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ABSTRACT

Background. Osteogenesis imperfecta (OI) is a genetic disorder of connective tissues caused by an abnormality in the synthesis or processing of type I collagen. The combination of OI and inflammatory arthritis is rare. Our literature review identified 5 cases of OI-related inflammatory arthritis, but only 2 of these cases have been reported in children.

Case Report. We present 3 cases diagnosed with OI and juvenile idiopathic arthritis (JIA). Two were diagnosed with enthesitis-associated arthritis, and one was diagnosed with oligoarticular JIA with laboratory findings and a magnetic resonance imaging examination. Only one of the patients had a previously diagnosed OI. For the others, whole gene sequence analysis was performed, and a mutation in the collagen type I alpha 1 *(COL1A1)* gene was detected. Identifying and treating inflammatory arthritis in our patients with OI improved their joint pain.

Conclusion. Musculoskeletal pain is a common issue in individuals with OI and JIA. Considering children with OI may also develop arthritis, early diagnosis, and accurate treatment may be crucial. Recognizing the rare association between JIA and OI is important, as investigating this relationship could help alleviate the disease burden. Thorough evaluation and prompt diagnosis of JIA in patients with OI can significantly reduce the impact of the disease.

Key words: osteogenesis imperfecta, juvenile idiopathic arthritis, arthralgia, bone mineral density, recurrent fractures.

Juvenile idiopathic arthritis (JIA) is the most common rheumatologic disease in childhood. The incidence and prevalence reported in European and North American populations ranged from 2 to 20 and 16 to 150 per 100,000, respectively.[1] A multifactorial interplay between immune, genetic, and environmental factors shapes the etiopathogenesis of JIA. The primary characteristic of JIA is tissue destruction with joint inflammation.[2] The synovium becomes thickened due to the uncontrolled proliferation of various cells, including synoviocytes and immune cells such as B cells, T cells, natural killer cells, macrophages, neutrophils, plasma cells, and dendritic cells. These immune cells infiltrate the sub-lining layer of the synovium. This immune response not only contributes to ongoing joint inflammation but also causes long-term structural damage, including cartilage degradation and bone erosion.[3] Disease classification is determined according to the criteria set by the International League of

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Associations for Rheumatology (ILAR) and is divided into seven subtypes.[1]

Osteogenesis imperfecta (OI), also known as "brittle bone disease," is a rare inherited connective tissue disorder. The estimated incidence is one in 10,000 to 20,000 births. It is often a disease caused by pathogenic variants in either collagen type I alpha 1 *(COL1A1)* or collagen type I alpha 2 *(COL1A2)*, which encode components of type I collagen. Recent studies have identified defects in multiple genes that encode proteins involved in the synthesis, secretion, processing, and post-translational modification of type I collagen. Additionally, mutations in proteins that regulate the differentiation and activity of bone-forming cells have been shown to cause OI. For instance, in cases of type I collagen mutations or mutations that affect the proteins involved in its biosynthetic pathway, osteoblasts frequently exhibit enlarged endoplasmic reticulum (ER) cisternae and signs of ER stress. This leads to a decreased secretion of type I collagen into the extracellular matrix, increased mineralization of the matrix, and impaired communication between the matrix and cells. This results in less than normal bone trabeculation, thinner bones and therefore brittle bones.[4] The clinical phenotypes of OI vary from perinatal death to mild forms without fractures. The revised Nosology and Classification of Genetic Skeletal Disorders defines five clinical forms of OI: non-deforming with persistently blue sclera (OI type I), perinatal lethal (OI type II), progressively deforming (OI type III), moderate (OI type IV), and with calcification of the interosseous membranes and/or hypertrophic callus (OI type V). However, patients have some common clinical features, such as an increased risk of fractures, skeletal fragility, and varying degrees of bone deformities.

The disease can affect any tissues that have type I collagen.[5] Joint findings such as arthralgia and deformities due to fractures may be missed if there is an accompanying diagnosis of JIA.

JIA and OI are distinct diseases, each with heterogeneous clinical manifestations. The concurrence of OI and JIA in a patient is extremely rare due to several factors, including the differences in their underlying pathophysiological mechanisms, genetic causes, and specific clinical features, which typically do not overlap.[6] OI involves structural bone abnormalities resulting from mutations in the collagen synthesis pathway, whereas JIA is primarily characterized by autoimmune-induced joint inflammation. While JIA can result in long-term bone damage, primarily through mechanisms of joint inflammation and bone erosion, its primary pathological process is synovitis, which is different from the bone-related issues associated with OI. The absence of significant inflammatory processes in OI, combined with the absence of common genetic pathways, further reduces the likelihood of these two diseases occurring simultaneously. So far, two cases of polyarticular JIA and three cases of seropositive rheumatoid arthritis (RA) have been reported with OI.[6-9] We report three cases of JIA associated with OI and review the literature.

Case Presentations

Case 1

A 17-year-old boy was admitted to our Pediatric Rheumatology Clinic due to hip joint pain and morning stiffness persisting for three years. His medical history revealed seven recurrent fractures of the upper extremities, three of which required surgery. According to the family pedigree, many family members had also experienced recurrent fractures (Fig. 1). During the physical examination, the patient's height was measured at 176 cm (55th percentile) and his weight at 66 kg (35th percentile). The patient exhibited blue sclera, pectus excavatum, restricted motion in the right hip joint, sacroiliac joint pain, positive bilateral flexion abduction external rotation (FABER) and Schober test results, as well as surgical

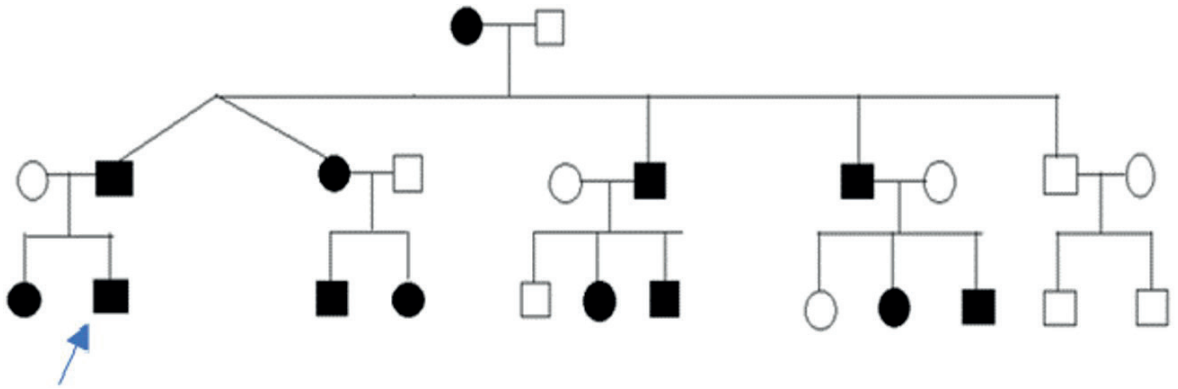

Fig. 1. A pedigree analysis of the family showed that individuals had a history of recurrent fractures and blue sclera.

scar marks on his arms (Fig. 2). The brain stem evoked response auditory (BERA) test revealed mild sensorineural hearing loss. Laboratory parameters included white blood cell (WBC): 7,130/mm^3, hemoglobin (Hb): 13.7 g/dL, platelet (PLT): 223,000/mm^3, erythrocyte sedimentation rate (ESR) 7 mm/hour, and C-reactive protein (CRP): 7.1 mg/L (average: 5 mg/L). He had normal vitamin D, alkaline phosphatase (ALP), parathyroid hormone, phosphorus, and calcium levels. Human leukocyte antigen-B27 (HLA-B27) was positive. Rheumatoid factor (RF) and antinuclear antibodies (ANA) were negative. X-rays of the hip and radius showed a significant decrease in bone density and trabecular prominence (Fig. 3). Bone mineral density (BMD) revealed the Z-score of the lumbar spine (L1-L4) was -3. The patient was diagnosed with OI after a heterozygous pathogenic variant (NM_000088.3) was detected in the *COL1A1* gene c.3749del (p.Gly1250Alafs*81) by whole exome sequencing. Intravenous zoledronate treatment was given every six months. Magnetic resonance imaging (MRI) revealed bilaterally chronic sacroiliitis (Fig. 4). Methotrexate treatment was started with the

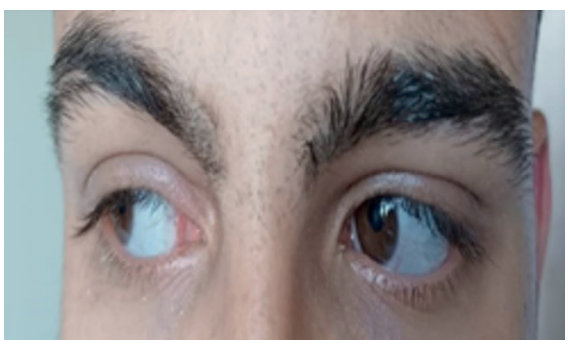

Fig. 2. Blue sclera in our patient diagnosed with OI and ERA.

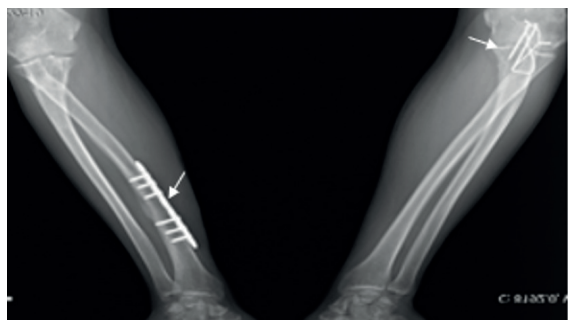

Fig. 3. Implants (arrows) were inserted in previous fracture operations in the left radius and right ulna on x-ray imaging.

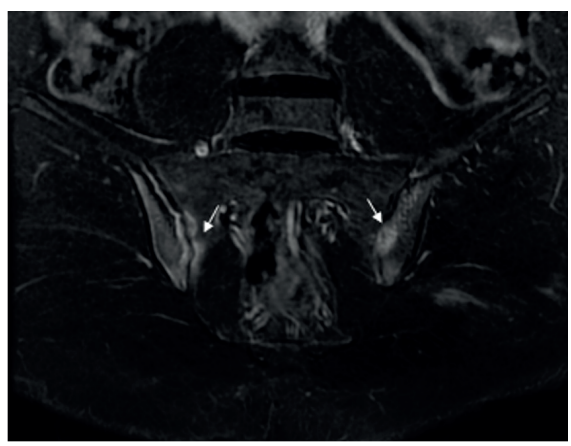

Fig. 4. Postcontrast coronal T1 sequence revealed bilaterally chronic sacroiliitis (arrows).

diagnosis of enthesitis-related arthritis (ERA), and adalimumab was added three months later due to inadequate improvement in symptoms. In the 6th month of rheumatological treatment, the patient's hip pain and morning stiffness subsided, and he continued his daily life easily.

Case 2

A 6-year-old boy was admitted to the Pediatric Rheumatology Clinic with complaints of swelling, pain, and morning stiffness in his right ankle, persisting for one month. His medical history included a history of fractures in the right elbow, the fifth finger of the right hand, and vertebrae at different times. On physical examination, his weight was 18 kg (15th percentile), and her height was 108 cm (3rd percentile). He had blue sclera, swelling, and pain in the right ankle and right knee, and increased heat. His hearing function was normal. Laboratory values included WBC: 10,300/mm^3, Hb: 10.3 g/dL, PLT: 878,000/mm^3, ESR: 66 mm/hour, CRP: 38 mg/L, ALP: 234 U/L, normal parathyroid hormone, calcium and phosphorus levels, and negative ANA. The BMD Z-score of the lumbar spine (L1-L4) was -2.53, and a genetic examination was requested with a preliminary diagnosis of osteogenesis imperfecta. MRI showed dense effusion in the right ankle joint space and tendon sheaths. After other possible causes were ruled out, methotrexate treatment was started with the diagnosis of oligoarticular JIA. Genetic analysis identified a pathogenic, frameshifting, heterozygous novel c.3571delCinsTTCGA (chr17:48264244) mutation in the 48th exon of the *COL1A1* gene, leading to a diagnosis of OI. Intravenous pamidronate treatment was given every three months. In the first year of follow-up, re-swelling and pain developed in the right knee and right ankle. Ultrasonography revealed effusion. Sulfasalazine was added to the treatment, and an intra-articular corticosteroid injection was administered to the knee. Methotrexate dose was increased to 20 mg/m^2/week subcutaneously and sulfasalazine to 1500 mg/day oral dose. Etanercept was added to the patient's treatment, whose arthritis persisted, and inflammation markers remained high in the second year of follow-up. The disease became inactive with etanercept treatment. One year later, he had arthritis in his right ankle. MRI showed effusion, tenosynovitis, and bone marrow edema in the navicular bone. Etanercept was switched to adalimumab. In the fourth year of follow-up, when active arthritis in the right ankle recurred, adalimumab and sulfasalazine treatments were stopped, and tocilizumab was started. Joint pain regressed, and inflammatory markers returned to normal levels.

Case 3

A 7-year-old boy with OI and familial Mediterranean fever (FMF) was admitted to the Pediatric Rheumatology Clinic. He complained of pain and limping in his right hip that had been going on for two years. His medical history included recurrent fractures in the arms, shoulders, and right hip since the age of 1 year. He was treated with pamidronate with the diagnosis of OI. After pamidronate treatment, his bone fracture numbers had decreased. On physical examination, his weight was 21 kg (25th percentile), and height was 117 cm (25th percentile). He had blue sclera, restricted motion in the right hip joint, and sacroiliac joint pain. His hearing function was normal. The laboratory results included WBC: 9,400/mm^3, Hb: 12.1 g/dL, PLT: 200,000/mm^3, ESR of 44 mm/hour, normal CRP, calcium, phosphorus, negative ANA, RF, and HLA-B27. Genetic analysis identified G>T change in intron 1, at the binding site of the specificity protein (Sp) 1 transcription factor of the *COL1A1* gene. The last measured BMD Z-score of the lumbar spine (L1-L4) was -0.6. MRI showed focal bone marrow edema, sclerosis, and erosive changes in the sacroiliac joint surface. He was diagnosed with ERA, and methotrexate was added to the treatment. The hip pain regressed one month later, and inflammatory markers returned to normal. Vertebral X-ray and lumbar MRI performed at the 9th month of treatment were normal. Sacroiliac MRI showed minimal bone

marrow edema in the left iliac wing and marked improvement compared to the previous MRI. Methotrexate treatment was given for one year.

A written consent form was obtained from the families for this publication.

Discussion

The association of OI and JIA was initially described in 2013.[4] The coexistence of these two conditions causes challenges in diagnosis and treatment because managing OI and JIA requires different approaches. Additionally, subtypes of JIA may differ among individuals with OI. Our literature review identified 5 cases of OI-related inflammatory arthritis, as listed in Table I. Only 2 of these cases have been reported in children. The median age of onset of complaints was 3.5 years for OI, 46 years for arthritis, and a 1/4 male/female ratio. Arthritis can occur at any stage of the OI disease. In 2 of the cases, the diagnosis of OI was made earlier than arthritis; in 2 of the cases, the diagnosis of arthritis was made earlier than in OI; and in 1 of the cases, the diagnosis of OI and arthritis were made at the same time. As seen in these cases, it is important to consider JIA when diagnosing OI patients, even if there is a variable temporal relationship and a long-time interval between the diagnosis of the two diseases. More studies are needed to better understand the causes and treatment of the relationship between these two diseases affecting bones and joints.

The management of these two conditions requires multidisciplinary and individualized treatment. Management of OI is primarily supportive and symptomatic, accompanied by physical therapy and orthopedic surgery. The main treatment goals for OI include improving bone strength, decreasing pain, reducing the risk of fractures, improving mobility, and preventing long-term complications. Bisphosphonate therapy is the most commonly used bone-directed pharmacologic treatment.[3] Bisphosphonates were beneficial in the treatment of our patients, in terms of bone pain relief and fractures. Annual bone densitometry is recommended to monitor the effectiveness of treatment.

In JIA, ongoing joint inflammation restricts the individual's ability to function in daily life. Treatment starts with non-steroidal anti-inflammatory drugs (NSAIDs), followed by disease-modifying anti-rheumatic drugs (DMARDs), that are commonly methotrexate, and/or corticosteroid injections. Systemic administration of high-dose corticosteroids has a good short-term effect but does not affect long-term disease outcomes. Moreover, long-term administration is associated with severe side effects, including osteoporosis, immunosuppression, growth suppression, and metabolic effects.[3] Patients should be evaluated every 3 months during treatment until the treatment goal is reached. Methotrexate is one of the most commonly used DMARDs in children. It may cause osteopenia in pediatric patients with malignancy; however, low-dose methotrexate used for inflammatory diseases does not adversely affect bone mass.[10] Biological treatment using etanercept and infliximab in children with JIA leads to a reduction in disease activity. The beneficial effects of treatment with tumor necrosis factor-alpha (TNF-α) antibodies on the skeleton have also been documented. Simonini was the first to demonstrate an increase in bone mass after one year of etanercept treatment in children with JIA. The reduction in bone loss was associated with a therapeutic response and decreased disease activity.[11] Etanercept also promotes linear growth in children with JIA.[12] In a study conducted with adults, there was an increase in bone formation and a decrease in bone resorption during the 12-month treatment of rheumatoid arthritis patients with TNF-α blockers.[13] Therefore, it can be said that biological agents positively affect bone health in OI patients with JIA.

Most cases of OI are caused by mutations in the *COL1A1* or *COL1A2* genes, which encode collagen type 1. The link between collagen and integrins plays a vital role in RA pathogenesis. Furthermore, endoplasmic reticulum stresses,

Table I. Review of cases of osteogenesis imperfecta and inflammatory arthritis.

Authors, years & reference	Gender	Age at diagnosis of OI	Age at diagnosis of JIA/RA	Onset types of arthritis	Joint manifestations	Genetic result	BMD Z-score	Laboratory tests
Bica et al. 2013[6]	F	53 years	15 years	Polyarticular JIA	Symmetric polyarthritis of large and small joints and temporomandibular joint involvement	NA	NA	Elevated ESR, RF (+)
Damian et al. 2020[7]								
Case 1	F	39 years	46 years	Seropositive RA	Joint pain and stiffness in lower limbs	*COL1A1* heterozygous pathogenic variant, c.3399del, p.Ala1134Profs*105	NA	Elevated ESR and CRP
Case 2	F	In adolescence	70 years	Seropositive RA	Joint pain in knees, ankles, and shoulders	*COL1A1* heterozygous pathogenic variant, c.3399del, p.Ala1134Profs*105	NA	Elevated ESR and CRP
Emna et al. 2022[8]	M	15 years	8 years	Polyarticular JIA	NA	NA	-4.2	Normal CRP
Mormile et al. 2022[9]	F	43 years	43 years	Seropositive RA	NA	Pathogenic heterozygous missense variant in *COL1A1* gene, NM_000088:c.769G>A, p.(Gly257Arg) (rs72645321; HGMD ID: CM960320)	NA	NA
Present case 1	M	17 years	17 years	ERA	Limitation of range of motion and joint pain in sacroiliac	Pathogenic heterozygous variant in *COL1A1* gene, NM_000088.3, c.3749del, p.Gly1250Alafs*81	-3	Normal ESR and CRP, positive HLA-B27
Present case 2	M	6 years	6 years	Oligoarticular JIA	Joint pain and swelling in knee and ankle	Pathogenic heterozygous variant in *COL1A1* gene, c.3571delCinsTTCGA (chr17:48264244)	-2.53	Elevated ESR and CRP
Present case 3	M	3 years	7 years	ERA	Limitation of range of motion and joint pain in sacroiliac	G>T mutation of intron 1 at the binding site of the Sp1 transcription factor of the *COL1A1* gene	-0.6	Elevated ESR

BMD, bone mineral density; CRP, C-reactive protein; ERA, enthesitis-related arthritis; ESR, erythrocyte sedimentation rate; HLA, human leukocyte antigen; JIA, juvenile idiopathic arthritis; NA, Not available; OI, osteogenesis imperfecta; RA, rheumatoid arthritis; RF, rheumatoid factor; SI, sacroileitis.

possibly caused by misfolding or excess proteins, have been described in both RA and OI, which can activate inflammation by driving cells to apoptosis or autoantigen formation.[14] These findings may lead us to believe systemic inflammation plays an important role in developing OI and RA. According to laboratory findings in the available literature, inflammatory markers were elevated in 3 (60%) patients but not in 1 (20%) patients. In our patients, inflammatory markers were elevated in 2 patients yet normal in 1 patient.

However, a reciprocal interaction exists between bone remodeling and inflammatory pathways in OI and RA. In the OI mouse model, the pro-inflammatory cytokines TNF-α and interleukin-1α are elevated, splenomegaly and increased osteoclast progenitors in the spleen suggest chronic inflammation.[15] Monocytes in OI highly express TNF-α and synthesize the receptor activator of the nuclear factor kappa B (RANK) ligand (RANKL), which is an important factor of bone erosions in RA.[16] In addition, It is conceivable that extracellular matrix irregularity due to structural abnormalities and repeated traumas, including fractures, may trigger arthritis in OI, similar to the onset of post-traumatic arthritis.[17]

Fracture risk increases in both OI and JIA by different mechanisms. Spontaneous fractures occur in OI patients with deterioration of the bone matrix. On the other hand, fractures in JIA may occur due to increased inflammatory cytokines, decreased secondary bone mass, physical inactivity, or osteoporosis caused by corticosteroids.[18] JIA is an important differential diagnosis in patients with spontaneous fractures. Patients with OI generally do not have elevated inflammatory markers or autoantibody positivity, and clinical symptoms include blue sclera, arthralgia, joint hypermobility, and tendon rupture.[19] OI can lead to deformities in the hands characterized by swan neck deformities and reversible contractures, and this condition can be confused with JIA.[20]

Patients with OI and JIA may have joint manifestations such as arthralgia and deformities due to fractures caused by OI. This may cause the diagnosis of JIA accompanying OI to be missed. Therefore, we believe it is important to be aware of the coexistence of these two diseases. The presence of morning stiffness and synovitis detected in our cases and the positive response to disease-modifying anti-rheumatic drugs (DMARDs) and biological treatments support the accuracy of our JIA diagnosis.

Since the association of JIA and OI has rarely been reported, we think this association is coincidental. Further studies are needed to understand the relationship between these two diseases better. It should be remembered that joint inflammation may develop in addition to existing bone pathology in patients diagnosed with OI. Evaluating and monitoring this condition is essential to determine the appropriate management and treatment approach. Therefore, in patients diagnosed with OI with joint complaints, a joint MRI examination should be performed in addition to a plain X-ray. In our patients, finding and treating JIA with OI improved joint pain. Because musculoskeletal involvement affects the quality of life in these patients, increasing awareness of the possible relationship between OI and inflammatory arthritis may help improve the quality of life.

Ethical approval

The study was approved by Uludağ University Ethics Committee (date: 07.02.2024, number: 2024-1/26). Informed consent was obtained from the parents of the child.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: HKZ, SSK; data collection: HKZ, TA, AUB, SS; analysis and interpretation of results: HKZ, SSK, OK;

draft manuscript preparation: HKZ, SSK. All authors reviewed the results and approved the final version of the manuscript.

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The authors declare that there is no conflict of interest.

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Case Report

A giant ovarian mucinous cystadenoma in a postmenarchal adolescent girl

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ABSTRACT

Background. Giant mucinous cystadenomas are rare in adolescents and young adults.

Case presentation. We report a mucinous cystadenoma in a 16-year-old postmenarchal girl presented with abdominal distention and pain, and elevated serum CA-125 levels. Radiological evaluations showed a large cystic mass originating from the right ovary. The patient underwent successful surgery with complete resection of the tumor without rupture and the histopathological examination confirmed the diagnosis of a benign mucinous cystadenoma.

Conclusion. The case emphasizes the importance of early diagnosis and the need for total surgical resection without rupture to ensure a favorable outcome in such cases and close follow-up is recommended.

Key words: mucinous cystadenoma, ovary, adolescent, abdominal mass.

Ovarian tumors frequently occur in the second decade of childhood and the majority are germ cell tumors.[1-5] Epithelial tumors account for 15-20% of pediatric ovarian tumors and they are mostly benign. The most common type of benign epithelial ovarian tumor is cystadenoma, with approximately three-quarters being serous and one-quarter mucinous.[3,6,7] Mucinous cystadenomas (MCA) are quite rare in children and adolescents.[7-9]

Almost all cases of MCA commonly present with complaints of abdominal distention and pain due to a rapidly growing large cystic mass.[2,3,6,7,10] The goal of therapeutic surgery is the complete removal of the mass without rupture, preserving normal ovarian tissue whenever possible.[8,11] We present here a postmenarchal adolescent patient with a giant mucinous cystadenoma of the ovary that was completely resected.

Case Presentation

A 16-year-old postmenarchal girl was admitted to the pediatric emergency department with one-week history of progressive abdominal distention and periumbilical colicky pain; no history of constipation, diarrhea or vomiting was noted, and she was otherwise in good health. The patient's most recent menstrual period was 45 days earlier, but she mentioned

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a small amount of spotting every day. On physical examination, the abdomen was tense and dull to percussion and markedly distended by a huge mass extending from the pelvis to the epigastric area; the bowel sounds were diminished, and no tenderness was noted. Initial laboratory examinations revealed normal complete blood count, serum biochemistry, alpha-feto protein and beta-HCG; serum CA-125 level was elevated at 168.4 U/mL (normal <35 U/mL). An abdominal ultrasound showed a huge multilocular cystic mass extending from the pelvis to the epigastric area which was considered to originate from the right ovary causing dilatation in the right renal collecting system. An abdominal magnetic resonance imaging (MRI) revealed a 28.5×21.1×12.2 cm cystic mass originating from the right ovary with septations; no solid component was detected (Fig. 1).

The patient underwent laparotomic oophorectomy, and the huge abdominal mass was totally resected without rupture. Abdominal exploration showed that the left ovary, tuba, and uterus were intact (Fig. 2). The patient had an uneventful postoperative course and was discharged. A gross examination of the resected mass revealed a 27.5×21.5×13 cm cystic mass with an intact capsule and multiple cysts filled with mucinous fluid; solid or papillary components were not detected in the walls of the cysts. The histopathologic examination reported a mucinous cystadenoma with seven reactive sampled lymph nodes (Fig. 3). Cytological examination of the peritoneal fluid showed mesothelial cells, macrophages, and lymphocytes with no tumor cells.

The patient was followed closely, and no further intervention or treatment was needed. The serum CA-125 level was normal in the

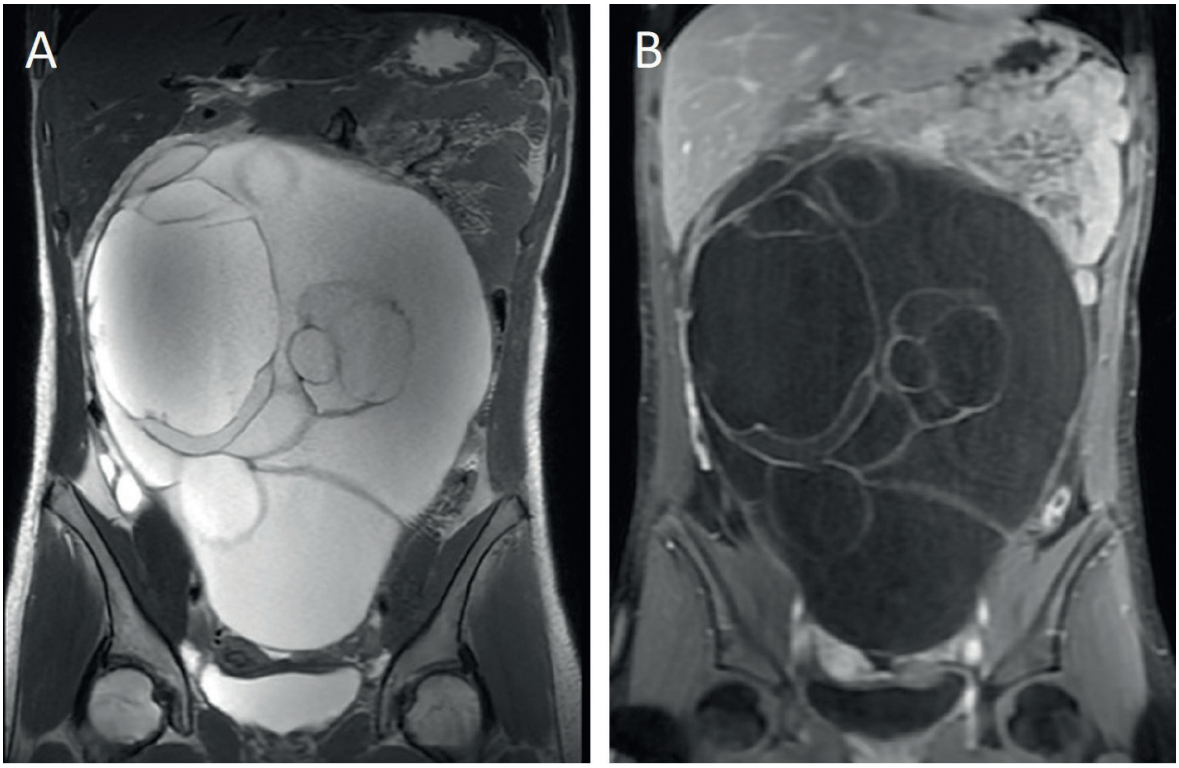

Fig. 1. (A) Coronal T2-weighted MR image shows a large multiloculated cystic lesion with septations. **(B)** Coronal post-contrast T1-weighted MR image demonstrates contrast enhancement of thin septa. There is no evidence of a solid mural component.

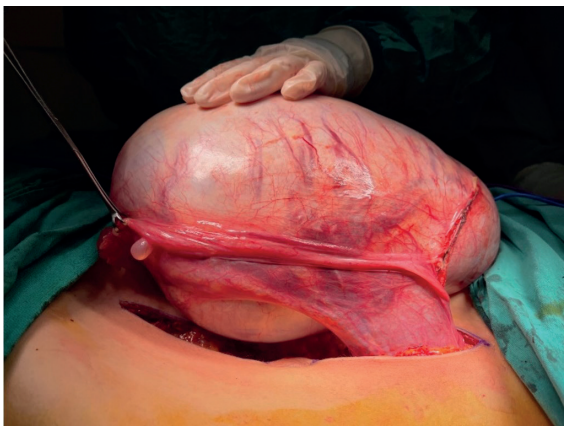

Fig. 2. Intraoperative findings show a right ovarian mass of 27.5×21.5×13 cm, which was resected totally without rupture (laparotomy).

third postoperative week with a value of 30.3 U/mL. Postoperatively, she had no major complaints and her menstrual periods were regular for the 18 months after surgery. The most recent abdominal ultrasound showed no evidence of residual or recurrent tumor, and a right oophorectomy with normal findings. The patient is under regular follow-up for 20 months after surgery.

Informed consent for the publication was obtained from the patient and her parents.

Discussion

Ovarian tumors are rare in the pediatric population, accounting for only 1% of all pediatric malignancies, with three-quarters being benign.[5,12,13] Epithelial neoplasms form a minority of ovarian tumors in childhood, the incidence increases with age, and most occur in the postmenarchal period suggesting that epithelial ovarian tumors are stimulated by hormones.[3,4,8,10] Most ovarian epithelial tumors are cystadenomas, mucinous types being less common than the serous and the vast majority are benign.[3,6,14-17] In this report, we present a postmenarchal patient with typical clinical, radiological, and pathological characteristics of MCA.

Patients with MCAs commonly present with a variety of clinical symptoms caused by the tumoral mass, such as increased intra-abdominal pressure, distention, pain, vomiting, constipation, pressure on the visceral structures, and sometimes ovarian torsion and rupture.[3,5-7,10] Almost all patients have a palpable abdominal mass on examination. Our patient had progressive abdominal distention and pain owing to the rapidly growing tumor in accordance with the literature.

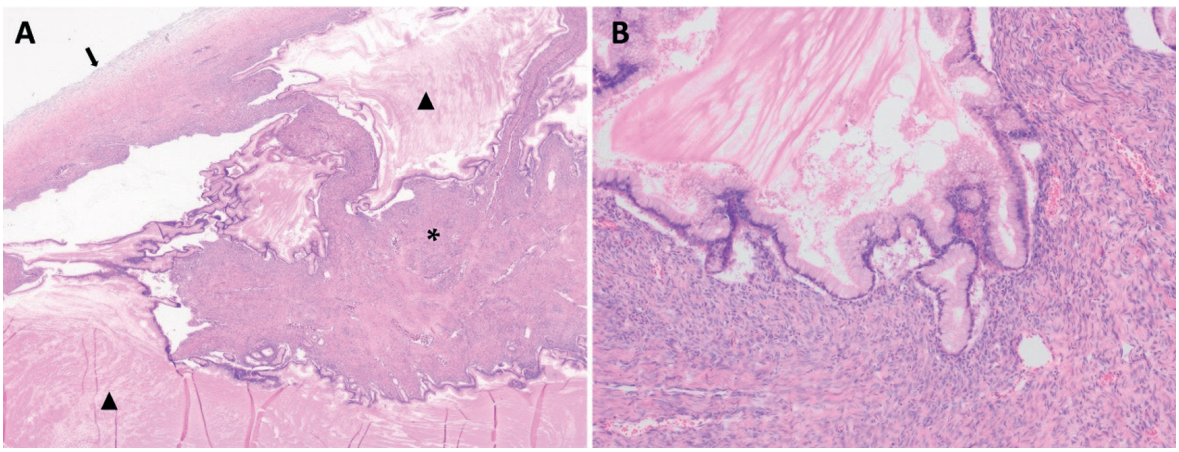

Fig. 3. Histopathological images. **(A)** Ovarian mucinous cystadenoma with large cysts filled with abundant mucin (arrowheads) and an intact capsule (arrow); multicystic neoplasm harbors ovarian stroma (asterisk) in numerous septae (HE, original magnification x25). **(B)** Cysts lined by non-stratified / single layered mucinous epithelial cells without prominent cytologic atypia (HE, original magnification x200).

Although most pediatric ovarian masses are benign, early diagnosis is important to prevent complications like ovarian torsion or rupture and to rule out malignant pathologies. Transabdominal ultrasonography is the initial imaging modality to evaluate abdominal masses in the pediatric population. Magnetic resonance imaging or computed tomography are other modalities for further evaluation, differential diagnosis, and management decisions. Radiological assessment of tumor size and complexity may provide valuable insights for stratifying the risk of malignancy in pediatric ovarian tumors prior to histopathological evaluation.[10,18]

Mucinous cystadenomas appear as a large cystic mass, and the majority are multilocular, characterized by a gelatinous intracystic fluid rich in mucus. The imaging characteristics of MCAs closely reflect their gross pathological features. On cross-sectional imaging, MCA is typically a large unilateral multilocular cystic mass with thin septations and heterogeneous internal components, and also the variable appearance of the cyst fluid owing to differences in the mucin content. Features commonly associated with malignancy include a mass size greater than 10 cm and the presence of solid components, whereas the presence of a simple cyst is highly suggestive of benign pathology.[10,15,18]

Reports suggest that MCAs are usually unilateral but can grow large, with a mean size of 16-20 cm.[12,16] The tumors reported in adolescent patients by Biçer et al.[19], Cevik et al.[3] and Vizza et al.[20] had maximum diameters of 45, 40 and 40 cm, respectively. Watanabe et al.[21] reported the largest mucinous cystadenoma case in the pediatric literature with a weight of 11.8 kg. Unfortunately, for our patient the tumor weight was not recorded, but, the largest diameter was measured as 27.5 cm which might be one of the largest tumors reported.

Mucinous ovarian tumors are histopathologically divided into 3 groups: MCA (70%), borderline MCA (10%), and mucinous carcinoma (20%).[12,18] MCAs are the most common, usually unilateral, and benign. Microscopically, the tumor consists of cystic spaces lined by tall columnar epithelium with mucinous differentiation.[12,18] Consistent with common MCAs, our patient had a mucinous multicystic tumor filled with abundant mucin. Cysts were lined by a single layered epithelium without any complex feature and there was no solid area in the cysts' walls (Fig. 3).

Serum tumor markers are important in the diagnosis and follow-up of ovarian tumors, elevated levels are usually associated with malignant pathologies.[13,22] In our case serum beta-human chorionic gonadotropin (HCG) and α-fetoprotein (AFP) levels were normal, but the CA-125 level was high in the preoperative tests and decreased to normal levels postoperatively. Li et al.[10] reported that CA-125 was elevated in 27/58 adolescents with ovarian mucinous tumors. The serum tumor marker CA-125 is specific for epithelial differentiation and plays an important role in the diagnosis and follow-up of epithelial ovarian cancers. The serum CA-125 level can be elevated owing to increased production by the cancer cells which is released from the damaged tumor epithelium at sites of adhesions and epithelial shearing stresses. The levels may also be elevated due to irritation, inflammation, or mechanical stretch of the peritoneal surfaces.[23,24] In our patient, the grossly enlarged tumor may have stretched the entire peritoneum, potentially contributing to the elevated serum CA-125 levels. Similar cases have been reported who had very large benign ovarian tumors and elevated serum CA-125 levels.[3,19,25] In our patient, despite the elevated serum CA-125 level, histopathological characteristics led to a diagnosis of benign MCA.

Ovarian epithelial tumors in children and adolescents are usually unilateral and benign. Ovarian surgery in children may affect future fertility owing to the removal of the normal ovary or adhesion formation. The current recommended treatment is typically unilateral oophorectomy or salpingo-oophorectomy or

cystectomy. If identified and possible, normal ovarian tissue should be preserved.[14] The size of the tumor, while not indicative of malignancy, combined with the concern for recurrence often leads the surgeon to perform an oophorectomy.[8]

During surgery, the large tumor mass should be removed in its entirety without rupture. It is not always possible to accomplish ovary sparing surgery, since MCAs are commonly too big. Cowan et al.[8] reported that a significant number of adolescents were treated with a minimally invasive, ovary-sparing approach for large benign MCAs, and this approach was feasible and safe with no evidence of recurrence. In our patient, the tumor was very large, and it was not possible to identify the normal ipsilateral ovary. So, the tumor was removed totally without rupture.

The outcome of MCA is favorable when the tumor is removed without rupture. In the literature, there have been limited cases of recurrent MCA.[11,14,25] Intraoperative rupture or spillage of the cyst contents might increase the risk of recurrence. Ben-Ami et al.[11] reported recurrence in three patients who experienced intraoperative cyst rupture. During surgery, the condition of the contralateral ovary is also important. If the opposite ovary is grossly normal a biopsy is not indicated. Postoperatively, the patients need to be under follow-up regardless of tumor type due to the possibility of recurrence.[11,25] If tumor markers are elevated before surgery, they can be included in the follow-up investigations.

In conclusion, ovarian mucinous cystadenomas are rarely seen in pediatric patients and radiological evaluations play a crucial role in diagnosis, staging, and treatment planning. Total surgical resection without rupture is the mainstay of management. The patients need close follow up after the surgery.

Ethical approval

Informed consent was obtained from the patient and her parents for the publication.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: BA, SE, HNÖ, MÜ, EA, ÖT, BY; data collection: BA, SE, HNÖ, MÜ, EA, ÖT, BY; draft manuscript preparation: BA, BY. All authors reviewed the results and approved the final version of the article.

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The authors declare that there is no conflict of interest.

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Diabetes care: role of the adolescent medicine specialists

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To the Editor,

We read with great interest the article titled "Adaptation of the Problem Areas in Diabetes-Teen [PAID-T] Scale into Turkish and examination of its psychometric properties: a validity and reliability study" recently published in your esteemed journal.[1] The authors provide an insightful discussion on diabetes distress in adolescents with type 1 diabetes mellitus (T1DM), emphasizing the reliability and moderate validity of the Turkish PAID-T scale. We commend the authors for their effort to adapt a validated tool to the Turkish context, which will undoubtedly contribute to improving psychosocial care in pediatric endocrinology.

As the study demonstrated, adolescents with T1DM frequently experience emotional distress linked to concerns about diabetes-related complications, feelings of isolation, and the pressure to adhere to strict treatment regimens. This distress not only affects their mental health, with higher rates of anxiety and depression, but also impairs their ability to manage their condition effectively, as adolescents experiencing higher levels of emotional stress demonstrate poorer glycemic control.[2] While the study effectively highlights diabetes distress as a critical issue, it does not address the broader role that adolescent medicine specialists can play in managing interconnected challenges. Adolescent medicine specialists are uniquely positioned to address the multifaceted needs of adolescents with chronic illnesses. By incorporating screening for mental health conditions such as depression and eating disorders, addressing challenges related to adherence to treatment challenges, and evaluating readiness for transition into adult care, we can provide a holistic approach that complements the work of pediatric endocrinologists. Instruments like the Turkish PAID-T, along with the Diabetes Eating Problem Survey[3], can serve as valuable tools within this broader framework, helping to identify diabetes distress as part of a comprehensive assessment of adolescent health. By recognizing the mental health challenges associated with diabetes, we can create a caring environment that supports adolescents more effectively, improving their overall quality of life and diabetes outcomes.

To strengthen the impact of future research, we recommend exploring how diabetes distress interacts with other psychosocial and behavioral factors. Additionally, collaborative efforts between pediatric endocrinologists and adolescent medicine specialists could enhance the implementation of targeted interventions, ensuring that adolescents with T1DM receive the support they need during this critical developmental period.

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Thank you for the opportunity to contribute to this important conversation.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: ESK, MPK, SA; draft manuscript preparation: ESK, MPK, SA. All authors reviewed the results and approved the final version of the article.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Improving linear discriminant analysis effect size analysis to enhance its reliability in small sample sizes

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Dear Editor,

With great interest, I read the recent article entitled "Characterization of lingual microbiota in pediatric geographic tongue" published in The Turkish Journal of Pediatrics.[1] The study provides valuable insights into the potential role of oral microbiota dysbiosis in the pathogenesis of geographic tongue in children. I would like to comment on the use of Linear discriminant analysis Effect Size (LEfSe) for analyzing the microbiota data and the considerations when applying this method to small sample sizes.

LEfSe is a Python-based software tool that integrates statistical testing with biological consistency estimation to identify features that are significantly enriched in one or more investigator-defined groups.[2] While LEfSe has been widely employed in thousands of microbiome studies, recent research indicates that the method is susceptible to false positives.[3] LEfSe does not perform false discovery rate (FDR) correction, leading to the identification of a large number of false positives in the absence of a distinguishing signal. This can mislead research conclusions and reduce the reliability of the study. Cho et al. also discovered that LEfSe frequently exhibits type I errors exceeding 5%, indicating a potential to erroneously identify non-significant genes as differentially expressed.[4] LEfSe method exhibits instability in sparse datasets, being susceptible to the degree of data sparsity.[5] Therefore, caution is warranted when interpreting results obtained from LEfSe analysis of sparse microbiome data, and appropriate validation is necessary.

If the authors intend to retain this approach, it is advisable to acknowledge the limitations in the interpretation of the results as a potential weakness. Additionally, it should be noted that the methodology section of the paper does not reference the application of FDR correction. Given that LEfSe is employed, it is strongly recommended to implement FDR correction to mitigate the risk of false positives. To mitigate potential biases inherent in single-method analyses, LEfSe-derived results should be rigorously cross-validated with outcomes from complementary approaches, such as ANCOM-BC (for compositionally aware analysis) and ALDEx2 (for sparse data robustness).[6,7] Consensus features identified across these independent frameworks are prioritized as high-confidence biomarkers, thereby reducing false discovery risks. Furthermore, to enhance the robustness of the findings, it is recommended to reuse publicly available data by downloading similar cohort data from NCBI SRA or EBI MGnify to expand the sample size.

In conclusion, this study revealed the association between pediatric geographic tongue and dysbiosis of the lingual microbiota, providing new insights into the pathogenesis of geographic tongue. While LEfSe is a valuable tool for microbiome analysis, its limitations,

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particularly in small sample sizes and sparse datasets, necessitate careful interpretation and complementary validation.

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Conflict of interest

The authors declare that there is no conflict of interest.

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SHORT COMMUNICATIONS

CASE REPORTS

LETTERS TO THE EDITOR

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