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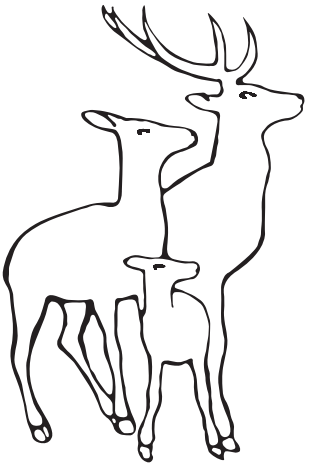

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The Turkish Journal of Pediatrics 2025; 67: 443-444
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Editorial

Gaza's children and the unbearable cost of war — a pediatric perspective on a public health emergency

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In armed conflict, children are often portrayed as innocent bystanders. Yet data from Gaza reveal they are not only victims—they are among the primary casualties. As pediatric professionals, we have a duty to both bear witness and act.

In this issue of *The Turkish Journal of Pediatrics*, we publish a critical contribution: *"Excess mortality and disease burden due to conflict in Gaza: focus on the 0–14 age group"* written by Gökler ME. This study quantifies the impact of the post-October 7, 2023 conflict on child health using disability-adjusted life years (DALYs). The results are staggering, revealing an overwhelming rise in the burden of disease and premature death among children. The analysis shows a dramatic surge in the loss of healthy life years, emphasizing the severe impact of the conflict on child survival and long-term well-being.[1]

This context is critical: recent conflicts in Gaza have produced the highest child casualty rates recorded in modern times.[2] According to data from the United Nations Office for the Coordination of Humanitarian Affairs as of July 23, 2025, the military offensive in Gaza has resulted in the deaths of more than 59,000 Palestinians, injured over 143,000 individuals, and led to the displacement of nearly the entire population of 2.1 million residents.[3] It is important to note that the actual burden may be even higher than reported. Due to the extensive destruction of infrastructure, the Gaza Health Ministry faces increasing challenges in data collection, which likely results in underreporting of both mortality and morbidity figures.[4] Since March 2, 2025, humanitarian aid access to Gaza has been severely restricted, with a complete blockade in place until May 19. Following the breakdown of a ceasefire on March 18, Israeli forces have resumed the use of explosive weaponry in their operations targeting Gaza.[5]

The conflict's impact extends beyond injury and mortality to include the systemic breakdown of health service delivery. Reports from humanitarian organizations operating in Gaza indicate that hospitals are now rationing food for inpatients, with only one or two meals provided per day—and expectations that food services may be entirely suspended in the coming weeks.[5] This level of deprivation not only compounds the physical and psychological toll on children but also reflects the collapse of basic care standards amid siege conditions.

When viewed together, the DALY analysis and the data being reported from the area illustrate both the quantitative scale and qualitative depth of suffering among Gaza's children. Beyond immediate injuries and mortality, the long-term implications for child development, mental health, and community resilience are incalculable.

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We urge the pediatric community, global health institutions, and humanitarian actors to amplify these data. This is not just a regional crisis—it is a global moral and public health emergency. A lasting and effective response must center on children's rights to health, protection, and recovery, and must be accompanied by a ceasefire, unimpeded humanitarian access, and sustainable rebuilding of Gaza's health infrastructure.

We commend the authors of the DALY study and other teams who continue to document and deliver care under unimaginable conditions. Their work ensures that the pediatric burden of war is no longer invisible.

Author contribution

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

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Review

Cat, dog, and horse allergies: emerging new insights

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ABSTRACT

Animal allergens, particularly those from cats, dogs, and horses, are significant risk factors for the development of allergic diseases in childhood. Managing animal allergies requires allergen avoidance and, when this is not feasible, specific immunotherapy. Patient history remains the cornerstone of diagnosis, providing the foundation for diagnostic algorithms. Extract-based tests, such as skin prick tests and specific IgE measurements, are essential for confirmation and screening. However, traditional extract-based diagnostic methods have notable limitations, as they are unable to distinguish between primary sensitization and immunological cross-sensitization, and also has the potential for both false negatives and false positives. Polysensitization may arise from either multiple independent sensitizations (co-sensitization) or cross-sensitizations, between homologous allergens. Due to complex cross-reactivity and polysensitization in mammals, extract-based tests are often insufficient in determining the true allergen, so molecular allergen testing should be used. Even with molecular testing, there is no consensus on how to define complex and intriguing sensitization patterns in mammals. In this report, we review the literature on cat, dog, and horse allergies and propose a novel approach to identifying complex sensitization patterns based on the current state of knowledge. We recommend that the evaluation of cat, dog, and horse allergies should begin with investigating genuine sensitization to Fel d 1, Can f 4/5, and Equ c 4, respectively. As a subsequent step, we propose a practical approach to determine primary allergen sensitization within the lipocalin group. Secondary sensitizations should then be evaluated in the context of recent contact history and presenting symptoms. While serum albumin is less strongly associated with true animal allergies, we suggest that it may serve as a complementary marker when considered alongside cross-reactive food allergen molecules.

Key words: allergy, allergen molecule, cat, dog, horse, sensitization.

Exposure to Animal Allergens

Humans have coexisted with cats, dogs, and horses since the dawn of civilization. While their traditional roles in pest control, protection, and transportation have diminished with technological advancements, modern life has not reduced our contact with these animals. On the contrary, they have become increasingly integrated into our homes and daily lives. Developing with pets can contribute to a child's self-esteem and confidence as well as developing non-verbal communication and empathy. Pet ownership rates vary in different parts of the world due to regional and cultural differences. Up to 50% of European households own a pet. Although the number of pet owners is one of the strongest predictors of increased allergen levels, high allergen levels have also been found in places where there are no pets (e.g. schools and public places). Animal allergens can become ubiquitous through passive transfer via human clothing or hair spread by pet owners.[1] In addition, there are significant

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stray animal populations in many parts of the world, including the Eastern Mediterranean region. Stray animals shed allergens (e.g. hair, dander, saliva and urine) into the environment, resulting in widespread exposure in public and residential areas. Stray animals, in lesser quantities, contribute to widespread environmental exposure to allergens. Although adoption rates are low (5% for dogs and 14% for cats), the high number of stray animals in Turkey indicates a high level of exposure.[2]

Prevalence of Sensitization to Cats, Dogs and Horses

Although there are regional differences in sensitization to furry animals, there has been an increase in sensitization in recent years. Sensitization to cats and dogs was found in 26% and 27% of adults in Europe, respectively.[3] In the United States, the prevalence of cat sensitization in subjects aged 6 years and older was 12.1%, whereas the incidence of dog sensitization was 11.8%.[4]

Horse sensitization is expected to be higher in those who have close contact with horses for professional or recreational purposes. In one study, horse sensitization was found to be 4.3% in the general population compared to 12.8% in grooms.[5] However, indirect exposure is also important in the equine sensitization. Another study found that 5.4% of patients were sensitized to horses, although most of the patients did not have contact with them. There are several possible routes of exposure to equine allergens, including airborne dispersion and indirect exposure through clothing.[6]

The prevalence of sensitization is modified by the age of the subject, with an increase during childhood and adolescence.[7] However, it should be emphasized that prevalence studies were carried out using extract-based tests. If the extract's constituents or the strength changes over time, this could lead to an apparent difference in prevalence.[8]

Impact of Pet Allergens on Allergic Diseases

Allergens from cats, dogs and horses are major risk factors for the development of asthma and allergic rhinitis. Sensitization to some allergen molecules and higher levels of IgE to allergen molecules are associated with an increased risk of allergic diseases. For example, asthmatic children with cat allergy have higher Fel d 1 sIgE levels than children with rhinitis alone.[9] One study reported that sensitization to Can f 2 and Equ c 1 was more common in severe asthma. IgE levels to Equ c 1 -a horse allergen molecule- correlated with asthma control. Children with higher levels of IgE antibodies to cats, dogs and horses had severe asthma and more bronchial hyperresponsiveness.[10] Sensitization to furry animal allergen molecules is an important predictor of asthma outcome and an indicator of severity.[11] It was reported that sensitization to more components was associated with increased airway inflammation.[11,12] One study reported that multi-sensitization towards lipocalin, kallikrein and secretoglobin molecules was more common among severe asthmatics compared to children with controlled asthma. These subjects also had higher blood eosinophils, higher fractional exhaled nitric oxide and increased bronchial hyperresponsiveness.[13]

Treatment is based on identifying the allergens causing symptoms and counseling patients to avoid these allergens. Reducing allergen exposure often requires removing the cat or dog from the home, which underscores the importance of accurately identifying the true allergen causing clinical symptoms. However, in most cases, removing the animal is not feasible due to the emotional bond between the animal and the child, as well as the parents. In such cases, specific immunotherapy is a valid option, but it is critical to identify children who are most likely to benefit from this therapy. Due to complex cross-reactivity and polysensitization among mammalian allergens, determining the true allergen can often be challenging.[8,14] In recent years, new strategies have been developed to neutralize Fel d1 at its

source. Since the biological function of Fel d 1 in cats is currently unknown, studies have focused on developing approaches that neutralize Fel d 1 after it is produced, without altering its natural production. Satyaraj et al showed that feeding cats a diet with an egg product ingredient containing anti-Fel d 1 IgY reduces environmental Fel d 1 levels and produces a significant improvement in the nasal and ocular symptoms cat-allergic humans.[15]

Molecular Allergy Diagnostics for Animal Allergies

Patient history remains the cornerstone of diagnosis where diagnostic algorithms converge. Extract-based tests, such as skin prick tests and specific IgE measurements, are essential for confirmation and screening. Traditional extract-based diagnostics have major limitations. Firstly, there is no standardization of the allergens used as substrates. As a result, the concentration of allergens in extracts varies widely. Extract-based tests may sometimes lack certain allergens or be contaminated with irrelevant ones. This is likely to lead to false negative or false positive results, which could have a negative impact on the accuracy of the diagnosis of animal allergy. For example, patients with a history of allergy but no evidence of sensitization on extract-based tests may require molecular testing.[14,16,17]

One of the major diagnostic challenges in animal allergy is that in routine testing, up to 75% of subjects sensitized to animal dander are also sensitized to two or more different species.[18] Conventional allergy testing with whole extracts can detect polysensitization well but cannot differentiate between primary sensitization and immunological cross-reactivity. Polysensitization can be based on multiple independent sensitizations (co-sensitization) or cross-sensitizations between homologous allergens. This can lead to false positive results. Patients who are found sensitized to extract-based tests but have no clear clinical symptoms or history of exposure to the detected allergen require further evaluation. The analysis and assignment of complex sensitization patterns are only possible with the growing availability of recombinant or purified native animal allergens for singleplex and multiplex testing. Therefore, in most cases, molecular allergology evaluation is necessary.[16,17]

In addition, component resolved diagnostic tests provide insight into the severity and outcome of asthma and allergic rhinitis.[11] Nwaru et al investigated 1872 adults for cat, dog and horse allergen serum IgE levels. They used cluster analysis to derive distinct sensitization clusters and show their association with asthma, rhinitis and markers of asthma severity in adults. Sensitization to furry animal allergen components is an important predictor of asthma, rhinitis, and markers of asthma severity with increased blood eosinophils, fractional exhaled nitric oxide, and airway hyperreactivity.[11]

Allergen molecule sensitizations measured by singleplex arrays are generally preferred to multiplex arrays due to their ability to provide precise, quantitative sensitization data, although they are limited by the number of available animal allergens. While multiplex platforms offer several advantages, including a comprehensive view of sensitizations, reduced serum requirements, and cost-effectiveness when testing for more than 12 allergens, they also have drawbacks, such as lower analytical sensitivity-particularly at low total IgE levels-semiquantitative sensitization data, and potential interference from IgG.[19,20]

Animal Allergen Molecules

The cat, dog, and horse allergen molecules identified to date, along with their respective groups, are listed in Table I. Fel d 1 is a glycoprotein belonging to the secretoglobin family. Fel d 1 is a thermostable protein that is found in the saliva, anal glands, sebaceous glands, skin and fur of cats. Fel d 1 spreads easily. Not all cats shed Fel d 1 at the same rate, hormonal status modifies its production. For

Table I. Cat, dog and horse allergen molecules according to Allergen Nomenclature by WHO/IUIS Allergen Nomenclature Sub-Committee (https://www.allergen.org).

Allergen molecule	Biochemical name
Cat	
Fel d 1	Secretoglobin
Fel d 2	Serum albumin
Fel d 3	Cystatin-A
Fel d 4	Lipocalin
Fel d 5	Immunoglobulin A
Fel d 6	Immunoglobulin M
Fel d 7	Lipocalin
Fel d 8	Latherin
Dog	
Can f 1	Lipocalin
Can f 2	Lipocalin
Can f 3	Serum albumin
Can f 4	Lipocalin
Can f 5	Kallikrein
Can f 6	Lipocalin
Can f 7	Niemann Pick type C2
Can f 8	Cystatin
Horse	
Equ c 1	Lipocalin
Equ c 2	Lipocalin
Equ c 3	Serum albumin
Equ c 4	Latherin
Equ c 6	Lysozyme

example, males have been shown to produce more Fel d 1 than females. In addition, neutered males produce less Fel d 1 than unneutered males.[21] Fel d 1 is a major cat allergen. Fel d 1-specific IgE has been shown to be as reliable as IgE to cat extract for the diagnosis of cat allergy.[9,22]

Can f 4 is a major dog allergen from the lipocalin family. It is associated with true dog allergy which has been demonstrated in some studies.[8] In a recent study[23], it was reported that sensitizations to Can f 4 indicates genuine sensitizations to dogs.

Can f 5 has been identified as the major dog allergen, a prostatic kallikrein expressed in the prostate gland and is consequently only present in male dogs. In addition, castration of male dogs has been shown to drastically reduce its production. Can f 5 is mainly found in the urine of male dogs, but also in extracts of dog hair and dander. Direct contact with the male dog is important for sensitization as it does not spread as easily as lipocalins.[24] Recently, Schoos et al. showed that patients allergic to male dogs and monosensitized to Can f 5 tolerated a conjunctival challenge with female dog extract.[25]

Horses produce latherin, a highly surface active, non-glycosylated protein. Latherin was detected in horse skin and salivary glands.[26] Up to 77% of patients with horse allergy are sensitized to Equ c 4, a latherin protein.[27] Equ c 4 may be a specific marker for horse allergy but this needs to be further evaluated.[8] In a recent study[23] it was reported that sensitizations to Equ c 4 differed from other horse allergen molecule sensitizations, indicating genuine sensitizations to horses.

Fel d 1, a secretoglobin protein, is the primary cause of IgE-mediated reactions in cat-allergic individuals. While dogs do not produce Fel d 1, a structurally similar protein in dog hair extracts has been identified, which may explain dual sensitization to both cat and dog allergens.[8] In a pediatric study[23], sensitization to the Fel d 1-like protein was observed in only a subset of Fel d 1-sensitized individuals (38 of 95), with more than 50% of these (22 of 38) not sensitized to other dog allergens. Quantitative analysis confirmed that Fel d 1 sensitization predominated in all dually sensitized individuals. The authors suggested that the Fel d 1-like protein in dogs likely results from cross-sensitization to Fel d 1 in cats. The clinical significance of Fel d 1-like protein sensitization warrants further investigation to elucidate its specific role in dog allergy.[16,23,28]

Lipocalins are the most important group of inhaled animal allergens. They are small, secreted molecules that are easily spread in

indoor environments. They are produced in the secretory glands or liver and are found in saliva, urine and hair dander. They share a characteristic tertiary structure with a central β-barrel formed by 8 anti-parallel β-strands. Lipocalin sequence identities between family members can be as low as 15%, while some lipocalins have much higher sequence identities, up to 67%. Due to pairs with high sequence identity, lipocalins may contribute to allergic cross-sensitizations between different species. Fel d 4 and Fel d 7 in cats, Can f 1, Can f 2, and Can f 6 in dogs, Equ c 1 in horses are allergen molecules belonging to the lipocalin family with cross-sensitization potential.[8,29]

Serum albumins are mainly respiratory allergens, but sensitization to serum albumins may be more complex and involve multiple routes of exposure. They are abundant in blood, but are also present in milk, saliva, dander and meat. It is highly likely that contact with aminal dander is the major source of health problems associated with serum albumin in humans. Serum albumins are considered minor allergens. Due to their high sequence similarity (up to 70%), cross-sensitization is one of the most important features of these allergens. In many cases, individuals allergic to serum albumin are polysensitized to different animals. Serum albumins are thermolabile proteins and their allergenicity is inactivated by heat. Bos d 6 in cattle, Can f 3 in dogs, Equ c 3 in horses, Fel d 2 in cats, Sus s 1 in pigs are examples of mammalian serum albumin allergens.[8,30]

There are other allergen molecules that belong to other allergen families in animals. For example, Fel d 3 and Can f 8 belong to the cystatin A protein family. Recently, Niemann-Pick type C proteins have been identified in both cats and dogs.[8]

Co-sensitization has to be distinguished from cross-sensitization. It is important to recognize that IgE-cross-sensitization may not always imply clinical cross-reactivity. When cross-sensitized lipocalin allergens have high sequence homology, patients may experience symptoms of all these allergen sources. Certain lipocalins (Can f 6, Fel d 4, Equ c 1; Fel d 7 and Can f 1) share a high sequence identity and serve as markers of cross-sensitization. However, there is limited data on symptoms clearly related to cross-sensitized molecules as monosensitization to these allergen molecules seems to be rare.[8,16]

Many animal allergens are pan-allergens, and most lipocalins and serum albumins exhibit complex cross-sensitization patterns, contributing significantly to polysensitization. However, the interpretation of sensitizations in the diagnosis of cat, dog, and horse allergies remains a topic of debate. While there is general consensus among experts and guidelines on the diagnostic approach, inconsistencies remain in the recommendations.[8,31-34]

Regarding cat allergy, there is a consensus that Fel d 1 is the genuine allergen, and in most cases, detecting sensitization to Fel d 1 is sufficient for diagnosis. Typically, sensitization to Fel d 4 and/or Fel d 7 develops after Fel d 1 sensitization[8,23] a phenomenon known as molecular spreading.[35] However, in rare cases, sensitization to cat lipocalins may precede Fel d 1 sensitization. Current diagnostic algorithms do not clearly define when to consider cat allergy in the absence of Fel d 1 sensitization, even if sensitization to Fel d 4 and/or Fel d 7 is present, which cross-reacts with Equ c 1 and Can f 1, respectively.[14,16,23,31,33,36,37]

For dog allergy, guidelines consistently recognize Can f 5 as a marker of genuine sensitization to male dogs and Can f 1/6 as major contributors to dog sensitization.[8,14,16,23,31,34,36-39] While Can f 1 and/or Can f 6 sensitizations may indicate dog allergy in the absence of other lipocalin sensitizations, their association with dog allergy is inconsistent when other lipocalin sensitizations are present. Additionally, there is no consensus on which allergen molecule sensitizations should be investigated in the absence of Can f 5 sensitization. Given the high cross-sensitization within the animal lipocalin group, it may be logical to evaluate all

furry animal lipocalins (Fel d 4/7, Can f 1/2/6, Equ c 1) simultaneously to identify the primary sensitizer.

Regarding horse allergy, diagnostic algorithms consistently identify Equ c 1 as the major allergen.[8,27] Recent findings suggest that Equ c 4 may be a genuine sensitizer for horses[23] representing genuine sensitization that has not been widely recognized to date. Additionally, Equ c 3, a serum albumin, has limited information regarding its association with horse allergy. While serum albumins are known to exhibit high cross-reactivity among different animal species (Fel d 2, Can f 3, Equ c 3)[8], the extent to which sensitization to Equ c 3 alone indicates a related animal allergy and the possibility of cross-sensitization with foods, particularly in children (Sus s 1, Bos d 5), has not been sufficiently studied.

These findings suggest that all furry animal lipocalin and serum albumin sensitizations should be evaluated simultaneously. Therefore, the diagnostic algorithm should not be restricted to a single animal allergy but should encompass a comprehensive evaluation of cross-reactive furry animals, including cats, dogs, and horses.

Further studies are needed to better understand the complex cross-sensitization properties utilizing multiplex assays and to accurately demonstrate patterns of polysensitization and disease-specific sensitization patterns.[16] With this aim, in a recent study[23] conducted a comprehensive analysis of animal allergen molecules using correlation and hierarchical clustering techniques to elucidate the relationship between cat, dog and horse allergen molecules and to detect primary sensitization, cross-sensitization and co-sensitization. They suggested that a unified algorithm should be used to accurately assess cat, dog and horse allergy, prioritising identification of genuine allergen sensitization first and then elucidating lipocalin sensitization patterns. It was stated that sensitization to Fel d 1, Can f 4/5 and Equ c 4 indicated genuine allergen sensitization for cat, dog and horse allergy, respectively. Although serum albumin is less associated with genuine animal allergy, it has been suggested that it may serve as a complementary marker together with cross-reactive food allergen molecules. They suggested that focusing on the lipocalin and serum albumin groups followed by the assessment of allergy history in the assessment of primary sensitization.[23] One of the most critical challenges is to identify the primary sensitizing allergen. A practical and effective approach involves determining the most reactive allergen molecule within the allergen group based on quantitative levels, while considering the coefficient of variation of the measurement method.[31,40,41] Identification of the primary sensitizer strongly indicates an allergy to the specific animal in question, but it can also lead to secondary sensitizations due to molecular structural similarities. These secondary sensitizations may or may not represent a true allergic response like the primary sensitizer; therefore, it is essential to carefully consider exposure histories to the relevant animal. Given the need to examine a broad panel of allergen molecules to identify the primary sensitizer within the lipocalin and serum albumin groups, multiplex microarrays offer significant advantages, including cost-effectiveness and reduced serum requirements. However, it should be noted that multiplex arrays provide semiquantitative data and are not as precise as singleplex assays.[19]

A New Algorithm is Required

Our proposed algorithm differs from previous ones in five key aspects (Fig. 1). First, we suggest a combined top-down and bottom-up approach. The bottom-up approach involves confirming allergies through extract-based assays in patients with a suggestive history of animal allergy. Additionally, the top-down approach emphasizes molecular allergology evaluation in most patients for a more precise diagnosis, particularly in patients with a clinical history but no sensitization through extract-based assays, in patients with sensitization but no close contact or allergy history, in patients

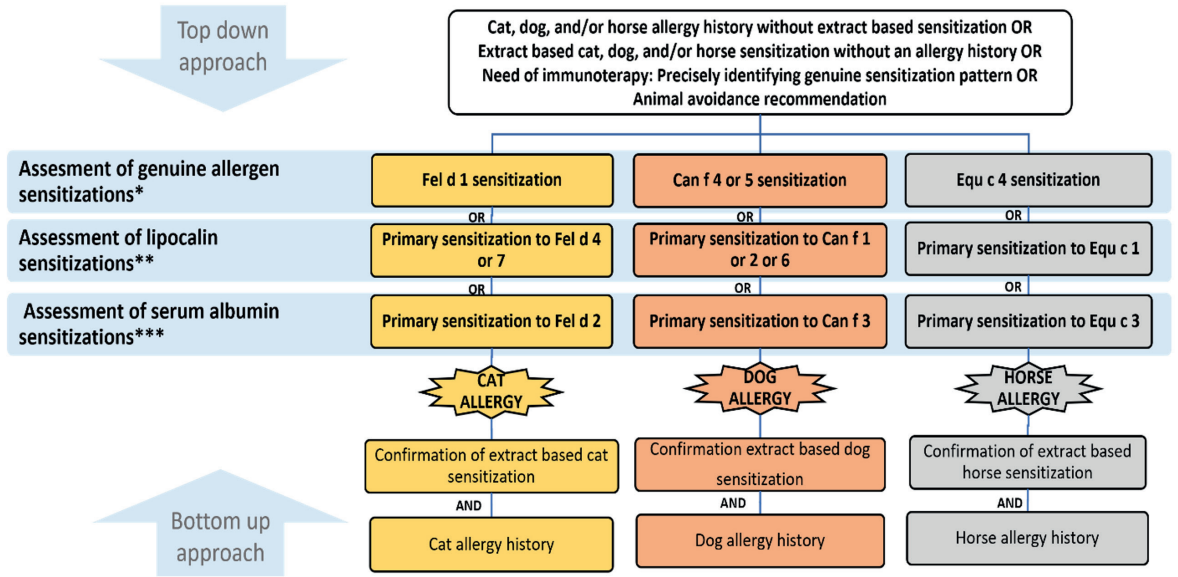

Fig. 1. Diagnostic algorithm for cat, dog, and/or horse allergy.

*: The presence of any allergen sensitization listed in the boxes indicates an allergy diagnosis to the corresponding animal.

**: The primary sensitization is defined as any allergen with the highest quantitative sensitization within the lipocalin group (Fel d 4/7, Can f 1/2/6, and Equ c 1).

***: The primary sensitization is defined as any allergen with the highest quantitative sensitization within the serum albumin group (Fel d 2, Can f 3, Equ c 3, Bos d 6, Sus s 1)

requiring immunotherapy, and in patients needing recommendations regarding avoidance or keeping the animal. Second, it identifies Can f 4 and Equ c 4 as additional genuine sensitizers, alongside Fel d 1 and Can f 5. Third, it assumes that genuine and primary sensitizations are the underlying causes of the relevant animal allergies. Fourth, it evaluates cat, dog, and horse allergies within a unified framework, recognizing the presence of numerous allergen molecules with a high likelihood of cross-sensitization. Fifth, it emphasizes the use of multiplex platforms, despite their limitations, due to the necessity of assessing sensitizations to a large number of allergen molecules. However, further studies may be needed to validate and enhance the utility of this algorithm. Its limitations include the exclusion of certain allergen sensitizations, reliance on assumptions not supported by clinical data but rather on hierarchical clustering of sensitizations, and the prioritization of primary sensitization based on quantitative levels, which may carry the potential risk of overdiagnosis.

Management of Cat, Dog and Horse Allergy

As with any allergic condition, the best course of action would be to avoid the offending animal completely. However, this has emotional consequences and is often not possible. There are also animal allergens in environments where animals are not present. There are some measures that focus on reducing exposure to allergens while keeping contact with the animal. These measures are less effective but may be more practical. Regular washing of the pet, keeping the pet out of the bedroom, air cleaning with HEPA filters, regular use and maintenance of high efficiency vacuum cleaners, application of topical lotions to the animal's fur and a combination of these measures. However, the measures described do not ensure clinical benefit. The effects of neutering and spaying dogs and cats have been inconsistent, and no specific recommendations have been made in this regard.[42]

Allergen immunotherapy (AIT) is emerging as a potential alternative treatment. However,

there is no consensus in the literature regarding cat, dog or horse AIT. Evidence on the efficacy and safety of cat AIT is limited, with no high-quality data on its cost-effectiveness. Some patients, particularly those with moderate-to-severe disease inadequately controlled by allergen avoidance and pharmacotherapy, or those monosensitized to Fel d 1, may benefit from this treatment modality.[43] The medical literature on dog extract immunotherapy shows inconsistent and conflicting results regarding clinical efficacy. These outcomes have been attributed to the poor-quality of extracts and the inherently complex profile of dog allergens.[44] Similarly, evidence on the safety and efficacy of horse immunotherapy is scarce. AIT with horse extract is not supported by experts, and no consensus has been reached regarding its benefits.[27]

Conclusion

Animal allergens, particularly from cats, dogs, and horses, present significant diagnostic challenges due to complex cross-sensitization and polysensitization patterns. A stepwise approach, beginning with the identification of genuine sensitization and followed by the determination of the primary sensitizer through molecular allergen testing using multiplex microarrays, can enhance diagnostic accuracy and guide effective management strategies.

Author contribution

Review conception and design: BEŞ; Literature review: BEŞ, BK, MO; Draft manuscript preparation: BEŞ, BK, MO. 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

Excess mortality and disease burden due to conflict in Gaza: focus on the 0-14 age group

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ABSTRACT

Background. The ongoing conflict in Gaza continues to take an unbearable toll, with particularly severe impacts on children. Measuring the burden of conflict-related disease in Gaza in terms of disability-adjusted life years (DALYs) is important in terms of showing this effect. The aim of this study was to calculate the conflict-related DALY in Gaza among children aged 0-14 years, following the October 7 events and compare these values with global and expected values.

Methods. We estimated the age and gender distribution of individuals killed or injured in Gaza, and calculated the DALYs, including Years of Life Lost (YLL) and Years Lived with Disability (YLD), attributable to the conflict. These estimates were then compared to the Institute for Health Metrics and Evaluation data for Palestine and global averages. The study also evaluated the DALY/YLD ratio and excess mortality rate.

Results. The DALY per 100,000 population was 160,745.01 (156,986.01-164,503.99) for males, 175,784.51 (170,812.52-180,756.50) for females, and 168,111.39 (164,009.17-172,213.62) overall. The daily DALY burden experienced by Gaza due to conflict indicates an increase of 181.05% compared to Palestinian estimates. The increase was calculated as 115.39% for YLL and 4,268.25% for YLD. Compared to global data for conflict and terrorism, the increases in daily DALY, YLL, and YLD values in Gaza were 1,918.08%, 1,316.32%, and 8,537.50%, respectively. The data calculated in our study indicate that the daily DALY/YLD ratio for the 0-14 age group in Gaza was 333.21 with a p-score of 6,952.0%.

Conclusion. To reduce the devastating effects of violence, such as conflict and terrorism, on children's health, more effective measures should be taken at the international level and preventive strategies should be developed.

Key words: conflict, disability adjusted life years (DALY), excess mortality, Gaza, 0-14 years.

Wars represent some of the most devastating and consequential events in human history, with their adverse effects extending far beyond immediate casualties in conflict zones.[1] The indirect effects of war, including the destruction of infrastructure, the disruption of healthcare services, the loss of economic resources, and forced displacement, have a significant impact on social structures. This leads to long-term losses in both the short and long term.[2] Additionally, wars promote the transmission of diseases and increase the prevalence of existing health issues.[3] The data published by the International Institute for Strategic Studies (IISS) indicates that the number and length of armed conflicts have increased over the 21st century.[4] Factors, such as political infighting, ethnic and religious animosity, economic disparities, and external assistance, have led to the increased intensity of conflicts, particularly in the Middle East, Africa, and Asia.

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The first recent instances of ongoing disputes were in Ukraine. The ongoing conflict in Ukraine has had a significant negative impact on the population of Ukraine. The healthcare system has been particularly affected. Recent estimates indicate that approximately 6.5 million people have been forced to move worldwide, and numerous healthcare facilities have been targeted.[5,6] The recent instance of this is the Palestinian-Israeli conflict. The Gaza Strip has historically faced severe humanitarian crises due to ongoing conflicts and political instability, however, the events following October 7, 2023, have led to a situation that is one of the most severe humanitarian crises in history, specifically affecting civilians, including children and women.

The events in Gaza have led to problems with healthcare services, infrastructure destruction, financial loss, and have directly affected healthcare. The suspension of healthcare services in Gaza allows diseases to spread and makes it difficult to treat chronic conditions.[7] Trouble with housing, constant travel, overcrowded conditions, and lack of access to fresh water have had a significant impact on the disease burden in Gaza during the ongoing conflict. The definitions of Disability-Adjusted Life Years (DALY), Years of Life Lost (YLL), and Years Lived with Disability (YLD) illustrate the complexity of estimating the public health burden associated with inadequate health services and conflict settings. In the context of the Gaza conflicts, indirect deaths have led to a substantial increase in YLL.[8] Particularly high rates of mortality among civilians contribute to increased YLLs.[2,9] Adversities in accessing healthcare services during disputes, tardy responses to emergency situations, and inability to address chronic diseases all contribute to increased fatalities.[7] Additionally, the long-term effects of bombings and armed conflict are increasing YLD.[10] These factors negatively affect the effective operation of health infrastructure and services, posing a significant threat to public health.

The aim of this study was to calculate the conflict-related DALY, YLL, and YLD in Gaza among children aged 0-14 years following the October 7 events and compare these values with global and expected values.

Materials and Methods

This descriptive study was conducted on the data of Palestinian citizens who were killed or injured due to the ongoing Gaza-Israel war between October 2023 and June 2024. According to the Ministry of Health in Gaza, at least 42,652 Palestinians were killed and 103,053 injured in Gaza between October 7, 2023 and the afternoon of October 7, 2024.[11]

DALYs were calculated as the sum of YLL due to premature mortality and YLD. YLLs were estimated by multiplying the number of deaths by the standard life expectancy at the age of death, based on Global Burden of Disease (GBD) reference life tables. YLDs were calculated by multiplying the number of incident cases by the average duration of the condition and a disability weight reflecting the severity of the health outcome. These metrics were applied to age- and sex-specific data to reflect the burden within the affected population. We calculated the DALY, YLL and YLD of the conflict in the Gaza. The 2023 projection of the United Nations Fund for Population Activities (UNFPA) for the Gaza population was used to calculate the rate per 100,000 population.[12] The DALY was calculated by adding YLLs and YLDs. One DALY equals one lost year of healthy life. YLLs were estimated and determined from age and gender stratified mortality rates (YLL=N*L, where N is the number of deaths per year and L is the life expectancy at age of death in years).[13] For the analysis of the number of deaths (predicted deaths: N) by age in this study, we used a list published by the Palestinian Ministry of Health, covering the period between October 7 and October 26, 2023 at 15:00, which, according to the source, included only people who were brought to health facilities and morgues.[14-16] In this list, the distribution of 6,747 people

with known ages in the 0-14 years range was obtained. Of the deceased individuals on the list, 2,258 (33.4%) were aged 0-14 years. Once this data was obtained, the estimated number of deaths and injuries by gender of individuals aged 0-14 years was estimated according to the number of deaths and injuries published by the Palestinian authorities on July 17, 2024.[11] Life Expectancy at Birth (L) in Palestine was based on data published by the Palestinian Bureau of Statistics for the year 2023 (male: 73.30, female: 75.5).[17] YLDs (YLD=I×DW×L) were calculated by multiplying the number of cases resulting from conflict and terrorism by the disability weight assigned to this health outcome. In the formula, I represents the number of new cases resulting from the conflict and terrorism during the study period, while DW denotes the disability weight reflecting the severity of the health outcome on a scale from 0 (perfect health) to 1 (equivalent to death).The estimation of the number of cases (I) was based on the age distribution in the above list and the number of injured as announced by the Palestinian authorities on October 7, 2024.[11] The DW value (0.273) was calculated by averaging the minimum and maximum values of the disability weights due to mass shooting as specified in Daniel G. Arce's study.[18] In the YLL formula, L refers to the standard life expectancy at the age of death (i.e., the number of years a person would have lived had they not died prematurely). In contrast, in the YLD formula, L represents the average duration of disability (i.e., the time period an individual lives with a health condition or injury before recovery or death). Although the same symbol is used, its interpretation differs in each context.

The Institute for Health Metrics and Evaluation (IHME), which conducts GBD studies, categorises diseases into three broad groups according to their main causes. The third category of causes includes injury-related health problems, which are subdivided into transport injuries, unintentional injuries, self-harm and interpersonal violence. Conflict and terrorism are included as a subgroup within the subcategory of self-harm and interpersonal violence. We compared YLL, YLD and DALYs by cause (conflict and terrorism- all causes) and over time.[19,20] In presenting past and estimated data, we used the GBD interactive data visualization tools (GBD Foresight Visualization and GBD Results). The data were evaluated daily and Absolute Change Relative Change was calculated. The following formulas were used in the analysis: Absolute Change = Value at Time 2 − Value at Time 1, Relative Change (%) = (Value at Time 2 − Value at Time 1) / Value at Time 1 × 100, Rate per 100,000 population = (Number of cases / Total population) × 100,000.

When converting to daily data, DALY, YLD and YLL variables calculated for Gaza were divided by 365 days (October 7, 2023 to October 7, 2024). In addition, the global and Palestinian 2023 estimate data were divided into 365 days. For the estimation of DALY, YLL, and YLD, 95% confidence intervals (CIs) were calculated using standard statistical approaches to uncertainty quantification, grounded in the propagation of error framework. Specifically, the standard error of the aggregate burden estimates was computed based on the empirical variance of the underlying input parameters (e.g., number of deaths, incidence of injuries, and disability weights). Assuming approximate normality of the sampling distribution, two-sided 95% confidence intervals were constructed using the Student's *t*-distribution. This approach is consistent with conventional methods employed in global burden of disease analyses and facilitates the quantification of uncertainty arising from data variability.[21]

The study also calculated the excess mortality rate. This rate refers to the extent to which all-cause mortality during a crisis exceeds the expected baseline under normal conditions. Excess mortality [P-score = (Reported deaths-Projected deaths) /Projected deaths×100] was measured as the percentage difference between the reported and estimated number of deaths.[22]

The study was conducted in accordance with the ethical standards of the Helsinki Declaration. Ethical approval and informed consent were not required for this study as it was based on secondary analysis of publicly available, aggregated data that did not contain any personal identifiers. Nevertheless, we acknowledge the ethical responsibility involved in reporting conflict-related mortality data with sensitivity and accuracy, recognizing the profound human impact behind these figures.

Results

According to the UNFPA 2023 projection, the total population in the 0-14 age group is 898,707, 458,531 males and 440,176 females. According to this analysis, 14,246 (1.58%) children in the 0-14 age group are predicted to have been killed. The distribution of YLL, YLD and DALY by gender in the 0-14 age group in Gaza is shown in Table I. While YLL, YLD, and DALY values are relatively lower for male children, these values are higher for female children.

For the year 2023, the expected DALY per 100,000 population was calculated as 87.41 (29.45-154.05) globally, while for Palestine it was 922.66 (24.77-6,240.73). In the same year, the expected YLL per 100,000 population was found to be 80.04 (23.39-145.97) globally, and 908.31 (9.67-6,226.94) in Palestine. Additionally, for 2023, the expected YLD per 100,000 population was determined to be 7.37 (3.35-21.07) globally, while in Palestine it was 14.34 (8.75-22.36). Table II provides the estimated values for daily DALY, YLL, and YLD due to conflict, as well as all causes, for the age group 0-14 years globally and in Palestine. These values show a significant increase when compared to the estimates for Palestine and the global estimates. The daily DALY burden experienced in Gaza due to conflict indicates an increase of 181.05% compared to Palestinian estimates. The increase was calculated as 115.39% for YLL and 4268.25% for YLD. Compared to global data for conflict and terrorism the increases in daily DALY, YLL, and YLD values in Gaza are 1,918.08%, 1,316.32%, and 8,537.50%, respectively. Additionally, when assessed across all causes, daily values

Table I. Distribution of YLL, YLD, and DALY by gender in children aged 0–14 years in Gaza.

Gaza, 0-14 years	Male (95% CI)	Female (95% CI)	Total (95% CI)
Population[1]	458,531.00	440,176.00	898,707.00
Total YLLs	466,633.81 (455,721.67–477,545.93)	484,037.28 (470,346.49–497,728.07)	950,671.09 (927,488.18–973,853.96)
Years of life lost (YLLs) (per 100,000 population)	101,770.01 (99,390.14–104,149.88)	109,961.24 (106,851.03–113,071.45)	105,782.09 (103,202.51–108,361.67)
Total YLDs	270,410.98 (264,087.49–276,734.49)	289,746.79 (281,551.43–297,942.17)	560,157.77 (546,473.51–573,842.01)
Years lived with disability (YLDs) (per 100,000 population)	58,975.00 (57,595.88–60,354.12)	65,823.27 (63,961.49–67,685.05)	62,329.30 (60,806.64–63,851.96)
Total DALY	737,044.79 (719,809.14–754,280.42)	773,784.07 (751,897.91–795,670.23)	1,510.828,86 (1,473,961.88–1,547,695.86)
DALY (per 100,000 population)	160,745.01 (156,986.01–164,503.99)	175,784.51 (170,812.52–180,756.50)	168,111.39 (164,009.17–172,213.62)

DALY: disability-adjusted life years, YLD: years lived with disability, YLL: years of life lost.

Table II. Distribution of Daily DALY, YLL, YLD values and changes due to conflict in Gaza with Palestine and Global 2023 estimate data on conflict and all causes for 0-14 years group.

	DALY (95% CI)	YLL (95% CI)	YLD (95% CI)
2023 Estimate Palestine (Conflict and terrorism, 0-14 years, per 100 000 population, per day)	2.53 (0.07–17.10)	2.49 (0.03–17.06)	0.04 (0.02–0.06)
2023 Estimate Global (Conflict and terrorism, 0-14 years, per 100 000 population, per day)	0.24 (0.08–0.42)	0.22 (0.06–0.40)	0.02 (0.01–0.06)
2023 Estimate Palestine (All causes, 0-14 years, per 100 000 population, per day)	33.24 (26.42–47.87)	22.81 (17.08–37.57)	10.47 (7.61–14.18)
2023 Estimate Global (All causes, 0-14 years, per 100 000 population, per day)	74.78 (65.79–86.33)	62.50 (53.87–72.91)	12.37 (8.98–16.46)
Gaza (0-14 years, per 100 000 population, per day)	460.58 (449.34–471.82)	289.81 (282.75–296.88)	170.77 (166.59–174.94)
Absolute change; Relative change[1]	458.05; 181.05	287.32; 115.39	170.73; 4.268.25
Absolute change; Relative change[2]	460.34; 1.918.08	289.59; 1.316.32	170.75; 8.537.50
Absolute change; Relative change[3]	427.34; 12.85	267.00; 11.71	160.30; 15.31
Absolute change; Relative change[4]	385.80; 5.16	227.31; 3.64	158.40; 12.81

[1]Comparison between 2023 Estimate Palestine (Conflict and terrorism) and Gaza
[2]Comparison between 2023 Estimate Global (Conflict and terrorism) and Gaza
[3]Comparison between 2023 Estimate Palestine (All causes) and Gaza
[4]Comparison n between 2023 Estimate Global (All causes) and Gaza.
DALY: disability-adjusted life years, YLD: years lived with disability, YLL: years of life lost.

of DALY, YLL, and YLD in Gaza were found to be higher than those in the broader Palestinian population, with increases of 12.85%, 11.71%, and 15.31%, respectively. Compared to global data for all causes, the increases in daily DALY, YLL, and YLD values in Gaza were 5.16%, 3.64%, and 12.81%, respectively (Fig. 1). These losses represent an additional burden of 0.28% to the globally expected total DALY (Global: 544,679,488.54; Gaza: 1,510,828.86), 0.21% to YLLs (Global: 455,247,949.56; Gaza: 950,671.09), and 0.62% to YLDs (Global: 90,096,459.72; Gaza: 560,157.77). When evaluating the losses due to conflict, the losses experienced by the 0-14 age group account for an additional burden of 86.63% of the globally expected DALY (Global: 1,743,877.45; Gaza: 1,510,828.86), 59,53% of YLLs (Global: 1,596,805.74; Gaza: 950,671.09), and 380.87% of YLDs (Global: 147,071.72; Gaza: 560,157.77).

Upon evaluation of the daily DALY/YLD ratio, it is anticipated that the expected DALY/YLD ratio due to conflict and terrorism for children aged 0-14 years in Palestine will be 1.58, while the global average for this age group is estimated to be 8.33. When all causes are considered, the estimated rate for children aged 0-14 years in Palestine was 31.50, while the global average is 16.54. The data calculated in our study indicate that the daily DALY/YLD ratio for this age group in Gaza is 333.21, which is above both the

Palestinian and global average. Although the expected values for Palestine are high compared to the global data, the disability experienced due to the war is found to be higher than the expected value for Palestine.

For 2023, the total number of projected deaths for the 0-14 age group in Palestine due to conflict and terrorism is 199.91[20] while the total predicted deaths amount to 14,246, resulting in an overall p-score of 6,952.0% (Table III).

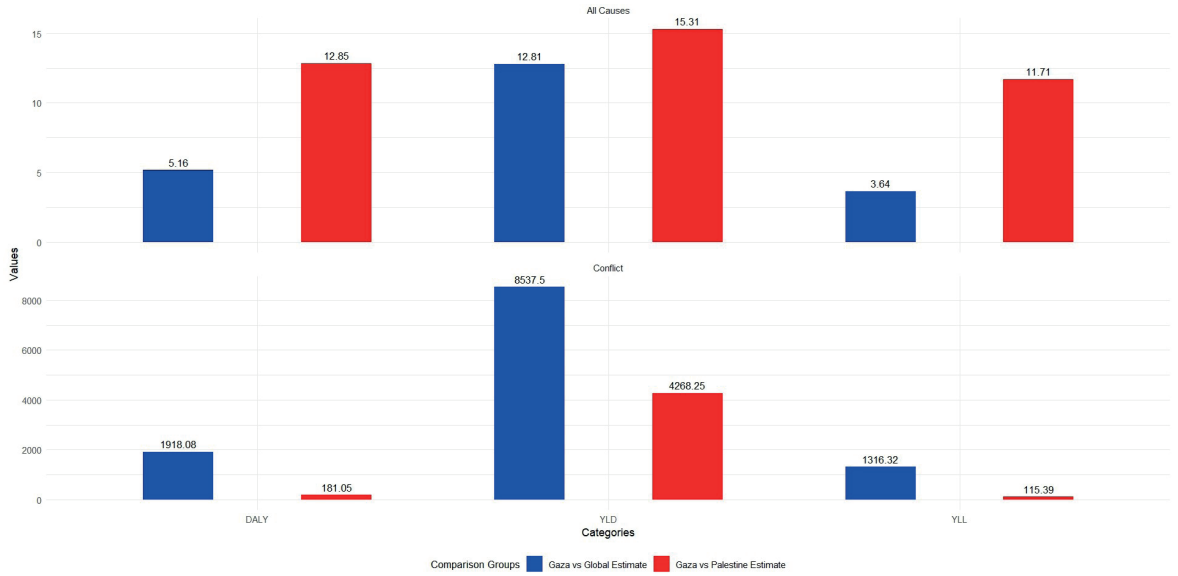

Fig. 1. Relative change in DALY, YLL, and YLD in Gaza (Oct 2023–Oct 2024), compared to 2023 estimates for Palestine and global data across conflict and terrorism and all causes.
DALY: disability-adjusted life years, YLD: years lived with disability, YLL: years of life lost.

Table III. Excess mortality in Gaza.

Years Group	Projected deaths[1]	Predicted deaths (N)	Excess mortality p-score[2]
0	13	970	7,361.5
1	14	999	7,035.7
2	16	1,075	6,618.8
3	15	1,007	6,613.3
4	16	1,162	7,162.5
5	15	1,041	6,840.0
6	12	939	7,725.0
7	12	928	7,633.3
8	12	850	6,983.3
9	11	821	7,363.6
10	15	1,072	7,046.7
11	13	937	7,107.7
12	13	886	6,715.4
13	14	842	5,914.3
14	11	716	6,409.1
Total	200	14,246	6,952.0

[1]2023 Projected Palestine death 0-14 years because of conflict and terrorism
[2]P-score (a measure of excess mortality)= (Predicted deaths- Projected deaths)/ Projected deaths×100

Among the individual age groups, the highest p-score is observed in age group 6, with a p-score of 7,725.0%. Other age groups also show considerable excess mortality, with p-scores ranging from 5,914.3% to 7,725.0%.

Discussion

This study highlights how children aged 0-14 years in the Gaza Strip have been affected in terms of the burden of disease following the attacks. The findings demonstrate that state violence substantially contributes to the loss of DALYs. The war imposed an additional burden of one in a million on the globally expected DALYs in the 0-14 age group. In addition, while the daily DALYs lost due to war are approximately five times the expected rate at the global level, it is approximately thirteen times the expected value for Palestine. The values for YLL and YLD calculated by considering all causes were found to be eleven and fifteen times higher than the expected values for Palestine, compared to four and twelve times, respectively, at the global level. A notable increase was observed in the rates when the conflict data analysis was examined.

On the other hand, the results of this study reveal a significant excess of predicted deaths due to conflict in the 0-14 age group in Palestine compared to reported deaths (p score: 6,952.0%). These excess mortality rates point to the need to reassess and improve health and humanitarian policies. One year after the beginning of the Israeli-Gaza war, the health system is severely strained. Numerous mass casualty incidents have led to a critical shortage of hospital beds, with many hospitals out of service. Both the inadequate functioning of hospitals and the inadequate provision of primary health care in the region are causing life crises secondary to the destruction caused by the war.[23] International humanitarian law, which should guarantee the rights of civilians and medical personnel during armed conflicts, has been repeatedly violated.[24] These high excess death rates may be due to deliberate targeting of civilians.[25]

According to figures provided by international organizations, approximately 1 million cases of acute respiratory infections, over 500,000 cases of acute watery diarrhea and over 100,000 cases of acute jaundice syndrome have been reported. Furthermore, supply problems in the provision of medical supplies, medicines, imaging and laboratory services are putting the lives of the critically ill and wounded at risk.[26] Adding to the risk, the lack of access to clean water for both drinking and use continues to pose major health risks. The effects of these problems are exacerbated by poor living conditions and overcrowding in Gaza, making it difficult for health care providers to respond effectively. Repeated displacement, insecurity and access restrictions lead to malnutrition among children and women in need of nutrition services. In screening surveys, 6.82% of children were reported to be diagnosed with malnutrition.[27] In addition, repeated displacement makes it difficult to monitor and track these cases. It has been reported that even under the most optimistic ceasefire conditions, the number of excess deaths will continue to be significant due to the time required to improve water, sanitation and shelter conditions, reduce malnutrition and restore health services.[9]

In our study, the frequency of years lived with disability was found to be very high when compared to both conflict-induced and all causes. Furthermore, the daily DALY/YLD ratio in Gaza was above both the Palestinian and global averages. The high DALY and YLD rates in this study reflect the severe and long-lasting impact of the conflict on the health of children in Gaza. A YLD rate of over 300 per 100,000 indicates not only high levels of disability, but also long-term pressure on an already fragile health system. This war has led to the formation of a generation that will continue their lives with disability, especially in the 0-14 age group. Beyond numerical estimates, these indicators underscore the importance of sustained healthcare access, rehabilitation services and long-term support for affected children. Addressing these broader implications

is essential for a proper understanding of the public health burden of war. In addition to physical trauma, psychological trauma is another important reason that will increase this burden. It reveals how important rehabilitation is in the post-war health system. This situation draws attention to the potential burden that health problems arising from post-war disability may create on the health system.

The findings of this study demonstrate that the impact of conflict on children is far-reaching and significant. A study on the health burden and attributable economic damage of conflict and terrorism in the region indicates that from 1990 to 2019, death rates among children under five increased by 337%, and among those aged 5-14 by 35.7% due to conflict. This increase has been compounded by a number of additional factors, including a lack of adequate food and clean water, failures in healthcare services, and the collapse of health facilities, which have resulted in an additional burden on surviving children.[28] While this study provides a direct measurement of the disease burden attributable to conflict, it is important to note that the indirect health consequences are likely to be even greater. In addition to mortality and injury, indirect effects such as psychological trauma, disruption in chronic disease management, and reduced access to essential health services contribute significantly to the overall public health burden. These consequences, though not quantified in the present analysis, are expected to intensify as the conflict persists and should be considered critical components of the long-term health impact, particularly among vulnerable groups such as children and individuals with pre-existing conditions.

This study has several limitations. The first one is that we were not able to make predictions for the age distribution due to the lack of accurate reporting of mortality data. The data cover only a 19-day window (October 7–26, 2023) and reflect cases reported through the Palestinian Ministry of Health from hospitals and morgues. While this source remains the primary provider of health data in the region, the ongoing conflict poses major obstacles to data completeness and accuracy, including restricted access, disruptions in reporting, and potential underreporting or duplication. To estimate the annual burden, we applied a cautious extrapolation approach based on patterns from previous conflicts and available surveillance data. Although necessary given the urgency and lack of longer-term data, this introduces uncertainty. Variability in conflict dynamics and health service accessibility likely influence the reliability of these projections, which is why confidence intervals were included to reflect potential margins of error. This analysis focuses solely on the burden related to conflict and terrorism, excluding other health issues such as non-communicable diseases that may be indirectly affected by the war.

Despite these constraints, the findings provide an important early assessment of the conflict's health impact and underscore the need for robust data infrastructure and international health monitoring in crisis settings. Second, only the conflict and terrorism cause was calculated. The impact of death and injury from other causes (e.g., non-communicable diseases and chronic diseases) on the burden of disease is not included in the calculation.

Despite these limitations, this analysis provides valuable insights into the immediate impact of the conflict. More effective measures should be taken at national and international levels to reduce the devastating effects of violence, such as conflict and terrorism, on children's health, and the ongoing war in Gaza should end as soon as possible.

Ethical approval

The study was conducted in accordance with the ethical standards of the Helsinki Declaration. Ethical approval and informed consent were not required for this study as it was based on secondary analysis of publicly available, aggregated data that did not contain any personal identifiers.

Author contribution

The author confirm contribution to the paper as follows: Study conception and design: MEG; data collection: MEG; analysis and interpretation of results: MEG; draft manuscript preparation: MEG. The author 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

Clinical spectrum of pediatric neutropenia: mostly benign, but not to be overlooked

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ABSTRACT

Background. Neutropenia is a common laboratory finding in children, therefore it is a common referral reason to pediatric hematology units. This study hypothesizes that most neutropenic children do not require pediatric hematology consultation, and that key clinical indicators can guide the need for referral.

Methods. Medical records of 180 patients who were admitted to a tertiary reference center, were evaluated in terms of demographical data, physical examination findings, laboratory findings, and outcome measures. The patients enrolled in the study had newly diagnosed or incidental neutropenia and did not meet the criteria for chronic neutropenia. Neutropenia was classified based on absolute neutrophil count (ANC) as follows: mild (1000–1499/mm^3), moderate (500–999/mm^3), severe (200–499/mm^3), and very severe (<200/mm^3).

Results. Of the 180 patients enrolled, 51.7% were male, with a mean age of 4.8 years (min-max: 1 week- 17 years). 12 patients (6.7%) were diagnosed with congenital neutropenia. The median age for patients diagnosed with congenital neutropenia was 12 months, whereas it was 47 months for those with post-infectious neutropenia (p=0.037). 64.4% of patients had no known prior disease, and 45% were incidentally found to have neutropenia. The average ANC was 732/mm^3, with 26.1% having mild, 47.2% moderate, 19.4% severe, and 7.2% very severe neutropenia. Etiological causes included post-infectious (53.9%), idiopathic/immune (25.6%), congenital (6.7%), and drug-related (6.7%) neutropenia. The median ANC for congenital neutropenia patients was 200/mm^3, and their infection rates were significantly higher than the other groups (p=0.001). The mean follow-up period was 10 months, with 69.4% of patients having normal ANC at the last follow-up.

Conclusions. Despite the increased frequency of neutropenia in childhood, a vast majority of the cases have a benign and transient clinical course.

Key words: pediatric neutropenia, congenital neutropenia, immune neutropenia, post-infectious neutropenia.

Neutropenia is one of the most common causes of referral to pediatric hematology outpatient clinics, defined as a decrease in the number of neutrophils in the peripheral blood. Although the normal values of absolute neutrophil count (ANC) vary according to age and race, the lower limit is considered to be 5000/mm^3 during the first 24 hours of life, 2500/mm^3 for term/near-term neonates 72-240 hours after delivery, 1000/mm^3 for preterm infants, and then 1000/mm^3 during the first year of life. After the first year of life, the lower limit for ANC is regarded as 1500/mm^3.[1-4] The underlying etiology can range from mild to life-threatening conditions. In the clinical evaluation, the presence of a serious underlying bone marrow-related disease and whether the patient has an increased risk of life-threatening infection due to neutropenia are important considerations. Neutropenia can lead to a serious course in immunocompromised

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patients with oncological diagnoses. In contrast, milder and transient conditions may play a role in the etiology of immunocompetent individuals.[3]

Depending whether it lasts shorter or longer than 3 months, neutropenia can be classified as acute or chronic. The etiologic causes of neutropenia can be divided into two main groups; acquired and congenital. Acquired neutropenia is more common than congenital neutropenia.[4] The most common underlying causes are infections, drugs and chronic benign neutropenia. The most common cause of chronic neutropenia in childhood is chronic benign neutropenia and the annual incidence is reported as 1/100,000. In this type of neutropenia, spontaneous remission is observed in almost all patients, within a median duration of 20 months. In addition, nutritional causes, metabolic and immunologic disorders can be listed as other causes of acquired neutropenia. On the other hand, congenital neutropenia represents a heterogeneous group of diseases and is characterized by moderate or severe neutropenia observed in intermittent episodes or persistently. Although rare, it can progress with recurrent infections and life-threatening conditions. The inheritance is autosomal recessive in the vast majority of cases.[5,6]

In the current cohort, we aimed to evaluate the patients with neutropenia who were admitted to our unit, which is a tertiary reference center. Our hypothesis was that the majority of patients referred to pediatric hematology outpatient clinics have benign and reversible causes of neutropenia, which will resolve without the need for follow-up and treatment by a pediatric hematologist.

Materials and Methods

In our study, the medical records of 180 patients who were referred to our clinic between January 2011 and December 2021, a 10-year period, due to neutropenia were assessed. It should be noted that the patients included in this study were referred upon first detection of neutropenia and had not yet fulfilled the diagnostic criteria for chronic neutropenia (defined as neutropenia persisting for more than 3 months). Age, gender, primary complaint and physical examination findings, personal and family histories, laboratory findings on admission and clinical follow-up, infections during follow-up, hospitalizations, drug use, diagnostic tests, diagnoses, whether there is a recovery in neutrophil counts, time of recovery and follow-up results were evaluated.

Neutropenia was classified based on ANC as follows: mild (1000–1499/mm^3), moderate (500–999/mm^3), severe (200–499/mm^3), and very severe (<200/mm^3).[1]Transient neutropenia accompanied by symptoms of infections and/or positive for infectious tests, in the absence of other underlying causes, was defined as infectious-related neutropenia. Immune-mediated neutropenia was also diagnosed with increased mature neutrophils in bone marrow aspiration (BMA) in patients with no accompanying signs and symptoms.

The current study was approved by the Dokuz Eylül University Ethics Committee (ethical approval number: 2022/04-04) and performed in accordance with the principles of Helsinki Declaration.

Statistical analysis

In the present study, clinical results were evaluated using descriptive statistical methods. The quantitative characteristics of the patients are shown with numbers (n) and frequencies (%) in the text and tables. Comparative evaluations between independent groups in terms of hospitalization, need for treatment and frequency of infection were assessed with non-parametric tests. Normal distribution of data was evaluated using the Kolmogorov–Smirnov test. Descriptive statistics; number and percentage for categorical variables; for numerical variables, data that provided normal distribution parameters were given as mean±standard deviation, and for data that

did not comply with normal distribution, they were given as median (minimum-maximum value). Comparison of data was made with Student's t test for variables with normal distribution, and with Mann-Whitney U test for those with non-normal distribution. Pearson chi-square (χ^2) test was used to compare categorical data. The relationship between the parameters was investigated with the Pearson or Spearman correlation test. Factors shown to be effective on the dependent variable were tested with multiple logistic regression analysis to reveal estimated risk ratios. In cases where the dependent variable did not show a normal distribution between groups, the non-parametric test, Kruskal-Wallis test, was applied. Statistical analyses were performed using Statistical Package for Social Sciences (SPSS 22, Chicago, IL, USA).

Results

Of the 180 patients, 87 (48.3%) were female and 93 (51.7%) were male. The mean age at admission was 4.8±4.87 years and the median age was 2.8 years (range: 1 week- 17 years). The median age of the patients diagnosed with congenital neutropenia was 12 months (range: 1 week- 150 months). On the contrary, the median age of the patients with post-infectious neutropenia was 47 months (range 1 month-214 months). The difference between the two groups was statistically significant with a p value of 0.037.

On admission, 116 patients (64.4%) had no known disease before. Eighty-one patients (45%) were referred with incidentally detected neutropenia and had no complaints on admission. The remaining patients were assessed for acute infectious processes and had findings such as a runny nose, fever, and cough. No pathological features were found on physical examination in 83.9% (n=151) of the patients at admission. Only 16 patients (8.8%) had neutropenia before admission. Among these patients, the median first time of neutropenia detection was observed as 3 months (range: 1 month-16 months). Some of these patients were evaluated for etiology in other centers. Of the physicians who referred patients to our unit, 87.8% (n=158) were pediatricians.

The mean ANC was 732±372/mm^3 and the median was 700/mm^3 (range: 100-1400/mm^3) on admission. Considering the ANC categories, 47 patients (26.1%) were in the mild neutropenia group, 85 patients (47.2%) in the moderate, 35 patients (19.4%) in the severe, and only 13 patients (7.2%) were in the very severe neutropenia group. Whole blood count findings and clinical characteristics on admission are presented in Table I. Lymphocytosis was observed in 40% (n=72) of patients on peripheral blood smear. Evaluating the monocyte and eosinophil counts, the mean absolute monocyte count (AMC) was 1265±174/mm^3 for congenital neutropenia patients (n=12, 6.7%), whereas it was 534±336/mm^3 for the others (n=168, 93.3%). The congenital neutropenia group had a mean absolute eosinophil count (AEC) of 652±238/mm^3 whilst the remaining had a mean AEC of 218±326/mm^3. Although an apparent difference was observed, statistical analysis could not be

Table I. Clinical characteristics and laboratory results on admission.

Female gender, n (%)	87 (48.3%)
Age (years)	4.8±4.87
Complaints on admission, n (%)	
No complaints	81 (45%)
Fever	61 (33.8%)
Viral infection	38 (21.1%)
Hemoglobin (g/dL)	11.5 ± 1.39
White blood cell count (/mm^3)	4,970 ± 2,466
Absolute neutrophil count (/mm^3)	732 ± 372
Lymphocyte count (/mm^3)	3,466 ± 2,260
Monocyte count (/mm^3)	583 ± 473
Platelet count (/mm^3)	285,000 ± 139.000
Severity of neutropenia, n (%)	
Very severe (ANC <200/mm^3)	13 (7.2%)
Severe (ANC 200-499/mm^3)	35 (19.4%)
Moderate (ANC 500-999/mm^3)	85 (47.2%)
Mild (ANC 1000-1499/mm^3)	47 (26.1%)

ANC: Absolute neutrophil count.

performed due to the limited sample size of the congenital neutropenia group.

Viral serology tests were obtained in 58.3% (n: 105) of the patients, and was negative in 73.3% (77) of them. Eighteen patients (64.2%) were positive for Epstein-Barr virus, while 3 were positive for cytomegalovirus, 2 for rubella, 1 for toxoplasmosis, 1 for varicella zoster, 2 for rhinovirus and 1 for influenza type B.

BMA was obtained in 60 (33.3%) of 180 patients, revealing an increased number of mature neutrophils in the marrow in 46 patients (25.6%), which supported the diagnosis of immune neutropenia. Of the remaining, 12 (6.7%) disclosed maturation arrest in the myeloid lineage, giving rise to the diagnosis of congenital neutropenia, and 2 were normocellular.

Of the patients, 53.9% (n=97) were diagnosed with post-infectious, 25.6% (n=46) with idiopathic/immune, and only 6.7% (n=12) with congenital neutropenia. In addition, drug-related neutropenia was observed in 12 patients (6.7%), and vitamin B12 deficiency-related neutropenia was observed in 5 patients (2.7%). Underlying rheumatological, genetic, and metabolic diseases such as celiac disease, Tay-Sachs disease, glycogen storage disease type 1b and IgA vasculitis (Henoch-Schönlein purpura) were detected in 4.4% (n=8) of the patients. None of the patients were diagnosed with a malignancy. A detailed summary of etiological causes is present in Table II.

Patients with the diagnosis of congenital neutropenia (n=12) had a median ANC of 200/mm^3 (100-800/mm^3) on admission, whereas the median ANC values for patients diagnosed with post-infectious and immune neutropenia were 800/mm^3 (100-1400/mm^3) and 750/mm^3 (100-1400/mm^3), respectively. This difference was statistically significant ($p<0.001$). Regarding the complaints of the congenital neutropenia group, recurrent fever, skin, gastrointestinal and pulmonary infections were detected. The number of infections (median: 4) of the patients diagnosed with congenital neutropenia was found to be significantly higher than the other groups (p:0.001) in the Kruskal-Wallis analyses. The confirmed diagnoses of congenital neutropenia included 1 patient each of Shwachman-Diamond syndrome, Kostmann syndrome, ELANE-related neutropenia, and G6PC3-related neutropenia. The remaining patients, despite negative results on the genetic panel used in our center, were diagnosed with congenital neutropenia based on decreased myeloid production observed in BMA, increased susceptibility to infections, and the presence of definitive infectious foci. All patients diagnosed with congenital neutropenia received granulocyte colony stimulating factor (G-CSF) therapy.

Table II. Etiological causes of neutropenia in our patients.

Etiological cause	n (%)
Infections	97 (53.9%)
EBV	18 (18.5%)
CMV	3 (3.09%)
Rubella	2 (2.06%)
Toxoplasmosis	1 (1.03%)
Varicella zoster	1 (1.03%)
Rhinovirus	2 (2.06%)
Influenza type B	1 (1.03%)
Undetermined	69 (71.1%)
Idiopathic/immune	46 (25.6%)
Congenital neutropenia	12 (6.7%)
Drug-related	12 (6.7%)
Vitamin B12 deficiency-related	5 (2.7%)
Other causes	8 (4.4%)
Celiac disease	2 (25%)
Tay-Sachs disease	2 (25%)
Glycogen storage disease type 1b	2 (25%)
IgA vasculitis (HSP)	2 (25%)

CMV: Cytomegalovirus, EBV: Epstein-Barr virüs, HSP: Henoch-Schönlein purpura.

The mean follow-up period was 10 ±14 months. Ninety-three patients (51.7%) did not need hematological follow-up. The median duration of neutropenia in these patients was 2 months (range: 1 week- 15 months). Twenty-two patients

(12.2%) are still being followed up. At the last follow-up, ANC was within normal limits in 69.4% (n=125) of the patients. Seventy-nine (63.2%) of these patients had post-infectious and 26 (20.8%) had with immune neutropenia.

Discussion

Neutrophils are produced from multipotent myeloid stem cells through granulopoiesis. They play a role in the immune response against mainly bacteria, and also contribute to the innate immunity.[1,7,8] Neutropenia is a common laboratory finding in children and also a common reason for referral to pediatric hematology units. Distinguishing between benign and severe pathological mechanisms of neutropenia is essential for protecting patients from infections, assessing their risk of malignant transformation, and guiding the implementation of appropriate supportive care and curative strategies.[1-5]

In clinical practice, neutropenia is usually grouped as mild, moderate, severe, and very severe, as in our study. It should be noted that this classification system is used in the non-infant period, since neutrophil normal values are different in the first year of life.[1,2] In addition to numerical grading of the depth of neutropenia, the age at which neutropenia arose and the underlying pathophysiology should be evaluated and thus diagnostic tests should be planned with this holistic approach.[4,6,9]

In the current study, the median age of all the patients enrolled in study was 4.8 years, whereas the median age of patients diagnosed with congenital neutropenia was 12 months. Consistent with the literature, congenital neutropenia cases are usually diagnosed in the early infancy period.[10] However, studies mentioning an older diagnostic age have also been reported.[5,7,11]

A family history of benign neutropenia may point to familial benign neutropenia. In addition, a consanguineous marriage may be a sign of congenital neutropenia or neutropenia due to a metabolic disorder.[12,13] Signs and symptoms on admission have a great value in directing the diagnostic process. Although a vast majority of pediatric patients presenting with neutropenia have transient and post-infectious neutropenia, a thorough patient and family history, along with a comprehensive physical examination, should be performed. In the present study, most of the patients had no complaints before and neutropenia was determined incidentally. Repetitive fever, oral-mucosal infections, unexpected skin lesions, recurrent pulmonary and serious infections that result in hospitalization are common signs for congenital neutropenias.[14] Similarly, in the present study, patients with congenital neutropenia were admitted with the signs and symptoms of serious infections.

Most of the underlying causes of neutropenia are acquired, benign, and transient. Congenital neutropenias and malignant etiologies are much less common.[6,9,14] In our study, similar to the literature, only 6.7% (n=12) of the patients were diagnosed with congenital neutropenia, whereas the remaining were diagnosed with secondary neutropenia, consisting of post-infectious, immune, drug-related, vitamin B12 deficiency and other rare causes of neutropenia. Furthermore, none of them were diagnosed with a malignancy. The findings of this study are consistent with the existing literature. It is important to emphasize that severe congenital neutropenia syndromes carry a significant risk of malignant transformation, with reported frequencies as high as 22%. Therefore, annual BMA should be undertaken in follow-up.[4,15-17] In patients with congenital neutropenia, maturation arrest of myeloid cells should be seen and dysplastic changes should be checked for myelodysplastic syndrome. Although not statistically proven, AMC and AEC were observed to be increased in congenital neutropenia patients regarding the enhanced phagocytic activity of these cell lines in the chronic decline of neutrophils. Anemia and thrombocytopenia were not observed in the majority of patients. Physical examination

findings such as lymphadenopathy, hepatomegaly, or splenomegaly were recorded in only a minority of cases, and none of the patients were diagnosed with hematologic malignancy. These findings suggest that in the absence of additional hematologic abnormalities or abnormal physical exam findings, neutropenia is more likely to be benign and transient.

As demonstrated in our study, infectious agents are the most common cause of transient neutropenia, typically presenting within the first week and resolving by the third week of illness. The mechanisms underlying post-infectious neutropenia are regarded as a decrease in production and an increase in demolition. This form of neutropenia is benign and frequently the agent is a virus, however, other bacterial or fungal agents can also develop neutropenia.[18,19] In our study, the most common cause of infections were viruses. The second most common cause was immune neutropenia. Although anti-neutrophil antibodies could not be assessed, the BMA of the patients were compatible with immune neutropenia, demonstrating an increased number of mature neutrophils. The clinical course of these patients were mild and reversible as reported in the literature.[20]

Regarding the other etiological causes, drug-related neutropenia was observed in 6.7% of our patients. The frequency is approximately 10% for childhood.[2,21] Almost all drugs can cause neutropenia, antimicrobials, anticonvulsants, antipsychotics and antipyretics being the most common ones. In our study, antiepileptics were responsible for 75% (n:9/12) of the cases. The primary mechanism is more complicated, consisting of both bone marrow toxicity and immune mediated mechanisms.[22,23] In general, these patients do not need any supportive care or treatment, owing to the resolution of neutropenia upon the withdrawal of the drug, similar to our study.[24,25]

Another important cause of neutropenia in childhood is nutritional deficiencies, especially vitamin B12, copper and folic acid deficiencies. The main mechanism is ineffective myelopoiesis and neutropenia alleviates after supplementation with the deficient nutrient.[2,26] Among our patients, 2.7% (n=5) had vitamin B12 deficiency-related neutropenia and their clinical course was benign. Also, there are several metabolic causes of neutropenia in childhood, including glycogen storage diseases, organic acidemias, and Shwachman-Diamond syndrome.[27,28] These conditions may require specialized management and follow-up due to their chronic nature and potential for severe complications, especially in regions where consanguineous marriage is frequent.

This study has several limitations. First, it was a retrospective single-center study, which may limit the generalizability of the findings to other populations or clinical settings. Second, anti-neutrophil antibody testing, which is important for confirming autoimmune neutropenia, could not be performed due to technical limitations. Third, although viral serologies were obtained in more than half of the patients, molecular diagnostic tests (such as PCR panels) were not routinely used, which may have led to underdiagnosis of certain viral etiologies. Additionally, the small number of patients with congenital neutropenia limited the ability to perform comprehensive statistical analyses or draw robust conclusions regarding this subgroup. Finally, long-term outcomes were not assessed beyond the average follow-up duration, which may have led to underestimation of late-onset complications or relapses.

In conclusion, this study highlights that pediatric neutropenia often does not require long-term follow-up by pediatric hematologists. One pivotal point to emphasize is that the patients in this cohort were referred at the time of first detection of neutropenia, and did not meet the definition of chronic neutropenia. As such, the findings of this study may not be directly applicable to patients with persistent neutropenia lasting longer than 3 months,

and should be interpreted within this context. Among the 180 patients, the majority had no significant underlying conditions, and 45% were incidentally diagnosed without any symptoms. The most common etiological cause was post-infectious neutropenia, accounting for 53.9% of cases. At the last follow-up, 69.4% of patients had normal ANC levels, and only 12.2% required ongoing follow-up. Importantly, congenital neutropenia patients had higher infection rates and required more intensive management. In infants younger than one year, the presence of recurrent mucocutaneous or organ-specific infections—particularly involving the gingiva, perianal region, or skin—together with laboratory findings such as severe neutropenia (ANC <500/mm^3), monocytosis, and eosinophilia, should raise suspicion for chronic neutropenia of congenital origin. Taken together, these findings suggest that pediatric neutropenia is often benign and self-limiting, particularly in cases related to transient or post-infectious causes. However, clinicians should maintain a high index of suspicion for inherited or persistent neutropenias, especially in infants presenting with recurrent infections, severe neutropenia, or abnormal leukocyte differentials. The identification of such clinical and laboratory predictors is critical for timely referral and management by pediatric hematologists.

Ethical approval

The study was approved by the Institutional Review Board of Dokuz Eylül University (approval number: 2022/04-04).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: ŞA, HÖ; data collection: ŞA, ÖT; analysis and interpretation of results: ŞA, HÖ, ÖT, ŞY; draft manuscript preparation: ŞA, ÖT, ŞY. 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

Association of problematic internet use with health-related daily habits in adolescents: evidence from a school-based survey

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ABSTRACT

Background. With the development of technology, easier access to the internet and its excessive use have led to problematic internet use (PIU). The prevalence of PIU and its association with lifestyle behaviors in adolescents have become subjects of increasing academic interest. This study aimed to determine the prevalence of PIU among Turkish high school students and to investigate its association with sleep, physical activity and dietary habits.

Methods. This cross-sectional study was conducted on high school students between October 2019 and March 2020. Participants completed a questionnaire regarding demographic characteristics, internet use, lifestyle habits and the "Young Internet Addiction Test-Short Form (YIAT-SF)".

Results. Among the total 951 participants, the mean age was 15.3±1.0 years, 42.3% were female, and the prevalence of PIU was determined as 12.1%. It was shown that having daily internet usage time ≥2 hours on weekdays, having at least one type of sleep problem, having breakfast less than 3 days per week, eating salty snacks ≥3 days per week, consuming sugary-carbonated drinks ≥3 days per week were associated with PIU.

Conclusions. PIU is a widespread public health problem that is negatively associated with the daily health habits of adolescents. There is a need for nationwide school screening programs for this problem and rehabilitation of adolescents diagnosed with PIU.

Key words: problematic internet use, internet addiction, sleep, dietary habits.

"Problematic Internet Use (PIU)" is characterized as the individual's inability to control internet use, resulting in feelings of distress and functional impairment in daily activities.[1] It has also been reported that adolescents are the most at-risk group for PIU. This may be because adolescents are more prone to risky behaviors and resort to addictive applications to cope with anxiety, disappointment, and failure, to satisfy the need for excitement or the feeling of invincibility, or because the internet offers adolescents the opportunity to distance themselves from their own identities and feel free.[2]

Studies reporting the prevalence of PIU have shown marked differences around the world.[3] According to the results of a recent meta-analysis, the global prevalence of PIU exhibits substantial variation across geographical regions and

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demonstrates a progressive increase over time. The highest prevalence rates were reported in the World Health Organization African Region (34.53%) and Eastern Mediterranean Region (30.11%), whereas the lowest rates were observed in the Americas Region (11.06%) and the European Region (11.06%). In the same meta-analysis, the prevalence of PIU among adolescents was reported to range from 10.60% to 13.70%.[4]

As the prevalence of PIU has increased, its effect on health-related behaviors has become more prominent.[5] Kim et al.[6] demonstrated an association between PIU and negative dietary behaviors such as skipping meals, snacking, inadequate fruit and vegetable intakes, consumption of carbonated soft drinks and fast food and negative lifestyle habits such as decreased physical activity. Koças et al.[7] reported that internet addiction was associated with worsening sleep quality among high school students. Previous studies also reported lower rates of PIU among those who engaged in more physical activity and found an association between PIU and poor physical health.[8,9]

The number of studies simultaneously investigating the relationship between sleep patterns, physical activity, and nutritional habits in conjunction with PIU remains limited. The present study aimed to assess the associations between these three health-related daily behaviors and PIU, as well as to determine the prevalence of PIU among high school students residing in the Inner Aegean Region of Türkiye.

Materials and Methods

Study design

This cross-sectional study was conducted in high schools between October 01, 2019 and March 13, 2020. Permission to carry out the study was approved by the local ethics committee. All study procedures were performed in accordance with the Declaration of Helsinki.

Sample size and selection

The minimum sample size was calculated as 886 with a 99.9% confidence interval (CI) using "OpenEpi (https://www.openepi.com/SampleSize/SSPropor.htm) calculator" according to $n = [DEFF*Np(1-p)]/ [(d^2/Z^2_{1-\alpha/2}*(N-1)+p*(1-p)]$ equation. Among the parameters, N (Total number of high school students living in Afyonkarahisar province) was accepted as 13862; p as 12±5% (previously reported percentage frequency of internet addiction risk among high school students);[10] d (confidence limit) as 1%; DEFF (design effect) as 2 and $Z^2_{1-\alpha/2}$ as 1.96. Taking into account the 20% non-participation margin, a total of 1051 students were planned to be included in the study. After determining the schools with different socioeconomic levels in cooperation with the Research and Development unit of the National Education Directorate, the schools were selected using the cluster sampling method. A total of 6 schools were randomly identified: one private school with a high socioeconomic level, two schools with a medium socioeconomic level, and three schools with a low socioeconomic level. In order to reach 175 students from each school, two classes from the 9th, 10th, 11th and 12th grades were selected. Students who agreed to participate in the research after obtaining parental and student consent, were included in the study. Students whose parents or themselves did not agree to participate in the research, those without internet access and those who had a history of chronic mental illness (such as obsessive-compulsive disease, anxiety disorder) or physical illness (such as diabetes, cardiac diseases) that could affect daily health-related habits such as diatary habits and physical activity were excluded from the study.

Data collection

After explaining the purpose and procedure of the research, written consent forms for adolescents and parents were distributed to students who verbally agreed to participate. The next day, the students, whose consent was

obtained, were asked to fill out the survey form and a scale to test PIU.

Measures

Questions regarding internet usage habits and lifestyle habits were prepared based on previous studies[6,7,11] and the lifetime status of habits was questioned in terms of their presence/absence or frequency. The following questions were asked in the questionnaire: (i) Demographic characteristics of students and their families (age, sex, residence, education level of parents). The age variable was evaluated as under or over 15 years, and a distinction was made between 9th-10th grades and 11th-12th grades. (ii) Internet usage characteristics (year of internet use, average daily internet usage time on weekdays and weekends, presence or absence of usage of internet cafe) (iii) Sleep characteristics (daily sleep duration; according to the recommendations of the US National Sleep Foundation, the short sleep duration limit was considered to be <8 hours[12] and presence or absence of sleep problems; difficulty falling asleep, frequent interruption of sleep, difficulty waking up in the morning, feeling sleepy in the morning) (iv) Using a mobile phone other than an alarm for the first time after waking up in the morning (v) Regular sports habit (regularly participating in licensed or unlicensed individual or team sports) and regular physical activity status (exercise for at least one hour a day, on certain days of the week), and (vi) Nutritional characteristics (how many days per week they skipped breakfast, skipped meals, consumed salty or sugary snacks, consumed sugary sodas and, whether they consumed caffeinated beverages and energy drinks). The adequacy of the questions was checked with a preliminary study with 20 high school students and the questionnaire was given its final form.

In our research, Youth Internet Addiction Test-Short Form (YIAT-SF) was used to test PIU. The original form of the "Internet Addiction Test (IAT)" was developed by Young[13] converted into a short form by Pawlikowski et al.[14] and adapted into Turkish by Kutlu et al.[15] YIAT-SF consists of 12 items and is a five-point Likert (1 = Never, to 5 = Very often) type scale. The Turkish version of the YIAT-SF has been previously shown to be reliable and valid for adolescents.[15] There is no reverse scored items in the scale. High scores from the scale indicate a high level of internet addiction. To calculate the frequency of internet addiction among participants, the cutoff score was accepted as 36, as recommended in the literature.[14,16] Scores of 36 and above indicated that the students had PIU.

Statistical analysis

IBM-SPSS 26.0 (SPSS Inc., Chicago, IL, USA) program was used for statistical analysis. Normal distribution of the data was evaluated with the Shapiro-Wilk test. Categorical variables were expressed as n (%) and normally distributed data from continuous variables were expressed as mean ± standard deviation. All variables were first tested with bivariate analysis. Participants were analyzed in two groups according to the presence or absence of PIU. Chi-square test or Fisher's exact test was used to compare the percentage distributions of categorical data between groups. Then multiple logistic regression analysis was performed to test the relations of the risk of PIU with internet and health variables by adjusting for age, sex, maternal education status, paternal education status and residence. Variables with a p value <0.05 in the univariate analyses were included in the multiple logistic regression backward step model. Multiple logistic regression results are reported as adjusted odds ratios (AOR) with 95% CI. A $p<0.05$ level was considered significant.

Results

A total of 1051 students participated in the study. Data from a total of 951 participants were analyzed by excluding students with chronic diseases (n=32) and incompletely filled out questionnaires (n=68). The mean age of the students was 15.3±1.0 years. Of the students, 42.3% (n=402) were female. The prevalence of PIU

among participants was determined as 12.1%. Table I shows the distribution of participants' demographic and internet usage characteristics according to PIU status. The proportion of more than >5 years of internet use (p=0.001), daily internet usage time more than 2 hours per week on weekdays (p<0.001) and weekends (p<0.001), use of internet cafes (p=0.015) were significantly higher in participants with PIU.

Table II shows the comparison of participants' health-related daily habits with PIU. The frequency of having at least one type of sleep problem (p<0.001), use of a mobile phone other than an alarm within the first hour after waking up (p<0.001), skipping breakfast (p<0.001) or other daily meals (p<0.001), eating sugary snacks (p=0.004) and salty snacks (p<0.001), consuming sugary-carbonated drinks (p<0.001), caffeinated drinks (p=0.015), and energy drinks (p=0.001) were significantly higher in participants with PIU.

By adjusting the age, sex, maternal education, paternal education and residence, risk of PIU presence according to internet-related (Year of internet use >5 vs. ≤5 years, daily internet usage time [weekdays] ≥2 vs. <2 hours, daily internet usage time [weekend] ≥2 vs. <2 hours, usage of internet cafe) and health-related variables (having at least one type of sleep problem, time of mobile phone use after waking up in the morning ≤1 vs. >1 hour, having breakfast <3 vs. ≥3 days/week, skipping daily meals ≥3 vs. <3 days/week, eating sugary snacks ≥3 vs. <3 days/week, eating salty snacks ≥3 vs. <3 days/week, consuming sugary-carbonated drinks ≥3 vs. <3 days/week), consuming caffeinated drinks, consuming energy drinks) was tested with multiple logistic regression analysis with

Table I. Distribution of participants' demographic and internet-related variables according to the presence of problematic internet use (N=951)

Variables		Total, n (%)*	PIU present, n (%)	PIU absent, n (%)*	P value (χ^2 test)
Age	<15 years	548 (57.6)	69 (60.0)	479 (57.3)	0.58
	≥15 years	403 (42.4)	46 (40.0)	357 (42.7)	
Sex	Female	402 (42.3)	54 (47.0)	348 (41.6)	0.28
	Male	549 (57.7)	61 (53.0)	488 (58.4)	
Maternal education	< high school	529 (55.6)	60 (52.2)	469 (56.1)	0.43
	≥ high school	422 (44.4)	55 (47.8)	367 (43.9)	
Paternal education	< high school	331 (34.8)	38 (33.0)	293 (35.0)	0.67
	≥ high school	620 (65.2)	77 (67.0)	543 (65.0)	
Residence	Urban	789 (83)	97 (84.3)	692 (82.8)	0.67
	Rural	162 (17)	18 (15.7)	144 (17.2)	
Year of internet use	≤5 years	531 (55.8)	48 (41.7)	483 (57.8)	<0.01
	>5 years	420 (44.2)	67 (58.3)	353 (42.2)	
Daily internet usage time (weekdays)	<2 hours	539 (56.7)	29 (25.2)	510 (61.0)	<0.001
	≥2 hours	412 (43.3)	86 (74.8)	326 (39.0)	
Daily internet usage time (weekend)	<2 hours	293 (30.8)	18 (15.7)	275 (32.9)	<0.001
	≥2 hours	658 (69.2)	97 (84.3)	561 (67.1)	
Usage of internet cafe	Yes	34 (3.6)	9 (7.8)	25 (3.0)	0.02**
	No	917 (96.4)	106 (92.2)	811 (97.0)	
YIAT-SF score	Non-PIU (<36)	836 (87.9)	-	-	
	PIU (≥36)	115 (12.1)	-	-	

PIU: Problematic internet use, YIAT-SF: Young Internet Addiction Test - Short Form
*Column percentage
**Fisher's exact test

backward stepwise model (Table III). Having daily internet usage time ≥2 hours on weekdays (adjusted odds ratio [AOR]: 3.45, 95% CI: 2.17-5.51), having at least one type of sleep problem (AOR: 2.04, 95% CI: 1.29-3.25), having breakfast less than 3 days per week (AOR: 2.22, 95% CI: 1.45-3.40), eating salty snacks ≥3 days per week (AOR: 1.98, 95% CI: 1.27-3.10), consuming sugary-carbonated drinks ≥3 days per week (AOR: 1.70, 95% CI: 1.06-2.61) were associated with PIU.

Discussion

This study showed that the prevalence of PIU among high school students is 12.1%. This is consistent with the worldwide prevalence of PIU for adolescents ranging from 10.60% to 13.70% reported by Meng et al.[4] However, it is slightly below the prevalence reported as 21.1% in the Black Sea region and 18.5% in the Marmara region in Türkiye.[8,9] The difference in the prevalence of PIU among high school students living in different geographical

Table II. Comparison of participants' health-related variables according to the presence of problematic internet use (N=951)

Variables		Total, n (%)*	PIU present, n (%)	PIU absent, n (%)*	P value (χ^2 test)
Daily sleep time	< 8 hours	787 (82.8)	93 (80.9)	694 (83.0)	0.57
	≥ 8 hours	164 (17.2)	22 (19.1)	142 (17.0)	
Having at least one type of sleep problem[a]	Yes	498 (52.4)	84 (73.0)	414 (49.5)	<0.001
	No	453 (47.6)	31 (27.0)	422 (50.5)	
Time of mobile phone use after waking up in the morning[b]	≤1 hour	623 (65.5)	93 (80.9)	530 (63.4)	<0.001
	>1hour	328 (34.5)	22 (19.1)	306 (36.6)	
Doing sports regularly	Yes	332 (34.9)	34 (29.6)	298 (35.6)	0.20
	No	619 (65.1)	81 (70.4)	538 (64.4)	
Regular physical activity status	Yes	556 (58.5)	65 (56.5)	491 (58.7)	0.65
	No	395 (41.5)	50 (43.5)	345 (41.3)	
Having breakfast	<3 day/week	306 (32.2)	61 (53.0)	245 (29.3)	<0.001
	≥3 day/week	645 (67.8)	54 (47.0)	591(70.7)	
Skipping daily meals	<3 day/week	810 (85.2)	86 (74.8)	724 (86.6)	<0.01
	≥3 day/week	141 (14.8)	29 (25.2)	112 (13.4)	
Eating sugary snacks	<3 day/week	493 (51.8)	45 (39.1)	448 (53.6)	<0.01
	≥3 day/week	458 (48.2)	70 (60.9)	388 (46.4)	
Eating salty snacks	<3 day/week	679 (71.3)	56 (48.7)	623 (74.5)	<0.001
	≥3 day/week	272 (28.7)	59 (51.3)	213 (25.5)	
Consuming sugary-carbonated drinks	<3 day/week	705 (74.1)	61 (53.0)	644 (77.0)	<0.001
	≥3 day/week	246 (25.9)	54 (47.0)	192 (23.0)	
Consuming caffeinated drinks	Yes	429 (45.1)	64 (55.7)	365 (43.7)	0.02
	No	522 (54.9)	51 (44.3)	471 (56.3)	
Consuming energy drinks	Yes	76 (8.0)	18 (15.7)	58 (6.9)	<0.01
	No	875 (92.0)	97 (84.3)	778 (93.1)	

PIU: Problematic internet use
*Column percentage
[a] Type of sleep problems are difficulty falling asleep, frequent interruption of sleep, difficulty waking up in the morning, feeling sleepy in the morning
[b] Time of using a mobile phone other than an alarm for the first time after waking up in the morning

Table III. Risk of problematic internet use presence according to internet-related and health-related variables, multiple logistic regression*

Variables	AOR	95% CI	p
Daily internet usage time (weekdays) (≥2 vs. <2 hours)	3.45	2.17-5.51	<0.001
Having at least one type of sleep problem[a] (Yes vs. No)	2.04	1.29-3.25	0.002
Having breakfast (<3 vs. ≥3 days/week)	2.22	1.45-3.40	<0.001
Eating salty snacks (≥3 vs. <3 days/week)	1.98	1.27-3.10	0.002
Consuming sugary-carbonated drinks (≥3 vs. <3 days/week)	1.70	1.06-2.61	0.027

AOR: adjusted odds ratio, CI: confidence interval
*Controlled for age, sex, maternal education, paternal education and residence
[a]Type of sleep problems are difficulty falling asleep, frequent interruption of sleep, difficulty waking up in the morning, feeling sleepy in the morning

regions in Türkiye may be due to the variability in the ability of families with different sociocultural norms to cope with excessive use of the internet. It may also be due to the use of different scales to detect PIU in these studies. However, Burkauskas et al.[17] reported that although the same instrument (IAT) and cutoff score (≥50) were used, the prevalence of PIU differed even in studies from the same country or region. Regional differences in prevalence can be assessed more clearly with a national, multicenter, longitudinal study using more robust measurement tools that test the diagnosis of internet addiction.

In our study, we found that the frequency of PIU did not change with age and sex. In previous studies, findings regarding the association between age, sex and PIU have varied. Some recent studies have reported that older high school students are more likely to have PIU.[3,18] However, in the study of Seyrek et al.[19] no significant association was found between internet addiction and age and sex. Khan et al.[20] found that internet addiction in medical school students did not differ between sexes. Cam et al.[8] also reported no significant correlation between PIU severity and sex. Dafour et al. showed that boys spent significantly more time online than girls, that a greater proportion of girls used social networks intensively, while boys used multiplayer online role-playing games, and that there was no significant difference between sexes in terms of internet addiction risk.[21] However, some previous studies also determined that the internet addiction rate was significantly higher in males.[3,22-24]

In this study, the ratio of PIU was found to be significantly higher in participants who used the internet for more than two hours on weekdays, but no similar finding was found for weekends. In a study conducted in South Africa in 2019, Salubi et al.[25] showed that 34.8% of the participants used the internet for more than ten hours a day, and the time spent on the internet was associated with internet addiction. In a study conducted among university students in India in 2020, Jain et al.[26] examined the association between internet addiction and the time spent on the internet per day. Internet use of two hours or more per day was found to be significantly associated with internet addiction. Şaşmaz et al.[22] also reported that internet addiction was related to the duration of daily computer use.

The present study determined that students with PIU were more likely to have at least one type of sleep problem such as difficulty falling asleep, frequent interruption of sleep, difficulty waking up in the morning, and feeling sleepy in the morning, but it was shown that the frequency of PIU did not change with sleep duration. Kojima et al.[5] examined the association between health-related behaviors and internet use in Japanese adolescents and found a significant association between PIU and sleeping after midnight. Park et al.[27] investigated the association between depressive

symptoms and PIU and sleep problems and reported that sleep-wake behavior problems, insomnia, and excessive daytime sleepiness increased as IAT scores increased in the non-depressed group. In their study examining the association between sleep habits and problems and internet addiction in Japanese adolescents, Kawabe et al.[28] showed that night sleep time was shorter, bedtime and morning waking time were later in the addicted group. Yang et al.[29] examined PIU and accompanying sleep problems in adolescents and showed that the risk of sleep disorders increased in the presence of PIU. It is known that excessive internet use undermines the time devoted to sleep and other life habits and therefore causes sleep problems. Yang et al.[29] also referred to "Time displacement theory" to clarify the association between PIU and sleep problems, which is used to explain that internet use generally reduces the time devoted to other activities.

Findings from previous studies examining the association between physical activity and PIU vary. Khan et al.[20] showed that medical students with low physical activity had a higher frequency of internet addiction than students with regular physical activity. It was also reported that physically active students are less likely to be problematic internet users among South Korean adolescents.[30] On the other hand, Dang et al.[11] reported that physical activity had no significant association with internet addiction in Vietnamese youth and adolescents. Similar to the present study, Haripriya et al.[31] showed that there was an association between smartphone addiction and sleep quality, but not with physical activity. In our study, we did not find a relationship between PIU and students' regular sports and regular physical activity habits. This may be due to the fact that students use the internet in their remaining free time, even if they do regular sports or physical activity. It may also be due to the confounding effects of other variables that affect physical activity habits, such as family income level, suitability of school and home environment for physical activity, and excessive school workload.

This study found that poorer breakfast habits, consuming more salty snacks and sugary-carbonated drinks were associated with PIU. A recent study observed a positive correlation between PIU and disordered eating attitudes[32] and a meta-analysis study identified internet addiction as a determinant of eating disorders.[33] Gür et al.[34] found a significant association between internet addiction scores and physical behavior problems (going to bed late, skipping meals, eating in front of the computer) in secondary school students. Kojima et al.[5] also found a significant association between PIU and skipping breakfast. Kim et al.[6] found a higher frequency of skipping dinner and consuming frequent snacks in high-risk internet users. They did not show any significant difference in favorite snacks according to the level of internet addiction. Similarly, a previous study found that use of social networking sites was associated with an increased likelihood of skipping breakfast and consuming sugar-sweetened beverages and energy drinks.[35] The reasons for the association between PIU and unhealthy eating habits may be due to increased exposure to unhealthy food advertisements via the internet, easier consumption of unhealthy snacks such as sugary drinks or chips while on the internet, and missing healthy meal times because of excessive time spent on the internet. Prospective studies are needed to elucidate the mechanisms of this relationship.

A key strength of this study is the simultaneous investigation of the associations between sleep patterns, physical activity, dietary habits, and PIU within a relatively large sample. By including these variables in the same model and applying logistic regression analysis, potential confounding effects were controlled, allowing a clearer delineation of their independent relationships with PIU.

There are also some limitations in our study that should be taken into account. The first limitation is that the study was conducted in

a single province and therefore the results are not generalizable. The second limitation of this study is that we did not use validated scales to test sleep, physical or sports activity, and dietary habits. Additionally, this study only measured general internet addiction. Specific addictions that may vary by gender, such as online gaming and problematic use of social media, were not measured, which constitutes a third limitation of the study. Future studies should examine the source of the problem, such as for what purposes adolescents use the internet, what their motivations are for using the internet, and how addictive devices such as game consoles or smartphones affect the total duration of internet use.

Conclusion

In this study, we determined that PIU is common among high school students and observed that PIU is associated with some health-related life habits. Having at least one type of sleep problem, skipping breakfast, and consuming salty snacks and sugary-carbonated drinks more were found to be associated with PIU.

PIU has become a new public health problem with a high prevalence that negatively affects the daily health habits of adolescents. It would be appropriate to address the action plans to be implemented to solve this problem in two ways. First, awareness of this issue should be raised among individuals who have frequent contact with adolescents, including parents, clinicians, and teachers. Providing families with guidance on developing a family media plan and implementing bedroom screen-time recommendations, delivered through clinicians or educators, as well as conducting screenings for PIU using validated tools within school counseling services to facilitate referral to professional help, may contribute to addressing the problem. In addition, both national and international health policy developers and implementers need to show the necessary sensitivity to this issue, and the implementation of large-scale measures globally should not be delayed.

Ethical approval

The study was approved by Afyonkarahisar Health Sciences University's Clinical Research Ethics Committee (date: 14.06.2019, number: 2011-KAEK-2). Written informed consent was provided by each participant. All study procedures were performed in accordance with the Declaration of Helsinki.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: EÇ, AO; data collection: EÇ, AO; analysis and interpretation of results: EÇ, AO, AB; draft manuscript preparation: EÇ, AO, AB. 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

Mevalonate kinase deficiency in a familial Mediterranean fever endemic region: a single-center experience

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ABSTRACT

Background. We aimed to document childhood onset mevalonate kinase deficiency (MKD) and to explore treatment responses and diagnostic challenges in regions endemic to familial Mediterranean fever (FMF).

Methods. This retrospective study included patients under 18 years of age, diagnosed with MKD and followed for at least six months at the pediatric rheumatology department of Istanbul University - Cerrahpaşa Medical Faculty between 2016 and 2024.

Results. Of 33 patients, 51.5% were female, with a median age of symptom onset at 6 (2-17.3) months. Eight patients had a history of tonsillectomy, and seven exhibited an underlying exon 10 Mediterranean FeVer (*MEFV*) gene mutation. The mean diagnostic delay was 67.6 months, which was longer for those with exon 10 mutations (95.0 months) and those with a history of tonsillectomy (99.5 months). The median duration of attacks was 5 (4-7) days. The median frequency of attacks was 12 (10-24) per year. The most prevalent clinical findings observed during these attacks included malaise (87.8%), arthralgia (69.6%), abdominal pain (63.6%), cervical lymphadenopathy (63.6%), diarrhea (54.5%), and maculopapular rash (51.5%). A total of 30 patients (90.9%) identified pre-attack triggers. Among the patients evaluated, 19 (57.5%) were homozygous for V377I, and 7 (21.2%) had V377I biallelic heterozygous mutation in *MVK* gene. Cytopenia was observed in 18 patients (54.5%) during episodes, including anemia (39.3%), lymphopenia (24.2%), leukopenia (12.1%), and neutropenia (9%).

Conclusions. Patients presenting with periodic fever suggestive of FMF who exhibit atypical features should be evaluated for MKD. Further genetic testing should be performed when atypical clinical findings are present, even in those carrying pathogenic variants in exon 10 of the *MEFV* gene.

Key words: hereditary autoinflammatory diseases, familial Mediterranean fever, mevalonate kinase deficiency, tonsillectomy, periodic fever.

Recurrent fevers are characterized by inflammatory episodes, interspersed with overall well-being intervals.[1] These fevers can arise from various etiologies, including infectious, malignant, autoimmune, inflammatory, and genetic causes, which complicates diagnosis. Following the identification of the MEditerranean FeVer (*MEFV*) gene in 1997, the concept of autoinflammation was introduced to highlight a group of diseases characterized

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by abnormal activation of the innate immune system occurring independently of pathogens and without the presence of circulating autoantibodies or self-reactive cells.[2,3] Monogenic defects in innate immunity have shown that inflammasome activity and interleukin-1 (IL-1) release are compromised in rare conditions known as hereditary autoinflammatory diseases (HAIDs). They are characterized by recurrent inflammation affecting the skin, joints, gastrointestinal tract, central nervous system, and other tissues, with interleukin (IL)-1β as the primary mediator of inflammation.[4] The understanding of HAIDs continues to expand with advancements in sequencing technology, and many recent developments have blurred the lines between autoimmunity, immunodeficiency, and autoinflammation.[2]

Familial Mediterranean fever (FMF) and mevalonate kinase deficiency (MKD) are the two most common HAIDs, sharing overlapping features such as recurrent fever, abdominal pain, arthralgia, and arthritis.[3,5] FMF is particularly prevalent in high-risk populations—including Armenians, Sephardic Jews, Turks, and Arabs—where *MEFV* mutation carrier frequencies range from 1 in 5 to 1 in 7, defining these regions as endemic.[4,6-8] In contrast, MKD predominantly affects individuals of Western European descent and results from biallelic mutations in the mevalonate kinase (*MVK*) gene.[9,10] While both diseases share clinical features, MKD is typically characterized by febrile episodes lasting 3 to 7 days, often triggered in infancy—particularly following vaccinations—accompanied by a rash, diarrhea, mucosal ulcers, and cervical lymphadenopathy.[5,10,11] Notably, bilateral lymphadenitis may lead to misdiagnosis as periodic fever, aphthous stomatitis, pharyngitis, and adenitis (PFAPA) syndrome, particularly in patients unresponsive to tonsillectomy.[12-14]

Despite the development of various classification criteria for HAIDs, diagnostic delay remains common in patients with recurrent inflammatory episodes, increasing the risk of complications such as AA amyloidosis.[15] Colchicine is a highly effective therapy for FMF, significantly reducing attack frequency and preventing amyloidosis, even in cases with partial clinical response. Compared to FMF, colchicine has been found to be ineffective in MKD. The prevalence of AA amyloidosis is roughly estimated to be about 6% of all MKD cases.[16] The evidence-based therapy for MKD is IL-1 blockade with canakinumab, which is the treatment of choice for patients experiencing frequent disease flares.[17]

In our study, we aimed to document the clinical characteristics of our patients with childhood-onset MKD, evaluate the treatment response, and investigate the reasons for diagnostic challenges in FMF-endemic regions to raise awareness among clinicians.

Materials and Methods

Study population

This study included patients under the age of 18 years who were diagnosed with MKD and were followed up for at least six months at the pediatric rheumatology department of Istanbul University - Cerrahpaşa Medical Faculty between 2016 and 2024. Among the patients enrolled in the study, 29 individuals met the diagnostic criteria for MKD according to the Eurofever/PRINTO classification for hereditary recurrent fevers.[1] These criteria include the presence of either homozygous or biallelic heterozygous mutation in the *MVK* gene, accompanied by at least one of the following clinical manifestations: gastrointestinal symptoms, cervical lymphadenitis, or aphthous stomatitis.

Additionally, four patients exhibited a heterozygous mutation in the *MVK* gene and fulfilled the diagnostic criteria for mevalonate kinase deficiency as outlined in the Eurofever/PRINTO clinical classification for PFAPA and hereditary recurrent fevers.[1] The criteria for this diagnosis include the presence of at least three of the following six features: age at onset less than one-year, gastrointestinal symptoms,

painful lymphadenopathy, aphthous stomatitis, specific triggering factors, and maculopapular rash.

Data collection

Demographic data, including age at onset, age at diagnosis, gender, and family history, were systematically recorded. Clinical manifestations were assessed, focusing on the type and duration of febrile episodes and the frequency of attacks per year. Laboratory evaluations were conducted before and after diagnosis, as well as during the most recent follow-up visit. Laboratory tests included the assessment of inflammatory markers, such as complete blood cell count, C-reactive protein (CRP) level, and erythrocyte sedimentation rate (ESR), along with mutation analysis. Next-generation sequencing analysis method was performed for genetic analysis. Based on the results of the genetic testing, *MVK* genotypes were documented in order of frequency. Previous diagnoses and treatment regimens were also noted, and the number of attacks following the initiation of treatment was recorded. Our center did not measure immunoglobulin D or urine mevalonate during episodes.

The standard colchicine dose was adjusted according to patients' ages, following EULAR recommendations for the management of FMF: ≤ 0.5 mg/day for children under 5 years, 0.5–1.0 mg/day for children aged 5–10 years, and 1.0–1.5 mg/day for those over 10 years of age, including adults. We assessed the response to colchicine over a follow-up period of at least 6 months, in line with EULAR recommendations.[18]

Anakinra was initially administered subcutaneously at a dosage of 2 mg/kg for patients weighing ≤50 kg or 100 mg for those over 50 kg, once daily, according to our clinic's drug label. For patients in clinical remission, dosing was extended after one month, with a transition to canakinumab after one to three months. Canakinumab was prescribed subcutaneously at a dose of 2-4 mg/kg for patients weighing ≤40 kg or 150 mg for those over 40 kg, every eight weeks. For patients in clinical remission, the dosing interval was further extended after six months.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 28, and figures were generated using GraphPad Prism software. Categorical variables were presented as frequencies and percentages. Continuous variables were expressed as mean ± standard deviation (SD) if normally distributed, and as median with interquartile range (IQR, 25th–75th percentiles) if not. The distribution of continuous variables was assessed using the Kolmogorov–Smirnov test. Comparisons between groups (patients with and without exon 10 *MEFV* mutations) were conducted using the chi-square test or Fisher's exact test for categorical variables, as appropriate. For continuous variables, the Mann–Whitney U test was used. A p-value < 0.05 was considered statistically significant.

Ethical approval

The study was approved by the Istanbul University-Cerrahpasa Ethics Committee (E-83045809- 604.01-910551).

Results

Demographic characteristics and medical history of patients

Of the 33 patients, 17 (51.5%) were female, and 16 (48.5%) were male. The median age at symptom onset was 6 (2-17.3) months. The mean age at diagnosis, follow-up period, and age at the last visit were 86.7±57.6, 47.5±46.5, and 134.3±67.2 months, respectively (Table I).

Among the patients, 12 (36.3%) reported a family history of periodic autoinflammatory diseases: two with MKD alone, two with both MKD and FMF; and eight with FMF alone. Eight patients (24.2%) underwent tonsillectomy

for PFAPA syndrome prior to the diagnosis of MKD.

The mean diagnostic delay was 67.6±56.4 months. This delay was prolonged to 95.0±76.2 months in patients with *MEFV* exon 10 mutation, compared to 58.4 ±48.4 months in those without such mutations. The median diagnostic delay for patients with a history of tonsillectomy was 99.5±47.2 months, while it was 57±56 months for those without (Table I, Fig. 1).

Clinical features

All patients reported fever during the episodes (attacks). The most prevalent clinical findings observed during these attacks included malaise in 29 patients (87.8%), arthralgia in 23 (69.6%), abdominal pain in 21 (63.6%), and cervical lymphadenopathy in 21 (63.6%). Additionally, diarrhea was reported in 18 patients (54.5%), and maculopapular rash was observed in 17 patients (51.5%) (Fig. 2, Table II). Less frequently noted symptoms included myalgia (n=10; 30.3%), vomiting (n=6; 18.1%), oral aphthae (n=4; 12.1%), headache (n=4; 12.1%), febrile seizures (n=3; 9%), arthritis (n=2; 6%), chest pain (n=2; 6%), and hepatosplenomegaly (n=2; 6%).

Table I. Demographic characteristics of patients with mevalonate kinase deficiency (N=33).

Gender	
Female	17 (51.5 %)
Male	16 (48.5 %)
Age at last visit (months)	134.3 ± 67.22
Age at symptom onset (months)	6 (2-17.3)
Age at diagnosis (months)	86.7 ± 57.6
Follow up duration (month)	47.5 ± 46.5
Diagnostic delay (months)	67.6 ± 56.4
MEFV (+) (n=7)	95.0 ± 76.2
MEFV (-) (n=26)	58.4 ± 48.4
Tonsillectomy (+) (n=8)	99.5 ± 47.2
Tonsillectomy (-) (n=25)	57 ± 56
MVK genotype	
V377I/V377I	19 (57.5 %)
V377I/another *MVK*	7 (21.2 %)
Another *MVK*	5 (15.1%)
With exon 10 *MEFV* mutation	7 (21.2%)

Data presented as n (%), mean ± standard deviation, or median (Q1-Q3); *MEFV*: Mediterranean fever; *MVK*: mevalonate kinase.

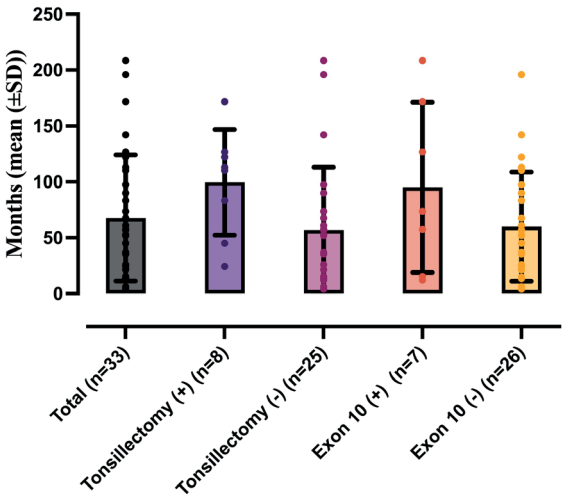

Fig. 1. Diagnostic delays in the study group based on tonsillectomy history and the presence of *MEFV* exon 10 mutation.

Exon 10-positive: patients with *MEFV* exon 10 mutation; Exon 10-negative: patients without *MEFV* exon 10 mutation; Tonsillectomy positive: patients with a history of tonsillectomy prior to the mevalonate kinase deficiency diagnosis; Tonsillectomy negative: patients with no history of tonsillectomy prior to mevalonate kinase deficiency diagnosis.

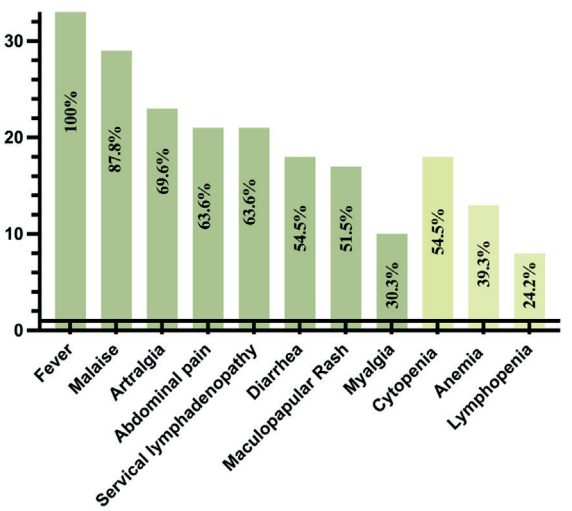

Fig. 2. Clinical and laboratory features observed during attacks in patients with mevalonate kinase deficiency.

Table II. Comparison of clinical and laboratory features between patients with and without MEFV exon 10 mutations.

	Total N=33	*MEFV* exon 10 -positive N=7	*MEFV* exon 10-negative N=26	P value
Clinical symptoms				
Fever	33 (100%)	7 (100%)	26 (100%)	1
Malaise	29 (87.8%)	6 (85.7%)	23 (88.4%)	0.64
Artralgia	23 (69.6%)	4 (57.1%)	19 (73%)	0.72
Abdominal pain	21 (63.6%)	4 (57.1%)	17 (65.3%)	0.96
Servical lymphadenopathy	21 (63.6%)	4 (57.1%)	17 (65.3%)	0.96
Diarrea	18 (54.5%)	4 (57.1%)	14 (53.8%)	0.78
Maculopapular rash	17 (51.5%)	1 (14.2%)	16 (61.5%)	0.07
Myalgia	10 (30.3%)	1 (14.2%)	9 (34.6%)	0.5
Cytopenia	18 (54.5%)	3 (42.8%)	15 (57.7%)	0.78
Anemia	13 (39.3%)	2 (28.5%)	11 (42.3%)	0.82
Lymphopenia	8 (24.2%)	1 (14.2%)	7 (26.9%)	0.84
Hemoglobulin (g/dL)	11.5 (10.8-12.4)	11.7 (11.4-12.5)	11.4 (10.7-12.4)	0.53
WBC (10^6/L)	8550 (6425-11363)	7200 (6400-10000)	8600 (5750-11700)	0.68
Platelet count (10^6/L)	315000 (255000-433750)	370000(277000-435000)	311000 (247500-433500)	0.33
ESR (mm/h)	12.5 (5-41.7)	12 (8-20)	13 (5-42)	0.87
CRP (mg/L)	12.4 (1-62.2)	1.4 (0.8-42)	22 (1.1-97.5)	0.33
Colchicine response	6/28 (21.4%)	0/7 (0%)	6/21 (28.5%)	0.28
Attack duration (days)	5 (4-7)	5 (4-7)	5 (4-7)	0.75
Attack number per year, before diagnosis	12 (10-24)	12 (8-24)	12 (10-24)	0.92
Attack number per year, at last visit	1 (0-2)	2 (0-2)	1 (0-2)	0.87

Data presented as n (%), or median (Q1-Q3).
CRP: C-reactive protein; ESR: erythrocyte sedimentation rate; *MEFV*: Mediterranean fever; *MVK*: mevalonate kinase; WBC: white blood cell.

One patient was diagnosed with renal failure resulting from renal AA amyloidosis.

When comparing the clinical manifestations between *MEFV* exon 10 mutation-positive and -negative subgroups, no statistically significant differences were observed in the frequency of fever, malaise, arthralgia, abdominal pain, cervical lymphadenopathy, diarrhea, or myalgia ($p > 0.05$). A maculopapular rash appeared less frequently in the *MEFV* exon 10 mutation-positive group (14.2% vs. 61.5%), but the difference did not reach statistical significance ($p = 0.07$; Table II).

A total of 30 patients (90.9%) identified a pre-attack trigger, with the majority reporting an infection as a trigger (n=30; 90.9%). Other identified triggers included vaccination (n=22; 66.6%) and stress (n=2; 6%). The median duration of attacks was noted to be 5 (4-7) days. Before treatment, the median frequency of attack was 12 (10-24) per year (Table II).

Laboratory parameters and genetic testing

Among the patients evaluated, their *MVK* gene analysis results were as follows:

- 19 (57.5%) were homozygous for the V377I mutation.
- 7 (21.2%) had V377I in compound heterozygosity with E93Fs (n=2), I268T (n=2), G18R (n=1), G144V (n=1), or G202R (n=1).
- 3 (9%) were homozygous for other *MVK* gene mutations, including I268V (n=1), R388X (n=1), and exon 3 deletion (n=1).
- 4 (12.1%) displayed heterozygous *MVK* mutations, including V377I (n=2), S52N (n=1), and D170D (n=1).
- 7 (21.2%) patients had heterozygous mutations in the *MEFV* gene, including V726A (n = 4), M694V (n = 1), M694I (n = 1), and M680I (n = 1).

Cytopenia was observed in 18 patients (54.5%) during attacks. Anemia was particularly prevalent, affecting 13 patients (39.3%), while leukopenia was noted in four patients (12.1%), neutropenia in three patients (9%), and lymphopenia in eight patients (24.2%). Patients with cytopenia exhibited normal blood parameters between episodes, and there were no indications of recurrent or refractory infections suggestive of immunodeficiency.

The median white blood cell (WBC) count at diagnosis was 8550/mm³ (6425–11363), and the median platelet count at diagnosis was 315,000/ mm³ (255,000–433,750). The median hemoglobin (Hb) value was 11.5 (10.8-12.4) g/dL. Median C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) values were 12.4 (1-62.2) mg/L and 12.5 (5-41.7) mm/h at diagnosis (Table II).

Hematological findings, including cytopenia, anemia, and lymphopenia, were similarly distributed between groups stratified by *MEFV* exon 10 mutation status, with no statistically significant differences observed ($p > 0.05$). Additionally, laboratory parameters such as Hb level, WBC count, platelet count, ESR, and CRP were comparable between the groups (Table II).

Treatment response

Colchicine was initiated as the first-line treatment in 28 patients (84.8%) suspected of having an autoinflammatory disease prior to the confirmation of MKD. Of these patients, 22 (78.5%) were colchicine unresponsive, and six patients (21.5%) were colchicine responsive. The colchicine response rate was 0% among patients with exon 10 *MEFV* mutations, compared to 28.5% in those without these mutation. Although the response was lower in the exon 10-positive group, the difference was not statistically significant ($p = 0.28$; Table II).

After the diagnosis, three patients continued colchicine therapy, 12 were started on anakinra, and 18 patients commenced treatment with canakinumab. Of the 12 patients initially receiving anakinra, 11 switched to canakinumab due to its ease of use, while one patient discontinued treatment because of irregular follow-up. During the last visit, 28 patients were on canakinumab, and four were on colchicine (Fig. 3). Among those receiving colchicine therapy, three had homozygous V377I mutations, while one had a heterozygous S52N mutation.

At the final visit, the median number of attacks per year was 1 (0-2). There was no statistically significant difference between the two groups based on the *MEFV* exon 10 mutation status ($p>0.05$; Table II). All four patients under colchicine were attack-free at the last visit (one patient had subclinical inflammation). The 28 patients treated with canakinumab received treatment every 2-3 months, and the median number of attacks per year was 1 (0-3).

Discussion

In this retrospective cohort study, we evaluated the clinical features of 33 MKD patients who were followed up in a single center. To our

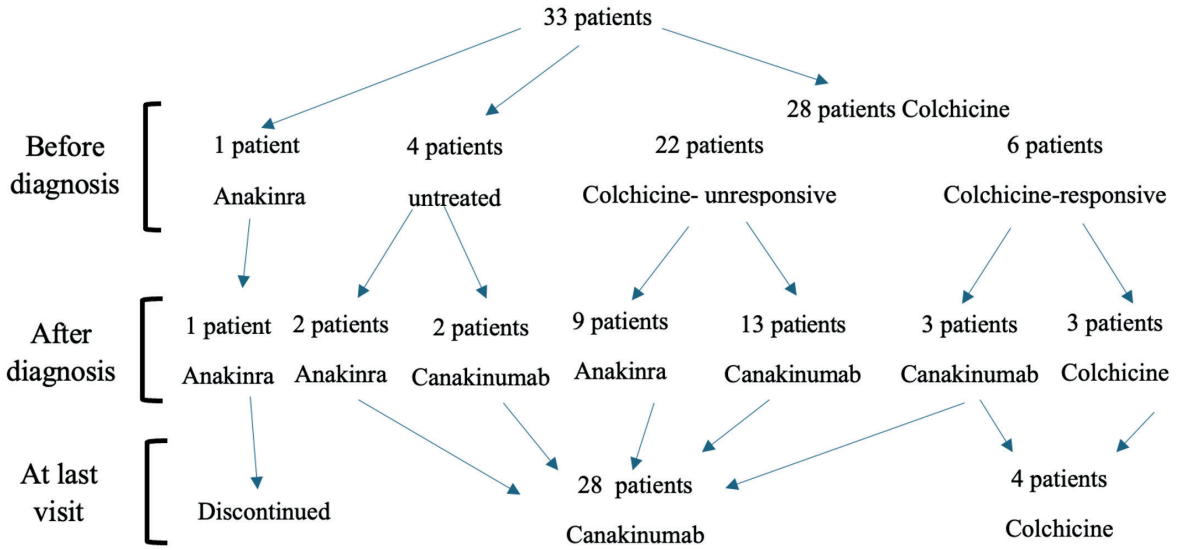

Fig. 3. Comprehensive treatment chart of study participants.

a) Before diagnosis: Empirical treatments were initiated based on clinical suspicion of autoinflammatory disease prior to genetic confirmation. Responses to colchicine prior to diagnosis were noted.

b) After diagnosis: Treatment strategies were adjusted or initiated following molecular diagnosis.

c) At last visit: Therapeutic status at most recent follow-up.

knowledge, this study represents the largest cohort of MKD patients reported in single-center studies so far. Notably, we documented the presence of the *MEFV* exon 10 variant in seven patients and a history of tonsillectomy due to a diagnosis of PFAPA syndrome in another 8 patients, both of which may contribute to delays in the diagnosis of MKD. Through this research, we aim to enhance awareness regarding the diagnosis of autoinflammatory diseases other than FMF in endemic populations.

Recent advancements in understanding the pathophysiology and genetics of AIDs are facilitating the identification of novel phenotypes. FMF and MKD represent the two most prevalent examples of hereditary autoinflammatory diseases characterized by autosomal recessive inheritance. Specifically, FMF is the most common hereditary autoinflammatory disease among Mediterranean populations, which makes diagnosing rare hereditary autoinflammatory diseases in these groups more challenging.[8,12] While MKD is recognized globally, it is more prevalent among individuals of Northern European descent. Mutations in the *MVK* gene cause MKD by resulting in uncontrolled activation of the pyrin. This triggers caspase-1, resulting in the release of IL-1β, a potent inducer of fever and inflammation.[19] Several case reports in the literature describe patients with overlapping features of MKD and FMF.[5,20,21] These studies involve patients with a wide range of clinical presentations. In the report by Çakan et al.[5] both patients exhibited symptom onset before the age of one and were unresponsive to colchicine therapy. Interestingly, a study by Moussa et al.[20] evaluated five siblings carrying mutations associated with both MKD and FMF, and found that only two were symptomatic, while the remaining three were asymptomatic.

PFAPA syndrome is recognised as the most prevalent autoinflammatory disease among children. Although the attacks are frequently manifested by tonsillitis and pharyngitis accompanied by fever, aphthous stomatitis, cervical lymphadenopathy, headache, rash, arthralgia, and abdominal pain may also be observed. A study by Gozen et al.[13] reported a response rate to tonsillectomy of 83.2% in

patients diagnosed with PFAPA syndrome. In our cohort, eight patients exhibited resistance to tonsillectomy and experienced a longer diagnostic delay for MKD. Therefore, the diagnosis of MKD should be considered in cases of recurrent fevers with atypical presentations who are not responsive to tonsillectomy.[14]

Our study largely confirms the clinical characteristics of MKD patients described in previous reports. Similarly to studies in the literature, the gender distribution was equal, and the onset age of symptoms was in the first 6 months.[22-24] In the study by Hilst et al.[22] approximately 50% of 103 MKD patients reported 7 to 12 annual attacks. Ter Haar et al.[23] noted a median frequency of 12 attacks per year, while our study recorded a mean of 15.3 attacks per year. The most common genetic mutation was V377I, similar to the literature, and 78% of the patients carried this mutation.[22-24] Diagnostic delay begins to decrease as awareness of the disease increases. In the study conducted by Hilst et al.[22] the mean diagnostic delay was reported as 13.9 years, with 13 patients (12.6%) being misdiagnosed with FMF. Meanwhile, Ter Haar et al.[23] found that the median diagnostic delay was 6 years, which closely aligns with our cohort's findings.

The most prevalent clinical findings observed during these attacks included malaise (87.8%), arthralgia (69.6%), abdominal pain (63.6%), cervical lymphadenopathy (63.6%), diarrhea (54.5%), and maculopapular rash (51.5%), in accordance with previous reports.[22-24] However, in contrast to other studies, oral aphthae, which is also included in the diagnostic criteria for MKD, was found less frequently in our patients. There were no chronic patients except for one with AA amyloidosis at diagnosis, one with an episode of macrophage activation syndrome, and three with epilepsy. In the last published MKD multicenter cohort of 114 patients, one had recurrent macrophage activation syndrome, and five had AA amyloidosis.[23]

In our study, 22 of 28 patients who were initiated on first-line colchicine treatment were unresponsive at 78.5%. However, at the last follow-up visit, four patients remained free of attacks while on colchicine. In the study by Ter Haar et al.[23] colchicine was initiated in 21 patients; one responded, and 13 were unresponsive. Partial response to colchicine was 15.9% in the study by Hilst et al.[22] Zhang found a complete non-response of 80% in their literature review.[11] Although there are case-based colchicine-responsive cases in the literature[25], they have not yet been studied, and studies show that there was no response in the early period. Canakinumab, a fully human anti-IL-1β monoclonal antibody, has been shown to control inflammation and prevent flares effectively in patients with MKD.[26-28] In the study by Jeyaratnam et al.[19] a median flare rate of 0 was reported, with 83% of patients experiencing either 0 or 1 flare during the study period, compared to a median flare rate of 12 prior to enrollment.

The main limitation of this study is its retrospective nature, consisting of patients with a median disease duration of 47.5±46.5 months. Clinical findings and other data were obtained from the patients' medical records. The number of patients was insufficient to yield statistically significant results due to the rarity of the disease and the fact that data were collected from a single center. Urine mevalonate, serum amyloid A, and immunoglobulin D levels could not be measured during the attack.

Conclusion

In FMF-endemic regions, FMF remains the most frequently diagnosed autoinflammatory disease in patients with periodic fever. Nevertheless, despite broader awareness and the increasing use of comprehensive genetic panels, FMF is often presumed as the initial diagnosis—even when *MEFV* mutation status is inconclusive.

Clinicians should be highly suspicious of MKD in colchicine-resistant patients, particularly

those with heterozygous *MEFV* mutations or atypical clinical features. In these cases, further molecular investigations beyond *MEFV* should be considered. Furthermore, patients with atypical phenotypes who carry a single HAID-associated gene variant may benefit from broader genetic evaluation to identify potential overlapping or coexisting mutations. A more comprehensive and gene-inclusive diagnostic approach may enhance early diagnosis and facilitate the development of targeted treatment strategies.

Ethical approval

The study was approved by İstanbul University-Cerrahpasa Institutional Review Board (date: 09.02.2024, number: 910551).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: EKK, AA, OK; data collection: EKK, UG, AG, NA, EA, EA; analysis and interpretation of results: EKK; draft manuscript preparation: EKK, FH, MY, AA, SS, KB, OK. 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 reliability and validity study of the Turkish version of Brief Measure of Eating Compulsivity (MEC) among adolescents

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ABSTRACT

Background. Food addiction has been increasingly recognized as a contributing factor to obesity and eating disorders. Compulsive eating, characterized by an uncontrollable urge to consume food despite adverse consequences, shares behavioral similarities with substance addiction. This study aims to adapt the Brief Measure of Eating Compulsivity (MEC) into Turkish and evaluate its validity and reliability in the adolescent population.

Methods. The study included a sample of 89 adolescents aged 12-18 years. Participants without chronic medical or psychiatric conditions affecting eating behaviors were included. The adaptation process involved translation, back-translation, and expert evaluations to ensure cultural and linguistic appropriateness. The psychometric properties of the Turkish MEC were assessed through internal consistency, exploratory factor analysis, and criterion validity using the Yale Food Addiction Scale (YFAS).

Results. The internal consistency of the Turkish MEC was 0.89, with item-total correlations ranging from 0.56 to 0.72. Factor analysis supported a single-factor structure explaining 52.6% of the variance. Convergent validity was established through a significant positive correlation with YFAS scores ($r = 0.57$, $p < 0.001$). Criterion validity analysis demonstrated significantly higher MEC scores in individuals classified as food addicts by YFAS ($p = 0.025$). Additionally, significant differences in MEC scores were observed across body mass index categories ($p = 0.010$), with higher scores in adolescents with obesity compared to the normal-weight group.

Conclusions. The Turkish version of the MEC demonstrated strong reliability and validity among adolescents, supporting its use in assessing compulsive eating behaviors. Given the increasing prevalence of obesity and eating disorders in Turkish youth, this tool provides a valuable resource for early detection and intervention in research and clinical settings.

Key words: compulsive eating, obesity, adolescents.

The impact of food addiction on obesity and eating disorders has become a prominent focus in contemporary research. Central areas of inquiry include the excessive intake of certain foods, especially those rich in carbohydrates and fats, and the psychological determinants affecting eating behaviors.[1,2] The observed resemblance between substance addiction and compulsive overeating broadly supports the concept of food addiction. Compulsiveness, characterized by an uncontrollable urge to continue a behavior despite its adverse consequences, is a

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crucial feature of addiction.[3] This is exemplified by analogous patterns in both conditions, including persistent engagement in behaviors despite adverse outcomes, withdrawal symptoms, obsessive preoccupation, increasing consumption, guilt after excessive intake and repetitive behaviors.[4,5]

The causes of obesity include, but are not limited to genetic predisposition, poor dietary habits, lack of physical activity, and medical conditions.[6] Notably, some individuals with obesity may exhibit eating patterns akin to those observed in substance addiction. This observation has resulted in the hypothesis that food addiction might also contribute to the development of obesity.[7,8] Consequently, compulsive overeating is regarded as a factor that both contributes to and perpetuates obesity in affected individuals.

It is essential to adapt objective measurement tools into the Turkish language, which facilitates healthcare professionals' ability to discuss eating behaviors and habits with their patients, thereby guiding research and practice in this domain. Reviewing the relevant literature in Türkiye, the single validated self-report measure is the Yale Food Addiction Scale (YFAS).[9] The YFAS primarily evaluates food addiction based on substance dependence criteria, which may not fully capture the compulsive dimension of disordered eating behaviors. Considering the removal of the distinction between abuse and dependence in the 5th edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5)[10] and the crucial role of compulsivity in assessing addiction, Shroder et al.[11] identified a need for a concise, user-friendly instrument that could effectively assess the compulsive component of food addiction and could be used in both research and clinical settings. The Brief Measure of Eating Compulsivity (MEC) was developed as a short and effective tool to assess a key aspect of food addiction—eating compulsivity. The 10-item scale (MEC-10) was initially validated in a sample of 65 individuals with a body mass index (BMI) of 30 or higher, demonstrating strong internal consistency and excellent test-retest reliability. Its brevity and psychometric strength make it suitable for clinical and research applications. Given the rising prevalence of obesity within the adolescent population in Türkiye[12-14], it was deemed necessary to adapt a brief yet comprehensive measure, such as the MEC, to address the gap in assessment tools within this specific age group. Therefore, the aim of this study was to examine the validity and reliability of MEC, designed to assess compulsive eating, a significant facet of eating behaviors.

Materials and Methods

Participants

The study was conducted at Hacettepe University, Division of Adolescent Medicine. The sample comprises 89 adolescents aged 12-18 who attend the adolescent medicine outpatient clinic. Adolescents without chronic medical conditions (such as diabetes mellitus or other endocrine disorders) and/or psychiatric diagnoses (including eating disorders) were included in the study. Other exclusion criteria include cognitive impairments affecting comprehension and completion of the questionnaire, using prescription/nonprescription drugs, or having a substance abuse problem that may cause compulsive eating behavior. Adolescents whose BMI was between the 5th and 85th percentiles and below the 5th percentile according to age and gender were included in the normal weight and underweight groups, respectively. Adolescents whose BMI was between the 85th-95th and above the 95th percentile according to age and gender were included in the overweight and obesity groups, respectively. Ethics board approval of the study (GO/19/770) was obtained from the Hacettepe University Non-Interventional Clinical Research Ethics Committee. Written informed consent was obtained from parents, and written assent was obtained from participants.

Measurement tools

Brief Measure of Eating Compulsivity (MEC): This measure was developed by Schroder et all.[11] It is a ten-item, five-point Likert scale (0 = definitely false and 4 = definitely true). The compulsive eating score is defined as the sum of the item scores and a higher score indicates an elevated level of compulsive eating. The internal consistency (Cronbach alpha) of the original scale was found to be 0.94. Factor analysis revealed that the scale explains 67.4% of the total variance, with items loading onto a single factor. The test-retest reliability coefficient was 0.92. The validity study showed that the score from the MEC effectively predicted the group identified as food addicts by the Yale Food Addiction Scale. The Spearman's correlation between the two scales was 0.727 ($p < 0.001$).

Yale Food Addiction Scale (YFAS): The Yale Food Addiction Scale is employed to identify individuals' addiction to high-fat and sugary foods, paralleling the diagnostic criteria for substance addiction. This scale, designed to assess food addiction, comprises 27 items and demonstrates substantial internal consistency, as indicated by a Cronbach's alpha coefficient of 0.93. Furthermore, the scale's discriminant validity is statistically significant, evidenced by the observed difference between clinical and non-clinical groups ($t = 10.662$, $p < 0.002$). The Turkish adaptation of the scale was performed by Bayraktar, Erkman, and Kurtuluş[15] and demonstrated statistically significant internal consistency (Cronbach's alpha = 0.93) and discriminant validity ($t = 10.662$, $p < 0.002$).

Procedure

To conduct the Turkish adaptation study, including translation, validity, and reliability of the MEC within an adolescent sample, the research team first secured prior approval from Rita Shroeder for the Turkish validation of the scale with Turkish adolescents. After obtaining the permission, the scale was independently translated from English to Turkish by two individuals proficient in both languages. These translators were familiar with the subject matter under investigation and thoroughly understood the construct assessed by the test. Subsequently, two professors of pediatrics evaluated and provided necessary recommendations for these translations for accuracy and cultural relevance. The research team reviewed these evaluations to establish a consensus translation, which was subsequently back-translated into English by another professor in psychology proficient in both languages and found to be compatible with the original scale. Then the final Turkish version was obtained to use for the validation. Participants were asked to fill out both the MEC and YFAS under supervision, ensuring that they could seek clarification if they encountered any difficulties in understanding the items.

Statistical analysis

Statistical Package for Social Sciences (SPSS Inc. Chicago IL) is used to analyze data, version 22.0, with a significance level set at $p \leq 0.05$ for all statistical procedures. Among the 94 participants, those who left 5% or more of the scale items blank were identified using the NMISS function, which calculates the number of missing observations for each variable, enabling us to assess data completeness and ensure that our analyses appropriately accounted for any incomplete cases, and 5 participants were removed from the data set. All subsequent analyses were conducted on this final sample of 89 participants. The suitability of the variables to a normal distribution was assessed using Kolmogorov-Smirnov test. Descriptive statistics for continuous variables are presented as mean ± standard deviation (SD), or median and interquartile range (IQR), while categorical variables are expressed as percentages. For the scale scores that deviated from a normal distribution, the Mann-Whitney U and Kruskal-Wallis tests were employed for comparisons. The Cronbach's alpha coefficient and item-total correlations were computed to determine the internal consistency. Additionally, the split-half reliability was assessed, and the Spearman-Brown coefficient was calculated to evaluate

the consistency between the two halves of the scale. Exploratory factor analysis (EFA) method was used to evaluate the scale's factor structure. Spearman's correlational analysis was employed to investigate the relationship between MEC and BMI. The Kruskal-Wallis test was used to examine the differences in MEC scores across BMI groups. The Mann-Whitney U test was used to investigate the difference between the MEC mean scores of the groups based on the YFAS food addiction score.

Results

Demographics and anthropometric data

The mean age of the sample (n=89) was 14.9 ± 1.65 years, and 65.2% were female. Participants' mean weight was 66.09 ± 17.72 kg (min-max: 34-113 kg), and the mean height was 162.9 ± 8.62 cm (min-max: 134-182 cm). BMI Z score ranged from -3.63 to 2.43, with a mean of 0.64 ± 1.33. In the BMI distribution, 5% of the participants were classified as underweight, 33% as normal weight, 24% as overweight and 27% as obese.

Reliability analysis

The median score of MEC was 13 (IQR=14). The Cronbach's alpha value was calculated as an indicator of the internal consistency coefficient and found to be 0.89. Cronbach's alpha values exceeding 0.80 indicate high internal consistency.[16] The split-half reliability of the 10-item scale was assessed using the Spearman-Brown coefficient. The Spearman-Brown coefficient for equal length was 0.89, indicating good internal consistency for the scale. In addition to assessing the internal consistency coefficient, item-total score correlations were analyzed. The item-total correlation values ranged from 0.56 to 0.72. All item-total test correlation coefficients were above 0.40. Table I shows the summary of the psychometric properties of the MEC items.

Validity analysis

Before evaluating factor structure, the Kaiser-Meyer Olkin (KMO) and Bartlett tests were used. The KMO value of 0.89 (higher than 0.050) and a statistically significant result on the Bartlett test ($x= 418.73$, $df=45$, $p<0.00$) were found. These showed that the data were appropriate for factor analysis. The EFA (Principal Component Analysis, varimax rotation) showed a single factor that accounted for 52.6% of the total variance.

Table I. Psychometric properties of Brief Measure of Eating Compulsivity (MEC).

	Mean	Standard deviation	Item total correlation	Cronbach if removed	Factor loading
I have urges to eat a lot of the time	1.37	1.26	0.563	0.894	0.636
I feel disturbed about my urges to eat	1.09	1.21	0.636	0.899	0.711
I have very little control over my eating	1.21	1.20	0.586	0.892	0.661
I often fear losing control of my eating	0.96	1.18	0.621	0.890	0.711
I am not able to control how much I eat in the presence of any food	1.39	1.16	0.680	0.886	0.754
I often feel out of control around certain foods	1.17	1.22	0.676	0.887	0.753
Food is like a drug to me	1.04	1.19	0.640	0.889	0.714
It worries me how little control I have over my eating	0.94	1.16	0.683	0.886	0.765
When I come across a very tasty food I can't stop thinking about it	1.56	1.41	666	0.888	0.737
I feel defeated by food	1.13	1.22	0.725	0.883	0.797

Convergent validity

To evaluate the convergent validity of the MEC, Spearman's correlational coefficient between MEC and YFAS was analyzed, and it was found that the coefficients for the relationship were statistically significant (Spearman's r=0.57, p<.001).

Criterion validity

A significant difference (U = 433.5, p = 0.025) was observed between the mean MEC scores of individuals diagnosed with food addiction (MEC mean rank = 65.93) and those without such a diagnosis (MEC mean rank = 43.21), as assessed by the YFAS. The mean MEC score for those diagnosed with food addiction is significantly higher.

Analysis of BMI group differences according to MEC scores revealed a significant difference ($\chi^2_{(3)}$=11.31, p=0.010) in MEC scores between the normal weight group (MEC mean rank =35.71) and the group with obesity (MEC mean rank = 57.56). The mean MEC score for the obese group was significantly higher than that of the normal weight group.

Discussion

This study's results provide strong evidence for the reliability and validity of the Turkish version of MEC, supporting its use in assessing compulsive eating behaviors among Turkish adolescents. The Cronbach's alpha coefficient of 0.89 indicates a high degree of internal consistency and the item-total score correlations ranged from 0.56 to 0.72, providing additional support for the reliability.[16,17] The strong item-total correlations further support the internal consistency of the scale, indicating that the items measure a common underlying construct.[17]

The EFA revealed a single-factor structure, which aligns with the conceptual framework of the original scale, suggesting that compulsive eating is a unidimensional construct within this population.[11] The significant positive correlation between MEC scores and the YFAS indicates that the MEC effectively measures a construct closely related to food addiction, consistent with previous research demonstrating the association between compulsive eating behaviors and food addiction symptoms.[18] This result confirms that the scale captures behaviors characteristic of compulsive eating as intended and reinforces its relevance for both research and clinical settings. Adolescents with food addiction, as identified by the YFAS, had significantly higher MEC scores than those without food addiction. This distinction demonstrates that the MEC can differentiate between individuals based on their level of compulsive eating behavior.[11]

Adolescence is a critical period for physical, emotional, and cognitive development, during which unhealthy eating behaviors can become deeply ingrained and contribute to the development of long-term health risks, including obesity, eating disorders and metabolic disorders.[19-21] As the findings suggest, adolescents with higher compulsive eating tendencies are more likely to have elevated BMI and meet the criteria for food addiction. This underscores the importance of identifying these behaviors early, as untreated compulsive eating can lead to obesity and escalate into more severe eating disorders.[22] Moreover, differences in MEC scores across BMI categories provide further evidence of the scale's sensitivity.[11] The higher MEC scores among adolescents with obesity reflect the strong association between compulsive eating and obesity, highlighting the importance of assessing these behaviors in this population, while avoiding stigmatization. Given that obesity rates among Turkish youth are rising[12-14], MEC can play a crucial role in the early detection of adolescents with compulsive eating and can promote targeted intervention since the treatment approach differs from obesity with other root causes.

Compulsive eating -characterized by a loss of control over eating- is a key component of several eating disorders, such as binge eating disorder.[4,23] Assessing compulsive eating behaviors is critical, especially considering the

DSM-5's removal of the distinction between abuse and dependence, shifting the focus toward compulsivity as a core feature of addiction.[24] It is essential to have reliable and valid tools to assess compulsive eating, which plays a key role in disordered eating behaviors. Our findings provide initial evidence that the Turkish version of the MEC is a suitable instrument for both research and clinical applications, offering a concise way to assess eating compulsivity in this age group.

Early intervention is essential for preventing the progression of disordered eating into clinical eating disorders, which are associated with serious physical and psychological consequences.[25,26] Eating disorders can impair social functioning, and mental health, leading to depression, anxiety, and other medical complications, some of which can lead to life-threatening conditions.[27,28] By identifying compulsive eating behaviors during adolescence, healthcare providers can intervene earlier, reducing the risk of developing full-blown eating disorders and obesity. In many developing countries, like Türkiye where awareness among caregivers and healthcare professionals are not high, and health services for eating disorders are still limited, having culturally adapted, psychometrically sound tools like the MEC is crucial. These tools allow for more accurate assessments in both research and clinical settings, enabling clinicians and researchers to better understand the unique patterns of disordered eating in adolescents. Furthermore, integrating assessments of compulsive eating into routine health screenings could enhance preventive efforts, providing opportunities for education, counseling, and behavioral interventions that promote healthy eating habits.

The MEC relies on self-reported data, which may introduce bias due to participants underreporting or overreporting their eating behaviors, particularly with sensitive topics like compulsive eating. The study employed a cross-sectional design, which limits the ability to assess changes in compulsive eating behaviors over time or determine causality between compulsive eating, BMI, and food addiction. Unlike the original scale development study, test-retest reliability could not be evaluated in this study due to difficulties in re-contacting the hospital-based outpatient sample within a standard retest interval. Although the sample provided meaningful insights, the study's findings may not be generalizable to all adolescents in Türkiye. Although the MEC was adapted to Turkish, certain cultural nuances around food, eating behaviors, and addiction may not be fully captured. While the MEC showed significant associations with the YFAS, the study did not include other clinical assessments or interviews to provide a more comprehensive evaluation of compulsive eating or related mental health issues.

In conclusion, the Turkish adaptation of the MEC offers an effective, valid and reliable instrument to assess compulsive eating behaviors among adolescents in Türkiye. Its ability to distinguish between individuals with and without food addiction, as well as its alignment with related constructs like YFAS, underscores its relevance for both research on eating behaviors and clinical interventions targeting obesity and disordered eating.

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Ethical approval

The study was approved by Hacettepe University Non-Interventional Clinical Research Ethics Committee (number: GO/19/770).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: NK, ZT, MPK; data collection: ZT, MPK; analysis and interpretation of results: ZT, NK, MPK; draft manuscript preparation: ZT. 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

Barriers and facilitators of pediatric adherence to antiretroviral therapy: perspectives from caregivers in Türkiye

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ABSTRACT

Aim. This study aimed to describe barriers and facilitators of the adherence of children with human immunodeficiency virus (HIV) to antiretroviral therapy (ART) from the perspectives of their caregivers.

Methods. In-depth interviews were held with the caregivers of 15 children. The collected data were analyzed using thematic analysis procedures. The Consolidated Criteria for Reporting Qualitative Research (COREQ) were followed in the study.

Results. The perspectives of caregivers were categorized under four themes and subthemes. Barriers and facilitators of the adherence of children with HIV to ART were categorized into 4 main themes: (1) medication-related, (2) child-related, (3) caregiver-related, and (4) health system-related. The results indicated that understanding the factors that influence pediatric ART adherence is critical to the development of adequate strategies. In addition, disclosure of HIV status to the child is also an important factor affecting drug administration in the social environment.

Conclusions. To ensure adherence to lifelong ART, targeted caregiver support through continuous supervision, clear guidance on drug preparation, and strategies for effective administration should be integrated into context-specific interventions that address the combined influence of factors related to the child, medication, healthcare, and of the sociocultural environment.

Key words: antiretroviral therapy, adherence, children, barriers, facilitators, human immunodeficiency virus (HIV).

Human immunodeficiency virus (HIV) continues to affect millions of people worldwide. According to UNICEF (2023), an estimated 39 million individuals were living with HIV in 2022, including 2.58 million children and adolescents under the age of 19.[1] In Türkiye, the number of individuals diagnosed with HIV has been progressively increasing each year.[2] To prevent acquired immune deficiency syndrome (AIDS), antiretroviral therapy (ART) should be provided effectively, and the individual's adherence to treatment should be at a rate of

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95-100%.[3,4] Adherence to ART decreases drug resistance, reduces the risk of HIV transmission, lowers HIV-related morbidity rates, and improves the patient's quality of life.[3,5-7] A child' s adherence to ART is a significant challenge for caregivers because it is influenced by many factors that make it easier or more complex[8] such as the child's behavior, the tolerability of medications, the quality of health services, the disclosure of HIV status, and the fear of stigma.[9-11] To understand the barriers to and facilitators of ART adherence in children is essential for achieving the Sustainable Development Goals (SDGs), especially SDG #3: Good health and well-being (United Nations, n.d.).[12]

Despite the increase in the number of children diagnosed with HIV in Türkiye[2], current studies on ART compliance are limited to adults.[13-15] Therefore, this study aimed to identify barriers to and facilitators of children's adherence to ART from the perspectives of caregivers in Türkiye.

Material and Methods

Study design

A phenomenological qualitative research approach was used in this study. The authors followed the COREQ Checklist to ensure accurate and complete reporting.[16]

Settings and participants

From November 2022 to January 2023, we conducted semi-structured interviews with caregivers of children and adolescents on ART at a Pediatric Infectious Diseases Clinic of İstanbul University Hospital in Istanbul. A purposive sampling method was used to identify participants. Eligible caregivers were responsible for the primary care of pediatric patients diagnosed with HIV and on ART for at least 3 months, and voluntarily agreed to participate in the study. Three researchers (AD, NMA, ED) contacted the participants by phone, explaining the purpose of the study and asking whether they would like to participate in the study. The term used in this study, "participant", refers to caregivers.

Data collection

An in-depth interview method was employed to collect the data by using a semi-structured interview form. This open-ended interview questionnaire was developed by the researcher team based on the relevant literature.[17-19] Examples of the questions that were used are shown in Table I.

Each interview was audio-recorded and lasted 30-45 minutes. One of the authors, with prior experience in in-depth interviews (ÖŞ), conducted the face-to-face interviews in the outpatient clinic room with only the interviewer and the participant present. Privacy was ensured by giving an anonymous ID.

Data analysis

The recordings were transcribed verbatim by one of the researchers (ÖŞ). The data were analyzed using the six-step thematic analysis method.[20] At the beginning of the data analysis process, two researchers (ÖŞ, AA) independently listened to the audio recordings and checked the transcripts for corrections for any mistakes. This was to assure methodological accuracy and ensure that the transcripts correctly expressed the statements of the participants. Once the raw data were gathered, three researchers (ÖŞ, AA, SB) independently created their codes and themes by reading the raw data. Then, they came together and identified the similarities and differences between the themes and subthemes they determined through codes. The researchers utilized code meanings and code frequencies to assess data saturation.[21] After 15 caregivers were interviewed, it was determined that there was no new code, the data reached the saturation point, it was decided that the data were sufficient, and no more interviews were held. The transcripts were sent to the

Table I. Interview questions

Interview topic: Providing care to a child with HIV
Questions:
How has your child's disease changed your life?
How is your child's care going?
What is the most difficult situation you have experienced in caring for your child?
When you need support in this process, how do you get support?
Interview topic: Antiretroviral therapy
Questions:
How is your child's treatment going? Can you share your experiences regarding your child's treatment?
How do you manage your child's treatment regime?
What types of difficulties do you experience with your child's treatment/use of medication?
What do you think are the factors that make it easier or more difficult for your child to take medication? How did you find solutions?

researchers participating in the study (AS, ND, NMA, ED , SHT) for their opinions and recommendations, and the final revision was made. Each author provided final approval for the publication of this version and agreed to be considered accountable for all aspects of the work.

Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki, and the study was approved by the İstanbul University's Social Sciences Ethics Review Board (1109334/22.08.2022). Additionally, institutional permission was obtained before the study. Before the interview, informed consent was taken from all participants for participation to the research and record audio of interviews. Due to the sensitive nature of the research topic, before each interview, the researcher reminded the participants that participation was voluntary and that they could cease participation at any time during the interview.

Results

The study analyzed data from 15 participants. Table II summarizes the sociodemographic characteristics of the participants and the pediatric patients they cared for. Qualitative analysis results are presented as themes and subthemes below and in Fig. 1.

Barriers to adherence to antiretroviral treatment

Theme 1: Medication-related barriers

From the perspectives of the participants, the 'structure and preparation of drugs', 'negative experiences with medications', and 'the numbers and timing of medications' were barriers to children's adherence to ART.

Structure and preparation of drugs. The participants reported that they had difficulty in calculating the dose by snapping the tablets into smaller pieces (lopinavir + ritonavir/Kaletra®), and they stated that the long dissolution period of the drug prolonged its preparation time (10/15).

'…but there is one called "Kaletra" (lopinavir + ritonavir/Kaletra®). We developed some methods at home because the drug is very hard to break. At first, we tried hitting it with a hammer or something, but it didn't work. Then, my wife found something [some method].' (P-8, father of 5-year-old girl)

Negative experiences with medicines. According to the statements of the participants, due to the

Table II. Sociodemographic characteristics of caregivers and pediatric patients

	Caregiver's characteristics					Child' s characteristics			
	Gender	Age (yr)	Level of education	HIV status	Relation to child	Age	Sex	Route of infection	Time since treatment initiation
P-1	F	37	University	Positive	Mother	16 yr	F	Perinatal	15 yr
P-2	F	22	High school	Positive	Mother	42 mo	M	Perinatal	30 mo
P-3	F	43	Primary-secondary school	Positive	Mother	20 mo	F	Perinatal	12 mo
P-4	F	38	Primary-secondary school	Positive	Mother	4 yr	M	Perinatal	4 yr
P-5	F	53	Primary-secondary school	Positive	Mother	14 yr	M	Perinatal	13 yr
P-6	F	46	Primary-secondary school	Positive	Mother	7 yr	M	Perinatal	4 mo
P-7	F	45	University	Negative	Legal guardian	17 yr	M	Unknown	26 mo
P-8	M	42	University	Positive	Father	5 yr	F	Perinatal	4 yr
P-9	F	24	University	Negative	Legal guardian	16 yr	F	Unknown	4 mo
P-10	F	27	High school	Positive	Mother	10 yr	M	Perinatal	3 yr
P-11	F	58	High school	Negative	Mother	17 yr	M	Sexual	18 mo
P-12	F	28	Primary-secondary school	Positive	Mother	5 yr	F	Perinatal	5 yr
P-13	F	47	Primary-secondary school	Negative	Mother	17 yr	M	Blood transfusion	9 yr
P-14	F	33	Primary school	Positive	Mother	42 mo	M	Perinatal	30 mo
P-15	F	23	High school	Positive	Mother	30 mo	M	Perinatal	30 mo

F: female, HIV: human immunodeficiency virus, M: male, mo: months, yr: years.

bitter taste of the medicine, it was very difficult to administer, and their children started to feel nausea and vomiting as soon as they tasted it (9/15).

'He immediately vomited after taking the medication, and I was nearby holding a bucket. He gets nauseous because it tastes so bad.' (P-14, mother of 3.5-year-old boy)

Numbers and timing of medications. The participants stated that the number of medications was too much, and therefore, they had difficulty in arranging medication times at occasions such as before going to bed or school (6/15).

'He takes a lot of medications. When he goes to school in the morning, I give him the medication to take [there]. He can't have breakfast because he goes early. ... I tell him you must take it at this time, but he could skip it [without my knowledge].' (P-5, mother of 15-year-old boy)

'...if he was very sleepy that day and was very tired. There were times when I couldn't give him the medicine because he was too sleepy.' (P-4, mother of 4-year-old boy)

Length of the treatment. The participants emphasized that taking drugs continuously and coming to the hospital often for treatment caused boredom in children (2/15).

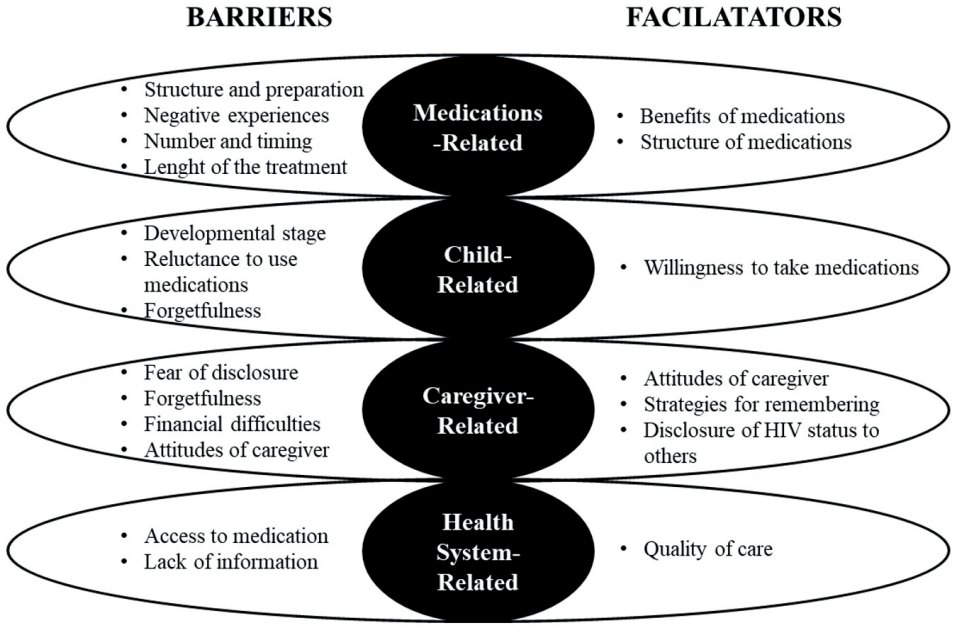

Fig. 1. Barriers to and facilitators of pediatric adherence to antiretroviral therapy.

'He is upset because he is constantly being treated. He regularly gives blood tests… He is tired. He doesn't want to go anymore.' (P-10, mother of 10-year-old boy)

Theme 2: Child-related barriers

Based on the statements of the participants, the 'child's developmental stage', 'forgetfulness', and 'unwillingness to take medication' were found to negatively affect children's compliance with ART.

Child's developmental stage. It was determined that children had different behaviours that made medication compliance difficult according to their developmental stage. The participants reported that young children ran away when they saw the medicine, while preschool children asked why they were taking the medicine (6/15).

'He immediately hides when he sees me holding the medication. I must give it to him regularly. It was and is hard...' (P-15, mother of 2.5-year-old boy)

'We can't explain it because he is very young. He says, mom, other children don't take [medication] every day.' (P-4, mother of 4-year-old boy)

Reluctance to use medicine. The participants revealed that children expressed reluctance to take medication when they felt better and asked why they had to continue using the medicine (5/15).

'If I give him the boxes of medicine, he knows all of them, he can take them, but sometimes he says that's enough. He says he doesn't have anything [of concern in terms of health]. …especially when he feels much better, he says, he's no different from others.' (P-7, legal guardian of a 17-year-old boy)

Forgetfulness. The participants said that their children's treatment was disrupted due to forgetfulness (3/15).

'This one is very forgetful, extremely forgetful. I even recall him not taking his medication for a week…' (P-11, mother of 17-year-old boy)

Theme 3: Caregiver-related barriers

From the perspectives of the participants, the 'fear of disclosure', 'caregiver's attitude', and 'forgetting and financial difficulties' were associated with children's adherence to ART.

Fear of disclosure. It was revealed that most of the participants did not disclose the diagnosis to their child or other relatives. They reported that they hid the medicine and removed the labels from the boxes, especially when they went on a vacation or had guests because they were concerned about stigma (13/15).

'… the only difficulty we have is that we don't tell anyone. When someone comes to our house, we always hide all the medicine, we take out the boxes, labels, etc. just in case they accidentally see them. Sometimes, when we visit our friends or relatives, we can't give [our child] the medicine there either.' (P-8, father of 5-year-old girl)

Forgetfulness. The participants stated that they had a lot of responsibilities because they had to take care of household chores and their other children, and they occasionally forgot to give their child medication (5/15).

'…I have to attend to my other child. I forget about it [administering medication], I am dealing with other things, sometimes I forget.' (P-14, mother of 3.5-year-old boy)

Financial difficulties. Although the medicines they used were free of charge, the participants stated that in some cases, they had to pay a fee (4/15).

'They have pills there but not syrup. I purchase the syrup from abroad. It's a bit expensive, let's say four bottles, 800 euros, they last for three months (Lopinavir/ritonavir, syrup). So, [I purchase it] as long as I can...' (P-2, mother of 42-month-old boy)

Attitudes of caregivers. According to the participants, they often had difficulties in the treatment of their children; some participants tried hard and used different methods to give their children the medication, while some gave up when they were unable to have their children take the medicine (1/15).

'He doesn't take [the medicine] in any way, I mean, I've tried in every way. I've given up these days.' (P-2, mother of 42-month-old boy)

Theme 4: Healthcare system-related barriers

The participants stated that 'access to medication' and 'lack of information' were barriers to their children's adherence to ART.

Access to medication. It was determined that the participants had problems in access to drugs that were prescribed with a medical report and that were available free of charge from pharmacies (4/15).

'It's been a week since we were able to get the tablet. There was a problem with the medical report.' (P-6, mother of 7-year-old boy)

Lack of information. The participants stated that they had received insufficient information about the preparation steps of medicines, such as how to calculate the dose and how to administer it (4/15).

'At first, we had a very hard time. Then we talked to the doctor, and he said you can put it in her formula. I had already found this on my own (this method) and I could not give it in any other way.' (P-3, mother of 20-month-old girl)

Facilitators of Adherence to Antiretroviral Treatment

Theme 1: Medication-related facilitators

It was determined that for the participants, 'benefits of medication' and 'the structure of medicines' facilitated the compliance of their children with ART.

Benefits of medication. The participants stated that they observed the positive effects of the medication and that these effects were influential in their administration of the medication to children regularly (5/15).

'There's no problem if he takes the medicine. He needs to take the medicine for his survival and immunity.' (P-11, mother of 17-year-ol boy)

Structure of drugs. It was found that the children of the participants found it easier to take drugs in the form of syrups compared to tablets (2/15).

'There is no difficulty with syrups. She already has [other] syrups with a spoon, there is no problem with syrups.' (P-3, mother of 20-month-old girl)

Theme 2: Child-related facilitators

Willingness to take medication. Some participants stated that their children were used to the treatment and were willing to take their medication (2/15).

'...it's very bitter, but she got used to it, and now she takes the medicine herself.' *(P-8, father of 5-year-old girl)*

Theme 3: Caregiver-related facilitators

Caregiver-related facilitators were identified as 'caregiver's attitudes', 'strategies for remembering', and 'disclosure of HIV status to others.'

Attitudes of the caregiver. It was stated by the participants that they had a lot of difficulties while administering the medication, but most of them had their children take the medication even if they had to persuade them to take it (7/15).

'I say to him, if he doesn't take the medicine, I would turn off the cartoon [on TV]' (P-14, mother of 42-month-old boy)

Strategies for remembering. Some participants reported that they used some strategies to have their children take their medication (4/15).

'We set an alarm on the phone, otherwise, we forget.' (P-13, mother of 17-year-old boy)

'You know, if I go somewhere, I always carry my spares in my bag in case the schedule is disrupted. In case I need to go somewhere urgently, I immediately grab it before leaving.' (P-3, mother of 20-month-old girl)

Disclosure of HIV status to others. The participants who disclosed the HIV diagnosis of their children stated that they received support from their close relatives in the treatment of their children (2/15).

'He doesn't take his medicine with anyone else. Either with me or with my brother. My mom, my brother, my whole family, they all support me.' (P-2, mother of 42-month-old boy)

Theme 4: Healthcare system-related facilitators

Quality of care. Some participants expressed their view that they were satisfied with the quality of service they received, and their children were well cared for (3/15).

'The healthcare workers here are very good. God bless them all. They are very protective. Like a family.' (P-10, mother of 10-year-old boy)

Discussion

This study explores the barriers and facilitators to pediatric ART adherence in Türkiye, highlighting caregivers' challenges and the need for targeted interventions.

Barriers to adherence

Medication-related barriers were the most frequently stated barriers by the participants. Since the children had difficulty taking the drugs in pill form, they were more exposed to their bitter taste and thus experienced nausea and vomiting. The participants of this study often tried to disguise the bitter taste of medicines by mixing them with other foods, which was similar to other methods mentioned in previous studies[22], but children

still have negative experiences with ART, such as nausea and vomiting. Our results, as in previous studies, showed that factors leading to difficulties in drug administration such as drug size[17,23], negative experiences including side effects[8,17,23,24], the numbers and timing of taking medication[17,23] affect children's adherence to ART. Similar to the results of studies with adolescents, activities of daily living, such as school or sleep prevented adolescents from taking their medication at the right time.[18] Therefore, long treatment durations may cause fatigue and boredom in children.[8,17,25]

The World Health Organization (WHO) has consistently revised its treatment protocols for individuals living with HIV in response to emerging evidence and clinical needs. In line with these efforts, the Paediatric Drug Optimization (PADO) initiative was launched in 2013 to establish strategic priorities for the development of antiretroviral therapies tailored to children.[26] This initiative has substantially contributed to the advancement of several high-priority pediatric ART formulations, including dispersible fixed-dose combinations (FDC) and long active injectable options. However, these formulations of dispersible FDCs remain largely inaccessible in many low- and middle-income countries (LMICs), including Türkiye.[27] The United States Food and Drug Administration (FDA) approved cabotegravir–rilpivirine (CAB/RPV) in 2021 as the first long-acting injectable treatment for adults living with HIV.[28] However, the development of such injectable therapies has also been identified as a long-term priority under the PADO-3 framework.[29] Children have difficulty taking oral medications, especially tablets, due to factors such as taste, smell, and the need for daily intake, which negatively affects medication adherence. Therefore, it is argued that long-acting injectable therapies should also be prioritized within pediatric HIV treatment research.

This gap between global drug development and local availability becomes particularly problematic during the formulation of individualized treatment plans for children. For example, lopinavir/ritonavir (LPV/r), one of the very few ART currently available in a liquid formulation for children, has been reported to have poor palatability in the literature[30,31]; however, one caregiver in our study stated that they were unable to administer the tablet form to their child and therefore resorted to obtaining the syrup formulation from abroad at a high cost. Therefore, ART regimens should be individualized, and access to appropriate pediatric drug formulations must be improved. If palatable options are not available, caregivers should be supported with strategies to increase children's compliance with unpleasant-tasting medications, such as numbing the taste with ice chips or masking the taste with sweet or tangy foods.[30]

The developmental stage of a child affects their behaviors of adherence to ART. Similar with our results, caregivers have reported that children aged 1-4 years refuse and avoid medication during administration whereas preschool children inquire as to why they are taking medication.[23,32] According to the participants of our study, adolescents who knew their diagnosis but were not fully informed were reluctant to take their medication, especially when they were asymptomatic or felt well. This is thought to be closely related to the explanation of the diagnosis. Recent studies have shown that the disclosure of HIV status to adolescents and the closest members of the family plays an important role in improving medication adherence.[9-11,33] The disclosure of HIV status to children helps them better understand their need to adhere to their medication regimen.[5,9,10,18] Nevertheless, HIV and AIDS are still taboo in Türkiye, and they are perceived as fatal. Thus, parents may be unwilling to disclose this condition to their children and other family members. This is also supported by the fact that sexual relationships are one of the transmission routes of HIV, and sexuality is not yet a topic that is easily discussed in Turkish society.

Caregivers may experience self-stigma when they internalize the societal stigma toward people living with HIV, which can lead to

feelings of guilt and shame. These emotions may result in withdrawal from social environments, delaying taking or giving medication in front of family members or friends, avoiding seeking support, and eventually becoming socially isolated. Consistent with previous research, our results showed that caregivers removed medication labels, skipped doses, or inadvertently missed medication times for fear of disclosure or stigmatization.[22] Disruptions in medication regimens are known to negatively affect adherence to ART.[3,5,34] Although this study did not include clinical data such as viral load measurements or drug resistance testing, the adherence-related challenges reported by caregivers such as fear of stigma and lack of social support and emotional distress are known to contribute to poor treatment outcomes. Previous mixed-method studies have shown that similar psychosocial and structural barriers can lead to virological failure and increase the risk of drug resistance in pediatric and adolescent populations living with HIV.[35,36]

The barriers related to the health system were stated by the participants of this study as delays in the electronic system for accessing drugs for free and their lack of information counseling on drug preparation. Drug reports and the pharmacy system need to be synchronized to avoid delays in accessing these drugs. Otherwise, treatment may be interrupted, and parents may have to purchase these medications and experience financial difficulties. Additionally, combined antiretroviral therapies may contribute to stock-outs and pose challenges for treatment adherence in young children.[27] However, beyond adherence, access to these medications is a fundamental right for children. Therefore, it is essential for health systems to develop proactive strategies that can anticipate and address delays in electronic systems or stock-out situations. Previous studies have suggested that lack of access and inadequate counseling on the use of medications negatively affect compliance with treatment.[3,18,22]

Facilitators of adherence

According to the results of this study, the themes that facilitated children's adherence to ART varied. Medication-related facilitators included the caregiver's observation of the benefits of ART on children and the syrup form. The benefits of ART are known to positively affect children's adherence to treatment.[35] The observation of the positive effects of drugs on children is thought to be a source of motivation for caregivers to continue their children's treatment regularly.[37] Secondly, although it is known that the syrup form of drugs is an important factor in treatment compliance in terms of taste and easy administration[23] very few children were able to access syrup forms in Türkiye because these forms were not covered by the healthcare system. This also posed financial challenges as a barrier for parents in ensuring their children's adherence to ART. It is vital to facilitate access to the syrup form of ART and improve the taste and size of pills so that children can take them easily. Considering all the barriers and facilitators related to medication, one of the most important interventions to facilitate adherence to ART is access to the syrup form.

Many studies have reported the importance of caregiver support for optimal ART adherence.[10,11,19,38,39] Consistent with previous results, caregivers made sure that their children adhered to treatment by persuasion or bargaining. Moreover, caregiver strategies such as reminders of medication time, setting an alarm, or carrying a spare bag facilitated adherence to ART in both young children and adolescents.[17,18] During clinic visits, frequent reminders provided to caregivers about the routine use of techniques such as setting alarms to remember medication times or carrying spare bags may positively affect children's adherence to ART.

As mentioned earlier, some caregivers of children whose treatment was followed up in the same clinic reported the quality of health services as a facilitator, while some caregivers

reported their lack of information about the health system as a barrier to their children's adherence to ART. This finding suggested that children and caregivers followed in the same clinic may not benefit from the same quality of care. Previous studies have shown that family-centered care help improve adherence to ART.[3,10]

Strengths and limitations

This study includes data based on the self-reports of caregivers. The possibility of bias in parental/caregiver statements constitutes a limitation of our study. In terms of the generalizability of the results, our sample represented a small group of 15 caregivers from a single urban center, which limits the transferability of findings to rural settings or diverse healthcare institutions across Türkiye. Additionally, the study did not examine how intersecting social determinants such as maternal age, education, economic status collectively influence adherence, which may be better addressed in future research. The strength of our study is that it is the first study to our knowledge, conducted in Türkiye to investigate the situations that facilitate and complicate the adherence of children to ART from the perspectives of their caregivers. Our study also sheds light on the experiences of caregivers regarding children's adherence to ART in Türkiye.

Conclusion and recommendations

Adherence to ART in the pediatric population is a complex and dynamic process. Caregivers identified more barriers than facilitators to children's adherence to ART, with the most significant barriers related to negative experiences and medication forms. Factors such as the child's developmental stage and the disclosure status of the diagnosis to the child or their environment also played a critical role. Not disclosing the diagnosis to the child often leads to uncertainty and reluctance about treatment, while withholding the diagnosis from the community can disrupt medication schedules due to challenges in administering medication in social settings. These findings highlight the need to further investigate adolescents' self-perceptions of HIV and ART, particularly in relation to disclosure and treatment autonomy. Caregivers, as central figures in managing pediatric HIV treatment, face significant difficulties in ensuring adherence. Health professionals, in their educator role, should provide targeted support to caregivers, offering strategies to address these challenges and improve medication administration. Effective treatment efforts must consider the interconnected influences of the child, caregiver(s), medication regimens, healthcare system, and sociocultural factors. Further research is essential to explore pediatric adherence to ART in Türkiye, particularly using larger, more diverse samples, to develop comprehensive and context-specific interventions. Employing mixed-methods designs could further enrich findings by integrating in-depth qualitative insights with measurable adherence outcomes.

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Ethical approval

The study was approved by Istanbul University's Social Sciences Ethics Review Board (1109334/22.08.2022). Additionally, institutional permission was obtained before the study. Before the interview, informed consent was taken from all participants for participation to the research and record audio of interviews. Due to the sensitive nature of the research topic, before each interview, the researcher reminded the participants that participation was voluntary and that they could cease participation at any time during the interview.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: AA, ÖŞ, AS; data collection: ÖŞ, AD, NMA, ED; analysis and interpretation of results: all authors; draft manuscript preparation: ÖŞ, SB. 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

Recognizing the overlooked: rethinking autism spectrum disorder symptom presentation in girls

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ABSTRACT

Background. Autism spectrum disorder (ASD) is more frequently diagnosed in boys than in girls, possibly due to gender-based differences in symptom presentation or referral patterns. This study investigates gender-related variations in symptom severity and clinical presentation among preschool children referred for suspected ASD.

Methods. This study included 125 children (boys: n=103; girls: n=22) aged 2–5 years suspected of having ASD. The Childhood Autism Rating Scale (CARS) was used to evaluate autism-related symptoms, focusing on presenting complaints and gender-specific differences in nonverbal communication and social interaction.

Results. Girls had a significantly younger median age at assessment (28 months) compared to boys (33 months, p=0.03). In the minimal to no symptoms group, girls had significantly higher total CARS scores (median 26 vs. 22.5, $p < 0.001$) and elevated ratings in domains such as nonverbal communication (p=0.03), relationship to people (p=0.01), imitation ($p < 0.001$), and visual response ($p < 0.001$). In the severe group, girls also showed significantly higher scores in adaptation to change, taste, smel, and touch response and use, and fear or nervousness. Effect sizes ranged from small to strong. A negative correlation was found between assessment age and total CARS score ($r= –0.45$, $p < 0.01$), particularly among girls.

Conclusion. This study highlights that girls may exhibit more prominent symptoms by the time they are referred for clinical evaluation, raising concerns about missed or delayed recognition of milder symptom profiles.

Key words: autism spectrum disorder, sex characteristics, female, child, preschool, diagnosis.

Autism spectrum disorder (ASD) is an early-onset neurodevelopmental disorder characterized by difficulties in social interaction and communication skills, as well as restricted and repetitive behaviors.[1] In recent years, the prevalence of autism has significantly increased, based on data from 2016, ASD prevalence estimates stand at 18.5 per 1000 (1 in 54) children by the age of 8, with rates among boys being 4.3 times higher than among girls.[2]

The difference in prevalence between boys and girls may be attributed to factors such as gender bias in diagnosis or genuinely better adaptation/compensation in girls. Considering that diagnosis heavily relies on a comprehensive assessment of personal history and direct observation of behaviors, and early diagnosis and intervention are critical in autism, the disproportionate diagnosis in males compared to females emerges as an issue warranting

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closer scrutiny. Several hypotheses have been formulated to explore whether male-specific risk factors and female-specific protective factors underlie this bias. However, it is important to note that the male risk and female protective factors are not mutually exclusive. It is suggested that both may contribute to the discrepancy observed in ASD diagnosis.[3] Research indicates that a greater number of concurrent behavioral or cognitive difficulties may need to be present in girls for the disorder to be identified.[4] While in girls, ASD incidence remained low until age 10, then increased, peaking in early adolescence; for boys, incidence sharply increased from birth, peaking at age 4, remaining steady until age 15, then declining. It is also supported by some evidence that adult women are seeking and receiving autism diagnoses to a greater extent than men.[5,6] It is possible that females have been even more disregarded at a younger age compared to their male counterparts, and indeed, current evidence supports the existence of a "female-typical autism presentation".[4] Understanding how sex and gender affect clinical presentation, biology, developmental trajectory, and treatment response is not only crucial for accurate diagnostic assessment but also effective intervention planning, and promoting societal gender equity.[7]

While recent literature increasingly addresses gender differences in autism, there remains a critical gap in identifying the more subtle and subthreshold symptom presentations often observed in girls. These may include milder or masked social communication difficulties and fewer observable restricted behaviors, particularly during early childhood.[8,9] Such nuanced presentations may not meet the conventional diagnostic threshold, yet still cause functional impairment and delay in intervention.[4,10] Standardized diagnostic tools, predominantly validated in male populations, may overlook these less overt manifestations, contributing to the underrecognition of ASD in females.

In this study, our aim was to investigate whether there were gender-related differences in the presenting complaints and observational assessments using standard measures among children suspected of having ASD, including those with subtle symptoms, given the critical importance of early diagnosis. We hypothesized that symptom severity and presentation patterns would differ between girls and boys, particularly across ASD severity strata, as measured by item-level scores on a standardized assessment.

Materials and Methods

To investigate sex differences in core symptoms and referral characteristics of ASD, we enrolled children with suspected ASD who were assessed using the Childhood Autism Rating Scale (CARS). The study was approved by the Ethics Committee of Hacettepe University, in accordance with the Declaration of Helsinki.

Participants and study procedure

The target population of the study consisted of children who were assessed using the CARS. These children had initially been referred to the Division of Developmental Pediatrics by general pediatricians or family physicians. In our division, developmental pediatricians perform the initial clinical evaluation using a structured form that encompasses detailed information on developmental milestones, behavioral concerns, and family observations. Following this evaluation, the CARS is administered to children for whom autism-related signs or parental concerns raise suspicion of ASD. In this context, children aged 24-60 months who were evaluated using CARS at the Hacettepe University İhsan Doğramacı Children's Hospital, Division of Developmental Pediatrics between December 2020 and December 2023 were included in the study, The exclusion criteria included: (1) a history of receiving special education for more than one month, (2) a history of other neurological or genetic disorders, such as Rett syndrome, cerebral palsy, epilepsy, or severe head injury, (3) hearing or visual impairment.

The participants' basic sociodemographic data, presenting complaints, and CARS scores were obtained retrospectively from the patient records. The CARS assessment is based on inter-rater agreement between two observers, who evaluate the child–caregiver dyad through a mirrored playroom and also engage directly with the child and caregiver through structured interaction and interview. This scale was developed in 1971 by Schopler and Reichler to diagnose and assess autism. This scale consists of 15 items and is completed by clinicians based on interviews with families, gathering information from relevant individuals, and observing the child. The items include relationship to people, imitation, emotional response, body use, object use, adaptation to change, visual response, listening response, taste, smell, and touch response and use, fear or nervousness, verbal communication, nonverbal communication, activity level, level and consistency of intellectual response, and general impressions. A total score on the scale ranging from 30 to 36.5 indicates mild-to-moderate autism, while a score between 37 and 60 indicates severe autism. The validity and reliability study of the Turkish version was conducted.[11,12]

Statistical analysis

Statistical analysis was conducted using SPSS version 19.0 software (SPSS Inc). The normality of continuous data was assessed using both statistical tests and visual methods such as histograms and Q-Q plots. Continuous variables were summarized using means and standard deviations (SD) or medians and interquartile ranges (IQR). Categorical variables are summarized using numbers and percentages. Categorical variables were compared using the $\chi 2$ test or Fisher's exact test. Differences in continuous variables among independent groups were assessed using the independent samples t-test for two groups. The Mann-Whitney U test compared continuous variables that were not normally distributed. Spearman's rank correlation coefficient evaluated correlations between non-normally distributed variables. A significance level of $p \leq 0.05$ was considered statistically significant.

Results

The study included 125 patients. Table I presents the sociodemographic characteristics of the study participants, showing no significant differences between girls (n=22) and boys (n=103) in terms of gestational age, maternal age, and paternal age. However, there was a statistically significant difference in the assessment age (p=0.03), with girls having a median age of 28 months and boys 33 months. Other factors, such as maternal and paternal education levels, and birth order, were similarly distributed between genders.

Speech delay was the primary complaint in 95.2% of girls (n=20) and 81.6% of boys (n=84), with no significant difference between groups. Non-response to name was reported as the main complaint in 9.8% of all cases (n=12), while lack of eye contact appeared as the primary complaint in 7.4% (n=9), with no significant difference observed based on gender ($p > 0.05$).

Among the girls who participated in the study, 50% (n=11) and 51.5% (n=53) of the boys had minimal to no symptoms of ASD according to CARS. There was no significant gender difference in the ASD severity group distributions (p>0.05, Table II).

When evaluating autism symptoms according to severity and gender, it was found that in the group with minimal to no symptoms of ASD, the median total CARS score for girls was significantly higher than that for boys (26 vs. 22.5; p=0.00), with a large effect size (r=0.819). The median assessment age for girls in this group was also significantly lower (28 vs. 34 months; p=0.02). Girls showed significantly higher scores on CARS items 1, 2, and 7, with moderate to strong effect sizes (r=0.524, 0.404, and 0.394, respectively). Although not all comparisons reached statistical significance, items 5, 8, and 12 also demonstrated moderate effect sizes, suggesting meaningful differences.

Table I. Basic sociodemographic characteristics.

Variable	Girls (n=22)	Boys (n=103)	Total (n=125)	p	Effect size
Assessment age (months); median (IQR)	28 (25-33)	33 (29-41)	32 (27-38)	**0.03***	0.192
Gestational age (weeks), median (IQR)	39 (38-40)	38 (38-40)	38 (38-40)	0.23	0.108
Maternal age (years), mean ± SD	32 ± 5.7	32 ± 5.6	32.02 ± 5.75	0.72	0.083
Maternal education, n (%)				1	0
< High school	10 (47.6%)	41 (46.6%)	51 (46.8%)		
≥ High school	11 (52.4%)	47 (53.4%)	58 (53.2%)		
Paternal age (years), mean ± SD	36 ± 6.6	36 ± 5.2	36 ± 9	0.87	0.04
Paternal education, n (%)				0.63	0.03
< High school	12 (57.1%)	42 (50%)	53 (51.4%)		
≥ High school	9 (42.9%)	42 (50%)	51 (48.6%)		
Birth order, n (%)				0.14	0.18
First	15 (68.2%)	54 (52.9%)	69 (55.6%)		
Second	4 (18.2%)	41 (40.2%)	45 (36.3%)		
Others	3 (13.6%)	7 (6.9%)	10 (8%)		

*$p < 0.05$.
Statistical tests: Mann–Whitney U test was used for comparisons of non-normally distributed continuous variables (assessment age and gestational age). Independent samples t-test was used for normally distributed continuous variables (maternal and paternal age). Categorical variables (parental education, birth order) were compared using chi-square tests or Fisher's exact test where appropriate. Effect sizes are reported as Cohen's d for parametric comparisons and r for non-parametric tests. Effect sizes are reported as r for non-parametric tests, Cohen's d for parametric comparisons, and Cramér's V for categorical variables.
IQR: interquartile range, SD: standard deviation.

Furthermore, correlation analyses revealed that younger assessment age was associated with higher scores on items 1, 2, 3, 5, 6, 12, and 15, as well as the total CARS score (r= –0.37, –0.41, –0.30, –0.34, –0.27, –0.34, –0.32, –0.45 respectively; $p < 0.05$). Similarly, among girls with severe symptoms, it was noted that their scores on items 6, 9, 10, and total scores of the CARS were significantly higher than those of boys. These differences had small to moderate effect sizes (r=0.25–0.31, Table II).

Discussion

During toddlerhood, the earliest stage for diagnosing autism in children, understanding gender differences is crucial.[13] In this study, children aged 2-5 years who were suspected of having ASD based on parental concern and clinical observation were systematically evaluated, and it was found that some autism symptom scores were found to be higher in girls across varying severity levels, with different symptoms being more pronounced in each group. To the best of our knowledge, this study is the first to investigate gender-related symptom differences assessed via the CARS in a preschool-aged clinical population in Türkiye.

There is conflicting evidence regarding whether boys and girls with ASD exhibit differences in symptom severity. Cognitive differences may complicate the comparison of symptoms since symptom severity often correlates with impairment levels. Despite controlling for IQ, studies have produced inconsistent results. While some research indicates similar scores in observational assessments, other studies have identified sex-related differences in ASD symptom severity and profiles, even when IQ is accounted for.[14] In this study, girls in both the minimal to no symptoms group and the severe symptoms group had significantly higher total scores than boys, while no significant difference was observed in the moderate symptom group.

Table II. Autism spectrum disorder related symptoms according to gender and severity of autism.

	Minimal to no symptoms of ASD (CARS score 15-29.5)				Mild to moderate symptoms of ASD (CARS score 30-36.5)				Severe symptoms of ASD (CARS score 37-60)			
	Girls (n:11)	Boys (n:53)	p	Effect size (r)	Girls (n:7)	Boys (n:36)	p	Effect size (r)	Girls (n:4)	Boys (n:14)	p	Effect size (r)
Assessment age (months)	27 (24-30)	34 (28.5-39.5)	**0.02***		28 (24-32)	31 (25-37)	0.33	0.147	36 (31-40.5)	36 (32-42)	1	0
CARS Scores												
1. Relationship to people	2 (2-2.5)	2 (1.5-2)	**0.01***	0.524	3 (2.5-3.5)	3 (2.5-3.5)	0.98	0.02	3.5 (3-4.5)	3 (2.5-3.5)	0.69	0.049
2. Imitation	2 (1.5-2.5)	1 (1-1.5)	**0.0***	0.404	2.25 (1.5-3.5)	2.75 (2-3.5)	0.23	0.182	3 (2.5-4)	3.25 (3-3.5)	0.43	0.099
3. Emotional response	1.5 (1-2)	1.5 (1-2)	0.8	0.539	2.5 (2-3)	2 (1.5-3)	0.27	0.170	3 (3-3.25)	3 (3-3)	0.42	0.101
4. Body use	1 (1-1)	1 (1-1.5)	0.36	0.3	2 (1.5-3)	2 (1.5-3)	0.25	0.176	3 (3-3)	2.75 (2.25-3.75)	0.51	0.083
5. Object use	2 (1.5-2.5)	1.5 (1-2)	0.07	0.478	2 (2-2)	2 (1.5-3)	0.13	0.231	2.75 (2-3)	3 (2.5-3.5)	0.46	0.092
6. Adaptation to change	1 (0.5-1.5)	1 (1-1.5)	0.37	0.194	1 (0.5-1.5)	1.75 (1-2.5)	0.07	0.279	3.25 (2.75-4)	2.25 (2-2.5)	**0.01***	**0.307**
7. Visual response	2 (2-2.5)	1.5 (1-2.5)	**0.00***	0.394	2.5 (2.5-3)	2.25 (1.5-3)	0.55	0.091	2.5 (1.5-3.5)	2.5 (2.25-2.75)	0.43	0.098
8. Listening response	2 (2-2)	1.5 (1-2.5)	0.07	0.379	2.5 (2.5-3)	2.5 (2-3)	0.67	0.0639	3 (3-3.25)	3 (2.75-3.25)	0.22	0.154
9. Taste, smell, and touch response and use	1 (1-1)	1 (1-1)	0.98	0.062	1 (1-1.5)	1 (1-2)	0.63	0.073	2.75 (2-3.5)	1 (1-1.5)	**0.02***	**0.285**
10. Fear and nervousness	1 (1-1)	1 (1-1)	0.84	0.122	1.25 (1-1.5)	1 (1-2)	0.94	0.012	2.75 (2-4)	1.25 (1-2.25)	**0.02***	**0.304**
11. Verbal communication	2 (1-3)	2.5 (1.5-3)	0.53	0.253	3.25 (2.5-3.5)	3 (2.5-3.5)	0.80	0.04	3.25 (2.5-5)	4 (3.5-4.5)	0.13	0.191
12. Nonverbal communication	2 (2-2.5)	1.5 (1-2.5)	**0.03***	0.36	3 (2.5-3.5)	3 (2-4)	0.76	0.047	3.25 (2.5-5)	3 (3-3)	0.4	0.106
13.Activity level	1 (0-1.5)	1 (1-1.5)	0.27	0.031	2 (1.5-2.5)	1 (1-2)	0.29	0.163	2 (1.5-3)	1.5 (1-2)	0.35	0.117
14.Level and consistency of intellectual response	1 (0.5-1)	1 (0.5-1)	0.28	0.357	2 (1.5-3)	1.75 (1-2.5)	0.47	0.109	2.5 (2-3)	2.75 (2.25-3.25)	0.62	0.063
15. General impressions	2 (1.5-2.5)	1.5 (1-2)	**0.04***	0.086	2.25 (1.5-3)	2.5 (2-3)	0.26	0.172	3.25 (2.25-4.25)	3 (3-3)	0.57	0.072
Total Scores	26 (25-27)	22.5 (20-25)	**0.0***	0.819	32 (29-35)	32.75 (30.5-35)	0.29	0.161	42.5 (41-45.5)	38.75 (35.25-42.25)	**0.05***	**0.247**

*p<0.05.

Data presented as median (interquartile range). All comparisons were conducted using the Mann–Whitney U test due to the small sample sizes and non-normal distribution of the data. Effect sizes are reported as r.

CARS: Childhood Autism Rating Scale.

When examining gender differences in ASD symptoms, communication skills emerge as one of the most notable areas of difference. While typically developing girls have been shown to have better early communication skills, such as better receptive language skills and using more words for communication compared to boys during the infant period, this slight advantage isn't observed in the ASD group.[15] During the toddlerhood period, studies have reported variable results depending on whether they rely on observational data or parent reports. While parents often report that girls with ASD reach language milestones earlier, findings from direct clinical measurements of language in children diagnosed during toddlerhood demonstrate similar or worse linguistic and verbal abilities compared to boys. Additionally, it has been shown that the acquisition of gestures and pragmatics was more impaired in the female subgroup than in the male subgroup of children with ASD, aged between 2 and 7 years old.[16] In our study, the finding that the nonverbal communication scores of girls were higher than those of boys in the minimal to no symptoms of ASD group not only aligns with the literature but also points to a very important and distinct aspect. Given that girls are generally expected to perform better in communication domains, the fact that those who did seek hospital evaluation still exhibited higher symptom scores may indicate a selection bias.

Another significant symptom domain believed to vary by gender notable findings concerns repetitive and restrictive behaviors. While data suggest a higher prevalence of these behaviors in males among older age groups, studies similar to ours have reported no significant gender differences in children under six years of age.[17] Conversely, data from the Autism Treatment Network suggest that females under six years old, with at least average IQ, do not consistently display significantly fewer stereotyped behaviors compared to their male counterparts.[18] The tendency for girls to engage in gender-typical play may lead to these behaviors being overlooked in girls during toddlerhood.[19]

Girls are often diagnosed later and at lower rates than boys, and some studies suggest that they may exhibit more complex or subtle social communication profiles.[13] In our data, girls with minimal to no symptoms scored higher than boys in several areas including nonverbal communication, relationship to people, imitation, and visual response. This observation suggests that girls with subtler symptoms — who may still be experiencing challenges — might not have been referred for clinical assessment at all, potentially representing only the tip of the iceberg. While our findings are preliminary and limited to a small clinical sample, they align with this perspective and may contribute to a better understanding of gender-related presentation differences. These observations highlight the need for future research on the development of diagnostic tools that are better attuned to gender-related nuances.

This study is particularly valuable as it includes a detailed assessment of subthreshold ASD symptoms in girls. It provides a structured comparison across symptom severity levels and incorporates item-level analysis using standardized tools, which strengthens the internal consistency of findings. Moreover, it is one of the few studies focusing on early clinical presentation in a preschool-aged sample, a period when timely recognition is especially critical for developmental outcomes. Studies from Turkey specifically investigating gender-related symptom differences in early childhood autism remain scarce. One early study compared clinical features of autistic girls and boys and suggested that girls may present with distinct symptom profiles, but national literature has offered limited updates since. Our findings aim to contribute updated and contextually relevant evidence to this underexplored area.[20]

However, several limitations should be acknowledged. First, the sample consisted of

clinically referred children, which limits the generalizability of the findings to the broader population. In the minimal to no symptoms group, girls had a younger median age, and there was a negative correlation between age and symptom severity, suggesting the need for further analysis. Although girls in this group were significantly younger at the time of assessment, this may reflect earlier referral due to more overt concerns rather than underrecognition. However, this pattern may also mask the risk that girls with milder symptom profiles are overlooked entirely, a possibility that underscores the complexity of interpreting gender-related diagnostic trends. Due to the small sample size and non-normal distribution, advanced statistical tests could not be performed. Although the CARS is a widely used and validated tool, it may not be sensitive enough to detect mild or subtle symptoms, especially in girls. Additionally, other standardized diagnostic instruments were not available in our clinic during the study period, which limits the assessment to a single scale. Despite the limited number of female participants in our, the female-to-male ratio (approximately 1:5) reflects the gender distribution commonly reported in clinical ASD samples.[2] Although we focused on symptom severity to examine gender-related differences, the smaller number of girls in each severity range limited the statistical power of our analyses. This remains an important limitation, and future studies with larger and more balanced samples are needed to confirm and build upon these findings. Nevertheless, the study's focus on early clinical presentation and its attempt to explore symptom variability among early ages contribute valuable insights to the literature and may inform future gender-sensitive assessment strategies. In light of these limitations, future studies with larger and more balanced samples, ideally drawn from population-based cohorts, are needed to more effectively address the research question

In summary, our findings suggest that girls may be referred for clinical evaluation only when their symptoms are more pronounced. In the group with minimal to no symptoms, girls had significantly higher total scores and elevated ratings in domains such as nonverbal communication, imitation, and social interaction. These results point to a potential referral bias and underscore the risk that milder difficulties in girls may go unnoticed. Although the sample size was limited, particularly for females, this study highlights the importance of early, gender-sensitive approaches and calls for further research using larger and more balanced samples.

Given the critical importance of early diagnosis, it is essential that girls are not overlooked, ensuring they gain timely access to interventions, which is of significant importance for society as a whole.

Ethical approval

The study was approved by Ethics Committee of Hacettepe University (2023/04-18).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: AMY, EÖ, ENÖ; Data collection: HCİ, ECÇ, EÖ, ŞK; Analysis and interpretation of results: AMY, EÖ, ECÇ; Draft manuscript preparation: AMY, HCİ, ŞK, EÖ. 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

Spastic cerebral palsy and quality of life in children aged 6-12 years: exploring key associated factors

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ABSTRACT

Background. Children with cerebral palsy (CP) may experience epilepsy and challenges with movement, posture, cognition, and musculoskeletal development, which can impact their quality of life (QOL). In this study, we investigated the relationship between demographic and clinical variables as well as QOL in children with spastic CP.

Methods. Children aged 6 to 12 years with CP who were followed-up at our tertiary center were included in this cross-sectional study, regardless of the cause. They were categorized into groups based on their gestational age, motor function levels, accompanying conditions such as epilepsy and intellectual disability, and demographic variables, including mothers' education and income levels. Subsequently, the QOL scores of these groups were compared. Among the 9-12 age group, those with sufficient intellectual capacity completed the QOL questionnaire by both the mothers and patients themselves. The Children's Sleep Habits Questionnaire (CSHQ) was evaluated and compared with the QOL scores of the patients.

Results. A total of 71 patients were included in the study (42 males, 59%). Children whose mothers were more educated and had higher in income level, who were ambulatory with hemiplegia, and did not have epilepsy had significantly better QOL scores. Those with better CSHQ scores were found to have significantly better QOL scores. Additionally, the responses of mothers and patients within the 9-12 age group were highly compatible.

Conclusion. Children with CP face challenges impacting their daily lives and overall QOL. Our study identified factors linked to the QOL of children with spastic CP and showed that their integration into CP management could enhance their well-being.

Key words: cerebral palsy, epilepsy, quality of life, sleep disorder.

Cerebral palsy (CP) is a neurological condition characterized by motor and postural impairments that result from a non-progressive brain injury in the fetal or infantile stage of development.[1] This condition often leads to limitations in physical activity, and it frequently accompanies sensory, cognitive, communication, and behavioral difficulties, as well as epilepsy and secondary musculoskeletal problems.[2]

The World Health Organization Quality of Life (WHOQOL) Group defines quality of life as an individual's personal perception of their position in life, taking into account their cultural and value systems, goals, expectations, standards, and concerns.[3] Various scales are used to assess quality of life (QOL) in children with CP. The first questionnaire designed for this purpose is Cerebral Palsy Quality of Life

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for Children (CPQOL-Child), and it involves domains for both children and caregivers.[4]

The CPQOL-Child questionnaire measures a child's well-being rather than their ill-being. Atasavun Uysal et al.[5] confirmed the reliability and validity of this questionnaire by translating it into their native language of Turkish for both the caregiver and child versions. Although there are several studies reporting poorer QOL in children with CP, limited number of studies comprehensively evaluate the factors affecting quality of life alongside all the neurological variables of patients.[6-9]

The most commonly observed form of CP is the spastic type, which can exhibit a wide range of clinical manifestations and may be accompanied by coexisting disorders. Thus, our research concentrated on children with spastic CP. We posited that the QOL for children with CP is not uniform. With this premise in mind, we intended to investigate the relationship between these factors and quality of life of children with CP by examining their clinical and demographic characteristics, including sociocultural factors, the distribution of spasticity, motor functions, intellectual levels, and comorbid conditions such as epilepsy, autism, and sleep disorders. Our secondary aim was to compare parents' and children's perspectives on QOL.

Materials and Methods

Patients and data collection

We planned a cross-sectional study between January 2020 and March 2021 at our tertiary center. Data regarding the QOL of the patients were collected by using CPQOL-Child. The questionnaire was administered during face-to-face appointments of the patients who visited our child neurology outpatient clinic for their regular follow-up. Demographic features including birth weight, gestational age, income and education levels of the parents were reviewed, and the Children's Sleep Habits Questionnaire (CSHQ) was also completed. Data of clinical and laboratory findings were obtained from their medical records, retrospectively.

We included the patients aged 6-12 years with spastic CP whose follow-up duration was longer than 2 years, whose height, weight, and body mass index (BMI) were within the normal limits, and who had a caregiver who was knowledgeable and available to provide information required for the QOL assessment. On the other hand, those who had not undergone proper brain magnetic resonance imaging (MRI), those for whom appropriate metabolic, genetic, and other differential diagnostic evaluations had not been completed, and those who had received botulinum toxin A injection or underwent any surgical procedure in the prior 6 months were excluded from the study.

The study was approved by institutional review board of Istanbul University-Cerrahpasa, Cerrahpasa Medical School (08/07/20-29430533-604.01-01-86126). The study complied with the recommendations of the Declaration of Helsinki for human biomedical research.

The classification of demographic data

Patients were grouped based on their birth weight into normal (2,500 g and above), low (2,500-1,500 grams), and very low (less than 1,500 g). Patients born at 37 weeks or later were classified as term, whereas those born before 37 weeks were classified as premature.

Since all of the patients' caregivers were mothers, the mothers were interviewed. Their age at the time of childbirth and educational level were recorded. Primary or elementary school graduates were categorized as low, while high school and university graduates were classified as high levels of education.

Each year, the Turkish government establishes a minimum monthly income to determine the poverty and hunger threshold. In our study, children's families were classified based on

income: Those below the hunger threshold were determined as low-income, between the hunger and poverty thresholds as medium-income, and above the poverty threshold as high-income groups.

The classification of cerebral palsy

Patients were classified into different groups based on the specific pattern of their spasticity, which included spastic quadriplegia, spastic diplegia, and spastic hemiplegia. The functional status of the patients was evaluated using the Gross Motor Function Classification System (GMFCS) and Bimanual Fine Motor Function (BFMF) classification systems.[10,11] By categorizing patients according to the severity levels of both functions, a better understanding of their motor skills was achieved. Specifically, patients with GMFCS and BFMF levels 1, 2 and 3 were grouped as having mild to moderate motor impairment, while those with GMFCS and BFMF levels 4 and 5 were grouped as having severe motor impairment.

Cerebral palsy associated disorders

In addition to clinical examinations, the Wechsler Intelligence Scale for Children-Revised (WISC-R) test was administered to assess the intellectual level and cognitive capacity of all patients. Patients with a WISC-R score of 70 and above were considered to have a normal intellectual level and cognitive capacity, while the others were defined as having intellectual disability and insufficient cognitive ability. DSM-V criteria were used to diagnose autism.[12] All patients were administered a one-hour wake-sleep video electroencephalography (EEG) examination, during which the 10/20 international electrode placement system was used to record EEG activity. Patients were assessed retrospectively for epilepsy diagnosis based on the current guidelines established by the International League Against Epilepsy (ILAE).[13]

Sleep disturbances

CSHQ, which is already shown to be valid and reliable in children living in Türkiye was used to assess whether the patients had sleep disturbances.[14,15] CSHQ is a parent questionnaire consisting of 33 multiple-choice and three open-ended questions. In addition to bedtime, morning wake-up time and total daily sleep duration, there are eight subscales reflecting different sleep domains in the questionnaire as follows: bedtime resistance, sleep onset delay, sleep duration, sleep anxiety, night waking, parasomnias, sleep-disordered breathing and daytime sleepiness. The caregivers of patients were requested to complete this questionnaire by assessing the previous week. The overall score ranged between 33 and 99, and a threshold of 41 was used to determine the presence of any sleep disruption.

Cerebral Palsy Quality of Life for Children Questionnaire

We assessed the QOL of our patients by applying the CPQOL-Child questionnaire which was validated and found to be reliable in the children of our country.[16] The CPQOL-Child questionnaire has also been used as self- and proxy-administered in different ethnic groups and has been found to be valid and reliable.[17,18] The questionnaire, which was completed by the caregivers, comprises seven domains: social well-being and acceptance, participation and physical health, functioning, emotional well-being and self-esteem, the consequences of disability and pain, family health, and accessibility to services. Among these, the first five domains were also administered to children aged 9–12 years, provided that they had sufficient cognitive ability to understand the questions. Therefore, while the full questionnaire was answered by caregivers of all children aged 6–12 years, only the child-directed section (covering the first five domains) was applied to children aged 9–12 years.

Statistical methods

Statistical analyses were performed using the NCSS (Number Cruncher Statistical System) software. Descriptive statistics included mean, standard deviation, median, frequency, percentage, minimum, and maximum values. The distribution of all numerical variables was assessed using the Shapiro-Wilk test and graphical methods, and all were found to follow a normal distribution. Therefore, comparisons between two groups were conducted using the Student's t-test, and the results were presented as mean ± standard deviation. Correlations between numerical variables were assessed using Pearson correlation analysis. A p-value of <0.05 was considered statistically significant. In addition, due to the relatively small sample size, effect sizes (Cohen's d) were calculated. Cohen's d values of 0.2, 0.5, and 0.8 were considered to indicate small, medium, and large effects, respectively, while values above 0.8 were interpreted as very large effects.

Results

Demographic data

One hundred patients were admitted for our study. Nevertheless, 29 of these individuals did not attend their follow up visits due to the COVID-19 pandemic during the data collection period. Consequently, 71 patients were included in the study (42 males, 59%) with a mean age of 8.34 ± 2.18 years. While almost half of our patients (n=35, 49.3%) were premature, less than half had normal birth weight (normal birth weight: n=34,47.9%, low birth weight: n=18, 24.4%, very low birth weight: n=19, 26.8%).

The median age of the mothers at the time of delivery were 28 (min-max: 15-48) years old. Most of the mothers (n=54, 76.1%) had low level of education. While the majority had medium income (n=47, 66.2%), and almost one third of the parents had low income (n=23, 32.4%). There was only one patient whose family had a high income level (1.4%).

The relationship between CPQOL scores and demographic-neurologic findings

Clinical features, EEG, and brain MRI findings of the patients are summarized in Table I. Overall CPQOL parent-proxy and CPQOL child-proxy scores of the patients were 348.7 ± 82.1, and 331.8 ± 44.1, respectively. The CPQOL parent-proxy scores were significantly better in the high-educated mother group than in the low-educated mother group (387.1 ± 64.2 vs. 336.6 ± 83.9; p=0.026; Cohen's d=0.6) and similarly they were better in the medium-income group than the low-income group (366.1 ± 79.6 vs. 312.3 ± 76.4; p=0.009; Cohen's d=0.68). Detailed data are given in Table II. The CPQOL scores were not significantly different between the normal, low, and very low birth weight groups. However, the scores were significantly better in preterm-born patients than in those with term gestational age (374.74 ± 79.6 vs. 323.44 ± 77.45; p=0.008) (The significant different domains were as follows: "Feelings about functioning", "Participation and physical health", "Emotional well-being", "Family health"). According to the classifications of both GMF (287.07 ± 69.9 vs. 393.85 ± 57.72; p=0.001) and BFMF (273.04 ± 66.1 vs. 337.38 ± 59.68; p=0.001), the patients in the severe group had significantly poorer QOL scores compared to the mild-moderate group. Furthermore, those with epilepsy (371.8 ± 77.59 vs. 315.31 ± 78.03; p=0.002; Cohen's d=0.72) and those with intellectual disability (405.48 ± 51.68 vs. 309.55 ± 76.42; p=0.001; Cohen's d=1.4) had significantly poorer QOL scores than those without. Detailed data are given in Table III.

Comparison of the relationship between CSHQ scores and CPQOL scores

The median CSHQ score of our patients was 45 (33-69), and 78.9% (n=56) of them had sleep disorders. There was a significant negative correlation between the CSHQ and CPQOL scores of patients (r=-0.237; p=0.047) (The significant different domains were as follow: "Bedtime resistance", "Delay starting sleep", "Duration sleep", "Parasomnia", "The amount of sleep"). Detailed data are given in Table IV.

Table I. Neurological examination and laboratory findings of children with spastic cerebral palsy (N: 71).

Examination / laboratory findings		n (%)
Spasticity limb involvement	Hemiplegia	17 (23.9%)
	Diplegia	30 (42.2%)
	Quadriplegia	24 (33.8%)
GMFCS level	Mild or moderate	41 (57.7%)
	Severe	30 (42.3%)
BFMF level	Mild or moderate	47 (66.2%)
	Severe	24 (33.8%)
Intellectual disability	Yes	29 (40.8%)
	No	42 (59.2%)
Autism	Yes	60 (84.5%)
	No	11 (15.5%)
Brain MRI	PVL	28 (39.4%)
	HIE	12 (16.9%)
	Vascular	8 (11.2%)
	Ischemic infarction	6 (8.4%)
	Bleeding	2 (2.8%)
	Congenital malformation	5 (7%)
	Encephalomalacia / porencephaly	5 (7%)
	CNS infection	4 (5.6%)
	*Operated brain tumor	3 (4.2%)
	Normal	6 (8.5%)
Epilepsy	Absence of epilepsy	42 (59.1%)
	Presence of epilepsy	29 (40.8%)

BFMF: Bimanual Fine Motor Function; CNS: central nervous system; EA: electrical activity; GMFCS: Gross Motor Function Classification System; HIE: hypoxic ischemic encephalopathy; MRG: magnetic resonance imaging; PVL: periventricular leukomalacia.
*:Patients underwent brain tumor surgery after the diagnosis of cerebral palsy.

Table II. Comparison of quality of life scores of the mothers according to education and income levels.

	Education Level				Income Level			
Domain of CPQOL-Child	Low (n=54)	High (n=17)	p	Cohen's d	Low (n=23)	Middle (n=47)	p	Cohen's d
Overall	336.6±83.9	387.1±64.2	**0.026**	0.6	312.3±76.4	366.1±79.6	**0.009**	0.68
Social well-being and acceptance	66.9±23.4	80.5±13.3	**0.004**	0.6	63.2±22.3	73.6±21.5	0.064	0.58
Feelings about functioning	64.2±23.3	76.2±22	0.065	0.5	58.3±21.7	71.3±23.2	**0.027**	0.57
Participation and physical health	53.8±24.3	67.4±19.3	**0.047**	0.58	46±22.3	62.3±22.9	**0.005**	0.72
Emotional well-being	37.6±10.7	42±6.9	0.177	0.4	34.9±9.1	40.4±10	**0.012**	0.56
Access to services	49.5±14	51.4±18.5	0.647	0.12	46.7±15.6	51.5±14.8	0.214	0.31
Pain and feeling about disability	31.8±11.4	30.6±13.3	0.730	0.1	31.7±11	31.4±12.3	0.915	0.02
Family health	24.3±7.1	25.7±8.6	0.502	0.7	21.2±8.1	26.33±6.6	**0.007**	0.7

CPQOL: Cerebral palsy quality of life.

Table III. Comparison of quality of life scores based on presence of epilepsy and intellectual disability in patients with spastic cerebral palsy.

	Epilepsy				Intellectual disability			
	Yes (n=29)	No (n=42)	p	Cohen's d	Yes (n=42)	No (n=29)	p	Cohen's d
QOL Child	315.31±78.03	371.80±77.59	**0.002**	0.72	309.55±76.42	405.48±51.68	**0.001**	**1.4**
Social WB and acceptance	58.79±23	78.14±17.93	**0.001**	**0.96**	60.57±22.92	84.24±10.82	**0.001**	**1.2**
Feelings about functioning	57.79±20.92	73.61±23.09	**0.004**	0.71	56.76±21.11	82.21±17.96	**0.001**	**1.2**
Participation and PH	48.03±22.56	63.31±22.96	**0.007**	0.67	45.98±21.98	73.14±16.27	**0.001**	**1.3**
Emotional WB	34.13±9.40	41.81±9.37	**0.001**	**0.81**	34±9.74	45.45±5.82	**0.001**	**1.3**
Access to services	48.6±14.11	50.76±15.94	0.607	0.14	47.33±13.89	53.83±16.3	0.076	0.4
Pain and feeling about disability	33.82±10.08	29.92±12.76	0.175	0.33	32.14±11.84	30.62±12.01	0.598	0.12
Family health	24.20±6.67	25.02±8.07	0.493	0.1	23.14±7.14	26.93±7.55	**0.035**	0.5

CP: Cerebral palsy; PH: physical health; QOL: quality of life; WB: well-being.

Comparison of parent proxy and child proxy CPQOL results

There was a significant positive correlation between the overall scores obtained from the QOL questionnaire by patients who were able to complete the questionnaire themselves (n:18) and their mothers (r=0.942; p=0.001). This correlation was significant in all domains except the disability pain and impact. In the disability pain and impact domain, the mothers' scores were significantly higher than the children's scores (32.5 (17-50) ; 29 (8-68)).

Discussion

We utilized the CPQOL-Child questionnaire in order to evaluate the QOL for children aged 6 to 12 years who had spastic cerebral palsy. Besides, we examined the relationship between demographic, neurologic and sleep disorder variables as well as quality of life. Our findings showed that higher income levels, higher maternal education, and the lack of comorbid conditions such as epilepsy, intellectual disability, and sleep disorders had a significant positive effect on QOL. Additionally, in our subanalysis, we found that social participation played a crucial role in influencing these results.

Although education is primarily a social issue, it is crucial for mothers of children with CP to be informed about their child's condition and care in terms of rehabilitation strategies. Limitations in motor functions caused by CP require additional support from families for children with CP and this can only be achieved through family education. We found that the QOL of children of mothers with low education level was poorer than that of children of mothers with high education level. Additionally, the effect size was medium according to the differences in education level. We conclude that this result is important and should be supported by studies with a larger number of patients. Moreover, when we analyzed the QOL scale answered by mothers with low education level, we found a significant decrease in the participation and physical health sub-parameters, in which social welfare and acceptance as well as motor limitations were predominantly evaluated. With these results, we have shown that the participation of children with spastic CP in society is closely related to family education.

Table IV. Correlation of quality of life scale scores and sleep scale scores in patients with spastic cerebral palsy.

CSHQ scores		CPQOL-Child							
		QOL child	Social WB and acceptance	Functionality	Participation and PH	Emotional WB and self-esteem	Access and service	The pain and impact of disability	Family health
Total sleep score	r	-0.237	-0.016	-0.275	-0.236	-0.151	-0.162	0.133	-0.247
	p	0.047	0.896	0.020	0.047	0.209	0.176	0.269	0.038
Bedtime resistance	r	0.138	0.271	0.075	0.058	-0.006	0.100	-0.171	0.085
	p	0.251	0.022	0.536	0.629	0.957	0.405	0.155	0.480
Delay in starting to sleep	r	0.454	0.355	0.437	0.463	0.418	0.061	-0.089	0.155
	p	0.001	0.002	0.001	0.001	0.001	0.615	0.458	0.198
Duration of sleep	r	0.296	0.303	0.194	0.271	0.205	0.074	-0.029	0.179
	p	0.012	0.010	0.104	0.022	0.086	0.537	0.808	0.135
Sleep anxiety	r	-0.182	-0.069	-0.240	-0.244	-0.196	-0.086	0.119	-0.127
	p	0.129	0.566	0.044	0.041	0.101	0.477	0.323	0.290
Wake up at night	r	-0.246	-0.192	-0.179	-0.235	-0.169	-0.106	0.017	0.022
	p	0.038	0.109	0.135	0.048	0.160	0.379	0.891	0.858
Parasomnia	r	-0.604	-0.393	-0.581	-0.593	-0.570	-0.248	0.134	-0.374
	p	0.001	0.001	0.001	0.001	0.001	0.037	0.266	0.001
RD during sleep	r	-0.126	-0.102	-0.112	-0.152	-0.159	0.010	-0.129	0.024
	p	0.296	0.395	0.354	0.206	0.185	0.932	0.285	0.844
Day time sleep	r	-0.015	0.029	-0.048	-0.080	0.075	-0.016	0.227	-0.127
	p	0.904	0.807	0.693	0.505	0.532	0.893	0.056	0.291
Amount of sleep	r	0.320	0.390	0.331	0.287	0.291	0.041	-0.164	0.174
	p	0.006	0.001	0.005	0.015	0.014	0.736	0.172	0.147

CP: Cerebral palsy; CPQOL-Child: Cerebral Palsy Quality of Life Questionnaire for Children; CSHQ: Child Sleep Health Questionnaire; PH: physical health; QOL: quality of life; RD: respiratory disorder; WB: well-being.

Similarly, the QOL of the mothers with a low level of education was observed to be significantly poorer than the mothers with a higher level of education in a study conducted by Fadwa et al [19], in Sudan. However, in contrast to us, they did not perform a subanalysis based on the questionnaire.

The QOL scores in the low income group was significantly poorer than in the high income group in our study. Additionally, the effect size was medium according to the differences in income level. This finding is consistent with a study by Power et al.[20], which assessed the QOL of CP patients in low- and middle-income countries. On the contrary, our study showed that variables such as access to hospital, communication with the child's physician, and access to rehabilitation services such as physical therapy, speech therapy and occupational therapy were not affected by financial income. In our country, rehabilitation programs for these patients are provided free of charge. Additional fees may be charged when necessary. Despite having access to these health services, low-income patients still face challenges in areas such as physical and emotional well-being, self-esteem and family health.

In recent years, advancements in neonatal care have led to an increase in the survival rates of preterm and low birth weight babies. Despite this progress, there has been a corresponding rise in the likelihood of conditions such as intracranial hemorrhage, sepsis, and hypoglycemia that may negatively impact brain development. Despite this, we showed that patients with CP born preterm had a better QOL than patients with CP born at term. This may be due to the fact that our patients with CP born preterm may have shown a better clinical course than patients with CP born at term. Therefore, we suggest that QOL scores may be a more complicated outcome than assumed and can be affected by a combination of several other findings including demographic and clinical features.

The study focused on the topographic subgroup of CP patients with spastic movement disorders. Since our results may be affected by subgroup types, a more homogeneous group was selected. Our results were consistent with the expectation that patients with hemiplegia would have better QOL than other types including quadriplegia and diplegia due to their better ambulation ability. Similarly, Öcal Eriman et al.[21] also found higher QOL in patients with hemiplegia. In our study, in addition to this finding, it was found that some sub-parameters of QOL such as pain, concerns about the disease, feeling of disability, physical health and happiness of the family were not affected by type of CP.

When the motor capacities of the patients were analyzed, it was seen that those with severe GMF and BFMF levels had significantly poorer QOL, which was consistent with the existing literature.[9,19] Patients with severe motor dysfunction had significantly lower levels of functioning, physical health and participation. Similar to our data, a study by Simeonsson et al.[22] found that children with severe disabilities participated less in social activities than those with mild disabilities. On the other hand, the scores on the QOL scale regarding the impact of disability and pain in the group of patients with severe motor dysfunction were similar to those of the group with mild to moderate motor dysfunction. Interestingly, this data showed that the level of motor impairment was not directly related to the amount of pain perceived. These findings overlap with the findings of a study conducted by Badia et al.[16]

Epilepsy is known to frequently co-occur with CP. Studies show that 15 to 60% of children with CP also have epilepsy.[23] Consistent with the literature, we found that the rate of epilepsy in our patient cohort was 40.8%. Patients with CP accompanied by epilepsy had a poorer QOL compared to those without epilepsy. Both conditions affect not only individuals but also their families. In this context, a study by Terra et al.[24] evaluated the QOL of mothers of children with CP and epilepsy was found to be lower than that of mothers of CP patients only. Although we did not evaluate the mothers, QOL scores were significantly poorer in the patients with

epilepsy then in the patients without epilepsy in our study. However, we did not observe a significant difference in QOL scores between patients with and without epilepsy in the sub-dimensions of family health, access and service.

Furthermore, it is well-established that a substantial proportion of individuals with CP also have intellectual disabilities, with estimates ranging from 30 to 50%.[25] Our study revealed an even higher prevalence, as more than half of the participants displayed cognitive impairment. We found that patients with intellectual disability had significantly poorer QOL compared to those with normal intellectual functioning. Also, the effect size was very large according to the differences in intellectual functioning. When the sub-domains that cause low QOL were examined; similar to the results of a study conducted by Arnaud et al.[26], mothers of patients with intellectual disability specifically reported that their children were not sufficiently socially accepted. Blasco et al.[9] approached the patients from a neuropsychological perspective. They evaluated visual perception, executive functions, memory, psychological adjustment, and general intellectual functions of the patients. They utilized specific tests and scales, such as Raven's Color Progressive Matrices (RCPM) for general intellectual functioning, the Face Recognition Test (FRT), and the Arrows subtest of the NEPSY-II for visual perception. As a result, they demonstrated the significant impact of neuropsychological factors, including executive functions, on the QOL of children with CP.[9]

First, we hypothesized that children with CP are more likely to have sleep disturbances than their healthy peers due to motor dysfunction limiting their ability to move and change position in bed which can cause more awakenings, pain and airway obstruction during sleep. In line with our predictions, it has been reported that sleep disorders are four times more common in children with CP than in children with normal development.[27-30] Previous studies have also shown a high prevalence of parasomnia in patients with behavioral problems and intellectual disabilities.[31,32] Although there was no relationship between intelligence level and sleep disorders, sleep disturbances were significantly more common in children with CP in our study. Furthermore, there was a significant positive correlation between the sleep disturbance frequency and both of the severity of motor functions and QOL scores. Therefore, our study, which evaluated sleep disturbances, QOL and motor function levels together, may provide valuable insights for the literature. The points we highlight may be particularly helpful for clinicians working with children with CP in assessing the overall well-being of their patients.

This study also aimed to compare parents' and children's perspectives on QOL, as emphasized by Swift et al.[33] We found that all the subdomains but "the pain and impact of disability" were highly consistent between the responses of children and their caregivers. This finding points out that although mothers are quite reliable sources for the data obtained regarding QOL, pain assessment is highly dependent upon the patient itself. Thus, we suggest that if the patient is unable to complete the questionnaire and we have to ask the mothers, we must be aware of possible exaggerated responses regarding the pain of the patients.

There are several noteworthy limitations of our study. The main limitation was the very low frequency of child responses, with lower than one fifth of the patients able to answer the questionnaire, therefore our data was mostly based on the mother's responses. Another limitation was the highly heterogeneous cohort which varied greatly in terms of topography and severity of the patients' neuromotor impairment.

In conclusion, the optimal care for a child with spastic CP should take into account family and environmental factors. Improving financial support for families, increasing mothers' education levels, providing early and effective physiotherapy for walking, developing gross and fine motor skills, implementing

personalized intellectual stimulation strategies from an early age, and promptly addressing sleep disorders and epilepsy can significantly improve a patient's QOL. Relying solely on parents' perspectives may ignore the valuable information that children can provide and vice versa. Therefore, asking both the children and their caregivers might offer a reliable method to evaluate the QOL of these patients.

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Ethical approval

The study was approved by Institutional Review Board (08/07/20-29430533-604.01-01-86126) of Istanbul University-Cerrahpasa, Cerrahpasa Medical School. The study complied with the recommendations of the Declaration of Helsinki for human biomedical research. The caregivers provided informed consent on behalf of all the participants.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: LK, SG, SS; data collection: LK; analysis and interpretation of results: LK, SS; draft manuscript preparation: LK. 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

Airway obstruction and gender affect arterial stiffness in children with cystic fibrosis

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ABSTRACT

Background. Vascular changes are observed in children with cystic fibrosis (cwCF), and gender-specific differences may impact arterial stiffness. We aimed to compare arterial stiffness and clinical parameters based on gender in cwCF and to determine the factors affecting arterial stiffness in cwCF.

Methods. Fifty-eight cwCF were included. Pulmonary function, lean body mass, handgrip strength, and peak oxygen uptake (VO_{2peak}) were assessed using a cardiopulmonary exercise test. Arterial stiffness (pulse wave velocity [PWV] and augmentation index [AIx@75]) and hemodynamic parameters (resting heart rate [HR] and stroke volume [SV]) were measured using brachial pulse waves. Endothelial function (ICAM-1, sVCAM-1, sE-selectin, VEGF-A, ET-1) was evaluated using blood samples.

Results. Female cwCF had significantly lower VO_{2peak}, SV, and PWV, and higher resting HR, AIx@75, and AIx@75-z-score than male cwCF ($p<0.05$). AIx@75-z-score was associated with gender ($r=0.516$, $p<0.001$), age ($r= -0.345$, $p=0.008$), lean body mass ($r= -0.451$, $p<0.001$), forced expiratory volume in one second (FEV_1)-z-score ($r= -0.332$, $p=0.011$), handgrip strength ($r= -0.466$, $p<0.001$), and VO_{2peak} ($r= -0.459$, $p<0.001$) and peak workload ($r= -0.527$, $p<0.001$). AIx@75-z-score was not associated with ICAM-1, sVCAM-1, sE-selectin, VEGF-A, or ET-1 ($p>0.05$). The FEV_1-z-score and gender explained 34.6% of the variance in AIx@75-z-score ($p<0.05$).

Conclusions. Female cwCF have more impaired hemodynamics, less maximal exercise capacity, and increased arterial stiffness, indicating a higher cardiovascular risk compared to male cwCF. FEV_1 and gender affect arterial stiffness in cwCF. Further studies are necessary to uncover the underlying factors for arterial stiffness and endothelial dysfunction and their clinical effects in cwCF.

Key words: cystic fibrosis, maximal exercise capacity, endothelial dysfunction, arterial stiffness, pulse wave velocity.

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Cystic fibrosis (CF) is a rare genetic disease resulting from mutations in the CF transmembrane conductance regulator (*CFTR*) gene.[1] The CFTR is detected in endothelial cells derived from multiple organ systems, including the lung microvasculature.[2] Impaired function of the CFTR in children with CF (cwCF) is associated with elevated cytokines and other inflammatory markers.[3] CFTR activity plays a key role in maintaining vascular homeostasis, especially during an inflammatory response by the vascular endothelium.[4] Increased endothelial permeability was observed in CF patients compared to healthy controls.[5] Increased oxidative stress, inflammation, endothelial dysfunction, and life expectancy may increase cardiovascular risk.[4]

Arterial stiffness and endothelial dysfunction highlight different dimensions of vascular disease.[6] The influence of systemic inflammation on endothelial function may contribute to the development of arterial stiffness.[7] Airway inflammation has been implicated in causing endothelial dysfunction in the pulmonary circulation, which could contribute to systemic endothelial dysfunction.[7] A reduction in pulmonary function may disrupt endothelial barrier function, which is directly affected by vascular wall stiffening.[8] Few studies reported increased arterial stiffness[9-12] and endothelial dysfunction[13,14] in cwCF compared to healthy children.[10] cwCF have enhanced aortic stiffness and wall thickness compared to controls.[12] Increased arterial stiffness and endothelial dysfunction are associated with pulmonary function.[9,13] These findings indicate that vascular changes observed in cwCF begin in early childhood. The arterial stiffness and endothelial function in cwCF have become even more critical, considering recent improvements in survival rates.

Arterial stiffness may be influenced by intrinsic gender differences.[15] To date, no studies have directly compared arterial stiffness and endothelial function between female and male cwCF, despite evidence showing increased arterial stiffness in cwCF compared to healthy peers.[9] The relationship between arterial stiffness and endothelial function, pulmonary function, and exercise capacity remains unclear. Therefore, we aimed (a) to compare the arterial stiffness, endothelial function, and clinical parameters, including physical characteristics, pulmonary function, peripheral muscle strength, and exercise capacity, between female and male cwCF and (b) to identify the factors affecting arterial stiffness in cwCF.

Material and Methods

Study design and population

All assessments were completed within a single day, with data collection taking place in the morning from 9 a.m. to 12 p.m. Ethical approval was obtained from the Hacettepe University, Non-Interventional Clinical Research Ethics Committee (Approval date: 07.01.2020, approval number: GO 19/1156). All participants and their parents signed informed consent forms. The study was registered on ClinicalTrials.gov (NCT04259983) and conducted in accordance with the Declaration of Helsinki.

Participants and procedures

This cross-sectional study was conducted between January 2020 and December 2023 at the Cardiopulmonary Rehabilitation Unit of the Hacettepe University, Faculty of Physical Therapy and Rehabilitation, in collaboration with the Hacettepe University, Faculty of Medicine (Department of Pediatric Pulmonology and Department of Physiology) and Faculty of Pharmacy (Department of Pharmaceutical Toxicology). Sixty-eight cwCF, aged 10–18 years, who were diagnosed and followed at the Department of Pediatric Pulmonology, Hacettepe University Faculty of Medicine, and referred to the Cardiopulmonary Rehabilitation Unit, were screened. The inclusion criteria were being 10–18 years old, clinically stable, able to cooperate with assessments, with forced expiratory volume in one second (FEV_1) >40% predicted, not having experienced any

exacerbations at least for three months, using regular medication for at least 12 months, and having no medication changes for at least three weeks. Exclusion criteria were having a resting oxygen saturation (SpO_2) <92%, a history of smoking, having pulmonary surgery, having use of vasoactive drugs or oral steroids, having CF-related diabetes, having advanced orthopedic, neurologic, and cardiovascular diseases, and having a lower extremity injury (e.g., strain, sprain, or fracture) in the past six months.

Assessments

Age, gender, mutations, and medications were recorded. Lean body mass was evaluated using a skinfold caliper (Baseline Medical Skinfold Caliper, Fabrication Enterprises, NY, USA). Three measurements were taken from the biceps, triceps, subscapular, and supra iliac regions, and the mean values of the right side were used for analysis.[16]

Forced vital capacity (FVC), FEV_1, peak expiratory flow (PEF), and forced expiratory flow from 25%–75% ($FEF_{25–75\%}$) were recorded from the medical records.[17] Handgrip strength was measured using a portable dynamometer (Jamar, Nottinghamshire, UK). The right and left sides were measured thrice, and the best value was recorded.[18]

A cardiopulmonary exercise test (CPET) using Godfrey protocol[19] was performed on an electronically braked bicycle ergometer (Lode, Corival CPET, Groningen, The Netherlands).[13] The test was terminated in the instances of voluntary exhaustion, inability to maintain a 60-rpm cadence, or reaching the peak heart rate (HR_{peak}) and respiratory exchange ratio (RER) >1.03. The RER and peak oxygen consumption (VO_{2peak}) were determined using gas exchange analysis (Quark CPET, COSMED, Rome, Italy) and HR_{peak} and peak workload (W_{peak}) were recorded.

Evaluation of arterial stiffness

A portable device was used to evaluate arterial stiffness using brachial pulse waves (Tel-O-Graph BT, IEM GmbH, Aachen, Germany).[20] The Tel-O-Graph, which uses an oscillometric principle, was employed to measure arterial stiffness. It enables blood pressure measurement with automatic transmission. A Bluetooth connection was established between the device and the data analysis software (Hypertension Management Software Client Server, HMS CS, Achen, Germany). Three consecutive measurements were taken for each child with CF, and the highest reading was used for evaluation. Pulse wave velocity (PWV), augmentation index normalized to heart rate with 75 beats/min (AIx@75), resting heart rate (HR), and stroke volume (SV) were evaluated.[21] Augmentation index is an integrated measure that reflects both arterial wave reflection and systemic arterial stiffness.[22] The AIx@75 was determined by assessing the aortic pressure wave and calculating the augmentation pressure, which is the difference between the peak of the reflected wave (P2) and the peak of the incident wave (P1). This value is expressed as a percentage of the central pulse pressure (cPP), calculated using the formula: AIx@75=(P2−P1)/cPP×100. We measured AIx@75 to minimize the influence of mean arterial pressure, age, gender, and HR on the augmentation index.[9] Arterial stiffness measurements were performed after 12 hours of overnight fasting and before inhaler therapy in a sitting position after the patient had rested for 15 minutes in a quiet room.[9]

Evaluation of endothelial function

Blood samples were collected to assess endothelial function by measuring the levels of intercellular adhesion molecule-1 (ICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), soluble endothelium-selectin (sE-selectin), vascular endothelial cell growth factor-A (VEGF-A), and endothelin-1 (ET-1). Samples were drawn via forearm venous puncture, collected into heparinized vacutainer tubes, and kept in sterile containers. Plasma

samples were obtained through 10 minutes of centrifugation at 2000 rpm. All plasma samples were aliquoted into 2 mL Eppendorf tubes and stored at −80 °C until analyzed.[23] Measurements of ICAM-1, sVCAM-1, sE-selectin, VEGF-A, and ET-1 levels were detected in plasma samples using an ELISA kit (Bioassay Technology Laboratory, Shanghai, China) following the manufacturer's instructions with slight modifications. The absorbance of samples was read at λ=450 nm against a standard curve using a SpectraMax® M5 Microplate Reader (Molecular Devices LLC, San Jose, CA, USA). All experiments with indicators of endothelial dysfunction parameters were conducted with technical duplicates.

Statistical analyses

SPSS version 27.0 (IBM Corp. IBM SPSS Statistics for Windows, Armonk, NY, USA) was used for statistical analysis. Normality was checked using the Shapiro–Wilk test, and descriptive statistics were calculated. The analysis was performed using measured values, except for population-based pulmonary function test z-scores. Z-scores for weight,[24] height[24], heart rate[25], systolic and diastolic blood pressure[26], PWV[26], and AIx@75[25] were calculated. Data were presented as the median, interquartile range, mean and standard deviations, frequencies, and percentages, as appropriate. Student's t-test or Mann-Whitney U test was used for the comparison, considering normality. Associations between AIx@75-z-score and the variables were analyzed using Pearson's correlation coefficients. The statistical significance was set at $p<0.05$. A multiple linear regression analysis was performed. The scatter plots were created using GraphPad Prism v.8.0.2 (GraphPad Software, San Diego, CA, USA) and used to investigate the associations between the AIx@75-z-score and gender, age, lean body mass, FEV_1-z-score, handgrip strength, VO_{2peak}, and W_{peak}. The variables showing a univariate association with the AIx@75-z-score ($p<0.05$) were initially entered into the multiple regression analysis (gender, handgrip strength, lean body mass, VO_{2peak}, FEV_1-z-score, and age).[27] Since there was no statistical significance found regarding handgrip strength, VO_{2peak}, and age with the model ($p>0.05$), the final model was established using gender and FEV_1-z-score. For the final model, assumptions for variables were tested (normal distribution, heteroscedasticity, multicollinearity). The assumption of homoscedasticity, referring to the constancy of the residuals' variance, was evaluated using a scatter plot of the residuals against the predicted values. The outcome dependent variable (AIx@75-z-score) was normally distributed, and the independent variables (FEV_1-z-score and gender) did not violate assumptions. The post-hoc power was calculated using the G*Power program 3.1.9.7 (Franz Faul, Kiel University, Kiel, Germany) based on the comparison of AIx@75-z-score between female and male cwCF. The effect size and post-hoc power were found to be 1.19 and 99.38%, respectively.

Results

Sixty-eight cwCF were screened. Ten cwCF were excluded for the following reasons: missing data (n=2), declining to participate (n=6), and being identified as outliers (n=2). Therefore, 58 cwCF were included in the final analysis. Our study included 27 female and 31 male cwCF. Physical characteristics, *CFTR* mutations, lung treatments, pulmonary function, peripheral muscle strength, and cardiopulmonary exercise testing findings in cwCF are presented in Table I. The maximal exercise test was terminated in 56 cwCF due to a RER >1.03 (n=56) and in two cwCF due to an inability to maintain a 60-rpm cadence. None of the study participants were receiving modulator therapy or blood pressure medications.

The age, weight-z-score, height-z-score, body mass index, lean body mass, mutations, lung treatments, FEV_1, and FEV_1%predicted, PEF, $FEF_{25-75\%}$, handgrip strength, RER, HR_{peak}, and HR_{peak} %predicted values were similar between female and male cwCF ($p>0.05$). Female cwCF had significantly lower FVC

Table I. Physical characteristics, mutations, lung treatments, pulmonary function, peripheral muscle strength, and cardiopulmonary exercise testing in children with cystic fibrosis.

Variables	All cwCF (n=58)	Female cwCF (n=27)	Male cwCF (n=31)	p
Age (years)	13.00 (12.00-15.25)	13.00 (12.00-16.00)	13.00 (12.00-15.00)	0.906[u]
CF diagnosis (months)	4.00 (2.50-7.00)	4.00 (3.00-7.00)	4.00 (2.50-8.00)	0.950[u]
Weight (kg)	47.34±12.75	45.99±13.03	48.52±12.59	0.456[t]
Weight-z-score	-0.16±1.04	-0.21±0.94	-0.11±1.14	0.733[t]
Height (cm)	154.12±11.91	151.62±10.26	156.29±12.96	0.139[t]
Height-z-score	-0.35±0.95	-0.44±1.04	-0.27±0.88	0.502[t]
BMI (kg/m^2)	19.62±3.37	19.62±3.32	19.62±3.46	0.999[t]
BMI-z-score	0.00±1.00	-0.00±0.98	0.00±1.02	0.999[t]
Lean body mass (kg)	36.39±8.66	34.52±7.22	38.01±9.57	0.127[t]
Mutations				0.636[p]
F508del homozygous, n (%)	9 (15.5)	5 (18.5)	4 (12.9)	
F508del heterozygous, n (%)	11 (19.0)	6 (22.2)	5 (16.1)	
Other mutations, n (%)	38 (65.5)	16 (59.3)	22 (71.0)	
Lung treatments				
Pharmacological treatments				
Inhaled antibiotics, n (%)	6 (10.3)	1 (3.7)	5 (16.1)	0.201[f]
Dornase alpha, n (%)	51 (87.9)	26 (96.3)	25 (80.6)	0.108[f]
Inhaled corticosteroids, n (%)	3 (5.2)	1 (3.7)	2 (6.5)	1.000[f]
Bronchodilators, n (%)	6 (10.3)	3 (11.1)	3 (9.7)	1.000[f]
Hypertonic saline, n (%)	9 (15.5)	4 (14.8)	5 (16.1)	1.000[f]
Airway clearance techniques	45 (77.6)	23 (85.2)	22 (71.0)	0.225[f]
Pulmonary function testing				
FVC (L)	3.03±0.92	2.73±0.74	3.28±0.99	**0.022**[*t]
FVC-z-score	-0.50±1.77	-0.46±1.45	-0.53±2.04	0.884[t]
FEV_1 (L)	2.61±0.82	2.38±0.69	2.82±0.88	0.045[t]
FEV_1-z-score	-0.21±1.77	-0.26±1.67	-0.18±1.87	0.865[t]
PEF (L)	5.18 (4.31-6.56)	5.25±1.57	5.95±2.15	0.169[t]
PEF-z-score	-0.40±1.23	-0.51±1.32	-0.31±1.17	0.544[t]
$FEF_{25-75\%}$ (L)	2.84 (2.16-4.06)	2.96±1.30	3.19±1.33	0.511[t]
$FEF_{25-75\%}$-z-score	-0.51±1.94	-0.49±2.18	-0.52±1.74	0.942[t]
Peripheral muscle strength				
Handgrip strength (N)	230.44 (176.50-284.37)	225.53 (156.89-264.76)	235.34 (205.92-294.18)	0.103[u]
Cardiopulmonary exercise testing				
VO_{2peak} (mL/min)	1209.19±395.22	1000.52±289.23	1390.94±388.53	**<0.001**[*t]
W_{peak} (Watt)	100.00 (75.00-120.00)	80.00 (60.00-100.00)	105.00 (90.00-140.00)	**0.002**[u]
RER	1.21±0.13	1.20±0.13	1.22±0.14	0.548[t]
HR_{peak} (bpm)	174.65±12.85	173.44±11.94	175.70±13.71	0.508[t]
HR_{peak} %predicted (%)	89.56±6.59	88.94±6.12	90.10±7.03	0.508[t]

Data are presented as median (interquartile range) or mean±standard deviation considering normality.
*p<0.05. [u]Mann-Whitney U test, [t]Student's t test, [p]Pearson Chi-Square test, [f]Fishers's exact test.
BMI: body mass index, cwCF: children with cystic fibrosis, $FEF_{25\ 75\%}$: forced expiratory flow from 25 to 75%, FEV_1: forced expiratory volume in one second, FVC: forced vital capacity, HR_{peak}: peak heart rate, PEF: peak expiratory flow, VO_{2peak}: peak oxygen uptake, W_{peak}: peak workload.

(mean±SD=2.73±0.74 L in females vs. 3.28±0.99 L in males; p=0.022), VO_{2peak} (mean±SD= 1000.52±289.23 mL/min in females vs. 1390.94±388.53 mL/min in males; p<0.001), and W_{peak} (median [interquartile range]=80.00 [60.00-100.00] Watt in females vs. 105.00 [90.00-140.00] Watt in males, p=0.002) compared to male cwCF. A comparison of arterial stiffness, hemodynamics, and endothelial function in female and male cwCF is presented in Table II. Female cwCF had significantly higher HR, AIx@75, and AIx@75-z-score with lower SV and PWV compared to those of male cwCF (p<0.05, Table II). ICAM-1, sVCAM-1, sE-selectin, VEGF-A, and ET-1 levels were similar between female and male cwCF (p>0.05, Table II).

AIx@75-z-score showed a moderate correlation with gender (r=0.516, p<0.001), weak correlation with age (r= −0.345, p=0.008) and FEV_1-z-score (r= −0.332, p=0.011), and moderate correlation with lean body mass (r= −0.451, p<0.001), handgrip strength (r= −0.466, p<0.001), VO_{2peak} (r= −0.459, p<0.001), and W_{peak} (r= −0.527, p<0.001). AIx@75-z-score was not associated with ICAM-1, sVCAM-1, sE-selectin, VEGF-A, and ET-1 (p>0.05).

The scatter plots showing associations between AIx@75-z-score and age, lean body mass, FEV_1-

Table II. A comparison of arterial stiffness and endothelial function according to gender in children with cystic fibrosis.

Variables	cwCF (n=58)	Females (n=27)	Males (n=31)	p
Hemodynamics				
Heart rate (bpm)	93.47±15.57	99.30±13.03	88.39±16.00	**0.007*[t]**
Heart rate-z-score	1.22±1.36	1.81±1.19	0.71±1.32	**0.002*[t]**
SBP (mmHg)	100.29±9.49	97.96±8.89	102.32±9.67	0.081[t]
SBP-z-score	-1.75±1.21	-2.08±1.19	-1.46±1.16	**0.049*[t]**
DBP (mmHg)	63.76±5.78	63.00 (61.00-67.00)	63.00 (60.00-65.00)	0.458[u]
DBP-z-score	-0.25±0.85	-0.43 (-0.73-0.15)	-0.31 (-0.75- -0.01)	0.679[u]
SV (mL)	45.90 (36.65-59.40)	40.94±10.29	55.64±14.90	**<0.001*[t]**
Arterial stiffness				
PWV (m/s)	4.15±0.30	4.06±0.28	4.22±0.29	**0.039*[t]**
PWV-z-score	-2.26±1.45	-2.45±1.22	-2.09±1.62	0.356[t]
AIx@75 (%)	31.22±11.67	37.30±9.15	25.94±11.15	**<0.001*[t]**
AIx@75-z-score	1.12±1.48	1.94±1.14	0.41±1.39	**<0.001*[t]**
Endothelial function				
ICAM-1 (ng/L)	435.09 (224.45-660.47)	425.33 (222.70-669.52)	440.42 (225.04-659.50)	0.981[u]
sVCAM-1 (ng/mL)	1.53 (0.74-2.38)	1.44 (0.74-2.86)	1.55 (0.73-2.08)	0.858[u]
sE-selectin (ng/mL)	8.61 (5.69-14.22)	7.90 (5.33-14.90)	8.86 (5.71-13.99)	0.785[u]
VEGF-A (ng/L)	51.17 (35.35-75.56)	60.38 (33.54-92.47)	50.98 (38.00-72.30)	0.714[u]
ET-1 (ng/L)	23.28 (10.56-45.50)	26.26 (10.32-47.83)	18.01 (10.64-44.73)	0.809[u]

Data are presented as median (interquartile range) or mean±standard deviation considering normality.
*p<0.05. [t]Student's t-test. [u]Mann-Whitney U test.
AIx@75: augmentation index normalized to heart rate of 75 bpm, cwCF: children with cystic fibrosis, DBP: diastolic blood pressure, ET-1: endothelin 1, ICAM -1: intercellular adhesion molecule 1, PWV: pulse wave velocity, SBP: systolic blood pressure, sE-selectin: soluble endothelium-selectin, SV: Stroke volume, sVCAM-1: soluble vascular cell adhesion molecule 1, VEGF-A: vascular endothelial cell growth factor A.

z-score, handgrip strength, VO_{2peak}, and W_{peak} are shown in Fig. 1. AIx@75-z-score was negatively associated with age (explaining 11.9% of the variance in AIx@75-z-score), lean body mass (explaining 20.4% of the variance in AIx@75-z-score), FEV_1-z-score (explaining 11.0% of the variance in AIx@75-z-score), handgrip strength (explaining 21.7% of the variance in AIx@75-z-score), VO_{2peak} (explaining 21.1% of the variance in AIx@75-z-score), and W_{peak} (explaining 27.8% of the variance in AIx@75-z-score). A scatter plot of the predictive values of AIx@75-z-score against residuals is presented in Fig. 2. The residuals appeared to be randomly distributed without any specific pattern, indicating that there was no evidence of heteroscedasticity.

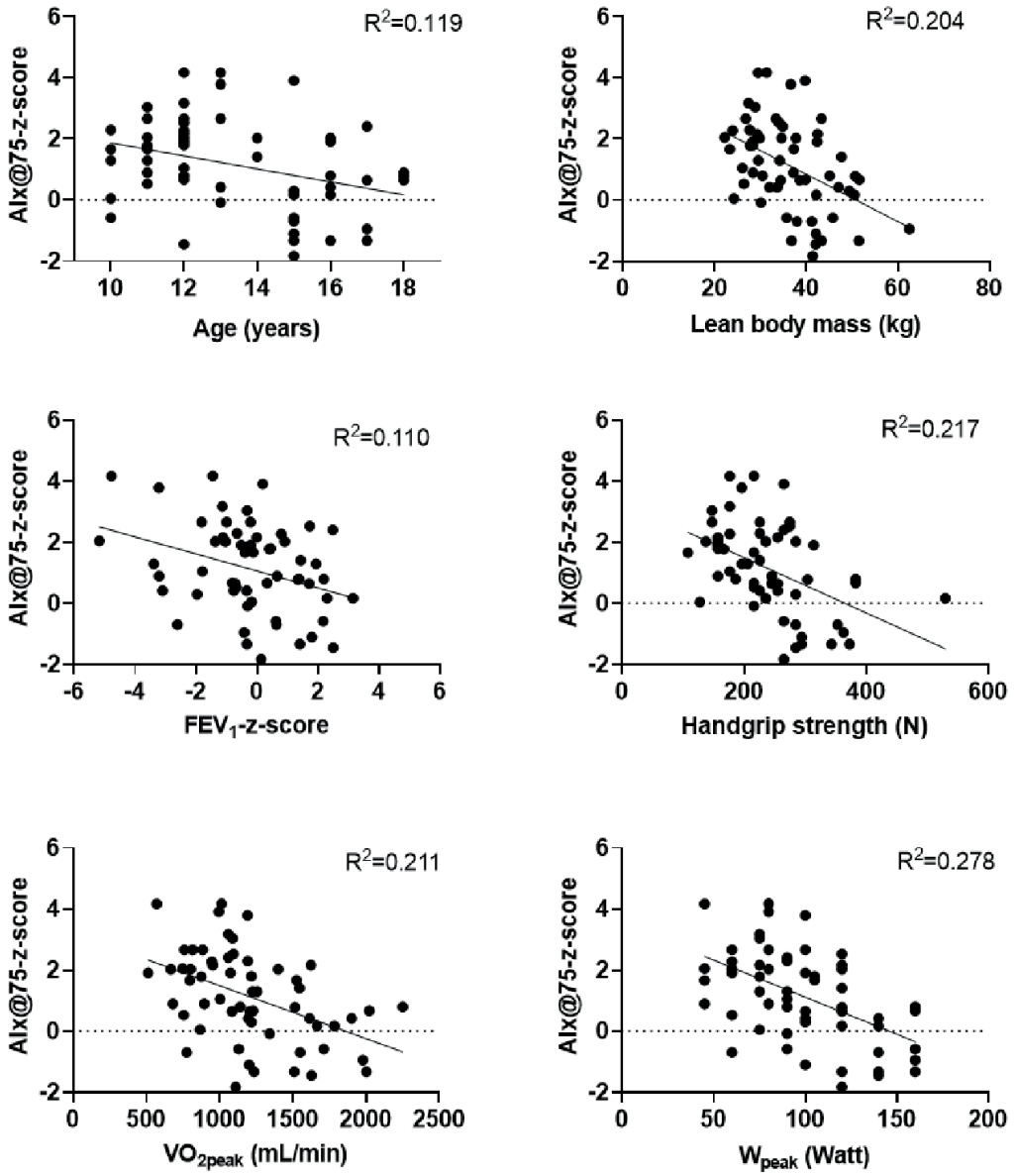

Fig. 1. Scatter plots showing associations between AIx@75-z-score and age, lean body mass, FEV_1-z-score, handgrip strength, VO_{2peak}, and W_{peak}.

AIx@75: augmentation index normalized to heart rate of 75 bpm, FEV_1: forced expiratory volume in one second, VO_{2peak}: peak oxygen uptake, W_{peak}: peak workload.

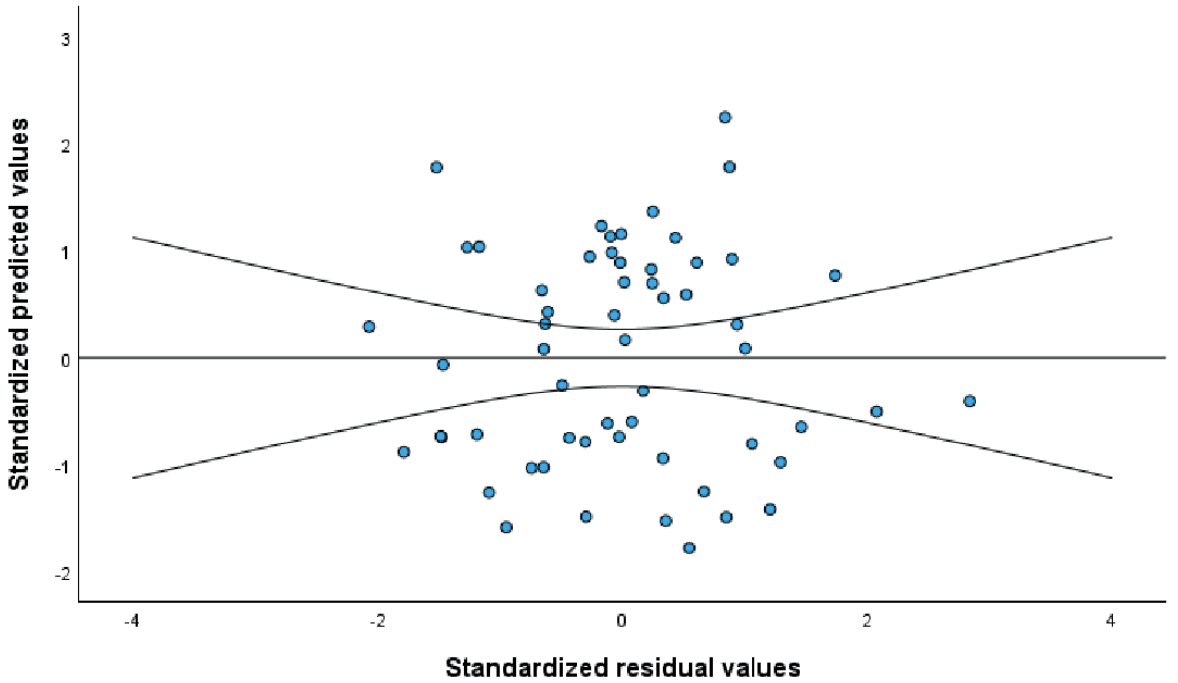

Fig. 2. Scatter plot of the predictive values of AIx@75-z-score against residuals.
AIx@75: augmentation index normalized to heart rate of 75 bpm.

Table III. Initial model with gender and handgrip strength, lean body mass, VO_{2peak}, FEV_1-z-score, and age as predictors of the AIx@75-z-score.

Initial model	Unstandardized B	Standardized β	p	95% CI	VIF
Constant	2.635		**0.009***	0.689-4.580	
Gender (F/M)	1.655	0.559	**<0.001***	0.877-2.432	1.638
Handgrip strength (N)	<0.001	-0.007	0.973	-0.008-0.007	3.513
Lean body mass (kg)	-0.042	-0.244	0.254	-0.115-0.031	4.264
VO_{2peak} (mL/min)	0.001	0.193	0.297	-0.001-0.002	3.198
FEV_1-z-score	-0.213	-0.254	**0.035***	-0.410- -0.016	1.303
Age (years)	-0.122	-0.198	0.226	-0.321-0.078	2.484

Initial model: $F_{(6\text{-}51)}$=7.415, p<0.001, R^2=0.466, Adjusted R^2=0.403.
AIx@75: augmentation index normalized to heart rate of 75 bpm, CI: confidence interval, FEV_1: forced expiratory volume in one second, VIF: variance inflation factor, VO_{2peak}: peak oxygen uptake.

The initial model is gender, handgrip strength, lean body mass, VO_{2peak}, FEV_1-z-score, and age (Table III). The final regression model with FEV_1-z-score and gender explained 34.6% of the variance in AIx@75-z-score with statistical significance (Adjusted R^2=0.346; Table IV, p<0.05) as shown in the following equation:

AIx@75-z-score = 0.368 + 1.506 × Gender (females=1, males=0) − 0.269 × FEV_1-z-score

When examining the individual relationships, the regression coefficients for gender and FEV_1-z-score were statistically significant (p<0.05, Table IV). Females had 1.506 units

Table IV. Final model summary with gender and z-score of FEV_1 as predictors of AIx@75.

Final model$^{\varphi}$	Unstandardized B	Standardized β	p	95% CI	VIF
Constant	0.368		0.095	-0.066-0.803	
Gender	1.506	0.509	**<0.001***	0.871-2.141	1.001
FEV_1-z-score	-0.269	-0.320	**0.004***	-0.450--0.089	1.001

*p<0.05. $F_{(2\text{-}55)}$=16.082, R^2=0.369, Adjusted R^2=0.346. $^{\varphi}$Model summary with gender and FEV_1-z-score as predictors.
AIx@75: augmentation index normalized to heart rate of 75 bpm, CI: confidence interval, FEV_1: forced expiratory volume in one second, VIF: variance inflation factor.

higher AIx@75-z-scores than males. For a 1-unit increase in FEV_1-z-score, AIx@75-z-score decreased by 0.269 units (Table IV).

Discussion

The present study reveals that airway obstruction (FEV_1) and gender are the factors affecting arterial stiffness in cwCF. Female cwCF have higher arterial stiffness, impaired hemodynamics (resting HR and SV), and lower maximal exercise capacity, indicating a higher cardiovascular risk than male cwCF.

Regarding comparing hemodynamic parameters, arterial stiffness, and endothelial dysfunction between genders, female cwCF had a higher resting HR than males. The increase in resting HR may be a way to compensate for the decrease in SV. The SV decrease may result from the changes in the contractile properties of the heart since a reduction in both right and left ventricular function is reported in cwCF.[10] The higher resting HR and lower SV in female cwCF might have led to lower VO_{2peak} and W_{peak} values in females compared to male cwCF.

Endothelial cell adhesion molecules of ICAM-1, VCAM-1, and sE-selectin play a role in the activation of inflammatory cells, their uptake and passage from vascular structures to the airways, and the development of airway inflammation.[28] Angiogenesis is stimulated by tissue hypoxia, and VEGF-A is a potent angiogenic factor induced by inflammation and tissue hypoxia.[29] Since the lungs clear ET-1, loss of functional pulmonary vascular channels and, thus, the decreased endothelial surface area may contribute to the decreased ability of the lung to remove ET-1[30] and the increased circulating ET-1 levels. Even though we did not observe any significant association between AIx@75 and endothelial markers as well as the differences between female and male cwCF in terms of endothelial markers, vascular endothelial dysfunction has been demonstrated in cwCF and adult CF relative to healthy peers.[13,14] We believe that relatively younger age[13], absence of cardiovascular disease risk factors such as CF-related diabetes and cardiovascular disease, colonization status, and relatively preserved airway function in most of the cwCF could be the main factors responsible for this finding.[28] Further follow-up studies may clarify the clinical appearance and predictive value of endothelial dysfunction and its underlying factors with advancing age and disease severity in CF.

We observed that cwCF had relatively high AIx@75 values when compared with a study including healthy children with similar mean age (mean age=13.50±2.41, 37.30±9.15% in female and 25.94±11.15% in male cwCF vs. mean age=13.53±3.17 years, 22.60±8.00% in female and 21.80±7.97% in male healthy children).[25] Furthermore, our findings revealed relatively low PWV values when compared with that of healthy individuals aged between 10-29 years (4.15±0.30 vs. 4.87±0.40 m/s [range: 4.25-5.25]).[31] Despite low PWV, high AIx@75 in cwCF may result from the distinct mechanisms of each index, i.e., PWV reflects aortic stiffness, while AIx@75 reflects peripheral arterial tone.[32] Factors such as inflammation and endothelial dysfunction may influence AIx@75 independently of PWV.[32]

Since PWV-z-score revealed no difference between female and male cwCF, we determined the individual contributors to AIx@75-z-score as AIx@75 is considered a more sensitive indicator of arterial aging in younger individuals than PWV.[33] The regression analysis showed that FEV_1-z-score and gender accounted for the change in AIx@75-z-score in cwCF. Regarding gender as a factor affecting AIx@75, compared with males of the same age, prepubescent females have stiffer large arteries, suggesting inherent genetic gender differences.[15] When compared to males of the same age, prepubertal females were shown to have stiffer large arteries, which suggested natural genetic gender differences.[15] The augmentation index decreases gradually with age in both genders, although the decrease significantly slows at the onset of puberty.[34] Arterial stiffness is higher in females than in males during both the pubertal and post-pubertal periods as well as at 18 years of age.[34] Differences in hormonal factors, metabolic and vascular responses may influence gender-based differences in cwCF.[35] Gender differences in arterial stiffness are important for prognosis, as greater arterial stiffness has been shown to be associated with mortality, and this relationship is nearly twice as strong in females compared to males.[36] In line with the literature, the higher AIx@75 and AIx@75-z-score in female cwCF compared with male cwCF in our study may indicate a higher risk in female cwCF for cardiovascular diseases. Being an independent factor, FEV_1-z-score indicates that more severe airway disease is associated with greater arterial stiffness. FEV_1 indicates the elastic fiber content of the lungs. In contrast, arterial stiffness reflects the aorta's fragmentation of elastin and medial collagen content, essential structural proteins playing a role in the elastic recoil of the lungs and arteries.[37] Since there is a balance between elastin and collagen production and their degradation, any variations in the volume and structure of these proteins result in dysfunction.[37] A previous study showed a negative association between arterial stiffness and $FEF_{25–75\%}$.[9] An association between pulmonary function and arterial stiffness at an early age might signify whether the basis for the association is developmental or genetic.

Maintaining a higher lean body mass can positively impact the cardiovascular health of young individuals.[38] Handgrip strength, a surrogate measure of overall muscle strength, is considered a biomarker of aging.[39] Low handgrip strength is associated with increased arterial stiffness across a wide age range, independent of gender and cardiovascular comorbidity.[39] Lower aerobic capacity is related to higher resting HR and cardiovascular risk factors[40] and increased arterial stiffness in children.[41,42] Maturity and growth may influence the relationship between increased arterial stiffness and cardiorespiratory fitness at early ages.[43] The associations between AIx@75-z-score and lean body mass, handgrip strength, and VO_{2peak} support that promoting a high lean body mass, handgrip strength, and cardiorespiratory fitness in cwCF may have preventative roles against the development of arterial stiffness.

To the best of our knowledge, the present study is the first to comprehensively and timely evaluate vascular parameters in cwCF using a combined approach of oscillometric devices (reducing the human factor) and biochemical markers (different aspects of endothelial function) and investigate their relationship with clinical parameters, including exercise capacity and pulmonary function. Moreover, the present study enables a comparison of arterial stiffness and endothelial markers between female and male cwCF.

This study is subject to several limitations. First, the study's cross-sectional nature prevented the observation of the clinical course of the endothelial function. A follow-up longitudinal study can highlight the clinical course of vascular function in cwCF. Second, our study included cwCF aged between 10-18 years with no cardiovascular diseases and mostly preserved lung function, i.e., only eight participants (13.8%) had an FEV_1% predicted

lower than 80%. While enabling a homogenous distribution in the study sample and mitigating the confounding effects, this situation may have masked potential associations, including the association between arterial stiffness and endothelial function. We did not evaluate the pubertal status of cwCF, and it was a limitation. Further studies with a wider sample size, incorporating pubertal status and adult participants considering cardiovascular comorbidities as well as follow-up studies, can provide a broader understanding of arterial stiffness and endothelial functions in CF.

In conclusion, female cwCF has higher resting HR, lower SV, lower VO_{2peak}, and higher arterial stiffness, indicating a higher cardiovascular risk than males. Therefore, FEV_1 and gender affect arterial stiffness in cwCF. Further follow-up studies with a larger sample size, including participants with cardiovascular comorbidities in CF, may help uncover the underlying factors for arterial stiffness and endothelial dysfunction and their effects in cwCF.

Ethical approval

The study was approved by Hacettepe University, Non-Interventional Clinical Research Ethics Committee (Approval date: 07.01.2020, approval number: GO 19/1156). This study was registered at ClinicalTrials.gov with identifier number NCT04259983. Informed consent forms signed by all participants and their parents.

Author contribution

Study conception and design: SS, ACO, MAE, YK, MTB, SS, NE, EEGY, DII; data collection: SS, ACO, MAE, YK, NE, EEGY; analysis and interpretation of results: SS, ACO, YK, DII; draft manuscript preparation: SS, ACO, YK, MTB, SS, NE, EEGY, DII. 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

Expression and clinical correlation of cathepsin S, programmed cell death-1 ligand 1, and BRAFV600E mutation in children with Langerhans cell histiocytosis

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ABSTRACT

Background. The expression and clinical correlation of BRAFV600E mutation and programmed cell death-1 ligand 1 (PD-L1) in children with Langerhans cell histiocytosis (LCH) have been reported, but the conclusions of previous studies are inconsistent. In addition, it has been reported that elevated cathepsin S (CTSS) expression is associated with various cancers. However, there is currently no research on the correlation between CTSS and LCH. The aim of this study was to reassess the clinical correlation of BRAFV600E mutation and PD-L1 in pediatric LCH and to investigate the expression and clinical correlation of CTSS in children with LCH.

Methods. A total of 35 tissue samples were analyzed for the BRAFV600E gene mutation using droplet digital polymerase chain reaction, and 31 tissue samples were examined for CTSS and PD-L1 by immunohistochemistry. In addition, the clinical characteristics and prognosis of these 35 pediatric patients were analyzed and summarized.

Results. The incidence of BRAFV600E gene mutation was 34.3% (12/35). The occurrence of BRAFV600E gene mutation was significantly associated with age ≤ 2 years and involvement of central nervous system risk sites (66.7% and 72.7%, respectively). The expression rate of PD-L1 was 35.5% (11/31), and it was significantly correlated with cutaneous involvement (100%, 3/3). PD-L1 expression was unrelated to BRAFV600E gene mutation. Neither BRAFV600E gene mutation nor PD-L1 expression had a significant impact on disease progression/reactivation and initial 6-week treatment response. CTSS was expressed positively in the lesion tissues of all 31 children with LCH. The H-scores of CTSS were significantly associated with age ≤ 2 years. CTSS had no significant effect on the initial 6-week treatment response, disease progression/reactivation, BRAFV600E gene mutation, or PD-L1 expression.

Conclusions. CTSS is positively expressed in LCH, and its expression level is associated with onset age ≤ 2 years. BRAFV600E gene mutation, PD-L1, and CTSS may not be associated with the prognosis of LCH.

Key words: Langerhans cell histiocytosis, cathepsin S, BRAFV600E, programmed cell death-1 ligand 1 (PD-L1).

Langerhans cell histiocytosis (LCH) is an inflammatory myeloid neoplasm[1], which affects children at an annual rate of approximately 3-9 per million[2] and adults at a rate of approximately 1-2 per million.[3] The clinical manifestations of LCH exhibit high heterogeneity, involving various systems throughout the body, ranging from spontaneously regressing isolated bone lesions or skin lesions to multisystem disease with involvement of risk organs (RO+) that can be life-threatening. The 5-year overall survival rate has reached over 80%, but the incidence

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of disease progression/reactivation still ranges between 30 and 50%.[4-6] Currently, LCH is believed to be a myeloid neoplasm driven by abnormal activation of the mitogen-activated protein kinase pathway.[7] The most common mutated gene in this signaling pathway is BRAFV600E, with an incidence of 36-60%.[8-12] In addition, inhibitors of the mitogen-activated protein kinase pathway (dabrafenib and trametinib) are effective and safe for LCH.[13]

The correlation between BRAFV600E mutation and clinical features, treatment response, and clinical outcomes of LCH that has been reported in different studies is inconsistent. An international cohort study[12] reported an association between BRAFV600E and younger age at diagnosis and a higher prevalence of multisystem involvement, high-risk disease, and cutaneous involvement. In the entire cohort, BRAFV600E was associated with decreased event-free survival rates. A study[9] conducted in Türkiye also demonstrated that BRAFV600E mutation was significantly associated with multisystem involvement, younger age (< 2 years), and cutaneous or special organ involvement, and was an independent predictor of disease recurrence. However, research conducted in Japan revealed that the BRAFV600E mutation showed no correlation with gender, age at diagnosis, disease severity, response to frontline treatment, recurrence, or sequelae related to central nervous system (CNS).[8]

Immune checkpoint programmed cell death-1 and its ligand programmed cell death-1 ligand 1 (PD-L1) are associated with the pathogenesis of various malignancies. The positive expression rate of PD-L1 in LCH was reported to be 32%, and increased PD-L1 expression acted as an independent predictor of poor disease-free survival.[14] However, Tandon et al.[15] reported no significant correlation between PD-L1 expression and clinical outcomes of childhood LCH. Therefore, further research is needed to investigate the correlation of PD-L1 expression with clinical features, treatment response, and clinical outcomes of LCH.

Thus far, no biomarkers for risk stratification have been identified in LCH.[1] It is necessary to actively seek new tumor biomarkers to improve risk stratification, identify potential molecular targeted therapies, and reduce the incidence of disease reactivation and sequelae (such as diabetes insipidus, growth retardation, sclerosing cholangitis, etc.).

Recently, it has been reported that increased cathepsin S (CTSS) expression is associated with different types of cancers.[16,17] CTSS is a papain-type cysteine protease that is widely present in various cells.[18] Typically, the secretion of CTSS occurs via vesicle exocytosis induced by elevated intracellular Ca^{2+} levels, resulting in lysosome fusion with the plasma membrane and subsequent release of their contents into the extracellular space.[19] CTSS plays roles in antigen presentation, cell signaling transduction, and the promotion of chemokine or cytokine release.[20] It is also involved in regulating multiple pathophysiological processes, including immune response modulation, angiogenesis and remodeling, as well as the promotion of tumor cell proliferation and metastasis.[18] The hydrolytic cleavage of specific proteins by CTSS contributes to pro-tumorigenic conditions, influences signaling pathways, and promotes tumor cell metastasis.[16] However, to the best of our knowledge no studies have yet explored the relationship between CTSS and LCH. Therefore, we hypothesize that there is a relationship between CTSS and LCH.

Herein, this study reassessed the expression and clinical correlation of BRAFV600E mutations and PD-L1 in children with LCH, meanwhile, investigating the expression of CTSS in pediatric LCH and its relationship with the clinical characteristics, treatment response, and prognosis of LCH.

Materials and Methods

Patients

A total of 35 children with LCH admitted for inpatient treatment at the Department

of Pediatric Hematology and Oncology, the Affiliated Hospital of Qingdao University from January 1, 2018, to September 30, 2023, were selected for the study. The diagnosis of LCH relied on the pathological biopsy results of affected tissues, with confirmation by immunohistochemical staining for CD1a and CD207 (Langerin) positivity.[7] Patients were classified into single-system involvement and multisystem involvement based on the number of affected systems. Risk organs (RO) included the hematopoietic system / bone marrow, liver, and spleen.[6,7] CNS-risk sites included craniofacial, ocular, auricular, and oral regions.[6,7] This study was approved by the Medical Ethics Committee of the Affiliated Hospital of Qingdao University and complied with the requirements of the Helsinki Declaration revised in 2013, with informed consent obtained from the guardians of the children.

LCH treatment regimens and assessment criteria for treatment response

Treatment for all patients with LCH followed a stratified approach based on clinical risk, and the chemotherapy regimen was LCH-III.[7] Targeted therapy with dabrafenib (initiated during the induction phase and continued throughout the entire treatment course) was added to the treatment for patients with BRAFV600E mutation. The chemotherapy regimen for patients with disease progression/reactivation was the BCH-LCH 2014 protocol (a combination of cytarabine, vindesine, and dexamethasone).[6] Treatment response was categorized as follows: 1) nonactive disease, 2) active disease/better (AD-better), 3) AD/intermediate (AD-intermediate), and 4) AD/worse (AD-worse).[7] Response to treatment included nonactive disease and AD-better. Disease progression comprised AD-intermediate and AD-worse. Reactivation was defined as the reappearance of signs and symptoms of active disease after complete remission or after a disease control period lasting more than 3 months following maintenance therapy.[6]

Droplet digital polymerase chain reaction (DDPCR) for BRAFV600E mutation detection

The BRAFV600E mutation was detected by DDPCR (the most sensitive method[21]). Tissue DNA was extracted from unstained paraffin-embedded tissue at diagnosis using the GeneRead DNA FFPE Kit (180134, QIAGEN, Hilden, Germany). BRAFV600E mutation detection was performed using the DDPCR System (SG-2000, Suzhou RainSure Scientific CO., LTD., Suzhou, Jiangsu, China). DDPCR-related reagents were purchased from Suzhou RainSure Scientific CO., LTD. The reaction program included a pre-denaturation step at 95 °C for 10 min, followed by 40 amplification cycles of 95.3 °C for 30 s and 57 °C for 1 min, and a final extension at 98 °C for 10 min, with a holding step at 20 °C for 2 min. The detection limit was set at 0.1%. Primers and probes were designed using AlleleID (version 6.0, PREMIER Biosoft, Palo Alto, CA, USA) as follows:

Forward primer:
5′-TGCTTGCTCTGATAGGAAAATGA-3′

Reverse primer:
5′-CCATCCACAAAATGGATCCAGAC-3′

Wild-type probe: FAM-
AGCTACAGTGAAATC-MGB

Mutant probe: VIC-
AGCTACAGAGAAATCTC-MGB

Immunohistochemistry (IHC) for PD-L1 and CTSS

IHC was performe d on formalin-fixed paraffin-embedded tissue sections. Continuous formalin-fixed paraffin-embedded tissue sections (4-μm thick) were deparaffinized and hydrated. After heat-induced antigen retrieval, endogenous peroxidase was blocked with 3% H_2O_2. Non-specific antigens were blocked with 5% goat serum. The sections were then incubated with anti-PD-L1 rabbit monoclonal antibody (SP142, working solution, 08008540001, Roche Diagnostics GmbH, Tucson, AZ, USA) and

rabbit monoclonal antibody against CTSS (EPR5128, 1:250, ab134157, Abcam, Cambridge, U.K.) at 37 °C for 60 min. Subsequently, the sections were incubated with HRP-conjugated goat anti-rabbit secondary antibody (1:250, ab6721, Abcam, Cambridge, U.K.) at 37 °C for 30 min. After incubation, the sections were stained with diaminobenzidine chromogen solution (ab64238, Abcam, Cambridge, U.K.) and observed under an optical microscope. All experiments were repeated twice. Selected cases underwent dual IHC using the VECTASTAIN® ABC kit (PK-6200 and AK-5200, Vecort Laboratories, Burlingame, CA, USA) and substrate kits (diaminobenzidine substrate kit, SK-4100 and VECTOR Red Substrate kit, SK-5100, Vecort Laboratories, Burlingame, CA, USA) according to the manufacturer's instructions for CTSS (ab134157, Abcam, Cambridge, U.K., 1:250) and Langerin (1:500, bs-2650R, Beijing Biosynthesis Biotechnology Co., Ltd., Beijing, China) staining.

IHC was performed on tissue samples from 31 patients, as the remaining 4 patients had insufficient tissue samples. Immunohistochemical results were assessed by experienced pathologists. PD-L1 staining in > 5% of total cells was considered positive.[22] A semi-quantitative scoring system using the H-score was applied. The staining percentage (0-100%) and intensity (0-3: 0, negative; 1, weak; 2, moderate; 3, strong) of CTSS in the lesions were evaluated, and the H-score was calculated using the following formula (0-300): H-score = (% of cells of weak intensity × 1) + (percentage of cells of moderate intensity × 2) + (percentage of cells of strong intensity × 3).[23]

Statistical analysis

Data analysis was performed using IBM SPSS Statistics (version 26, International Business Machines Corporation, Amonk, NY, USA). Continuous variables are expressed as mean ± standard deviation ($\bar{x}\pm s$). For intergroup quantitative comparisons, t-test was used if the data met the normal distribution, and the Mann-Whitney U test was used if the normal distribution assumption was violated. Qualitative data are reported as percentages, and the Fisher's exact test was employed. Multivariate analysis was conducted by logistic regression analysis. A p-value < 0.05 was considered statistically significant.

Results

General clinical features

Bone involvement (94.3%) was the most common. Multisystem involvement occurred in 65.7% of cases. RO involvement was observed in 22.9% of cases, with the liver being the most frequently affected organ (14.3%). Liver involvement mainly manifested as hepatomegaly, with two cases progressing to cholangitis (one with intrahepatic bile duct stones). BRAFV600E gene mutation was detected in 12 cases (34.3%), with 83.3% (10/12) of them receiving targeted therapy (dabrafenib). The overall rate of response to treatment for the 35 patients was 85.7%. The incidence of disease progression/reactivation was 14.3% (5/35). See Table I for basic clinical characteristics.

Association of BRAFV600E gene mutation with clinical features, treatment response, and prognosis

The incidence of BRAFV600E gene mutation was 34.3% (12/35). The mutation rates in the ≤ 2 years age group and > 2 years age group were 66.7% and 17.4%, respectively, with a statistically significant difference ($P < 0.05$). The mutation rates in the CNS-risk site involvement group and non-CNS-risk site involvement group were 72.7% and 16.7%, respectively, with a statistically significant difference ($P < 0.05$). BRAFV600E gene mutation was not associated with gender, RO+, multisystem involvement, CNS involvement, bone involvement, skin involvement, lymph node involvement, pituitary involvement, initial 6-week treatment response, or disease progression/reactivation, with no statistically significant differences ($P > 0.05$); see Table II for details.

Table I. General clinical characteristics of 35 children with LCH.

Characteristics	Number	Percentage (%)
Gender		
Male	19	54.3
Female	16	45.7
Age (years)		
≤ 2	12	34.3
> 2	23	65.7
Involvement of sites		
Skin	4	11.4
Bone	33	94.3
CNS-risk site	11	31.4
CNS	8	22.9
Liver	5	14.3
Spleen	4	11.4
Hematopoietic system/bone marrow	3	8.6
Lung	4	11.4
Lymph node	6	17.1
Endocrine system (pituitary)	3	8.6
Involvement of systems		
Single system	12	34.3
Multisystem	23	65.7
Risk organ involvement		
Yes	8	22.9
No	27	77.1
Complication: HLH	1	2.9
Targeted therapy (dabrafenib)	10	28.6
Response to treatment	30	85.7
Treatment with dabrafenib	7	70.0
Treatment without dabrafenib	23	92.0
Initial 6-week treatment response	29	82.9
Treatment with dabrafenib	6	60.0
Treatment without dabrafenib	23	92.0
Progress/reactivation	5	14.3
Risk organ involvement	2	40.0
Without risk organ involvement	3	60.0
Deaths	0	0

CNS, central nervous system; HLH, hemophagocytic lymphohistiocytosis; LCH, Langerhans cell histiocytosis.

Association of PD-L1 expression with clinical features, treatment response, and prognosis

The expression rate of PD-L1 was 35.5% (11/31). The expression rates in the skin involvement group and non-skin involvement group were 100% (3/3) and 28.6% (8/28), respectively, with a statistically significant difference (P = 0.037). PD-L1 expression showed no correlation with gender, age at onset, BRAFV600E gene mutation, RO+, multisystem involvement, CNS involvement, CNS-risk site involvement, bone involvement, lymph node involvement, pituitary involvement, initial 6-week treatment response, or disease progression/reactivation, with no statistically significant differences (P > 0.05); see Table III for details.

Relationship of CTSS expression with clinical features, treatment response, and prognosis

CTSS showed positive expression in tissues of all 31 children (Fig. 1). The expression levels of CTSS in the ≤ 2 years age group and > 2 years age

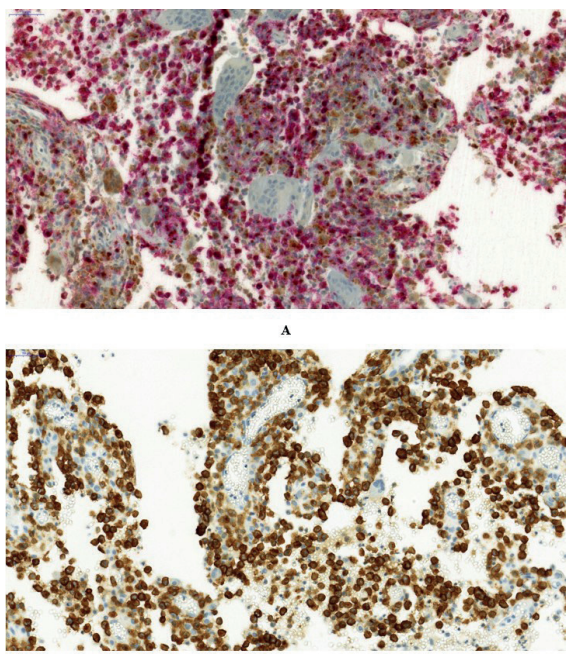

Fig. 1. Immunohistochemical staining: **(A)** Dual immunohistochemical staining, CD207+ (red), cathepsin S (CTSS)+ (brown), original magnification x400. **(B)** Immunohistochemical staining, CTSS+ (brown), original magnification x400.

Table II. The correlation between BRAFV600E gene mutations and clinical characteristics, treatment response, and prognosis in children with LCH (n=35).

Factors	BRAFV600E (+), n (%)	BRAFV600E (-), n (%)	P value*
Gender			0.311
Male	5 (26.3)	14 (73.7)	
Female	7 (43.8)	9 (56.2)	
Age (years)			0.007
≤ 2	8 (66.7)	4 (33.3)	
> 2	4 (17.4)	19 (82.6)	
Risk organ involvement			0.11
Yes	6 (75.0)	2 (25.0)	
No	6 (22.2)	21 (77.8)	
Multisystem involvement			0.149
Yes	10 (43.5)	13 (56.5)	
No	2 (16.7)	10 (83.3)	
CNS-risk site involvement			0.002
Yes	8 (72.7)	3 (27.3)	
No	4 (16.7)	20 (83.3)	
CNS involvement			0.216
Yes	1 (12.5)	7 (87.5)	
No	11 (40.7)	16 (59.3)	
Bone involvement			1.000
Yes	11 (33.3)	22 (66.7)	
No	1 (50.0)	1 (50.0)	
Skin involvement			0.106
Yes	3 (75.0)	1 (25.0)	
No	9 (31.0)	22 (69.0)	
Lymph node involvement			0.151
Yes	4 (66.7)	2 (33.3)	
No	8 (29.6)	21 (70.4)	
Endocrine system (pituitary) involvement			0.266
Yes	2 (66.7)	1 (33.3)	
No	10 (31.3)	22 (68.7)	
Initial 6-week treatment response			0.151
Response	8 (27.6)	21 (72.4)	
No response	4 (66.7)	2 (33.3)	
Progress/reactivation			0.313
Yes	3 (60.0)	2 (40.0)	
No	9 (30.0)	21 (70.0)	

(+), mutation positive; (-), mutation negative CNS, central nervous system; LCH, Langerhans cell histiocytosis.
* Fisher's exact test.

Table III. The correlation between PD-L1 expression and clinical characteristics, treatment response, and prognosis in children with LCH (n=31).

Factors	PD-L1 (+), n (%)	PD-L1 (-), n (%)	P value*
Gender			0.716
Male	5 (31.3)	11 (68.7)	
Female	6 (40.0)	9 (60.0)	
Age (years)			0.423
≤ 2	5 (50.0)	5 (50.0)	
> 2	6 (28.6)	15 (71.4)	
BRAFV600E gene mutation			0.452
(+)	5 (45.5)	6 (54.5)	
(-)	6 (30.0)	14 (70.0)	
Risk organ involvement			0.405
Yes	4 (50.0)	4 (50.0)	
No	7 (30.4)	16 (69.6)	
Multisystem involvement			1.000
Yes	8 (38.1)	13 (61.9)	
No	3 (30.0)	7 (70.0)	
CNS-risk site involvement			1.000
Yes	4 (40.0)	6 (60.0)	
No	7 (33.3)	14 (66.7)	
CNS involvement			1.000
Yes	3 (37.5)	5 (62.5)	
No	8 (34.8)	15 (65.2)	
Bone involvement			0.355
Yes	10 (33.3)	20 (66.7)	
No	1 (100)	0 (0)	
Skin involvement			0.037
Yes	3 (100)	0 (0)	
No	8 (28.6)	20 (71.4)	
Lymph node involvement			1.000
Yes	2 (40.0)	3 (60.0)	
No	9 (34.6)	17 (65.4)	
Endocrine system (pituitary) involvement			0.281
Yes	2 (66.7)	1 (33.3)	
No	9 (32.1)	19 (67.8)	
Initial 6-week treatment response			0.638
Response	8 (32.0)	17 (68.0)	
No response	3 (50.0)	3 (50.0)	
Progress/reactivation			0.317
Yes	3 (60.0)	2 (40.0)	
No	8 (30.8)	18 (69.2)	

(+), expression positive; (-), expression negative; CNS, central nervous system;LCH, Langerhans cell histiocytosis; PD-L1, programmed cell death-1 ligand 1.
* Fisher's exact test.

group were 216.00 ± 35.65 and 169.05 ± 63.79, respectively, with a statistically significant difference ($P < 0.05$). CTSS expression showed no correlation with gender, multisystem involvement, RO+, CNS-risk site involvement, CNS involvement, bone involvement, skin involvement, lymph node involvement, pituitary involvement, initial 6-week treatment response, disease progression/reactivation, BRAFV600E gene mutation, or PD-L1 expression, with no statistically significant differences ($P > 0.05$, Table IV).

Characteristics of children aged ≤ 2 years

The incidence of multisystem involvement in the ≤ 2 years age group and > 2 years age group was 91.7% and 52.2%, respectively ($P < 0.05$). The incidence of risk organ involvement in the ≤ 2 years age group and > 2 years age group was 58.3% and 4.3%, respectively ($P < 0.05$). The mutation rates of BRAFV600E in the ≤ 2 years age group and > 2 years age group were 66.7% and 17.4%, respectively ($P < 0.05$). The expression levels of CTSS in the ≤ 2 years age group and > 2 years age group were 216.00 ± 35.65 and 169.05 ± 63.79, respectively ($P < 0.05$), all exhibiting statistically significant differences (details presented in Table IV and TableV).

Multivariate analysis of the relationship of the prognosis (progression / reactivation) of LCH with CTSS, BRAFV600E gene mutations, and PD-L1

The results of the logistic regression analysis showed that CTSS expression, BRAFV600E gene mutations, and PD-L1 expression in tissue were not associated with the prognosis (progression/ reactivation) of LCH, with no statistically significant differences ($P>0.05$).

Discussion

In our study, the incidence of the BRAFV600E gene mutation was 34.3%, and it was significantly associated with age ≤ 2 years and involvement of CNS risk sites. The positive expression rate of PD-L1 was 35.5%, and it was significantly correlated with cutaneous involvement (notably, only three children exhibited cutaneous involvement). CTSS was positively expressed in the lesion tissues of all children with LCH. The H-scores of CTSS were significantly associated with age ≤ 2 years.

The reported incidence of tissue BRAFV600E gene mutations vary among different research centers. The reported incidence of BRAFV600E gene mutations in China ranges from 30 to 60%, and the incidence in East Asian populations may be lower than that in other ethnic groups.[10,11,14,24] In this study, the incidence of BRAFV600E gene mutations was 34.3%, which is consistent with the aforementioned research findings but lower than the results reported in an international cohort study (50.7%).[12] This may be due to the preservation time of the specimen, the abundance of tumor tissue within it, or the relatively small sample size. BRAFV600E gene mutations were correlated with onset age ≤ 2 years, consistent with the findings of previous studies.[24] Additionally, this study found a correlation between BRAFV600E mutation and CNS-risk site involvement, consistent with the results of a previous study.[12] BRAFV600E gene mutation was not associated with RO+, multisystem involvement, initial 6-week treatment response, or disease progression / reactivation in this study, consistent with previous research reports.[11,14,25] A recent study by Tandon et al.[15] reported no significant correlation between BRAFV600E expression and clinical outcomes (early treatment response, reactivation rate, and late sequelae) in children with LCH, which is consistent with the findings of this study. A previous study[14] reported a significant correlation between BRAFV600E gene mutation and increased PD-L1 expression; however, this association was not observed in this study. Additional investigation is required to confirm the correlation between BRAFV600E and PD-L1. Therefore, BRAFV600E gene mutation may not be used as a biomarker for risk stratification in LCH currently.

The effect of adding dabrafenib on the induction response and outcomes during follow-up

Table IV. The relationship between CTSS expression and clinical characteristics, treatment response, and prognosis in children with LCH (n=31).

Factors	Number (%)	CTSS expression (H-score, mean ± standard deviation)	T value	P value
Gender			-0.576	0.569
Male	16 (51.6)	178.13 ± 56.83		
Female	15 (48.4)	190.67 ± 64.42		
Age (years)			2.16	0.039
≤ 2	10 (32.3)	216.00 ± 35.65		
> 2	21 (67.7)	169.05 ± 63.79		
BRAFV600E gene mutation			0.271	0.789
Positive	11 (35.5)	188.18 ± 44.23		
Negative	20 (64.5)	182.00 ± 68.02		
PD-L1 expression			1.501	0.144
Positive	11 (35.5)	202.73 ± 39.01		
Negative	20 (64.5)	174.00 ± 67.54		
Risk organ involvement			1.823	0.079
Yes	8 (25.8)	216.25 ± 39.62		
No	23 (74.2)	173.04 ± 62.41		
Multisystem involvement			-1.698	0.117
Yes	21 (67.7)	199.05 ± 41.58		
No	10 (32.3)	153.00 ± 80.84		
CNS-risk site involvement			0.494	0.625
Yes	10 (32.3)	192.00 ± 51.81		
No	21 (67.7)	180.48 ± 64.30		
CNS involvement			0.517	0.609
Yes	8 (25.8)	193.75 ± 45.02		
No	23 (74.2)	180.87 ± 64.87		
Skin involvement			-0.125	0.901
Yes	3 (9.7)	180.00 ± 34.64		
No	28 (90.3)	184.64 ± 62.45		
Lymph node involvement			1.138	0.264
Yes	5 (16.1)	212.00 ± 47.64		
No	26 (83.9)	178.85 ± 61.34		
Endocrine system (pituitary) involvement			-1.941	0.192
Yes	3 (9.7)	153.33 ± 41.63		
No	28 (90.3)	200.00 ± 0.00		
Initial 6-week treatment response			-0.486	0.631
Response	25 (80.6)	181.60 ± 64.27		
No response	6 (19.4)	195.00 ± 39.37		
Progress/reactivation			0.072	0.943
Yes	5 (16.1)	186.00 ± 36.47		
No	26 (83.9)	183.85 ± 64.00		

LCH, Langerhans cell histiocytosis; CTSS, Cathepsin S; PD-L1, programmed cell death-1 ligand 1; CNS, central nervous system; (+), positive; (-), negative.

Table V. Characteristics of children aged ≤ 2 years (n=35).

Characteristics	≤ 2 years, n (%)	>2 years, n (%)	P value*
Multisystem involvement			0.027
Yes	11 (91.7)	12 (52.2)	
No	1 (8.3)	11(47.8)	
Risk organ involvement			<0.001
Yes	7 (58.3)	1 (4.3)	
No	5 (41.7)	22 (95.7)	
BRAFV600E gene mutation			0.007
Yes	8 (66.7)	4 (17.4)	
No	4 (33.3)	19 (82.6)	

CNS, central nervous system; LCH, Langerhans cell histiocytosis.
* Fisher's exact test.

requires confirmation through a clinical trial. In this study, only 12 cases (due to the relatively small sample size) were BRAFV600E mutation-positive, and 2 of these cases did not receive dabrafenib treatment. Consequently, our study did not perform statistical analysis or evaluation of the therapeutic effect of dabrafenib.

The expression of PD-L1 in CD11C cells within lung lesions of LCH mice was significantly higher.[26] A study[22] conducted in Japan found that the expression rates of programmed cell death-1 and PD-L1 in six patients with musculoskeletal LCH were 16.6% and 83.3%, respectively, suggesting that the programmed cell death-1 / PD-L1 immune checkpoint molecules may play a role in the microenvironment of musculoskeletal LCH. Another study[14] with a large sample size reported that the positive expression rate of PD-L1 in LCH was 32%, showing no association with age, gender, multisystem involvement, or RO+. This is consistent with the findings of our study, where the PD-L1 expression rate was 35.5%. This study showed that PD-L1 expression was not correlated with the initial 6-week treatment response and progression/ reactivation of LCH, consistent with recent findings by Tandon et al.[15] where they found that PD-L1 was not significantly correlated with the clinical outcomes (early treatment response, reactivation rate, and late sequelae) of children with LCH. However, Zeng et al.[14] reported that *BRAF* mutation and increased PD-L1 expression were independent predictors of adverse disease-free survival, and BRAFV600E mutation was significantly correlated with increased PD-L1 expression. This study found a correlation between PD-L1 expression and skin involvement (however, there were only three cases of children with skin involvement) but not with BRAFV600E mutation, bone, lymph node, CNS or CNS-risk site involvement. Therefore, the correlation between PD-L1 and LCH requires further research, and currently, it may not be used as a biomarker for risk stratification in LCH.

CTSS is primarily found in the lysosomal/ endosomal compartment of antigen-presenting cells (such as B cells, macrophages, and dendritic cells), but it can also be produced by epithelial cells, smooth muscle cells, endothelial cells, and neutrophils.[19] Moreover, CTSS can also originate from tumor cells themselves or from other cell types in the tumor microenvironment, such as endothelial cells, macrophages, and T cells.[16] The pathological feature of LCH is granulomatous lesions, consisting of CD1a+ and CD207+ histiocytes/ dendritic cells and abundant inflammatory background cells, including T cells, neutrophils, eosinophils, B cells, monocytes, macrophages, and multinucleated giant cells[1], while the predominant cellular populations in the immune microenvironment are M2-polarized macrophages and regulatory T cells.[27] Therefore,

immunohistochemical detection in this study showed that CTSS was expressed in all 31 LCH patients, and it was associated with age ≤ 2 years. CTSS from infiltrating immune cells and endothelial cells in the tumor further influences the tumor microenvironment[16], and studies have confirmed that CTSS mutations induce a tumor-promoting immune microenvironment in follicular lymphoma.[28] Thus, considering the expression of CTSS in LCH lesion tissues, we speculate that CTSS may be involved in the pathogenesis of LCH.

Given the expression of CTSS in LCH lesion tissues, CTSS may potentially serve as a therapeutic target. The reasons are as follows: Firstly, loss of CTSS activity reduces lymphoma growth by limiting communication with CD4+ T follicular helper cells, while inducing antigen diversification and activation of CD8+ T cells.[29] Secondly, CTSS inhibition has non-redundant therapeutic potential in enhancing anti-tumor immune responses in indolent and invasive lymphomas.[29] Finally, targeting CTSS can induce autophagy by activating the epidermal growth factor receptor (EGFR) / rat sarcoma virus oncogene (Ras) / mitogen-activated protein kinase (MEK) / extracellular signal-regulated kinase (ERK) signaling pathway[30], indicating that it is a potential therapeutic target.[17]

The limitations of this study mainly include the relatively small sample size (associated with the rarity of the disease) and the possibility of admission rate bias. Future research should focus on multi-center studies with larger sample sizes or the construction of LCH cell lines/ animal models to further investigate the role of the CTSS gene in the pathogenesis of LCH.

In summary, CTSS is expressed positively in LCH, and its expression level is associated with age ≤ 2 years. BRAFV600E gene mutation, PD-L1, and CTSS may not be associated with the prognosis of LCH.

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Ethical approval

The study was approved by Medical Ethics Committee of the Affiliated Hospital of Qingdao University (date: 19.06.2023, number: QYFY WZLL 27894). Informed consent was obtained from their guardians.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: YN, SS, LW, LS; data collection: YN, SS, YW; analysis and interpretation of results: YN, SS, YW; draft manuscript preparation: YN. 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

Clinical and laboratory characteristics of children with leukemia: a 34-year single-center experience

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ABSTRACT

Backround. Leukemia is the most common childhood malignancy and often presents with nonspecific symptoms, which may lead to delays in diagnosis. Early recognition of clinical signs and laboratory abnormalities is essential to ensure timely referral and improve outcomes. This study assesses the clinical and laboratory characteristics of pediatric patients with acute and relapsed leukemia, points out key considerations during diagnosis, and investigates potential factors contributing to delayed diagnosis.

Methods. A retrospective analysis was performed on pediatric patients diagnosed with leukemia at a tertiary care hospital between the years 1986 and 2020. Early diagnosis was defined as a diagnosis made within 20 days of symptom onset.

Results. Among the 378 patients, fatigue was the most frequently reported symptom, followed by fever and bone or joint pain. Common laboratory abnormalities included anemia (83%), thrombocytopenia (80%), and leukocytosis (46%). Bone or joint pain ($p< 0.001$), mucosal bleeding ($p=0.013$), and pallor ($p=0.005$) were significantly associated with late diagnosis. In contrast, lymphadenopathy ($p=0.014$) and bone tenderness ($p=0.024$) were linked to earlier recognition. Among laboratory findings, low hemoglobin levels were associated with early diagnosis ($p=0.023$) and elevated platelet count was also significantly related to delayed diagnosis ($p=0.028$). In relapsed leukemia cases, abnormal blood count findings were common, and neurological symptoms were observed more frequently compared to acute leukemia patients.

Conclusions. Fatigue, fever, and bone or joint pain were identified as the most common presenting symptoms in acute leukemia cases, while hepatomegaly, splenomegaly, and lymphadenopathy were the predominant physical findings. Bone or joint pain, mucosal bleeding, and pallor were associated with late diagnosis, whereas lymphadenopathy and bone tenderness appeared to facilitate earlier recognition.

Key words: childhood leukemia, clinical presentation, diagnostic delay, relapsed leukemia.

Leukemia is the most common childhood cancer, accounting for one-third of all childhood cancer cases.[1]Acute leukemias are divided into two main groups: acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML).[2] Relapsed leukemia is defined as the reappearance of disease in patients who have previously achieved remission.[3] The initial symptoms, physical examination findings, and laboratory values of leukemias can mimic those of many other diseases. This often leads to misdiagnosis and delays in the diagnostic process.[4] Low awareness of clinical symptoms and appropriate diagnostic tests may also

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contribute to delays in diagnosis.[5] Therefore, early identification of symptoms, presence of specific physical findings and proper evaluation of laboratory tests are of critical importance. Herein, data at presentation of newly diagnosed leukemia cases and leukemic relapse cases over a 34-year period were analyzed. In this descriptive study, we defined the characteristics of patients who would be referred to the pediatric hematology department from primary health care institutions and the patients who would be at risk of relapse in the follow-up of leukemia.

Materials and Methods

Study design and participants

This study was approved by the Dokuz Eylül University Hospital Network Ethics Committee (Number of approval: 2020/15-36). In this study, pediatric patients diagnosed with acute leukemia and relapsed leukemia between the years 1986 and 2020 in the Division of Pediatric Hematology, Department of Pediatrics, Faculty of Medicine, Dokuz Eylül University were included. Symptoms, findings and laboratory parameters of the patients at the time of diagnosis were retrospectively analyzed.

Patients with acute leukemia were classified into two subgroups: ALL and AML. Relapsed cases were evaluated in comparison with patients newly diagnosed with acute leukemia.

The categorization of early and late diagnosis in the acute leukemia group was based on the median time to diagnosis calculated for the entire study population. Patients with a diagnostic interval shorter than the median value were assigned to the early diagnosis group, while those with an interval equal to or longer than the median were assigned to the late diagnosis group.

Symptom onset was assessed according to the history provided by the family. The time at which all presenting symptoms were first reported at the initial visit was considered the onset point.

Since there is a wide variation in age, normal blood count values were determined for each age group.[6]

Statistical evaluation

IBM SPSS Statistics 24.0 (SPSS Inc., Chicago, IL, USA) program was used for statistical evaluation. Frequency distributions were evaluated as number and percentage, and continuous variables (measurements) were evaluated as mean ± standard deviation. Shapiro–Wilk test was used to determine whether the data was normally distributed. For numerical variables, if the assumptions of parametric tests were met, the results were presented as mean ± standard deviation; if not, the median (minimum-maximum, interquartile range [IQR]) was reported. For categorical variables, the frequency (n) and percentage (%) were provided. In the comparison of continuous variables for two groups, Student-t test was used if there was a normal distribution, and Mann-Whitney U test if there was no normal distribution. Categorical variables were compared using Pearson chi-square or Fisher's exact test. Reported p values were considered significant if <0.05. Logistic regression analysis was performed to identify clinical predictors of late diagnosis. In addition to symptom-based variables, similar regression models were planned for physical examination findings and laboratory parameters to assess their independent associations with delayed diagnosis.

Results

Of the total 423 patients in this study, 378 patients were diagnosed with newly diagnosed acute leukemia and 45 patients with relapsed leukemia. In the acute leukemia group, 313 (83%) patients had ALL, and its frequency aligned with previous studies.[7,8] The median age of patients with acute leukemia was 58.5 months (3-216 months, IQR: 91 months) for ALL

and 142.5 months (1 month-214 months, IQR: 89 months) for AML. The ages of diagnosis closely resembled those observed in earlier studies.[9-11]

Seventy-three patients with acute leukemia (19.3%) were referred with a suspicion of leukemia from various hospital departments. The majority were referred from the pediatric emergency department (26 patients, 6.9%), followed by orthopedics (10 patients, 2.6%), otorhinolaryngology (8 patients, 2.1%), and rheumatology (6 patients, 1.6%).

A total of 378 patients diagnosed with acute leukemia were included in the study. The mean age of these patients (196 males, 52%) was 71.5 months (1-216 months, IQR: 104 months). The median time from the onset of the first symptom to diagnosis was 20 days (2-350 days, IQR: 23 days). The most extreme outlier was a patient who had initially presented with the same symptoms—including fever, fatigue, approximately 10% weight loss over the preceding month, and bone pain—approximately ten months prior, followed by a recurrence of these symptoms. Fatigue was the most frequently reported symptom, followed by fever, bone or joint pain, and cutaneous signs of bleeding. The frequencies of symptoms were compared between ALL and AML patients. Bone or joint pain was more common in ALL patients and neurologic symptoms were more common in AML patients. Also, asymptomatic patients were more common in the AML group. Table I presents the distribution of symptoms among two groups. In addition to these, less common presenting symptoms among all patients with acute leukemia included cough (11 patients), gingival hyperplasia (7 patients), diarrhea (6 patients), hematuria (4 patients), night sweats (3 patients), and chest pain (2 patients).

Among the physical examination findings at the time of admission, extramedullary involvement findings such as hepatomegaly, splenomegaly and lymphadenopathy (LAP) were common. Extramedullary involvement was observed more often in the ALL group, compared to the AML group. Pallor was the most frequent physical examination finding in the AML group. The comparative physical examination findings for the two groups are summarized in Table II.

Anemia was present in 313 patients (82.8%), thrombocytopenia in 304 patients (80.4%), and both in 267 patients (70.6%). Leukocytosis was observed more commonly compared to leukopenia. The frequencies of abnormal complete blood count (CBC) findings are summarized in Table III.

Laboratory tests revealed that liver enzymes and phosphate levels were significantly higher in the ALL group. In the AML group, mean

Table I. Distribution of symptoms in acute leukemias (N=378).

Symptoms	ALL (N=313), n (%)	AML (N=65), n (%)	p value
Fatigue	147 (46.9)	32 (49.2)	0.756
Fever	143 (45.6)	29 (44.6)	0.875
Bone or joint pain	118 (37.6)	7 (10.7)	<0.001
Weight loss	40 (12.7)	12 (18.4)	0.226
Mucosal bleeding	25 (7.9)	9 (13.8)	0.133
Skin bleeding	52 (16.6)	13 (20)	0.51
Neurological	19 (6)	9 (13.8)	0.029
Neck swelling	51 (16.2)	6 (9.2)	0.148
Pallor	58 (18.8)	12 (18.4)	0.99
Abdominal pain	27 (8.75)	2 (30.6)	0.054
Asymptomatic	8 (2.5)	3 (4.6)	<0.001

ALL: acute lymphoblastic leukemia, AML: acute myeloid leukemia.

Table II. Physical examination findings in acute leukemia (N=378).

Findings	ALL (N=313), n (%)	AML (N=65), n (%)	p value
Hepatomegaly	215 (69)	32 (49)	0.002
Splenomegaly	191 (61)	23 (35)	<0.001
Lymphadenopathy	154 (49)	18 (27)	0.001
Pallor	162 (51)	34 (49)	0.975
Ecchymosis	44 (14)	11 (16)	0.565
Petechiae	51 (16)	8 (12)	0.410
Testicular enlargement, firmness	4 (1.2)	1 (1.5)	0.082
Normal Findings	11 (3.5)	4 (6.1)	0.330

ALL: acute lymphoblastic leukemia, AML: acute myeloid leukemia.

Table III. Complete blood count abnormalities in acute leukemia (N=378).

Findings	n (%)
Anemia	313 (82.8)
Thrombocytopenia	304 (80.4)
Anemia and thrombocytopenia	267 (70.6)
Leukocytosis	176 (46.5)
Leukocytosis and thrombocytopenia	154 (40.7)
Anemia and leukocytosis	142 (37.5)
Anemia, leukocytosis and thrombocytopenia	130 (34.3)
Leukopenia	85 (22.4)
Anemia and leukopenia	74 (19.5)
Leukopenia and thrombocytopenia	65 (17.1)
Anemia, leukopenia and thrombocytopenia (pancytopenia)	57 (15.0)

corpuscular volumes were recorded to be higher. No significant difference was observed between the other parameters. Table IV summarizes all laboratory parameters in acute leukemia.

Comparison between the early and late diagnosis groups revealed similar median ages at diagnosis, with 78.5 months in the early group and 84 months in the late group. Laboratory parameters, including leukocyte count, neutrophil count, hemoglobin level, and mean corpuscular volume, showed no significant differences between the groups. Thrombocyte and lactate dehydrogenase (LDH) levels were also comparable. Additionally, biochemical markers—including uric acid, aspartate aminotransferase, alanine aminotransferase, calcium, and phosphate—were similar in both groups.

In terms of clinical symptoms, fatigue, fever, and neck swelling occurred at similar rates across both groups. However, bone or joint pain, and weight loss were notably more prevalent in the late diagnosis group. Other symptoms, such as skin/ mucosal bleeding, neurological symptoms, and pallor, do not show substantial differences between the groups (see Table V).

In the logistic regression analysis, several clinical features were significantly associated with late diagnosis. Bone or joint pain (B = 1.357, $p < 0.001$, odds ratio [OR] = 3.886), mucosal bleeding (B = 1.076, p = 0.013, OR = 2.932), and pallor (B = 0.851, p = 0.005, OR = 2.341) were independently associated with a significantly increased likelihood of late diagnosis. When physical examination findings were analyzed separately, lymphadenopathy (B =-0.544, p

Table IV. Laboratory findings in patients with ALL and AML (N=378).

Parameters	ALL (N=313)	AML (N=65)	p value
Leukocytes (x10^9 /L)	11.3 (0.34-595)	17.9 (0.8-637)	0.3
Neutrophils (x10^9 /L)	1.1 (0-213)	1.5 (0.1-173)	0.087
Hemoglobin (g/dL)	7.9 (2.1-16.4)	8.7 (3.5-12.3)	0.172
MCV (fL)	82 (54.2-144)	88.7 (71-110)	<0.001
Thrombocytes (x10^9 /L)	59 (2-833)	48 (5-505)	0.590
LDH (IU/L)	638 (139-15700)	646 (210-3329)	0.880
Uric acid (mg/dL)	4.8 (0.5-20.3)	4.2 (0.5-11.8)	0.102
AST (IU/L)	32 (6-2017)	23 (10-86)	<0.001
ALT (IU/L)	18 (5-1588)	13 (2-158)	<0.002
Calcium (mg/dL)	9.3 (2.3-14.3)	9.1 (2.5-10.3)	0.152
Phosphate (mg/dL)	4.7 (0.7-9.5)	4.4 (1.5-6.6)	0.002

Data presented as median (range); ALL: acute lymphoblastic leukemia, ALT: alanine aminotransferase, AML: acute myeloid leukemia, AST: aspartate aminotransferase, LDH: lactate dehydrogenase, MCV: mean corpuscular volume.

Table V. Comparatison of the characteristics of patients with early vs. late diagnosis.

Parameters	Early diagnosis (N=199)	Late diagnosis (N=179)	p value
Age (month)	78.5 (1-216)	84 (3-208)	0.11
Leukocytes (x10^9 /L)	9.05 (0.34-637)	9.7 (1.2-595)	0.605
Neutrophils (x10^9 /L)	1.1 (0-140)	1.4 (0.1-134)	0.418
Hemoglobin (g/dL)	8.3 (4.6-13.7)	8.3 (3-16.4)	0.145
MCV (fL)	82.2 (54.2-100.0)	84 (63-103.8)	0.240
Thrombocytes (x10^9 /L)	66.5 (5-833)	75 (2-559)	0.474
LDH (IU/L)	572 (211-15700)	699 (237-4378)	0.134
Uric acid (mg/dL)	4.3 (0.5-20)	5 (1.7-20.3)	0.142
AST (IU/L)	30 (6-267)	25 (11-273)	0.162
ALT (IU/L)	15.5 (6-900)	15 (5-213)	0.204
Calcium (mg/dL)	9.3 (7-14.3)	9.4 (6.9-11.1)	0.826
Phosphate (mg/dL)	4.7 (1.6-9.5)	4.7 (0.7-6.8)	0.509
Fatigue	56 (50.9%)	80 (52.6%)	0.8
Fever	51(46.4%)	70 (46.1%)	>0.99
Bone or joint pain	19 (17.3%)	62 (40.8%)	<0.001
Weight loss	9 (8.3%)	34 (22.4%)	0.002
Mucosal bleeding	7 (6.4%)	20 (13.2%)	0.099
Skin bleeding	27 (24.5%)	25 (16.4%)	0.110
Neurological	11 (10%)	13 (8.6%)	0.829
Neck swelling	16 (14.5%)	29 (19.1%)	0.407
Pallor	18 (16.4%)	40 (26.3%)	0.070

Data presented as median (range) for numerical variables and as n (%) for categorical variables.
ALL: acute lymphoblastic leukemia, ALT: alanine aminotransferase, AML: acute myeloid leukemia, AST: aspartate aminotransferase, LDH: lactate dehydrogenase, MCV: mean corpuscular volume.

= 0.014, OR = 0.580) and bone tenderness (B= -0.573, p=0.024, OR=0.564) were significantly associated with a decreased likelihood of late diagnosis. Among laboratory parameters, lower hemoglobin levels (B= -1.127, p = 0.023, OR = 0.880) and higher platelet counts (B=0.001, p=0.028, OR=1.003) were significantly associated with late diagnosis. Neutrophil count approached statistical significance (B = -0.017, p = 0.072, OR = 0.983), indicating a potential trend.

There were 45 patients (27 males, 60%) diagnosed with relapsed leukemia. Twenty-one (46.6%) of these patients were on maintenance therapy and 24 (53.4%) were diagnosed after the end of chemotherapy.

Our findings revealed that 37.8% of patients with relapse were diagnosed with relapse even though they were asymptomatic during routine follow-up. The majority of patients (42 out of 45, 93.3%) experienced relapse within the first five years following initial diagnosis. Among patients diagnosed with relapse, the most frequent complaints were related to the neurologic system, accounting for 18%. Three patients with relapse presented with testicular enlargement. The newly diagnosed leukemia group showed a higher frequency of symptoms like fatigue, fever, bone or joint pain, and weight loss, whereas the relapsed leukemia group had more instances of neurologic and testicular involvement. The distribution of symptoms for both groups is summarized in Table VI.

Patients with relapsed leukemia had less significant physical examination findings compared to those with newly diagnosed leukemia. The presence of hepatomegaly was observed in 10 patients (22%), splenomegaly in 7 patients (16%), and LAP in 3 patients (7%) with relapse. Testicular enlargement was observed in 4 patients (9%), surpassing its frequency in the newly diagnosed leukemia group (p=0.01).

Thrombocytopenia (26 cases, 58%) and anemia (16 cases, 36%) were the predominant CBC results among relapsed patients. Pathological CBC findings were observed in all symptomatic patients and in 55% of those without symptoms. Elevated uric acid and LDH levels due to tumor lysis syndrome were observed in 18 patients (40%).

Discussion

Acute leukemia is the most common malignancy in children. Since access to diagnostic facilities is more difficult especially in developing countries, the time to diagnosis varies from that in developed countries.[5,12] Therefore, presenting symptoms, physical examination findings and first-line investigations should guide access to diagnostic facilities.

Table VI. Distribution of symptoms in leukemias (N=423).

Symptoms	Newly diagnosed leukemias (N=378), n (%)	Relapsed leukemias (N=45), n (%)	p value
Fatigue	179 (47.5)	5 (11.1)	<0.001
Fever	172 (45.5)	7 (15.6)	<0.001
Bone or joint pain	125 (33.1)	7 (15.6)	0.017
Weight loss	52 (13.8)	1 (2.2)	0.029
Skin bleeding	65 (17.2)	2 (4.4)	0.029
Neurological symptoms	28 (7.4)	8 (17.8)	0.018
Neck swelling	57 (15.1)	0 (0)	0.002
Pallor	70 (18.5)	0 (0)	<0.001
Testicular enlargement	0 (0)	3 (6.7)	0.001
Asymptomatic	11 (2.9)	17 (37.8)	<0.001

In the present study, fatigue was the most common symptom among patients diagnosed with acute leukemia (48.8%), followed by fever (46%), bone or joint pain (31%), and skin hemorrhage (17%). A recent study of 203 patients with ALL revealed that the most common symptoms were fatigue, fever, and bone or joint pain.[13] A study conducted in our country, Türkiye showed that the top three symptoms were consistent with those of the present study, although fever was the leading cause for presentation.[14] Another study conducted in Colombia identified pallor as the most frequently reported symptom, followed by anorexia and weight loss as the second and third most common symptoms, respectively.[15] The most frequent presenting symptom in a study of 101 pediatric ALL patients from Saudi Arabia was fever, which was observed in one third of the cases. Fatigue and bone pain were also among the primary symptoms reported.[16] In another study conducted in Türkiye, ALL was identified as the most common malignancy among children presenting with rheumatological symptoms[17], underlining the importance of careful interpretation of clinical features in this patient group.

Another study from Saudi Arabia examined the presenting symptoms of 30 children with AML. The patients were highly symptomatic, with bone pain being the most common presenting symptom. The next most frequently reported symptoms were high fever and a history of bleeding.[18] Our study involved the evaluation of 313 patients with ALL and 65 patients with AML. Both groups experienced fatigue and fever as the most common symptoms. Differences in the timing of presentation may explain the variation in symptom frequency.

In a recent study, 96.1% of patients diagnosed with acute leukemia had abnormal physical examination findings. This high rate highlights the importance of careful physical examination. In our 313 patients, the most common abnormal physical examination finding was hepatomegaly (65.3%). Splenomegaly (56.6%) and LAP (45.5%) were the other common abnormal findings, respectively. The present study's findings aligned with the physical examination results in the study by Pérez et al.[13] These three findings were more frequently reported in another study conducted in our country.[14]

Physical examination often reveals abnormal findings, but laboratory tests must be considered because there are a wide variety of different underlying diseases. CBC findings revealed that anemia was common (83%) in newly diagnosed leukemia patients. Thrombocytopenia was the second most common abnormal CBC finding in 304 patients (80%). Anemia and thrombocytopenia were present in 267 patients (71%). In the study by Pérez et al. evaluating CBC results, anemia and thrombocytopenia were prominent among other findings. The combination of the two was observed in more than half of the patients.[13] These findings emphasize that the differential diagnosis of acute leukemia should be considered in patients with anemia and thrombocytopenia. These two laboratory findings can serve as a guide for referring patients to the hematology department, particularly from primary healthcare institutions.

Early and late diagnosis thresholds vary across studies. Thresholds for early and late diagnosis vary across studies. For example, a study from our country defined early diagnosis as within 15 days of symptom onset[19], whereas studies from China and Japan used cutoffs of 24 and 30 days, respectively.[20,21] These discrepancies underscore the absence of a universally accepted definition. In our study, we selected 20 days—the median time to diagnosis—as the threshold, in order to ensure comparable sample sizes between the early and late diagnosis groups. The median time to diagnosis reported in the literature ranges between 18.5 and 21 days[4,22], which is consistent with the findings in our study.

Our study highlights the importance of identifying clinical and laboratory features associated with early and late diagnoses of acute leukemia.

Although the mean values of laboratory parameters were similar between the early and late diagnosis groups, multivariate regression analysis revealed that anemia was independently associated with a decreased likelihood of delayed diagnosis, while elevated platelet counts were linked to an increased risk. Our finding contrasts with previous literature. For example, a recent study in Türkiye reported elevated leukocyte and LDH levels, along with lower platelet counts late diagnosis group.[19] These results suggest that peripheral blood abnormalities tend to become more pronounced in delayed cases. However, in our cohort—which included a larger sample size—we observed no such trend, indicating that delayed diagnosis may not consistently be associated with more evident hematological changes. Similarly, in a study by Dai et al. involving 419 patients with ALL, white blood cell counts and hemoglobin levels were found to be comparable between the early and late diagnosis groups, whereas platelet counts were higher in the late diagnosis group.[20] In light of these findings, it may be inferred that patients with more marked laboratory abnormalities are more likely to receive an earlier diagnosis. Accordingly, the observation that peripheral blood parameters were similar between early and late diagnosis groups in these two larger studies —despite the expectation of more pronounced abnormalities in delayed cases— may also be attributed to this tendency. This may lead to the suggestion that greater clinical awareness is directed toward laboratory parameters. In our study, we also investigated clinical symptoms and physical examination findings that might contribute to diagnostic delay. Notably, certain clinical symptoms, including bone or joint pain, mucosal bleeding, and pallor, emerged as significant indicators of delayed diagnosis. In contrast, physical findings such as lymphadenopathy and bone tenderness appeared to be more frequent in patients diagnosed earlier, suggesting that the presence of specific physical signs may facilitate timely recognition.

Our findings, showing similar frequencies of fatigue, fever, and lymphadenopathy between groups, further highlight the diagnostic challenges and the often subtle nature of presenting symptoms.

Despite advances in modern chemotherapy, relapsed leukemia remains the leading cause of mortality among children diagnosed with acute leukemia.[23] In the present study, relapse occurred in 45 (11.9%) out of 378 patients with acute leukemia. Among the patients, 19 (42%) experienced relapse while undergoing treatment, while 26 (%58) had relapsed during post-chemotherapy follow-up. In this study, 17 asymptomatic cases (37.8%) were diagnosed with relapsed leukemia based on physical examination and laboratory findings at routine follow-up. This result underlines the importance of follow-up. Among the laboratory tests, especially CBC revealed frequently abnormal results in symptomatic and asymptomatic patients. Once again, in this study the significance of routine follow-up with CBC was highlighted, as also reported in previous studies.[24] Neurologic symptoms were the most common symptom in relapsed cases. We recorded this as a result that could lead to an increased focus on neurologic symptoms at routine follow-up.

Our study has some limitations, including its retrospective design and single-center setting. Moreover, we were unable to assess several potential contributors to diagnostic delay — such as misinterpretation of symptoms by patients or parents, delayed presentation to healthcare facilities, parental education level, socioeconomic status, the patient's pubertal status, and the specialty of the first physician consulted outside the hospital— due to lack of available data.

Conclusions

The present study revealed that fatigue, fever, and bone or joint pain were the primary symptoms observed in children with acute leukemia. The most common physical

examination findings in acute leukemia patients were hepatomegaly, splenomegaly, and lymphadenopathy. The most frequently observed abnormalities in the CBC of acute leukemia patients were anemia and thrombocytopenia. Clinical symptoms such as bone or joint pain, mucosal bleeding, and pallor were associated with late diagnosis, whereas physical signs like lymphadenopathy and bone tenderness were linked to earlier recognition. In addition, elevated platelet counts may indicate an increased risk of delayed diagnosis, whereas low hemoglobin levels appear to be associated with a lower likelihood of diagnostic delay. Abnormal CBC findings were commonly observed at the time of relapsed leukemia diagnosis. Moreover, neurological symptoms emerged as the leading cause for hospital admission in these cases. In addition to the complaints, a careful physical examination and evaluation of the CBC are important in both diagnosis of initial leukemia and the detection of relapse.

Ethical approval

The study was approved by Dokuz Eylül University Hospital Network Ethics Committee (date: 06.07.2020 number: 2020/15-36).

Author contribution

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

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The authors declare that there is no conflict of interest.

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Short Communication

Ivermecatin induces cytotoxic effects in SUP-B15 cell line

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ABSTRACT

Background. Glucocorticoids remain the primary treatment for acute lymphoblastic leukemia (ALL) in children. However, glucocorticoid-resistant ALL exhibits increased mortality rates. To overcome resistance and improve management strategies, alternative therapeutic agents are required. Ivermectin (IVM), widely used as an anthelmintic agent, has been reported to possess anticancer properties through various mechanisms. These properties suggest IVM as a potential alternative treatment for ALL. This study aims to evaluate the role of IVM in inducing cytotoxic effects in the ALL cell line SUP-B15.

Methods. The ALL cell line SUP-B15 was examined following treatment with IVM at concentrations of 5, 10, and 20 μM. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to assess cytotoxic effects of IVM, including measurements of optical density, cell inhibition, and cell viability.

Results. IVM demonstrated inhibitory effects on SUP-B15 cells starting at a low dose of 5 μM. The half maximal inhibitory concentration (IC_{50}) of IVM was 5 μM, showing an inhibitory effect of 54.18 ± 0.03% (95% confidence interval: 54.11-54.25%; $p < 0.001$). Treatment with 10 μM IVM demonstrated the most significant inhibitory effect (59.05 ± 0.1%) and the lowest cell viability (40.95 ± 0.01%).

Conclusions. IVM holds potential as a promising alternative therapeutic agent for the treatment of ALL.

Key words: acute lymphoblastic leukemia, apoptosis, ivermectin, glucocorticoid resistance.

Acute lymphoblastic leukemia (ALL) is a malignancy of lymphoblasts, characterized by their uncontrolled and abnormal proliferation, which disrupts bone marrow production. This pathological process leads to the replacement of healthy bone marrow with leukemic cells, resulting in clinical manifestations associated with anemia, neutropenia, and thrombocytopenia.[1] In 2020, more than 60,000 new leukemia cases and over 25,000 leukemia-related deaths were reported.[2] ALL is the most reported type of leukemia, accounting for 70% of pediatric leukemia cases.[3] The condition predominantly affects children under 15 years, with the highest incidence observed between ages 1 and 4.[4] The 5-year survival rate of ALL is high in high-income countries, reported to be over 90%. Nevertheless, in low- and middle-income countries, this figure is substantially lower, where overall survival and disease-free

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survival rates are reported at 69.9% and 67.8%, respectively.[3] Despite the high survival rate of ALL, numerous cases have reported poor prognoses influenced by treatment-related factors.[5]

Chemotherapy remains the first-line treatment for ALL, aiming to achieve remission through induction, intensification, and maintenance phases.[6] The treatment regimen incorporates a range of cytostatic agents, with glucocorticoids (GCs) recognized as the most effective in inhibiting proliferation and inducing apoptosis in malignant cells.[6,7] Currently, GCs, particularly prednisone and dexamethasone, are the primary therapeutic agents for the majority of lymphoid neoplasms in both pediatric and adult populations. GCs predominantly impact lymphoid tissue by inhibiting cell proliferation and inducing apoptosis.[8] The cytotoxic properties of GCs have shown effectiveness in the treatment of ALL. Nonetheless, the emergence of GC resistance, observed both in vitro and in vivo, has been identified as a negative prognostic indicator in ALL.[9]

Resistance to GC has been a challenge in the management of ALL, as it results in inadequate treatment responses.[10] Consequently, the risk of relapse increases, necessitating higher chemotherapy dosages and potentially resulting in severe adverse effects.[11] Various studies have investigated alternative therapeutic strategies to manage GC resistance. One such potential alternative is ivermectin (IVM), which has been reported to exhibit anticancer properties through various mechanisms.[12] One mechanism involves the induction of mitochondrial dysfunction and oxidative stress, which promotes increased apoptosis of leukemia cells, particularly in acute and chronic myeloid leukemia.[13,14] In a leukemia xenograft model, IVM was shown to induce apoptosis and inhibit cell proliferation by enhancing the cleavage of poly(ADP-ribose) polymerase (PARP) and caspases.[15] Another study also reported the efficacy of IVM in promoting apoptosis in esophageal cancer cells by elevating the BAX/BCL-2 ratio.[16]

This study aimed to investigate the role of IVM in inducing cytotoxic effects in the ALL cell line SUP-B15.

Materials and Methods

This study was approved by the Research Ethical Committee of the Faculty of Medicine, Universitas Sumatera Utara (approval number: 665/KEPK/USU/2023) and conducted according to the principles outlined in the Declaration of Helsinki. In this study, we examined the cytotoxic effects of IVM against SUP-B15 cells. The SUP-B15 cells were B lymphoblast cells derived from the bone marrow of an 8-year-old Caucasian male patient with ALL.

Cell cultures

SUP-B15 cells (ATCC, Manassas, USA) were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (GIBCO, Berlin, Germany), 2 mM L-glutamine, 50 μg/mL streptomycin, and 50 μg/mL penicillin in an incubator set at 37 °C and 5% CO_2 concentration. The culture process was carried out in 24-well plates. To regulate confounding factors, including variations in culture conditions, this study followed a standard protocol of cell culturing and the cell culture was considered successful when the cell density ranged from 1.5 to 3 × 10^6 cells/mL.

Measurement of cytotoxic activity

Cytotoxic activity was measured in SUP-B15 cells by assessing optical density, inhibition, and cell viability using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The study was conducted in accordance with a standard protocol to mitigate confounding factors that affect cell viability, including the duration of the incubation period and the density of cells per well. The cells were categorized into 4 experimental groups: (1) IVM 5 μM; (2) IVM 10 μM; (3) IVM 20 μM; (4) negative controls. SUP-B15 cells were seeded in 96-well plates at a density of 2 × 10^4 cells/well

and incubated at 37 °C under 5% CO_2. After 24 hours of incubation, the cells were treated with IVM (5, 10, and 20 μM). Negative controls were obtained by excluding treatment from the wells. After 72 hours of treatment, the MTT assay was performed by adding 10 μL of MTT (5 mg/mL) to each well. Following a 4-hour incubation at 37 °C, absorbance was measured at a wavelength of 595 nm.

Statistical analysis

Data were processed and analyzed using the Statistical Product Service Solution (SPSS) software for Windows. The data were subjected to the Shapiro–Wilk normality test. A Levene's test for homogeneity of variance was performed to assess the variability of the data. One-way analysis of variance (ANOVA) was performed to determine the relationship between the variables tested, followed by a post-hoc test. A p-value <0.05 was considered statistically significant.

Results

IVM exhibits cytotoxic effects on SUP-B15 cells

The cytotoxicity of IVM on SUP-B15 cells is shown in Fig. 1. IVM exerted a significant inhibitory effect on SUP-B15 cells compared to the control, starting from a low dose of 5 μM. Fig. 2 shows the IVM dose-response curve of the SUP-B15 cell viability, normalized according to the absorbance of the MTT assay data. The half maximal inhibitory concentration (IC_{50}) of IVM was 3.435 μM. The curve took the form of a sigmoidal curve of the logarithmic inhibition model, which indicated increasing IVM concentrations caused a significant decrease in cell viability until reaching a plateau point approaching 0%. IVM at 10 μM produced the strongest inhibitory effect at 59.05 ± 0.10% (95% confidence interval [CI]: 58.80-59.29%; p < 0.001) and the lowest cell viability at 40.95 ± 0.01% (95% CI: 40.93-40.97%; p < 0.001). Fig. 3 presents the results of the post-hoc Bonferroni test. Optical

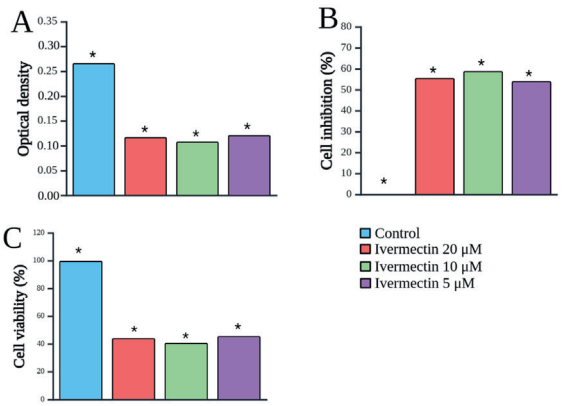

Fig. 1. Cytotoxicity activity of ivermectin on SUP-B15 cells.

*significant at p < 0.001.

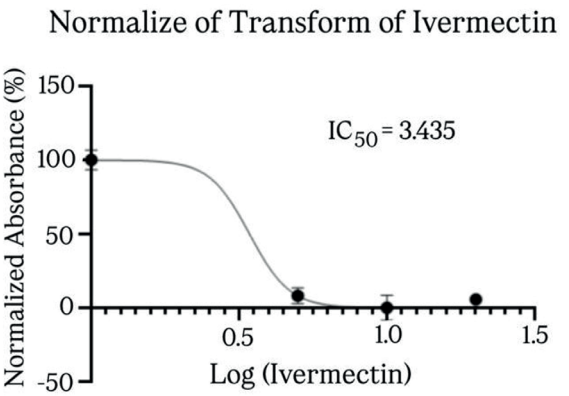

Fig. 2. Dose-response curve of ivermectin on cell viability based on MTT assay.

density measurements yielded a p-value of <0.001 for the correlation between the IVM and control groups. However, no significant differences were observed among IVM doses (5, 10, and 20 μM) in the optical density test. All treatment correlations yielded a p-value <0.001 for inhibition and viability, indicating significant differences between all treatment groups and the control in both assessments.

Discussion

The results of this study demonstrated that IVM exhibited cytotoxic effects on SUP-B15 cells in vitro, with observable effects starting at a low dose of 5 μM. These findings are consistent with a previous study that reported IVM to inhibit the

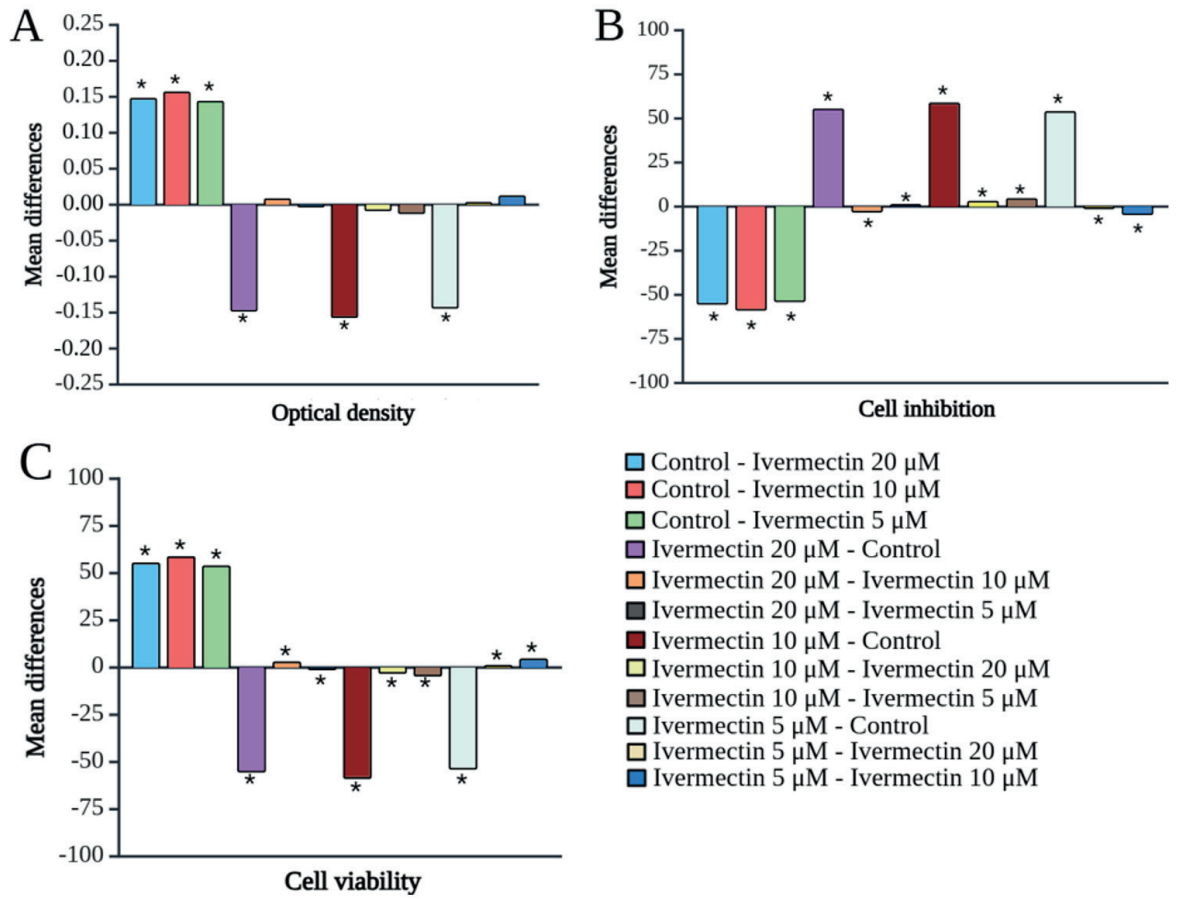

Fig. 3. Post-hoc Bonferroni test for cytotoxicity activity of ivermectin on SUP-B15 cells.
*significant at $p < 0.001$.

proliferation of colorectal cancer cells at a similar dose of 5 µM.[17] IVM has been reported to exert an inhibitory effect on malignant cells through various mechanisms.[12] Liu et al. reported that IVM inhibited malignant cell proliferation by inducing autophagy and apoptosis.[18] Furthermore, IVM induced apoptosis and delayed the proliferation of malignant cells by increasing the cleavage of PARP and caspases.[15] Another study reported that IVM can induce cell apoptosis even in the absence of receptor-interacting serine/threonine protein kinase 1 (RIPK1) and effector caspases, further supporting its potential as a potent anticancer drug.[19] Xu et al. also demonstrated that IVM significantly induced reactive oxygen species (ROS) accumulation, inhibited the activation of the NF-κB signaling pathway, and elevated the BAX/BCL-2 ratio, thereby effectively reducing the proliferation of esophageal cancer cells.[16] Elevated BAX and caspase-3 expressions, along with reduced BCL-2 expression, are known to promote cell apoptosis, ultimately inhibiting the proliferation of ALL cells.[20,21]

In this study, IVM at 10 µM concentration was reported to yield the strongest inhibitory effect on SUP-B15 cells. This finding contrasts with the pharmacokinetics of IVM, which typically exhibit antiparasitic and antiviral effects in a dose-dependent manner.[22,23] Interestingly, 10 µM IVM was more cytotoxic than 20 µM because the cytotoxic effects were exhibited through the induction of oxidative stress.[14] While oxidative

stress triggered apoptosis in malignant cells, it also initiated and promoted the development of malignant cells. Excessive doses of IVM can result in a significant accumulation of oxidative stress, potentially contributing to the initiation and proliferation of malignant cells.[24] Therefore, a concentration of 10 μM IVM was determined as the optimal dose to demonstrate cytotoxic effects in leukemia.

Limitations and suggestions for further research

A limitation of this study was the utilization of a single cell line due to budgetary limitations. The utilization of a single cell line limited the generalizability of IVM's efficacy on ALL. Research involving several cell lines and studies comparing IVM's efficacy against standard ALL treatments such as GCs or chemotherapeutic agents could provide better evidences regarding the efficacy of IVM on ALL. Further investigation of cell cycle alterations is necessary to improve our understanding of the apoptosis induced by IVM. Additional in vivo or an animal model study is recommended for better clinical applicability. This study provides a basis for subsequent in vitro investigations into the role of IVM in inducing cytotoxic effects in ALL.

Conclusions

IVM is increasingly recognized for its diverse therapeutic functions beyond its established antiparasitic properties. The cytotoxic effects of IVM against ALL were demonstrated in this study, and the findings suggest that IVM holds potential as an alternative therapeutic approach for ALL.

Ethical approval

The study was approved by the Research Ethical Committee of the Faculty of Medicine, Universitas Sumatera Utara (clearance number: 665/KEPK/USU/2023) and was conducted according to the Declaration of Helsinki.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: ORS, ASW, APP, DE, IDGU, RA, INDY, MR; data collection: ORS, ASW, APP, DE, IDGU, RA, INDY, MR; analysis and interpretation of results: ORS, ASW, APP, DE, IDGU, RA, INDY, MR; draft manuscript preparation: ORS, ASW, APP, DE, IDGU, RA, INDY, MR. 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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Case Report

Successful percutaneous retrieval of fractured umbilical artery catheter in a very low birth weight preterm neonate

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ABSTRACT

Background. Umbilical arterial catheterisation is a common intervention performed in the neonatal intensive care unit (NICU) especially in extremely preterm and extremely low birth weight neonates. Rarely catheter fracture or breakage can occur, leaving behind part of the catheter in the aorta. A handful of cases have been reported in the literature, with the majority being managed surgically. There is no well-established protocol or consensus on the optimal management of such situations, leaving clinicians to rely on innovative and individualized approaches to address this critical situation.

Case Presentation. We report the successful percutaneous retrieval of a fractured umbilical artery catheter (UAC) from the aorta of a very low birth weight preterm neonate born at 28 weeks of gestation, who was undergoing treatment for neonatal sepsis at our hospital's NICU. The fractured catheter was retrieved via the right femoral artery using an improvised snare created by double-folding and inserting a 300 cm, 0.014-inch coronary guidewire into a Judkins Right (JR) 6F coronary guide catheter, forming a loop that protruded from its distal end. Following retrieval, the patient exhibited transiently weak pulses and decreased limb movement in the accessed limb. A duplex colour Doppler scan was performed which ruled out thrombosis or dissection. It was attributed to a spasm of the common femoral artery, which recovered eventually.

Conclusion. This case demonstrates the feasibility and effectiveness of successful percutaneous removal of the fractured UAC from the aorta in very low birth weight preterm neonates and underscores the importance of innovative use of improvising hardware, appropriating it to the small size of the patient.

Key words: umbilical artery catheter, catheter fracture, percutaneous retrieval, improvised snare technique.

Umbilical arterial catheterisation is a commonly performed procedure in the neonatal intensive care unit (NICU), especially for extremely preterm and extremely low birth weight neonates. While it is a critical intervention for monitoring and managing these fragile patients, it carries inherent risks. Potential complications include catheter tip malposition, occlusion, bacterial infections, thrombosis, perforation of vessels during insertion, and vascular compromise of lower extremities or abdominal organs.[1,2] In rare and particularly challenging cases, the catheter itself may become fractured, leading to internal migration or displacement. The options for removal of these broken fragments include surgical removal or percutaneous snaring. There is no well-established protocol or consensus on the optimal management of such cases, leaving clinicians to rely on innovative and individualized approaches to address this

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critical situation. In this report, we present a case of a very low birth weight neonate with a fractured umbilical artery catheter (UAC) lying in the descending aorta-common iliac-internal iliac artery.

Case Presentation

With informed consent obtained from the legal guardians, we present the case of a first-born female infant from an artificially conceived twin pregnancy, delivered at 28 weeks of gestation via emergency caesarean section due to prolonged premature rupture of membranes. She was undergoing treatment in the NICU at our hospital due to very low birth weight (1030 grams), respiratory distress and early onset neonatal sepsis. Appearance, Pulse, Grimace, Activity, and Respiration (APGAR) scores at 1 minute and 5 minutes after birth were 5/10 and 9/10, respectively. The neonate was intubated in the delivery room as she did not cry immediately after birth and was on invasive mechanical ventilation for 7 hours. Early rescue surfactant was administered through an endotracheal tube. Two 3.5 Fr umbilical catheters (Vygon™) were inserted for routine blood sampling, parenteral feeding and invasive blood pressure monitoring on day 1 of life with one catheter each in the umbilical vein and umbilical artery. On day 4, both catheters were removed. However, part of the UAC, which got fractured during removal, was left in the aorta/common iliac artery and went unnoticed. Chest X-ray performed on day 22 of life to evaluate the cause of apnoea episodes revealed a fractured UAC catheter lying in the neonate's aorta. The neonate weighed 1300 g on this day. Investigations done before the procedure ruled out infection with a leucocyte count of 10.7 x 10^9/L, 77% neutrophils, C-reactive protein level of 0.57 mg/L and a sterile blood culture. Owing to a high surgical risk, the neonate was referred to us for percutaneous catheter retrieval.

Under general anaesthesia a 20-Gauge intravenous cannula was used to gain right femoral artery access, through which a 0.035-inch hydrophilic guidewire (Terumo™), followed by a 6F Radifocus radial sheath (Terumo™) was introduced. Thirty units of unfractionated heparin (UFH) were administered to prevent catheter-related thrombosis. An improvised snare was prepared by double folding a 300 cm 0.014-inch Balanced-Middle-Weight (Abbott™) coronary guidewire which was inserted in a Judkins Right (JR) 6F coronary guide catheter creating a loop protruding through its distal end (Fig. 1A). The broken catheter was caught within the loop at the level of the descending thoracic aorta and was snared out through the femoral sheaths (Fig. 2, Fig. 3; also see Video 1 in the Supplementary Materials). During the procedure, there was a blood loss of 10-15 mL (15% of the total blood volume of the neonate), which was replaced by packed red blood cell transfusion. Weak pulses with signs of hypoperfusion were noticed in the right

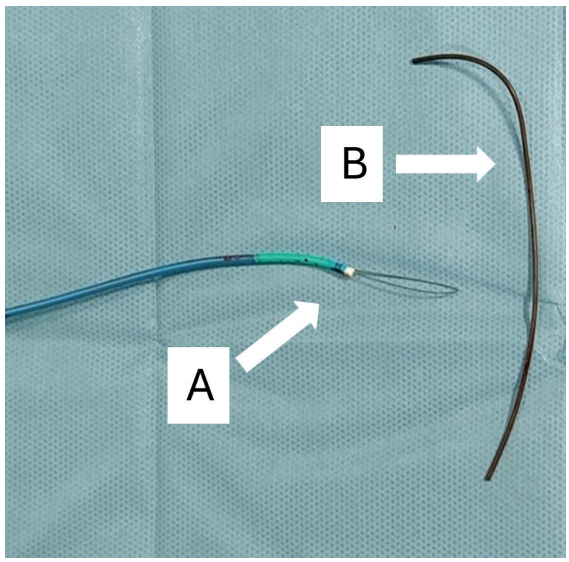

Fig. 1. Improvised snare made from Judkin's Right catheter with a 0.014 inch x 300 cm angioplasty guidewire folded into a loop (A); The UAC after removal from the body (B).

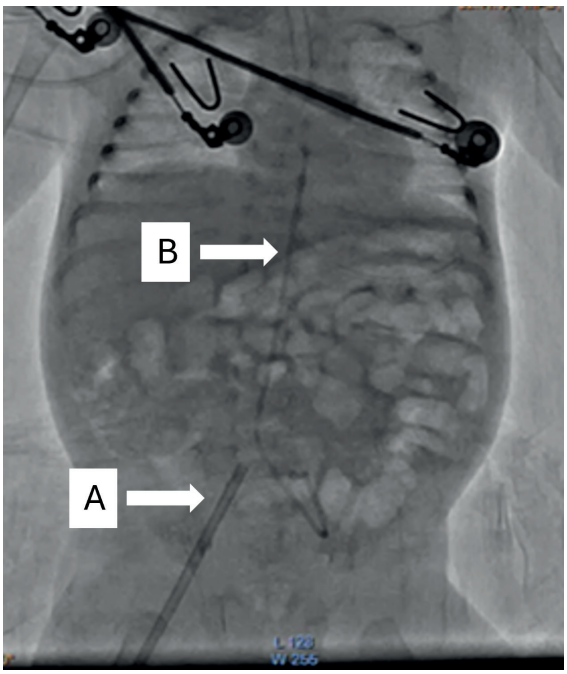

Fig. 2. Femoral sheath in situ (A), and umbilical artery catheter (B), lying in the aorta.

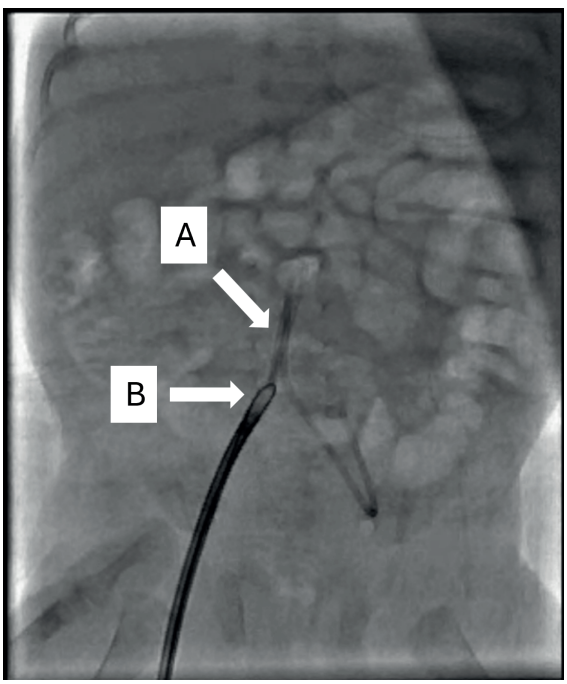

Fig. 3. Umbilical artery catheter being snared out using improvised snare; captured foreign body (A), snare loop pulling the umbilical artery catheter (B).

lower limb post procedure which was managed with warm packs and a pre-emptive continuous infusion of UFH (28 U/kg/hour); the latter was discontinued once a duplex colour Doppler scan ruled out thrombosis or dissection. Hence, the hypoperfusion was finally attributed to the spasm of the common femoral artery, which later recovered spontaneously. Invasive mechanical ventilation was continued for 2 more days and switched to non-invasive ventilator support, subsequently which the patient was weaned off 23 days later. Subsequently, the neonate developed late-onset neonatal sepsis due to *Klebsiella pneumoniae*, which was successfully treated with antibiotics. The neonate was discharged finally on the 51st day of life. Further outpatient examination revealed functionally normal right lower limb with well palpable distal pulses. The neonate is doing well with a follow-up of 5 months.

Discussion

Umbilical venous and arterial catheterisations are commonly employed in NICUs to establish prolonged venous access and provide direct arterial access for blood sampling and monitoring, particularly in infants with very low birth weight. Although inserting a UAC is conceptually straightforward, at times it can present significant challenges in clinical settings. Optimal positioning involves the catheter advancing through the umbilicus, descending via the umbilical artery, continuing through the anterior branch of the internal iliac artery, into the common iliac artery, and finally entering the aorta.[3] The proximal catheter tip is placed usually in one of the two positions: between the sixth and ninth thoracic vertebrae or at the level of the third or fourth lumbar vertebrae.[4] A meta analysis concluded that a higher position of the tip should be preferred to a lower one to avoid vascular complications.[5] Numerous dreaded complications like aortic

dissection, aortic pseudoaneurysm, aortic thrombosis, renal artery thrombosis, umbilical artery perforation, fracture/breakage have been reported in the literature.[6-11] Levit et al.[12] reported an overall complication rate of 2.5% among 2035 UAC usage in the NICU over an 11 year period. The most common complication was catheter breakage or fracture, accounting for approximately 40% of all reported complications. The potential reasons for this complication could be inadvertent damage to the catheter during insertion, fixation and removal, particularly from needles or scissors.[10] Contributing factors may include catheter material used (Polyurethane has more mechanical strength compared to silicone.), manufacturing defects or prolonged use, which can lead to structural weakening of the catheter.[11]

After an extensive literature search, we could find only 14 cases of umbilical artery retrieval prior to ours. The majority were retrieved by a surgical approach and only 4, including ours, were successful in a percutaneous approach.[13-15] The major problems in adopting a percutaneous approach are difficult vascular access in fragile neonates, local complications post procedure, and limited availability of necessary hardware and technical expertise.[16-18]

If the stump is visible or palpable at the local site, then local surgical exploration and retrieval should be attempted as done by Doodnath et al.[16] and Maggioni et al.[17] in their respective cases. In our case, the possibility of local exploration to pull out the UAC from the umbilical area was ruled out since roentgenogram had already shown that its proximal end had embolised into the common iliac artery.

Most of the authors relied upon surgical retrieval with or without local imaging assistance. Lackey et al. were the first to report retrieval of a UAC from the thoracic aorta using a surgical approach.[19] In a similar case, Murphy et al. employed a transumbilical surgical approach for catheter retrieval.[20] In another case, Dennis et al. reported a 7-day-old neonate with a fractured UAC, where imaging revealed the catheter extended into the umbilical cord stump.[11] In this case, despite undergoing exploratory laparotomy and ileal resection, the broken catheter could not be removed surgically, and it was later found to have embolized into the aorta. The operators eventually used intra-operative ultrasound guidance to locate the catheter, which was successfully removed through an aortotomy following aortic cross-clamping. Aortotomy would require cross clamping, hampering perfusion to distal organs with possible grave consequences of ischemia to the spinal cord, limbs and other organs. In another noteworthy case, Uwaifo et al.[15] used transcarotid access for retrieval of a UAC from the descending thoracic aorta after failed infraumbilical surgical exploration and transductal approach through a femoral venous access. In this case, the ductus arteriosus was closed and there was lower limb ischemia to begin with, which might have forced them to prefer transcarotid access; however, carotid access may pose a risk of ischemic injury to the brain as well as difficulty achieving hemostasis since there is no bony support behind the vessel against which compression can be easily achieved. In our case there were no signs of lower limb hypoperfusion, therefore we could safely introduce a 6F radial-access sheath which was wide enough to accommodate the UAC even if it was folded or bent on itself at the point where it was captured by the improvised snare.

As neonates, particularly those with low birth weight, are more susceptible to catheter-associated risks, continuous monitoring and early detection are crucial in preventing catastrophic outcomes. Measuring the length of the catheter after routine retrieval, early removal after usage, avoiding tight sutures around the catheter, monitoring the proximal tip position periodically with an ultrasound or X-ray can be done to prevent this catastrophic complication.[4] Retrieving foreign bodies using commercially available snares, due to their

large diameters, may cause damage to the small vessels in neonates, especially those who are very low birth weight.

Conclusion

The successful percutaneous retrieval of the fractured UAC from the aorta using a make-shift snare improvised from coronary angioplasty hardware in this very low birth weight neonate demonstrates the feasibility and effectiveness of minimally invasive techniques in managing rare and complex complications in neonatal care. Despite the challenges posed by the unexpected catheter fracture and subsequent migration, prompt diagnosis and intervention led to a favourable outcome. A team effort from cardiology, neonatology and anaesthesia helped in achieving this goal.

Supplementary materials

Supplementary materials for this article are available online at https://doi.org/10.24953/turkjpediatr.2025.6075

Video 1. Cine loop demonstrating retrieval of fractured umbilical artery catheter using snare improvised from coronary hardware.

Ethical approval

As per our institutional protocol, ethical committee approval is not required for case reports. Informed consent was obtained from the legal guardians of our patient presented in our case report.

Author contribution

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

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Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

A pediatric-onset case of chronic kidney disease caused by a novel sporadic *ACTN4* variant and literature review

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ABSTRACT

Background. The *α-actinin-4 (ACTN4)* gene encodes an actin-binding protein, which plays a crucial role in maintaining the structure and function of podocytes. Previous studies have confirmed that *ACTN4* mutations can lead to focal segmental glomerulosclerosis-1 (FSGS1), a rare disease primarily manifesting in adolescence or adulthood, characterized by mild to moderate proteinuria, with some cases progressing slowly to end-stage renal disease.

Case Presentation. We report a 12.5-year-old boy who presented with non-nephrotic range proteinuria, hyperuricemia, markedly reduced bilateral kidney volume, and stage 3 chronic kidney disease (CKD). An ophthalmic examination revealed optic disc dysplasia in the right eye. The results of whole-exome sequencing revealed a de novo variant in the *ACTN4*, a previously unreported variant.

Conclusions. We reported a novel sporadic *ACTN4* variant and reviewed previously reported cases. Through analysis of the genotypes and clinical phenotypes of reported cases, we found that *ACTN4* variants may not always present as FSGS1, and there was significant phenotypic heterogeneity among individuals. Notably, mutations affecting residues 260-265 are associated with collapsing glomerulopathy and rapid progression to end-stage kidney disease in prior studies, whereas the p.Ala278del variant in our case, located outside this region, exhibited stable CKD3. This suggests domain-specific genotype-phenotype correlations. However, this association requires further validation through additional cases and experiments. Our findings may have significant implications for clinical diagnosis, prognosis assessment, and scientific research on kidney diseases related to *ACTN4* variants.

Key words: *ACTN4*, variant, focal segmental glomerulosclerosis, proteinuria, eye.

With the continuous development of genomics research, a deeper understanding has been gained of renal diseases caused by monogenic inheritance. Research has found that up to 30% of patients with steroid-resistant nephrotic syndrome are caused by monogenic diseases.[1] The *α-actinin-4 (ACTN4)* gene is located on human chromosome 19 and is involved in encoding the cytoskeleton and actin-binding protein ACTN4. It is crucial for maintaining podocytes' normal structure and function. Additionally, variants in *ACTN4* have been confirmed as a monogenic cause of steroid-resistant nephrotic syndrome.[2,3]

It has been confirmed that mutations in *ACTN4* can lead to autosomal dominant focal segmental glomerulosclerosis-1 (FSGS1), a rare disease with a relatively high probability of familial occurrence. Onset usually occurs during

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adolescence or later and is characterized by mild to moderate proteinuria accompanied by renal dysfunction, with some cases progressing slowly to end-stage kidney disease (ESKD).[2] Currently, there are approximately 107 reported cases of renal disease caused by *ACTN4* mutations, including 28 mutation types.[1-16] Since Kaplan et al. first reported in 2000, it has been generally considered that *ACTN4* mutations are associated with FSGS1. However, in recent years, there have been reports of sporadic *ACTN4* variants.[4-6,9] Here, we report a case of sporadic *ACTN4* variant causing chronic kidney disease (CKD) in a child. The variant in this case has not been reported previously, and this patient is currently the only reported case with concurrent ocular lesions. Our case report and literature review may have expanded the phenotypic and genotypic spectrum associated with *ACTN4* variants. We also identified a potential correlation between genotype and phenotype. Moreover, some of our findings require further validation through additional cases and functional studies, thereby providing new directions for future research on *ACTN4*-related disorders.

Case Presentation

A 12.5-year-old boy presented to our hospital due to persistently elevated serum creatinine and uric acid levels over one year, during which no specific treatment was administered. He did not present any manifestations such as hematuria, foamy urine, enuresis, oliguria, urinary frequency, urgency, edema, rash, or pallor. The patient had no significant past medical history. His father had hyperuricemia managed with febuxostat, though specific pre-treatment uric acid levels were unavailable due to incomplete medical records. No abnormalities were found in the father's urine routine examination, and his estimated glomerular filtration rate (eGFR), calculated using the CKD-EPI equation, was within the normal range (90-120 mL/min/1.73 m²). Moreover, there is no history of consanguineous marriage or other family history. There were no apparent abnormalities in the boy's physical examination. A 24-hour proteinuria of 9.5 mg/m²/hour was detected, consistent with non-nephrotic range proteinuria. Renal function tests showed elevated serum creatinine (maximum 1.5mg/dL) and uric acid (maximum 11.6 mg/dL) levels. The patient's eGFR, calculated using the Schwartz formula, was 59 mL/min/1.73 m², consistent with CKD stage 3a according to the KDIGO 2024 Clinical Practice Guideline.[17] Ultrasonography of the urinary system revealed significant bilateral renal volume reduction (right kidney 7.7 × 3.5 × 3.1 cm, left kidney 8.3 × 3.8 × 3.6 cm) with enhanced echogenicity of the renal parenchyma. Ophthalmic examination revealed refractive errors and right optic disc dysplasia. The patient exhibited no clinical features suggestive of systemic lupus erythematosus (SLE). Serological testing showed no evidence of SLE-associated autoantibodies or hypocomplementemia. Screening tests for blood lipids, blood glucose, serum albumin, coagulation function, immunological disorders, and infectious diseases (such as hepatitis B, tuberculosis, and human immunodeficiency virus) showed no abnormalities. Malignancy was excluded through tumor markers and chest-abdomen-pelvis computed tomography scans.

Considering the insidious onset of the patient's condition and the absence of apparent triggers leading to CKD3, along with his father's hyperuricemia, further genetic examination was conducted. Genomic DNA was extracted from the proband and parents using the Qiagen Blood DNA Mini Kit (Qiagen, Germany). Whole-exome sequencing (WES) was performed by MyGenostics (Beijing, China) using the GenCap® Exome Enrichment V6.0 probe (51 Mb target region, covering ~23,000 genes). Sequencing was conducted on an Illumina NovaSeq 6000 platform with 200× mean coverage depth, ensuring >99% of target

regions achieved ≥20× coverage. Raw reads were filtered using CutAdapter to remove low-quality sequences. Clean reads were aligned to the GRCh37/hg19 reference genome using BWA. GATK was used for single nucleotide variation and inserts and deletions. Variants were annotated using ANNOVAR and filtered against population databases (1000 Genomes, Exome Variant Server, ExAC) with a minor allele frequency <0.1%. SIFT, PolyPhen2, MutationTaster, and GERP were used for in silico analysis.

WES revealed a novel heterozygous variant in *ACTN4* (NM_004924.6:c.832_834del, p.Ala278del). In silico tools (SIFT, PolyPhen2, MutationTaster, and GERP) predicted uncertain significance for the p.Ala278del variant. The variant was highly associated with the patient's clinical phenotype, and no other variants related to the patient's phenotype were detected. His parents do not have variants at this locus or any other variants. The variant is considered to be a de novo variant in the patient (Fig. 1). According to the standards of the American College of Medical Genetics and Genomic, this variant is classified as likely pathogenic (PS2+PM2_Supporting+PM4).[18]

Over 18 months of follow-up, the patient's renal function remained stable with persistent non-nephrotic proteinuria. No corticosteroids or immunosuppressive agents were administered, as clinical progression was indolent and genetic testing indicated an *ACTN4* variant associated with steroid resistance. Informed consent for publication was obtained from the patient's legal guardian.

Discussion

Podocytes serve as critical structures maintaining the renal filtration barrier. Mutations in *ACTN4* can cause podocyte dysfunction, thereby initiating the development of CKD. Kos et al. found that knockout mice lacking the *ACTN4* showed disappearance of foot processes and reduced expression of ACTN4 in podocytes. Most knockout mice died around the perinatal period while surviving mice exhibited proteinuria and FSGS.[19] This indicates the crucial role of *ACTN4* in renal function. Actin in podocytes is crucial for the construction and maintenance of the cytoskeleton. ACTN4 encoded by the *ACTN4* is an actin-binding protein primarily localized to the foot processes, playing a significant role in regulating the actin cytoskeleton of podocytes. Studies have indicated that mutations in *ACTN4* lead to the mislocalization of the encoded protein and the formation of intracellular aggregates. This results in instability of the formed ACTN4, increased affinity with actin, and reduced dissociation and degradation rates, ultimately disrupting the actin cytoskeleton in podocytes.[2,3,6,11,20] Consequently, mutations in *ACTN4* disrupt podocytes' normal structure and function, causing renal disease.

We reviewed previously reported and clinically significant cases with *ACTN4* variants (Supplementary Table I). A systematic search of PubMed and Google Scholar was performed using the terms "ACTN4 AND mutation" and "ACTN4 AND variant". Articles were restricted to those published in the English language. Studies were included if they (1) confirmed *ACTN4* variants via genetic testing, (2) provided renal manifestations, and (3) excluded non-human or phenotype-unclear cases. A total of 107 cases (including ours) were eligible for analysis. Previously, our understanding of renal disease caused by *ACTN4* variants mainly focused on the development of FSGS1, characterized by familial aggregation, and later onset (mainly in adolescents or adults), with some cases progressing slowly to ESKD.[2,3] However, through a comprehensive review of the clinical characteristics of previously reported cases, particularly considering some newer reports in recent years involving phenotypes different from those previously described, we

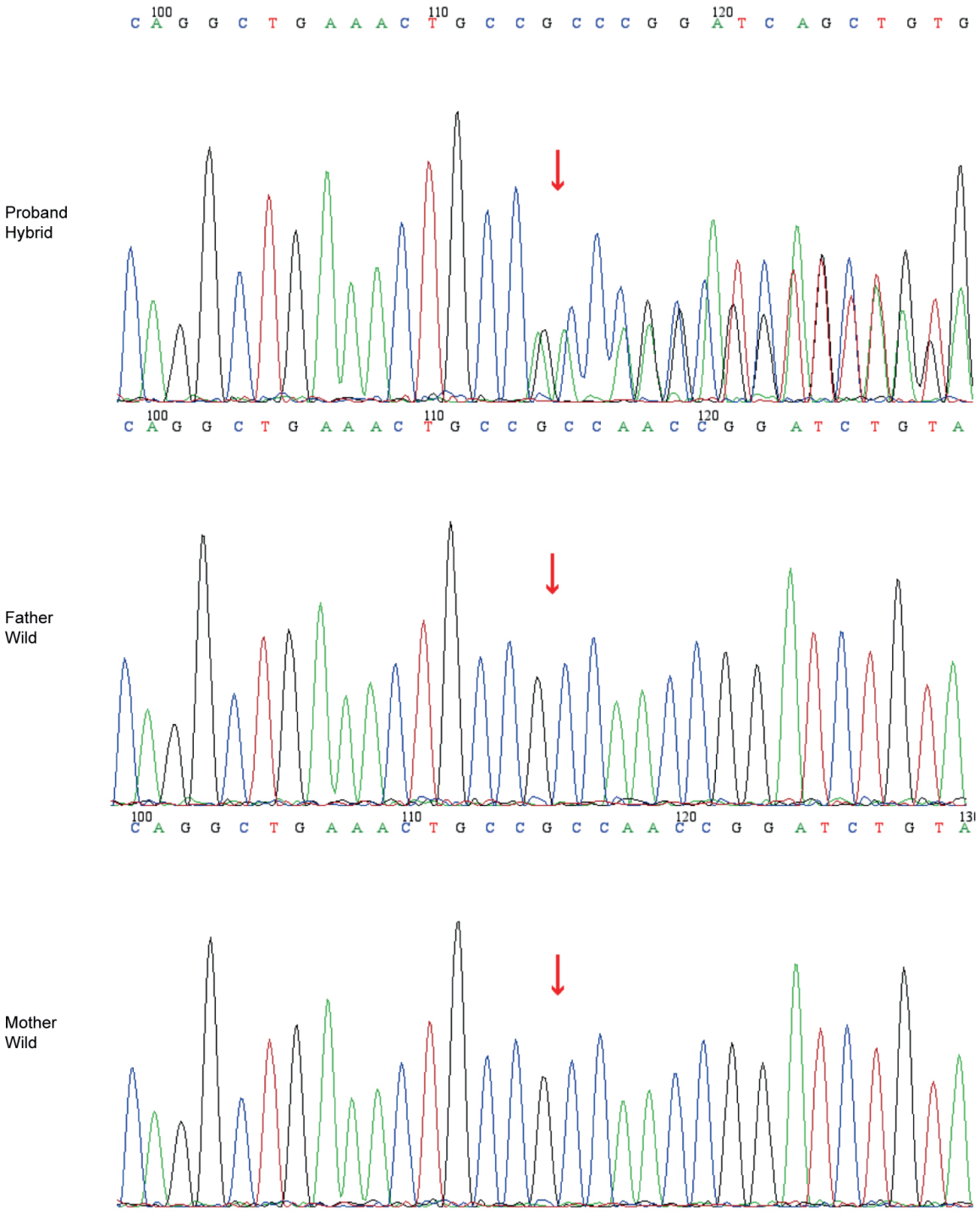

Fig. 1. Sanger sequencing of the proband and his parents.

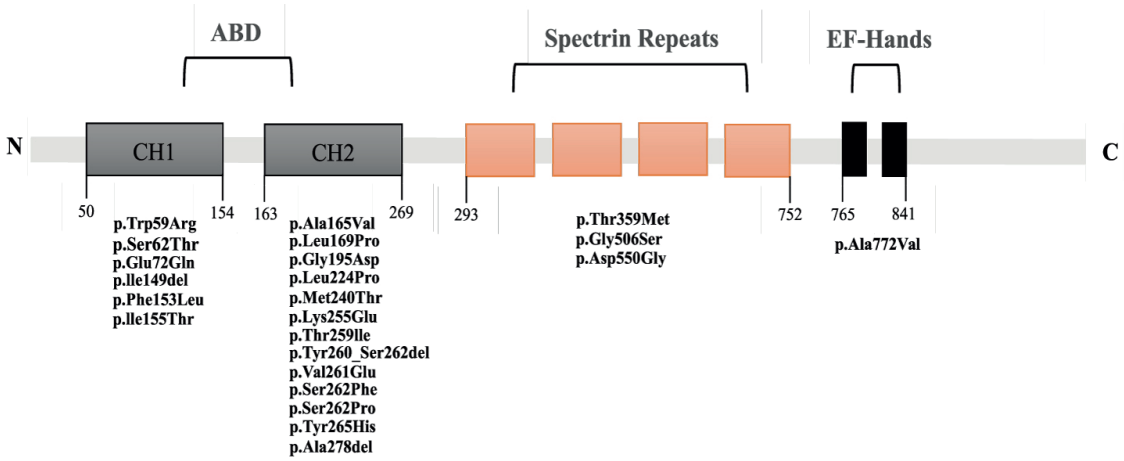

Fig. 2. The structure diagram of ACTN4, along with the reported variant sites.

found that *ACTN4* variants may not solely manifest as FSGS1, and there is significant heterogeneity in individual clinical phenotypes. It mainly includes the following aspects: 1. Variants may not always be inherited within families. Among the 107 reported cases: 18 were sporadic; 3 had unknown family history; and the remaining 86 were familial.[1,4-6,9-13] The 18 sporadic cases had no family history of renal disease, with onset age ranging from 3.7 to 17 years old, mainly presenting with proteinuria or nephrotic syndrome, almost all progressing to ESKD. 2. Some patients developed the disease before adolescence (10 cases occurred before adolescence, with the youngest onset age being three years old).[3,5-7,9,11] 3. There were patients with rapid progression (nine cases progressed to ESKD within three years of onset, with one case progressing to ESKD within six months). 4. Patients with childhood-onset disease progressed to ESKD more rapidly than those with adult-onset disease.[1,3,4,6,8-10,12] However, the mechanisms underlying the heterogeneity of clinical phenotypes remain unclear and warrant further investigation.

ACTN4 comprises three main structural domains: 1. The N-terminal actin-binding domain (ABD), which comprises two calponin-homology (CH) domains; 2. The spectrin domain (composed of four repeat sequences); 3. A pair of C-terminal EF-hands.[21] We have listed the amino acid sequence positions on the ACTN4 protein corresponding to the reported variant sites (Fig. 2). It can be observed that variants reported so far are more common in the ABD region. At the same time, there are fewer reports of mutations in the spectrin domain. To date, six cases of mutations in the spectrin domain have been reported, all in Asian populations: five Chinese (including the present case) and one Japanese.[5,15] It suggests that the spectrin domain may be a hotspot variant region in Asian populations. While this clustering could suggest ethnic-specific factors, current evidence is insufficient to confirm a biological basis. Larger multi-ethnic cohorts and functional studies are needed to distinguish technical biases (e.g., regional diagnostic practices) from true genetic predisposition.

Review of reported *ACTN4* mutations (Supplementary Table I) reveals a potential genotype-phenotype correlation: Pathogenic mutations within residues 260-265 (e.g., p.Ser262Phe, p.Val261Glu, p.Tyr265His, p.Tyr260_Ser262del [denoting deletion of residues Tyr260 through Ser262]; cases 9, 11, 13, 14, 21, 25, 30 in Supplementary Table I) are strongly associated with collapsing glomerulopathy and rapid progression to ESKD (0.5-3 years), as documented in prior studies.[1,6-9,12]

For example, Kakajiwala et al. described a child with p.Ser262Phe who progressed to ESKD within six months[6], while Feng et al. reported a p.Tyr265His variant linked to collapsing lesions and ESKD in six months.[12] In contrast, our patient's novel p.Ala278del variant, located outside this region, presented with stable CKD3 over 18 months, suggesting that mutation position may modulate phenotypic severity. We hypothesize that the 260-265 region, which overlaps with the actin-binding domain, may be critical for cytoskeletal stability, and variants here could disrupt actin dynamics more severely than those in other domains. This effect likely arises because slower degradation of ACTN4-actin complexes in these variants leads to abnormal protein clumps inside podocytes. These aggregates destabilize the cytoskeleton, accelerating disease progression.[11] However, confirming such an association between genotype and phenotype necessitates further investigation with additional cases and experimental validation in the future. Moreover, the limited clinical data on mutations in other domains (e.g., spectrin domain) precludes a systematic comparison of domain-specific phenotypic differences. Future studies with larger cohorts and functional validation are needed to confirm whether mutation location directly dictates clinical severity.

Due to the similarity in development, structure, and physiology between the kidneys and eyes, ocular involvement is relatively common in hereditary renal diseases.[22,23] To our knowledge, this is the first reported case associating an *ACTN4* variant with ocular manifestations. Retina, lens, and ocular muscle abnormalities have been observed in mouse models with *ACTN4* variants.[24] Human Protein Atlas showing low but detectable ACTN4 expression in human retina. While ACTN4 is primarily characterized in podocytes, its potential role in ocular tissues remains speculative. This case highlights the phenotypic expansion of *ACTN4*-related disorders, though the causal link between the variant and ocular manifestations has yet to be established. Future studies could validate the ocular localization of ACTN4 and explore whether this variant exerts pleiotropic effects beyond the kidney.

Previously reported cases of *ACTN4* variants with clinical treatment outcomes, except for two cases with variants occurring at splice sites[5], all were resistant to steroids. In the patient Odenthal et al. reported, proteinuria was significantly alleviated upon initial use of cyclosporine. It may be attributed to the impact of such immunosuppressive agents on the cytoskeleton and attenuation of immune response. The study suggests that cyclosporine may help delay renal replacement therapy or renal transplantation.[11] While this suggests a potential role for cytoskeletal modulation, its applicability to *ACTN4*-related nephropathy remains unproven. Importantly, cyclosporine's nephrotoxicity risk necessitates cautious patient selection. Further studies are needed to define its utility in *ACTN4*-related disorders.

As the significantly reduced bilateral kidney volume observed in the patient upon presentation and the heightened risk of renal biopsy-related bleeding, coupled with the manifestation of sclerosing nephropathy in most cases, renal biopsy was not performed in the case we reported. The patient presented with persistent proteinuria accompanied by renal insufficiency without a significant family history. However, genetic testing revealed a novel variant in the *ACTN4*. It suggests that patients with unexplained CKD may harbor genetic abnormalities despite lacking a clear family history of renal disease. Therefore, physicians should not refrain from genetic testing simply because patients lack a family history of renal disease. For patients who cannot undergo renal biopsy, genetic testing not only aids in making a definitive diagnosis but also guides treatment and facilitates planning for future renal transplantation. Our summary of previous cases shows that the majority of patients were steroid-resistant. Additionally, the probability of monogenic diseases in steroid-resistant nephrotic syndrome patients is not low. Early genetic testing for such patients

can help avoid excessive use of corticosteroids. Hence, genetic testing methods are necessary to assist clinicians in disease management.

This study has limitations, as functional validation of the p.Ala278del variant was not conducted. Moreover, the variant's uncertain prediction by in silico tools likely stems from their limited training data for in-frame deletions. However, the following aspects might support its pathogenicity: this variant is de novo and has not been detected in the parents or population databases; the patient's clinical presentation is consistent with *ACTN4*-related nephropathies; and this variant meets the criteria for 'likely pathogenic' (PS2+PM2_Supporting+PM4). Future studies could prioritize in vitro assays and structural modeling to confirm its pathogenic mechanism.

To the best of our knowledge, this is the first clinical report of a pediatric patient harboring a novel sporadic *ACTN4* variant presenting with CKD3 and concurrent ocular involvement. While murine models and low-level retinal ACTN4 expression suggest plausible biological links between ACTN4 dysfunction and ocular phenotypes, the causal relationship in humans remains speculative. This case highlights the potential phenotypic expansion of *ACTN4*-related disorders and underscores the need for functional studies to validate ocular pathogenicity mechanisms. Moreover, our literature review reveals phenotypic heterogeneity in *ACTN4*-related nephropathies and potential genotype-phenotype correlation. These findings are essential for clinical diagnosis, treatment, and prognosis assessment and provide direction for future research on this disease.

Supplementary materials

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Ethical approval

Informed consent was obtained from the patient's legal guardian for publication.

Author contribution

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

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Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

A rare pediatric case of immune thrombocytopenia attributed to brucellosis

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ABSTRACT

Background. Brucellosis is a zoonotic infection transmitted to humans by ingestion of contaminated unpasteurized dairy products or via direct or indirect contact with infected animals. It is characterized by nonspecific symptoms like fever and joint pain, and laboratory findings including anemia, leukopenia, thrombocytopenia, or rarely pancytopenia. Here we report a case of brucellosis with thrombocytopenia that did not improve despite anti-brucella treatment and required intravenous immunoglobulin treatment.

Case Presentation. A six-year-old boy from a brucellosis-endemic area presented with fever and fatigue. Initial laboratory tests showed moderate thrombocytopenia and a *Brucella* agglutination titer of 1/320. *Brucella spp.* was isolated from blood culture. Rifampicin, trimethoprim-sulfamethoxazole (TMP-SMX), and gentamicin treatment were given to the patient, and clinical improvement followed, with normalization of blood count. However, on day 10, severe thrombocytopenia with epistaxis and ecchymosis developed, suggestive of immune thrombocytopenia (ITP). Intravenous immunoglobulin at a dose of 1000 mg/kg was given, resulting in a rise in platelet count. The patient was discharged with rifampicin and TMP-SMX. During follow-up, his platelet levels returned to normal without the need for additional immunoglobulin, suggesting resolution of *Brucella*-related immune thrombocytopenia.

Conclusion. Brucellosis should be kept in mind in the differential diagnosis of thrombocytopenia in endemic regions. If there is no response to antimicrobial treatment in brucellosis patients presenting with thrombocytopenia, immune thrombocytopenia should be considered.

Key words: *Brucella spp*., brucellosis, immune thrombocytopenic purpura, thrombocytopenia.

Brucellosis is a zoonotic infection transmitted to humans by ingestion of contaminated unpasteurized dairy products or via direct or indirect contact with infected animals. The causative agents are *Brucella spp*., which are facultative intracellular bacteria that can multiply within phagocytic cells.[1] Brucellosis presents with nonspecific symptoms such as fever, malaise, anorexia, and arthralgia, and a broad spectrum of laboratory manifestations such as anemia, leukopenia, thrombocytopenia, or less frequently pancytopenia.[2] *Brucella*-related thrombocytopenia is a rare and mild hematological finding, does not lead to bleeding complications, and tends to resolve with appropriate antibiotic therapy. However, in less common scenarios, an immune-mediated mechanism may cause a marked decrease in platelet counts, either at the onset of infection or during treatment, sometimes accompanied by clinically significant hemorrhagic symptoms.[3]

In this report, we present a case of brucellosis complicated by thrombocytopenia, which

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persisted despite anti-*Brucella* therapy, but subsequently improved following intravenous immunoglobulin (IVIG) treatment.

Case Presentation

A six-year-old boy applied to a local hospital with complaints of fever, fatigue and joint pain lasting for 3 days. His complete blood count revealed moderate thrombocytopenia. This is an area where brucellosis is endemic and it was reported that this patient had ingesting unpasteurized dairy products. The patient's *Brucella* tube agglutination titer was positive with a titer of 1/320. The patient received rifampicin and trimethoprim-sulfamethoxazole (TMP-SMX) combination as anti-*Brucella* treatment regimen in the local hospital. Nevertheless, the patient's fever persisted for 3 days. In addition, platelet values in his complete blood count gradually decreased, and along with the development of leukopenia and anemia. Therefore, the patient was referred to our hospital, where a pediatric infectious diseases and hematology specialist was also present, to exclude hematological malignancies and infection-induced hemophagocytic lymphohistiocytosis (HLH).

The patient had fever, fatigue, and joint pain for 6 days when he was admitted to our hospital. On physical examination, he had a fever (38.7 °C) and tachycardia (116/min, in sinus rhythm), other vital signs were normal. The spleen was palpated 4 cm below the left costal margin, and the liver was palpated 1.5 cm below the right costal margin. There was no lymphadenopathy, petechiae, or purpura. The rest of the physical examination was essentially unremarkable.

Laboratory test results were as follows: hemoglobin: 10.8 g/dL (normal range, 12-16 g/dL), white blood cell count (WBC): 2.22×10^3/μL (normal range, 4-10×10^3/μL), absolute neutrophil count (ANC): 0.86×10^3/μL (normal range, 1.5-8×10^3/μL), absolute lymphocyte count (ALC): 1.23×10^3/μL (normal range, 1-4.8×10^3/μL), platelet count: 60×10^3/μL (normal range, 150-400×10^3/μL), erythrocyte sedimentation rate: 44 mm/h (normal range 0-20 mm/h), C-reactive protein: 130 mg/L (normal range, 0-5 mg/L), ferritin: 681 ng/mL (normal range, 20-300 ng/mL). The peripheral blood smear made by direct finger-prick sampling revealed platelet clusters, typically forming groups of 5–10, with otherwise normal platelet morphology. No atypical cells or blasts were identified. Biochemical parameters in serum (alanine and aspartate transaminases, sodium, potassium, creatinine, uric acid) were within normal limits, and there was no hypertriglyceridemia. Laboratory findings were not fully supportive of infection-induced HLH. The patient fulfilled 3 (splenomegaly, cytopenias, hyperferritinemia) out of the 8 diagnostic criteria defined in the HLH-2004 guidelines.[4] Although leukemia was considered in the differential diagnosis due to the patient's fever, hepatosplenomegaly and cytopenia, *Brucella* was suspected based on the positive *Brucella* test results and the patient's clinical history and symptoms, thus bone marrow aspiration was deemed unnecessary.

The patient was treated with rifampicin (20 mg/kg/d, p.o.), TMP-SMX (8 mg TMP/kg/d, p.o.), and gentamicin (7.5 mg/kg/d, i.v.). The patient's fever decreased on the second day after his admission to our hospital. There were no signs of petechiae, purpura, or ecchymosis bleeding. *Brucella spp*. were isolated in his hemoculture. In the complete blood count taken on the 7th day of treatment in our facility, it was seen that the platelet count increased to 138×10^3/μL, neutropenia and lymphopenia had also improved.

While the patient was under anti-*Brucella* treatment and his clinical findings were improving, his platelet count gradually decreased to 9×10^3/μL on the 10th day of hospitalization in our facility. At that time, the complete blood count showed hemoglobin: 11.6 g/dL, WBC: 6.09×10^3/μL, ANC: 1.7×10^3/μL, and ALC: 3.78×10^3/μL. The peripheral blood smear obtained from a direct finger-prick showed no platelet clumping; instead, only occasional

single platelets were observed. Again, no atypical cells or blasts were present. These findings effectively ruled out EDTA-dependent pseudothrombocytopenia. At the same time, the patient also had epistaxis and ecchymosis. He was afebrile, with a body temperature of 36.8 °C. Physical examination revealed multiple ecchymoses on the trunk and lower extremities. The spleen was palpable 3 cm below the left costal margin, and the liver was palpable 1 cm below the right costal margin. Other systemic examinations were within normal limits. Based on these new findings, the patient was considered to have immune thrombocytopenia. Along with the treatment against brucellosis, he was placed on intravenous immunoglobulin (IVIG) at 1 g/kg/day for one day. The day after IVIG platelet counts increased to 29 $\times 10^3/\mu L$. Gentamicin was administered for 2 weeks. The patient was discharged on the 14th day with rifampicin and TMP-SMX as maintenance therapy, which were continued for a total of 6 weeks. Laboratory parameters during treatment and follow-up are presented in Table I.

In the outpatient clinic follow-ups, the patient's platelet count increased to 338 $\times 10^3/\mu L$. He did not require IVIG again during the follow-up. Chronological changes in platelet levels and the treatment timeline of the patient are shown in Fig. 1.

Informed consent was obtained from the patient's parents for the publication.

Discussion

Brucellosis is a multisystemic disease that affects many systems, including the skeletal, central nervous, cardiovascular, and reticuloendothelial systems. Hematological abnormalities, such as anemia, lymphocytosis, thrombocytopenia and pancytopenia occurring in brucellosis in children have been reported in the literature.[2,5] Anemia is more frequently associated with acute brucellosis, but pancytopenia and thrombocytopenia are less often seen. The incidence of thrombocytopenia in children with brucellosis varies between 5-14%.[2,5] Many different mechanisms of thrombocytopenia observed during the clinical course of brucellosis have been described, including hypersplenism, bone marrow suppression, or immune-mediated.[6-8]

Table I. Laboratory investigations and clinical findings during hospitalization and after discharge

	Day 1	Day 3	Day 7	Day 10	Day 11	Day 21	Day 45
Hb (g/dL)	10.8	11.2	11.9	11.6	10.6	12.3	12.7
WBC ($\times 10^3/\mu L$)	2.22	2.92	4.79	6.09	4.96	6.17	8.83
ANC ($\times 10^3/\mu L$)	0.86	0.76	1.87	1.70	1.55	2.17	3.73
ALC ($\times 10^3/\mu L$)	1.23	1.94	2.32	3.78	2.90	3.43	4.14
PLT ($\times 10^3/\mu L$)	60	27	138	9	29	234	338
AST (U/L)	47	45	61	31	27	30	31
ALT (U/L)	29	30	48	28	18	14	12
LDH (U/L)	-	432	346	339	341	310	-
Na (mEq/L)	137	139	137	136	-	-	139
Ferritin (ng/mL)	-	681.1	-	-	48.9		
CRP (mg/L)	130.1	90.1	6	3.8	2.1	1.1	1.3
Body temperature (°C)	38.9	36.9	36.3	36.8	36.5	-	-
Epistaxis and ecchymosis	-	-	-	+	-	-	-

ALC, absolute lymphocyte count; ALT, alanine aminotransferase; ANC, absolute neutrophil count; AST, aspartate aminotransferase; CRP, C-reactive protein; Hb, Hemoglobin; LDH, lactate dehydrogenase; PLT, platelets; WBC, white blood cell count.

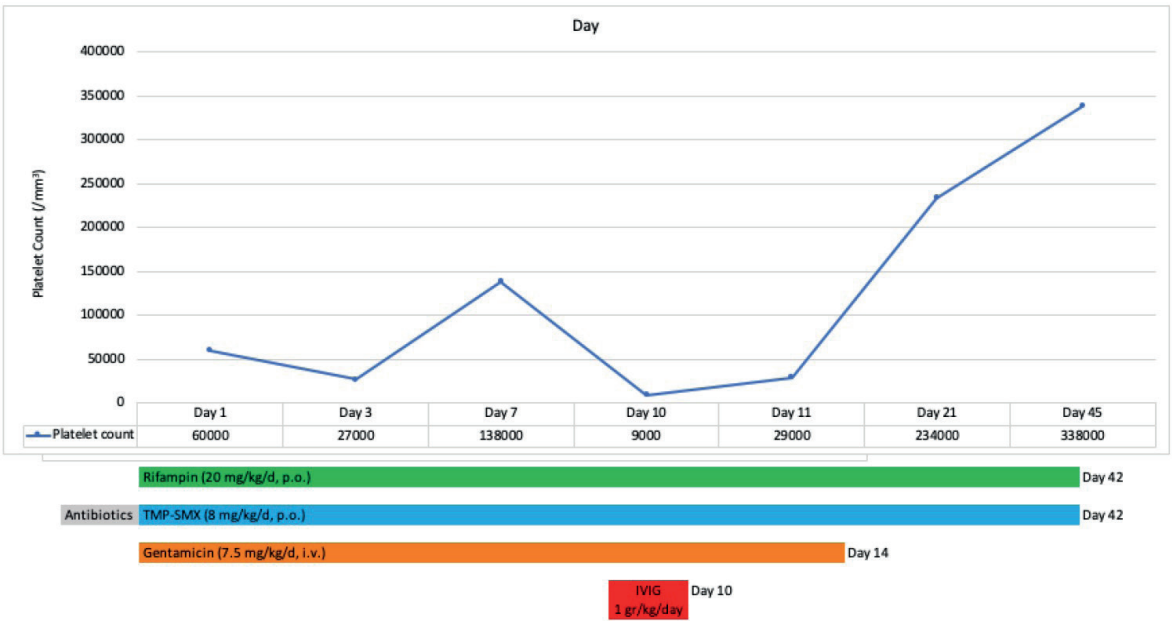

Fig. 1. Chronological changes in platelet levels and treatment timeline of the patient
IVIG: intravenous immunoglobulin, TMP-SMX: trimethoprim-sulfamethoxazole

There is evidence that *Brucella* infection can prompt a systematic autoimmune response.[9] This autoimmune stimulation may manifest as autoimmune hemolysis or platelet destruction. Several adult cases of immune thrombocytopenia associated with *Brucella* infection have been reported in the literature.[9-11] *Brucella*-specific antibiotics and corticosteroids were started together for an 85-year-old woman patient with generalized purpura, rhinorrhagia, and severe thrombocytopenia.[9] Another patient with immune thrombocytopenia due to brucellosis initially received *Brucella*-specific antibiotics only. Then corticosteroids were added to the treatment because thrombocytopenia did not improve.[10] In another series of seven adult patients, thrombocytopenia resolved completely after appropriate antibiotic treatment for brucellosis was given to the patients. No additional treatment was required.[11] It is noteworthy that in these reports involving adult patients, corticosteroid treatment was added to anti-*Brucella* antibiotics when thrombocytopenia did not improve or when purpura or bleeding was present.

In a study evaluating 14 children with brucellosis who presented with hematological manifestations, immune thrombocytopenia was detected in 5 of the patients (35.7%).[6] The ages of these patients ranged from 3 to 10 years. The platelet counts of these patients ranged from 1 to 5 $\times 10^3/\mu L$. In the case series, all patients with brucellosis-induced immune thrombocytopenia had severe thrombocytopenia with symptoms. Therefore, along with the anti-*Brucella* treatment, IVIG was given at 1 g/kg/day for 2 days.[6] IVIG therapy exhibits a more rapid effect compared to steroids, as it inhibits the phagocytosis of antibody-coated platelets and reduces complement-mediated platelet destruction.[12] In our patient, pancytopenia resolved after anti-*Brucella* antibiotic treatment was started. However, ecchymosis and epistaxis developed a few days later, and thrombocytopenia recurred. Due to the presence of mucosal bleeding, the need for a more rapid increase in platelet count was considered and IVIG therapy was administered. Similar to the cases in the literature, our patient benefited from IVIG treatment in terms of clinical and laboratory findings.

Table II. Reported cases of pediatric immune thrombocytopenia associated with brucellosis in literature.

Article	Patient sex and age	Bleeding manifestations	Platelet count ($10^3/\mu L$)	Initial diagnosis	Time between diagnoses of brucellosis and ITP	*Brucella* treatment regimen (Duration)	ITP treatment regimen (Dose)	Response
Makis et al.[3]	F, 5.5 y	Purpura, petechiae, gingival bleeding	1	Brusellosis	3 day	TMP-SMX, rifampicin (6 weeks)	IVIG (1 g/kg/day for 2 days)	Resolved
Şanal et al.[13]	M, 12 y	Purpura, petechiae, mucosal bleeding, epistaxis	4	Brucellosis	3 day	Doxycycline, rifampicin (6 weeks), gentamicin (N/A)	IVIG (1 gr/kg/day) and methylprednisolone (30mg/kg)	Resolved
Sevinç et al.[14]	M, 16 y	Purpura	1	ITP	8 day	TMP-SMX, rifampicin, ciproflaxacin (N/A)	Methylprednisolone (30mg/kg)	Resolved
Tsirka et al.[15]	M, 11 y	Petechiae, ecchymosis	8	ITP	5 day	Doxycycline, rifampicin, gentamicin (N/A)	IVIG (N/A)	Resolved
Qiu et al.[16]	F, 2 y	Purpura	12	ITP	7 day	TMP-SMX, rifampicin (N/A)	IVIG (2 g/kg)	Resolved

F, female; ITP, immune thrombocytopenia; IVIG, intravenous immunoglobulin; M, male; N/A, Not applicable; TMP-SMX, Trimethoprim-Sulfamethoxazole; y, years.

A review of pediatric cases of immune thrombocytopenia (ITP) associated with brucellosis in the literature reveals a notable diagnostic variability (Table II). In several cases, including our own, patients were initially diagnosed with brucellosis and received appropriate antimicrobial therapy; however, when thrombocytopenia persisted or worsened, a subsequent diagnosis of ITP was made.[3,13] Conversely, there are also reports in which ITP was the initial presumed diagnosis, but brucellosis was later identified following further evaluation.[14-16] In both groups of cases, favorable clinical outcomes were achieved with appropriate anti-*Brucella* therapy in combination with IVIG and/or corticosteroid treatment.

Conclusion

Brucellosis should be kept in mind in the differential diagnosis of thrombocytopenia in endemic regions. On the other hand, if there is no response to anti-*Brucella* treatment in brucellosis patients presenting with thrombocytopenia, immune mediated thrombocytopenia should be considered.

Ethical approval

Informed consent was obtained from the patient's for the publication.

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: EK, MB; data collection, and analysis: EK, MB; literature review: EK; draft manuscript preparation: EK. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

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Case Report

A child with ulcerative colitis presenting with delirium: a case report

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ABSTRACT

Background. Delirium in patients with ulcerative colitis may be seen, especially in the elderly and in patients hospitalized for a long time. In children, Wernicke's encephalopathy may occur due to thiamine deficiency in both ulcerative colitis and Crohn's disease. We present a patient with ulcerative colitis who presented with delirium as the first symptom, did not respond to steroid treatment and improved with anti-tumor necrosis factor-alpha treatment.

Case Presentation. A 14-year-old male child presented with complaints of nonsensical speech and hallucinations for two days. He also had 2-3 loose, non-bloody stools per day. Neurological examination was normal. There was no electrolyte abnormality or vitamin deficiency. The patient's diarrhea gradually increased and became bloody, and was ultimately diagnosed with ulcerative colitis. The patient, who did not respond to steroid treatment, completely recovered with anti-tumor necrosis factor treatment.

Conclusions. We report a patient with ulcerative colitis who presented with delirium as the first symptom. The absence of electrolyte imbalance or vitamin deficiency in our patient suggests that inflammation is the cause of this condition. To the best of our knowledge, this is the first description of the relationship between inflammatory bowel disease and delirium in children.

Key words: ulcerative colitis, delirium, children.

Inflammatory bowel diseases (IBD) are chronic inflammatory conditions affecting the digestive tract and are divided into two main groups: ulcerative colitis (UC) and Crohn's disease (CD). IBDs may also affect extraintestinal organs such as joints, eyes, mouth and skin. Extraintestinal manifestations are mostly caused by immunologic mechanisms, but may also be due to nutritional and metabolic complications, prothrombotic state and side effects of medications. Neurologic involvement is relatively less common. Neurologic complications have been reported 3% in adult IBD patients.[1] Data on neurologic manifestations of IBD in children are limited and mostly consist of case reports. However, new and rare symptoms and complications have been reported in parallel with the increasing prevalence of this disease in children. Epilepsy, cerebrovascular disease, neuropathies, and neuropsychiatric disorders are the most common.[2]

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Delirium is a neurocognitive disorder with rapid onset and fluctuating course, characterized by attention deficit, cognitive impairment and dysfunction, decreased ability to sustain and focus attention, damage to cognition and disorientation.[3] Delirium usually occurs within a short period of time after an intense inflammatory stress event in vulnerable individuals.[4] In IBD, alterations in brain activity, involvement of the brain-gut axis, malnutrition and inflammatory process may increase the risk of delirium. In a study conducted in geriatric (>65 years) IBD patients, the rate of delirium was found to be 0.3% and delirium picture was found to occur in patients who were hospitalized for a long time and whose nutrition was impaired.[5] In this article, a child who presented with delirium and was subsequently diagnosed with UC is described.

Case Presentation

A 14-year-old male patient with no medical history presented with complaints of nonsensical speech and hallucinations for two days. He had diarrhea for two days and had no complaints of abdominal pain, vomiting or fever. His diarrhea was 2-3 times a day, watery and there was no nocturnal defecation. On examination, his height was 1.60 meters (z score: -0.77) and weight was 48.1 kg (z score: -0.93), and body mass index was 18.79 kg/m^2 (z score: -0.65). He was agitated, aggressive and had poor time-space orientation. Other neurologic and systemic examinations were normal. Laboratory tests revealed low hemoglobin (9.6 g/dL), ferritin (12.9 μg/L), 25-hydroxy vitamin D (9.93 μg/L) and high C-reactive protein (48.8 mg/L). Other biochemical values (including electrolytes and albumin), urine and stool analysis and ammonia level were normal. Glucose, protein and immunoglobulin levels in the cerebrospinal fluid (CSF) were normal. Further laboratory investigations, including thyroid function tests, anti-nuclear antibodies, anti-double stranded deoxyribonucleic acid, complement (C3 and C4) levels and viral markers were negative. Electroencephalogram, brain magnetic resonance (MR) imaging and MR angiography were normal.

Vitamin B1 level was not evaluated because of the technical inadequacies in our hospital. The family was asked for a detailed list of the meals eaten by the child for three days. Using the "Computer Assisted Nutrition Program, Nutrition Information Systems Package Program (BeBiS, Version 7.2)", adequate vitamin B1 intake was determined. Brain MRI was also normal and Wernicke's encephalopathy was not considered. However, while investigating, the patient was hospitalized and immediately started on 250 mg intravenous thiamine three times a day for three days, followed by 100 mg daily. Intravenous immunoglobulin (1 g/kg/day) was administered for 5 days due to the inability to rule out autoimmune encephalopathy. As no clinical improvement was observed, pulse steroid therapy with methylprednisolone (30 mg/kg/day) was initiated for 5 days, followed by maintenance at 2 mg/kg/day. However, no response was obtained to this treatment either. Thereupon, plasma exchange was performed a total of 5 times. However, the patient's delirium state continued. In addition, diarrhea increased and became bloody. Fecal calprotectin measured >300 μg/g and anti-neutrophil cytoplasmic antibodies were positive. Subsequently, a colonoscopy was performed in the third week of admission. We observed that the terminal ileum mucosa appeared normal. The submucosal vascular network had disappeared from the rectum to the cecum and the mucosa was granular and fragile. There were also multiple ulcers with exudate in the rectum and sigmoid colon (Mayo endoscopic score 3) (Fig. 1). Histopathologic examination revealed cryptitis, multiple crypt abscesses and intense lymphoplasmacytic inflammation (Fig. 2).

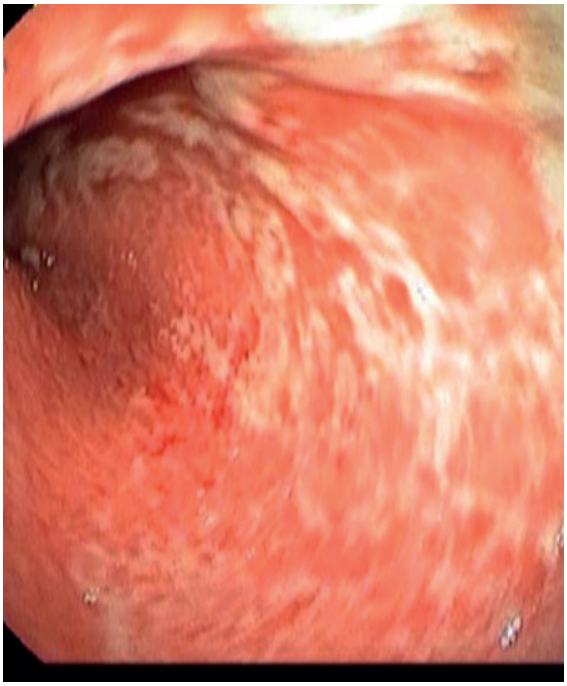

Fig. 1. Colonoscopic findings of the sigmoid colon. There is loss of the submucosal vascular network and there are multiple ulcers with exudate on the mucosa.

The patient was diagnosed with UC and a tumor necrosis factor-alpha (TNF-α) antagonist (infliximab) was started at a dose of 5 mg/kg at weeks 0, 2 and 6 and then every 8 weeks. After the 2nd dose, the delirium and diarrhea improved. After the 3rd dose, the patient had a Pediatric Ulcerative Colitis Activity Index (PUCAI) score of 5 (55 before treatment) and fecal calprotectin < 20 μg/g. The patient received infliximab treatment for two years and had no complaints during follow-up.

Discussion

We report a patient with UC who presented with delirium without any previous symptoms. UC may rarely present with neuropsychiatric symptoms. In the literature, delirium due to UC is usually associated with electrolyte disturbances or vitamin deficiencies, but the absence of these factors in our patient suggests that systemic inflammation may have led

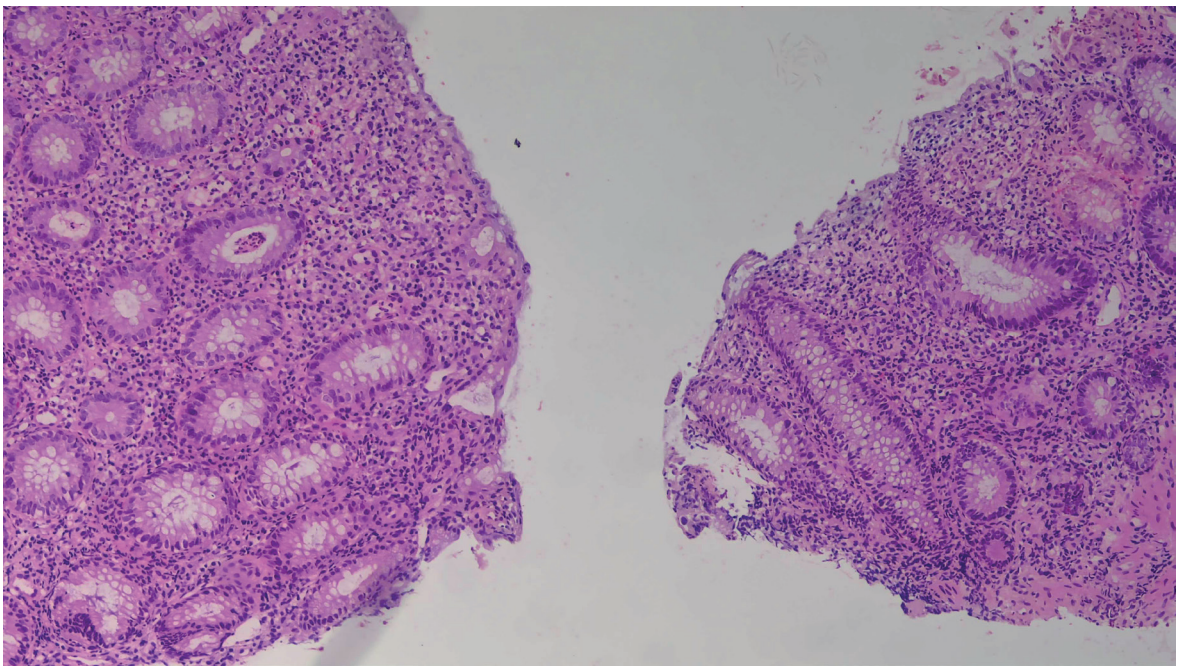

Fig. 2. Intense inflammatory infiltration consisting of lymphocytes, plasma cells, eosinophils and neutrophils is present in the lamina propria, and cryptitis and crypt abscess are observed (H&E, original magnification x10).

directly to neurological involvement. The fact that the patient showed resistance to steroid treatment but rapidly improved with an anti-TNF agent points to the critical role of TNF-α-mediated cytokine storm in the pathogenesis.

The etiology of UC is mostly thought to be related to various factors, including genetics, environment, infection and immune regulation disorders.[6] Cytokines act as important signals in the initiation and maintenance of UC. Increased TNF-α levels in UC have proinflammatory effects on both the intestinal and central nervous systems. It has been suggested that neuronal damage associated with delirium may be linked to the inflammatory cascade triggered by TNF-α in the brain-gut axis.[7,8] In this case, the patient did not respond to steroid treatment and his clinical condition rapidly improved with anti-TNF therapy. Anti-TNF therapy suppressed the cytokine storm by targeting TNF-α and controlled both gastrointestinal and neurological symptoms. Anemia and elevated acute phase reactant levels in our patient also indicate the severity of systemic inflammation. Increased proinflammatory cytokines such as TNF-α and interleukin-6 may lead to bone marrow suppression and stimulation of hepatic acute phase protein synthesis.

The management of extra-intestinal symptoms in IBD (most commonly in the joints, skin, hepatobiliary system and eyes) has been significantly altered by the use of anti-TNF drugs. A systematic review supported the benefits of anti-TNFs in the treatment of extraintestinal symptoms through the analysis of 9 interventional trials and 13 non-interventional trials.[9] However, we could not find any data in the literature on the ameliorating effect of anti-TNF therapy on neurologic symptoms in UC patients. Our case shows that anti-TNF therapy has the potential to improve the neurologic manifestations of IBDs.

In conclusion, we present a patient with UC who presented with delirium as the first symptom and improved with anti-TNF therapy. Anti-TNF therapy may suppress the gastrointestinal symptoms of UC as well as the effects of inflammation on the brain and may help improve neurologic symptoms such as delirium. However, more clinical trials are needed to better understand the effect of anti-TNF therapy on neurologic symptoms.

Ethical approval

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

Author contribution

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

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Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

An alternative approach to diagnosis and treatment of intractable paroxysmal sneezing in a child

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ABSTRACT

Background. Intractable paroxysmal sneezing is a rare and diagnostically challenging condition in children, often mimicking organic diseases. While it is often addressed as psychogenic in the literature, our case presented findings suggestive of a tic disorder, highlighting the need for a broader diagnostic perspective.

Case Presentation. An 11-year-old girl was referred to the child and adolescent psychiatry clinic with a one-year history of persistent and fluctuating sneezing episodes. Despite comprehensive evaluations by pediatric neurology, allergy, and otolaryngology services, no significant pathology was identified. While the symptoms initially appeared psychogenic due to their onset following a school change and exacerbation during periods of heightened stress, a detailed assessment revealed findings suggestive of a tic disorder, including the fluctuating nature of the symptoms, their absence during sleep, and transient suppressibility. Partial symptomatic relief observed with metoclopramide, a dopamine antagonist, led to the initiation of risperidone therapy (0.25–0.5 mg/day), which resulted in significant clinical improvement.

Conclusions. This case illustrates the complex interplay between psychogenic stressors and tic-like manifestations in pediatric intractable paroxysmal sneezing. The positive response to risperidone underscores the potential role of dopamine antagonist treatments in managing such cases. A multidisciplinary approach is crucial for accurate diagnosis and effective management, ultimately enhancing patient quality of life.

Key words: intractable paroxysmal sneezing, tic disorder, pediatric, psychogenic, dopamine antagonist.

Sneezing is a physiological defense mechanism triggered by irritation of the nasal mucosa from allergies, infections, or local pathologies.[1] However, intractable paroxysmal sneezing (IPS) is rare and predominantly affects female adolescents.[2-4] Unlike typical sneezing, IPS is generally considered to be of psychogenic origin and is characterized by recurrent, treatment-resistant sneezing episodes.[2,5]

Individuals with IPS do not sneeze during sleep and sneeze with their eyes open. Psychogenic sneezing is further characterized by the absence of a full inspiratory phase, minimal nasal secretions, and generally normal findings on physical examination. Additionally, while a significant psychiatric history is frequently observed in these patients, it is not invariably present.[2]

Tic disorders, among the most common movement disorders in childhood, manifest as semi-involuntary, sudden, rapid, and repetitive movements, facial expressions, gestures, or vocalizations.[6] In severe cases of tic disorders, pharmacological treatment

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with antipsychotic agents has been reported to be particularly effective.[7,8] Interestingly, otolaryngologic symptoms, including coughing and sneezing, have also been described as potential manifestations of tic disorders in prior reports.[9] Similarly, in a case marked by intense coughing, transient tic disorder was considered in the differential diagnosis, and complete remission was achieved with haloperidol treatment.[10] Furthermore, recent research has proposed shared genetic and etiological factors underlying the coexistence of allergic diseases and tic disorders, indicating a common neurobiological substrate.[11] Despite these observations, the conceptualization of IPS within a tic disorder framework remains scarce.

Reframing IPS as a tic-related manifestation may refine diagnostic algorithms, and expand therapeutic horizons to include targeted neuroleptic strategies. To the best of our knowledge, this is among the rare cases in which IPS has been re-evaluated as a possible tic disorder, with favorable response to dopamine antagonist treatment.

We present the case of an 11-year-old girl with IPS, initially suspected to be psychogenic but displaying tic-like features. Her symptoms improved significantly with low-dose risperidone. This case underscores the importance of integrating neuropsychiatric perspectives into the evaluation of refractory sneezing and advocates for a multidisciplinary approach to optimize patient outcomes.

Case Presentation

An 11-year-old girl was referred to the Child and Adolescent Psychiatry Clinic at Ankara Bilkent City Hospital with a one-year history of IPS, characterized by a fluctuating course. Prior evaluations by pediatric neurology, allergy, and otolaryngology specialists—both at other centers and at our institution—had failed to reveal any significant pathology, prompting her referral to psychiatry.

Her symptoms commenced on the first day at her new school, after experiencing peer bullying at her previous institution. On that initial day, she developed consecutive sneezing bouts that persisted throughout school hours but were notably absent during sleep. During academic terms, her sneezing peaked each morning before classes; intensified during exams and subsided substantially during summer vacations, occasionally decreasing to fewer than twenty sneezes per day. At home, she could experience intervals of up to three hours without sneezing. These observations suggested a strong contextual component, wherein school-related stressors appeared to exacerbate her symptoms.

The patient reported that while she could temporarily suppress the sneezing, it would soon recur with greater intensity. She described a premonitory sensation accompanied by nasal itching prior to sneezing, and noted that her symptoms worsened in dusty environments and after consuming spicy foods. The frequency of her sneezing exceeded three times per minute, amounting to over 100 episodes daily.

Aside from sneezing, she had no upper respiratory symptoms. Nonetheless, the severity and unpredictability of her sneezing significantly disrupted daily functioning—she missed multiple school days, frequently exited formal examinations prematurely due to acute sneezing attacks, and made recurrent visits to the emergency department. Academically, her performance had been age-appropriate prior to symptom onset, but she later reported considerable anxiety and guilt stemming from absenteeism and diminished exam participation. She rated her distress at being unable to control or suppress the sneezing as 7 out of 10 on a visual analog scale. She actively tried to suppress her symptoms and appeared embarrassed and anxious during episodes.

A psychiatric interview and mental status examination revealed an anxious affect, with the patient expressing profound distress regarding

her inability to adapt to school demands. The sneezing episodes disrupted her speech and were preceded by a brief inspiratory phase, minimal nasal secretions, and a faint grunt. Video recordings confirmed that her eyes remained open during sneezing, with limited facial expression changes. Lip-tremor vocal tics were observed in conjunction with some sneezes; no additional motor or vocal tics were detected.

Comorbidity was evaluated through a comprehensive psychiatric interview and standardized self-report tools. The patient completed the Screen for Child Anxiety Related Emotional Disorders (SCARED), scoring 4; the Children's Depression Inventory (CDI), scoring 6; and the Children's Somatization Inventory-24 (CSI-24), scoring 12 —all within the non-clinical range. Additionally, the Child Behavior Checklist, completed by the caregiver, did not indicate any clinically significant elevations across syndrome scales or broadband indices. No prominent psychopathology was identified, including internalizing, externalizing, or somatoform symptoms.

Her past medical history was notable only for seasonal pollen allergy, which had not required pharmacological treatment. There was no personal or family history of atopic conditions, tic disorders, or neurological illnesses. Initial allergy evaluations included trials of antibiotics, montelukast, cetirizine, and intranasal corticosteroids, with only minimal, transient improvement. No clinically significant sensitivities were detected on external allergy testing. Serum IgE was measured at 191 IU/mL, and immunoglobulin levels were as follows: IgA: 0.66 g/L, IgG: 6,400 mg/L, and IgM within normal limits. Neurological examination was also unremarkable, with no focal findings or signs of central nervous system involvement. Cranial magnetic resonance imaging performed at another center had shown no abnormalities.

A detailed review of her history revealed that metoclopramide, administered during an emergency visit, had provided notable symptomatic relief. Given the dopamine antagonist properties of metoclopramide, treatment with risperidone was initiated at 0.25 mg/day, subsequently titrated up to 0.5 mg/day. Within two weeks, sneezing ceased entirely at home and decreased to fewer than 15–20 mild episodes per day while at school. By the one-month follow-up, symptoms had nearly remitted both at home and in the school setting, prompting maintenance of risperidone at 0.5 mg/day. Over the ensuing four months of monotherapy—without adjunctive psychotropic medications or psychotherapy—the patient attended school consistently, completed examinations without interruption, and reported marked improvements in mood, concentration, and anticipatory anxiety related to sneezing at school. She resumed normal peer interactions without shame or avoidance behaviors. No significant adverse effects emerged, and she remains under regular follow-up in our clinic. The family provided a written consent form for this publication.

Discussion

This case highlights the intricate relationship between psychogenic stressors and tic-like manifestations in pediatric IPS, emphasizing both the diagnostic challenges and therapeutic opportunities inherent in this rare condition. Most reported IPS cases occur in adolescent girls and are frequently linked to psychosocial triggers.[2] In several documented cases, symptom remission was achieved through the removal of psychological stressors and the use of supportive psychotherapy, further supporting the functional nature of these sneezing episodes.[3,12] Accordingly, our initial diagnostic considerations centered on a psychogenic origin, as the onset and course of her symptoms—triggered by peer bullying at her previous school and exacerbated during school hours and examinations— supported this explanation.

However, the fluctuating and suppressible nature of her symptoms, their absence during

sleep, accompanying lip-tremor vocal tics, and the presence of premonitory sensory awareness pointed toward a tic disorder variant. It is plausible that psychogenic stressors acted as triggers, exacerbating these tic-like manifestations.[13,14] Notably, these persistent sneezing episodes did not serve as a means of secondary gain; rather, they emerged as a significant stressor that markedly impaired her daily functioning.

The overlapping pathophysiology and clinical features of psychogenic sneezing and tic disorders further complicate the diagnostic process. A previous case report demonstrated a significant reduction in psychogenic sneezing with haloperidol—a potent dopamine D2 receptor antagonist—supporting the hypothesis that dopamine dysregulation plays a role in symptom manifestation.[11] Similarly, the partial symptomatic relief provided by metoclopramide, another dopamine antagonist, influenced our decision to initiate risperidone therapy. Following risperidone initiation, a substantial reduction in sneezing episodes was observed, bolstering the notion that this condition may represent a variant of tic disorder.

The diagnostic process for functional respiratory disorders is often protracted and challenging due to their clinical resemblance to organic diseases. As highlighted in a previous review, key differentiating features include the absence of nocturnal symptoms, sudden onset, variable duration, rapid regression, and a lack of response to standard pharmacotherapy[11] with diagnostic workups typically yielding normal findings. In our patient, these criteria were notably present, underscoring the importance of early and accurate diagnosis to prevent unnecessary and potentially harmful treatments.

In conclusion, this case illustrates that persistent sneezing in pediatric patients may arise from a confluence of psychogenic factors and tic disorder phenomena. The marked improvement in symptoms with risperidone suggests that dopamine antagonist medications could be an effective treatment modality for similar cases. These findings underscore the importance of a multidisciplinary approach—including child and adolescent psychiatry—in managing IPS to enhance patients' quality of life and overall prognosis. Additional studies are essential to unravel the underlying mechanisms of IPS and to devise more effective therapeutic interventions.

Ethical approval

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

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: CK, GIE; data collection: CK. analysis and interpretation of results: GIE; draft manuscript preparation: CK, GIE. 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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Evaluation of serum procalcitonin as a diagnostic tool to differentiate bacterial sepsis from rheumatic flare-ups in children with rheumatic disorders

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Dear Editor,

I read the article published in The Turkish Journal of Pediatrics by Majumder et al. with great interest, which concerned the evaluation of serum procalcitonin (PCT) to differentiate bacterial sepsis from rheumatic flare-ups. The study provides valuable insights into the diagnostic challenges faced by clinicians when managing febrile episodes in children with rheumatic diseases, particularly juvenile idiopathic arthritis (JIA) and systemic lupus erythematosus (SLE).[1]

The authors' focus on comparing the diagnostic utility of PCT with traditional inflammatory markers such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) is both timely and clinically relevant. The findings that PCT, with a cut-off at 0.275 ng/mL, demonstrated superior sensitivity (94.7%) and specificity (74.3%) in differentiating bacterial sepsis from disease flare-ups is particularly noteworthy. This underscores the potential of PCT as a more reliable biomarker in guiding clinical decisions, especially in children with low disease activity.

However, we would like to highlight a few points. First, while this study highlights the diagnostic superiority of PCT, it would be useful to discuss the potential limitations of PCT in clinical practice. For instance, could factors such as renal impairment or concurrent viral infections affect PCT levels, and how might these confounders influence its diagnostic accuracy?[2]

Second, the study population was relatively small (n=73), with a majority of patients diagnosed with JIA (56.2%) and SLE (38.3%). It would be interesting to see if these findings are generalizable to a larger and more diverse cohort, including children with other rheumatic disorders or those with comorbidities.

Thirdly, although the cross-sectional study design and the exclusion of non-bacterial infections enhance the study's internal validity, real-world clinical practice frequently involves cases with ambiguous or mixed diagnostic presentations.[3] Future studies could investigate the utility of PCT in more complex contexts, such as mixed infections where flares overlap with underlying chronic conditions.

Furthermore, the study stratified patients into flare-up and sepsis groups based on disease activity scores (Juvenile Arthritis Disease Activity Score [JADAS-27] or Systemic Lupus Erythematosus Disease Activity Index [SLEDAI]) and the presence of a bacterial focus. Although this approach is methodologically rigorous, future studies should investigate whether PCT levels correlate with clinical disease activity scores independent of concurrent infection. Such data could provide

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additional clarity on the interplay between inflammation due to rheumatic activity and infection.

Lastly, the study's conclusion that PCT is a better diagnostic tool than CRP or ESR is well-supported by the data. However, it would be helpful to discuss the cost-effectiveness and accessibility of PCT testing, particularly in resource-limited settings where CRP and ESR remain widely used due to their affordability and availability.[4]

In conclusion, this study makes a significant contribution to the literature by highlighting the diagnostic utility of PCT in differentiating bacterial sepsis from rheumatic flare-ups in children, and also significantly advances pediatric rheumatology practice. I commend the authors for their rigorous methodology and insightful findings. I look forward to further research in this area, particularly studies that address the limitations and practical implications of incorporating PCT into routine clinical practice.

Thank you for considering my comments. I believe this article will spark important discussions among clinicians and researchers alike, ultimately improving the care of children with rheumatic disorders.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Authors' reply to the letter: "Evaluation of serum procalcitonin as a diagnostic tool to differentiate bacterial sepsis from rheumatic flare-ups in children with rheumatic disorders"

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Dear Editor,

We are grateful to the reader for their thoughtful engagement with our article[1] and for highlighting both the clinical relevance and broader implications of our findings through the communication.[2] We address the points raised as follows:

Influence of confounders on procalcitonin (PCT) levels (e.g., renal impairment or viral infections):

We agree that certain conditions may affect PCT levels. Renal dysfunction, particularly severe acute kidney injury, has been associated with falsely elevated PCT due to reduced clearance. However, none of the children included in our study had clinical or biochemical evidence of significant renal impairment at admission. Additionally, we excluded children with confirmed viral or non-bacterial infections such as dengue, malaria, or COVID-19, to reduce diagnostic ambiguity. Nevertheless, we acknowledge that in real-world scenarios, mixed infections and underlying renal pathology can confound interpretation, and thus, PCT should be interpreted in conjunction with clinical judgment and other markers.

Sample size and generalizability:

Our prospective study included 73 febrile children with previously diagnosed rheumatic diseases, the majority being cases of juvenile idiopathic arthritis and systemic lupus erythematosus, the most prevalent paediatric rheumatic disorders.[3] While the sample size reflects the constraints of single-centre and pandemic-era data collection, it does provide robust preliminary evidence. We fully agree that multicentre studies with more diverse rheumatological profiles (e.g., vasculitides, mixed connective tissue disease) and larger cohorts are necessary to generalize the findings further. This remains a focus for future research.

Mixed presentations and real-world clinical complexity:

This is a valuable point raised by the reader. While we deliberately excluded cases with overlapping clinical features or diagnostic ambiguity to maintain internal validity, we recognize that real-life scenarios often involve complex, overlapping disease manifestations. Future prospective studies incorporating mixed or evolving presentations — including macrophage activation syndrome and viral co-infections — will better reflect clinical

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practice and test the robustness of PCT-based differentiation in such contexts.

Correlation between PCT and disease activity scores:

Our study did not aim to directly correlate PCT values with Juvenile Arthritis Disease Activity Score (JADAS-27) or Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) scores in isolation from infection. The major barrier in such analysis is the heterogeneity of the initial disease activity across the study population. The flare-up criteria in both diseases are dependent on the initial severity scores; therefore, a specific value (of JADAS-27 or SLEDAI), taken at a cross-section can neither detect nor exclude the flare-up. The durations of the diseases since onset were not uniform. To date, no relationship between biomarker levels and disease severity scores could be formulated, even with the common inflammatory markers like C-reactive protein (CRP), or erythrocyte sedimentation rate (ESR). Considering these features, we did not intend to establish any correlation between PCT level and the scores. However, we agree that such correlation might offer insight into whether rising PCT levels correlate with flare severity in the absence of infection, if any. Exploring this relationship could potentially refine interpretation thresholds for PCT in the context of disease activity and deserves exploration in follow-up studies.

Cost-effectiveness and feasibility in resource-limited settings:

Cost-effectiveness is a pertinent concern. While PCT testing is costlier and less widely available than CRP or ESR, the potential for timely and accurate differentiation between flare and infection could reduce unnecessary antibiotic use and hospital stays, ultimately offsetting the upfront cost. Moreover, point-of-care PCT testing is becoming increasingly accessible.[4] Nevertheless, cost-benefit studies in resource-constrained settings such as India are warranted before widespread adoption.

We thank the reader once again for raising these important and constructive points. We hope our responses provide clarity and emphasize both the significance of the study and the directions for future research. We remain committed to contributing to the evolving discourse on the use of biomarkers PCT in paediatric rheumatology.

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Conflict of interest

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