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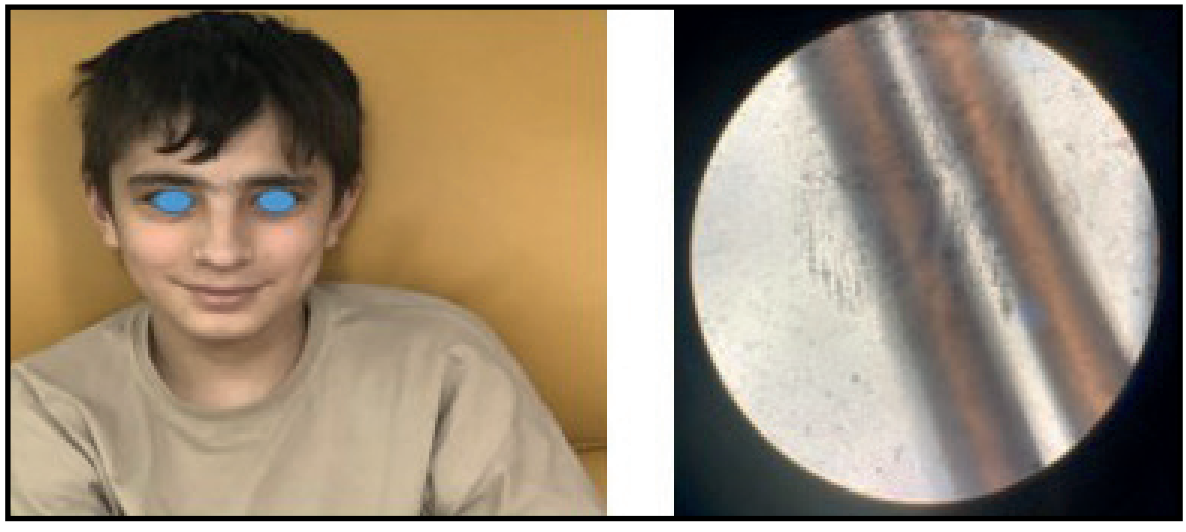

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Prof. Ahmet Anık, MD
Department of Pediatrics, Division of Pediatric Endocrinology, Aydın Adnan Menderes University, Medical Faculty, Aydın, Türkiye
ahmet.anik@adu.edu.tr - https://orcid.org/0000-0002-7729-7872

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Review

Exploring the role of artificial intelligence (AI) in pediatric dermatology for underserved communities in the United States

Jasmine Jaing[1], Sandhya J. Kadam[2]

[1]School of Osteopathic Medicine, A.T. Still University, Mesa, USA
[2]Pediatrics Department, Family HealthCare Network, Visalia, USA

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ABSTRACT

Skin rashes and lesions are among the most common health concerns in pediatric patients, yet access to pediatric dermatologists remains limited. This shortage contributes to delays in clinical diagnoses, suboptimal treatment, and reduced quality of life for affected patients. Artificial intelligence (AI), particularly image-based diagnostic and decision-support tools, offers a promising approach to augment care delivery in regions where specialist access is scarce.

AI has the potential to improve diagnostic accuracy and enhance triage for common pediatric skin conditions. By assisting clinicians in identifying disorders and recommending evidence-based management pathways, AI can reduce the time to intervention. Recent studies have demonstrated that AI can achieve diagnostic accuracy comparable to that of specialists and improve clinician performance when used as a support tool. To ensure these benefits are equitably distributed, however, AI tools must be designed with attention to social determinants of health, including disparities in digital access, bias in algorithm training, and the need for cultural competence.

This review examines the current applications of AI in pediatric dermatology, with a focus on its role in rural and primary care settings. It also explores how AI intersects with the social determinants of health and the psychosocial well-being of children, including stigma, mental health, and quality of life. Equitable design and careful implementation will determine whether AI can effectively assist pediatric dermatologic care or exacerbate existing disparities.

Our analysis draws on peer-reviewed literature and publicly available AI tools from PubMed and Google Scholar, using Boolean operations.

Keywords: Artificial intelligence (AI), pediatric dermatology, social determinants of health, quality of life, underserved communities, health equity

INTRODUCTION

Pediatric skin conditions, such as atopic dermatitis, acne, and warts, are a frequent complaint in primary care visits, accounting for up to 30% of encounters in some studies. Despite this high demand, access to pediatric dermatologists remains scarce across the United States.[1] Contributing factors include a small number of training programs, financial disincentives, geographic maldistribution, and long wait times. Pediatric dermatology only became a formally recognized subspecialty in 2004 and has since struggled to grow due to both workforce shortages and economic constraints. Many pediatric residents who develop an interest in dermatology find they are not competitive for dermatology residency. In contrast, dermatology residents are often discouraged from pursuing pediatric subspecialization due to lower reimbursement compared to fields like cosmetic dermatology.[2]

✉ Jasmine Jaing ▪ sa212442@atsu.edu

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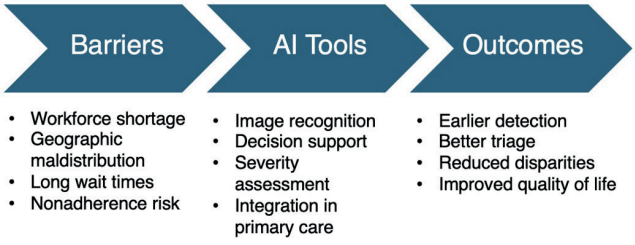

Figure 1. AI in pediatric derm conditions in underserved communities[1-20]

Structural barriers further compound this shortage. Insurance restrictions and long travel distances disproportionately impact underserved communities, particularly Medicaid patients. While many dermatologists are listed as Medicaid providers, 44% decline to accept new pediatric patients.[3] Pediatric dermatologists also tend to cluster in urban areas, leaving patients in rural areas with longer travel times and longer wait times. For example, the average clinic wait time exceeds six weeks, with 25% patients waiting over 10 weeks.[4] Families traveling more than 20 miles were over seven times more likely to be nonadherent to treatment plans, resulting in worse health outcomes.[5] These systemic barriers highlight the urgent need for innovative strategies to expand access to dermatologic care for children, especially in underserved communities (Figure 1).

MATERIALS AND METHODS

For this review, we searched the electronic PubMed/Medline and Google Scholar databases for peer-reviewed articles on AI and pediatric dermatology, preferably published within the last 5 years. The keywords used were: "Artificial intelligence (AI), pediatric dermatology, social determinants of health, quality of life, underserved communities, health equity." AND, OR, and NOT Boolean operators were used to refine searches by combining or excluding keywords. As a result, a total of 45 articles were identified. The aim was to synthesize existing evidence and offer critical analysis and insights to inform providers. The papers were evaluated based on their titles, abstracts, and complete texts, with the primary inclusion criteria being the description of AI use in dermatology. The most important exclusion criterion was that the article addressed diseases other than AI use in skin conditions. Papers that were not written in the English language were also excluded. An independent search was performed by all authors in the mentioned databases. Disagreements in the selection of literature were resolved through discussion and consensus among authors. Inter-rater agreement was not formally quantified, but it was achieved before inclusion. The included articles met all inclusion and exclusion criteria, including those written specifically for children under 18 years of age. One author reviewed the reference lists of each retrieved article to identify other articles that could be selected per the eligibility criteria. The main author began collecting data by reviewing the literature in January 2025. However, the final review of data sources was conducted in June 2025 by both authors. The review included 20 articles after the final evaluation. Please refer to Table 1 for the main article conclusions.

AI diagnostic applications

Artificial intelligence (AI) describes a system of computers that uses innovative algorithms and computational models to simulate aspects of human thinking and analysis. With recent advancements in big data, processing power, and algorithm development, AI is being increasingly integrated into everyday life, with promising abilities in healthcare. From identifying at-risk patients to preventive care screenings, AI has the potential to expand clinicians' reach while improving efficiency. Healthcare providers must balance the application of AI to ensure it complements, rather than replaces, the human touch in patient care.[6]

AI has the potential to both alleviate and exacerbate health inequities. This is impacted by factors including access, trust, dehumanization, autonomy, bias, and community effects, as summarized in Table 2.

These factors are especially relevant for rural and underserved communities, which are already disproportionately affected by limited access to specialists. When this is taken into consideration, AI can extend dermatologic expertise into settings where pediatric dermatologists are scarce.[7]

Several studies illustrate the promise of AI in pediatric dermatology. A 2021 randomized clinical trial investigated how AI tools can help primary care providers accurately diagnose skin conditions. The AI model used a convolutional neural network to assess over 16,000 images across almost 420 skin conditions, with diagnoses confirmed by

Table 1. Summary of literature review

	Article	Authors	Conclusion
1	Pediatric dermatology: past, present, and future	Prindaville B, Antaya RJ, Siegfried EC.	Pediatric dermatology workforce shortage is due to limited training pathways and geographic maldistribution
2	Geographic distribution and characteristics of the pediatric dermatology workforce in the United States	Sinha S, Lin G, Zubkov M, Wu R, Feng H.	Majority of the pediatric dermatology workforce is clustered in urban regions
3	Pediatric access to dermatologists: Medicaid versus private insurance	Chaudhry SB, Armbrecht ES, Shin Y, et al.	Pediatric patients with Medicaid have reduced access to dermatologists
4	Pediatric dermatology workforce in the United States	Prindaville B, Horii KA, Siegfried EC, Brandling-Bennett H.	Geographic maldistribution of pediatric dermatologists in the US causes long wait times and further care shortages
5	Distance traveled affects adherence to treatment and follow-up plans for patients with infantile hemangioma	Desrosiers AS, Ibrahim JM, Jacks SK.	Greater travel distance to a pediatric dermatologist is associated with lower treatment adherence and reduced follow-up attendance
6	Artificial intelligence in healthcare: An essential guide for health leaders	Chen M, Decary M.	AI can augment clinician decision-making, improve efficiency, and support care delivery when integrated into clinical workflows
7	Artificial intelligence and health inequities in primary care: a systematic scoping review and framework.	D'Elia A, Gabbay M, Rodgers S, et al.	AI can improve primary care but may exacerbate health inequities if not implemented thoughtfully
8	Development and Assessment of an Artificial Intelligence-Based Tool for Skin Condition Diagnosis by Primary Care Physicians and Nurse Practitioners in Teledermatology Practices	Jain A, Way D, Gupta V, et al.	AI diagnostic tool improved clinician accuracy and agreement with dermatologists in teledermatology
9	Infantile hemangioma. Part 1: Epidemiology, pathogenesis, clinical presentation and assessment	Rodríguez Bandera AI, Sebaratnam DF, Wargon O, Wong LF.	Review of infantile hemangioma and the importance of early recognition and assessment
10	Development of an artificial intelligence algorithm for the diagnosis of infantile hemangiomas	Zhang AJ, Lindberg N, Chamlin SL, et al.	AI algorithm that accurately classifies infantile hemangiomas from clinical images
11	Assessing the performance of artificial intelligence models in evaluating inflammatory skin disease severity: a systematic review and meta-analysis	Cai ZR, Kim J, Rezaei SJ, et al.	AI models show strong performance in assessing severity of common inflammatory skin diseases and reliably distinguish them compared to dermatologist scoring systems
12	Early intervention and disease modification in atopic dermatitis-the current state of the field and barriers to progress	Jacobson ME, Seshadri RS, Morimoto R, et al.	Early diagnosis and intervention in pediatric atopic dermatitis can improve long-term outcomes
13	Enhanced early skin treatment for atopic dermatitis in infants reduces food allergy	Yamamoto-Hanada K, Kobayashi T, Mikami M, et al.	Early skin treatment for infant atopic dermatitis significantly reduced the incidence of hen's egg allergy compared with conventional reactive treatment
14	Disparities in dermatology AI performance on a diverse, curated clinical image set	Daneshjou R, Vodrahalli K, Novoa RA, et al.	AI models showed decreased performance on diverse image datasets for darker skin tones and rare conditions
15	Disparities in Health Care and the Digital Divide	Saeed SA, Masters RM.	Digital access gaps contribute to persistent health care disparities, limiting the reach and benefits for underserved populations

Table 1. Continued

	Article	Authors	Conclusion
16	Stigmatization and Mental Health Impact of Chronic Pediatric Skin Disorders	Paller AS, Rangel SM, Chamlin SL, et al.	Chronic pediatric skin disorders are strongly associated with stigma, which correlates with reduced quality of life, increased anxiety and depression, and poorer peer relationships in affected children
17	Risk of Mental Disorders in Children and Adolescents With Atopic Dermatitis: A Systematic Review and Meta-Analysis	Xie QW, Dai X, Tang X, Chan CHY, Chan CLW.	Children and adolescents with atopic dermatitis have a significantly increased risk of developing mental health disorders
18	Ethical considerations for artificial intelligence in dermatology: a scoping review	Gordon ER, Trager MH, Kontos D, et al.	Identifies key ethical principles for AI in dermatology such as fairness, inclusivity, and transparency
19	Lack of Transparency and Potential Bias in Artificial Intelligence Data Sets and Algorithms: A Scoping Review	Daneshjou R, Smith MP, Sun MD, Rotemberg V, Zou J.	Highlights the lack of transparency and potential for bias in AI datasets and algorithms
20	Challenges of artificial intelligence in medicine and dermatology	Grzybowski A, Jin K, Wu H.	Identifies key challenges for AI in medicine and dermatology including bias, lack of transparency, ethical concerns, data security, and unequal access

board-certified dermatologists. In this study, 40 primary care providers, including 20 physicians and 20 nurse practitioners, submitted images of skin disorders found in the clinic to the tool and then received differential diagnoses. AI-aided clinical assessments agreed with a dermatologist's assessment, with an improvement of 47% to 58% ($p<0.001$). This was found to be equivalent to 1 additional correct diagnosis per 8 to 10 cases.[8] Such tools can help providers triage pediatric skin conditions more effectively by prioritizing urgent referrals and reducing delays in care, especially for malignant or rapidly proliferating lesions. Infantile hemangiomas, for example, are one of the most common pediatric tumors and have the potential to cause permanent disfigurement and comorbid conditions due to their rapid growth.[9] To address this concern, a similar algorithm was trained with over 5,800 images of facial infantile hemangiomas diagnosed by a dermatologist. The algorithm achieved 91.7% accuracy in diagnosing the lesion, with a sensitivity of 93%.[10] Identifying such lesions correctly is vital for timely intervention, especially in vulnerable regions like the face.

AI for severity assessment

A meta-analysis evaluated the performance of AI tools in assessing the severity of common skin conditions, such as acne, eczema, and warts. The results showed strong diagnostic performance, with AI models achieving a pooled sensitivity of 80.5% and reliably distinguishing moderate-to-severe disease from dermatologist scoring systems such as the Eczema Area and Severity Index (97.3%), Investigator's Global Assessment (78.9%), or Hayashi grading (89.7%).[11] For children in underserved areas, this sensitivity ensures that significant disease is not overlooked. Early identification through AI-assisted screening could allow prompt initiation of therapies such as topical corticosteroids for atopic dermatitis, which are known to reduce allergic sensitization and improve quality of life.[12] For example, treatment with topical corticosteroids for secondary prevention decreased the risk of developing an allergy to chicken egg compared with conventional therapy (31.4% vs 41.9%, $p = 0.0028$).[13] Thus, earlier identification of skin disease with AI tools can not only alleviate symptoms but also decrease the risk of other potential health conditions.

Social determinants and equity considerations

The use of AI in pediatric dermatology can only be integrated into healthcare effectively if it is designed with accessible and inclusive datasets. Rural and underserved communities are particularly vulnerable to inequities if algorithms are not created with inclusivity in mind. These datasets must include information on the social determinants of health, such as race, geographic location, socioeconomic status,

and digital access. A 2022 study highlighted this risk using the Diverse Dermatology Images dataset, which includes a broad spectrum of skin tones and uncommon biopsy-confirmed dermatologic conditions. Compared to standard datasets, AI performance in correctly distinguishing between correct and incorrect diagnoses decreased by 27-36%, with the most significant declines observed in darker skin tones and presentations of rare diseases. Without deliberate inclusion of such populations, AI risks perpetuating existing disparities rather than reducing them.[14]

Barriers to accessibility also extend to technology itself. For AI to best be utilized in healthcare, there must be digital literacy, reliable internet service, and readily available devices within the community. Without these factors, the use of AI is severely limited. If left unaddressed, these challenges risk excluding the very populations most in need of care, particularly those living in rural communities where access to technology may be scarce. To prevent this, infrastructure changes are necessary to fund technological services and provide community education on these technologies.[15]

Psychosocial implications

Chronic skin disorders often begin in childhood and influence both physical health and psychological and social well-being. Stigmatization, or the association of discriminatory and false views with a person, can have profound effects on children with visible skin disease, whether self-perceived or imposed by others. In a cohort of 1671 children aged 8-17 with chronic skin conditions, 56.4% reported high visibility of disease, and 43.8% reported at least moderate stigma. Acne, atopic dermatitis, and vitiligo were among the most visible conditions. Stigma was strongly associated with a reduction in quality of life, an increase in depression and anxiety ($p<0.001$), and poor peer relationships.[16] Furthermore, a related study found that children with atopic dermatitis had 65.2% increased odds of developing a mental health disorder when compared to peers without the skin condition. Specifically, children with atopic dermatitis had a significantly higher risk of comorbid conditions of ADHD, sleep disorders, and depression ($p<0.001$). Race further contributed to these odds, with minority children experiencing even greater risk. These findings highlight the psychosocial burden of pediatric skin conditions and emphasize the importance of early detection and intervention.[17]

AI has the potential to reduce the visibility and severity of disease by assisting providers in early identification of skin disorders, enabling more efficient management. By treating the disease at the beginning of the clinical course, it can reduce the risk of psychosocial distress that the disease would have caused. In addition, patients and families can be connected earlier with appropriate mental health support and resources, integrating dermatologic care with broader child well-being.

Challenges and ethical considerations

While AI holds promise in pediatric dermatology, its integration raises challenges for ensuring safety and equity in clinical practice. Algorithmic bias remains a significant concern given that children of color are underrepresented in training datasets.[18] A recent review of dermatologic AI studies found that 20% indicated data on race or ethnicity, and only 10% included information on skin tone, indicating a lack of transparency within data.[19] Other challenges include privacy concerns, informed consent, and the protection of minors, given the reliance on sensitive image and health data. For example, many AI algorithms have limited explainability in how the tool reached a clinical diagnosis, which may impede shared decision-making and

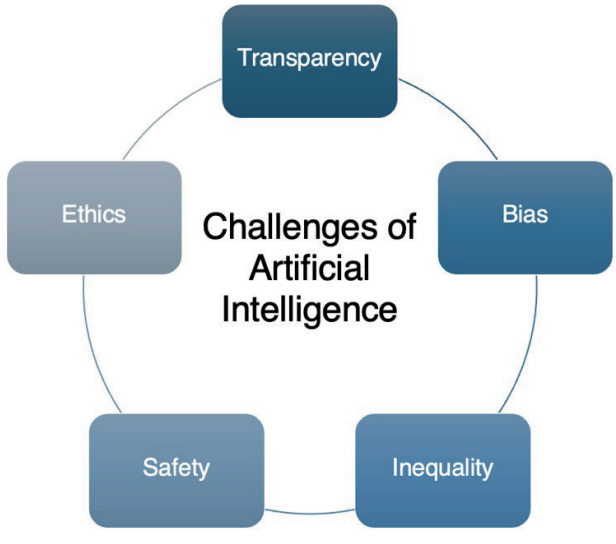

Figure 2. The challenges of Artificial Intelligence[20]

concerns of consent for not only minors, but all patients.[20] These challenges are summarized in Figure 2, created by the authors for this review.

Underserved communities may face additional barriers, such as poor internet access and low image quality, which further limit their effectiveness. When implementing new technologies, providers should work with patients to understand their level of understanding of technology. Educating patients on how to tailor the use of AI to their needs correctly is the first step towards reducing the challenges faced in these communities. Building trust with communities and designing diverse algorithms are vital steps to ensure AI is improving care rather than deepening disparities.[20]

DISCUSSION

This review demonstrates that AI has the potential to close gaps in pediatric dermatologic care by increasing access to specialist-level support in rural and underserved communities. Across the literature, AI technologies show promising diagnostic performance, with broader significance in their ability to reduce triage delays, enabling prompt therapeutic interventions. Current evidence suggests that AI functions best when complementing clinician judgement, rather than replacing it. Integrating AI into pediatric workflows may help primary care providers triage more effectively, reduce unnecessary referrals, and initiate appropriate management earlier. This is particularly impactful for addressing children's disease course, quality of life, and psychosocial outcomes. A proposed model for AI integration for pediatric skin conditions is summarized in Figure 3, created by the authors for this review.

This review has several limitations that should be considered when interpreting its findings. Current existing literature reviews mainly focus on adult dermatology or the general use of AI. In comparison, this review investigates disparities that are specific to the pediatric population, as children have specific disease patterns, developmental milestones, and psychosocial considerations that are often underrepresented in AI training datasets. The few studies that validate the use of AI tools in pediatric or rural clinics limit their generalizability and raise questions about the equitable implementation of AI. Another limitation is that this review includes only studies in English and may be geographically biased toward the United States, which may hinder understanding of AI use in underserved regions. The narrative nature of this review also introduces the possibility of selection bias, as a formal meta-analysis

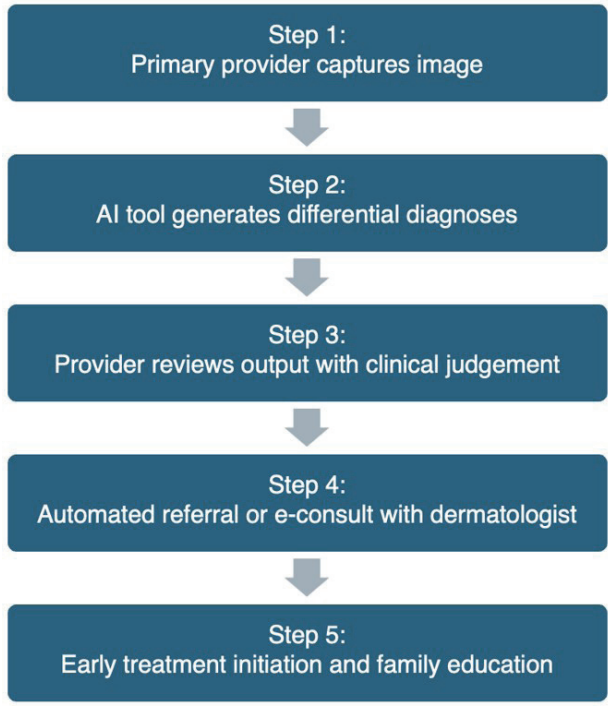

Figure 3. Proposed framework for integrating AI into pediatric dermatology workflows in rural primary care

was not feasible given the study designs and outcomes. Furthermore, as AI technologies continue to evolve, newer models or studies may have emerged since the time of the literature collection.

Thus, clinicians should consider using AI to aid diagnosis and disease management, and policymakers should prioritize funding equitable AI technologies. Future research should focus on creating diverse training datasets to minimize bias, strengthening the integration of AI into electronic health records, and developing technological infrastructure in rural and underserved communities. Longitudinal studies that investigate clinical adherence, health outcomes, and patient satisfaction can provide essential information to guide responsible, equitable adoption of AI in pediatric dermatology.

CONCLUSION

Artificial intelligence offers a unique opportunity to improve the quality of pediatric dermatologic care, particularly for children in rural and underserved communities. By extending specialist support into primary care, AI has the potential to improve access, accuracy, and timeliness of patient care. Attaining these benefits will require a

continued commitment to the development of equitable models, careful clinical integration, and ongoing evaluation. With appropriate safeguards, AI can strengthen pediatric dermatologic care and help ensure that all children receive timely, high-quality care regardless of geographic or socioeconomic barriers.

Author contribution

The authors declare contribution to the paper as follows: Study conception and design: JJ, SK; data collection: JJ, SK; analysis and interpretation of results: JJ, SK; draft manuscript preparation: JJ, SK. 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

Molecular diagnostic success of targeted next-generation sequencing (NGS) in 101 pediatric patients with inborn errors of immunity

Yasemin Kendir Demirkol[1], Burcu Yeter[1], Zeynep Meriç[2], Esra Yücel[2], Ayça Kıykım[2], Volkan Okur[3]

[1]Department of Pediatric Genetics, Health Science University, Ümraniye Training and Research Hospital, İstanbul, Türkiye
[2]Department of Pediatric Allergy and Immunology, Faculty of Medicine, İstanbul University-Cerrahpaşa, İstanbul, Türkiye
[3]Molecular Diagnostics, New York Genome Center, New York, USA

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ABSTRACT

Objective: Inborn errors of immunity (IEIs) comprise a genetically heterogeneous group of disorders predisposing individuals to recurrent and severe infections, autoimmunity, and immune dysregulation. Next-generation sequencing (NGS) has greatly improved diagnostic efficiency by allowing simultaneous analysis of multiple genes. This study aimed to evaluate the molecular diagnostic yield and characterize the variant spectrum in patients with suspected IEIs using a targeted NGS panel.

Methods: A total of 101 pediatric patients clinically diagnosed with IEIs and referred to the Pediatric Genetics Department Ümraniye Training and Research Hospital between 2018 and 2021 were included in the study. Genetic analysis was performed using a targeted NGS panel encompassing 260 genes associated with IEIs. Variants were interpreted according to American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) and Clinical Genome (ClinGen) Sequence Variant Interpretation (SVI) guidelines. Single-nucleotide variants (SNVs) were confirmed by Sanger sequencing, and copy number variants (CNVs) were validated using array-based comparative genomic hybridization (array-CGH).

Results: Pathogenic or likely pathogenic (P/LP) variants were identified in 25 of 101 patients (24.7%), yielding 26 distinct variants across 21 genes, including one patient with two variants in a compound heterozygous state. Among these, 18 were homozygous, 3 heterozygous, 3 hemizygous, and 1 compound heterozygous. The most frequently affected genes were *RAG1*, *DCLRE1C*, *SPINK5*, and *STAT1*. Three novel variants were identified, expanding the known mutational spectrum of IEIs. In addition, several variants initially identified in this cohort were later reported by our group, highlighting the contribution of our study to the expanding genetic spectrum of IEIs in Türkiye. Most variants exhibited autosomal recessive inheritance, consistent with the high consanguinity rate in the study population.

Conclusion: In our IEI cohort, targeted NGS achieved a 24.7% molecular diagnostic yield and successfully identified both known and novel pathogenic variants across a broad spectrum of genes. These findings highlight the diagnostic value of targeted NGS in genetically and clinically heterogeneous conditions such as IEIs and underscore the importance of population-specific variant databases for improving variant interpretation and optimizing patient care.

Keywords: Inborn errors of immunity, next-generation sequencing, children, diagnosis

✉ Yasemin Kendir Demirkol ▪ dryasminkendir@yahoo.com

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INTRODUCTION

Inborn errors of immunity (IEIs) are a heterogeneous group of disorders caused by genetic defects in immune system development and function.[1,2] These conditions predispose affected individuals to recurrent and severe infections, autoimmunity, malignancies, and autoinflammatory complications. The 2024 classification by the International Union of Immunological Societies (IUIS) Expert Committee recognizes 508 distinct genetic defects and further documents 17 phenocopy conditions. This update incorporates 67 newly defined monogenic IEIs and 2 novel phenocopies, thereby expanding both genotypic and phenotypic categorization and providing an updated framework for the design of diagnostic panels.[3]

The global prevalence of IEIs is approximately 1 in 10,000 live births, affecting more than 6 million individuals worldwide. However, 70–90% of patients remain undiagnosed due to clinical heterogeneity and limited access to advanced genetic testing.[4,5] In populations with high consanguinity, such as Türkiye (20–35%), the frequency of autosomal recessive IEIs is significantly increased.[5,6] While this contributes to a higher disease burden, it also enhances the likelihood of identifying homozygous or compound heterozygous variants, thereby improving the diagnostic yield of genetic testing. Given the extensive genetic heterogeneity of IEIs, conventional single-gene sequencing approaches are often slow and insufficient for diagnosis.

Next-generation sequencing (NGS) has transformed the field by enabling simultaneous analysis of multiple genes.[7] Targeted NGS panels have been shown to be cost-effective first-line tests, offering high coverage, shorter turnaround times, and fewer incidental findings.[8] More recently, large-scale studies indicate that whole-exome sequencing (WES) provides diagnostic yield equal to or greater than that of other approaches and is increasingly cost-effective, with the added advantages of reanalysis potential and novel gene discovery. Targeted panels, however, remain useful in well-defined clinical scenarios requiring rapid results, deeper coverage, or in settings where access to WES is limited.[9] Reported diagnostic yields of targeted NGS panels in PID cohorts range from 15% to 70%, depending on patient selection and disease category. Notably, higher yields are observed in severe combined immunodeficiency (SCID), with reported rates of 58–90%.[7] Türkiye's high consanguinity rate may further enhance diagnostic yield, as suggested by several studies: targeted sequencing in SCID patients yielded a 32% diagnostic rate, while WES-based approaches achieved 41–63%.[5,10,11] Globally, recent large-scale analyses have reported a 42% overall diagnostic yield, rising to 58% in patients with a positive family history, and cost-effectiveness analyses often favor WES approaches.[12] In smaller clinical exome studies, complementary analyses further increased diagnostic yield from 31% to 42%.[2]

In this study, we aimed to evaluate the molecular diagnostic success rate, characterize the spectrum of identified variants, and assess the clinical implications of genetic diagnosis for patient management and counseling, based on our experience at our institution using a targeted NGS panel in a cohort of 101 pediatric patients with suspected IEIs.

MATERIALS and METHODS

Study population

A total of 101 pediatric patients with a clinical diagnosis of IEIs who were referred for genetic testing between 2018 and 2021 were included in this study. Among these, 25 patients had likely pathogenic or pathogenic (P) variants according to the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) and Clinical Genome (ClinGen) guidelines (see Genetic and Variant Analysis section). Ethical approval for the study was obtained from the Health Science University, Ümraniye Training and Research Hospital Ethics Committee (approval number: B10.1.T.K.H.4.34.H.GP.0.01/392).

Genetic and variant analysis

Genomic DNA was extracted from EDTA-anticoagulated peripheral blood using a semi-automated system (Qiagen) in accordance with the manufacturer's protocol. DNA quality and concentration were assessed using spectrophotometric and fluorometric methods. Library preparation was performed with the Clinical Exome Solution Kit (Sophia Genetics, Switzerland), targeting 260 genes associated with primary immunodeficiency (gene list provided in Supplementary Material). Sequencing was conducted on the NextSeq 500 platform (Illumina, San Diego, CA, USA).

Bioinformatic analysis, including alignment, variant calling, and annotation, was performed using Sophia DDM software (version 5.2) with the NCBI Build 37 (hg19) human genome reference. Variants within ±10 bp of exon–intron boundaries

and with ≥50× read depth were analyzed, while low-quality or off-target variants were excluded. All called variants were visually verified in Integrative Genomics Viewer (IGV). Detected single nucleotide (SNV) and copy number (CNV) variants were classified as LP or P according to the ACMG/AMP guidelines,[13] updated recommendations by the ClinGen Sequence Variant Interpretation (SVI) Working Group (https://clinicalgenome.org/working-groups/sequence-variant-interpretation), and gene and disease-specific specifications developed by ClinGen Expert Panels available through the ClinGen Clinical Specification Portal (https://cspec.genome.network/cspec/ui/svi/). Sanger sequencing was performed for variant confirmation, with primer details and reaction settings available upon request.

All CNVs identified by NGS were confirmed by array-based comparative genomic hybridization (array-CGH) analysis.

RESULTS

A total of 101 pediatric patients with a clinical diagnosis of IEIs were analyzed using a targeted NGS panel encompassing 260 genes associated with immune disorders. Genetic analysis identified LP or P variants in 25 patients (24.7%), representing the molecular diagnostic yield of the study cohort. Of the 25 genetically confirmed patients, 13 were male, and 12 were female. The median age at symptom onset was 6 months (range: 0–180 months) among patients with available data. Parental consanguinity was documented in 10 of the 25 families (40.0%), consistent with the high background consanguinity rate reported for Türkiye. In total, 26 distinct variants were detected among these 25 patients, one of whom carried two variants in a compound heterozygous state. Among the identified variants, 18 (69.2%) were homozygous, 3 (11.5%) were heterozygous, and 3 (11.5%) were hemizygous. Most variants were SNVs or small insertions/deletions (indels), while CNVs were detected in two patients (8.0%) and confirmed using array-based comparative genomic hybridization (array-CGH).

The identified disease-causing variants were distributed across 21 genes. The most frequently affected genes were *RAG1*, *DCLRE1C*, *SPINK5*, and *STAT1*, each detected in more than one individual. Most variants showed an autosomal recessive inheritance pattern, consistent with the high rate of parental consanguinity documented in our cohort, and this likely contributed to the relatively high proportion of homozygous variants identified. Of the 26 variants, 8 (30.8%) were classified as LP and 18 (69.2%) as P according to the ACMG/AMP and ClinGen SVI guidelines. Three novel variants (11.5%) were identified. The major clinical diagnostic categories observed among genetically confirmed patients were combined immunodeficiency (CID) (8 patients), severe combined immunodeficiency (SCID) (5 patients), and Mendelian susceptibility to mycobacterial disease (MSMD) (4 patients), followed by immune dysregulation, agammaglobulinemia, and autoinflammatory disorders. For an overview of the immunological phenotype categories (e.g., T-B+NK+ SCID, T-B-NK+ SCID, CID, agammaglobulinemia, immune dysregulation, autoinflammatory disease) and their corresponding genes, the 25 genetically confirmed patients are summarized in Supplementary Table 1.

Segregation analysis was performed in all available family members, confirming biallelic inheritance, X-linked transmission, or de novo occurrence in each case. Identified variants and corresponding disease categories are summarized in Table 1, which also includes detailed clinical and laboratory findings to enable genotype–phenotype correlation.

DISCUSSION

In this study, we evaluated the molecular diagnostic yield and variant spectrum of a targeted NGS panel in 101 pediatric patients with clinically diagnosed IEIs. P or LP variants were detected in 25 patients (24.7%), distributed across 21 genes, including 3 novel variants. In addition, five variants (patients 2, 4, 16, 21, and 22) were initially identified in this cohort and later reported by our group, highlighting the contribution of our study to the expanding genetic spectrum of IEIs in Türkiye. The findings confirm the diagnostic utility of targeted NGS panels as a first-line genetic test in IEIs cohorts, particularly in populations with high rates of consanguinity or in regions where access to comprehensive approaches such as WES or whole genome sequencing (WGS) is limited due to cost or infrastructure constraints. In our cohort, combined immunodeficiencies, including SCID, represented the largest subgroup, in line with previous reports from Türkiye.[5,10,11] Patients with *RAG1*/*RAG2*, *DCLRE1C*, *CIITA*, *DOCK8*, and *FOXN1* defects illustrated the broad clinical spectrum of CID/SCID, ranging from classic T-B−NK+ SCID to leaky or syndromic forms with dysmorphic features, growth delay, or skeletal and ectodermal anomalies. Immune dysregulation phenotypes were also prominent, particularly in patients with *FAS*, *LRBA*, *FOXP3*, and *NFKB2* variants, reflecting the growing

Table 1. Summary of identified gene variants, clinical features, and laboratory findings

Patient	Primary Diagnosis	Gene/ Transcript	Variant	Zygosity	Inheritance †	ACMG classification	Clinical Findings	Laboratory Findings	Reported Previously (Reference)
1	ALPS	*FAS* NM_000043.6	c.869C>T p.(Ala290Val)	homozygous	biallelic	Likely Pathogenic	Recurrent fever and infections, lymphoproliferation, hepatosplenomegaly, and T-cell lymphoma.	Elevated IgG and IgE levels, hypergammaglobulinemia, increased DNT cells, high vitamin B12, abnormal CD4/ CD8 ratio.	[14]
2	Immune dysregulation	*LRBA* NM_001364905.1	c.6372del p.(F2124Lfs*29)	homozygous	biallelic	Pathogenic	Recurrent respiratory infections, chronic diarrhea, malabsorption, recurrent otitis media, bronchiectasis, hyperthyroidism, food allergy, arthralgia, and splenomegaly.	Panhypogammaglobulinemia, lymphopenia, pancytopenia, inverted CD4/CD8 ratio, anemia.	*[15]
3	SCID	*RAG2* NM_000536.4	c.233G>C p.(Cys78Ser)	homozygous	biallelic	Likely Pathogenic	Eczema, recurrent wheezing, chronic diarrhea, recurrent respiratory infections, otitis media, arthritis, ptosis, adrenal mass, oral candidiasis, aphthous ulcers, and history of hemolytic anemia.	Decreased IgG, IgE, and IgA levels; normal IgM; lymphopenia with reduced $CD3^+$, $CD4^+$, $CD8^+$ T lymphocytes and NK cells.	[16]
4	MHC class II deficiency	*CIITA* NM_000246.4	c.2879T>A p.(Leu960Gln)	homozygous	biallelic	Likely Pathogenic	Upper respiratory tract infection and cytomegalovirus infection.	Decreased $CD4^+$ T lymphocytes, reduced recent thymic emigrants, and low human leukocyte antigen–DR expression.	*[17]
5	SCID	*DCLRE1C*	Exon1-3 deletion[&]	homozygous	biallelic	Pathogenic	Recurrent respiratory infections, diarrhea, and eczema; small perimembranous ventricular septal defect; two siblings died due to Artemis-SCID; underwent HSCT at 9 months of age	Decreased IgA and IgG levels, normal IgM; lymphopenia, reduced $CD4^+$ T cells, $CD8^+$ T cells, and B cells.	[18,19]
6	SCID	*DCLRE1C* NM_001033855.3	c.632G>T p.(Gly211Val)	homozygous	biallelic	Pathogenic	Recurrent infections, recurrent otitis media, frequent febrile episodes, thrombus in the left middle cerebral artery, moyamoya disease, growth retardation, microcephaly, and congenital hypothyroidism; underwent HSCT at 46 months of age.	Decreased IgA and IgE levels; IgG unknown; reduced $CD4^+$ T cells, $CD8^+$ T cells, and B lymphocytes.	[9,19]
7	MSMD	*STAT1* NM_007315.4	c.1154C>T p.(Thr385Met)	heterozygous	*de novo*	Pathogenic	Chronic mucocutaneous candidiasis, recurrent aphthous ulcers, recurrent respiratory infections, pertussis, acneiform rash, growth retardation, dermatomycosis, and autoimmune hemolytic anemia.	Lymphopenia, low IgM, and inverted CD4/CD8 ratio.	[20]
8	MSMD	*STAT1* NM_007315.4	c.71A>G p.(Asp24Gly)	heterozygous	*de novo*	Likely Pathogenic	Recurrent fever, moniliasis, history of seizures, miliary tuberculosis, onychomycosis, and cheilitis.	Lymphopenia, elevated IgE, and decreased IgG.	Novel
9	Agammaglobulinemia	*BTK* NM_000061.3	c.900_903del p.(Gly302Valfs*28)	hemizygous	maternal	Pathogenic	Recurrent upper respiratory tract infections and sinusitis, short stature due to growth hormone deficiency, and chronic diarrhea.	Panhypogammaglobulinemia and decreased $CD19^+$ B lymphocytes.	[21]
10	SCID	*RAG1* NM_000448.3	c.1682G>A p.(Arg561His)	homozygous	biallelic	Pathogenic	Recurrent upper respiratory tract infections, diaper dermatitis, cutaneous granulomas, recurrent warts, chronic diarrhea, moniliasis, and history of molluscum contagiosum; underwent HSCT.	Lymphopenia, panhypogammaglobulinemia, and T-, B-, and NK-cell lymphopenia.	[16,22]

ALPS: Autoimmune lymphoproliferative syndrome, MSMD: Mendelian susceptibility to mycobacterial disease, SCID: Severe combined immune deficiency. MHC: Major Histocompatibility Complex CID: Combined immune deficiency. CMC: chronic mucocutaneous candidiasis IVIG: Intravenous immunoglobulin; IgG: Immunoglobulin G; IgA: Immunoglobulin A; IgE: Immunoglobulin E; IgM: Immunoglobulin M; DNT: Double negative T.
HSCT: Hematopoietic stem cell transplantation. BCG: Bacillus Calmette–Guérin; CD: Cluster of differentiation
[&]Chromosomal microarray done for confirmation.
[†]Segregation analysis (parental ± sibling testing) was performed in all cases to confirm the reported mode of inheritance (biallelic, X-linked, or de novo
[*]Patients 15, 17, 27, 31, and 32 were first identified in our cohort and subsequently reported in the literature by our group or collaborators. These cases are therefore not classified as novel but were unpublished at the time of detection.

Table 1. Continued

Patient	Primary Diagnosis	Gene/ Transcript	Variant	Zygosity	Inheritance †	ACMG classification	Clinical Findings	Laboratory Findings	Reported Previously (Reference)
11	SCID	*RAG1* NM_000448.3	c.1767C>G p.(Tyr589*)	homozygous	biallelic	Pathogenic	Generalized dermatitis, erythroderma, chronic diarrhea, diaper dermatitis, axillary lymphadenopathy, and history of omphalitis; underwent HSCT from an unrelated donor at 8 months of age.	Decreased $CD19^+$ B cells and $CD45RA^+$ naïve T cells, increased memory T cells ($CD45RO^+$), eosinophilia, and decreased IgG, IgA, and IgE levels	[9]
12	Immuno-osseous dysplasia	*SMARCAL1* NM_014140.4	c.1939A>C p.(Lys647Gln)	homozygous	biallelic	Likely Pathogenic	Recurrent otitis media, history of small for gestational age birth, skeletal dysplasia, short stature, recurrent upper respiratory tract infections, cheilitis, hyperpigmented skin lesions, herpes zoster infection, proteinuria, focal segmental glomerulosclerosis, chronic kidney disease and hypertension.	Lymphopenia, decreased $CD4^+$ T cells, decreased IgG levels, and proteinuria.	[23]
13	MSMD	*IFNGR1* NM_000416.3	c.523del p.(Tyr175Metfs*2)	homozygous	biallelic	Pathogenic	BCG-associated lymphadenitis, hepatosplenomegaly, anemia, and history of disseminated BCG infection and pulmonary tuberculosis; interferon-gamma therapy was attempted without clinical benefit; underwent HSCT complicated by acute graft-versus-host disease and fulminant hepatitis.	Normal complete blood count, immunoglobulin levels, and immunophenotyping; Mycobacterium tuberculosis complex isolated from axillary and abdominal lymph nodes.	[24]
14	CID	*SPINK5* NM_006846.4	c.238dup p.(Ala80Glyfs*19) and c.1888-1G>A	Compound heterozygous	biallelic	Pathogenic/ Pathogenic	Eczema, erythroderma, alopecia, bamboo hair, congenital cytomegalovirus infection, growth retardation, chronic mucoid diarrhea, periorbital erythema, prolonged neonatal jaundice, oral candidiasis, and seborrheic dermatitis of the scalp.	Elevated IgE levels, eosinophilia, and decreased $CD19^+$ B cells.	[25,26]
15	CID	*SPINK5* NM_006846.4	c.1351dup p.(Cys451Leufs*6)	homozygous	biallelic	Pathogenic	Generalized erythroderma, recurrent otitis media, recurrent gram-negative sepsis.	Eosinophilia, elevated IgE levels, and mildly decreased $CD8^+$ T cells.	Novel
16	Immune dysregulation	*FOXP3* NM_014009.4	c.1040G>A p.(Arg347His)	hemizygous	maternal	Likely Pathogenic	Autoimmune hepatitis, jaundice, hepatosplenomegaly, chronic diarrhea, recurrent fever, cervical, mediastinal, and axillary lymphadenopathy, eczema, and asthma.	Lymphopenia, positive autoantibodies (antinuclear antibody and anti-smooth muscle antibody), decreased $CD3^+$, $CD4^+$, and $CD8^+$ T cells, and elevated IgG levels.	*[27]
17	CID	*TTC37* NM_014639.4	c.66C>G, p.(Tyr22*)	homozygous	biallelic	Pathogenic	Thin, fragile hair, growth retardation, interatrial septal aneurysm, and delayed neuromotor development.	Decreased $CD4^+$ T cells, negative vaccine responses, and normal immunoglobulin levels.	Novel
18	CMC	*AIRE* *NM_000383.4*	c.769C>T p.(Arg257*)	homozygous	biallelic	Pathogenic	Chronic mucocutaneous candidiasis, scalp dermatophyte infection, moniliasis, recurrent upper respiratory tract infections, bronchiectasis, and asthma.	Eosinophilia with normal immunophenotyping results.	[28]

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HSCT: Hematopoietic stem cell transplantation. BCG: Bacillus Calmette–Guérin; CD: Cluster of differentiation
[&]Chromosomal microarray done for confirmation.
[†]Segregation analysis (parental ± sibling testing) was performed in all cases to confirm the reported mode of inheritance (biallelic, X-linked, or de novo
[*]Patients 15, 17, 27, 31, and 32 were first identified in our cohort and subsequently reported in the literature by our group or collaborators. These cases are therefore not classified as novel but were unpublished at the time of detection.

Table 1. Continued

Patient	Primary Diagnosis	Gene/ Transcript	Variant	Zygosity	Inheritance †	ACMG classification	Clinical Findings	Laboratory Findings	Reported Previously (Reference)
19	Autoinflammatory Disorders	*LPIN2* NM_001375808.2	c.1673G>A p.(Trp558*)	homozygous	biallelic	Pathogenic	Abdominal pain, weight loss, diffuse pain, peritoneal thickening, and generalized lymphadenopathy; diagnosed with tuberculous peritonitis; mild mental retardation, speech disturbance, amnesia, and hepatomegaly.	Normal nitro blue tetrazolium test, immunophenotyping, and immunoglobulin levels.	ClinVar: Variation ID: 2760513
20	CID	*FOXN1* NM_001369369.1	c.880G>A p.(Val294Ile)	homozygous	biallelic	Pathogenic	Recurrent fever and diarrhea, alopecia, growth retardation, hyperpigmented skin lesions, perianal abscess, moniliasis, and delayed neurological and speech development; underwent HSCT in 2020.	Severe neutropenia, panhypogammaglobulinemia, decreased $CD3^+$, $CD4^+$, $CD8^+$, and NK lymphocytes, with increased B lymphocyte count.	[29,30]
21	CID	*MALT1* NM_006785.4	c.1202_1203insAAT p.(Leu401_ Leu402insIle)	homozygous	biallelic	Likely Pathogenic	Refractory seborrheic dermatitis, alopecia, chronic diarrhea, herpes simplex virus infection, history of sepsis, recurrent diaper dermatitis, multiple inguinal lymphadenopathies, multiple food allergies, and growth retardation; underwent HSCT at 13 months of age.	Eosinophilia, elevated IgE and IgM levels, decreased IgG and IgA levels, and reduced T regulatory cells and $CD19^+$ B cells.	*[31]
22	CID	*IKBKG* NM_001099857.5	c.64del p.(Ala22Glnfs*93)	hemizygous	maternal	Pathogenic	Chronic perforated otitis media, recurrent upper respiratory tract infections, growth retardation, pulmonary infection with atypical mycobacteria (Mycobacterium bovis), uveitis, juvenile idiopathic arthritis, and splenomegaly.	Lymphopenia, decreased class-switched B lymphocytes, $CD4^+$ T lymphopenia, elevated IgA levels, and decreased IgM levels	*[32]
23	CID	*DOCK8*	Exon2-26 deletion[&]	homozygous	biallelic	Pathogenic	Asthma, recurrent pneumonia, otitis media, sinusitis, hepatosplenomegaly, milk and egg allergy, eczema, hyperpigmented skin lesions, history of pulmonary tuberculosis, cytomegalovirus infection, Pneumocystis jirovecii pneumonia, and growth retardation; underwent HSCT from a sibling at 51 months of age.	Eosinophilia, elevated IgE levels, and decreased $CD19^+$ B cells	[9]
24	CID	*NFKB2* NM_001322934.2	c.2557C>T p.(Arg853*)	heterozygous	*de novo*	Pathogenic	History of acute immune thrombocytopenic purpura, asthma, recurrent upper respiratory tract infections, history of otitis media, past urinary incontinence with left pelvicalyceal ectasia, and adrenal insufficiency.	Panhypogammaglobulinemia with decreased class-switched B lymphocytes	[33]
25	MSMD	*TYK2* NM_003331.5	c.647del p.(Pro216Argfs*14)	homozygous	biallelic	Pathogenic	Recurrent upper respiratory tract infections, fever and generalized rash after measles-mumps-rubella vaccination, hepatosplenomegaly, and history of pulmonary tuberculosis.	Normal immunoglobulin levels and immunophenotyping results.	[34,35]

ALPS: Autoimmune lymphoproliferative syndrome, MSMD: Mendelian susceptibility to mycobacterial disease, SCID: Severe combined immune deficiency. MHC: Major Histocompatibility Complex CID: Combined immune deficiency. CMC: chronic mucocutaneous candidiasis IVIG: Intravenous immunoglobulin; IgG: Immunoglobulin G; IgA: Immunoglobulin A; IgE: Immunoglobulin E; IgM: Immunoglobulin M; DNT: Double negative T.
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[&]Chromosomal microarray done for confirmation.
[†]Segregation analysis (parental ± sibling testing) was performed in all cases to confirm the reported mode of inheritance (biallelic, X-linked, or de novo
[*]Patients 15, 17, 27, 31, and 32 were first identified in our cohort and subsequently reported in the literature by our group or collaborators. These cases are therefore not classified as novel but were unpublished at the time of detection.

recognition of primary immune regulatory disorders within the IUIS classification. These patients frequently presented with autoimmunity, lymphoproliferation, and enteropathy, underscoring the need for early genetic testing to guide targeted therapies, including hematopoietic stem cell transplantation in selected cases. The presence of *STAT1*, *IFNGR1*, and *TYK2* variants among our patients highlights the contribution of inborn errors of IFN-γ/IL-12/IL-23 signaling to Mendelian susceptibility to mycobacterial disease in our population. In addition, *AIRE*-related chronic mucocutaneous candidiasis and *LPIN2*-associated autoinflammatory disease further illustrate the diversity of IUIS categories captured by targeted NGS panels in a single-center pediatric cohort.

Comparison with previous studies

The diagnostic yield of 24.7% in our cohort is comparable to previous targeted NGS studies, which reported yields ranging from 15% to 31% depending on cohort size, gene content, and sequencing platform. Stoddard et al.[8] analyzed 173 IEI genes and achieved a 15% yield, Cifaldi et al.[36] screened 300 genes and reported 31%, while Rudilla et al.[2] applied clinical exome sequencing covering approximately 4,800 clinically relevant genes, including a large subset of IEIs-associated genes, and reported a 31% diagnostic yield that increased to 42% after complementary analyses.

A large international study by Platt et al. involving 878 patients with suspected IEIs demonstrated an overall diagnostic yield of 56–58% using a combined targeted NGS and WES strategy. While WES provided slightly higher diagnostic efficiency and allowed identification of novel disease genes, the authors emphasized that targeted panels remain a feasible first-line approach due to faster turnaround, high coverage, and lower data burden—particularly in settings where access to exome or genome sequencing is still limited.[9]

Although detailed information on consanguinity was not available for all patients, most of the identified variants displayed an autosomal recessive inheritance pattern. The predominance of autosomal recessive defects in this cohort may have contributed to the relatively high diagnostic yield, consistent with findings from populations characterized by increased parental relatedness.[6,11] This observation underscores the importance of accounting for population-specific genetic architecture when evaluating the efficiency and interpretation of NGS-based diagnostic approaches.

Novel variants and clinical implications

Three novel variants were identified in our cohort, expanding the mutational spectrum of genes associated with primary immunodeficiency. These variants were distributed across genes involved in major immunologic pathways, including *STAT1*, *SPINK5*, and *TTC37*. Variant interpretation was performed according to ACMG/AMP and ClinGen SVI guidelines and supported by literature review, segregation analysis, and close collaboration between clinical immunologists and molecular geneticists. The concordance between genotype and clinical phenotype further reinforced the pathogenicity of these variants.

The identification of novel variants in well-characterized PID genes highlights the genetic diversity of our study population and emphasizes the importance of regional studies for improving variant interpretation. Populations with high allelic heterogeneity, such as Türkiye, continue to contribute substantially to global variant databases and to the identification of population-specific mutations. Establishing collaborative diagnostic networks and integrating clinical expertise into molecular interpretation can enhance diagnostic accuracy and facilitate reclassification of uncertain variants over time.

From a clinical perspective, achieving a molecular diagnosis provides tangible benefits for patient care and family counseling. In our cohort, genetic findings directly impacted clinical management: 8 of the 25 genetically confirmed patients underwent hematopoietic stem cell transplantation, mainly those with severe or syndromic combined immunodeficiencies (including *RAG1*, *DCLRE1C*, *DOCK8*, *FOXN1*, and *MALT1* defects). Immunoglobulin replacement and/or antimicrobial prophylaxis were initiated or adjusted in 19 patients, and cascade genetic testing, together with formal genetic counseling, was offered to all families. In selected cases with immune dysregulation or MSMD (including *STAT1* and *IFNGR1*/*TYK2* defects), targeted immunomodulatory therapies such as interferon-γ or JAK inhibition were introduced, illustrating how multidisciplinary interpretation of genetic data supports precision medicine in primary immunodeficiencies and complements the case-based information summarized in Table 1.

Methodological advantages and limitations

The main advantage of targeted NGS panels lies in their ability to provide high sequencing depth, uniform coverage, and rapid turnaround time, making them particularly suitable for routine clinical diagnostics. In our study, the

260 gene panel achieved reliable coverage across all target regions and allowed the simultaneous detection of single-nucleotide variants and copy number variations, which were subsequently confirmed by array-CGH. The standardized use of ACMG/AMP and ClinGen SVI guidelines for variant classification ensured reproducibility and alignment with international diagnostic criteria. Furthermore, the collaborative workflow between clinicians and molecular geneticists facilitated accurate phenotype–genotype correlation and timely clinical reporting.

Despite these advantages, targeted NGS panels have inherent limitations. Because they are restricted to predefined gene sets, variants in genes not yet associated with IEIs at the time of panel design remain undetected. Deep intronic, regulatory, and structural variants are also beyond the reach of standard short-read sequencing methods. In addition, periodic updates are required to incorporate newly discovered IEI genes and maintain clinical relevance. Whole-exome or whole-genome sequencing can overcome some of these limitations by offering broader coverage and the possibility of reanalysis as new genes are identified. In addition, recent work has shown that systematic reanalysis of WES and WGS data with extended IEI gene panels and structural variant calling can provide an incremental increase in diagnostic yield in patients with suspected primary immunodeficiency.[37] However, these approaches remain more resource-intensive and are not yet accessible in all healthcare settings. In this context, extended IEI gene panels represent a practical alternative to whole-exome sequencing in many clinical settings, and both extended panels and clinical exome/WES, especially when combined with reanalysis and structural variant calling, can further increase diagnostic yield in patients who remain undiagnosed after initial targeted panel testing.

Nevertheless, targeted NGS panels continue to represent a practical and efficient first-line diagnostic tool for primary immunodeficiencies, especially in populations with well-defined clinical phenotypes or in regions where access to comprehensive genomic testing is limited.

Limitations

This study has several limitations. First, it was conducted at a single center with a limited cohort size, which may not fully represent the genetic heterogeneity of IEIs in the general population. Although variant interpretation followed standardized ACMG/AMP and ClinGen guidelines, functional validation of the novel variants was not performed, and their pathogenicity was inferred based on clinical correlation and segregation data.

Second, the targeted NGS panel was restricted to 260 known IEI-related genes, which represent approximately half of the currently recognized IEI genes. As a result, pathogenic variants in genes not included in this panel or discovered after the panel design, as well as deep intronic, promoter, regulatory, or structural variants, could not be detected and may partly explain the unsolved cases. Structural rearrangements and mosaic variants may also have been underrepresented due to the limitations of short-read sequencing technology.

Finally, while clinical genetic correlation was established for all reported variants, future studies incorporating functional validation, expanded gene content, and multicenter collaboration will be crucial to further enhance diagnostic yield and refine genotype–phenotype interpretation in IEIs cohorts.

CONCLUSION

Our study demonstrates that targeted next-generation sequencing is a reliable, efficient, and cost-effective approach for the molecular diagnosis of primary immunodeficiency diseases. Using a 260-gene panel, we achieved a diagnostic yield of 24.7% and identified three novel variants across 21 genes, expanding the known mutational spectrum of IEIs. The predominance of autosomal recessive inheritance patterns reflects the genetic characteristics of our population and underscores the importance of population-specific genetic studies. Through close collaboration between clinicians and molecular geneticists, the integration of genetic data with clinical evaluation enabled accurate diagnosis and informed patient management, including hematopoietic stem cell transplantation and targeted therapy. Although whole-exome and whole-genome sequencing offer broader genomic coverage and reanalysis potential, targeted NGS panels remain a practical first-line diagnostic tool, particularly in regions where access to comprehensive genomic testing is limited. Continued expansion of multicenter collaborations and inclusion of functional studies will further enhance diagnostic precision and contribute to personalized care for patients with IEIs.

Ethical approval

This study has been approved by the Health Science University, Ümraniye Training and Research Hospital Ethics Committee (approval date 23.10.2025, number B10.1.T. K.H.4.34.H.GP.0.01/392). Written informed consent was obtained from the participants.

Author contribution

The authors declare contribution to the paper as follows: Study conception and design: YKD, AK; data collection: YKD, AK, ZM, EY; analysis and interpretation of results: YKD, AK, ZM, BY, EY, VO; draft manuscript preparation: YKD, AK, ZM, BY, VO. 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

Co-occurrence of autoimmune conditions and familial autoimmunity in pediatric patients followed in a single pediatric rheumatology center

Dilara Ünal[1], Veysel Çam[1], Hülya Ercan Emreol[1], Özge Başaran[1]

[1]Department of Pediatric Rheumatology, Hacettepe University, Ankara, Türkiye

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ABSTRACT

Background: Pediatric autoimmune diseases (ADs) are more and more known as complicated conditions influenced by overlapping genetic and environmental factors; however, the coexistence of secondary ADs and their familial accumulation has not yet been explored thoroughly in pediatric populations. In this study we aimed to evaluate the occurrence and characteristics of secondary ADs among pediatric patients with autoimmune diseases followed in our pediatric rheumatology department and to examine the impact of a family history of autoimmunity on the development of secondary ADs.

Methods: We retrospectively reviewed the records of 488 pediatric patients followed in our pediatric rheumatology department who were diagnosed with autoimmune diseases. We have collected clinical, serological, and familial data. Secondary ADs were defined as newly diagnosed autoimmune diseases evolving after the initial diagnosis. Kaplan-Meier analysis and logistic regression were used to determine predictive factors.

Results: Secondary ADs were detected in 7% of patients. Systemic lupus erythematosus (SLE) (3.1%) was the most frequent, followed by autoimmune thyroid disease (0.8%), psoriasis (0.6%), and inflammatory bowel disease (0.6%). Among patients who developed SLE as a secondary diagnosis, the most frequent primary conditions were autoimmune hepatitis and immune thrombocytopenic purpura. Autoimmune hepatitis showed the strongest link with secondary AD development (OR=95.15, 95% CI: 19.07–474.70, $p < 0.001$). Patients with a positive family history of autoimmune diseases (FHADs) had a significantly higher likelihood of developing secondary autoimmune diseases (OR=5.11, 95% CI: 1.55–16.86, p=0.007). Meanwhile, we have seen that the probability of developing secondary ADs increased over time, reaching 26.6% over 15 years.

Conclusion: In this cohort, systemic lupus erythematosus was the most frequent secondary autoimmune disease, while secondary autoimmunity overall was more strongly associated with autoimmune hepatitis and a positive family history.

Keywords: children, autoimmunity, systemic lupus erythematosus, familial autoimmunity, autoimmune hepatitis

INTRODUCTION

Autoimmune diseases (ADs) are chronic disorders resulting from a breakdown of immunological tolerance to self-antigens and may affect either a single organ or multiple systems.[1] Despite their heterogeneity, ADs often present with overlapping clinical features such as arthralgia, arthritis, Raynaud's phenomenon, or fatigue. They are also frequently associated with various autoantibodies, as well as elevated proinflammatory cytokines such as TNF, IL-1, IL-6, IL-10, and IL-17.[2] From a pathophysiological perspective, immune-mediated tissue injury is driven by dysregulated B- and T-cell responses, accompanied by infiltration of macrophages, neutrophils, and dendritic cells. Subsets

✉ Ozge Basaran ▪ ozgesalor@yahoo.com

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of T helper cells, especially Th1 and Th17 cells, have been associated with intensifying the inflammatory cascade, whereas regulatory T cells play an essential role in maintaining tolerance. The defects of regulatory tools, together with altered cytokine signaling, are vital to the initiation and perpetuation of ADs.[1-3]

In the development of autoimmunity, environmental triggers have long been implicated. In particular, infectious agents play a central role, with Epstein–Barr virus and cytomegalovirus consistently linked to several autoimmune conditions.[4] Genetic predisposition is another major factor in disease vulnerability. As evidenced by twin and family studies, both common and disease-specific genetic factors contribute to autoimmunity.[1]

The most common ADs in children include juvenile idiopathic arthritis (JIA), type 1 diabetes mellitus (DM), autoimmune thyroiditis, inflammatory bowel disease, and celiac disease.[5,6] The global prevalence of ADs in children is anticipated at 5%, although it varies across regions.[3,5] These diseases are causing chronic morbidity, decreased quality of life, and increased healthcare costs, with some cases leading to high mortality rates.[7] Familial clustering of ADs is well known and suggests a genetic predisposition.[8-12] Studies have shown that relatives of patients with autoimmune diseases such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), or JIA have high rates of autoimmune conditions, indicating a shared genetic and environmental factor.[10,11,13]

This study aims to characterize the co-occurrence of secondary ADs and explore the role of familial autoimmunity among pediatric patients followed in our department.

MATERIALS AND METHODS

Study design and population

This retrospective cohort study was conducted at the Pediatric Rheumatology Department of Hacettepe University, Ankara, and included 488 pediatric patients diagnosed with autoimmune diseases between 2005 and 2024. All patients were under 18 years of age at the time of initial diagnosis and had at least 6 months of clinical follow up. This study has been approved by the Hacettepe University Ethics Committee (approval date 24.04.2025, number 25/286) and conducted in accordance with the principles of the Declaration of Helsinki. Written informed consent was obtained from the legal guardians of all patients.

Definitions

The diagnoses of ADs were established based on validated international classification criteria. JIA was diagnosed using the International League of Associations for Rheumatology (ILAR) criteria.[14] SLE was classified according to both the 2012 Systemic Lupus International Collaborating Clinics (SLICC) criteria and 2019 EULAR/ACR classification criteria.[15,16] Autoimmune hepatitis (AIH) was diagnosed based on the International Autoimmune Hepatitis Group (IAIHG) criteria and confirmed by liver biopsy findings.[17] Autoimmune thyroid disease (AITD) was diagnosed in the presence of elevated anti-thyroglobulin (TgA) and/or anti-thyroid peroxidase antibodies (TPOA), along with abnormal thyroid function tests. Celiac disease (CD) was diagnosed through positive anti-tissue transglutaminase (tTG) IgA and confirmed by small bowel biopsy. Inflammatory bowel disease (IBD) was confirmed by endoscopic and histopathological findings in patients with compatible clinical symptoms.

Other autoimmune conditions were defined according to the following established criteria: Juvenile Dermatomyositis (JDM) by the Bohan and Peter criteria and the 2017 EULAR/ACR classification criteria; Systemic Sclerosis (SS) by the 2013 ACR/EULAR classification criteria; and Sjögren's syndrome by the 2016 ACR/EULAR criteria.[18-21] Mixed connective tissue disease (MCTD) was diagnosed according to the Alarcón-Segovia criteria.[22] Vitiligo and psoriasis were diagnosed by dermatologists based on characteristic clinical findings, with histopathological confirmation when necessary. Immune thrombocytopenic purpura (ITP) was diagnosed using the 2009 International Working Group (IWG) criteria, defined as a platelet count below 100,000/mm³ in the absence of other causes of thrombocytopenia.[23] The diagnosis was made clinically based on exclusion, without reliance on disease-specific laboratory tests.

Secondary ADs were defined as a newly diagnosed autoimmune condition occurring after the initial diagnosis of a primary autoimmune disease, based on clinical findings, serological markers, and relevant classification criteria. The interval between primary and secondary diagnoses was recorded in years.

Family history of ADs was obtained from caregivers during clinical follow-up. Only first-degree relatives were considered. Reported conditions included autoimmune thyroid disease, rheumatoid arthritis, ankylosing spondylitis, psoriasis, celiac disease, juvenile idiopathic arthritis, type 1 diabetes mellitus, vitiligo, alopecia areata, inflammatory

bowel disease, systemic lupus erythematosus, and cutaneous lupus erythematosus.

Statistical analysis

To summarize the study data, descriptive statistics were used. Continuous variables are presented as mean ± standard deviation (SD) when normally distributed, and as median (25th–75th percentile) when non-normally distributed. Categorical variables are presented as frequencies and percentages. Normality was assessed using the Shapiro–Wilk test and visual inspection of histograms and Q–Q plots. Group comparisons were managed using the chi-square test or Fisher's exact test for categorical variables, and Student's t-test or Mann–Whitney U test for continuous variables, whichever was appropriate. Logistic regression analysis was used to evaluate associations between clinical variables and the development of secondary autoimmune diseases (SAIDs), results expressed as odds ratios (ORs) and 95% confidence intervals (CIs). We used Kaplan–Meier analysis to estimate the cumulative probability of SAID development over time. A two-sided p-value < 0.05 was considered statistically significant.

Table 1. Distribution of primary and secondary autoimmune diseases among pediatric patients (n=488)

Autoimmune Disease	Primary, n (%)	Secondary, n (%)
Oligoarticular JIA	127 (26.0)	–
Polyarticular JIA	41 (8.4)	1 (0.2)
Enthesitis-related JIA	40 (8.2)	2 (0.4)
Psoriatic arthritis	5 (1.0)	–
Juvenile Dermatomyositis	42 (8.6)	–
Systemic lupus erythematosus	121 (24.8)	15 (3.1)
Scleroderma	63 (12.9)	–
Sjögren's syndrome	8 (1.6)	1 (0.2)
Mixed connective tissue disease	7 (1.4)	–
Autoimmune hepatitis	10 (2.1)	2 (0.4)
Autoimmune thyroid disease	5 (1.0)	4 (0.8)
Inflammatory bowel disease	2 (0.4)	3 (0.6)
Psoriasis	11 (2.3)	3 (0.6)
Immune thrombocytopenic purpura	5 (1.0)	–
Type 1 diabetes mellitus	1 (0.2)	1 (0.2)
Vitiligo	–	2 (0.4)

JIA: juvenile idiopathic arthritis

RESULTS

This cohort involved 488 pediatric patients (353 females) diagnosed with several ADs. The mean age at primary AD onset was 8.89 ± 4.95 years. The median disease duration was 5.42 years (25th–75th percentile: 2.3–9.5). The most common AD in the cohort was JIA, which affected 213 patients (43.6%), with the most prevalent subtype being oligoarticular JIA (n=127), followed by polyarticular JIA (n=41).

Thirty-four patients (7%) had secondary ADs. The most frequent condition was SLE (n=15), followed by AITD, psoriasis, and IBD. Table 1 presents the detailed distribution of both primary and secondary autoimmune diseases observed in our cohort. The median disease duration was significantly longer in patients with secondary ADs than in those without (p=0.040). The median time between the diagnosis of the primary disease and the development of secondary ADs was 3.20 years (25th–75th percentile: 0.7–7.13). Since JIA was the most frequent AD in our cohort, no significant difference was found in the development of secondary autoimmunity concerning the diagnosis of oligoarticular JIA (p=0.250). Secondary autoimmunity was not reported in patients diagnosed with enthesitis-related arthritis (ERA) and psoriatic arthritis. Regarding specific primary diagnoses, a statistically significant difference was observed in patients with autoimmune hepatitis (p < 0.001), where 20.6% developed secondary ADs. Psoriasis and Sjögren's Syndrome were less frequently associated with secondary ADs in both groups (p=0.450 and p=0.560, respectively) (Table 2).

In patients with a primary diagnosis of IBD, the secondary diagnosis was ERA. Among the 5 patients diagnosed with ITP, 2 developed SLE as a secondary diagnosis. Of the 10 patients with a primary diagnosis of autoimmune hepatitis, 7 developed secondary autoimmune diseases, including 6 cases of SLE and one case of Sjögren's syndrome. In the 3 patients with a primary diagnosis of autoimmune thyroid diseases, secondary diseases included one case of SLE, a case of polyarticular JIA, and a case of psoriasis.

In our cohort, there were also cases of two autoimmune diseases coexisting at the time of diagnosis. One patient with a primary diagnosis of diabetes mellitus simultaneously developed autoimmune thyroid disease and enthesitis-related arthritis. In contrast, another patient with juvenile dermatomyositis was found to have concomitant diabetes mellitus and celiac disease as secondary autoimmune conditions. Among those who ended up developing a second autoimmune condition, three patients were diagnosed with psoriasis. Psoriasis appeared in three

Table 2. Comparison of primary autoimmune diagnoses and clinical characteristics in patients with and without secondary autoimmune diseases

Characteristics	With Secondary AD (n=34)	Without secondary AD (n=454)	p-value
Female, n (%)	22 (64.7)	331 (72.9)	0.300
Median age at onset (years)	9.5 (25th–75th percentile: 4.7-13.1)	8.9 (25th–75th percentile 4.6–13)	0.890
Median disease duration (years)	6.8 (25th–75th percentile 3.7–12.3)	5.1 (25th–75th percentile 2.3–9.3)	**0.040**
Systemic lupus erythematosus (%)	11.8	25.8	0.070
Juvenile idiopathic arthritis (%)	20.6	45.4	**0.005**
Scleroderma (%)	5.9	13.4	0.300
JDM (%)	2.9	0.9	0.300
Psoriasis (%)	0	2.4	0.450
Sjögren's syndrome (%)	0	1.8	0.560
Autoimmune hepatitis (%)	20.6	0.7	**<0.001**
Mixed connective tissue disease (%)	2.9	1.5	0.440
Immune thrombocytopenic purpura (%)	14.7	0	**<0.001**
Autoimmune thyroid disease (%)	14.7	0	**<0.001**

AD: Autoimmune disease; JDM: Juvenile dermatomyositis

Table 3. Laboratory autoantibody and immunological test results in pediatric autoimmune diseases

Test	Number Tested (n)	Positive, n (%)
ANA	349	249 (71.3%)
Anti-dsDNA	262	100 (38.2%)
ANCA	25	8 (32%)
HLA-B27	21	11 (52.4%)
Direct Coombs test	30	9 (30%)
TPOA	45	15 (33.3%)
TgA	45	10 (22.2%)

ANA: antinuclear antibody; dsDNA: double-stranded DNA; ANCA: anti-neutrophil cytoplasmic antibody; HLA: human leukocyte antigen; TPOA: anti-thyroid peroxidase antibody; TgA: anti-thyroglobulin antibody.

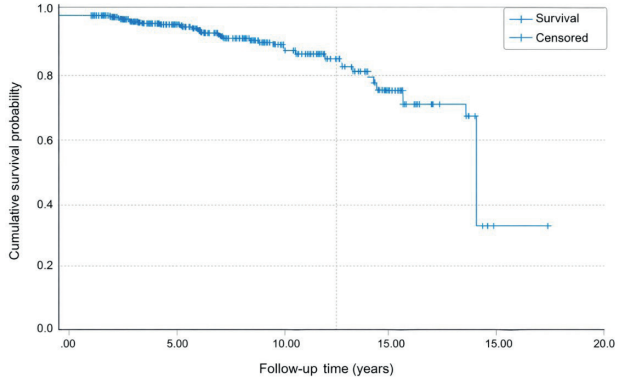

Figure 1. Kaplan–Meier curve for secondary autoimmune disease–free survival

children. Two of them were on anti-TNF drugs at the time. The third one had not used any biologics before. Also, one child with ERA ended up developing ulcerative colitis while being treated with infliximab. A total of 249 of the 349 patients (71.5%) were positive for antinuclear antibodies (ANA). The homogeneous pattern was the most frequent among ANA-positive patients, occurring in 28.3% of cases. This was followed by the granular pattern (25.5%) and the dense fine speckled pattern (6.28%). A full breakdown of the remaining autoantibody findings is presented in Table 3.

After univariate analyses, a logistic regression analysis revealed that certain primary autoimmune diseases significantly increased the likelihood of developing secondary autoimmune conditions. Primary diagnosis of autoimmune hepatitis showed the strongest association with the development of secondary autoimmune diseases (OR=95.15, 95% CI: 19.07–474.70, $p < 0.001$). Similarly, a positive family history of autoimmune diseases was significantly associated with the presence of secondary autoimmune conditions (OR=5.11, 95% CI: 1.55–16.86, p=0.007). The Kaplan-Meier estimates of the cumulative probability of developing a secondary autoimmune disease were 3.2% at 5 years (95% CI: 0.7%–5.7%), 9.6% at 10 years (95% CI: 2.8%–16.4%), and 26.6% at 15 years (95% CI: 2.4%–50.8%) (Figure 1).

Patients with a family history of autoimmune diseases have developed higher rates of secondary ADs compared to those without (13.8% versus 5.9%) (p=0.030). Among the cohort, 65 patients (13.3%) had at least one first-degree relative with a family history of autoimmune diseases (FHADs). The analysis revealed three main clusters of familial ADs in our cohort. AITD (4.9%), rheumatoid arthritis (RA) (2.1%), and DM (1.8%) were the most frequent autoimmune conditions found in the families of the patients. Table 4 presents the frequency of ADs observed in the families of pediatric patients.

Table 4. Frequency of autoimmune diseases among families of pediatric patients (n=488)

Autoimmune Disease	Frequency (n)	Percentage (%)
Autoimmune thyroid disease	24	4.9%
Rheumatoid arthritis	10	2.1%
Diabetes mellitus	9	1.8%
Ankylosing spondylitis	6	1.2%
Psoriasis	6	1.2%
Systemic lupus erythematosus	4	0.8%
Ulcerative colitis	3	0.6%
Psoriatic arthritis	2	0.4%
Other*	5	1.0%

The "Other*" category includes the following diseases, each with a frequency of 1 case (0.2%): Sarcoidosis, alopecia areata, cutaneous lupus erythematosus, celiac disease, and juvenile idiopathic arthritis. In 3 families, more than two diagnoses were present. Percentages are calculated based on the total study population (n=488). More than one autoimmune disease could be reported within the same family; therefore, percentages do not sum to 100%

The family history of ADs was further analyzed. The results indicated that 30 patients (6.1%) had a family history of autoimmunity in the mother, 19 patients (3.9%) had it in the father, 8 patients (1.6%) had a sibling with AD, and 8 patients (1.6%) had both parents and siblings affected by autoimmunity. Table 5 presents the clinical characteristics and disease features of pediatric patients with and without FHADs.

DISCUSSION

Autoimmune burden in juvenile idiopathic arthritis (JIA) was previously evaluated. But the occurrence of secondary autoimmune diseases across varied pediatric autoimmune conditions has not been clearly examined. This study gives a comprehensive assessment of secondary autoimmune diseases in this context and also insight into the role of familial autoimmunity.

We already know that in some cases, distinguishing between a new autoimmune diagnosis and a manifestation of the primary disease may be challenging. To minimize this potential misclassification, we required that all secondary diagnoses meet established international classification criteria and that satisfactory follow-up confirm their persistence as independent disease entities. Yet the possibility remains that some cases classified as secondary ADs may represent disease evolution rather than distinct

Table 5. Comparison of clinical characteristics in patients with and without a reported family history of autoimmunity

Characteristic	With FHADs (n=65)	Without FHADs (n=423)	p value
Female, n (%)	45 (69.2)	308 (72.8)	0.550
Median age at onset (years)	10.7 (25th–75th percentile 5.9–13.3)	8.7 (25th–75th percentile 4.4–12.9)	0.120
Median disease duration (years)	4 (25th–75th percentile 1.5–6.2)	5.8 (25th–75th percentile 2.5–10)	<0.001
Secondary autoimmune disease rate (%)	13.8	5.9	0.030
Systemic lupus erythematosus (%)	29.2	24.1	0.370
Juvenile idiopathic arthritis (%)	40.0	44.2	0.520
Scleroderma (%)	7.7	13.7	0.180
Psoriasis (%)	4.6	1.9	0.170
Sjögren's syndrome (%)	4.6	1.2	0.080
Autoimmune hepatitis (%)	0	2.4	0.370
Mixed connective tissue disease (%)	1.5	1.7	0.700
Immune thrombocytopenic purpura (%)	3.1	0.7	0.130
Autoimmune thyroid disease (%)	3.1	0.7	0.130

FHAD: family history of autoimmune diseases
Percentages are calculated within each column (n=65 for patients with FHADs and n=423 for patients without FHADs).

comorbid autoimmunity, which we admit as a limitation of our study.

In our cohort of 488 pediatric patients, 7% developed secondary ADs. Systemic lupus erythematosus was the most frequent one (3.1%), followed by AITD, psoriasis, and IBD (each 0.6%). Juvenile idiopathic arthritis being the most common primary diagnosis, secondary AD development was not significantly associated with oligoarticular JIA. This finding differs from the study by Tronconi et al., which reported a 15.2% secondary AD rate in JIA, primarily AITD (10.1%).[24] Our cohort showed a low prevalence of DM and celiac disease, but previous studies propose that clinicians should remain vigilant for additional autoimmune manifestations in patients with underlying endocrinopathies.[25]

Psoriasis was rarely a primary diagnosis with secondary ADs, but it was identified secondarily in three patients—two of whom had received anti-TNF therapy. This supports previous reports of psoriasis as a paradoxical effect of TNF inhibitors.[26] However, one patient developed psoriasis without prior biologic treatment, and we considered that psoriasis itself may increase the likelihood of additional autoimmune diseases. It is reasonable that both mechanisms coexist— specifically, paradoxical drug-induced lesions and true secondary autoimmunity. Wu et al. showed that psoriasis increases the likelihood of further autoimmune diseases, highlighting the importance of close monitoring.[27]

Inflammatory bowel disease (IBD) was identified as a secondary AD in three patients. None of them had an ERA, and only one had received anti-TNF therapy. This contrasts with van Straalen et al., who found that ERA and a family history of autoimmunity significantly increased IBD risk in JIA.[28] Our findings imply that IBD in non-ERA patients may involve distinct mechanisms.

Autoimmune hepatitis (AIH) showed the strongest association with secondary AD development (20.6%; $p < 0.001$), particularly with SLE and Sjögren's syndrome as secondary diagnoses. This finding supports the previous studies that note the link and overlap between AIH and other autoimmune disorders. A retrospective study conducted on patients diagnosed with Sjögren's syndrome found that 1.7% of patients with primary Sjögren's syndrome had AIH.[29] Given its high secondary ADs risk, patients with AIH need careful follow-up. Haslak et al. reported that ANA positivity in children rarely progressed to autoimmune disease, underscoring the need for reasonable interpretation in clinical practice.[30] The co-occurrence of multiple autoimmune diseases in pediatric patients may reflect a complex relationship of genetic susceptibility, shared immunopathogenic pathways, and environmental triggers. Familial autoimmunity in our cohort supports the role of common inherited risk factors, particularly within HLA and non-HLA loci, in predisposing to polyautoimmunity.[24]

Problems in how B and T cells behave, along with the failure of both central and peripheral immune tolerance, seem to play a role in the development of multiple autoimmune conditions.[3] Ongoing stimulations by infections may also contribute. Altogether, these overlapping mechanisms suggest that coexisting autoimmune diseases do not occur in isolation—they are likely part of a broader, shared disease process.

Familial autoimmunity was another key risk factor for secondary ADs development. Patients with a positive family history had a significantly higher secondary ADs rate. The most common familial autoimmune conditions were AITD (4.9%), rheumatoid arthritis (2.1%), and diabetes mellitus (1.8%). Though JIA patients with a family history of autoimmunity have been reported to show clustering of AITD, RA, and psoriasis, our findings showed no significant difference in SAID development between familial and non-familial JIA cases.[24] Similar patterns were observed in scleroderma and Sjögren's syndrome. Notably, while previous studies indicated familial aggregation in juvenile dermatomyositis (JDM), our JDM patients had no family history of autoimmunity, highlighting possible regional or population-specific differences.[31]

In our own group, ITP was seen in 14.7% of children with a family history of autoimmunity. Interestingly, two out of five of those ITP cases later developed SLE as a second autoimmune disease. This relatively high rate of disease progression is important from a clinical point of view and is consistent with earlier studies suggesting that some children with ITP may later develop SLE. In a national French cohort, 22% of ANA-positive children with autoimmune cytopenia ended up being diagnosed with SLE, which made up 4.4% of the whole AIC group.[32] These findings highlight why it's important to follow pediatric ITP patients closely over time, especially those with unusual antibody results or a family history of autoimmune conditions.

Our study has important strengths, including data from a relatively large pediatric cohort, systematic evaluation of a broad spectrum of autoimmune diseases (not just JIA), and

a long observation period. Additionally, the combination of detailed family history data provides valuable insight into the genetic and environmental background of pediatric autoimmunity. On the other hand, we admit certain limitations. The retrospective, single-center design may limit the generalizability of our findings. Some disease subgroups have relatively small sample sizes, which may reduce power to detect significant associations, and reliance on caregiver reports of family history may introduce recall bias. Therefore, caution should be kept when generalizing these frequencies to the general pediatric population.

In our large pediatric cohort, 7% of patients developed secondary autoimmune diseases, most commonly systemic lupus erythematosus. Autoimmune hepatitis and a positive family history emerged as key risk factors for additional autoimmunity. These findings highlight the need for long-term follow-up and careful monitoring of children with autoimmune diseases, particularly those with these risk factors.

Ethical approval

This study has been approved by the Hacettepe University Ethics Committee (approval date 24.04.2025, number 25/286). Written informed consent was obtained from the participants.

Author contribution

The authors declare contribution to the paper as follows: Study conception and design: DU, OB; data collection: DU, VC, HEE; analysis and interpretation of results: DU, OB; draft manuscript preparation: DU, VC, HEE, OB. 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

Pulmonary arterial capacitance in children with pulmonary arterial hypertension and response to the treatment

Selcan Öztürk[1], Serdar Kula[2]

[1]Division of Pediatric Neurology, Department of Pediatric Health and Disease, University of Health Sciences, Kayseri City Hospital, Kayseri, Türkiye
[2]Division of Pediatric Cardiology, Department of Pediatrics, Faculty of Medicine, Gazi University, Ankara, Türkiye

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ABSTRACT

Objective: The pulmonary arterial capacitance index (PACi) has recently emerged as a dynamic marker of pulmonary vascular compliance. However, its clinical relevance in pediatric pulmonary arterial hypertension (PAH), and particularly its relationship with functional capacity and exercise tolerance in congenital heart disease (CHD), remains unclear. This study explored these associations and assessed changes following PAH-specific therapy.

Materials and Methods: Thirty-five patients with CHD-associated PAH with a mean pulmonary artery pressure (mPAP)≥20 mmHg receiving PAH-specific therapy and 35 age-, sex-, and anthropometry-matched CHD controls without PAH were evaluated. Demographic characteristics, hemodynamic parameters, PACi, functional class, brain natriuretic peptide (BNP) levels, and six-minute walk test (6MWT) distances were compared. Pre- and post-treatment hemodynamic and clinical parameters were also analyzed in the PAH group.

Results: Ventricular septal defect was the most common CHD in both groups. Children with PAH had significantly higher mPAP and pulmonary vascular resistance index (PVRi) and lower PACi than controls. PACi was inversely correlated with mPAP (r=-0.383, p=0.023) and PVRi (r=-0.812, p<0.01) but exhibited no significant association with BNP or 6MWT distance. No significant improvement in PACi or PVRi was observed after treatment.

Conclusion: PACi may serve as an early indicator of pulmonary vascular stiffness in pediatric PAH. Its limited association with functional and exercise-based assessments likely reflects early disease stages, age-related variability, and measurement constraints. Persistently low PACi and PVRi despite therapy underscore the progressive nature of pediatric PAH and highlight the need for larger, long-term prospective studies.

Keywords: congenital heart disease, exercise tolerance, functional capacity, pulmonary arterial capacitance, pulmonary arterial hypertension

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a multifactorial and heterogeneous disorder defined by a mean pulmonary artery pressure (mPAP)≥20 mmHg, a pulmonary artery wedge pressure (PAWP)≤15 mmHg, and a pulmonary vascular resistance index (PVRi)≥3 Wood units·m² (WU·m²).[1-3] Early and accurate diagnosis, together with regular follow-up, is critical for guiding treatment decisions,

✉ Selcan Öztürk ▪ drselcanozturk@gmail.com

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assessing prognosis, and improving the quality of life of affected pediatric patients.[4]

PAH results in significant hemodynamic and histopathological changes in the pulmonary vasculature.[5] Parameters such as mPAP and PVRi have traditionally been used to evaluate these changes.[6] However, since these indices reflect only the static properties of pulmonary circulation, their prognostic value is limited.[7]

Consequently, attention has shifted toward dynamic parameters such as the pulmonary arterial capacitance index (PACi), defined as the ratio of stroke volume to pulmonary arterial pulse pressure and considered a measure of pulmonary vascular compliance.[8,9]

Adult PAH studies have associated reduced PACi with poor prognosis, and an inverse hyperbolic relationship between PACi and PVRi has been described. PVRi rises as PACi decreases, increasing right ventricular workload, hypertrophy, and ultimately right heart failure.[10,11] However, pediatric data regarding the relationship between PACi and exercise tolerance, functional capacity, and long-term outcomes remain limited.[8]

This study investigated the associations between PACi and PVRi, functional capacity, exercise tolerance, and serum brain natriuretic peptide (BNP) levels in children with PAH secondary to congenital heart disease (CHD) with left-to-right shunts. It also evaluated changes in these parameters before and after PAH-specific therapy.

A preliminary analysis of the research findings had previously been presented as a pediatric thesis (Yılmaz S. Pulmonary Arterial Capacitance in Children with Pulmonary Arterial Hypertension and Response to the Treatment. Gazi University, Faculty of Medicine, Department of Pediatric Health and Disease; 2014).[12]

MATERIALS AND METHODS

This retrospective study included pediatric patients diagnosed with CHD and PAH, who underwent diagnostic cardiac catheterization at the Gazi University, Faculty of Medicine, Department of Pediatric Cardiology, Türkiye. The study protocol was approved by the Gazi University Faculty of Medicine's Ethical Committee.

Study population

Data were collected from the hospital's electronic medical records and patient files. Patients were eligible if they were over three months of age, had a confirmed diagnosis of CHD, attended regular follow-up visits, and underwent diagnostic cardiac catheterization. Patients with persistent pulmonary hypertension of the newborn, idiopathic or heritable PAH, or neuromuscular disorders affecting functional assessment were excluded.

Two of the 40 patients with mPAP≥20 mmHg were excluded due to treatment non-compliance, two were lost to follow-up, and one had incomplete documentation. Thirty-five patients were included in the final analysis. A control group of 35 age-, sex-, and anthropometry-matched CHD patients without PAH, evaluated during the same period, was also included. These control patients underwent cardiac catheterization solely for shunt evaluation. Although they did not meet hemodynamic criteria for PAH, residual confounding related to underlying CHD physiology could not be completely excluded.

Treatment groups

The PAH patients were subdivided according to the treatment received:

- Monotherapy: Bosentan, iloprost, or sildenafil
- Combination therapy: Two or more PAH-specific drugs

Data collection

The following parameters were evaluated in the PAH group before and after therapy:

- Hemodynamic: mPAP, PVRi, and PACi
- Laboratory: serum BNP levels
- Clinical: six-minute walk test (6MWT) distance and New York Heart Association (NYHA) functional class
- Pediatric-specific: Ross classifications

Since the Ross classification does not routinely include direct measurements of peak oxygen consumption (%VO_2 max), this parameter was excluded from the analysis.

Pre-treatment values were obtained from the first catheterization at admission, while post-treatment values were obtained from follow-up catheterizations. Children younger than six years or physically unable to perform the 6MWT were excluded from exercise capacity evaluation. Fourteen children were eventually excluded from the 6MWT assessment because they were under 6 years of age.

Hemodynamic calculations

Hemodynamic data recorded during catheterization were used to calculate stroke volume and PVRi:[13]

Stroke volume (SV) (mL)=Cardiac output (CO) / Heart rate (HR)

The following formula was used to calculate the PACi, representing the ratio of volume change to pressure change in the pulmonary artery:[9]

PACi (mL/mmHg/m²)=Stroke volume (SV) / Pulmonary artery pulse pressure (systolic – diastolic PA pressure) / Body surface area (m²)

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences software (SPSS, IBM Corp., Armonk, NY, USA). Descriptive statistics were expressed as mean ± standard deviation (SD), median (minimum–maximum), frequency, and percentage values. Due to wide value ranges in some variables, non-parametric methods were predominantly used.

The normality of data distribution was assessed using both visual methods (histograms and probability plots) and analytical tests (Kolmogorov-Smirnov and Shapiro-Wilk). The Mann-Whitney U test was applied for non-normally distributed continuous variables in intergroup comparisons, while the chi-square test was used for categorical variables.

Pre- and post-treatment comparisons of continuous variables were performed using the Wilcoxon signed-rank test. Functional class comparisons using the modified Ross and NYHA classifications were evaluated with the McNemar-Bowker test and the Wilcoxon test.

Spearman's correlation analysis was conducted to assess relationships among PACi, mPAP, PVRi, BNP levels, and 6MWT distance.

A p-value of<0.05 was considered statistically significant.

RESULTS

Thirty-five patients were included in both the PAH and control groups. The control group consisted of 16 girls (45.7%) and 19 boys (54.3%), and the PAH group of 13 girls (37.1%) and 22 boys (62.9%). The most common congenital heart defect in both groups was a ventricular septal defect (Table 1).

Table 1. Distributions of Congenital Heart Defects in Patients with PAH and the Control Group

	Patient Group n=35 (%)	Control Group n=35 (%)
ASD	2 (5.7%)	10 (28.6%)
VSD	19 (54.2%)	16 (45.8%)
PDA	1 (2.9%)	6 (17.1%)
ASD+VSD	3 (8.5%)	-
ASD+VSD+PDA	3 (8.5%)	-
AVSD	1 (2.9%)	1(2.8%)
PFO	-	1(2.8%)
VSD+PFO+BAV	-	1(2.8%)
PA+ASD	1 (2.9%)	-
AP Window	1 (2.9%)	-
TAPVR+ASD	1 (2.9%)	-
AVSD+Pulmonary artery anomalies	1 (2.9%)	-
TGA+ASD+VSD	1 (2.9%)	-
TGA+Pulmonary artery banding	1 (2.9%)	-

Abbreviations: ASD: Atrial Septal Defect, VSD: Ventricular Septal Defect, PDA: Patent Ductus Arteriosus, AVSD: Atrioventricular Septal Defect, PFO: Patent Foramen Ovale, BAV: Bicuspid Aortic Valve, PA: Pulmonary Atresia, AP Window: Aortopulmonary Window, TAPVR: Total Anomalous Pulmonary Venous Return, TGA: Transposition of the Great Arteries.

Pre-treatment age, weight, and body surface area were significantly lower in the PAH group than in the controls (p=0.032, p=0.033, and p=0.033, respectively). All three parameters increased significantly after treatment. In terms of hemodynamics, the PAH group exhibited significantly higher systolic, diastolic, and mean pulmonary artery pressure (mPAP), and pulmonary vascular resistance index (PVRi), and lower pulmonary arterial capacitance index (PACi) than controls (p<0.05). The post-treatment cardiac index decreased significantly within the PAH group (p=0.006), while PACi remained lower than in the controls (Table 2).

Table 2. Patients' Pre- and Post-Treatment Demographic Characteristics and Hemodynamic Parameters				
		Patient Group	**Control Group**	**p1/p2**
Age (months)	Pre-treatment	48 (6-212)	132 (3-204)	0.032
	Post-treatment	85 (17-216)	132 (3-204)	0.541
		p^3=0.001		
Height (cm)	Pre-treatment	100 (63-177)	132 (67-139)	0.060
	Post-treatment	113 (75-177)	132 (67-139)	0.492
		p^3=0.001		
Weight (kg)	Pre-treatment	15 (5-75)	28 (5-63)	0.033
	Post-treatment	19.5 (7-76)	28 (5-63)	0.417
		p^3=0.001		
Body surface area (m^2)	Pre-treatment	0.62 (0.27-1.73)	1.01 (0.28-1.69)	0.033
	Post-treatment	0.77 (0.37-1.73)	1.01 (0.28-1.69)	0.414
		p^3=0.001		
Cardiac index (L/m^2)	Pre-treatment	7.24 (0.93-62.96)	4.94 (2.54-28.33)	0.285
	Post-treatment	5.56 (0.93-26.67)	4.94 (2.54-28.33)	0.643
		p^3=0.006		
Systolic PAP (mmHg)	Pre-treatment	98 (52-125)	25 (18-34)	0.001
	Post-treatment	102 (44-130)	25 (18-34)	0.001
		p^3=0.748		
Diastolic PAP (mmHg)	Pre-treatment	46 (11-80)	9 (5-14)	0.001
	Post-treatment	47 (9-90)	9 (5-14)	0.001
		p^3=0.738		
Mean PAP (mmHg)	Pre-treatment	70 (30-95)	16 (12-21)	0.001
	Post-treatment	71 (22-107)	16 (12-21)	0.001
		p^3=0.707		
PVRi (WU x m^2)	Pre-treatment	6.71 (0.66-65.61)	1.4 (0.15-2.61)	0.001
	Post-treatment	8.6 (0.82-58.02)	1.4 (0.15-2.61)	0.001
		p^3=0.028		
PACi ($mL/mmHg/m^2$)	Pre-treatment	1.5 (0.192-10.678)	2.91 (1.97-366.06)	0.001
	Post-treatment	1.1 (0.192-6.213)	2.91 (1.97-366.06)	0.001
		p^3=0.077		
Qp ($L/min/m^2$)	Pre-treatment	4.8 (1.61-17)	4.9 (3.2-17)	0.259
	Post-treatment	4.8 (1.61-12)	4.9 (3.2-17)	0.109
		p^3=0.193		
Qs ($L/min/m^2$)	Pre-treatment	3.5 (1.82-23.77)	3.8 (2.9-9.4)	0.251
	Post-treatment	3.5 (1.1-5.65)	3.8 (2.9-9.4)	0.078
		p^3=0.669		

p<0.05 Mann-Whitney U, p^1: Pre-treatment PAH patients-control group, p^2: Post-treatment PAH patients-control group, p^3: Pre-treatment- post-treatment PAH PAP: Pulmonary artery pressure, PVRi: Pulmonary vascular resistance index, PACi: Pulmonary artery capacitance index, Qp: Pulmonary blood flow, Qs: Systemic blood flow

Table 3. A comparison of BNP levels and six-minute walk test results between the patient and control groups

		Patient group median (min-max)	Control group	p1/p2
BNP levels	Pre-treatment	650 (60-3000)	35 (10-65)	0.001
	Post-treatment	498 (60-1288)	35 (10-65)	0.001
		p^3=0.328		
6-MWT values	Pre-treatment	420 (153-560)	540 (510-585)	0.001
	Post-treatment	450 (300-564)	540 (510-585)	0.001
		p^3=0.123		

p<0.05 Mann-Whitney U, p^1=Comparison between the pre-treatment group and the control group; p^2=Comparison between the post-treatment group and the control group; p^3=Comparison between pre-treatment and post-treatment values within the patient group.

BNP levels and Six-Minute Walk Test (6-MWT)

Due to the retrospective nature of the study, pre-treatment BNP levels were only available for 14 patients, and post-treatment levels for 21. BNP analyses were conducted using available-case data, and patients with missing BNP values were excluded from the relevant comparisons.

The BNP levels of the PAH group, both before and after PAH-specific therapy, differed significantly from those of the control group (p<0.05 for both; Table 3). Similarly, significant differences were observed between the PAH and control groups' 6MWT results at both time points (p< 0.05 for both).

Fourteen children were younger than 6 years and, therefore, ineligible to perform the 6MWT, which restricted exercise capacity analyses to the remaining patients.

However, no statistically significant difference was observed between pre- and post-treatment BNP levels or 6MWT distances within the PAH group (p>0.05) (Table 3).

Functional capacity

Functional capacity, assessed using the modified Ross and NYHA classifications, showed no significant differences between pre- and post-treatment evaluations (p=0.617 and p=0.123, respectively). Table 4 summarizes the distribution of functional classes. The majority of patients in the PAH group were in Ross Class II before (71.4%) and after treatment (71.4%), with only minor shifts between Class I and Class III. Similarly, NYHA classifications showed that the majority of patients remained in Class II at both time points (74.3% pre-treatment and 74.3% post-treatment). Only small proportions of patients were classified as Class I or III in either system, and no statistically significant changes were observed after therapy (p>0.05 for both the Ross and NYHA systems).

Table 4. Number of patients in the Ross and NYHA Classifications in the patient and control groups

		Pre-treatment	Post-treatment	p
Ross Classification	Class I	7	8	
	Class II	25	25	
	Class III	3	2	
	Ross	2 (1-3)	2 (1-3)	0.617
NYHA Classification	Class I	4	7	
	Class II	26	26	
	Class III	5	2	
	NYHA	2 (1-3)	2 (1-3)	0.109

p<0.05 Mann-Whitney U, p^1=Comparison of the Ross and NYHA classifications between the pre-treatment patient group and the control group, p^2=Comparison of the Ross and NYHA classifications between the post-treatment patient group and the control group, p^3=Comparison of the Ross and NYHA classifications between the patient group

Correlation analysis

The relationships between PACi, PVRi, and mPAP values and serum BNP levels and 6MWT results in the patient group were assessed using Spearman correlation analysis (Table 5).

Table 5. Correlations between PACi, mPAP, and PVRi values and BNP levels and 6-MWT distance in the patient groups

	mPAP	PACi	PVRi	BNP	6-MWT
PACi	r=-0.383* p=0.023	r=1.000 p=-	r=-0.812** p<0.01	r=0.4 p=0.072	r=0.139 p=0.701
PVRi	r=0.669** p<0.01	r=-0.812** p<0.01	r=1.000 p=-	r=-0.326 p=0.149	r=-0.588 p=0.074
mPAP	r=1.000 p=-	r=-0.383* p=0.023	r=0.669** p<0.01	r=-0.129 p=0.577	r=- 0.116 p=0.751
BNP	r=- 0.129 p=0.577	r=0.400 p=0.072	r=-0.326 p=0.149	r=1.000 p=-	r=-0.101 p=0.848
6-MWT	r=-0.116 p=0.751	r=0.139 p=0.701	r=-0.588 p=0.074	r=-0.101 p=0.848	r=1.000 p=-

*Significant at the 0.05 level (2-tailed)
**Significant at the 0.01 level (2-tailed)
mPAP: mean Pulmonary artery pressure, PACi: Pulmonary artery capacitance index, PVRi: Pulmonary vascular resistance index, BNP: Brain Natriuretic Peptide, 6-MWT: six-minute walk test.

Analysis revealed a statistically significant negative correlation between PACi and mean pulmonary artery pressure (mPAP) at the 0.05 significance level (r=–0.383, p=0.023), and between PACi and PVRi at the 0.01 level (r=–0.812, p<0.01). No significant correlation was observed between PACi and either BNP levels or 6MWT results.

Additionally, PVRi was positively correlated with mPAP at the 0.01 significance level (r=0.669, p<0.01) and negatively correlated with PACi at the 0.05 significance level. However, no significant correlation was observed between PVRi and either BNP or 6MWT values.

DISCUSSION

Pulmonary arterial hypertension remains a major cause of morbidity and mortality in both adults and children, particularly in those with CHD. As reported in previous pediatric cohorts, a ventricular septal defect was the most common lesion in our study population.[14]

The management of pediatric PAH requires accurate and dynamic prognostic markers. Traditional parameters such as mPAP and PVRi provide essential information but predominantly reflect static vascular properties.[15] In the present cohort, PVRi increased significantly after treatment despite stable functional capacity, suggesting that conventional markers may not fully capture early hemodynamic deterioration.

Pulmonary arterial capacitance index (PACi) has emerged as a dynamic indicator of pulmonary vascular compliance.[7-9] Consistent with previous studies, children with PAH in our cohort demonstrated significantly reduced PACi values compared with the controls, and PACi exhibited inverse correlations with mPAP and PVRi.[16] Recent pediatric data indicate that impaired pulmonary arterial compliance independently predicts short-term clinical deterioration, underscoring the prognostic relevance of compliance-based indices in childhood PAH.[17] Importantly, PACi did not improve after therapy. Taken together, these findings indicate that despite treatment, low PACi persisted, suggesting ongoing vascular remodeling and loss of arterial compliance.[18,19] The lack of improvement in PACi and PVRi after therapy may be attributable to several factors. Treatment escalation or optimization was not uniform across all patients, and some children remained on monotherapy due to age, tolerability, or clinical stability. Additionally, the follow-up intervals may have been insufficient to capture structural vascular changes, since pulmonary vascular remodeling often progresses slowly and may not be detectable over short-term catheterization periods. Some patients may also have had more advanced vascular disease at baseline, limiting the potential for measurable reversibility.

Biomarkers such as BNP and NT-proBNP are widely used for monitoring PAH and have been linked to hemodynamic status and exercise capacity.[20-22] In this study, no significant change in BNP values was observed after treatment, likely due to the limited sample size and incomplete data.

Previous retrospective evaluations of complications and cardiac risk factors have similarly been limited by small sample sizes, supporting the need for larger, prospective studies.[23] Nevertheless, a negative correlation between BNP levels and 6MWT distance is suggestive of its role as an indicator of functional limitation.

Although adult studies have demonstrated that lower PVRi and higher PACi are associated with longer 6MWT distances, our analysis showed no correlation between PACi and 6MWT or BNP levels.[24] This discrepancy may be attributed to age-related differences in exercise performance, limited patient numbers, and non-synchronous data collection. Larger studies with standardized assessments are now needed to clarify these relationships. Several factors may further explain the lack of association between PACi and BNP or 6MWT in this cohort. The limited size and incomplete BNP dataset substantially reduced statistical power, limiting our ability to detect moderate correlations. Additionally, BNP measurements, cardiac catheterization data, and 6MWT results were not collected synchronously, potentially masking short-term physiological changes in pulmonary vascular compliance. Exercise performance in children also exhibits wide age-related variability. Differences in stride length, motivation, cooperation, and developmental stage, particularly among younger children, can introduce significant noise into functional assessments. These methodological and age-related factors likely contributed to the absence of measurable correlations in this study.

The Ross and NYHA classifications were used to measure functional capacity in this study. The majority of patients were in FC II, and no significant functional improvement was observed during follow-up. The limited change in functional class may reflect several factors specific to pediatric PAH.[25,26] In contrast to adults, as noted in the REVEAL registry, children frequently exhibit variations in functional scores despite alterations in hemodynamic or biomarker parameters. Age-related differences in activity perception, developmental variability in exercise tolerance, and the inherently subjective nature of these scoring systems may all contribute to this discrepancy. In younger children, caregiver-reported assessments may obscure subtle changes, whereas older children may adapt to chronic symptoms and underreport limitations. Additionally, the relatively short follow-up period and small sample size in the present study may have reduced the likelihood of detecting clinically meaningful changes in functional class. Prospective, multicenter studies are now needed to validate pediatric functional scoring tools and to better define their role in monitoring disease progression and treatment response.

Study limitations

This study has several limitations. First, its retrospective design restricted access to complete clinical, laboratory, and functional data, particularly concerning BNP levels. The incomplete BNP dataset reduced the strength of correlation and longitudinal analyses and limited the interpretability of biomarker-hemodynamic relationships. Second, the relatively small sample size (n=35) substantially reduced statistical power and limited our ability to perform subgroup analyses, which should be considered when interpreting the findings. These factors may have affected the robustness and generalizability of the results. Additionally, since the control group consisted of CHD patients undergoing catheterization solely for shunt evaluation, residual confounding related to underlying cardiac physiology cannot be completely excluded. Further prospective, multicenter studies with larger and more homogeneous cohorts and standardized data collection are now needed to validate the study.

CONCLUSION

Pulmonary arterial hypertension in children remains a progressive condition despite targeted therapies, reflecting ongoing vascular remodeling and limited treatment options. Early identification of hemodynamic changes, particularly using parameters such as PACi, may enhance clinical assessment and guide management strategies.

Future prospective multicenter studies with larger cohorts are essential to clarify disease mechanisms and support the development of novel, targeted therapies for pediatric PAH.

Ethical approval

This study has been approved by the Gazi University Faculty of Medicine's Ethical Committee (approval date 2013, decision no. 2013-111).

Author contribution

The authors declare contribution to the paper as follows: Study conception and design: SO; data collection: SO, SK; analysis and interpretation of results: SO; draft manuscript preparation: SO, SK. 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

Serum immunoglobulins and lymphocyte subset levels and relation to treatment response in children with acute immune thrombocytopenia

Mehmet Cemal Dönmez[1], Mehmet Fatih Orhan[2], Öner Özdemir[3], Mustafa Büyükavcı[2,4]

[1]Department of Pediatrics, Faculty of Medicine, Sakarya University, Sakarya, Türkiye
[2]Department of Pediatric Hematology and Oncology, Faculty of Medicine, Sakarya University, Sakarya, Türkiye
[3]Department of Pediatric Allergy and Immunology, Faculty of Medicine, Sakarya University, Sakarya, Türkiye
[4]Department of Pediatric Hematology and Oncology, Faculty of Medicine, Necmettin Erbakan University, Konya, Türkiye

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ABSTRACT

Objective: In this study, we investigated the initial serum immunoglobulin and lymphocyte subset levels of children with immune thrombocytopenia (ITP), and their association with treatment response.

Methods: Thirty children with ITP were retrospectively analyzed. Immunoglobulin isotypes, IgG subtypes, and lymphocyte subset levels in the patients were compared with those of the age- and sex-matched control group and age-appropriate reference values. In addition, we investigated the relationship between immunological parameters and treatment responses.

Results: There was no statistically significant difference between the patient and control groups regarding immunoglobulin isotype and IgG subtype levels. However, IgG levels were below normal limits in 10 (33.3%) patients, and IgA and IgG2 levels were low in 2 patients with normal IgG levels. Younger age and low IgG level at diagnosis were associated with an increased treatment response on day 7.

Conclusion: Serum immunoglobulin levels at the time of diagnosis can help predict treatment response in the early period and detect subclinical immunodeficiency in children with acute ITP.

Keywords: childhood, acute immune thrombocytopenia, immunodeficiency

INTRODUCTION

Immune thrombocytopenia (ITP) is an autoimmune disease characterized by isolated thrombocytopenia and is the most common cause of thrombocytopenia in children. The incidence of ITP in children ranges from 1.9 to 9.5/100,000 children annually. A seasonal fluctuation is observed, peaking in late winter and early spring.[1-5]

Immune thrombocytopenia can be divided into three classes according to the duration of thrombocytopenia: It is called newly diagnosed ITP in the first three months after diagnosis, persistent ITP between 3 and 12 months after diagnosis, and chronic ITP if it lasts longer than 12 months.[6]

Primary immunodeficiencies are a group of diseases comprising over 500 diseases.[7] They can be seen at any age,

✉ Mustafa Büyükavcı ▪ buyukavci@hotmail.com

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but are much more common in childhood. There are delays of up to five years in diagnosing primary immunodeficiency patients, which may result in irreversible complications, especially bronchiectasis, after recurrent respiratory tract infections.[8-10]

In patients with primary immunodeficiency, susceptibility to autoimmune and infectious diseases increases. Common pathophysiological and genetic risk factors have been described for autoimmunity and primary immunodeficiency. Selective immunoglobulin A (IgA) deficiency is the most common primary immunodeficiency disease. The frequency of autoimmune diseases, including ITP, is higher in patients with selective IgA deficiency than in the general population.[8,11,12] In addition, autoimmune disease was found in approximately 20-30% of patients with common variable immunodeficiency, the second most common disease among all primary immunodeficiencies, and ITP has been detected in 10-12% of them.[8,13,14]

Autoimmunity may be the first manifestation in patients with primary immunodeficiency, and thrombocytopenia is the most common hematological finding.[8,15,16] Therefore, children with immune thrombocytopenia should be examined, considering that primary immunodeficiency may also be present.[16,17]

All these findings indicate that there may be some changes in the immune systems of children with ITP. We hypothesize that these changes, which may be related to disease onset, may also provide insight into disease prognosis. In this study, we investigated two issues: 1-Are the serum immunoglobulin levels and lymphocyte subsets at the time of diagnosis different from those of healthy children in children with acute ITP? 2- Is there a relationship between serum immunoglobulin and lymphocyte subset levels and treatment response?

MATERIALS AND METHODS

This retrospective study was carried out in the Sakarya University University Faculty of Medicine, Department of Pediatric Hematology and Oncology, and approved by the Ethics Committee of the Sakarya University Faculty of Medicine (March 30, 2021; E-71522473-050.01.04-21476-233). Patients under 18 years of age who were followed up with a diagnosis of immune thrombocytopenia in the Department of Pediatric Hematology and Oncology between 2018 and 2021 were included in the study. The diagnosis of immune thrombocytopenia was made based on the history, physical examination, and laboratory data. Children with platelet count <100,000/µL, average white blood cell count and hemoglobin values, and no findings suggestive of another cause for thrombocytopenia (a sign of hemolysis or blast on peripheral smear; positive direct Coombs test or antinuclear antibody; lymph node, spleen or liver enlargement; joint anomalies and presence of hyper or hypopigmented skin lesions; family or patient history of abnormal bleeding) as a result of examination and laboratory tests were defined as ITP. Patients with thrombocytopenia lasting less than one year were accepted as acute or persistent immune thrombocytopenia and included in the study. However, patients who had been symptomatic for longer than 1 year were considered to have chronic immune thrombocytopenia and were excluded from the study.

Demographics of the patients, laboratory test results at admission (platelet, white blood cell, neutrophil, and lymphocyte counts in peripheral blood; Immunoglobulin isotype, IgG subtype, complement C3, C4, and lymphocyte subset levels), treatment modalities, and platelet counts measured in the control periods (7, 15, and 30. days; 2, 3 and 6. months) were recorded. An increase in the platelet count to >30,000/µL was considered a positive response to treatment (responder), and <30,000/µL was considered a negative response (non-responder).[18] Patients were divided into two groups, “low” and “normal,” according to the age-appropriate reference ranges for lymphocyte subsets[19], immunoglobulin isotypes[20], IgG subtypes[21] and complement[22] levels. We evaluated values below the lower limit of 95% confidence interval (<-1.96 SD) as low.

The control group was selected from children referred to the Pediatric Immunology Outpatient Clinics at Sakarya University and were deemed healthy. In this study, only immunoglobulin isotype and IgG subtype levels were compared between the healthy control and patient groups.

Statistical analyses

The numerical variables used in the study were evaluated for normality using the Kolmogorov-Smirnov test. All variables except some had a normal distribution. Logarithmic transformation was performed on variables that did not follow a normal distribution, and the transformed data showed a normal distribution. All numerical variables were presented as mean ± standard deviation. Categorical variables were expressed as numbers and percentages. Numerical variables were compared using the independent samples t-test. Categorical variables were compared using the chi-square test. Repeated-measures ANOVA was used to

assess whether platelet counts changed during the control periods. Logistic regression analysis and the Backward Wald elimination model were applied to determine the factors affecting the treatment response rate in the control periods. Type I error (α) was predicted as 0.05 in the study, and it was considered statistically significant when the p-values were below 0.05. All statistical analyses were performed using software (IBM SPSS Statistics, Version 23.0, Armonk, NY: IBM Corp.).

RESULTS

The study was conducted on 30 children with ITP (70.03±57.07 months of age; 13 males, 17 females) and 30 age and sex-matched healthy controls (70.08±57.3 months of age; 14 males, 16 females).

There was no statistically significant difference between the patient and control groups regarding immunoglobulin isotype and IgG subtype levels. However, in 10 (33.3%) patients with a normal CD3+ cell ratio, IgG levels were below normal limits, and this was accompanied by at least one decrease in IgA or IgM according to the age-related reference values of serum immunoglobulin levels in healthy Turkish children. These patients were considered to have a suspected diagnosis of common variable immune deficiency. In addition, IgA and IgG2 levels were low in two of 14 patients with normal IgG levels but low levels of one IgG subtype. These patients may also have selective IgA deficiency. However, the diagnosis could not be confirmed in both groups.

Children with ITP were divided into "low" and "normal" subgroups according to immunoglobulin isotype and IgG subtype levels. At least one immunoglobulin isotype or subtype level was lower than the age-appropriate reference values, except in one patient. While serum IgA levels were below normal in 26.7% of patients, IgM levels were low in 60% of patients (Table 1).

Regarding lymphocyte subset levels, at least one subset value was below the age-appropriate reference range except in one patient. The percentage of CD3+CD8+ cells was lower than normal in 3.3% of patients, whereas 40% of patients had a lower-than-normal CD19+ cell population (Table 1).

There was a statistically significant difference ($p<0.001$) between the platelet counts measured at the time of the diagnosis and on the 7th day, 15th day, 30th day, second month, third month, and sixth month after the diagnosis (Figure 1). As a result of pairwise comparisons, it was determined that the mean platelet value at the time of diagnosis was statistically significantly lower than all other measurements except the 7th day; and the 7th, 30th day, and 2nd month values were lower than the 3rd and 6th months.

Table 1. Immunoglobulin isotypes, IgG subtypes, and lymphocyte subset levels of the patients according to the age-appropriate reference range

Immunoglobulin level		**n (%)**	**Lymphocyte subset levels**		**n (%)**
IgG	Low	13 (43.3)	CD3+ cells	Low	2 (6.7)
	Normal	17 (56.7)		Normal	28 (93.3)
IgA	Low	8 (26.7)	CD3+CD4+ cells	Low	4 (13.3)
	Normal	22 (73.3)		Normal	26 (86.7)
IgM	Low	18 (60.0)	CD3+CD8+ cells	Low	1 (3.3)
	Normal	12 (40.0)		Normal	29 (96.7)
IgG1	Low	16 (57.1)	CD4+ / CD8+ cells	Low	5 (16.7)
	Normal	12 (42.9)		Normal	25 (83.3)
IgG2	Low	16 (57.1)	CD19+ cells	Low	12 (40.0)
	Normal	12 (42.9)		Normal	18 (60.0)
IgG3	Low	8 (28.6)	NK cells	Low	5 (16.7)
	Normal	20 (71.4)		Normal	25 (83.3)
IgG4	Low	8 (28.6)			
	Normal	20 (71.4)			

CD3+: cluster of differentiation 3 positive; CD4+: cluster of differentiation 4 positive; CD8+: cluster of differentiation 8 positive; CD19+: cluster of differentiation 19 positive; IgA: immunoglobulin A; IgG: immunoglobulin G; IgG1: immunoglobulin G1; IgG2: immunoglobulin G2; IgG3: immunoglobulin G3; IgG4: immunoglobulin G4; IgM: immunoglobulin M; NK: natural killer.

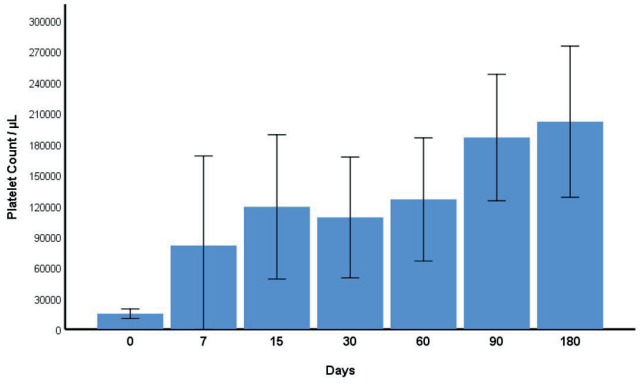

Figure 1. Platelet counts (per µL) in children with ITP at the time of diagnosis and during follow-up

Sixty-seven percent (67%), 68%, and 76% of patients responded to treatment (platelet count >30,000/µL) at the 7th, 15th, and 30th days, respectively, after treatment.

Ten (33.3%) children were treated with IVIG, eight (26.7%) with corticosteroids, and seven (23.3%) with sequential treatment (IVIG + corticosteroid); five (16.7%) were followed without treatment. The response rates of the treatment groups were similar (Table 2).

The age of the patients who responded to treatment on day seven was significantly lower than that of those who did not respond. However, there was no difference between the groups on days 15 and 30. There was no difference between the groups regarding white blood cell, neutrophil, and lymphocyte counts on days seven, 15, and 30.

Patients who did not respond to treatment on day seven had significantly higher IgG and IgA levels at diagnosis (973.6±311.8 mg/dL and 120.5±80.4 mg/dL) than responders (752.6±248.6 mg/dL and 70.3±49.7 mg/dL); however, IgM, IgG subtypes, and complement levels were similar in both groups (Table 3). There was no difference

Table 2. Comparison of the response rates of the treatment groups

	Response	IVIG n (%)	Corticosteroid n (%)	IVIG + Corticosteroid n (%)	W/O treatment n (%)	p*
7. day	Negative	1 (10.0)	2 (25.0)	5 (71.4)	2 (40.0)	0.061
	Positive	9 (90.0)	6 (75.0)	2 (28.6)	3 (60.0)	
15. day	Negative	1 (14.3)	2 (33.3)	4 (57.1)	1 (20.0)	0.337
	Positive	6 (85.7)	4 (66.7)	3 (42.9)	4 (80.0)	
30. day	Negative	1 (11.1)	1 (16.7)	3 (42.9)	1 (33.3)	0.474
	Positive	8 (88.9)	5 (83.3)	4 (57.1)	2 (66.7)	

* Fisher's Exact Test was used for statistics.
IVIG: intravenous immunoglobulin; W/O: without.

Table 3. Comparison of immunoglobulin isotypes, IgG subtypes, and complement levels at diagnosis according to seventh-day treatment responses (Mean±SD)

	Non-responder (n=10)	Responder (n=20)	p
IgG (mg/dL)	973.6±311.8	752.6±248.6	**0.044**
IgA (mg/dL)	120.5±80.4	70.3±49.7	**0.043**
IgM (mg/dL)	119.9±58.1	81.6±30.6	0.076
IgG1 (mg/dL)	742.3±268.4	603.3±235.1	0.166
IgG2 (mg/dL)	190.6±90.2	136.6±65.8	0.080
IgG3 (mg/dL)	71.3±37.5	63.2±25.3	0.502
IgG4 (mg/dL)	70±52.3	53.4±61.9	0.161
C3 (g/L)	1.3±0.2	1.2±0.3	0.499
C4 (g/L)	0.2±0.1	0.2±0.1	0.232

C3: complement component 3; C4: complement component 4; IgA: immunoglobulin A; IgG: immunoglobulin G; IgG1: immunoglobulin G1; IgG2: immunoglobulin G2; IgG3: immunoglobulin G3; IgG4: immunoglobulin G4; IgM: immunoglobulin M.

Table 4. Comparison of treatment responses on day 7 according to immunoglobulin levels at diagnosis

		Non-responder (n=10) n (%)	Responder (n=20) n (%)	p
IgG	Low	1 (10.0)	12 (60.0)	**0.017**
	Normal	9 (90.0)	8 (40.0)	
IgA	Low	1 (10.0)	7 (35.0)	0.210
	Normal	9 (90.0)	13 (65.0)	
IgM	Low	4 (40.0)	14 (70.0)	0.139
	Normal	6 (60.0)	6 (30.0)	
IgG1	Low	5 (50.0)	11 (61.0)	0.698
	Normal	5 (50.0)	7 (39.0)	
IgG2	Low	5 (50.0)	11 (61.0)	0.698
	Normal	5 (50.0)	7 (39.0)	
IgG3	Low	3 (30.0)	5 (28.0)	1.000
	Normal	7 (70.0)	13 (72.0)	
IgG4	Low	1 (10.0)	7 (39.0)	0.194
	Normal	9 (90.0)	11 (61.0)	

IgA: immunoglobulin A; IgG: immunoglobulin G; IgG1: immunoglobulin G1; IgG2: immunoglobulin G2; IgG3: immunoglobulin G3; IgG4: immunoglobulin G4; IgM: immunoglobulin M.

Table 5. Results of multivariate logistic regression analysis of factors affecting treatment response on day seven

	β	SE (β)	p	OR	95% CI (OR)
Age (month)	- 0.026	0.013	0.049	0.974	0.949 – 0.999
IgG	3.286	1.499	0.028	26.740	1.417 – 504.549
IgA	3.076	2.324	0.186	21.679	0.228 - 2062.178
IgG1	- 2.446	1.532	0.110	0.087	0.004 - 1.744
Constant	1.927	1.256	0.125		

Age at diagnosis, IgG1, IgG2, IgG3, IgG4 levels, and CD3+, CD3+CD4+, CD3+CD8+, CD19+ cells, and NK cell levels were included in the logistic regression model. The backward Wald elimination method was applied.
CI: confidence interval; IgA: immunoglobulin A; IgG: immunoglobulin G; IgG1: immunoglobulin G1; CI: Confidence interval; OR: odds ratio; SE: standard error.

between the two groups in immunoglobulin isotypes, IgG subtypes, or complement levels compared with responses at 15 and 30 days.

In cases with low IgG levels at diagnosis, the positive treatment response rate was significantly higher on day 7. There was no difference between patients with low IgA, IgM, and IgG subtype levels and those with normal levels. There was no difference in response rates between children with low and normal immunoglobulin levels on days 15 and 30 (Table 4).

The relationship between lymphocyte subsets and treatment responses was also examined. The lymphocyte subset levels of the patients who responded and did not respond to treatment on the 7th, 15th, and 30th days were not different. Similarly, patients with low and normal lymphocyte subset levels had similar response rates on days seven, 15, and 30.

In logistic regression analysis, lower patient age and IgG level were associated with an increased treatment response rate on day 7 (Table 5). However, no factors affected the treatment response rate on day 15 and day 30.

DISCUSSION

Almost all children with acute ITP had either low serum IgG or low lymphocyte subset levels. In only one patient, both immunoglobulin levels and lymphocyte subset ratios were within the normal range. The response rates for patients with low IgG levels at diagnosis were significantly higher on the seventh day of treatment. In logistic regression analysis, a low IgG level was associated with an increased treatment response rate on day 7.

Rahiminjad et al.[23] evaluated 36 patients with ITP, aged 3-51, and found no significant differences in immunoglobulin isotype and IgG subtype levels between the patient and control groups. However, they reported selective IgA deficiency in two (5.5%) patients and IgG subtype deficiency in four (11.1%) patients. In the present study, all three immunoglobulin isotype levels were normal in seven (23.3%) patients. However, one, two, and three different immunoglobulin isotype levels were below the reference values in 40%, 20%, and 16.7% of the patients, respectively. Ten (33.3%) patients had low IgG levels accompanied by low levels of at least one of the IgA and IgM. However, the CD3-positive cell ratios were normal. Although common variable immunodeficiency was suspected in these ten patients, the diagnosis could not be confirmed.

Serum immunoglobulin levels are among the most important screening tests for diagnosing primary and secondary immunodeficiencies. Common variable immunodeficiency progresses with low serum IgA or IgM levels in addition to low IgG.[8] The frequency of autoantibodies increases in patients with both isolated and combined IgA and IgG2 deficiency.[24] In this study, of the 17 patients with normal IgG isotype levels, 14 had a decrease in any of the IgG subtypes. IgA and IgG2 levels were low in 2 of these 14 patients. We thought these two children with normal IgG isotype levels might have selective IgA deficiency. Twenty percent of the healthy population may have low IgG subtypes and progress without clinical findings. These cases do not require treatment and should not be evaluated for immunodeficiency unless clinical findings are present.[23]

In a study examining lymphocyte distribution in the bone marrow of children with acute ITP, an increase in B cell precursor markers, such as CD10, CD19, and CD20, and a significant decrease in T cell markers (CD2, CD3, CD5, and CD7) were observed.[25] Zahran et al.[26] reported that the peripheral blood CD8+ and CD19+ cell ratios were significantly higher than the healthy group, and the CD4+ cell ratio was significantly lower in 40 children with acute ITP. In addition, there are differences in CD8+ and CD4+ T lymphocyte subpopulations. Central memory T (TCM) and CD8+ Naïve T cells are lower, and CD8+ T effector memory RA cells are higher; CD4+ TCM cells are lower; however, CD4+ T effector memory is higher in children with ITP than in controls.[27] Unlike the above studies, we did not compare the patient results with the control group. We only determined those that were low or high according to the reference values. In the present study, the rate of CD19+ cells was below the reference range in 40% of the patients, and the percentages of CD8+ and CD4+ cells were mainly within the normal range. Two children with a low CD19+ cell percentage were accompanied by a low T cell (CD3+ in one patient, CD3+, CD4+ in another) and low immunoglobulin levels. Although these two patients had no history of recurrent severe infections, they were referred for evaluation for combined immunodeficiency. As a result, 10 (33.3%) patients were referred to the Immunology and Allergy Clinic with suspicion of common variable immunodeficiency, two (6.6%) patients with suspicion of combined immunodeficiency, and two patients with suspicion of IgA - IgG subtype deficiency.

We found that patients who responded to treatment on day seven were significantly younger, and in the logistic regression analysis, increasing age was associated with a decreased response rate to treatment. However, this effect was not observed on days 15 and 30. Additionally, during any of the control periods, there was no difference in white blood cell, neutrophil, or lymphocyte counts at the time of diagnosis between the treatment-responsive and non-responsive groups, nor in gender. In a multicenter cohort study involving 705 pediatric patients, remission rates at 12 and 24 months increased with decreasing age.[28] Ahmed et al.[29] reported that children's age was not correlated with remission. Although white blood cell and lymphocyte counts in peripheral blood have been reported not to influence treatment success in adults with ITP, no study in children has demonstrated an association between these parameters and treatment response.[30]

Selective IgA deficiency was defined in 1% of the patients with ITP and common variable immunodeficiency in 1% of the patients, and it was found that elevated serum IgA and a decrease in serum IgM levels were associated with treatment-resistant ITP.[31] However, this association was mostly demonstrated in patients aged 65 and over, and no relationship was found between serum immunoglobulin levels and the course of ITP in children under 18 years of age. Other studies have shown that high IgA and low IgM

levels are associated with lower treatment success.[32] In both studies mentioned above, treatment response was evaluated cumulatively, and no comparison was made in terms of responses at different periods, as we did. We detected an inverse relationship between immunoglobulin G and A levels at diagnosis and treatment response on day seven. However, there were no significant differences in IgM, IgG subtypes, and lymphocyte subset levels between the groups that responded and those that did not respond to treatment on days 7, 15, and 30. In addition, we did not find a significant difference between groups in treatment responses of patients with normal and high IgA levels during the control periods.

Autoantibodies against platelets are observed in approximately two-thirds of patients with ITP, most commonly IgG.[3,33-36] In patients with ITP and high serum IgG levels, platelet surface IgG is also elevated, and the presence of high IgG on the platelet surface shortens platelet survival.[37] In the light of the above studies, it can be speculated that the patients with low serum IgG levels in the current study also had low antiplatelet antibody levels and therefore had better responses on day seven.

The literature has reported that many changes in the T cell line play a role in the pathogenesis of ITP.[38,39] In addition, regulatory T cells (Tregs) are lower in patients with ITP than in the healthy population, and there is a negative correlation with treatment response.[26,40-42] Zhao et al.[43] reported that adults with ITP who have higher levels of CD8+ cells or lower levels of CD4+/CD8+ cell ratio responded poorly to first-line steroid and IVIG treatments. However, Zahran et al.[26] found that children with a high cytotoxic CD8+ T cell population at baseline had a higher recovery rate within the first three months. This study did not find a relationship between lymphocyte subset proportions measured at diagnosis and treatment response within the first 30 days.

A limitation of our study is that the patients' serum immunoglobulin isotype and IgG subtype levels, and lymphocyte subgroup ratios, were evaluated only at the time of diagnosis. Since these measurements were not repeated, the diagnosis of immunodeficiency could not be confirmed, and the patients were referred to the Pediatric Immunology and Allergy Clinic for evaluation. In addition, the small sample size and the lack of evaluation of the treatment effects on clinical outcomes are other limitations of the study. In conclusion, serum immunoglobulin levels at the time of diagnosis can help predict treatment response in the early period and detect subclinical immunodeficiency in children with acute ITP.

Ethical approval

This study has been approved by the Ethics Committee of Sakarya University Faculty of Medicine (approval date 30.03.2021, number E-71522473-050.01.04-21476-233). Written informed consent was obtained from the participants.

Author contribution

The authors declare contribution to the paper as follows: Study conception and design: MFO, ÖÖ, MB; data collection: MFO, MCD, MB; analysis and interpretation of results: ÖÖ, MB; draft manuscript preparation: MFO, MB. 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

The evaluation of hepatitis B positivity in fully vaccinated children with juvenile idiopathic arthritis

Sümeyra Özdemir Çiçek[1], Nihal Şahin[2], Ayşenur Paç Kısaarslan[1], Meda Kondolot[3], Ruhan Düşünsel[4], Muammer Hakan Poyrazoğlu[1]

[1]Department of Pediatric Rheumatology, Faculty of Medicine, Erciyes University, Kayseri, Türkiye
[2]Department of Pediatric Rheumatology, Faculty of Medicine, Kocaeli University, Kocaeli, Türkiye
[3]Department of Social Pediatrics, Ankara Sami Ulus Research and Training Hospital, University of Health Sciences, Ankara, Türkiye
[4]Department of Pediatric Rheumatology, Faculty of Medicine, Yeditepe University, İstanbul, Türkiye

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ABSTRACT

Objectives: Biologic disease-modifying antirheumatic drugs (bDMARDs) have been frequently used to treat juvenile idiopathic arthritis (JIA) resistant to classical (c) DMARDs. There is concern that vaccines may reduce vaccine effectiveness due to their immunosuppressive effects. This study aimed to evaluate the prevalence of anti-hepatitis B surface (HBs) antibody positivity in JIA patients treated with bDMARDs and to compare it with that in JIA patients treated with cDMARDs and in healthy controls.

Materials and Methods: Anti-HBs antibody positivity and titers were compared between patients with JIA treated with bDMARDs or only cDMARDs, followed in our clinic, and healthy controls aged 2-20 years. All participants were vaccinated in infancy according to the routine vaccination schedule in our country. Anti-HBs titers ≥10 IU/L were considered seroprotective.

Results: Ninety-two JIA patients receiving cDMARDs, 92 receiving bDMARDs, and 91 healthy controls were included in the study. The median time from the last vaccination to the study was 12.5 (min-max:3.5-17.55) years in controls, 12.5 (min-max:1.75-19.5) years in patients with cDMARDs, and 13.0 (min-max:2.25-19.0) years in patients with bDMARDs (p=0.060). Anti-HBs was positive in 50 (54.9%) controls, 66 (71.7%) patients with cDMARDs, and 62 (67.4%) patients with bDMARDs (p=0.048). Age, time from vaccination to study, and duration of bDMARD use were found to be risk factors for anti-HBs negativity in univariate regression analyses. According to the multivariable analysis of these three variables, the duration of bDMARD use was an independent risk factor for anti-HBs negativity (OR: 1.023, 95% CI: 1.005-1.041, p=0.013). Anti-HBs negativity was associated with a duration of bDMARDs longer than 32 months (AUC: 0.658, 95%CI: 0.541-0.776, p=0.014) with 60.0% sensitivity, and 59.7% specificity.

Conclusions: A longer duration of bDMARDs was found to be a risk factor for anti-HBs negativity. Physicians should be careful in terms of anti-HBs negativity when the duration of biologics use is prolonged.

Keywords: Anti-HBs antibody, biologic drugs, bDMARDs, hepatitis B, juvenile idiopathic arthritis, tumor necrosis factor inhibitors.

INTRODUCTION

Juvenile idiopathic arthritis (JIA) is the most common cause of chronic arthritis in children. Immunosuppressants and biologics are the cornerstones of treatment, but both raise concerns about reducing the protective effects of vaccines.[1-3] Studies evaluating the effects of classical disease-modifying anti-rheumatic drugs (cDMARDs) and biological (b) DMARDs on vaccine responses in JIA patients are controversial.[4] It is known that not only bDMARDs

✉ Sümeyra Özdemir Çiçek ▪ smyra_ozdemir@hotmail.com

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and cDMARDs, but also the disease itself can reduce the immunogenicity of vaccines.[5,6]

In recent decades, neonatal immunization programs have been implemented in many countries, but hepatitis B virus (HBV) infection remains a major public health problem.[7] Hepatitis B vaccine has been included in the national immunization programs for all children and at-risk groups. It has been routinely administered in Turkey as three doses at birth, 1 month, and 6 months since 1998. The immunization program has significantly decreased the seroprevalence of hepatitis B surface antigen (HBsAg).[8]

The American College of Rheumatology (ACR) recommended antibody testing for HBV prior to initiating methotrexate or tumor necrosis factor α inhibitors (TNFi) for only JIA patients at risk of HBV infection.[9] The authors identified patients born in geographic regions with HBsAg prevalence ≥2% as a risk factor for HBV infection.[9] The prevalence of HBsAg positivity was determined 4.0% in our country, so our JIA patients are at risk for HBV infection.[10]

The European League Against Rheumatism (EULAR) and the Paediatric Rheumatology European Society (PRES) have recommended adhering to national immunization guidelines for inactivated vaccines, such as the HBV vaccine, in JIA patients receiving cDMARDs or bDMARDs.[1,2] Low seroprotective antibody titers have also been reported in JIA patients.[11] However, the immunization and treatment guidelines do not specify whether booster vaccination or routine screening for protective antibody levels is required in JIA patients after initiation of treatment.[1,2,9] Over the past decade, there has been increasing evidence that inactivated vaccines, including HBV, do not exacerbate the underlying disease or cause serious side effects, and studies have shown that the immunogenicity of the HBV vaccine is adequate.[2,3,12] In contrast, several studies have reported that bDMARDs such as TNFi reduce the immunogenicity of the HBV vaccine.[13,14]

Our study aimed to evaluate HBV vaccination responses in JIA patients treated with cDMARDs and bDMARDs and to compare them with those in healthy controls. We also investigated whether the use of bDMARDs influences seroprotective antibody levels in JIA patients.

MATERIALS AND METHODS

Our study was carried out between July 2018 and July 2019, and included 92 JIA patients treated with cDMARDs, 92 patients treated with bDMARDs, and 91 healthy controls aged 2-20 years. The flowchart of patients and group assignments is shown in Figure 1. All JIA patients who were followed for at least three months and attended the outpatient clinic during this period were included in the study. The control group consisted of age and sex-matched healthy children who applied to general pediatrics clinics for routine examination. Patients or healthy children who received one or more booster doses of HBV vaccine after infancy were excluded. All patients and controls were vaccinated with the hepatitis B vaccine in infancy (0, 1, and 6 months schedule). Vaccination schedules were confirmed from vaccination cards. The diagnosis of JIA was made according to the International League of Associations for Rheumatology (ILAR) classification criteria.[15] Demographic data, clinical characteristics, and treatment durations were obtained from the medical records of JIA patients. The Childhood Health Assessment Questionnaire (CHAQ) and the Juvenile Arthritis Disease Activity Score 27 (JADAS27) were recorded for patients at the last visit.

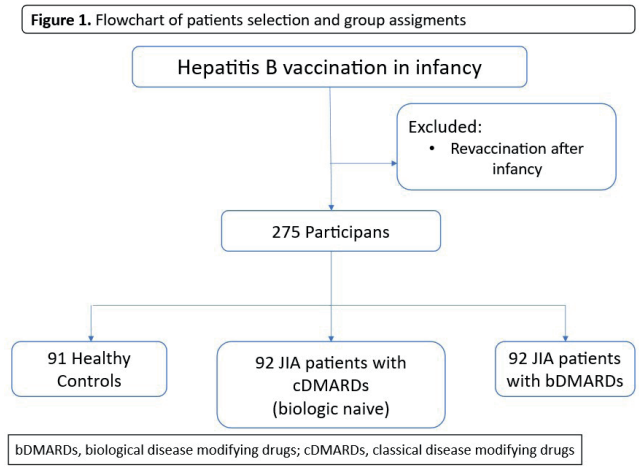

Figure 1. Flowchart of patients selection and group assigments

JIA patients were divided into two groups: patients treated with cDMARDs and with bDMARDs. Ninety-two JIA patients received standard therapies such as nonsteroidal anti-inflammatory drugs, systemic or intra-articular steroids, and cDMARDs (methotrexate, sulfasalazine, leflunomide, cyclosporine, hydroxychloroquine), and did not receive any bDMARDs treatment throughout the course of their disease. Ninety-two JIA patients received bDMARDs (anti-TNFα, anti-interleukin [IL]-6, anti-IL-1, or abatacept [cytotoxic T lymphocyte-associated antigen-4 immunoglobulin]) with/without other standard therapies and cDMARDs.

HBsAg, hepatitis B surface antibody (anti-HBs), and hepatitis B core antibody (anti-HBc) concentrations were measured using electrochemiluminescence immunoassay (ECLIA) (Cobas 8000, Roche Diagnostics, Germany). Anti-HBs titers ≥10 IU/L were considered seroprotective.[16] Patients who had an anti-HBs titer ≤10 IU/L were considered anti-HBs negative.

This study was carried out in accordance with the Declaration of Helsinki. Informed consent was obtained from all parents and patients, and from controls older than 18 years of age. This study was approved by the institutional ethics committee (2018/223)

Statistical analysis

Statistical analyses were performed using IBM SPSS 20 (SPSS, Inc., Chicago, IL). Categorical variables are presented as frequencies and percentages. The distribution of continuous variables was evaluated with the Kolmogorov-Smirnov test. Continuous variables are shown as mean and standard deviation (SD) if normally distributed, and median and minimum-maximum if abnormally distributed. The Mann-Whitney U test was used to compare two groups, and the Kruskal-Wallis test was used to compare three groups of continuous variables that were abnormally distributed. Logistic regression analysis was performed to investigate which factors were associated with anti-HBs negativity. The variables found to be significant in the univariate logistic regression analysis were subjected to multivariate regression analysis to determine the independent risk factors for anti-HBs negativity. The time interval between the last HBV vaccination and antibody testing varied among participants. We accounted for its potential effect by including the time from vaccination to the study as a covariate in a multivariate logistic regression model. Model calibration was evaluated using the Hosmer–Lemeshow goodness-of-fit test. Receiver operating characteristic (ROC) analysis was used to determine the optimal cutoff point for the duration of bDMARD treatment. A p-value of <0.05 was considered significant.

RESULTS

Ninety-two patients received bDMARDs, 92 patients received cDMARDs, and 91 healthy controls were included in the study. The median age was 13.0 (4.0-18.0) years in controls, 13.0 (2.25-20.0) years in cDMARDs group, and 13.5 (2.75-19.5) years in bDMARDs group (p=0.059). Forty-five (49.5%) of controls, 57 (62.0%) of patients treated with cDMARDs, and 58 (63.0%) of patients treated with bDMARDs were female (p=0.117). The median time from the last vaccination to the study was 12.5 (3.5-17.55) years in controls, 12.5 (1.75-19.5) years in patients with cDMARDs, and 13.0 (2.25-19.0) years in patients with bDMARDs (p=0.060).

Anti-HBs was positive in 50 (54.9%) controls, 66 (71.7%) patients with cDMARDs, and 62 (67.4%) patients with bDMARDs (p=0.048) (Table 1). The omnibus chi-square test indicated a significant association between the variables. However, Bonferroni-corrected post-hoc pairwise comparisons (three 2×2 sub-analyses) revealed no statistically significant differences between any specific category pairs (all p-values > 0.0167). This suggests that the overall chi-square significance reflects a distributed pattern across cells rather than a discrete pairwise difference.

Table 1. Comparison of the characteristics of healthy controls, patients with juvenile idiopathic arthritis treated with classical disease-modifying drugs, and with biological disease-modifying drugs

Variable	Control (n=91)	Patients with cDMARDs (n=92)	Patients with bDMARDs (n=92)	p value
Age, year	13.0 (4.0-18.0)	13.0 (2.25-20.0)	13.5 (2.75-19.5)	0.059
Gender				0.117
Female	45 (49.5)	57 (62.0)	58 (63.0)	
Male	46 (50.5)	35 (38.0)	34 (37.0)	
Time from last vaccination to the study, years	12.5 (3.5-17.55)	12.5 (1.75-19.5)	13.0 (2.25-19.0)	0.060
Anti-HBs				**0.048**
Positive (≥10 IU/L)	50 (54.9)	66 (71.7)	62 (67.4)	
Negative (<10 IU/L)	41 (45.1)	26 (28.3)	30 (32.6)	
Anti-HBs titer (IU/L)	12.95 (2-1000)	19.45 (2-1000)	26.68 (2-1000)	0.100

Values are summarized using median (min-max) and n (%). DMARDs, disease modifying anti-rheumatic drugs

Anti-HBs titers were not different among the three groups (p=0.100). None of the 275 participants had serological evidence of active or previous hepatitis B infection. HBsAg and anti-HBc were negative in all participants.

Demographic, clinical, and laboratory features of the patients are shown in Table 2. The median age at the study was 13.0 (2.25-20.0) years in the cDMARDs group and 13.5 (2.75-19.5) years in the bDMARDs group (p=0.018). The median age at diagnosis was 9.4 (1.33-16.0) years in the cDMARDs group and 8.38 (1.42-16.0) years in the bDMARDs group (p=0.481).

The time from vaccination to the study was 13.0 (2.75-19.0) versus 12.5 (1.75-19.5), and the follow-up time was 49.0 (5-172) versus 29.5 (3-132), respectively, in the

Table 2. Comparison of the characteristics of patients with juvenile idiopathic arthritis treated with classical disease modifying drugs and with biological disease modifying drugs

Variable	All JIA patients (n=184)	Patients with cDMARDs (n=92)	Patients with bDMARDs (n=92)	p value
Age, year	13.12 (2.25-20.0)	13.0 (2.25-20.0)	13.5 (2.75-19.5)	**0.018**
Gender				0.879
Female	115 (62.5)	57 (62.0)	58 (63.0)	
Male	69 (37.5)	35 (38.0)	34 (37.0)	
Age at diagnosis, years	8.89 (1.33-16.0)	9.4 (1.33-16.0)	8.38 (1.42-16.0)	0.481
Time from last vaccination to the study, years	12.62 (1.75-19.5)	12.5 (1.75-19.5)	13.0 (2.75-19.0)	**0.018**
Follow up, months	38.75 (3.0-172.0)	29.5 (3-132)	49.0 (5-172)	**<0.001**
JIA subtypes				0.001
Oligoarticular JIA	88 (47.8)	57 (62.0)	31 (33.7)	
ERA	42 (22.8)	18 (19.6)	24 (26.1)	
RF- polyarticular JIA	32 (17.4)	8 (8.7)	24 (26.1)	
RF+ polyarticular JIA	7 (3.8)	5 (5.4)	2 (2.2)	
Systemic JIA	11 (6.0)	4 (4.3)	7 (7.6)	
Psoriatic arthritis	3 (1.6)	-	3 (3.3)	
Undifferentiated	1 (0.5)	-	1 (1.1)	
ANA positivity (169/184)	84 (49.7)	43 (51.2)	41 (48.2)	0.701
HLA-B27 positivity (108/184)	25 (23.1)	8 (15.7)	17 (29.8)	0.082
Total number of involved joints in disease course	3 (1-30)	2 (1-25)	4 (1-30)	**<0.001**
Patients received steroids[#]	94 (51.1)	32 (34.8)	62 (67.4)	**<0.001**
Duration of steroids[#], months	3.1 (0.3-105.0)	1.9 (0.3-7)	4.12 (0.66-105)	**<0.001**
Cumulative steroid[#] dosage, grams	1.4 (0.08-22.38)	0.81 (0.08-5.62)	2.0 (0.15-22.38)	**0.001**
Patients received cDMARDs	177 (96.2)	86 (93.5)	91 (98.9)	0.118
Duration of cDMARDs, months	26.0 (1.0-140.0)	15.5 (1-105.0)	37.0 (1-140.0)	**<0.001**
Duration of bDMARDs, months	30.8 (1.33-115.0)	-	30.8 (1.33-115.0)	-
Active arthritis at last visit	0 (0-13)	0 (0-13)	0 (0-6)	0.127
CHAQ score at last visit	0 (0-1.88)	0 (0-1.0)	0 (0-1.88)	0.658
JADAS27 at last visit	0 (0-19.8)	0 (0-18.10)	0.05 (0-19.8)	0.696
Steroid[#] at last visit	8 (4.3)	4 (4.3)	4 (4.3)	1.000*
DMARDs at last visit	134 (72.8)	69 (75.0)	65 (70.7)	0.507
Biologics at last visit	76 (41.3)	-	76 (82.6)	-

Values are summarized using median (min-max) and n (%). ANA: anti-nuclear antibody; CHAQ: childhood health assessment questionnaire; DMARDs: disease modifying anti-rheumatic drugs; ERA: enthesitis related arthritis; HLA, human leukocyte antigen; JADAS, juvenile arthritis disease activity score; JIA: juvenile idiopathic arthritis; RF: rheumatoid factor.

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*Fisher's exact test

bDMARDs group compared with the cDMARDs group, p=0.018, p<0.001, respectively. Oligoarticular JIA patients constituted the majority of patients in both patient groups. JIA patient subtypes are shown in Table 2.

The number of involved joints, the number of patients who received steroids, and the total duration and dosage of steroid treatment were greater in the bDMARDs group (p<0.001). The median duration of cDMARD use was longer in the bDMARDs group (p < 0.001). The disease activity scores at the last visit were not different between cDMARDs and bDMARDs groups (p>0.05) (Table 2).

Sixty-three (68.5%) patients used only one bDMARD throughout the disease course, and 29 (31.5%) patients used more than one bDMARD due to drug switching because

Table 3. Comparison of anti-HBs positive and negative patients with juvenile idiopathic arthritis treated with and without biologics

Variable	Patients with cDMARDs (n=92)			Patients with bDMARDs (n=92)		
	Anti-HBs positive (n=66)	Anti-HBs negative (n=26)	p value	Anti-HBs positive (n=62)	Anti-HBs negative (n=30)	p value
Age, years	12.5 (2.25-20.0)	13.93 (7.25-18.0)	0.072	13.5 (3-19.5)	13.5 (2.75-19)	0.423
Gender			0.367			0.616
Female	39 (59.1)	18 (69.2)		38 (61.3)	20 (66.7)	
Male	27 (40.9)	8 (30.8)		24 (38.7)	10 (33.3)	
Age at diagnosis, years	8.08 (1.33-16.0)	10.6 (3.83-15.33)	0.033	8.5 (1.42-16.0)	7.96 (1.75-15.33)	0.960
Time from vaccine to the study, years	12.0 (1.75-19.5)	13.43 (6.75-17.5)	0.072	13.0 (2.5-19.0)	13.0 (2.25-18.5)	0.423
Follow up, months	29 (3-132)	32 (3-108)	0.983	45 (5-164)	61 (13-172)	0.159
JIA subtypes			0.533			0.076
Oligoarticular JIA	44 (66.7)	13 (50.0)		23 (37.1)	8 (26.7)	
ERA	11 (16.7)	7 (26.9)		17 (27.4)	7 (23.3)	
RF- polyarticular JIA	6 (9.1)	2 (7.7)		11 (17.7)	13 (43.3)	
RF+ polyarticular JIA	3 (4.5)	2 (7.7)		2 (3.2)	-	
Systemic JIA	2 (3.0)	2 (7.7)		6 (9.7)	1 (3.3)	
Psoriatic arthritis	-	-		3 (4.8)	-	
Undifferentiated	-	-		-	1 (3.3)	
ANA positivity (84/85)	33 (55.0)	10 (41.7)	0.269	28 (50.0)	13 (44.8)	0.651
HLA B27 positivity (51/57)	7 (18.4)	1 (7.7)	0.662*	12 (30.0)	5 (29.4)	0.965
Total number of involved joints in disease course	2.0 (1-25)	2.0 (1-17)	0.807	3 (1-30)	6 (1-26)	**0.007**
Patients received steroids[#]	21 (31.8)	11 (42.3)	0.342	44 (71.0)	18 (60.0)	0.293
Duration of steroids[#], months	1.8 (0.3-4.8)	2.0 (0.5-7.0)	0.647	4.02 (0.66-105)	3.67 (0.66-55.5)	0.994
Cumulative steroid[#] dosage, g	0.7 (0.08-2.7)	0.97 (0.22-5.62)	0.634	1.83 (0.23-11.14)	3.09 (0.15-22.38)	0.675
Patients receiving DMARDs	61 (92.4)	25 (96.2)	0.672*	61 (98.4)	30 (100)	1.000*
Duration of DMARDs, months	18.0 (1-105)	14.5 (1-98)	0.628	29.0 (1-140)	46.13 (9-114)	0.078
Duration of biological drugs, months	-	-	-	29.42 (1.33-100)	38.0 (7.5-115)	**0.014**
Active arthritis at last visit	0 (0-12)	0 (0-13)	0.855	0 (0-4)	0 (0-6)	0.138
CHAQ score at last visit	0 (0-1)	0.06 (0-0.75)	0.321	0 (0-1.25)	0 (0-1.88)	0.291
JADAS27 at last visit	0 (0-18.1)	1.5 (0-15)	0.190	0 (0-13.6)	1.0 (0-19.8)	0.105
Steroids[#] at last visit	1 (1.5)	3 (11.5)	0.067*	2 (3.2)	2 (6.7)	0.594*
DMARDs at last visit	50 (75.8)	19 (73.1)	0.789	44 (71.0)	21 (70.0)	0.924
Biologics at last visit	-	-	-	51 (82.3)	25 (83.3)	0.899

Values are summarized using median (min-max) and n (%). ANA, anti-nuclear antibody; CHAQ, childhood health assessment questionnaire; DMARDs, disease modifying anti-rheumatic drugs; ERA, enthesitis related arthritis; HLA, human leukocyte antigen; JADAS, juvenile arthritis disease activity score; JIA, juvenile idiopathic arthritis; RF, rheumatoid factor

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of treatment failure. During the course of the disease, 61 patients in the bDMARD group received etanercept, 32 received adalimumab, 16 received tocilizumab, 14 received infliximab, 5 received anakinra, 3 received canakinumab, and 2 patients received abatacept. Because the number of patients receiving non-TNFi biologics was very small, subgroup sizes were highly unbalanced. Therefore, no inferential statistical comparison between bDMARD subclasses was performed.

Patients treated with cDMARDs and bDMARDs were evaluated separately for anti-HBs positivity (Table 3). The age at diagnosis was higher in anti-HBs negative patients with cDMARDs [10.6 (3.83-15.33) versus 8.08 (1.33-16.0), p=0.033]. Other demographic, clinical, and laboratory features were not different between anti-HBs-positive and anti-HBs-negative patients in the cDMARDs group (p>0.05) (Table 3). The number of joints involved was higher [6 (1-26) versus 3 (1-30) joints, p=0.007], and the duration of treatment with bDMARDs was longer [38.0 (7.5-115) versus 29.42 (1.33-100) months, p=0.014] in anti-HBs negative patients treated with bDMARDs.

Age, time from vaccination to study, and duration of bDMARD use were found to be risk factors for anti-HBs negativity in univariate regression analyses (Table 4). According to the multivariable analysis of these three variables, the duration of bDMARDs was an independent risk factor for anti-HBs negativity (OR: 1.023, 95% CI: 1.005-1.041, p=0.013) (Table 4). Goodness-of-fit was assessed using the Hosmer–Lemeshow test, which indicated an adequate model fit (χ^2 = 10.586, p = 0.226).

Table 4. Factors affecting the anti-HBs negativity in patients with juvenile idiopathic arthritis (n=184)*

Variables	Univariate		Multivariate$	
	OR (95% CI)	p	OR (95% CI)	p
Age, years	1.093 (1.007-1.1856	0.034	1.034 (0.915-1.169)	0.593
Gender	1.398 (0.720-2.714)	0.322		
Age at diagnosis, years	1.057 (0.985-1.134)	0.126		
Duration of disease, months	1.004 (0.996-1.013)	0.324		
Time from vaccine to the study, years	1.093 (1.007-1.186)	0.034	1.034 (0.915-1.169)	0.593
Number of involved joints	1.032 (0.978-1.089)	0.252		
ANA positivity	0.691 (0.359-1.330)	0.269		
HLA B27 positivity	0.776 (0.276-2.182)	0.631		
Receiving steroid# therapy	1.041 (0.555-1.951)	0.900		
Duration of steroid# therapy, months	0.996 (0.965-1.029)	0.812		
Cumulative total steroid# dosage (grams)	1.113 (0.960-1.291)	0.155		
Receiving cDMARD therapy	2.705 (0.318-23.009)	0.362		
Duration of cDMARD therapy, months	1.007 (0.996-1.018)	0.223		
Receiving bDMARDs therapy	1.228 (0.655-2.305)	0.522		
Duration of bDMARDs therapy, months	1.023 (1.005-1.041)	0.013	1.023 (1.005-1.041)	0.013
Active arthritis at last visit	1.137 (0.965-1.339)	0.126		
CHAQ score at last visit	1.706 (0.549-5.302)	0.356		
JADAS27 at last visit	1.054 (0.979-1.134)	0.161		
Steroid# treatment at last visit	4.085 (0.941-17.730)	0.060		
cDMARDs treatment at last visit	0.904 (0.449-1.821)	0.778		
bDMARDs treatment at last visit	1.218 (0.645-2.297)	0.543		

ANA, anti-nuclear antibody; CHAQ, childhood health assessment questionnaire; DMARDs, disease modifying anti-rheumatic drugs; ERA, enthesitis related arthritis; HLA, human leukocyte antigen; JADAS, juvenile arthritis disease activity score; JIA, juvenile idiopathic arthritis; RF, rheumatoid factor

*All JIA patients were included in univariate and multivariate logistic regression analyses

$Age, time from vaccine to the study and duration of bDMARDs therapy variables were included to the multivariate logistic regression analysis

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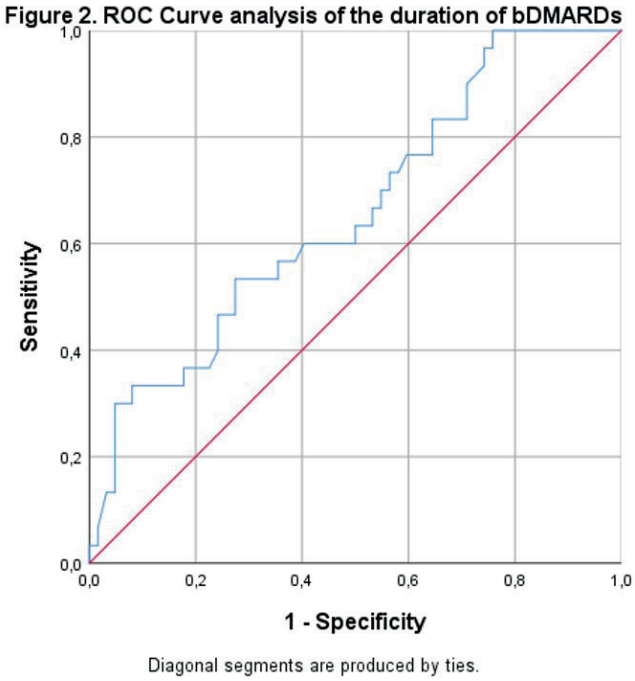

Figure 2. ROC Curve analysis of the duration of bDMARDs

ROC analysis was used to evaluate the optimal cutoff for the duration of bDMARD treatment for anti-HBs negativity. Anti-HBs negativity was associated with a duration of bDMARDs treatment longer than 32 months (AUC: 0.658, 95%CI: 0.541-0.776, p=0.014) with 60.0% sensitivity, and 59.7% specificity (Figure 2).

DISCUSSION

Our study showed that the use of bDMARDs is not a risk factor for anti-HBs negativity in JIA patients, whereas longer bDMARD use is a significant risk factor for decreased seroprotective antibody levels. Therefore, we believe that patients on bDMARDs should be followed more closely for anti-HBs negativity, especially if the duration of treatment is prolonged.

Previous studies have shown that anti-HBs positivity rates ranging 60.9-69.2% in healthy children, and the percentage of seroprotective anti-HBs levels decreases over time in healthy children vaccinated in infancy.[8,17-21] Anti-HBs positivity rate in healthy children was 54.9% in our study. Consistent with the literature, our study also showed that anti-HBs positivity decreases with age. However, interestingly, the percentage of anti-HBs-negative individuals was higher in the control group than in the JIA groups in our study. The lack of significant Bonferroni-adjusted pairwise differences implies that the observed association in the overall chi-square test may result from small, non-specific deviations across multiple cells rather than a strong difference between any two individual categories. In a previous study, Heijstek et al.[5] showed that patients with JIA had lower levels of mumps, rubella, diphtheria, and tetanus-specific antibodies, except for measles, compared to healthy controls. Interestingly, they found higher antibody levels in patients with active disease.[5] They could also not explain the reason, but they concluded that nonspecific immune activation during active disease plays a role in modulating antibody concentrations.[5] The immune activation in the disease course may have caused the anti-HBs positivity to be higher in our JIA patients than in controls. When we compared anti-HBs-negative and anti-HBs-positive patients with JIA, we could not find any difference in disease activity scores.

We found that anti-HBs titers did not differ between healthy controls, JIA patients on cDMARDs, and bDMARDs. Szczygielska et al. investigated the anti-HBs concentration in 56 JIA patients vaccinated in infancy and who had received bDMARDs for at least 3 months; 22 (39.3%) were anti-HBs negative, and 34 (60.7%) were seropositive.[11] The rate of anti-HBs positivity was similar to that in our study. However, they did not include healthy controls for comparison with JIA patients. In addition, no statistically significant correlation was found between the anti-HBs concentration and the time after the last vaccination, or the current age of the patients.[11]

Anti-HBs positivity rate was not lower in JIA patients treated with cDMARDs or bDMARDs in our study. Maritsi et al.[22] reported that anti-HBs antibody titers were lower in treatment-naïve JIA patients than in matched healthy children. Similarly, Çakmak et al. investigated HBV vaccine responses in treatment-naïve JIA patients and reported lower anti-HBs positivity in JIA patients than in healthy controls.[6] These findings suggested that the disease itself reduces anti-HBs positivity. But we could not evaluate treatment-naïve JIA patients.

Salinas GF et al.[14] evaluated HBV vaccine responses in SpA patients receiving only TNFi treatment, and none of these patients received concomitant immunosuppressive drugs. Serum samples were collected at baseline before HBV vaccination, and at 6, 10, 22, and 26 weeks after vaccination.[14] The median antibody titers in patients treated with or without TNFi were 10 IU/ml (0-115 IU/ml) and 595 IU/ml (65-2190 IU/ml) at week 26, respectively (p=0.005).[14]

They claimed that there was a strong suppression of the induction of T-cell-dependent antibodies against HBV in patients receiving TNFi treatment.[14] Their study evaluated anti-HBs levels at 6th months postvaccination, but we evaluated long-term antibody concentrations and did not find any significant difference in anti-HBs concentrations.

In our study, we did not find any differences in anti-HBs levels or positivity rates by JIA subtype. Kostik et al. investigated the risk factors for non-protective antibody levels against HBV, measles, mumps, rubella, and diphtheria. They reported that patients treated with bDMARDs had the lowest probability of having protective antibody levels against HBV, measles, mumps, and diphtheria compared to patients treated with MTX and NSAIDs.[3] In addition, they found that the most important predictor affecting anti-HBs antibodies was systemic JIA subtype.[3] Univariate logistic regression analysis showed that age, time from vaccination to the study, and duration of bDMARDs were statistically significant risk factors for anti-HBs negativity in our study. The time interval between the last HBV vaccination and antibody testing varied among participants. We included this variable in our multivariate regression model to avoid the potential misleading effect. We evaluated these three variables using multiple logistic regression analysis, and only the duration of bDMARDs was found to be an independent risk factor for anti-HBs negativity. We also compared anti-HBs-negative and anti-HBs-positive patients separately. We confirmed that the duration of bDMARD therapy was longer in anti-HBs-negative patients in the bDMARD group. The frequencies of memory B cells, antibody-secreting cells, and plasma cells associated with maintenance of anti-HBs antibody titers in healthy subjects.[23] Anolik et al. have shown that TNFi treatment reduces the percentage of memory B cells in the peripheral blood.[24] The reason why anti-HBs negativity was seen more frequently in our patients with a longer bDMARDs duration may be related to this aspect.

There are some limitations of our study. First, although the majority of bDMARDs received were TNFi, some patients received other bDMARDs. Second, we did not evaluate treatment-naïve JIA patients. Also, patients on bDMARDs may represent a subgroup with more severe disease, which could confound the results. But we included disease activity scores, the number of active joints, other treatments such as steroids and cDMARDs, and the duration of cDMARD use. These variables were not significant in univariate analysis. The strengths of our study are that it covers the long-term period after the vaccination and that the effects of both cDMARDs and bDMARDs on vaccine responses were also assessed.

CONCLUSION

To our knowledge, this is the first study to show a correlation between vaccine response and the duration of bDMARD use. The rate of anti-HBs positivity did not differ between patients treated with cDMARDs, bDMARDs, and healthy controls. However, the duration of biologic therapy seems to be a risk factor for anti-HBs negativity. Patients receiving bDMARDs should be carefully monitored for anti-HBs negativity, especially if the duration of treatment is prolonged. Prospective studies with larger, more homogeneous patient groups are needed.

Ethical approval

This study has been approved by the Erciyes University Clinical Researches Ethics Committee (approval date 18.04.2018, number: 2018/223). Informed consent was obtained from all parents and patients, and from controls older than 18 years.

Author contribution

The authors declare contribution to the paper as follows: Study conception and design: SÖÇ, APK, MK, MHP; data collection: SÖÇ, NŞ; analysis and interpretation of results: SÖÇ, NŞ, APK, RD; draft manuscript preparation: SÖÇ, APK. 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

Evaluation of teachers' first aid knowledge about children with seizures before and after video simulation training

Pınar Özkan Kart[1], Beril Dilber[2], Sinan Paslı[3], Nihal Yıldız[4], Ahmet Kağan Özkaya[5], Gülnur Esenülkü[1], Tülay Kamaşak[2], Elif Acar Arslan[6], Sevim Şahin[2], Ali Cansu[2]

[1]Department of Pediatric Neurology, Trabzon Kanuni Training and Research Hospital, Health Science University, Trabzon, Türkiye
[2]Department of Pediatric Neurology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Türkiye
[3]Department of Emergency Medicine, Faculty of Medicine, Karadeniz Technical University, Trabzon, Türkiye
[4]Department of Pediatric Neurology, Faculty of Medicine, Zonguldak Bülent Ecevit University, Zonguldak, Türkiye
[5]Department of Pediatric Emergency, Faculty of Medicine, Karadeniz Technical University, Trabzon, Türkiye
[6]Department of Pediatric Neurology, Faculty of Medicine Hospital, Biruni University, İstanbul, Türkiye

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ABSTRACT

Objective: This study aimed to evaluate the effect of training teachers using the video simulation method on their knowledge of epileptic seizure management and on their first-aid intervention skills.

Methods: A survey was distributed to 250 participating teachers working in both private and public schools. The survey included basic demographic information, teachers' awareness of epilepsy, and their knowledge of first aid measures. Subsequently, the teachers received a training session on general information about epilepsy, first response to epileptic seizures, and emergency procedures using the video simulation training method. Awareness and attitudes were reassessed using the same survey.

Results: The study involved 250 participants with an average age of 39.76 years, 62.8% female, and 78.4% working in the public sector. Participants with connections to individuals with epilepsy had significantly higher pre-training correct response scores (p=0.026, p=0.001). 72.8% had received first-aid training, and 26.6% had performed first aid, but only 6.1% considered their knowledge sufficient. Before training, 94.4% recognized epilepsy as a neurological disorder. The awareness of epilepsy being treatable increased from 70.7% to 90.4% (p<0.001). Knowledge of proper seizure interventions significantly improved, with correct responses to questions about safe positioning and jaw clenching rising from 52.4% to 78.4% and 52.4% to 90.8%, respectively (p<0.001). The total number of correct answers significantly increased after training, from 9.38 ± 4.18 to 11.59 ± 3.64 (p<0.001).

Conclusion: These findings indicate that video simulation training is an effective method for improving teachers' knowledge and first-aid skills in managing epileptic seizures, supporting the integration of structured simulation-based interventions into school-based emergency preparedness programs.

Keywords: epilepsy, first-aid management, school teachers, educational video

✉ Pınar Özkan Kart ▪ drpinar_ozkan@yahoo.com

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INTRODUCTION

Epilepsy is a common chronic neurological disorder and a significant public health issue.[1] Globally, 4–10% of children experience at least one seizure by age 16, with about one-third occurring in schools.[1,2] This highlights the critical role of teachers as first responders, responsible for ensuring the child's safety and providing seizure first aid.

Epilepsy is considered a neurological emergency due to the sudden and unpredictable onset of seizures, which can be life-threatening if not promptly and correctly managed. Seizures may be distressing for both the patient and observers, and inadequate first aid can lead to injury or death.[3] Special attention is needed in school and work settings, where trained individuals can ensure safety during seizures, provide appropriate post-seizure care, and seek medical assistance when necessary. Public education and awareness programs can promote safer social participation for individuals with epilepsy and help reduce disease-related stigma.[3,4] Children with epilepsy may face additional challenges at school, including social isolation, learning difficulties, and stigmatization.[5]

Teachers' knowledge of epilepsy and seizure first aid is crucial for student safety and well-being.[5] Studies indicate that teachers' misconceptions about epilepsy negatively impact the educational experiences of children with the condition.[3,6] Teachers' knowledge and attitudes can directly influence students' academic performance, development, and self-confidence. During a seizure, teachers are responsible for preventing injury, providing appropriate post-seizure care, and seeking medical assistance when necessary, as improper interventions may cause secondary harm.[7] Seizures lasting longer than five minutes or occurring consecutively indicate status epilepticus, which requires immediate medical attention. Early recognition and referral are crucial to prevent serious neurological and systemic complications.[8]

To enhance teachers' knowledge and preparedness in managing epileptic seizures, video simulation training offers an innovative instructional approach that combines visual demonstration with scenario-based learning. Video simulation training is widely used in clinical and emergency education to support both knowledge acquisition and practical skill development.[9] This approach incorporates elements such as interactive video content, branching scenarios, role-model demonstrations, and expert commentary, aiming to help learners develop both cognitive and affective skills within a safe and controlled setting.[9] Video simulation training is a hybrid model that fits naturally with existing training approaches.[10] In this study, a video simulation was created based on current evidence on epileptic seizure management.

The aim of this study was to innovatively enhance teachers' competencies in providing accurate first aid to students experiencing seizures and to investigate changes in their knowledge and attitudes toward seizure management before and after a video simulation–based training program specifically designed for effective seizure management in the school setting.

MATERIALS AND METHODS

Study design

This cross-sectional study was conducted between November 15 and December 31, 2022. Following approval from the ethics committee and obtaining the necessary permissions from the Ministry of National Education, 250 teachers working in private and public schools affiliated with the Provincial Directorate of National Education were included in the study. Data were collected using survey forms administered before and after a three-minute clinical simulation training video titled "First Approach to a Child Having a Seizure."

Sample size and sampling procedure

The target population and sample size were determined based on previous studies.[11] Using OpenEpi, the minimum sample size required to detect at least a 20% change between pre-test and post-test responses was calculated as 118, with 95% confidence and 90% power.[12] Due to higher-than-expected voluntary participation, all eligible teachers who consented were included, resulting in a final sample of 250 participants, which enhanced the study's statistical power.

Data collection tools

The data collection instrument was adapted from a prior study, with context-appropriate modifications to ensure its suitability for the current research objectives.[13] These modifications included adjustments to item wording, alignment with the target population, and refinement of response options for improved clarity. Following the adaptation process, the instrument was reviewed for content relevance and comprehensibility. It was then administered to volunteer teachers from the designated

schools, selected according to predetermined inclusion criteria, to obtain the data necessary for the study.

In the video simulation, seizure management was explained step by step and demonstrated on a mannequin experiencing a seizure, in accordance with evidence from the literature. The questionnaire was first administered to participants before the training video. Following this pre-test, the teachers watched the video simulation training video (Appendix 1). After viewing the video, the same questionnaire, consisting of identical items, was re-administered as a post-test to assess changes in knowledge. The first section of the 25-item questionnaire assessed participants' sociodemographic characteristics, including age, gender, length of professional experience, family history of epilepsy, prior knowledge about epilepsy, and first-aid knowledge. The second section evaluated teachers' knowledge of epilepsy and appropriate interventions during an epileptic seizure. For knowledge assessment, each correct response was scored as "1" and each incorrect response as "0"; unanswered items were excluded from scoring. The total score for all knowledge items was 21. To evaluate the effect of the training, the difference between pre-test and post-test correct response rates was analyzed, reflecting improvements in participants' knowledge and their ability to provide correct interventions during seizures.

Video simulation training

The video aimed to provide a standardized, structured, and reproducible educational experience for all participants. The educational content and seizure management steps in the video were aligned with current recommendations from the International League Against Epilepsy (ILAE) and internationally accepted first-aid principles for seizure management (https://www.cdc.gov/epilepsy/first-aid-for-seizures/index.html).[14] The video was simulated by a pediatric neurologist and clinical educators experienced in pediatric emergency care and simulation-based education. Prior to implementation, the video content was reviewed by two pediatric neurology experts and one simulation-based education specialist. They evaluated the scenario, seizure description, demonstrated interventions, and pedagogical coherence. Minor revisions were made based on their feedback to ensure adherence to best practices. The video clearly defined learning objectives, aiming to improve teachers' knowledge, first-aid skills, and appropriate decision-making during seizure events in the school setting.

Video content summary

In this study, the video simulation was developed based on current literature on seizure management and included both theoretical and practical components (Appendix 2). In the first 1 minute and 10 seconds, the video provided brief information on epilepsy, seizure types, treatment principles, and the importance of correct first aid. From 1:10 to 2:52, a child seizure scenario was narrated: a previously healthy boy suddenly collapses while playing, exhibiting limb stiffness, shaking, upward eye deviation, and jaw clenching. During this segment, seizure management steps were demonstrated on a manikin, highlighting correct interventions while verbally emphasizing actions to avoid. In the final segment (2:52–3:02), conditions requiring activation of emergency medical services were displayed on the screen and read aloud. This video provided a standardized, structured, and reproducible educational experience for all participants. The simulation used in this study is provided in Appendix 2.

Statistical analysis

Normality assumptions were examined with kurtosis and skewness values. If the kurtosis and skewness coefficients were within ±2, it was accepted that the data were in compliance with a normal distribution.[15] We used an independent two-sample t-test to compare the pre-test and post-test total correct answers between two groups, assuming a normal distribution. One-way analysis of variance (ANOVA) was used to compare pre- and post-test total correct answers, assuming a normal distribution across three or more groups. Variance homogeneity was examined using the Levene test, and the F test was evaluated when homogeneity was achieved. In cases of significant difference in ANOVA results, multiple comparisons were examined using the Duncan test under the assumption of homogeneity of variances. A paired two-sample t-test was used to compare the total correct numbers of pre-test and post-test with a normal distribution. Comparisons between pre-test and post-test in categorical data were examined using McNemar's test for two-time two-category data and the Stuart-Maxwell (Marginal Homogeneity) test for two-time three-category data. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 26.0 (IBM Corp., Armonk, NY, USA), and a p-value of <0.05 was considered statistically significant.

RESULTS

The study included 250 participants with a mean age of 39.76 years (range: 25–65). Of the participants, 62.8% were female, and 78.4% were employed in public schools. Regarding the educational level at which they taught, 44.8% worked in elementary schools, while 33.2% were primary school teachers. Descriptive statistics on the participants' demographic characteristics are presented in Table 1.

In the study, 0.8% of the participants reported having epilepsy, while 11.6% indicated that they had close relatives with the condition. Additionally, 30.4% stated that they had at least one student with epilepsy in their schools. Participants who had relatives or students with epilepsy demonstrated significantly higher mean pre-training correct response scores compared with those who did not (p = 0.026 and p = 0.001, respectively).

Table 1. Descriptive statistics of the participants' demographic characteristics

Characteristics	Mean ± SD n	Median (min.-max.) %
Age	39.76 ± 8.54	40 (25- 65)
Sex		
Female	157	62.8
Male	93	37.2
School type		
Public	196	78.4
Private	54	21.6
School education level		
Daycare/Preschool	17	6.8
Primary School	112	44.8
Middle School	86	34.4
High School	35	14
Teaching field		
Primary school teacher / Elementary school teacher	83	33.2
Special education teacher	27	10.8
Mathematics teacher	20	8
English language teacher	20	8
School counselor /Guidance and psychological counselor	15	6
Early childhood education teacher	14	5.6
Turkish language teacher	14	5.6
Religious culture and ethics teacher	11	4.4
Social studies teacher	7	2.8
Science teacher / general science teacher	7	2.8
Physical education teacher	5	2
Music teacher	5	2
Biology teacher	4	1.6
Chemistry teacher	3	1.2
Visual arts teacher	3	1.2
Technology and design teacher	3	1.2
Physics teacher	2	0.8
Geography teacher	2	0.8
Computer science teacher / Ict teacher	2	0.8
German/Arabic language teacher	2	0.8
Health sciences teacher	1	0.4
Years of teaching experience		
1-5	30	12
6-10	52	20.8
> 10	168	67.2
Do you have epilepsy?		
Yes	2	0.8
No	248	99.2
Do you know anyone with epilepsy?		
Yes	29	11.6
No	221	88.4
Do you have students with epilepsy?		
Yes	76	30.4
No	174	69.6
Have you ever received first aid training?		
Yes	182	72.8
No	68	27.2
Sufficient first aid knowledge?		
Yes	15	6.1
No	147	60.2
Partially	82	33.6

SD: Standard Deviation, min: minimum, max: maximum.

SD: Standard Deviation, min: minimum, max: maximum.

Among the participants, 26.6% (n= 66) reported having previously administered first aid. In response to the question, "Do you think your first-aid knowledge is sufficient?", 6.1% (n= 15) answered "Yes," 60.2% (n=147) answered "No," and 33.6% (n= 82) responded "Partially." Additionally, 72.8% (n=182) had received prior first-aid training, with most participants reporting having obtained it from various sources. Among those who had received first-aid training, 61.2% (n=153) had attended face-to-face courses or training, 9.6% (n=24) had self-taught using informal online resources (such as informational websites or videos), and 7.2% (n=18) had completed structured online courses or formal training programs.

According to the participants, 59.3% (n=147) had previously witnessed someone experiencing an epileptic seizure. When examining their reactions to witnessing an epileptic seizure, the following responses were noted: 11.6% reported feeling "fear," 12.4% "panic," 2% "I distanced myself from the scene," 6.8% "I did nothing," and 2% selected "other". On the other hand, in terms of positive responses, 33.2% stated that they "assisted," and 16.8% reported that they " immediately called the emergency medical services number (112)."

Analysis of participants' knowledge revealed significant improvements following the training (Table 2). Recognition of epilepsy as a treatable condition increased markedly (70.7% to 90.4%, p <0.001). Awareness of key seizure characteristics—including behavioral arrest, involuntary facial movements, limb contractions, and incoherent speech—also improved significantly ($p \leq 0.002$ for all). The proportion selecting "all of the above" rose from 15.6% to 28.0% (p = 0.001), while the percentage reporting no knowledge declined from 2.4% to 0% (p = 0.030). No significant changes were observed for generalized muscle contractions or memory impairment (p> 0.05). Overall, the training substantially enhanced teachers' understanding of epilepsy and seizure presentation.

Significant improvements were observed in participants' knowledge regarding safe positioning during an epileptic seizure (Table 3). The proportion selecting "move the person to a safe place" increased from 52.4% to 78.4% (p <0.001), and those choosing "turn the person onto their left side to facilitate breathing" rose from 45.6% to 84.4% (p <0.001). Similar significant gains were noted for "prevent vomit from entering the lungs" and "place a soft pillow under the head" (p <0.001). The percentage of participants reporting "I have no knowledge/no opinion" declined from 15.2% to 0% (p <0.001).

Table 2. Evaluation of participants' knowledge levels regarding epilepsy disease

Related questions	Pre-test		Post-test		p
	Unmarked	Marked	Unmarked	Marked	
Is epilepsy a treatable condition?					
Yes	176 (70.7)		226 (90.4)		**<0.001**[mc]
No	73 (29.3)		24 (9.6)		
What kind of condition is epilepsy?					
Psychological	4 (1.6)		6 (2.4)		0.050[sm]
Supernatural	2 (0.8)		2 (0.8)		
Neurological	236 (94.4)		241 (96.4)		
I have no knowledge	8 (3.2)		1 (0.4)		
How would you describe an epileptic seizure?					
Staring at a single point and appearing lost in thought	175 (70.3)	74 (29.7)	131 (52.4)	119 (47.6)	**<0.001**[mc]
Involuntary facial expressions and movements, such as lip biting	165 (66)	85 (34)	129 (51.6)	121 (48.4)	**0.002**[mc]
Generalized body stiffness and trembling	55 (22)	195 (78)	45 (18)	205 (82)	0.320[mc]
Stiffness and trembling in a single body part	207 (82.8)	43 (17.2)	162 (65.1)	87 (34.9)	**<0.001**[mc]
Incoherent speech and repetitive movements	217 (86.8)	33 (13.2)	195 (78)	55 (22)	**0.015**[mc]
Memory loss to the extent of distraction	191 (76.4)	59 (23.6)	171 (68.4)	79 (31.6)	0.065[mc]
None of the above	249 (99.6)	1 (0.4)	250 (100)	0 (0)	1.000[mc]
All of the above	211 (84.4)	39 (15.6)	180 (72)	70 (28)	**0.001**[mc]
I don't know	244 (97.6)	6 (2.4)	250 (100)	0 (0)	**0.031**[mc]

mc: McNemar test, sm: Stuart–Maxwell test, n (%), p-value of <0.05.

Following the training, participants' knowledge regarding jaw clenching during an epileptic seizure improved significantly (Table 3). The proportion selecting unsafe interventions—such as inserting objects into the mouth or forcibly opening it—decreased (p <0.001), while those choosing "provide a safe position and allow the seizure to stop" increased from 52.4% to 90.8% (p <0.001). The percentage reporting "I have no knowledge/no opinion" declined from 14% to 0% (p <0.001), and the selection of "all of the above" also decreased significantly (p = 0.035).

Table 3. Comparison of participants' knowledge levels on epileptic seizure management

Related questions	Pre-test		Post-test		p^{mc}
	Unmarked	Marked	Unmarked	Marked	
What actions should be taken to ensure a safe position for someone having an epileptic seizure?					
The individual should be moved to a safe location	119 (47.6)	131 (52.4)	54 (21.6)	196 (78.4)	<0.001
The person should be turned onto their left side to facilitate easier breathing	136 (54.4)	114 (45.6)	39 (15.6)	211 (84.4)	<0.001
The individual should be turned onto their left side to prevent aspiration of stomach contents in case of vomiting	130 (52.2)	119 (47.8)	85 (34)	165 (66)	<0.001
Clothing that may restrict breathing, such as ties, and breakable items like glasses should be removed	104 (41.6)	146 (58.4)	43 (17.2)	207 (82.8)	<0.001
The person should be positioned with their head lower than their feet	231 (92.4)	19 (7.6)	232 (92.8)	18 (7.2)	1.000
A soft pillow should be placed under the individual's head	197 (78.8)	53 (21.2)	78 (31.2)	172 (68.8)	<0.001
The person should be left in place without interference or intervention	234 (93.6)	16 (6.4)	243 (97.2)	7 (2.8)	0.078
I don't know / I have no opinion	212 (84.8)	38 (15.2)	250 (100)	0 (0)	<0.001
What should be done if an individual having an epileptic seizure has a clenched jaw?					
Attempt to open the mouth by inserting objects such as a fork or spoon	210 (84)	40 (16)	241 (96.4)	9 (3.6)	<0.001
Attempt to open the mouth with fingers	214 (85.9)	35 (14.1)	238 (95.2)	12 (4.8)	<0.001
Immediately call an ambulance	154 (61.6)	96 (38.4)	201 (80.4)	49 (19.6)	<0.001
Ensure a safe position and wait for the seizure to stop	119 (47.6)	131 (52.4)	23 (9.2)	227 (90.8)	<0.001
I don't know / I have no opinion	215 (86)	35 (14)	250 (100)	0 (0)	<0.001
Which of the following interventions are correct for an individual having an epileptic seizure?					
The person is exposed to the smell of onion or garlic	242 (96.8)	8 (3.2)	249 (99.6)	1 (0.4)	0.039
The person is exposed to the smell of cologne	235 (94)	5 (6)	247 (98.8)	3 (1.2)	0.008
A slap is applied	248 (99.2)	2 (0.8)	250 (100)	0 (0)	0.500
The body is shaken	246 (98.4)	4 (1.6)	249 (99.6)	1 (0.4)	0.375
Sugar water is given to the person	247 (98.8)	3 (1.2)	250 (100)	0 (0)	0.248
Water is poured on the person	249 (99.6)	1 (0.4)	249 (99.6)	1 (0.4)	1.000
Cardiopulmonary resuscitation is performed	250 (100)	0 (0)	249 (99.6)	1 (0.4)	1.000
Artificial respiration is given	248 (99.2)	2 (0.8)	249 (99.6)	1 (0.4)	1.000
A safe position is ensured, and the seizure is allowed to stop	66 (26.4)	184 (73.6)	5 (2)	245 (98)	<0.001
I don't know / I have no opinion	205 (82)	45 (18)	249 (99.6)	1 (0.4)	<0.001
When would you call an ambulance for an individual having an epileptic seizure?					
Immediately	115 (46)	135 (54)	225 (90)	25 (10)	<0.001
Never	248 (99.2)	2 (0.8)	248 (99.2)	2 (0.8)	1.000
If the seizure lasts longer than 5 minutes	164 (65.6)	86 (34.4)	39 (15.6)	211 (84.4)	<0.001
If there is discoloration or blueness in the skin	195 (78)	55 (22)	118 (47.2)	132 (52.8)	<0.001
If consciousness and respiration do not return to normal after the seizure	170 (68)	80 (32)	76 (30.4)	174 (69.6)	<0.001
If the individual has another seizure without regaining consciousness after the first one	185 (74)	65 (26)	90 (36)	160 (64)	<0.001
If confusion or disorientation persists an hour after the seizure	218 (87.2)	32 (12.8)	149 (59.6)	101 (40.4)	<0.001
If a serious injury occurred during the seizure	188 (75.2)	62 (24.8)	94 (37.6)	156 (62.4)	<0.001

mc: McNemar test, n (%), p-value of <0.05.

Table 4. Comparison of pre- and post-test total correct scores according to demographic characteristics

Characteristics	Pre-test	Test statistic	p	Post-test	Test statistic	p
Sex						
Female	9.8 ± 4.16	2.056	0.041[t]	11.54 ± 3.74	-0.298	0.766[t]
Male	8.68 ± 4.16			11.68 ± 3.5		
School Type						
Public	9.84 ± 4.09	3.355	0.001[t]	11.71 ± 3.7	1.002	0.317[t]
Private	7.72 ± 4.15			11.15 ± 3.43		
School education level						
Daycare/Preschool	12.41 ± 3.78[a]	3.547	0.015[f]	10.82 ± 3.64	0.844	0.471[f]
Primary School	9.37 ± 4.29[b]			11.56 ± 3.64		
Middle School	9.08 ± 3.69[b]			11.99 ± 3.59		
High School	8.69 ± 4.7[b]			11.06 ± 3.81		
Years of teaching experience						
1-5	10.17 ± 4.91	0.719	0.488[f]	11.73 ± 3.24	0.033	0.968[f]
6-10	9.52 ± 3.41			11.62 ± 3.87		
> 10	9.2 ± 4.27			11.55 ± 3.66		
Do you know anyone with epilepsy?						
Yes	11 ± 3.98	2.235	0.026[t]	11.1 ± 3.43	-0.761	0.447[t]
No	9.17 ± 4.17			11.65 ± 3.67		
Do you have students with epilepsy?						
Yes	10.72 ± 4.22	3.427	0.001[t]	11.78 ± 4	0.511	0.610[t]
No	8.79 ± 4.04			11.51 ± 3.49		

t: Independent samples t-test, f: One-way ANOVA, Test ist.: Test statistic, Mean± Standard deviation, a–b: Groups sharing the same letter do not differ significantly (Duncan test), p-value of <0.05.

The training led to significant improvements in participants' knowledge of correct interventions (Table 3). Selections of ineffective measures, such as "smelling onion or garlic" and "smelling cologne," decreased (p = 0.039 and p = 0.008), while choosing "provide a safe position and allow the seizure to stop" increased from 73.6% to 98% (p <0.001). The proportion reporting "I have no knowledge/no opinion" declined from 18% to 0.4% (p <0.001).

Participants' knowledge regarding emergency response improved significantly after the training (Table 3). While nearly all participants correctly identified 112 as the emergency number both before and after the training, responses about when to call an ambulance during a seizure changed markedly. The proportion selecting "immediately" decreased (p <0.001), whereas selecting "if the seizure lasts longer than 5 minutes" increased from 0.8% to 84.4% (p <0.001). Significant increases were also observed for conditions such as cyanosis, failure of consciousness or breathing to return to normal, subsequent seizures without recovery, prolonged dizziness, and severe injury (p <0.001).

A significant increase was observed in the total number of correct answers after the training (pre-training: 9.38±4.18; post-training: 11.59±3.64; p < 0.001), indicating an overall improvement in participants' knowledge (Figure 1). Before the training, significant differences in total correct answers were observed based on sex and institution type (Table 4).

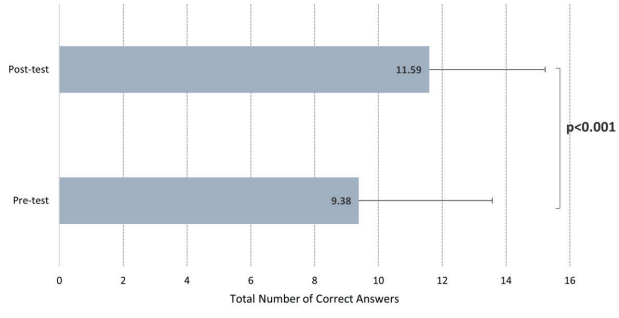

Figure 1. Comparison of teachers' average correct answers before and after training

Females scored higher than males (p= 0.041), and public-school teachers outperformed those in private institutions (9.84 vs. 7.72, p = 0.001). Before the training, significant differences in total correct answers were observed based on school level, family history of epilepsy, and having students with epilepsy. Preschool/kindergarten teachers scored highest (12.41), while high school teachers scored lowest (8.69) (p= 0.015). Participants with relatives or students with epilepsy also scored higher than those without (11 vs. 9, p=0.026; 10.72 vs. 8.79, p=0.001). No significant difference was found based on years of professional experience (p=0.488).

DISCUSSION

Epileptic seizures are acute clinical events that frequently require emergency intervention, and the implementation of correct first aid is essential to prevent injuries, reduce complications, and decrease mortality.[16] The findings of this study demonstrate that video simulation training significantly improved teachers' knowledge, attitudes, and intervention skills regarding epileptic seizures.

Teachers' knowledge of epilepsy was found to be limited prior to the training program, with a mean total of correct answers of 9.38 ± 4.18, which increased to 11.59 ± 3.64 following the training. These findings align with previous studies indicating the presence of knowledge gaps regarding epilepsy among teachers.[17] Consistent with previous national studies, our findings highlight that teachers' first-aid knowledge and competence in managing seizures remain moderate despite prior training. For instance, a Turkish study involving 291 teachers reported that while those with first-aid training scored higher on self-efficacy scales, overall knowledge was still at a moderate level, with participants most frequently encountering minor injuries, bleeding, and seizures.[18] Similarly, other studies in Turkey have demonstrated that teachers possess insufficient first-aid knowledge and exhibit limited awareness and practical skills in managing epilepsy and seizures.[19,20] These consistent findings underscore the need for innovative and structured educational interventions, such as video simulation training, to effectively enhance teachers' preparedness and confidence in responding to school-based emergencies.

When examining teachers' total knowledge pre- and post-test scores, higher baseline levels were observed among female teachers, those working in public schools, preschool and primary school teachers, participants with students with epilepsy, and those with individuals with epilepsy in their immediate environment. Our findings are consistent with the existing literature and suggest that prior exposure to epilepsy—whether through professional experience or personal familiarity—together with demographic and institutional factors, may contribute to higher baseline knowledge.[13,21,22] Pre-test scores differed across school levels, with preschool teachers demonstrating higher baseline knowledge, likely due to greater emphasis on child safety and first-aid training. Post-test scores showed no significant differences, indicating that video-simulation training effectively improved seizure first-aid knowledge across all groups, reducing initial disparities. These findings support the implementation of standardized training programs and align with previous studies showing higher first-aid knowledge among preschool and primary school teachers.[19,23] On the contrary, a nationwide study conducted with 291 teachers from various educational levels across all provinces of Türkiye, using an online questionnaire, similarly reported that teachers' first-aid knowledge levels were only moderate and that there were no significant differences between school levels.[18] These findings suggest that deficiencies in first-aid knowledge are not limited to a particular educational stage but represent a systemic need across the entire school system.

Alkhotani et al. reported that private school teachers demonstrated higher pre-education awareness (81.3%) than public school teachers (49.8%), whereas post-education results showed a reversal, with public school teachers (88.5%) outperforming private school teachers (62.5%).[24] This pattern suggests that education yields stronger gains among groups with lower baseline levels. In our study, public school teachers had significantly higher pre-test scores than private school teachers, whereas no significant difference remained in post-test scores. These findings collectively indicate that education reduces initial disparities between school types and contributes to leveling teachers' knowledge.

Although this study aimed to assess seizure first aid knowledge among teachers of both sexes, the majority of participants (62.8%) were female, consistent with previous literature reporting higher participation of female teachers in education- and health-related studies.[7,25] This greater interest among female teachers may reflect a higher awareness of the field. Another notable finding is that 33% of participants were primary school teachers. Given that this group is more likely to encounter neurological conditions such as epilepsy, it highlights the need for targeted seizure first-aid training for classroom teachers. Furthermore, guidance and special education teachers comprised 10.8% of the sample, indicating that this group frequently works

with students with learning disabilities or special needs and is therefore more likely to encounter seizures, underscoring the importance of providing them with appropriate first-aid training.

The study findings indicate that 94.4% of teachers recognized epilepsy as a neurological disorder; however, their overall knowledge of first aid, seizure types, seizure management, and related topics was insufficient. This suggests that while basic awareness of epilepsy exists, more in-depth education is needed to develop a comprehensive understanding and enhance teachers' practical skills. In a comparable study, 87.5% of teachers identified epilepsy as a neurological disorder, and 65.5% believed it to be treatable. Nevertheless, only 9.2% had received first-aid training, 52% reported that they would ensure safety and call for help during a seizure, and 64.8% indicated that they would position the student on their side and seek assistance after the seizure.[26] In our study, although over half of the participants had received first-aid training, 63.8% had completed it more than five years ago. Nearly half of the participants correctly answered questions on seizure response. Additionally, 59.3% had previously witnessed a seizure, with 33.2% assisting and 16.8% immediately calling emergency services, while 11.6% reported fear, 12.4% panic, and 6.8% inaction. These findings indicate that although many participants were willing to intervene, anxiety and insufficient knowledge contributed to passive or avoidant behaviors. Similarly, Al Muslim et al. conducted a study with 423 teachers and found that 61.5% of them had insufficient knowledge about epilepsy. However, nearly half of the participants answered questions about how to respond during a seizure correctly.[26] Many studies clearly indicate that there is a lack of sufficiently trained educators who have received first-aid training.[7,27,28] This may reflect reliance on online research or past experiences, highlighting the need to use reliable guidelines and sources for accurate information.

Before the training, most teachers demonstrated limited knowledge of epilepsy, with 70.7% recognizing it as a treatable condition and 78% identifying seizures primarily as generalized convulsions, consistent with previous literature.[24] Before the training, less common seizure manifestations—such as blankly at a fixed point, involuntary movements, lip biting, localized muscle contractions, incoherent speech, repetitive behaviors, and memory loss—were infrequently recognized. After the training, recognition of these symptoms significantly improved, highlighting the need for teachers to acquire comprehensive knowledge of different seizure types, including absence epilepsy, the most common form in children aged 6–11, accounting for approximately 18% of cases.[29] Teachers are crucial in recognizing absence seizures. Initially, only 29.7% identified a blank stare as a symptom; this increased to 47.6% after training. Nonetheless, most teachers still failed to associate blank stares with seizures, highlighting the need for further training on subtle seizure types.

Video simulation-based first-aid training significantly improved teachers' awareness and attitudes toward epilepsy. The proportion of participants correctly selecting "ensure a safe position and wait for the seizure to stop" when the jaw was clenched increased from 52.4% pre-training to 90.8% post-training. Similarly, correct responses regarding interventions to maintain a safe position increased significantly, indicating the training effectively enhanced knowledge and attitudes toward seizure management. İsler et al. reported that, before a modular training program, only 30% of healthcare professionals correctly identified the appropriate initial interventions for a child experiencing a seizure. After the training, this proportion increased to 70%, demonstrating the effectiveness of the program in improving seizure management skills among pediatric healthcare providers.[11]

Incorrect or delayed interventions during seizure first aid can lead to secondary injuries and serious complications, including status epilepticus.[30] A study in Cameroon found that despite high knowledge, medical students still exhibited inappropriate attitudes and practices toward epileptic seizures.[31] A systematic review revealed teachers' insufficient knowledge and negative attitudes toward epilepsy, including limited awareness of its emergency management.[24] This suggests that traditional beliefs and values established in society shape the perception of epilepsy. First-aid training and awareness campaigns based on accurate information can improve teachers' competence, positively impact attitudes, and enhance the safety and quality of life for individuals with epilepsy. Using diverse materials—such as video simulations, brochures, case examples, and online content—can further engage teachers and improve knowledge retention. Tavares et al. evaluated the impact of a low-cost program comprising a brief educational video and booklet on teachers' knowledge of epilepsy and seizure first aid.[32] This aligns with our findings, showing a significant increase in teachers' knowledge after training. The study also noted that the booklet alone had little impact, with the primary effect attributed to the video-based training.[32] These results indicate that brief video trainings effectively enhance epilepsy awareness and should be more widely

incorporated into educational programs, as supported by evidence on the benefits of multimedia-based learning.[33] In our study, video simulation training presented practically on a model by a health professional was supported by visual and auditory components. It is reported in the literature that similar educational videos have a high impact on learning.[11,33,34]

In comparison with many studies conducted in Türkiye, the present study offers a broader scope and more robust methodology. Most national studies have focused exclusively on teachers working in preschool or primary education and have relied primarily on survey-based assessments, many of which demonstrated that teachers' first-aid knowledge levels were below the desired competency.[20,35] In contrast, our study includes teachers from all educational stages and employs both a structured questionnaire and a video-simulation–based training intervention. Therefore, it not only assesses baseline knowledge but also evaluates the effectiveness of an educational intervention, providing a methodological contribution to the national literature and offering a more comprehensive understanding of teachers' seizure first-aid preparedness.

Methodological issues and limitations

This study has several methodological limitations. First, although the questionnaire was adapted from a previous study and reviewed by experts, formal construct validation and pilot testing were not conducted, and internal consistency (Cronbach's α) could not be calculated due to the heterogeneous item structure. Second, the post-video questionnaire was administered again after a short interval, which may have limited the assessment of longer-term learning effects. Third, reliance on structured questionnaires may not fully capture the complexity of knowledge related to seizure management. Finally, the intervention relied solely on a video-based format; incorporating additional educational materials could have provided more comprehensive learning outcomes. These limitations should be considered when interpreting the findings.

CONCLUSION

This study aimed to evaluate the awareness of epilepsy and first aid among teachers through video simulation training. After the training, a significant increase was observed in teachers' theoretical knowledge regarding intervention during seizures. The study results show that teachers improved their knowledge across all knowledge categories. This development provided by the training shows that teachers will be able to intervene in seizure cases more consciously and effectively in the future.

Ethical approval

This study has been approved by the Local Ethics Committee of Faculty of Medicine, Karadeniz Technical University (approval date 30.12.2001, number2021/319). Written informed consent was obtained from the participants.

Author contribution

The authors declare contribution to the paper as follows: Study conception and design: PÖK, AC, BD, SP, NY, AKÖ; data collection: PÖK, AC, BD, SP, NY, AKÖ, GE, TK, EAA, SS; analysis and interpretation of results: PÖK, AC; draft manuscript preparation: PÖK. 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

Are AI chatbots reliable sources for parents to ask about their concerns about childhood vaccination?

Zeynep Gizem Ergün Özdel[1], Esra Çiftci[2], Mustafa Hacımustafaoğlu[2]

[1]Division of Social Pediatrics, Department of Pediatrics, Faculty of Medicine, Bursa Uludağ University, Bursa, Türkiye
[2]Division of Pediatric Infectious Diseases, Department of Pediatrics, Faculty of Medicine, Bursa Uludağ University, Bursa, Türkiye

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ABSTRACT

Background: The increasing use of AI-powered chatbots for health-related inquiries has positioned them as potential tools in combating vaccine hesitancy among parents. However, the reliability of these tools in delivering accurate, consistent, and actionable information on childhood immunization remains underexplored.

Objective: This study aimed to assess how the most widely used AI chatbots guide parents seeking information about childhood vaccines, rather than comparing them against each other.

Methods: A cross-sectional comparative design was used. Three freely accessible, commonly used AI conversational agents were each presented with 9 frequently asked parental questions about childhood vaccinations. To maintain neutrality and avoid brand-based interpretation bias, these chatbots are anonymized in the study as AICB 1, AICB 2, and AICB 3. Responses were independently evaluated across four domains—accuracy, consistency, information sufficiency, and source reliability—using a 5-point Likert scale. Each chatbot was tested in two temporally distinct sessions to assess consistency.

Results: All chatbots generated scientifically accurate and temporally consistent responses. Mean composite scores were highest for AICB 1 (4.9), followed by AICB 2 (4.7) and AICB 3 (4.1). The performance difference was statistically significant ($H = 11.27$, $p < 0.01$). Despite the statistically significant differences between the agents, all three chatbots achieved high scores across the four evaluated dimensions.

Conclusion: We need to know that AI chatbots can offer accessible, generally reliable information on vaccines and provide more reliable, accurate data than profit-driven websites; they should be used as supplementary tools, especially when addressing sensitive public health topics like childhood immunization.

Keywords: artificial intelligence chatbots, vaccine hesitancy, digital health

INTRODUCTION

Internet use for health information seeking has grown exponentially over the last three decades. A synthesis of studies conducted between 1994 and 2018 shows that nearly 79% of parents use the Web to search for general health information concerning their children, with Google as the almost universal starting point for such queries.[1] Yet online searches do not necessarily alleviate anxiety; between 14% and 52% of parents report experiencing stress

✉ Zeynep Gizem Ergün Özdel ▪ zeynepgizem@uludag.edu.tr

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or worry after reading health content on the Web, and only 46 – 54% trust the information they find. 41% of parents report difficulty identifying trustworthy sources, and only half perceive the Web as a reliable medium.[1] In parallel, artificial intelligence (AI) has spread into everyday life, reshaping industries and decision-making at a global scale.[2] Türkiye mirrors these trends: AI applications now range from fraud detection in banking to AI-assisted diagnostic tools in healthcare.[3] Public awareness is high, and survey data places Türkiye among the top five countries for active use of generative AI chatbots (AICB).[4] Nevertheless, ethical challenges such as algorithmic bias and data privacy remain.[5]

AI chatbots are increasingly being used in healthcare to support patient education[6] and chronic disease management[7], and are becoming more prevalent, offering real-time, accessible, and personalized health information. Their use in vaccine communication, particularly among parents, has gained attention amid growing concerns about vaccine hesitancy and digital misinformation.[8,9]

Recent studies suggest that chatbots (ChatGPT-4.0, Google Gemini) can provide accurate and reliable scientific reference sources, aligned responses to vaccine-related questions, and effectively counteract misinformation.[10,11] Parents often appreciate their availability and ease of use.[12] However, concerns remain regarding their lack of empathy, context sensitivity, and the reliability of underlying data sources.[13]

Most studies indicate that chatbots provide scientifically grounded and reliable vaccine-related information, are particularly effective in addressing misinformation[8-11,13], and can respond to anti-vaccine conspiracy theories with clear, evidence-based explanations.[11] Users report high satisfaction with chatbots due to their speed, accessibility, and ability to provide personalized responses.[12-14]

Chatbots have been found to increase knowledge and promote positive attitudes toward vaccination, although their influence on vaccine uptake appears to be more limited.[8,13] Chatbots have outperformed medical students in answering complex vaccine-related questions in educational settings.[15] Despite their potential, most chatbots are relatively simplistic, and their long-term effects and ethical implications remain insufficiently studied.[13,16]

Therefore, AI chatbots are valuable tools that generally provide accurate, up-to-date information.[8,10,11,13] They counteract misinformation and also provide user satisfaction and accessibility[8,11,14,17] but they are not a substitute for expert consultation.[10,11] Official health authorities and expert medical advice should remain the primary sources of reliable guidance.

AI-powered conversational agents are increasingly consulted for medical queries. Their potential to deliver instant, comprehensible answers could be valuable to parents who struggle with online information overload. Our study, therefore, aims to determine whether AI chatbots are a reliable source of vaccine information for parents.

METHODS

Study design

This study employed a comparative cross-sectional design to evaluate the reliability of three widely used, free-to-access AI-powered conversational agents (AI Chatbots; abbreviated as AICB) in responding to nine frequently asked parental questions about childhood vaccination. Although product names are disclosed once for transparency, they are anonymized throughout the evaluation process to eliminate potential brand-related bias. Therefore, the chatbots were coded as AICB 1, AICB 2, and AICB 3 throughout the manuscript. Each chatbot was anonymized and referred to using the following codes:

AICB 1: GPT-4o (OpenAI, 2025)
AICB 2: Microsoft Copilot
AICB 3: Google Gemini Flash 2.0 (2025)

This approach allowed for an objective evaluation of the overall reliability, consistency, and sufficiency of chatbot-generated vaccine-related content without drawing attention to specific commercial platforms.

Selection rationale

All three AI chatbots were selected based on their widespread usage and accessibility in Türkiye, as confirmed by recent usage surveys, application download statistics, and national search engine market shares. Their public availability makes them plausible sources of health information for parents in the region.

Question set

Nine recurring questions on childhood vaccines were selected based on their high prevalence in pediatric clinical consultations and their frequency in the literature on vaccine hesitancy. These questions represent common parental concerns encountered in outpatient pediatric practice and have been cited in previous studies addressing vaccine misinformation, religious concerns, safety myths, and cultural skepticism.[18-20] The selected questions span medical safety (e.g., autism, immune system), religious and ethical concerns (e.g., pork products, permissibility), and public misinformation (e.g., COVID-19, natural alternatives). They were chosen to reflect a realistic and diverse range of issues that parents commonly raise when deciding on childhood vaccinations.

- The questions are as follows:
- Will vaccinating my children harm them?
- Do vaccines cause autism?
- Do vaccines weaken the immune system? (Do vaccines contain pork products?)
- Are the chemicals in vaccines dangerous?
- Can we rely on natural methods instead of vaccines?
- Is vaccination religiously permissible?
- Is the HPV vaccine necessary?
- Do COVID-19 vaccines trigger heart problems? (Regarding religious reasons, in both Islam and Judaism, the consumption of pork is strictly prohibited.)

Each question was submitted verbatim and in the same sequence to AICB 1, AICB 2, and AICB 3. An initial ("baseline") set of responses was collected (Table 1), and the entire question set was resubmitted, on average, five weeks later, to assess temporal consistency. The second set of responses was not re-evaluated in terms of the remaining three parameters: accuracy, information sufficiency, and source reliability.

Evaluation criteria and process

All chatbot responses were independently evaluated and scored on a 5-point Likert scale by two pediatric health specialists (a pediatric infectious disease specialist and a social pediatrics lecturer), both affiliated with a university medical faculty. Discrepancies between the two reviewers were resolved through discussion or adjudicated by a third

Table 1. The summarized answers given by each chatbot (AICB-1 – 3) to the nine parental questions

#	Questions	AICB-1 (summary)	AICB-2 (summary)	AICB-3 (summary)
1	Will vaccinating my children harm them?	No, protects; adverse events are mild	No, protects; emphasises national schedule	No, it protects; it warns against disease risk
2	Do vaccines cause autism?	No; Wakefield retracted; large cohort studies	No; research shows no link	No; 1998 study retracted; no evidence
3	Do vaccines weaken the immune system?	Strengthens immunity; explains the mechanism	Strengthens; multiple vaccines are safe	Strengthens; trains the immune system
4	Do vaccines contain pork products?	Some vaccines use pork-gelatin, which is permissible mainly	There are none in the Turkish schedule	Rare trace gelatin; alternatives exist
5	Are the chemicals in vaccines dangerous?	Thiomersal, aluminum is safe at trace doses	The same chemicals are safe; the dosage is tiny	Chemicals are strictly tested and safe
6	Can we rely on natural methods instead of vaccines?	Lifestyle helps but is not sufficient.	Same; vaccines are irreplaceable	Same; vaccines are essential
7	Is vaccination religiously permissible?	Islam, Christianity, and Judaism endorse vaccination.	Diyanet and other leaders approve	Many faith leaders support vaccination
8	Is the HPV vaccine necessary?	Yes; prevents HPV cancers; 9–26 y recommended	Yes; prevents multiple cancers; 9–26 y	Yes, it reduces cancer risk in early teens
9	Do COVID-19 vaccines trigger heart problems?	Very rare mild myocarditis; infection risk is higher	Very rare mild myocarditis; thrombosis data; infection risk is higher	There is no causal link; infection risk is higher

*Diyanet; The Directorate of Religious Affairs is a Turkish state institution responsible for providing Islamic guidance

expert. The final score was determined by a third reviewer, who is a professor of pediatric infectious diseases. This adjudicator considered the justifications provided by both reviewers, reviewed the relevant response(s), and then assigned a final score, which was accepted as the consensus rating.

Responses were independently evaluated across four criteria, each rated on a 5-point Likert scale (1 = very poor, 5 = excellent):

Accuracy: Whether the response aligns with up-to-date guidance from major health authorities (e.g., CDC, WHO, NHS).

Consistency: Internal coherence and stability between baseline and follow-up responses.

Information Sufficiency: Depth, breadth, and clarity of explanation.

Source Reliability: Use of reputable scientific sources and reliable references (e.g., governmental or institutional health websites, peer-reviewed, highly indexed journals) rather than unvetted online content. To specifically evaluate factual accuracy and hallucination risk, both reviewers cross-checked the key claims in each response against reliable sources, including WHO and CDC guidelines and relevant peer-reviewed literature. "Hallucination" was defined as the inclusion of fabricated references, unverifiable factual assertions, or clinically incorrect guidance. None of the chatbot responses in this study met the criteria for hallucination.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA). The Kruskal–Wallis test was employed with a significance threshold of $\alpha = 0.05$ to compare median scores across the three AI chatbots. Where significant differences were identified, Dunn's post hoc test was conducted to determine pairwise contrasts between systems.

Ethics statement

Our study did not involve any experiments on live human or animal subjects. It is based solely on publicly accessible chatbot interactions and does not include personal or identifiable data. Therefore, ethical approval was not required.

RESULTS

Temporal consistency

All three chatbots reproduced their core messages in the follow-up session; none reversed its stance, as shown in Table 2.

Overall, all three AICBs showed strong consistency between first and second responses, particularly regarding accuracy and scientific agreement. AICB 1 and AICB 2 provided highly stable and detailed answers, while AICB 3 exhibited slightly more variability but still remained scientifically sound.

Performance scores

As summarized in Table 3 above, AICB 1 demonstrated superior performance across all four evaluation domains, achieving a perfect mean score of 5.0 for Accuracy and high scores in Consistency (4.9), Information Sufficiency (4.8), and Source Reliability (4.9). AICB 2 also performed well, particularly in accuracy (4.8) and information sufficiency (4.7), although it had slightly lower consistency (4.6), suggesting occasional variations in tone or phrasing. AICB 3 showed adequate performance, but lower mean values—particularly in Information Sufficiency (3.9)—indicate that its responses, while generally correct, tended to lack the depth and comprehensiveness observed in the other agents.

Kruskal–Wallis testing indicated a significant difference among chatbots ($H = 11.27$, $p < 0.01$). Post hoc Dunn analysis showed AICB 1 > AICB 3 ($p < 0.01$) and AICB 2 > AICB 3 ($p = 0.03$); AICB 1 vs. AICB 2 was not statistically different ($p = 0.35$). Overall, AICB 1 was the most consistent and robust in delivering reliable, persuasive vaccine-related information across domains critical to public health communication.

DISCUSSION

This study assessed the reliability of freely accessible AI chatbots as information sources for parents seeking guidance on childhood vaccination. Our findings indicate that all three chatbots produced scientifically accurate and temporally consistent responses to common parental vaccine questions. AICB-1 demonstrated the most in-depth and evidence-based responses, often citing authoritative global health sources, which is crucial for building trust and confidence in vaccine information. AICB-2 effectively integrated local Turkish public health statements,

Table 2. Comparative summaries of two responses from three AI conversational agents to common parental vaccine questions

Question	AICB 1 (First Responses Summary)	AICB 1 (Second Responses Summary)	AICB 2 (First Responses Summary)	AICB 2 (Second Responses Summary)	AICB 3 (First Responses Summary)	AICB 3 (Second Responses Summary)
1) Do vaccines cause autism?	Fraudulent Wakefield study; no link (CDC/ WHO, Danish source, 2019).	Same reasoning; Wakefield retracted, Danish study.	No; studies show no link.	Same; no link.	No; many studies show no link; 1998 study retracted.	Same; fake study, consensus.
2) Do vaccines harm the immune system?	No harm; strengthens immunity (WHO/ CDC).	Same; strengthens immunity.	No; strengthens immunity, multiple vaccines safe.	Same; immunity strengthened.	No, it strengthens immunity.	Same; immunity trained.
3) Do vaccines contain pork products?	May contain purified gelatin; religiously approved.	Similar; may contain gelatin, generally approved.	No pork products in national vaccines.	Similar; no pork in vaccines.	May contain gelatin; generally acceptable.	Same; gelatin is possible, alternatives exist.
4) Will vaccinating my child harm them?	No harm; protects from deadly diseases.	Same; protects children and community.	No harm; provides protection.	Same emphasis.	On the contrary; protects.	Similar emphasis; protects.
5) Are vaccine ingredients harmful chemicals?	Safe ingredients (thimerosal, aluminum, formaldehyde).	Same ingredients; low-dose safety confirmed.	Safe in low doses.	Same; safe components.	Safe at controlled doses.	Same; non-toxic doses.
6) Can we rely on natural methods instead of vaccines?	Helps but doesn't replace vaccines.	Similarly, natural methods alone are insufficient.	Supportive but do not replace vaccines.	Same explanation.	Supports but does not replace vaccines.	Similarly, the vaccine is most effective.
7) Are vaccines religiously acceptable?	Approved by major religions.	Same; major religions endorse.	No barrier; important for health.	Same message.	Recommended by religious authorities.	Same; major religions agree.
8) Is the HPV vaccine necessary?	Yes; reduces cancer risk.	Same; prevents cancer, both sexes.	Yes; reduces cancer risk.	Similar detail; protective for all.	Yes; reduces cancer risk.	Same; recommended for both.
9) Do COVID-19 vaccines cause cardiovascular disease?	Rare myocarditis; infection risk is higher.	Same; infection risk is higher.	Rare myocarditis; infection risk is higher.	Same emphasis.	No evidence; infection risk is higher.	Same; infection risk is higher.

AI: Artificial Intelligence; AICB: Artificial Intelligence Chat Bot; WHO: World Health Organization; CDC: Centers for Disease Control and Prevention.

demonstrating the value of tailoring information to specific cultural and regional contexts. In contrast, AICB-3's responses were accurate but more superficial and lacked source citation. These results align with existing literature on the potential of AI chatbots in public health education[15] and vaccine communication.[8,16] Chatbots can serve as virtual health assistants, offering medical guidance and promoting public health education.[8] Our findings also support the idea that AI-driven chatbots can disseminate accurate vaccine information[16] and improve vaccine literacy, supported by scientific data evaluated by experts[8], which involves the ability to access, understand, appraise, and apply vaccination-related information for users.[8] The strong agreement between chatbots and trusted scientific sources on vaccine topics shows that AI chatbots can help support traditional ways of communicating about prevention.[9] Apart from these findings, commercially biased or misleading scientific information may also be

presented without proper oversight, alongside reliable and referenced scientific sources.[21]

The differing performance among chatbots underscores the need to evaluate each model carefully for specific public health use cases.[22,23] Evaluation should go beyond factual accuracy to also consider the depth of information, cultural relevance, and clarity of source attribution—key dimensions in vaccine-related communication. This is particularly important when addressing vaccine hesitancy, where personalized messaging and culturally adapted content can significantly influence parental attitudes, decisions, and literacy across diverse populations.[24]

All three AI chatbots delivered accurate, stable, and comprehensive vaccine information. Crucially, none propagated vaccine myths such as an autism link, which strengthens their potential as safe tools. Given that many parents rely on online information yet doubt its credibility, AI chatbots, when properly validated, may serve as first-line educational tools to mitigate vaccine hesitancy.[9,23] However, the observed variability in depth and source citation suggests the need for ongoing evaluation and refinement of AI systems for public-facing health education.

Parents traditionally rely on forward-looking search engines such as Google to gather information on pediatric vaccines.[9,23] Our data suggest that large language model chatbots add unique value beyond keyword search: conversational retrieval. Chatbots deliver concise, contextual answers without requiring users to sift through dozens of links, reducing cognitive load and time cost.[23]

Integrated synthesis. AICB 1 and AICB 2, in particular, aggregate multiple guidelines (CDC, WHO, national ministries) into a single narrative, whereas search engines typically present fragmented pages that parents must reconcile. The dialogue is iterative; parents can immediately request more straightforward wording or a follow-up explanation that search engines cannot provide. Reduce exposure to misinformation. Algorithmic hallucination is a risk, yet chatbots in this study produced zero significant factual errors, while ordinary search results still surface anti-vaccine blogs high in the ranking.[3,22,23]

AI chatbots, while helpful, present several significant risks in healthcare. First, there are concerns about opaque sourcing and transparency. While general-purpose AI models can contribute to judgment under uncertainty[23], there is a recognized need to provide greater transparency into algorithms and to explain decisions explicitly to human observers.[22,23] Ensuring AI systems are transparent, interpretable, and explainable is critical for user trust.[22,23] Secondly, these AI models can experience model drift. The tool itself can learn and evolve, potentially changing user confidence.[23] The output of Large Language Models (LLMs) like ChatGPT is non-deterministic, meaning applying the same prompt multiple times can result in different responses, which hinders reproducibility and consistency.[23] Thirdly, there's a significant risk of hallucinations, where LLMs can add statements not supported by the original text or generate plausible-sounding but false content.[23] These hallucinations are an intrinsic problem of generative models and are difficult to remedy.[23] In healthcare, instances of incorrect text passages and missing relevant medical information, or misinterpretations of medical terms, could lead patients to draw harmful conclusions, potentially resulting in physical and/or psychological harm.[23] Finally, privacy leakage is a major concern. When users, such as parents, share personal health information with chatbots, the information can be more detailed than that typically entered in online searches.[23] Uploading protected health information to proprietary services like ChatGPT might compromise patient privacy.[23] The ethical and legal issues surrounding data rights, privacy, and ownership are critical as AI becomes more integrated into health and healthcare.[22]

Limitations

This study, while providing valuable insights, is subject to several limitations that warrant consideration. Firstly, the analysis was confined to three publicly accessible AI chatbots and a predetermined set of nine frequently asked parental questions. To enhance the generalizability of findings, future research should broaden the scope to include a wider array of AI systems and question categories. Secondly, although rigorous efforts, including the use of a third reviewer for consensus, were undertaken to mitigate bias, the expert-based scoring methodology inherently involves subjective elements. Thirdly, while the study assessed temporal consistency between chatbot responses, the long-term stability of these models and their susceptibility to "model drift" remain unexplored. Finally, critical potential risks associated with chatbot deployment, such as the propensity for hallucinations, the lack of transparent sourcing, and the risk of privacy breaches during sensitive health discussions, necessitate ongoing investigation. These concerns are particularly pertinent as the real-world application of chatbots in health communication continues to expand. Future evaluations must rely on robust, evidence-based data from reputable institutions and peer-reviewed sources to ensure reliable knowledge generation. Despite these limitations, our study

provides valuable evidence on the potential of AI chatbots to serve as reliable information sources for parents seeking guidance on childhood vaccination. As AI technology evolves, it is essential to carefully evaluate and optimize chatbot performance to deliver accurate, comprehensive, and culturally appropriate vaccine information.[16] Further research should focus on comparative studies that examine how chatbot effectiveness may vary with question/argument design and implementation. Additionally, efforts should be made to address the digital divide and ensure equitable access to these potentially valuable tools.[17] Chatbots can hallucinate, update unpredictably, and raise privacy concerns.[22,23] Their outputs should supplement, not replace, professional medical advice. Regulatory frameworks, transparent sourcing, and user education are essential to maximize benefits while minimizing risk.[23]

This study found that AI chatbots generally provide accurate and consistent responses to common vaccine-related questions from hesitant parents. However, some answers lacked depth and did not include source citations. Chatbots should be viewed as supplementary resources rather than definitive sources of medical information. Enhancing their ability to guide users toward verified, expert-backed content remains essential.

Ethical approval

Our study did not involve any experiments on live human or animal subjects. It is based solely on publicly accessible chatbot interactions and does not include personal or identifiable data. Therefore, ethical approval was not required.

Author contribution

The authors declare contribution to the paper as follows: Study conception and design: ZGEÖ, MH; data collection: ZGEÖ; analysis and interpretation of results: ZGEÖ, EÇ, MH; draft manuscript preparation: ZGEÖ, EÇ, MH. 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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Original Article

Determinants of disease severity in pediatric protracted bacterial bronchitis

Evrim Hepkaya[1], Pelin Güler[2]

[1]Department of Pediatric Pulmonology, Ümraniye Training and Research Hospital, İstanbul, Türkiye
[2]Department of Pediatrics, Kartal Dr. Lütfi Kırdar Training and Research Hospital, İstanbul, Türkiye

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ABSTRACT

Background: Protracted bacterial bronchitis (PBB) is an increasingly recognized cause of chronic wet cough in children and may lead to bronchiectasis if not adequately treated. However, data regarding its clinical presentation and associated risk factors remain limited.

Objective: To evaluate the clinical, radiological, and microbiological characteristics of children diagnosed with PBB and to identify factors associated with disease severity and recurrence.

Methods: This cross-sectional study included 49 children followed in a pediatric pulmonology clinic with a diagnosis of PBB. Demographic features, comorbidities, sputum microbiology, pulmonary function tests, thoracic computed tomography (CT) findings, and serum vitamin D levels were analyzed. The association between clinical parameters and annual bronchitis frequency was evaluated using correlation analyses.

Results: Forty-nine children were analyzed (69.3% female), with a median age of 7 years. Asthma and atopy were present in 65.3% and 30.6% of patients, respectively. A pathogen was isolated in 63.3% of sputum samples, with Haemophilus influenzae being the most common agent (41.9%). A moderate inverse correlation was detected between serum vitamin D levels and the annual number of bronchitis episodes ($\rho = -0.56$; $p = 0.0016$). Spirometry values were mostly within normal limits; however, weak inverse correlations were observed between pulmonary function parameters and exacerbation frequency. Thoracic CT abnormalities were identified in 81.9% of patients, most commonly bronchial wall thickening.

Conclusion: PBB is frequently accompanied by asthma and recurrent infections. H. influenzae appears to be associated with increased disease burden. Low vitamin D levels may contribute to higher susceptibility to recurrent bronchitis. Early diagnosis and targeted microbiological evaluation are essential to prevent long-term pulmonary complications.

Keywords: bronchitis, bronchiectasis, Hemophilus influenzae, Vitamin D

INTRODUCTION

Protracted bacterial bronchitis (PBB) is now recognized as one of the most frequent causes of persistent wet cough in early childhood, yet it remains underdiagnosed in routine clinical practice. The condition is characterized by a chronic productive cough lasting longer than four weeks that cannot be explained by alternative diagnoses, including asthma, structural airway abnormalities, or immunodeficiency disorders. A key hallmark of PBB is complete symptom resolution following appropriate antibiotic therapy, supporting its bacterial origin.[1,2] The disease primarily affects the lower airways, where bacterial colonization—most commonly involving Haemophilus influenzae, Moraxella catarrhalis, and Streptococcus pneumoniae—induces sustained neutrophilic inflammation.[3] Mechanisms

✉ Evrim Hepkaya ▪ dr_ekarakaya@yahoo.com

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such as biofilm formation and impaired mucociliary clearance contribute to epithelial injury and excessive mucus production.[4] When inadequately treated, this ongoing inflammatory process may result in structural airway damage and progression toward bronchiectasis.[5]

Recurrent PBB, often defined as three or more episodes annually, has been associated with a greater likelihood of chronic suppurative lung disease, particularly among children infected with H. influenzae.[6,7] These observations emphasize the importance of early diagnosis, improved clinical awareness, and implementation of appropriate treatment strategies.

The aim of this study was to evaluate the clinical, microbiological, and radiological characteristics of children diagnosed with PBB and to identify factors associated with disease severity and recurrence.

MATERIALS AND METHODS

Study design and population

The study was designed as a cross-sectional study, evaluating data collected from patients diagnosed with PBB. A total of 49 patients who were systemically followed by the pediatric pulmonology department enrolled in the study. Patients with persistent productive cough were screened for underlying conditions such as cystic fibrosis, immunodeficiency, tuberculosis, primary ciliary dyskinesia, congenital cardiac and neuromuscular diseases, and foreign body aspiration. Those diagnosed with any of these conditions were excluded. PBB was defined when all three of the following criteria are fulfilled; 1) Presence of continuous chronic (>4 weeks' duration) wet or productive cough; 2) absence of symptoms or signs (i.e. dyspnea, exertional dyspnea, haemoptysis, respiratory distress, digital clubbing, chest wall deformity, failure to thrive) suggestive of other causes of wet or productive cough and 3) cough resolved following a 2–4-week course of an appropriate oral antibiotic.[8]

Variables and data collection

Demographic variables, including age, gender, age at diagnosis, annual number of bronchitis attacks, and serum vitamin D levels, were recorded. During outpatient visits, patients were systematically assessed for comorbidities, including prematurity, breastfeeding history, and prior diagnoses of pneumonia, asthma, or atopy.

Spirometry was performed in children aged over 6 years who were able to cooperate, using the same technician for all tests. The key parameters measured included forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), FEV_1/FVC ratio, and forced expiratory flow between 25% and 75% of the pulmonary volume (FEF25–75). Normal values were defined as >80% of the predicted value for FVC, FEV_1, and FEV_1/FVC, and >70% of the predicted value for FEF25–75.[9] A positive bronchodilator response (BDR) was defined as a relative increase of >10% in FEV_1 or FVC following bronchodilator administration.[10] Spontaneous or induced sputum samples were obtained and cultured from patients presenting with a productive cough. Bronchoscopy was performed in patients with radiological or clinical signs of infection who were unable to expectorate sputum, and in those with persistent radiological findings despite appropriate medical treatment. All patients with chronic cough were screened for pulmonary tuberculosis, cystic fibrosis, and immunodeficiency. Immunological evaluation included measurement of total serum immunoglobulins (IgA, IgG, IgM, and IgE), IgG subclasses, lymphocyte subpopulations, and antibody responses to vaccine antigens. A sweat test was performed using direct chloride measurement and interpreted according to established guidelines.[11] Screening for tuberculosis included the purified protein derivative (PPD) skin test; in cases with anergic PPD results, interferon-gamma release assays (IGRAs) were also used.[12] Patients with early-onset disease or severe bronchitis episodes who had negative results for these screening tests underwent further genetic analysis to investigate underlying conditions. Thoracic computed tomography (CT) was performed in children who showed abnormal findings on chest X-ray—such as bronchial wall thickening, atelectasis, or air trapping—or in those with persistent respiratory symptoms and recurrent bronchitis. Disease severity was assessed using data on the annual number of bronchitis attacks, accompanying comorbidities, microbiological findings, spirometry results, and radiological characteristics.

Data analysis

Statistical analyses were performed using SPSS version 29. Continuous variables were expressed as mean ± standard deviation (SD) for normally distributed data or as median with interquartile range (25th–75th percentiles) for non-normally distributed data. Categorical variables were expressed as frequencies and percentages. Normality was assessed using histograms, probability plots, and the Shapiro–Wilk test. Spearman's rank correlation coefficient (ρ) was utilized to assess the associations between ordinal

categorical variables, and for nominal categorical variables, the Chi-square test and Fisher's exact test were used. A two-tailed p-value <0.05 was considered statistically significant. A multivariable Poisson regression analysis was performed to evaluate the independent associations between serum vitamin D levels and annual bronchitis exacerbation frequency, adjusting for asthma status, sputum culture results, and pulmonary function parameters.

Ethical statement

Ethical approval for the study was obtained from the Umraniye Training and Research Hospital Ethics Committee (date: 18.12.2025, number: 2025-446), and the research was conducted in full accordance with the principles of the Declaration of Helsinki. Informed consent was thoroughly obtained, reflecting our adherence to the highest ethical standards in research. All participants' rights were strictly protected throughout the study, and no procedures or inquiries were conducted that could compromise the children's physical or psychological well-being. Written informed consent for participation was obtained from the parents during clinic visits, and official consent forms were duly signed.

RESULTS

A total of 49 patients were evaluated, of whom 71.4% (35/49) were female. The median age was 7 (3-15) years, while the median age at first symptom was 4.5 (0.5-15) years. Prematurity was observed in 10 (20.4%) patients, and breastfeeding for over 6 months was observed in 40 (81.6%) patients. Asthma was associated with PBB in 32 (65.3%) patients, and atopy was reported in 15 (30.6%) patients. Pneumonia history was reported in 20 (40.8%). The median vitamin D level was 16.9 ng/ml (6.7-112) among patients. Serum vitamin D levels were inversely correlated with the number of annual bronchitis attacks (Spearman rho = –0.56, p = 0.0016) (Table 1). In multivariable analysis, serum vitamin D levels were not independently associated with annual bronchitis exacerbation frequency (IRR 1.02, 95% CI 0.96–1.08; p = 0.521) (Table 2).

The median annual number of bronchitis attacks was 3 (1-15). A positive culture was obtained from 31 patients (63.3%). Haemophilus influenzae was the most common pathogen 13 (41.9%), while the others were 8 (25.8%) Streptococcus pneumoniae, 3 (9.6%) were methicillin-resistant Staphylococcus aureus, 3(9.6%) were coinfection with H. influenzae and S. pneumoniae, 2 (6.4%) were Klebsiella pneumonia, 1 (3.2%) Haemophilus parainfluenzae, and 1 (3.2%) patient Streptococcus pyogenes (Table 3). Bronchoscopy was performed in 3 (6.1%) patients with normal anatomical appearance and negative microbiological cultures.

Table 1. Characteristics of the study population

Sex (female), n (%)	35 (71.4%)
Age (yr), median (25th-75th)	7 (3-15)
Age at first symptom (yr), mean (±SD)	4.5 (0.5-15)
Annual bronchitis attacks, median (25th-75th)	3 (1-15)
Prematurity, n (%)	10 (20.4%)
Breastfeeding (> 6 m), n (%)	40 (81.6%)
Atopy, n (%)	15 (30.6%)
Asthma, n (%)	32 (65.3%)
History of pneumonia, n (%)	20 (40.8%)
25-OH Vit. D levels, ng/ml, median (25th-75th)	16.9 (6.7-112)

yr: year, m: month, SD: standard deviation, Vit: vitamin.

Out of the total, 23 patients were able to perform spirometry. The mean FEV1 was 84.94±11.68, FVC was 86.70±9.12, and the FEV1/FVC was 96.52±8.81 (Table 3). The associations between FEV1, FVC, and FEV1/FVC and the annual exacerbation frequency were examined using Pearson correlation analysis. The analysis demonstrated weak inverse correlations across all spirometry parameters (FEV1: r = –0.21; FVC: r = –0.15; FEV1/FVC: r = –0.13), indicating that higher exacerbation rates were associated with lower pulmonary function values.

Thirty-three patients were screened with Thorax CT scans. Bronchial wall thickness was the most common finding observed in 8 (24.2%) patients. Other findings were, consolidation in 7 (21.2%), atelectasis in 5 (15.1%), mosaic perfusion in 3 (9%), ground-glass opacities in 2 (6%), bronchial dilatation in 1 (3%) and interlobular septal thickness in 1 (3%) patient respectively, 6 (18.1%) of CT scans were normal (Table 4). Thorax CT scan findings were not associated with spirometry values (p>0.05). The majority (40/49) of the patients had a history of 3 bronchitis attacks in the previous year. Of them, 6 (15%) had≥ 10 bronchitis attacks. Patients with positive sputum cultures had more bronchiolitis attacks without a significant difference (4.74 vs 4.11, p>0.05). Regarding the association between sputum cultures, patients with methicillin-resistant Staphylococcus aureus (MRSA) had an annual mean of 10 bronchitis attacks, while patients with H. influenza had a mean of 5.08 attacks, and those with coinfection with H. influenza and S. pneumoniae had a mean of 4.66 attacks.

Table 2. Multivariable Poisson regression analysis for annual bronchitis exacerbations					
Variable	**β (Estimate)**	**SE**	**p value**	**IRR**	**95% CI for IRR**
Intercept	4.062	1.344	0.003	–	-
FEV_1 (% predicted)	−0.029	0.016	0.062	0.97	0.94 – 1.00
Serum Vitamin D (ng/mL)	0.019	0.030	0.521	1.02	0.96 – 1.08
Asthma (Yes vs No)	−1.398	0.779	0.073	0.25	0.05 – 1.16
Sputum Culture (Negative vs Positive)	−0.378	0.306	0.216	0.69	0.38 – 1.26

FEV1: forced expiratory volume in one second.

Table 3. Microbiological and spirometry findings of the patients	
Sputum samplings	**n (%)**
Hemophilus influenza	13 (41.9%)
Streptococcus pneumoniae	8 (25.8%)
MRSA	3 (9.6%)
Coinfection (H. influenza and S. pneumoniae)	3 (9.6%)
Klebsiella pneumoniae	2 (6.4%)
Hemophilus parainfluenza	1 (3.2%)
Streptococcus pyogenes	1 (3.2%)
Spirometry values	**mean±SD**
FEV1	84.9±11.68
FVC	86.70±9.12
FEV1/FVC	96.52±8.81

MRSA: methicillin resistant staphylococcus aureus, FEV1: forced expiratory volume in one second, FVC: forced vital capacity.

Table 4. Radiological findings of the patients	
	n (%)
Bronchial wall thickness	8 (24.2%)
Consolidation	7 (21.2%)
Atelectasis	5 (15.1%)
Mosaic perfusion	3 (9.1%)
Ground glass opacities	2 (6.1%)
Bronchial dilatation	1 (3.1%)
Interlobular septal thickness	1 (3.1%)
Normal	6 (18.1%)

DISCUSSION

This study provides an overview of the clinical, radiological, and microbiological characteristics of children diagnosed with protracted bacterial bronchitis (PBB). Although PBB has been increasingly recognized over the past decade, it remains an underdiagnosed cause of chronic wet cough in childhood. The demographic structure of our cohort, in which symptoms began in early childhood, and the median age at evaluation was 7 years, is consistent with the epidemiological profile reported in previous studies.[13,14] While some authors have noted a slight male predominance among affected children, our sample showed a higher proportion of females; this discrepancy may reflect regional differences or variations in referral patterns rather than a true sex-related predisposition.[5]

A key finding of our study was the high rate of positive sputum cultures, identified in nearly two-thirds of patients, supporting the central role of bacterial pathogens in PBB pathogenesis. Consistent with previous reports, H. influenzae was the most frequently isolated organism, followed by S. pneumoniae and MRSA.[15,16] The predominance of H. influenzae is attributed to its ability to induce neutrophilic inflammation, form biofilms, and persist in the lower airways. Recurrent PBB episodes (>3/year) and H. influenzae infection have been associated with bronchiectasis.[17] In our cohort, H. influenzae–positive patients had higher annual exacerbation rates, suggesting a more severe phenotype.[18] Although the difference between culture-positive and culture-negative groups was not statistically significant, the numerical trend toward higher exacerbation rates in culture-positive patients remains clinically relevant. Notably, patients with MRSA had the highest exacerbation burden. Given the association between PBB and chronic suppurative lung disease or early bronchiectasis, these findings underscore the importance of close follow-up and early microbiological evaluation.[19]

Spirometry results showed that pulmonary function was largely preserved in most patients, consistent with previous reports indicating normal lung mechanics in early or moderately severe PBB.[17,20] However, a weak inverse correlation was observed between spirometric parameters and the annual number of bronchitis exacerbations, suggesting that recurrent infections may gradually impair lung function despite initially normal measurements. Supporting this, one study reported that 24% of patients demonstrated at least one abnormal spirometric

parameter.[21] These findings highlight the importance of close follow-up, particularly in persistent cases.

Radiological evaluation revealed bronchial wall thickening as the most common CT abnormality, followed by consolidation and atelectasis. These imaging patterns align with structural changes typically attributed to chronic bacterial inflammation and impaired mucociliary clearance.[5,16] CT findings did not correlate with lung function measures in our study, which may be explained by the fact that structural changes often precede measurable declines in spirometric values. Previous studies also describe a similar dissociation between imaging abnormalities and pulmonary function in children with chronic wet cough.[16]

An additional noteworthy finding was the inverse association between vitamin D levels and annual bronchitis episodes. Vitamin D deficiency is well known to impair innate immune responses, particularly epithelial integrity and antimicrobial signaling pathways.[22] Ferri et al. demonstrated that serum vitamin D levels in children with bronchiectasis were significantly associated with disease severity.[23] Moreover, the use of vitamin D as an anti-inflammatory agent in cystic fibrosis has been hypothesized based on its beneficial effects on lung function.[24] In line with the literature, the moderate negative correlation observed in our study suggests that low vitamin D status may contribute to increased susceptibility to respiratory infections in this patient population and may represent a potentially contributing risk factor. Although a significant inverse correlation was observed, this association did not persist in the adjusted model, suggesting potential confounding effects. FEV_1 demonstrated a borderline inverse relationship with exacerbation frequency, indicating a possible trend toward lower attack rates with better lung function. These findings imply that while vitamin D deficiency may be associated with disease burden, it may not act as an independent determinant of exacerbation frequency in children with PBB.

Comorbid conditions such as asthma and atopy were common, observed in nearly two-thirds and one-third of patients, respectively. In a 5-year longitudinal study, asthma was present in 27.1% of children with PBB.[17] Similarly, another study reported that asthma and episodic wheezing were identified in 36% of patients presenting with prolonged cough.[25] The coexistence of asthma and PBB complicates clinical decision-making, as chronic cough in these patients may be erroneously attributed to asthma alone, potentially delaying appropriate antibiotic therapy. These findings reinforce the importance of distinguishing PBB from asthma-related cough and highlight the need for a systematic diagnostic approach.[2,26]

This study has several limitations. First, its cross-sectional design precludes causal inferences regarding the identified associations, particularly between vitamin D levels and disease severity, and the relatively small sample size from a single center limits generalizability.

Taken together, our results emphasize several key clinical implications. First, early recognition of PBB and timely initiation of appropriate antibiotic therapy remain fundamental to preventing disease progression. Second, identifying underlying pathogens—particularly in recurrent or severe cases—may provide valuable prognostic information. Finally, evaluation of potentially correctable contributing factors to disease severity, such as vitamin D deficiency, may offer additional opportunities to reduce exacerbation frequency. Long-term prospective studies are warranted to clarify the natural course of PBB, identify markers of recurrence, and determine which children are at the highest risk for developing bronchiectasis or other chronic airway diseases.

Ethical approval

This study has been approved by the Umraniye Training and Research Hospital Ethics Committee (approval date 18.12.2025, number 2025-446). Written informed consent was obtained from the participants.

Author contribution

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

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Case Report

Concurrent serum sickness–like reactions in fraternal twins following amoxicillin exposure

Kaley A. Desher[1]

[1]Department of Allergy & Immunology, Memorial Healthcare System, Hollywood, FL, USA

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ABSTRACT

Serum sickness–like reaction (SSLR) is an immunologic process most often triggered by β-lactam antibiotics or viral infections, typically presenting with urticarial rash, arthralgias, and low-grade fever. This report describes concurrent SSLR in 5-year-old fraternal twin girls after amoxicillin exposure for presumed viral pharyngitis. Twin A developed diffuse urticaria, facial and extremity swelling, arthralgias, and mucosal discomfort. Laboratory studies showed leukocytosis, elevated inflammatory markers, and detection of rhinovirus/enterovirus by PCR. She required hospitalization, systemic corticosteroids, and multimodal analgesia, with gradual improvement over several days. Twin B developed similar symptoms three days later, including rash, edema, and joint pain, but had a milder course managed with antihistamines and supportive care. The simultaneous occurrence of SSLR in siblings is rarely reported, and concurrent presentation in fraternal twins has not been described in the literature. This case highlights the interplay of shared environmental exposures, potential infectious triggers, and host susceptibility in SSLR, as well as the variability of disease expression even among related children. Awareness of SSLR in the differential diagnosis of rash and arthralgia following antibiotic exposure can prevent unnecessary investigations, guide supportive treatment, and reduce morbidity.

Keywords: serum sickness-like reaction, twins, amoxicillin, drug hypersensitivity, pediatrics

INTRODUCTION

Serum sickness–like reaction (SSLR) is an uncommon condition most often associated with β-lactam antibiotics, particularly amoxicillin.[1] It is clinically distinguished by the triad of urticarial or erythematous rash, joint involvement, and systemic symptoms such as fever or malaise.[2] Unlike classic serum sickness, hypocomplementemia is not typical. While classic serum sickness has been reported in twins following equine rabies immunoglobulin,[3] concurrent SSLR has not been previously described in siblings. To date, concurrent serum sickness-like reactions occurring in fraternal twins following amoxicillin exposure have not been reported. This presentation highlights the potential interplay between shared environmental exposures, infectious triggers, and individual host susceptibility in the pathogenesis of SSLR.

CASE PRESENTATION

Written informed consent was obtained from the patients' mother for publication. Both children were up to date on routine childhood immunizations, with no recent vaccinations administered prior to symptom onset.

A comparison of clinical features is shown in Table 1.

Twin A: A 5-year-old girl developed urticarial rash, facial, lip, and extremity edema, and severe arthralgias near the end of a 10-day course of amoxicillin prescribed empirically despite a negative rapid streptococcal antigen

✉ Kaley Ann Desher ▪ kdesher@mhs.net

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test. Laboratory evaluation showed leukocytosis with marked neutrophilia, mildly elevated CRP, unremarkable complement levels, and a positive rhinovirus/enterovirus PCR. Intramuscular epinephrine was administered in the emergency department due to initial concern for anaphylaxis in the setting of diffuse urticaria and angioedema. However, the absence of hypotension or respiratory compromise, lack of sustained response to epinephrine, and subsequent progression of severe arthralgias supported a diagnosis of SSLR rather than IgE-mediated hypersensitivity. Symptoms progressed despite scheduled cetirizine with adjunctive diphenhydramine and hydroxyzine, as well as nonsteroidal anti-inflammatory therapy with ketorolac and naproxen. She had worsening pain and limited mobility, which prompted escalation to systemic corticosteroids (intravenous methylprednisolone followed by oral prednisolone) and analgesia with oxycodone and hydrocodone-acetaminophen. She was hospitalized for five days with gradual improvement.

Twin B: Three days later, her fraternal twin developed diffuse urticaria, extremity edema, and arthralgias after completing approximately eight days of empiric amoxicillin for pharyngitis. Laboratory studies revealed mild neutrophilia without leukocytosis, normal inflammatory markers, and complement levels not decreased. She improved rapidly with cetirizine and hydroxyzine, along with acetaminophen and ibuprofen. A single dose of dexamethasone was given in the emergency department, but further steroids were avoided as symptoms resolved. Her hospitalization lasted two days.

DISCUSSION

Both patients developed characteristic findings of SSLR, including urticarial rash, edema, and arthralgias, approximately 8 days into amoxicillin therapy, consistent with the typical 7 to 10-day latency described in recent systematic reviews of pediatric SSLR.[1,2] The overall presentation was more compatible with SSLR than with other causes of rash and arthralgia, such as anaphylaxis, viral exanthem, erythema multiforme, IgA vasculitis, urticarial vasculitis, or classic serum sickness, which typically demonstrates hypocomplementemia and immune-complex-mediated vasculitis.[1] IgE-mediated anaphylaxis was considered less likely due to the absence of cardiorespiratory compromise and poor response to epinephrine, while normal complement levels excluded classic serum sickness and urticarial vasculitis. Viral exanthem, erythema multiforme, and IgA vasculitis were considered less consistent with the clinical course and absence of purpura or mucosal involvement.

The etiology in these cases remains uncertain. Although streptococcal infection cannot be definitively excluded, negative rapid tests make this less likely. Importantly, recent reports highlight how positive streptococcal testing can confound the diagnosis of SSLR by directing clinicians toward rheumatic or post-infectious syndromes.[4] Such diagnostic pitfalls underscore the need for careful interpretation of test results when clinical features better align with SSLR. Rhinovirus/enterovirus was detected in Twin A, supporting a possible infectious contribution. Viral testing was negative in Twin B, but this does not exclude infection. The respiratory viral panel evaluates a limited set

Table 1. Comparison of clinical features in Twin A and Twin B

Feature	Twin A (Patient A)	Twin B (Patient B)
Age/Sex	5-year female	5-year female
Clinical severity	Severe; prolonged course with impaired mobility	Mild-to-moderate; self-limited
Antibiotic exposure and timing	Onset near the end of a 10-day amoxicillin course	Onset three days after completing ~8 days of amoxicillin
Initial symptoms	Urticarial rash; facial/lip and extremity edema; arthralgias; severe pain; no response to epinephrine	Urticarial rash; extremity edema; arthralgias; milder pain
Key laboratory findings	Leukocytosis; CRP mildly elevated; C3 91 mg/dL, C4 24 mg/dL; rhinovirus/enterovirus positive	Mild neutrophilia; CRP normal; C3 121 mg/dL, C4 41 mg/dL
Hospital course	5 days; systemic corticosteroids, antihistamines, multimodal analgesia including opioids	2 days; antihistamines and analgesia; brief steroids then discontinued per allergy consultation
Outcome	Gradual resolution with tapering antihistamines	Rapid resolution

CRP: C-reactive protein.

of 18 pathogens and may also yield false-negative results for included targets depending on the timing of sampling and viral burden. Serum sickness-like reactions occurring during antibiotic treatment have been described as infection-associated urticarial syndromes with unpredictable recurrence, rather than reproducible drug hypersensitivity.[5] The simultaneous development of SSLR in fraternal twins raises the possibility of shared host susceptibility. Because these were dizygotic twins and no genetic analyses were performed, these observations support a role for shared environmental exposures and host susceptibility rather than a definitive genetic predisposition. Although classic serum sickness has been reported in identical twins following rabies immunoglobulin,[3] concurrent SSLR in siblings have not been previously described. The combination of shared environmental exposures, parallel viral illness, and similar timing of antibiotic exposure aligns with emerging models suggesting a multifactorial interplay among infection, drug exposure, and individual predisposition in SSLR pathogenesis.[5]

The differences in clinical severity between the twins also highlight the heterogeneity of SSLR and its implications for clinical management. Twin A experienced a more severe, protracted course requiring systemic corticosteroids and multimodal analgesia, whereas Twin B improved rapidly with supportive care alone, with a shorter hospital stay. This variability is consistent with a 10-year cohort study demonstrating that SSLR can range from mild, self-limited cases to more severe episodes necessitating inpatient management for pain control or impaired mobility.[6] Recent pediatric emergency department data similarly report that SSLR and SSLR-like illnesses are significant drivers of acute care utilization, with systemic symptoms such as joint pain and swelling associated with more prolonged or complicated courses.[7] A large acute-care cohort found that children with amoxicillin-associated reactions re-utilized emergency or urgent care services in approximately 10% of cases, with urticaria, angioedema, and systemic symptoms predicting repeat visits.[8]

Evidence supporting corticosteroid therapy remains limited. Steroids are frequently prescribed in moderate to severe cases, but studies show wide variability in practice and no clear evidence of accelerated recovery. Current recommendations, therefore, reserve corticosteroids for substantial discomfort, decreased mobility, or refractory symptoms.[1,6]

Management also includes planning for future antibiotic use. SSLR rarely represents a fixed β-lactam allergy, and lifelong avoidance is not routinely necessary.[9] Evaluation by an allergy specialist and consideration of supervised oral challenge can safely clarify tolerance in most children.[10] However, families should be counseled that recurrence is possible, as SSLR and SSLR-like reactions may reappear unpredictably even after a previously tolerated re-exposure.[5]

Conclusion: This dual presentation highlights the rare concurrence of SSLR in fraternal twins and the multifactorial pathogenesis involving host susceptibility, viral triggers, and antibiotic exposure. Recognition of SSLR within this broader framework can help clinicians avoid unnecessary investigations, guide appropriate therapy, and provide informed counseling regarding future β-lactam use.

Ethical approval

Written informed consent was obtained from the patients' mother for publication of these cases.

Author contribution

The author declares contribution to the paper as follows: Study conception and design: KD; data collection: KD; analysis and interpretation of results: KD; draft manuscript preparation: KD. The author reviewed the results and approved the final version of the article.

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Conflict of interest

The author declares that there is no conflict of interest.

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Letter to the Editor

Seeing the unseen: a late-onset Griscelli Syndrome Type 2 in a child without hypopigmentation

Kübra Baskın[1], Serap Kirkiz Kayalı[2], Gülsüm Kayhan[3], Emine Orulluoğlu[2], Bahar Büyükkaragöz[4], Ebru Azapağası[5], Zühre Kaya[2], Ülker Koçak[2]

[1]Department of Pediatric Immunology and Allergy, Faculty of Medicine, Gazi University, Ankara, Türkiye
[2]Department of Pediatric Hematology, Faculty of Medicine, Gazi University, Ankara, Türkiye
[3]Department of Medical Genetics, Faculty of Medicine, Gazi University, Ankara, Türkiye
[4]Department of Pediatric Nephrology, Faculty of Medicine, Gazi University, Ankara, Türkiye
[5]Department of Pediatric Intensive Care, Faculty of Medicine, Gazi University, Ankara, Türkiye

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To the Editor,

Autosomal recessive mutations in the RAB27A gene (located on chromosome 15q21 and encoding Rab27a) cause Griscelli syndrome type 2 (GS-2).[1] GS-2 is characterized by hypopigmentation and early-onset, potentially fatal hemophagocytic lymphohistiocytosis (HLH). Rab27a interaction with Munc 13-4 is fundamental for the secretion of lytic granules. Cytotoxic T lymphocytes and natural killer cells lacking Rab27a exhibit defective cytotoxicity and target cell killing.[1] Besides, the Rab27a-melanophilin-myosin VA complex is required for the release of melanosomes from melanocytes. Therefore, GS-2 patients also show partial oculocutaneous albinism with silver-grey hair and large pigment aggregates of melanosomes within melanocytes in hair shafts. Recently, GS-2 cases, both late onset and sine albinism, have been reported in the literature.[2-7] Here, we present a pediatric patient diagnosed with late-onset GS-2 without hypopigmentation.

A fourteen-year-old boy was admitted to the pediatric emergency department in his hometown due to a fever. Physical examination did not reveal pathologic findings except splenomegaly and mild oropharyngeal hyperemia. After receiving ceftriaxone, he had a sudden loss of consciousness and cardiac arrest. After resuscitation, he was intubated. Cranial imaging was performed and didn't reveal any pathologic findings supporting intracranial infections. Laboratory tests revealed haemolytic anemia haemoglobin 1.3 g/dL; direct Coombs test, 3+) and thrombocytopenia (platelet count, 21,000/mm^3). Because of his thrombocytopenia and clinical deterioration, lumbar punction was not performed. He received erythrocyte and platelet transfusions, inotropes, intravenous immunoglobulin (IVIG), and pulse corticosteroid therapy. On follow-up, he was referred to our pediatric intensive care unit (PICU) in need of plasmapheresis and continuous renal replacement therapy. On PICU admission, physical examination revealed splenomegaly, nonspecific rashes, and extensive mucosal bleeding. Aside from severe acute kidney injury findings requiring continuous venovenous hemodiafiltration, laboratory tests revealed anemia, thrombocytopenia, low haptoglobin, and elevated ferritin, triglyceride levels, positive direct Coombs, elevated liver enzymes, and bone marrow hemophagocytosis supporting the diagnosis of HLH (Table 1).[8] Because of his complicated clinical course, an immunology consultation was planned.

✉ Kubra Baskin ▪ akbaskin@hotmail.com

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Background history revealed that he was born to non-consanguineous parents, and over the last two years, he has been followed by splenomegaly and intermittent pancytopenia/bicytopenia. Neither he nor his siblings had fever episodes or neurological symptoms. Myelodysplastic syndrome, paroxysmal nocturnal hemoglobinuria, Gaucher disease, and Niemann-Pick disease were excluded. We evaluated our patient for possible causes for HLH (e.g., infections, malignancy, immunodeficiency) via immunologic work-up, viral serology, bacterial cultures, radiologic imaging, and bone marrow aspiration. Laboratory assessment revealed EBV viremia, low IgM levels, and low absolute lymphocyte subset counts (Table 1). He received rituximab, anakinra, IVIG, and steroid treatments. Exome sequencing (ES) was performed and identified compound heterozygous [c.488G>T (p.Ser163Ile), and c.-3A>C] variants of unknown significance in the RAB27A gene (NM_183235.3). Sanger sequencing validated that the c.488G>T (p. Ser163Ile) variant was maternally and the c.-3A>C variant was paternally segregated and found to be in trans configuration. Contrary to expectations, he did not have hypopigmentation (Figure 1). After discharge from PICU, he was treated according to HLH 94:2018 consensus recommendations.[9] Eight months after HLH treatment and remission, he was transplanted from his 10/10 HLA-matched elder sister (who had a heterozygous c.-3A>C

Table 1. Laboratory and immunologic assessments of the patient who previously received IVIG and steroid

	Patient	Reference ranges for age
Haemoglobin (g/dL)	**9.5**	11-14.5
Platelets (/mm^3)	**20000**	175000-332000
Absolute neutrophile count (/mm^3)	4800	1540-7040
Absolute lymphocyte count (/mm^3)	**1000**	(970-3260)
Ferritin (ng/mL)	**540**	10.9-135
Triglyceride level (mg/dL)	**286**	38-250
Direct Coombs	**Positive (3+)**	Negative
AST (U/L)	**3507**	17-44
ALT (U/L)	**932**	15-47
Haptoglobin (g/L)	**0.29**	0.3-2
Fibrinogen (mg/dl)	**110**	200-400
IgG (mg/dl)	1230	(907-1958)
IgA (mg/dl)	142	(96-465)
IgM (mg/dl)	**58.4**	(83-282)
T. Ig E (IU/mL)	73.4	(195)
Isohemagglutinin titer	Anti-A: 1/128 Anti-B: 1/32	≥1/8
EBV PCR (Viral load) (copy/ml)	**8360**	Negative
CD3+T cells (%, /mm^3)	58 **580**	(58-82) (1100-4100)
CD3+CD4+ T cells (%, /mm^3)	35 **350**	(26-48) (600-2400)
CD3+CD8+ T cells (%, /mm^3)	22 **220**	(16-32) (400-1500)
CD19+ B cells (%, /mm^3)	38 380	(10-30) (200-1400)
CD3-CD16+CD56+ NK cells (%, /mm^3)	**1** **10**	(8-30) (200-1000)

ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; EBV: Epstein-Barr virus; NK: Natural Killer cells; PCR: Polymerase Chain Reaction.
Bold values indicate abnormal laboratory levels.

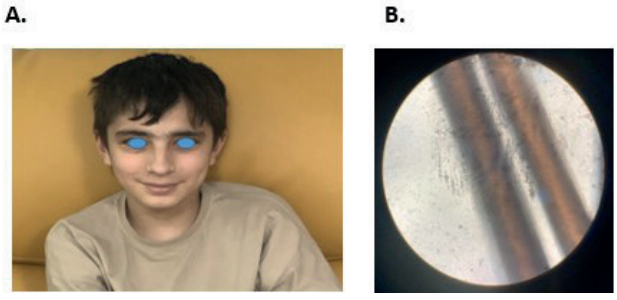

Figure 1. A. Patient (without albinism) and B. Microscopic imaging of his hair shaft (indicating normally distributed melanosomes)

variant of unknown significance in the RAB27A gene). His post-transplant course was uncomplicated. Neutrophil engraftment was achieved on day +14, and no profound thrombocytopenia was seen. Chimerism analysis of the peripheral blood showed full donor chimerism on day +21. He is now well at post-transplant 10 months with 100% chimerism.

Recently, a comprehensive dataset of 149 GS-2 patients diagnosed up to now was published.[7] According to this study, 31 patients (21%) did not have any signs of albinism, and 16 patients in the cohort (11%) developed initial symptoms at age 10 years or over (late-onset). The most common presenting feature in patients with late-onset was systemic HLH (n = 10, 63%). Sixty-nine percent of these patients (11/16) did not exhibit features of partial albinism. In addition, 5 of 8 patients with viral infections at HLH presentation had EBV viremia. Our patient's clinical features were also consistent with the findings in this publication.

In conclusion, our case also highlights that hypopigmentation can be absent in GS-2 and should not preclude the diagnosis. RAB27A mutations should be investigated in patients with suspected HLH disease without hypopigmentation.

Ethical approval

Both parents and patient provided the written informed consent.

Author contribution

The authors declare contribution to the paper as follows: Study conception and design: KB; data collection: KB, SKK, GK, EO, BB, EA, ZK, UK; analysis and interpretation of results: GK; Performing and confirming genetic analysis: GK; draft manuscript preparation: KB. All authors reviewed the results and approved the final version of the article.

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