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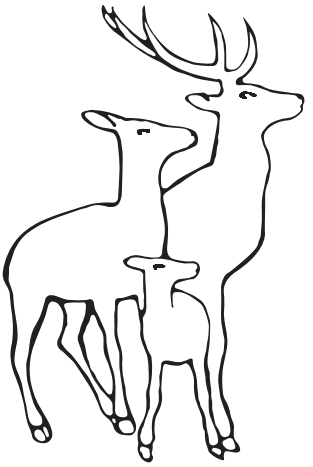

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The Turkish Journal of Pediatrics 2023; 65: 1-2
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The Türkiye-Syria Earthquake: a response from the editors of the Turkish Journal of Pediatrics

Ali Düzova, Sinem Akgül, Gülen Eda Utine, Yılmaz Yıldız

On February 6, 2023, two consecutive earthquakes near Kahramanmaraş, Türkiye sent shockwaves across hundreds of miles, forever altering the lives of millions across Türkiye and Syria. The countries were rocked by two earthquakes with magnitudes of 7.7 and 7.6 (Mw), separated by just nine hours, that were the region's strongest in nearly a century. It is estimated that more than a million people have been left homeless as more than 150,000 buildings collapsed or were rendered uninhabitable in Turkey alone as a result of the earthquakes. At the time of this writing, 21 days after the catastrophe, the official death toll has exceeded 40,000, with countless others injured, some of whom are still in critical condition.

The earthquakes exposed multiple, sometimes fatal shortcomings in construction, infrastructure, emergency preparedness and emergency response. After the disaster, it immediately became evident that the lessons to be learned from numerous recent deadly earthquakes, most notably the 1999 Gölcük (Marmara) earthquake near İstanbul in the densely-populated northwestern Türkiye, had not been effectively implemented. Many lives were lost as a result of buildings reduced to rubble, including hospitals that collapsed or were damaged preventing access to health care. Damaged roads and airports hindered rescue efforts and the shipment of urgently-needed personnel and supplies. The importance of standardization, regulation and oversight of construction projects by competent authorities cannot be overstated.

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In the immediate aftermath of the earthquakes, the limitations of local response teams in terms of trained staff, adequate equipment and coordination became apparent. Mobilization from surrounding areas proved impossible due to disruptions in transportation and the devastation of an extremely large region, leaving many settlements to fend for themselves, especially in the first few days after the earthquakes, when rapid search and rescue was crucial.

The pediatric community rushed to the aid of those in need. Those who were already in the affected area were among the first responders, providing care in partially collapsed buildings, with limited or no equipment, sometimes without electricity and in bitter cold. While some pediatricians volunteered, and went to the affected area immediately, working outdoors with search and rescue teams, in hospitals or makeshift tents, others remained at their posts and awaited the arrival of patients. Hospitals across the nation have been working tirelessly to provide life-saving care.

The central organization of the medical response, which initially requested surgeons but not pediatricians or internists to the affected area, highlights a well-known flaw in the approach to trauma: the misconception that trauma is exclusively in the domain of surgery. While the heroic and tireless efforts and accomplishments of orthopedic and trauma surgeons, neurosurgeons, cardiovascular surgeons, plastic surgeons, general surgeons, pediatric surgeons, among others, are invaluable, the medical complexities of patients and especially children with severe trauma and/or crush syndrome must not be underestimated. Early fluid therapy (even before extrication), treatment of electrolyte

and acid-base imbalances, acute kidney injury, respiratory failure, shock, sepsis and other conditions demonstrated the significant contributions of general pediatricians, pediatric emergency physicians, pediatric intensivists, pediatric nephrologists and others, both in the field and in referral centers across the country. Despite the fact that pediatricians have long been aware of their crucial roles in trauma teams, a multidisciplinary, data- and guideline-driven "trauma team activation" approach to major trauma should also be implemented by health care policy makers.

Long-term consequences of this dire event will start unraveling in the coming weeks, months and years. This natural disaster has struck a region already afflicted by a refugee crisis. Those affected are in urgent need of shelter, clean water, food security and acute medical care. Children and adolescents are among the most vulnerable when it comes to disasters. Numerous children are at risk for communicable diseases (diarrhea, pneumonia, scabies etc.), acute malnutrition and the vicious cycle between the two. Some have become amputees, and will require life-long physical rehabilitation, mental health care and socioeconomic support. Numerous children are orphaned and have lost their homes, schools and support systems. The disruption of their daily lives in the long-term increases their risk for school dropouts, mental health problems, child marriages, adolescent pregnancy, abuse, and violence. The environmental pollution caused by the earthquakes is also expected to have a greater impact on children, including but not limited to asbestos exposure from demolished old buildings. Multiple renowned health centers in the region have been damaged by the earthquake, which are expected to affect health care services in the near future.

Children will also be exposed to the potential remote effects of a disaster of this magnitude. In addition to the loss of many lives, loved ones, and homes, thousands of families will be displaced due to the destruction of personal relationships, businesses, opportunities, plans, and aspirations. Migration from the region may alter the social structure of the region for decades to come, and regions receiving migrants may experience difficulties accommodating them, including problems with housing, education, health care and social services. Parents, foster families, social workers, teachers, pediatricians, child psychologists and psychiatrists, physiotherapists and communities should work together to ensure the best possible health outcomes for these vulnerable children.

Currently, numerous pediatricians both in the field and in hospitals are working tirelessly to save severely injured children. International support from all around the globe arrived swiftly, and is greatly appreciated. As the editors of the Turkish Journal of Pediatrics, we extend our deepest condolences to the people of Türkiye and Syria, who have suffered immeasurable loss, grief, and sorrow and would like to thank all those who have worked and will continue to work for the health and safety of children in our nation.

We must emphasize that it is imperative for all authorities and policymakers to follow the path of reason and science, and heed the warnings and recommendations of the scientific and medical community in order to minimize the short- and long-term consequences of this disaster on children, and to take the necessary precautions to prevent such an earthquake from causing this much damage in the future.

Finally, we are calling on all pediatricians and other health care providers working with children to submit to the Turkish Journal of Pediatrics studies based on data from these earthquakes. Our journal will provide a platform for the dissemination of scientific data regarding child health in the aftermath of these earthquakes. We intend to expedite the review and publication of such manuscripts in order to facilitate rapid communication.

Ali Düzova, *Editor-in-Chief*
Sinem Akgül, *Associate Editor*
Gülen Eda Utine, *Associate Editor*
Yılmaz Yıldız, *Managing Editor*

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Original Article

Phenotypes of persistent hen's egg allergy in children and adolescents

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ABSTRACT

Background. Optimum management of food allergy (FA) includes consideration of co-allergies and multimorbidities and tolerance assessment. Documentation of FA practices may pave the way for better practice.

Methods. Patients aged 3-18 years, with persistent IgE-mediated hen's egg allergy were reviewed.

Results. A total of 102 children with a median age of 59 months (IQR= 40-84) (72.2% males) were included. All were diagnosed during infancy and the initial symptoms were atopic dermatitis (65.6%), urticaria (18.6%), and anaphylaxis (5.9%). Of the total population, 21 (20.6%) experienced anaphylaxis with hen's eggs, and 79.4%, 89.2%, and 30.4% had multiple FAs (≥2 food categories), ever atopic dermatitis, and asthma, respectively. The most common co-allergies were tree nuts, cow's milk, and seeds, respectively. From 52 heated egg yolk and 47 baked egg oral food challenges, 48 (92.3%) and 41 (87.2%) were found as tolerant, respectively. The baked egg nontolerant group had a greater egg white skin prick test diameter [9 mm (IQR: 6-11.5) vs. 6 mm (IQR: 4.5-9); (p=0.009)] and specific IgE [12.6 kU/L (IQR: 4.11-45.4) vs. 6.2 kU/L (IQR: 1.9-12.4) (p=0.009)], respectively. In the multivariate analysis, baked egg tolerance was more likely in those with egg yolk-tolerant subgroup (OR: 6.480, 95% CI: 2.524-16.638; p<0.001) and heated egg tolerance in those with baked egg tolerance (OR: 6.943, 95% CI: 1.554-31.017; p=0.011).

Conclusions. Persistent hen's egg allergy is characterized by multiple food allergies and age-related multimorbidities. Baked egg and heated egg yolk tolerance were more likely to be considered in a subgroup with a high expectation for finding a way to eliminate their allergy.

Key words: Hen's egg, allergy, atopic dermatitis, children, adolescents.

Hen's eggs are one of the most important basic foods of early childhood across many countries and cultures because of ease of access and affordability. However, it is also an important trigger of food allergies (FA) in early childhood. For example, in Turkey, it is the most common etiology of both immunoglobulin (Ig)-E–mediated food allergies[1] and food protein-induced enterocolitis syndrome[2] in early childhood, as well as the third cause of food-induced anaphylaxis.[3]

Though it varies according to both culture and study design, the prevalence of IgE-mediated FA is estimated to be between 0.5-2.5%.[4,5] However, egg sensitization rates are much higher than allergy prevalence; therefore, routine allergy testing is not recommended for those without a history of allergies and early-onset moderate-severe atopic dermatitis (AD)[6,7] because this may lead to unnecessary avoidance. The gold standard of IgE-mediated hen's egg allergy diagnosis is oral food challenge (OFC); however, this carries inherent risks as it is labor-intensive and time-consuming.[8] Half of those diagnosed as having IgE-mediated egg allergy

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have been reported to resolve itself by the age of 3 years and two-thirds by the age of 5 years.[4,9,10] Therefore, empirically, the age of 3 years can be regarded as an age when egg sensitivities are excluded, mild allergies are resolved and more persistent allergies remain.

It is important to document allergy practices to observe shortcomings and examine practice differences between centers. In this study, we aimed to document the co-allergies and comorbidities of children with persistent hen's egg allergies, how they were managed, and how different parts of the egg and different processed forms were consumed.

Material and Methods

Study Design and Subjects

In this retrospective study; patients aged between 3 and 18 years who were followed up regularly due to persistent IgE-mediated hen's egg allergy between January 2014 and December 2020 were included and those who had a resolved egg allergy within this period were excluded.

To evaluate age-related characteristics, the study population was grouped as preschoolers (age 3-5 years), school-aged children (age 6-11 years) and adolescents (age 12-18 years). Allergy to more than one type of food group was stated as multiple food allergies (≥2 food categories). The diagnosis of AD, allergic rhinitis (AR), and asthma/recurrent wheezing was made according to international guidelines.[11-13] The age of asthma diagnosis was determined as the start of asthma control therapy. The age of onset of AR was considered to be the age at which the patient showed signs of rhinitis when exposed to the aero-allergen to which they were sensitive. The analyses were made according to "current" status (atopic diseases during the past year visits as "current asthma," "current AD" and "current AR" or "inactive"), the patients' resolved atopic diseases as "inactive asthma", "inactive AD," and "inactive AR," or "never" status (no history of atopic diseases).[14]

Diagnostic Procedures

For skin prick tests (SPTs), allergen extracts (ALK®, Horsholm, Denmark) were applied on the volar surface of the forearm or back along with negative and positive controls. The mean wheal diameter was measured after 15 minutes by calculating the mean value of the longest diameter and the diameter perpendicular to it. According to the routine practice of the clinic, SPTs were performed if the patient did not use any drug such as antihistamine that could interfere with the SPT within the last 5 days. Allergen-specific IgE levels were measured using the Immuno-CAP method in the sera of the patients (Thermo Fisher Scientific, Uppsala, Sweden). A positive SPT (3 mm or more above the negative control) and specific IgE (≥0.35kU/L) was defined as usual. In the presence of positive SPT and/or sIgE, the diagnosis of hen egg allergy was based on either a positive OFC or a consistent and clear-cut history of IgE-mediated symptoms within 2 hours after the ingestion or in the presence of a positive SPT and/or sIgE suggesting clinical reactivity with >95% positive predictive value (PPV) (SPT ≥7 mm than the negative control and/or sIgE ≥ 7kU/L).[15]

The local ethics committee of Hacettepe University approved the retrospective study (Number: GO-20/1115, Date: November 17th, 2020).

Statistical Analysis

IBM SPSS Statistics for Windows v.22.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Values are shown as the median and interquartile range for data not normally distributed. The Mann-Whitney U test or Kruskal-Wallis test was used for comparisons and, Pearson's Chi-square ($\chi 2$) test or Fisher's exact test for between-group comparisons. Univariate and multivariate analyses were performed to predict persistence. Predictors that were significant based on univariate regression analysis ($P<0.2$) were then included as covariates in multiple regression analysis. The odds ratio

(OR) with 95% confidence intervals (CI) was calculated via uni- and multivariate analyses. All statistical tests were two-sided, and the level of statistical significance was set at $p<0.05$.

Results

Study Population

A total of 102 children with a median age of 59 months (IQR 40-84) (72.2% males) were enrolled into the study. The median follow-up period of the patients was 26 months (IQR:13.3-48.7). Of the study group, 53.9%, 42.2%, and 3.2% of the patients were preschool children, school-age children, and adolescents, respectively.

The median age at diagnosis was 6 months (IQR: 4-6). The initial symptoms were AD (65.6%), urticaria (18.6%), and anaphylaxis (5.9%) (Fig. 1). In 21 of these children (20.6%), there was a history of anaphylaxis after exposure to hen eggs. The rate of having experienced anaphylaxis with eggs was 10.9% (6/55) and 31.9% (15/47), in the preschool children andschool age children- adolescent groups (p=0.009), respectively (Table I).

Food Co-Allergies

Multiple food allergies were diagnosed in 79.4% of the study population. (Table I) The most common co-allergies were tree nuts (60.8%), cow's milk (38.2%), sesame seeds (38.2%), peanut (23.5%), legumes (11.8%), and wheat (7.8%), respectively. The most common tree nut co-allergies were hazelnut [46.1% (47/62)], cashew [45.1% (46/62)], and walnut [43.1% (44/62)], respectively. Food allergies according to baked egg tolerance (Fig. 2a) and age groups (Fig. 2b) are shown in Fig. 2.

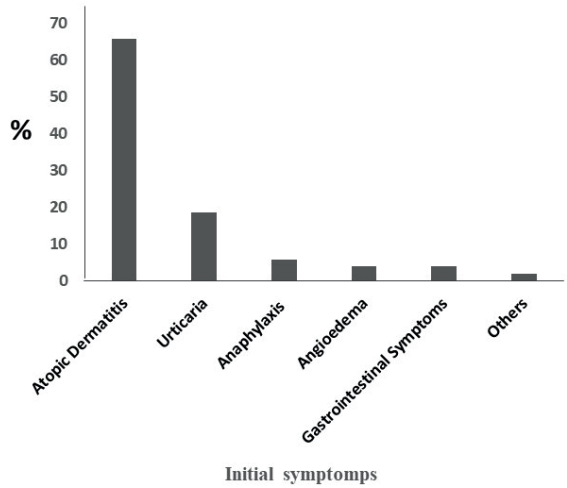

Fig. 1. The initial symptoms in egg allergy diagnosis

Multi-morbidities

When comorbidities were considered, 89.2% (n=91) and 30.4% (n=31) of the patients had a history of AD and asthma, whereas 34 (33.3%) and 29 (28.4%) of the 102 children had current AD and asthma , respectively. Three-quarters (75.8%) (22/29) of patients with asthma were school age children and adolescents group ($p<0.001$). A total of 27 patients (26.4%) had aeroallergen sensitization and compatible rhinitis symptoms with exposure to these allergens and most (85.1%) were from the

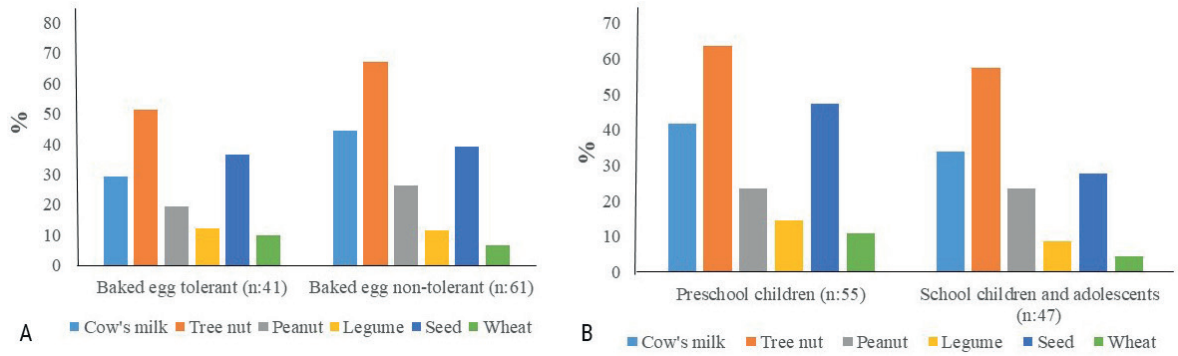

Fig. 2. Food allergies according to baked egg tolerance (Fig. 2a) and age groups (Fig. 2b)

Table I. The characteristics of the study group and its subgroups according to age and baked egg tolerance.

Patient characteristics	All (N=102)	Baked egg tolerance			Child's age		
		Tolerant (n=41)	Non-tolerant (n=61)	P	Preschool (n=55)	Older (n=47)	P
Age, month	59 (40-84)	49 (39-69)	61(41-94)		40 (36-48)	89 (72-108)	<0.001
Male gender, n (%)	74 (72.2)	32 (78)	42 (68.9)		41 (74.5)	33 (70.2)	
Total IgE, kU/L	421 (164-1018)	412 (126-688.3)	432 (175-1187)		369 (131.3-593)	517 (181-2010)	
Eosinophils, /μL	400 (253-793)	300 (200-700)	450 (300-825)		400 (300-700)	500 (200-900)	
Eosinophils, %	5.4 (3.1-8.6)	4.2 (2.4-7.2)	6.1 (3.3-8.7)		5.2 (3.2-6.8)	6.8 (3.1-9.3)	
Egg white sIgE, kU/L	7.78 (2.93-25.2)	6.2 (1.9-12.4)	12.6 (4.11-45.4)	0.009	7.64 (3.13-20.4)	10.3 (2.53-38)	
Egg yolk sIgE, kU/L	3.17 (0.71-8.6) [n=52]	1.58 (0.57-4.47) [n=17]	4.5 (0.71-11.6) [n=35]		3.42 (0.66-10.2) [n=29]	3.06 (0.71-7) [n=23]	
Ovomucoid sIgE, kU/L	2.93 (0.82-10.3) [n=28]	0.86 (0.45-4.5) [n=9]	4.57 (0.97-39.2) [n=19]	0.099	0.92 (0.44-6.08) [n=16]	4.63 (2.01-23.5) [n=12]	
Egg white SPT, mm wheal	7.5 (5-11)	6 (4.5-9)	9 (6-11.5)	0.009	8 (5.13-10.4)	7 (4.9-11)	
Egg yolk SPT, mm wheal	5 (3-8) [n=79]	3 (0-5) [n=29]	6.8 (4.3-8.62) [n=50]	<0.001	4.5 (2.3-7.8) [n=44]	5.5 (3.5-9) [n:35]	
Egg anaphylaxis, n (%)	21 (20.6)	3 (7.3)	18 (29.5)	0.007	6 (10.9)	15 (31.9)	0.009
Single FA, n (%)	21 (20.6)	12 (29.3)	9 (31.1)		11 (20)	10 (21.3)	
Multiple FA, n (%)							
2 FA	30 (29.4)	13 (31.7)	17 (27.9)		12 (21.8)	18 (38.3)	
3 FA	29 (28.4)	8 (19.5)	21 (34.4)		16 (29.1)	13 (27.7)	
≥4 FA	22 (21.6)	8 (19.5)	14 (23)		16 (29.1)	12 (12.8)	

Data are presented as median (interquartile range) unless indicated otherwise. FA: food allergy, SPT: skin prick test.

school-age children and adolescents group (p<0.001) (Table II).

Baked Egg and/or Egg Yolk Tolerance

Of the 102 children, 52 had heated egg yolk OFC and 48 (92.3%) were found to be tolerant. From 47 baked egg OFCs, 41 were found to be tolerant (87.2%). Overall, 47.1% (48/102) and 40.2% (41/102) were consuming egg yolk and baked egg regularly, respectively. There was no difference between the baked egg tolerant and nontolerant subgroups concerning current age, sex, presence of concomitant AD, eosinophil counts/percentages, and serum total IgE levels (Table I). However, in the baked egg nontolerant subgroup, there was higher egg white SPT [9 mm (6-11.5)] and specific IgE [12.6 kU/L (4.11-45.4)] compared with the tolerant subgroup (p=0.009). Ovomucoid-specific IgEs were evaluated in only 28 patients and there was a tendency of higher ovomucoid sIgE [4.57 kU/L (IQR: 0.97-39.2)] in the baked egg nontolerant group compared with the tolerant subgroup [0.86 kU/L (IQR: 0.45-4.5)] (p=0.099). The rate of having experienced anaphylaxis with egg was 29.5% (18/61) and 7.3% (3/41) in the baked-egg nontolerant and tolerant subgroups (p=0.007), respectively. Remarkably, the presence of multiple food allergies (≥2) was more frequent in the baked egg nontolerant group compared with the tolerant subgroup (p=0.075), but the difference was not statistically significant.

Multivariate analysis revealed that baked egg tolerance was more likely in those with egg yolk tolerance (OR: 6.480, 95% CI: [2.524-16.638]; p<0.001) and heated egg yolk tolerance in those with baked egg tolerance (OR: 6.943, 95% CI: [1.554-31.017]; p=0.011) as detailed in Table IIIa-IIIb.

Table II. The comorbid allergic diseases.

Comorbidity	All (N=102)	Baked egg tolerance			Child's age		
		Tolerant (n=41)	Non-tolerant (n=61)	P	Preschool (n=55)	Older (n=47)	P
Asthma							
Never	71 (69.6)	31 (75.6)	40 (65.5)	NS	47 (85.5)	24 (51)	<0.001
Inactive	2 (2)	1 (2.4)	1 (1.6)	NS	1 (1.8)	1 (2.1)	
Current	29 (28.4)	9 (22)	20 (32.7)	NS	7 (12.7)	22 (46.8)	<0.001
Atopic dermatitis							
Never	11 (10.8)	6 (14.6)	5 (8.2)	NS	3 (5.5)	8 (17)	
Inactive	57 (55.9)	24 (58.5)	33 (54)	NS	32 (58.2)	25 (53)	
Current	34 (33.3)	11 (26.8)	23 (37.7)	NS	21 (38.2)	13 (27.6)	
Allergic rhinitis							
Never	75 (73.5)	33 (80)	42 (68.9)	NS	50 (90.9)	24 (51)	<0.001
Current	27 (26.4)	8 (19.5)	19 (31.1)	NS	4 (7.2)	23 (48.9)	<0.001
Aeroallergen sensitivity							
Grass pollen	11 (10.7)	4 (9.7)	7 (11.4)	NS	0	11 (23.4)	0.016
Mite	12 (11.7)	2 (4.8)	10 (16.3)	NS	4 (7.2)	8 (17)	
Alternaria	6 (5.8)	2 (4.8)	4 (6.5)	NS	0	6 (12.7)	
Pet	9 (8.8)	1 (2.4)	8 (13)	NS	0	9 (19)	
Others	2 (1.9)	0	2 (3.2)	NS	0	2 (4.2)	

Data are presented as number (percentage). NS: non-significant.

Table IIIa. Predictors in univariate and multivariate analysis for baked egg tolerance.

	Univariate			Multivariate		
	OR	95% CI	P	OR	95% CI	P
Age	0.991	0.979-1.004	0.172			
Gender	0.622	0.249-1.555	0.310			
Current AD	0.606	0.255-1.436	0.255			
Egg white SPT	0.880	0.785-0.985	0.027			
Egg white sIgE	0.980	0.962-0.997	0.025			
Egg yolk tolerance	5.342	2.252-12.673	<0.001	6.480	2.524-16.638	<0.001

Table IIIb. Predictors in univariate and multivariate analysis for egg yolk tolerance.

	Univariate			Multivariate		
	OR	95% CI	P	OR	95% CI	P
Age	0.996	0.985-1.007	0.479			
Gender	0.526	0.215-1.291	0.161			
Current AD	0.584	0.252-1.351	0.209			
Egg yolk SPT	0.803	0.693-0.931	0.004			
Egg yolk sIgE	0.890	0.772-1.027	0.110			
Baked egg tolerance	5.342	2.252-12.673	<0.001	6.943	1.554-31.017	0.011

AD: atopic dermatitis, CI: confidence interval, OR: odds ratio, SPT: skin prick test.

Discussion

The management of FA has different goals: to confirm the diagnosis; to look for underlying causes; to identify co-allergies; to check comorbidities; to identify the consequences; to assess the predictors of the course of the disease and tolerance of processed forms; and to monitor components of disease activity, impact, and control. Due to limited data about persistent egg allergies in children besides infancy in the literature, our study concerning the characteristics of patients aged over 2 years with egg allergies deserves further awareness.[16-18]

In our study, 79.4% of children had multiple FAs. This high rate may be related to the fact that only patients aged over 24 months and those with persistent hen's egg allergies during the follow-up period were included in the study, those with mild allergies had resolved by this time, and persistent allergies were more likely to have multiple FAs. This rate is similar to other studies[17] and even higher than in some.[16,19,20] The fact that our center is a reference center for the country may have resulted in more referrals of patients with multiple and severe allergies and persistent cases. Multiple FAs is a critical problem because daily life restrictions can lead to many consequences in nutrition, growth, and mental health.

Nearly 90% of our patients had a history of AD, and more than one-third had current AD. Atopic dermatitis, beginning in the infantile period with a severe course, is commonly accompanied by FA, of which hen's egg allergy is the most common.[1] The most common FA is cow's milk allergy in patients diagnosed as having FA without AD.[1] This feature illustrates the importance of eczema in the clinical presentation and development of egg allergies. The rate of AD multi-morbidity in our study was higher than in previous studies.[17,21] Consistent data show that the number of FAs increase as the severity of AD increases, as does the persistence of AD.[22]

Egg white and egg yolk differ in their protein constituents and allergenicity. The main allergenic proteins identified in eggs have

different physicochemical characteristics and different allergenic potentials; therefore, thermal heat processing and digestibility have different impacts on the allergenic capacity of these proteins.[23] In this retrospective review, we showed that almost half of the patients were evaluated for the tolerance of baked egg and egg yolk. Potential reasons for these evaluations being performed only on half of the patients may include the reluctance of parents, a current or severe history of reactions in the past, avoiding hospital visits due to the COVID-19 outbreak[24], or the physician's or parent's desire not to take the risk of OFC due to high sensitivity.

In our study, we found that 92.3% of the patients who underwent OFC with egg yolk tolerated the egg yolk, and 47.1% of the entire group regularly consumed egg yolk. In fact, egg yolk is less allergenic than egg white, and more than 90% of patients with egg allergy can tolerate egg yolk, as shown in two recent studies.[25,26] Although we know that it is not possible to safely separate the egg yolk and egg white parts other than in hard-boiled eggs, it may be desirable for patients and parents to feel that the yolk is tolerated because it can support the hope that they can recover from the allergy. However, the documentation of practice is important in terms of reflecting the expectations of families and physicians.

Similarly, it was observed that 87.2% of the patients who underwent OFC with baked eggs could tolerate them, and as a result, 40.2% of the study group could consume baked eggs. Considering that 50-85% of patients with egg allergies have been reported to tolerate baked egg products[23,27-29], our low rate may be a result of the desire of patients and/or physicians. However, the measurement of ovomucoid sIgE is the variable that best reflects the ability to consume baked eggs, and the low rate of this assessment in our study group is actually due to the limitations of the social security system in Turkey for component-based sIgE testing. In particular, considering that most patients have multiple allergies, it seems necessary to develop reliable multiplex assays instead of singleplex measurements for both cost and blood sampling in these young patients. In addition, in the multivariate analysis, we found that patients who tolerated baked eggs were more likely to be from the egg yolk-consuming subgroup and vice versa, reflecting the presence of a subgroup of patients/parents with a high expectation of finding a way out of their allergy. Considering that patients who consume baked eggs can develop an earlier tolerance[30] and consumption of various egg products such as egg yolk and/or baked eggs increases the nutritional variety and quality of life of children, more efforts should be made in this regard.

Atopic dermatitis and subsequent egg allergy are considered the first steps of atopic march, and over time, these patients may develop asthma, aeroallergen sensitivity, and AR.[31] Indeed, when we classified the patients by age groups, we found that AR, asthma, and aeroallergen sensitization were more frequent in school-age children and adolescents ($p<0.001$), which is consistent with the predefined allergic march.[32-34]

Almost all egg allergies develop in the first year of life and generally have a better prognosis compared with many other food allergies. Although early studies report resolution rates of 52% and 66% at ages 3 and 5 years, respectively[9,10], more recent studies from tertiary referral centers show that the estimated tolerance rates are 4% at 4 years, 12% at 6 years, 37% at 10 years, and 68% at 16 years of age.[4,35] In this study, we included patients aged over 24 months with persistent egg allergies and nearly half of the study group (46.1%) were aged over 5 years.

The limitations of our study were that both egg yolk and baked egg challenges were not performed in all patients, the most likely reasons included the concerns of families and physicians, the COVID-19 pandemic period, and a low rate of component-specific IgE measurements. However, the strengths of this study include real-life practice, the production of baseline data for improving practice, and

documentation of a subgroup of patients/ parents seeking a way to eliminatethe allergy.

In conclusion, this study evaluated children and adolescents with persistent hen egg allergies, characterized by multiple food allergies and ever/current atopic dermatitis at a national reference centre. We showed that in this group, the possibility of heated egg yolk tolerance, and more importantly, baked egg tolerance, might not have been adequately addressed by both ovomucoid-specific IgE measurements and the relevant OFCs. Considering the evidence that encouraging the consumption of baked eggs can support the resolution of hen's egg allergies, it may shed light on how centers can reach a better level of practice.

Ethical approval

The local ethics committee of Hacettepe University approved the retrospective study (Number: GO-20/1115, Date: November 17th 2020).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: BEŞ; data collection: HÜ, STD, AA; analysis and interpretation of results: HÜ, ÜMŞ, ÖS, BEŞ; draft manuscript preparation: HÜ, BEŞ. 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

Correlation between anthropometric measurements of height and arm span in Indonesian children aged 7-12 years: a cross-sectional study

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ABSTRACT

Background. Height is an anthropometric measurement that serves as the most constant indicator of growth. In certain circumstances, arm span can be used as an alternative to height measurements. This study aims to analyze the correlation between anthropometric measurements of height and arm span in children aged 7-12 years.

Methods. A cross-sectional study was carried out from September to December 2019 in six elementary schools in Bandung. Children aged 7-12 years were recruited with a multistage cluster random sampling method. Children with scoliosis, contractures, and stunting were excluded from the study. Height and arm span were measured by two pediatricians.

Results. A total of 1,114 children, comprising 596 boys and 518 girls, fulfilled the inclusion criteria. The ratio of height to arm span was 0.98-1.01. The regression equation used to predict height through measurement of arm span in male subjects was Height = 21.8623 + 0.7634 x Arm span (cm) + 0.0791 x age (month); R2 = 94%; standard error of estimate (SEE): 2.66 and that in female subjects was Height = 21.2395 + 0.7779 x Arm span (cm) + 0.0701 x age (month); R2 = 95.4%; SEE: 2.39. The predicted height and the average actual height were not significantly different. There is a strong correlation between height and arm span in children aged 7-12 years.

Conclusions. Arm span can be used to predict the actual height of children aged 7-12 years and as an alternative measurement for growth.

Key words: anthropometric, arm span, height, children, growth.

Growth is an important indicator to assess children's nutritional and health status.[1,2] The growth of children can be assessed through history taking, physical examination, and anthropometric measurement. The anthropometric measurements that are routinely performed include the measurement of body weight, length/height, and head circumference.[3] Body length/height is considered crucial in assessing nutritional status, calculating body mass index (BMI), calculating drug doses, blood pressure, kidney function, lung function, and health monitoring.[4-6] According to World Health Organization (WHO) guidelines, the measurement of length/height for children aged above 2 years should be performed by having the children stand up straight barefoot with the back of the head, shoulders, buttocks, calves, and heels against the wall.[7] Due to several different circumstances, such as deformities of the lower limb, fractures, limited mobility, amputations, lower limb contractures, paraplegia, and pain, a proper measurement of height cannot be

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performed.[6,8-10] Therefore, other methods are required to measure height.[3,5]

Several anthropometric measurements such as arm span, crown-rump length, sitting height, and segmental length (knee height, upper arm length, tibial length can be used as alternatives.[5] Arm span measurement has been found to provide a good predictive value for height and has been widely used as an alternative to height measurement.[11-13] The arm span is measured from the tip of the right middle finger to the tip of the left middle finger, either in a sitting or standing position (CDC 2009), as long as the arms can be fully stretched. This measurement requires two examiners.[14,15] The limitation of this measurement is that it cannot be performed on children with contractures or spastic hands.[5]

The use of arm span measurement in predicting height has been widely studied. Forman et al.[5] reported that the correlation of arm span to body height (r=0.97) was stronger than that of ulnar length to body height (r=0.91). Yousafzai et al.[16] compared arm span, arm length, and tibial length to body height and found the highest correlation coefficients for arm span (r^2=0.93), compared to arm length (r^2=0.81) and tibial length (r^2=0.72). These studies showed that arm span has a very strong correlation in predicting height compared to the other measurements.

The Center for Disease Control and Prevention (CDC) reported that the ratio of arm span to height was 1:1.[14] Lee et al.[13] in Taiwan demonstrated that the ratio of arm span compared to height was 0.98-1.03. Meanwhile, Mazicioglu et al.[10] in Turkey found that the ratio of arm span and height on 50th percentile was 0.99-1.01. The ratio of height to arm span can vary according to age, gender, and ethnicity.[17]

Data from Indonesia's National Socioeconomic Survey in 2018 showed that there were 31.59% of children aged 0-17 years suffering from health complaints, of which 15.89% of them had limitations on daily activities due to illnesses. As many as 1.11% of children aged 2-17 years were found to have long-term physical, mental, intellectual, or sensory disabilities.[18] This study was, therefore, conducted to analyze the correlation between the anthropometric measurements of arm span to body height in children aged 7-12 years.

Material and Methods

Study Population

The subjects of this study were children aged 7-12 years who were recruited from six elementary schools in Bandung, Indonesia with multistage cluster random sampling, as follows: (1) 3 out of 30 districts in Bandung were selected. (2) Out of every district, 3 public and private elementary schools were selected. (3) Out of the selected school, one class was selected from every grade (grades 1-6), of which all students in the selected classes were selected to be the research subjects.

The inclusion criteria of the study were healthy children whose parents consented to the participation of their children in the study by signing an informed consent. The exclusion criteria included children with scoliosis, stunting, and contractures. Scoliosis is a disorder in which the spine curves laterally >10^o with rotation of the spine. For the examination of scoliosis we used the Adams forward bend test in a standing and bent-forward position in order to assess the spinal symmetry from the back and from the side of the subject. The subject was asked to stand and bend forward with hands on the waist. The examiner then assessed the spinal symmetry from the back and from the side of the patient. Any spinal abnormalities, e.g. a rib hump, were considered signs of scoliosis. Stunting was defined as height according to age <-2 SD. Meanwhile, contracture was defined as reduced passive range of motion due to limitation of joints, muscles and soft tissue.

By using an observational analytic design with a cross-sectional method, the study was conducted from September to December 2019.

Clinical Evaluation

The height and arm span were measured by using 200-cm iron measuring tapes from Nankai brand (approval number 4478/PKTN.4.7/12/2018) and Ikoala brand (approval number 4477/PKTN.4.7/12/2018), which had been calibrated at the Director of Metrology of Directorate General of Consumer Protection and Orderly Commerce in Bandung.

Height measurement was performed on the subject while standing using a 200-cm, Nankai-brand measuring tape. The subject was asked to stand up straight with the back of the head, shoulders, buttocks, calves and heels against the wall barefoot. Any headdresses were removed. The subjects were asked to face straight ahead on a Frankfort horizontal plane and then to take a deep breath. Height was measured with the help of a stature meter, which was pulled until it pressed down against the crown of the head. The results were documented with an accuracy of 0.1 cm. Arm span was measured by two examiners. The subjects were asked to stand up straight with the arms stretched perpendicular to the trunk, assisted by the examiner. The arms were stretched maximally. The length of arm span was measured from the tip of the right middle finger to the tip of the left middle finger. Measurements were performed at the back of the body. The results were documented with the accuracy of 0.1 cm. All measurements were taken twice and the results of the measurements were documented based on the average value of the two measurements.

Outcome Variables

The assessed variables included anthropometric data and general characteristics of the subject. The data for general characteristics were taken from the school records, which included age, gender, parents' educational level, parents' occupation, family income, and ethnicity. Anthropometric data included arm span, body height, and body weight.

Sample Size Calculation

The sample size was calculated using an 80% power test, with a minimum correlation of r=0.4 and a significance level of 5%. Using this formula, the minimum sample size for each age group was 54 subjects. Therefore, the total number of samples needed for six age groups of 7-12 years were 324 subjects. With the anticipation of a 10% dropout rate, the final number of samples needed were 360.

Statistical Analysis

If the data distribution was normal, correlations between numerical variables were analyzed using Pearson correlation. Multivariate analysis was performed using multiple linear regression and a p value <0.05 was considered to be statistically significant. A concordance correlation was used to assess the difference between the predicted height and the actual height.

Ethical Consideration

This study has been approved by the Research Ethics Committee of the Faculty of Medicine Universitas Padjadjaran (approval number 1171/UN6.KEP/EC/2019)

Validity of Anthropometric Measurements

Before starting the data collection, a validity test was performed to assess the intra- and inter-observer validity. The test was conducted by two investigators, in which each investigator recorded two measurements. The validity and reliability tests were performed on 30 samples. The validity test showed that the height and arm span measurements between the two investigators had no significant differences (p>0.05). The reliability test also showed a good correlation between the measurements performed by the two investigators.

Results

Based on the data from six selected schools, there were 1,279 children aged 7-12 years. Of those, 165 children were excluded from the study due to several reasons, including 145 children with a Z-score of height for age <-2 SD (stunting), 2 children with contractures, and 18 children who were absent during the data collection. Hence, a total of 1,114 children underwent measurements and data collection for basic characteristics and anthropometric measurements (body weight, height, and arm span). The data for basic characteristics were taken from the student data record. (Fig. 1).

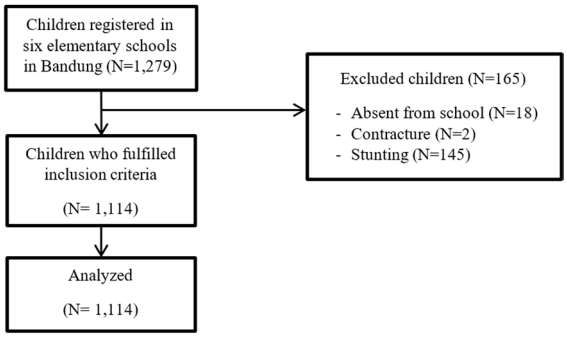

Fig. 1. Subject Selection Flow.

The sociodemographic characteristics and body mass index distribution of the children are shown in Table I. A total of 1,114 children were analyzed, of whom 596 were boys (53.5%) and 518 were girls (46.5%). For the distribution of age, most of the subjects were at the age of 11 years (20.9 %), followed by the age of 7 years (19.8%), and, finally, the age of 12 years (10.9%). Sundanese made up the majority of ethnic groups (85.1%). The majority of the subjects were categorized as having a normal nutritional status (86%).

The distribution of height and arm span of subjects were presented in figure 2 and figure 3. Both figures display the data distribution from +3 SD to -3 SD. The data in -2 SD and -3 SD of height and arm span does not indicate that those with stunting were recruited, rather it is the result of data distribution of the subjects enrolled.

Table I. Sociodemographic and body mass index distribution of the study children.

Characteristics	Total	%
Age (years)		
7 years (84-95 months)	221	19.8
8 years (96-107 months)	189	17.0
9 years (108-119 months)	170	15.3
10 years (120-131 months)	180	16.2
11 years (132-143 months)	233	20.9
12 years (144-155 months)	121	10.9
Gender		
Male	596	53.5
Female	518	46.5
Ethnic Group		
Sundanese	948	85.1
Javanese	74	6.6
Others	92	8.3
Body Mass Index		
> 2 SD	57	5.1
+ 1 – +2 SD	80	7.2
-2 – + 1 SD	958	86
-3 – <-2 SD	14	1.3
<-3 SD	5	0.4

Table II demonstrates the average value, range, and ratio of height and arm span for each age group. The ratio of height to arm span in this study was between 0.98-1.01. Height was found to be the same as arm span at the age of 7-10 years for both male and female subjects. At the ages of 11 and 12, arm span was found to be greater than body height (>1 cm), for both male and female subjects.

Table III shows the anthropometric measurements of body height, weight, arm span, and BMI in both male and female subjects. This table also shows no significant differences between male and female measurements ($p>0.05$).

Table IV shows the correlation between height and arm span as well as between height and age. The result showed a significant difference between the variables ($p <0.05$) with a strong correlation ($r>0.7$).

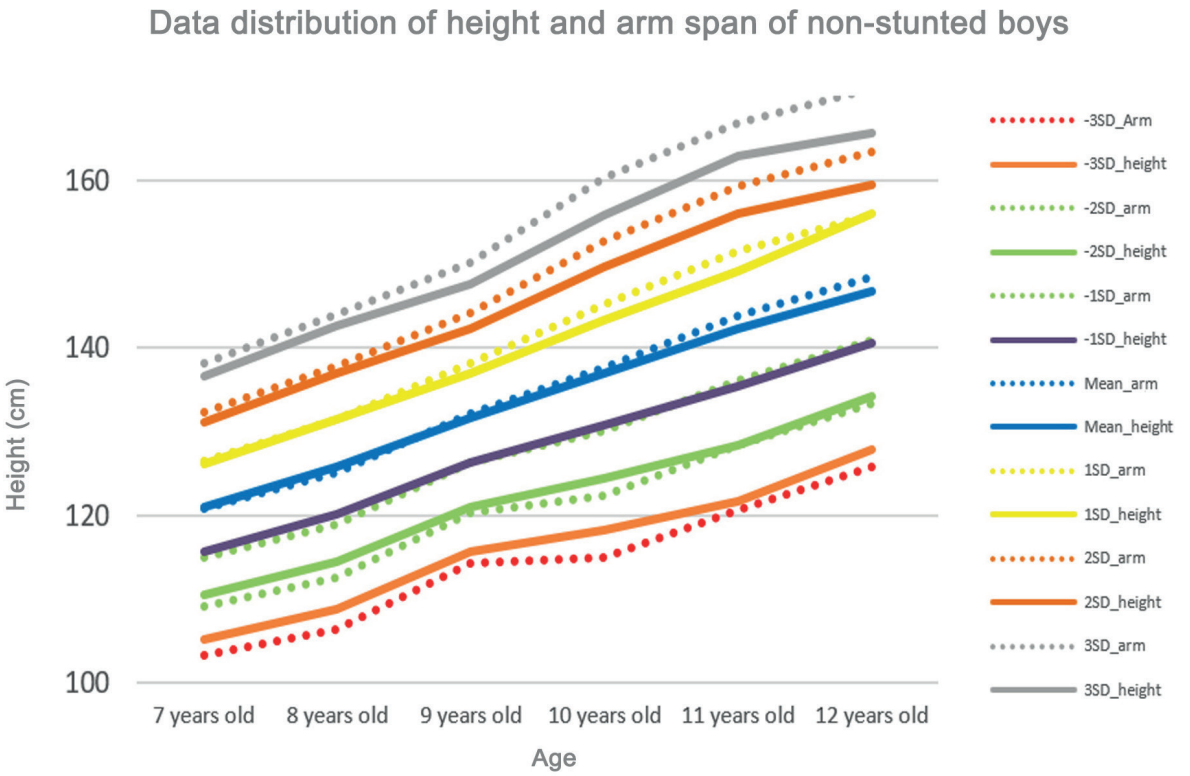

Fig. 2. Height and arm span of male subjects.

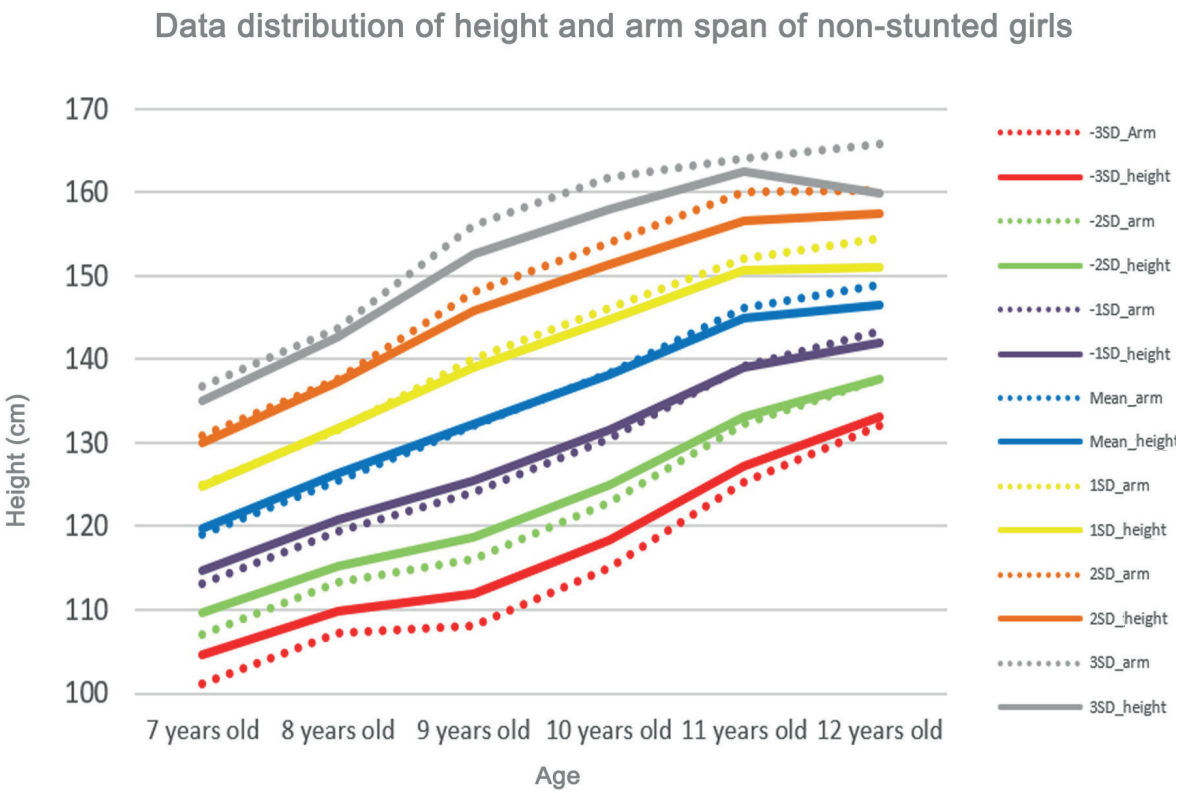

Fig. 3. Height and arm span of female subjects.

Table II. Distribution of height, arm span and height/arm span ratios according to age groups and gender.

Anthropometric Measurement	Child age (year)					
	7	8	9	10	11	12
Male	(n=129)	(n=95)	(n=90)	(n=85)	(n=127)	(n=70)
Height (cm)						
Mean (SD)	120.96 (5.23)	125.76 (5.66)	131.60 (5.34)	137.06 (6.30)	142.29 (6.88)	146.81 (6.31)
Range	111.4-137.8	117.2-147.1	122.70-147.9	125.8-155.8	131.2-161.0	135.0-166.4
Arm span (cm)						
Mean (SD)	120.74 (5.83)	125.20 (6.30)	132.25 (5.97)	137.66 (7.58)	143.79 (7.72)	148.41 (7.54)
Range	108.6-143.0	113.5-146.7	121.0-148.7	124.4-163.9	129.2-168.2	135.3-163.1
Height to arm span ratio	1.00	1.00	1.00	1.00	0.99	0.99
Height to arm span difference (HAD)	0.22	0.56	-0.65	-0.60	-1.50	-1.60
Female	(n=92)	(n=94)	(n=80)	(n=95)	(n=106)	(n=51)
Height (cm)						
Mean (SD)	119.79 (5.07)	126.26 (5.48)	132.21 (6.79)	138.15 (6.60)	144.88 (5.88)	146.49 (4.47)
Range	113.1-135.1	116.3-138.4	121.7-154.1	126.0-154.9	131.2-159.1	137.9-156.9
Arm span (cm)						
Mean (SD)	119.01 (5.95)	125.95 (6.11)	132.05 (7.99)	138.75 (7.81)	146.16 (6.95)	148.92 (5.62)
Range	105.6-135.5	112.7-139.5	119.1-158.9	124.1-158.0	132.4-169.9	137.8-163.4
Height to arm span ratio	1.01	1.00	1.00	1.00	0.99	0.98
Height to arm span difference (HAD)	0.78	0.31	0.16	-0.60	-1.28	-2.43

Table III. Comparison of anthropometric measurements in male and female children.

Anthopometric Measurements	Gender		p value*
	Male (n = 596)	Female (n=518)	
Height (cm)			0.180
Mean (SD)	133.2 (10.9)	134.0 (11.1)	
Range	111.4 – 166.4	113.1 – 159.1	
Weight (kg)			0.583
Mean (SD)	31.44 (10.35)	31.77 (9.85)	
Range	14.9 – 72.0	15.5 – 62.8	
Arm span (cm):			0.385
Mean (SD)	133.76 (12.0)	134.40 (12.46)	
Range	108.6 – 168.2	105.6 – 163.4	
Body Mass Index (kg/m^2)			0.672
Mean (SD)	17.40 (3.92)	17.31 (3.38)	
Range	11.08 – 50.07	11.27 – 31.02	

Table IV. Pearson bivariate analysis of height in correlation with arm span and age.

Characteristics	Height (cm)	
	R coefficient	p-value
Arm span (cm)	0.962	<0.001
Child age (year)	0.850	<0.001

In order to investigate the relationship between several variables (arm span, age, and gender) and height, a backward-type multiple regression analysis was performed. From the analysis, it was found that gender was not a statistically significant variable in predicting height; hence, it was opted out of the formula produced in this study. Table V shows the prediction formula of height based on arm span and age according to gender.

The equation of the male regression model (R^2 = 94%; Root Mean Square Error (RMSE) = 2.852; standard error of estimate (SEE)=2.66):

Height (cm)=21.8623+0.7634 × Arm Span(cm)+0.0791 × Age(month)

The equation of the female regression model (R^2 = 95.4%; RMSE = 2.402; SEE=2.39):

Height (cm)=21.2395+0.7779 × Arm Span(cm)+0.0701 × Age(month)

A concordance correlation test was performed in order to test the accuracy of the height prediction formula produced from this study when compared to the actual height. Table VI shows that the concordance correlation coefficient (CCC) of the male and female formula exhibited a high value of CCC (>0.95), precision (p> 0.95), and accuracy (Cb> 0.95).

Table VII demonstrates the average difference between the predicted height and actual height. It also shows that there was no significant difference between predicted height and actual height in the male and female formula (p>0.05). The differences in average values obtained were -0.003 cm in the male formula and -0.002 cm in the female formula.

Discussion

This study was conducted on 1,114 children aged 7-12 years. For optimum measurements,

Table V. Prediction formula of height based on arm span and age according to gender.

Gender	Independent Variable	Unstandardized Coefficients		t value	p-value
		B	Std. Error		
Male	Constant value	21.8623	1.4121	15.483	<0.001
	Arm span (cm)	0.7634	0.0162	47.173	<0.001
	Age (Month)	0.0791	0.0093	8.532	<0.001
Female	Constant value	21.2395	1.2902	16.463	<0.001
	Arm span (cm)	0.7779	0.0156	49.838	<0.001
	Age (Month)	0.0701	0.0099	7.079	<0.001

Dependent Variable: Height.

Table VI. Concordance correlation value of height prediction formula based on arm span in comparison to actual height.

This Study	N	Concordance correlation coefficient	Pearson ϱ (precision)	Bias correction factor Cb (accuracy)
Male Formula	596	0.9693 (0.9641 – 0.9738)	0.9698	0.9995
Female Formula	518	0.9764 (0.9721 – 0.9801)	0.9767	0.9997

Table VII. Difference between average height based on formula and actual height based on several prediction formula results.

	Average of Predicted Height (cm)	Difference in average (95% CI)	*p* value
Male			
(Actual Height Mean: 133.211 (10.898))			
Actual vs. this study (2020)	133.214 (10.569)	-0.003 (-0.217 – 0.211)	0.978
Female			
(Actual Height Mean: 134.012 (11.136))			
Actual vs. this study (2020)	134.014 (10.877)	-0.002 (-0.208 – 0.204)	0.986

we included only children who, based on their age, are expected to be capable of properly following measurement instructions. In addition, children aged 7-12 years old are elementary school children, and this eased the sample collection process in this study. The total number of subjects analyzed in this study exceeded the calculated minimum sample size as all students of every selected class were included as the subjects of this study. From a statistical point of view, larger samples are known to produce a greater power with a smaller standard error.[19] The average height and arm span of male subjects were found to be lower than those of female subjects even though no significant difference was found in this study ($p>0.05$). This was probably caused by earlier puberty in girls compared to boys.[20]

Several factors were found to affect height, including age and arm span. Strong correlations were found between height and arm span ($r=0.962$) and between age and arm span ($r=0.850$). The results of this study were in line with the study by Forman et al.[5], which reported a strong correlation between height and arm span ($r=0.97$), as well as the study by Maziciouglu et al.[10] ($r=0.95$).

Height was found to be the same as arm span in male subjects aged 7-10 years, but the arm span was found to exceed the height for the other age groups with a maximum difference of 1.6 cm. Whereas in female subjects, height was found to exceed the arm span at the age of 7 years, equal to arm span at age 8 to 10 years, but subsequently, arm span was found to exceed height with maximum difference of 2.43 cm. This result is different from the study by Zverev et al.[21] in Malawi, in which arm span was found to exceed height in all age groups between 6-15 years. The difference between arm span and height was about 2.9 cm in boys aged 6 years and 10.5 cm in boys aged 15 years. Whereas in girls, the difference was 10.3 cm at younger ages and 7.7 cm at the ages of 14 and 15 years. Turan et al.[22] in Turkey reported that the difference between arm span and height was about 2 cm in girls aged 4 years and later became the same at the age of 9 years. Whereas in boys, the difference was about -1.1 cm and gradually increased by about 2 cm after puberty.

The ratio of height to arm span in this study ranged between 0.98-1.01 in all age groups. The CDC reported that the height-to-arm-span ratio is 1:1 with an accurate measurement and normal growth.[14] Lee et al.[13] in Taipei found the results of the height-and-arm-span ratio varied from 0.98-1.03 in boys and from 0.99-1.03 in girls. A study by Alam et al.[23] in Uttar Pradesh, India demonstrated that the height-to-arm-span ratio varied from 0.98-0.99. The possible reason regarding the difference found in the height-to-arm span ratio and the differences among the studies is that the above studies were conducted locally with many different ethnicities, which can affect the children's growth. Several factors known to affect growth include genetic, hormonal and environmental factors (nutritional factors, physical activity, family factors, psychological factors).[14,22] This

reasoning is also in line with the study by Quanjer et al.[17], which found that the height-to-arm-span ratio might vary based on gender, age and race.

The results of this study do not indicate that the arm span ratio is equal to height as 1:1, thus arm span should not be used as a direct substitute for height. A study by Chhabra[24] compared the three estimates of height obtained from arm span: (1) by direct substitution using arm span, (2) the ratio of mean arm span-to-standing height, and (3) the regression equations. The study found that the error rates of ratios and regression equations were smaller (5-6%) than those of direct substitution (23.7%). Aggarwal et al.[25] also showed that the error rate was higher when the arm span was used as a direct substitute to height. The use of arm span directly to substitute height was found to produce an error of 16% compared to the use of the ratio (14%). The use of arm span in a prediction equation to calculate height leads to a smaller error rate compared to direct substitution. Error rates were found to be even smaller if height is predicted based on arm span and age.[17] A study by Mishra et al.[26] also showed that the regression equation provides a better estimation of height compared to the use of height-to-arm-span ratio.

The resulting regression equations, both in male and female subjects, provide a very high coefficient of determination (R^2> 94%) and a low SEE value (SEE=2.53). Zverev et al.[21] studied 626 children aged 6-15 years in Malawi and found the R^2=0.988, SEE=0.76. Mazicioglu et al.[10] in Turkey studied 5,358 children aged 6-17 years and found a correlation of R = 0.8310. Meanwhile, Mishra et al.[26] studied 1,465 children aged 6-11 years in India and found R^2=0.91 and SEE=2,96. The aforementioned studies showed a strong correlation between height and arm span, as well as a low SEE value. By conducting a correlation test comparing the actual height and the predictive height obtained from various existing regression equations, it was found that by using the regression equation obtained in this study, there was no significant difference between the predicted height and actual height (p> 0.05). The result of this study also showed that the average differences between the actual height and predicted height were of low values, which were -0.003 for male's height and -0.002 for female's height. The result of this study was also tested by using concordance correlation to see the level of accuracy and precision of the prediction formula on the actual height. The results obtained consecutively in male and female subjects were CCC=0.9693, p=0.9698 and Cb=0.9995 and CCC=0.9764, p=0.9767, Cb=0.9997, respectively. This might be due to the use of the "month" as the measurement of age. Age as an additional variable would strengthen the correlation between height and arm span. The regression equation will be specific for a certain age group and ethnicity.[16]

Studies in Indonesia are still limited. This study included a large number of subjects, 1,114 in total. This study also used "month" as the measurement of age, thus the results are expected to be more accurate. The limitation of the study is that the results of the study could not cover all ages, only for children aged 7-12 years. Therefore, the existing formula can not be used to estimate the alternative height in children aged younger than 7 years or older than 12 years. In addition, we did not asses the status of puberty of the subjects in this study.

To conclude, arm span and height are strongly correlated. Arm span can predict the actual height of children aged 7-12 years; thus, it can be used as an alternative measurement for growth.

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Ethical approval

This study has been approved by the Research Ethics Committee of the Faculty of Medicine Universitas Padjadjaran (approval number 1171/UN6.KEP/EC/2019).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: RR; data collection: RR, FR; analysis and interpretation of results: RR; draft manuscript preparation: RR, EF, MD, KR, RT. 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

Body image dissatisfaction among school children in Turkey and its potential effect on body esteem

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ABSTRACT

Background. This study aimed to validate the Turkish version of Collins' Body Figure Perceptions and Preferences (BFPP) scale. The second aim of this study was to investigate the relationship between body image dissatisfaction (BID) and body esteem (BE), and between body mass index (BMI) and BID, among Turkish children.

Methods. A descriptive cross-sectional study was conducted among 2066 4th grade children (mean age was 10.06 ± 0.37 years) in Ankara, Turkey. The Feel-Ideal Difference (FID) index from Collins' BFPP was used to assess the degree of BID. FID ranges from -6 to +6, with scores below or above 0 indicating BID. Collins' BFPP's test-retest reliability was evaluated in a subset of 641 children. The Turkish version of the BE Scale for Adolescents and Adults was used to evaluate the children' BE.

Results. More than half of the children were dissatisfied with their own body images (57.8% of girls vs. 42.2% of boys, $p<.05$). The lowest BE score in both genders was among adolescents who desired to be thinner ($p<.01$). The criterion-related validity of Collins' BFPP, in relation to BMI and weight, was at an acceptable level in girls (BMI rho= 0.69, weight rho= 0.66) and boys (BMI rho= 0.58, weight rho= 0.57), and was statistically significant in all cases ($p<.01$). The test-retest reliability coefficients of Collins' BFPP were found to be moderately high for both girls (rho=0.72) and boys (rho=0.70).

Conclusions. Collins' BFPP scale is a reliable and valid tool for Turkish children aged 9-11 years. This study demonstrates that more Turkish girls than boys were dissatisfied with their bodies. Children who were affected by overweight/obesity and underweight had a higher BID than those with a normal weight. It is important to evaluate adolescents' BE and BID in addition to their anthropometric measurements during their regular clinical follow-up.

Key words: children, adolescent, body image dissatisfaction, body esteem.

Body image was first defined as "the picture of our own body which we form in our minds, that is to say the way the body appears to ourselves" by German writer Schilder.[1,2]

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Slade[2,3] expanded the definition of body image as "the picture we have in our minds of the size, shape and form of our bodies; and our feelings concerning these characteristics and our constituent body parts." There are many different survey instruments for the evaluation of body image.[4] The most commonly used in pediatric research was developed by Collins[5,6], which consits of a figural drawing scale with 7 body figure charts to depict weight ranging

from very thin to obese for preadolescent girls and boys. Figural drawing scales have several advantages. These scales are suitable and understandable for children of different ages and can be answered easily and quickly by children.[7]

The description of body image consists of two main components: a perceptual component and an attitudinal component.[2,3] Body image dissatisfaction (BID) is the negative subjective evaluation of one's body shape (perceptual), whereas body esteem (BE) is characterized by feelings about how one looks or about one's overall appearance (attitudinal).[8,9] Research has shown that age and gender are the strongest correlates of BID and BE. Negative BID is widespread among girls; they generally want to be thinner than they are, even if they have a normal weight. Boys, on the other hand, are more likely to want to be heavier and more muscular. Girls who want to be thinner cut down on food intake and avoid sweets and fatty foods. Boys may also cut out sweets and fatty foods but eat more healthy foods and are likely to do more exercise. Besides commonly recognized social pressures around ideal body types, early maturation can also cause BID, especially in girls.[10] BID may contribute to depression, low self-esteem, obesity and eating disorders such as anorexia or bulimia.[11-17]

According to limited studies conducted in Turkey, BID has been seen as an important problem in adolescence. For example, Arslan et al.[18] found, among high school students in Istanbul, the percentage of BID was 46.8% overall. Most concerningly, while the prevalence of BID was 29.8% in 9th grade, this percentage increased to 78.9% in 12th grade. Elsewhere, studies have also shown that females have greater BID than males in Turkey.[19,20] There are also studies that have shown the relationship between BID and self-esteem in the early adolescent age group.[21-23] Tok and Gedik suggested the lack of validated measurements in Turkish is a significant constraint in this area of research in Turkey.[23]

This study has three objectives. The first objective is to establish the validity and test-retest reliability of the Turkish version of Collins' Body Figure Perceptions and Preferences (BFPP) scale in order to contribute to this vital area of study. The second aim was to investigate the association between body mass index (BMI) and BID, and the last aim was to evaluate the association between BE and BID.

Material and Methods

Participants and data collection

Approval from the Provincial Directorate of the Ministry of National Education was obtained to conduct the study in the selected schools. In addition, ethical approval was obtained from the Noninterventional Clinical Research Ethics Board at Hacettepe University, Turkey (GO 14/429-07). Each participating school in the study sent relevant information to parents. The consent of parents and the assent of children were obtained before data collection.

A cross-sectional, descriptive study was performed. This study was part of the Child Obesity Study of Ankara (COSA), which aimed to investigate the prevalence of obesity and related factors in Turkey. Ankara was stratified according to 3 socio-economic status (SES) levels using the criteria mentioned by Yüceşahin and Tuysuz.[24] The study was carried out in 46 schools in Ankara during the 2014-2015 school year. The schools were selected from each SES stratum using probability proportional-to-size methodology. The sample included 2066 4th grade students aged 9-11 years. Further details of the sampling design of the COSA study can be found in the article by Yardim et al.[25] A subsample of 641 children was used in the test-retest study.

Measures

Anthropometric measures

Child anthropometric measurements, including weight and height, were taken by trained

nursing school students using a Seca 813 weight scale and Seca 213 height board. Children's weight status was estimated using World Health Organization (WHO) cutoff points.[26] Based on BMI-for-age z-scores, underweight was defined as < -2, normal weight between ≥ -2 and ≤ +1, overweight > +1 and ≤ +2, and obesity > +2 standard deviation units.[27]

Body Image Dissatisfaction (BID) and Feel-Ideal Difference Index (FID)

Collins' BFPP scale[5] was used to assess BID. The scale includes two sets of 7 body figures for boys or girls, ranging from very thin to obese. The children were asked to mark the figure that most resembled their perceptions of how they looked in the first set of figures (Fig. 1). Children were then asked to choose their ideal body, i.e. the body they most wanted to resemble (their ideal figure), from the second set of figures chart (Fig. 2). The original study found good test-retest reliability in this instrument; the actual figure showed r = 0.71 and the ideal figure showed r =0.59.[5] Collins used weight and BMI for evaluation of criterion-related validity of the actual body figure chart. The criterion-related validity coefficients of the actual pictorial with weight (0.36, $p<0.05$) and with BMI (0.37, $p<0.05$) were found to have modest statistical significance.[5,28]

The FID index represents the difference between the actual and ideal figures and indicates the degree of body dissatisfaction.[29-31] For example, if the score of the perceived self-figure is 3 and the score of the ideal is 1, then the FID index of the child is 2. A larger positive score indicates the child wishes to be thinner, and a larger negative score indicates the child desires to be larger. The range of the FID index can be between -6 and +6. An FID index score equaling 0 shows there is no BID. FID index values were grouped into three categories: children wanting to be thinner than their actual body image (FID index >0), children wanting to be larger than actual body image (FID index <0), and children satisfied with their body image (FID index=0).

Body Esteem (BE) scale

Mendelson et al.[21] defined BE as the "self-evaluation and self-esteem of one's physical appearance." The BE Scale for Adolescents and Adults (BESAA) consists of three dimensions: appearance ("feelings about one's general appearance", Cronbach's α = 0.92), weight ("feelings about one's weight" Cronbach's α = 0.94), and attribution ("evaluations attributed to others about one's body and appearance" Cronbach's α = 0.81). Children's responded to the survey on a 5-point Likert scale, ranging from 0 (never) to 4 (always).[21] The validity and reliability of the Turkish version of the BESAA Scale for children was previously established by our team.[32] In that study, Cronbach's α for the weight, appearance, attribution subscales and the total scale were 0.85, 0.76, 0.69, and 0.85. Test-retest reliability showed r=0.68, 0.68, and 0.57 for the three subscales, respectively.[32]

Statistical analysis

Arithmetic mean, standard deviation, median, and minimum-maximum values were given as descriptive statistics for quantitative data. Criterion-related validity can be assessed by calculating the degree of correlation between the test score and a known standard criterion.[33] The actual weight and BMI were accepted as standard criteria. Spearman's rho correlation coefficient between pictorial figure selections and actual weight and BMI was used for evaluation of criterion-related validity of Collins' BFPP scale. Spearman's rho correlation coefficient was used for the assessment of test-retest reliability. Pearson χ^2 test was used to compare BMI groups in terms of the three FID index groups described above.[29-31] Pairwise comparisons between BMI groups in the each FID index group were examined by using Z-test with the Bonferroni correction. Since there were four levels of BMI groups and six pairwise comparisons, Bonferroni adjustment was applied for the significance level (adjusted significance value = 0.0083). The Shapiro-Wilk and Kolmogorov-Smirnov tests were used to determine the normality of BE

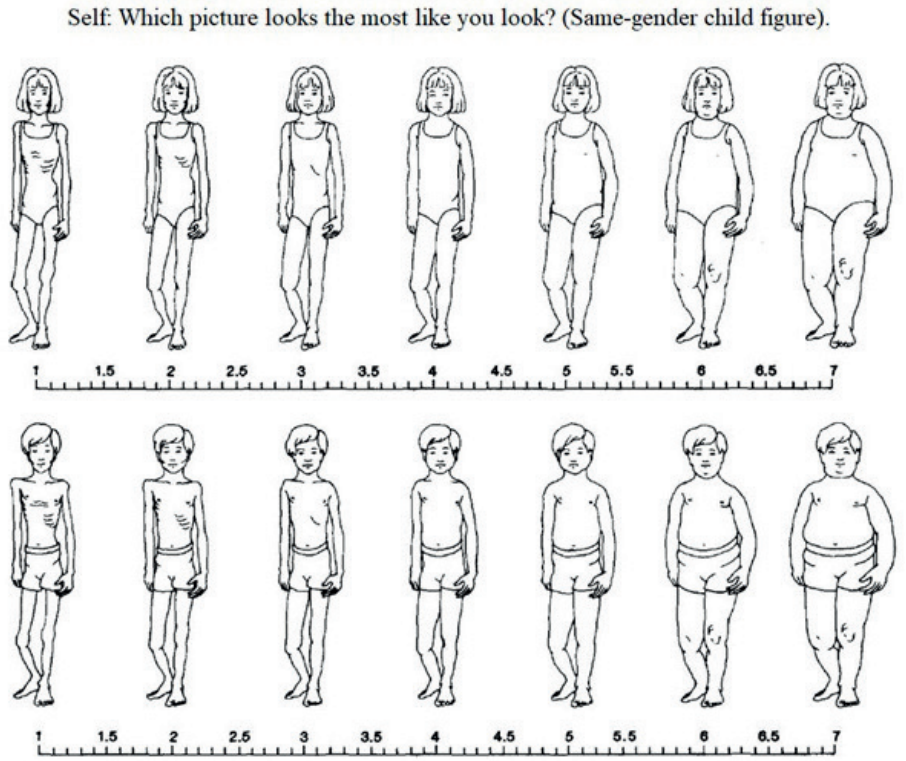

Fig. 1. Collins' instrument for BFPP scale for actual body size.

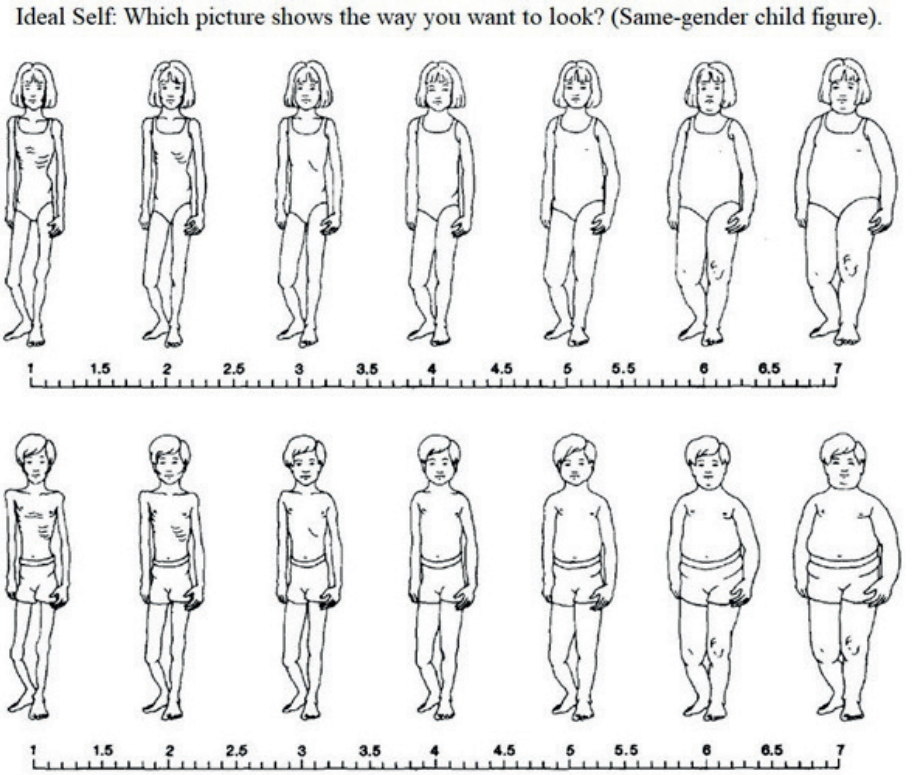

Fig. 2. Collins' instrument for BFPP scale for ideal body size.

scores. Because BE scores were not normally distributed, the Kruskal-Wallis test was used to compare BE scores among FID groups. Pairwise comparisons were performed using Dunn's test. The Mann Whitney-U test was used to determine differences between girls and boys in terms of the BE scores in each FID index group. The independent samples t-test was used to compare two proportions. The Statistical Package for the Social Sciences (SPSS) v.23.0 (Chicago, IL) was used for data analysis. A p value of less than 0.05 was considered to indicate a statistically significant difference.

Results

The study was conducted in 46 schools (15 schools from a low SES county, 17 from a medium SES county, and 14 from a high SES county). The full sample of children in this study (n = 2066) included 1100 (53.2%) children in low SES, 715 (34.6%) in medium SES, and 251 (12.2%) in high SES categories. Out of 2066 children, 53.1% were girls and 46.9% were boys. The mean age was 10.06 years (±0.37). Of the total sample, 2.2%, 59.1%, 20.2%, and 13.9% were of underweight, normal, overweight, and obese status, respectively, based on WHO BMI cutoff points.[26,27] The mean weight, height, and BMI were 35.9 kg (±8.7 kg) , 139.3cm (±6.5 cm), and 18 kg/m^2 (±3.5 kg/m^2) respectively.

Test-retest reliability and criterion-related validity of Collins' BFPP in Turkish children

A total number of 641 children aged 9-11 years, (296 girls, mean age was 9.65 years (±0.54); and 345 boys, mean age was 9.56 years (±0.59)) were included in the subsample for the test-retest study of Collins' BFPP scale. Among the sample, 243 (38.0%) children were from low SES, 205 (32.0%) from middle SES, and 193 (30.0%) from high SES regions.

The results of test-retest reliability and criterion-related validity for the Collins' BFPP scale are presented in Table I. The test-retest reliability coefficients of perceived self-figure were found to be moderately high for girls (rho = 0.72) and boys (rho = 0.70). Test-retest reliability coefficients of ideal self-figure were found to be moderate for girls (rho = 0.60) and boys (rho = 0.55). These test-retest associations were all significant (p<.01) The criterion-related validity analyses were conducted within the full sample. In both girls (BMI rho= 0.69, weight rho= 0.66) and boys the criterion-related validity of self-figure in relation to BMI and weight was acceptable and statistically significant (p<.01).

BID and BMI

Table II shows the distribution of FID by gender and BMI. Gender was significantly associated with BID (p<.05). Approximately 63.0% of girls and 53.0% of boys were dissatisfied with their own body image; 45.5% percent of girls wanted to be thinner and 17.1% wanted to be larger, whereas 30.7% of boys wanted to be thinner and 21.9% wanted to be larger. There was a statistically significant difference between boys (33.1%) and girls (24.2%) wanting to be larger in the normal BMI groups (p<.01).

A significant association was also found between BMI and FID index (p<.05). Children with an overweight or obese status were

Table I. Test-retest reliability and criterion-related validity of self-figure and ideal self-figure.

Figures	Test-retest Reliability Coefficient	Validity Coefficient with BMI	Validity Coefficient with Body Weight
Girl self-figure	0.72	0.69	0.66
Girl ideal self-figure	0.60	-	-
Boy self-figure	0.70	0.58	0.57
Boy ideal self-figure	0.55	-	-

BMI: Body mass index.
p<.01 for all values.

Table II. FID index groups by gender and BMI status.

	Gender						
	Girls		Boys		Total		p
FID index groups	n	%	n	%	n	%	
Want to be thinner	492	45.5	289	30.7	781	38.6	
Want to be the same	404	37.4	446	47.4	850	42.0	p<0.05
Want to be larger	185	17.1	206	21.9	391	19.3	
Total	1081	100.0	941	100.0	2022	100.0	

	Boys' BMI status										
	Underweight		Normal		Overweight		Obese		Total		
FID Index Groups	n	%	n	%	n	%	n	%	n	%	p
Want to be thinner	1	4.3[a]	75	14.3[a]	78	43.8[b]	115	68.0[c]	269	0.30	
Want to be the same	13	56.5[a,b]	275	52.6[b]	88	49.4[b]	50	29.6[a]	426	0.48	p<0.05
Want to be larger	9	39.1[a]	173	33.1[a]	12	6.7[b]	4	2.4[b]	198	0.22	
Total	23	100.0	523	100.0	178	0	169	100.0	893		

	Girls' BMI status										
	Underweight		Normal		Overweight		Obese		Total		
FID Index Groups	n	%	n	%	n	%	n	%	n	%	p
Want to be thinner	0	0.0[a]	196	29.1[b]	177	75.0[c]	105	94.6[d]	478	0.46	
Want to be the same	9	45.0[a,b]	315	46.7[b]	57	24.2[a]	6	5.4[c]	387	0.37	p<0.05
Want to be larger	11	55.0[a]	163	24.2[b]	2	0.8[c]	0	0.0[c]	176	0.17	
Total	20	100.0	674	100.0	236	100.0	111	100.0	1041	100.0	

BMI: body mass index, FID: feel-ideal difference.
BMI groups with different superscript in the same FID index group statistically significant differences via post-hoc Z test with Bonferroni correction.
A total of 132 (6.3%) of the children did not answer all the questions of the scales used in this study.

more dissatisfied with their own body image compared to those in the underweight and normal weight groups. Results showed that 94.6% of girls of obese status wanted to decrease their body size (75.0% of girls of overweight status). Among boys, 68.0% of those affected by obesity wanted to decrease their body size (43.8% of boys of were of overweight status). Although 33.1% of boys wanted to be larger in the normal BMI group, 24.2% of girls reported the same thing in the normal BMI group.

BID and BE

Table III shows the BE scores based on the 3 subscales (weight, appearance, attribution) and the overall scale (total score) by FID index groups. Kruskall-Wallis test results indicated that BID had a significant effect on all BE scores using the subscales and overall scale (p<.01) for both gender. According to the results of a post-hoc Dunn's test with Bonferroni correction, children with BID had lower BE scores in comparison with the "Want to be the same" group (p=.017). Among both girls and boys, the "Want to be the same" group had the highest overall BE scores.

There were some gender differences noted. In the "Want to be the same" FID group, all of the BE scales showed significantly higher scores in girls than in boys (p<.01). Similarly, in the "Want to be larger" FID group, with the exception of weight subscale score, all other BE scores of girls were significantly higher than those of boys (p<.01). In contrast, in the "Want to be thinner" FID group, only the weight subscale score was statistically different by gender; weight subscale scores for girls were lower than for boys (p<.01).

Table III. BE scores by FID index by gender.

Gender	BE Subscales	FID Index Groups	n	Mean (SD)	Median	Min-Max	p[a]
Girls	Weight	Want to be thinner[b,c]	452	10.9 (6.0)	11.0	0-20	<.01
		Want to be the same	375	15.5 (4.6)	17.0	0-20	
		Want to be larger[b]	173	13.8 (5.0)	14.0	0-20	
	Appearance	Want to be thinner[b,c]	453	14.2 (6.2)	15.0	0-24	<.01
		Want to be the same	373	18.3 (5.6)	20.0	0-24	
		Want to be larger	174	18.3 (5.1)	20.0	0-24	
	Attribution	Want to be thinner[b,c]	454	9.3 (3.7)	9.0	0-16	<.01
		Want to be the same	379	11.1 (3.5)	11.6	0-16	
		Want to be larger[b]	172	10.3 (3.7)	10.0	2-16	
	Total	Want to be thinner[b,c]	398	34.6 (12.5)	35.0	4-60	<.01
		Want to be the same	340	45.3 (10.2)	47.0	15-60	
		Want to be larger[b]	151	42.1 (9.7)	43.0	7-60	
Boys	Weight	Want to be thinner[b]	269	11.7 (6.1)	11.0	0-20	<.01
		Want to be the same	424	14.6 (5.3)	16.0	0-20	
		Want to be larger[b]	189	12.9 (5.4)	13.0	0-20	
	Appearance	Want to be thinner[b,c]	265	13.6 (6.7)	14.0	0-24	<.01
		Want to be the same	404	16.8 (6.0)	18.0	0-24	
		Want to be larger	185	15.8 (6.0)	16.0	0-24	
	Attribution	Want to be thinner[b]	265	9.1 (3.9)	9.0	0-16	<.01
		Want to be the same	423	10.3 (4.0)	11.0	0-16	
		Want to be larger	194	9.3 (4.2)	9.0	0-16	
	Total	Want to be thinner[b,c]	236	34.1 (12.8)	35.0	0-60	<.01
		Want to be the same	373	42.2 (10.6)	44.0	13-60	
		Want to be larger[b]	169	38.8 (9.6)	37.0	9-60	

BE: body esteem.
[a]: Kruskall-Wallis analysis of variance test for 3 groups.
[b]: Statistically significant according to Bonferroni correction (p =.017) compared with the "Want to be the same" group.
[c]: Statistically significant according to Bonferroni correction (p =.017) compared with the "Want to be larger" group.

Discussion

In this study, more than half of the adolescents aged 9-11 were dissatisfied with their own body image. We found that approximately 46% of girls and approximately 30.0% of boys wanted to be thinner. Our study demonstrated that as BMI increased, body dissatisfaction increased in both genders. The percentage of body dissatisfaction among girls affected by overweight, or obesity was higher than that of their male counterparts.

Our results support recent studies that have shown that body dissatisfaction is a common problem among early adolescents[34,35], and that girls are more dissatisfied with their bodies than boys.[5,12,28,36-38] We found that there is a relationship between BMI and BID among our participants who have rapid physical growth and psychological development. Body image and BE are developed in early adolescence. In addition, our finding showing that adolescents with obesity experience greater dissatisfaction with their bodies is also consistent with prior research.[39,40]

We examined BE scores for various body figure dissatisfaction groups. Our findings showed that in both girls and boys, the lowest scores of

BE occurred in the "Want to be thinner" group, whereas those without body dissatisfaction had the highest overall BE. Studies show that low BE is associated with restraint and emotional eating, emotional disturbances, as well as other psychological disorders.[9,41-44] The results of the present study indicate that both "Want to be thinner" and "Want to be larger" are risk factors of low BE.

Weight-related stigmatization is a widespread problem for children with obesity.[45-47] These children are frequently perceived as lazy, unhealthy, unattractive, and inactive by their peers.[47] Obesity-related stigmatization is associated with low self-esteem, depression, and body dissatisfaction.[2] Jendrzyca and Warschburger[46] found that weight status, body dissatisfaction and restrained eating directly influenced disordered eating in both genders aged 6-11 years. They also found that among girls who experienced weight stigma and exhibited eating disorder behaviors, 43.5% were affected by obesity; among boys, this percentage was 33.3%.

Television and other media can affect both the perception of body image and the ideal body image of children. Many theorists have opined that the media may play a central role in creating and exacerbating body dissatisfaction.[48] Ata et al.[49] found that females tend to report greater pressures from the media than males. According to Ricciardelli and McCabe[50], girls show a greater desire to be thin and are more likely to diet, while boys are more likely to be concerned with muscularity and a desire to increase the strength and size of their muscles. We found similar results in the current research, where a higher percentage of girls than boys wanted to be thin while a higher percentage of boys than girls wanted to increase their body size. That said, our study did not specifically examine the issue of muscularity, only body size. Future research should explore this aspect further among Turkish boys.

Our findings show that the Turkish version of Collins' BFPP scale has sufficient validity and reliability for early adolescents. Figural drawing scales for evaluating body perceptions and preferences provide several advantages, including ease of administration in group settings and ease of understanding by children.[7,28,51,52] It has been discovered that body dissatisfaction is related to lower BE scores, indicating that the relationship between body dissatisfaction and BE is a growing public health issue in our country.

Our study has several strengths, including the relatively large sample of early adolescents and measured anthropometry. The study was designed to provide the ability to examine Collins' BFPP test-retest reliability and its validity against both BMI and BE as key criteria, thus making a significant contribution to the literature on the Turkish pediatric population. A limitation of the study is that it only included early adolescents living in Ankara; thus, findings may not generalize to all children in Turkey, especially those in rural parts of the country. We also did not collect specific data on eating disorders, the internalization of masculinity or mental health. Future research is warranted to investigate these additional dimensions in relation to BID.

This research shows that Collins' BFPP scale is reliable and valid for use in Turkish children aged 9-11 years, at least in large metropolitan areas such as Ankara. We found that girls were more vulnerable in terms of BID than boys. Children who are overweight/obese and underweight have higher levels of body dissatisfaction than adolescents with normal BMI. In light of the known negative health consequences of BID, BID may be an important risk factor to assess in pediatric settings beyond BMI. This study brings additional awareness of the importance of BID to researchers and clinicians in Turkey and should simulate further investigation using Collins' BFPP in child health research in Turkey.

Ethical approval

Approval from the Provincial Directorate of the Ministry of National Education was obtained to conduct the study in the selected schools. In addition, ethical approval was obtained from the Noninterventional Clinical Research Ethics Board at Hacettepe University in Ankara, Turkey (GO 14/429-07).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: UEA, HÖ; data collection: UEA, HÖ, SÜ, MSY, HKÜ; analysis and interpretation of results: UEA, HÖ; draft manuscript preparation: UEA, HÖ, ÖA, TTKH. 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

Comparison of urine bisphenol A levels in transient tachypnea of the newborn and healthy newborns

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ABSTRACT

Background. To investigate the relationship between neonatal urine bisphenol A (BPA) levels and the prevalence and prognosis of transient tachypnea of the newborn (TTN).

Methods. This prospective study was conducted between January and April 2020 in the Neonatal Intensive Care Unit (NICU) of Gaziantep Cengiz Gökçek Obstetrics and Pediatric Hospital. The study group consisted of patients diagnosed with TTN and the control group was made up of healthy neonates housed together with their mothers. Urine samples were collected from the neonates within the first 6 hours postnatally.

Results. Urine BPA levels and urine BPA/creatinine levels were statistically higher in the TTN group ($P < 0.005$). The receiver operating characteristic (ROC) curve analysis determined the cut-off value of urine BPA for TTN to be 1.18 μg/L (95% confidence interval [CI]: 0.667-0.889, sensitivity: 78.1%, and specificity: 51.5%) and the cut-off value of urine BPA/creatinine to be 2.65 μg/g (95% CI: 0.727–0.930, sensitivity: 84.4%, and specificity: 66.7%). Furthermore, the ROC analysis indicated that the cut-off value of BPA for neonates requiring invasive respiratory support was 15.64 μg/L (95% CI: 0.568–1.000, sensitivity: 83.3%, and specificity: 96.2%) and the cut-off value for BPA/creatinine was 19.10 μg/g (95% CI: 0.777–1.000, sensitivity: 83.3%, and specificity: 84.6%) among the TTN patients.

Conclusions. BPA and BPA/creatinine values were higher in the urine of newborns diagnosed with TTN which is a fairly common cause of NICU hospitalization, in samples collected within the first 6 hours after birth, which may be a reflection of intrauterine factors.

Key words: Bisphenol A, urine, newborn, transient tachypnea of the newborn, ventilation.

Bisphenol A (BPA) is an endocrine-disrupting monomer, that was developed as a synthetic estrogen.[1,2] BPA is found in many products, including plastics, food packaging, toothpaste, and thermal receipts.[3] People are exposed to BPA through their diet, inhalation, or dermal exposure.[4]

In addition to its serious side effects on the endocrine system, recent studies have begun to focus on the effects of gestational BPA exposure on neonatal outcomes.[5,6] The negative effects of intrauterine BPA exposure on lung development were demonstrated in a mouse model study.[7] In addition, cell culture studies have shown that BPA negatively affects the lung development via the ERß/NF-kB/GR signaling pathway and that this pathway affects lung alveolar epithelium via the sodium ion channel.[8] Studies have concluded that BPA inhibits epithelial sodium channel (ENaC) expression.[7-9]

A different study on intrauterine exposure found that neonatal urine BPA levels were correlated with BPA levels in maternal serum, breast milk, the placenta, and the umbilical cord.[5]

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Transient tachypnea of the newborn (TTN) is a condition that requires minimal intervention and spontaneously regresses within 24–72 h. Although the actual prevalence is unknown, it is estimated that 0.33%–0.50% of neonates have transient tachypnea at birth. The major risk factors are cesarean delivery, macrosomia, maternal diabetes, maternal asthma, multiple pregnancies, being male, and preterm, early term, and post term birth.[10,11]

At birth, the ENaCs located on the apical surface of the pulmonary epithelium open and absorb Cl and Na, thus absorbing pulmonary fluid. One of the known causes of TTN physiopathology is the decreased activity or immaturity of lung ENaCs.[11]

With reference to these studies, the current study was designed to investigate the association between newborn urine BPA levels and the presence and prognosis of TTN. The study aimed to investigate the relationship between urinary BPA levels and the incidence of TTN by comparing the BPA levels in urine samples of neonates with TTN with those of healthy neonates collected within the first 6 hours postnatally.

Material and Methods

Patients

Study group

This prospective study included patients with TTN who were hospitalized in the Neonatal Intensive Care Unit (NICU) of Gaziantep Cengiz Gökçek Obstetrics and Pediatric Hospital, between January and April 2020. After obtaining written consent from their parents, a single urine sample was collected from neonates who had been diagnosed with TTN and admitted to the NICU. Urine samples were collected within the first 6 hours postnatally. The demographic and neonatal data of the patients were recorded. The inclusion criterion was being hospitalized in the neonatal ICU with TTN. The exclusion criteria were as follows: acute respiratory distress due to any reason other than TTN (congenital pneumonia, neonatal pneumonia, apnea, congenital diaphragmatic hernia, pulmonary hypertension, etc.), congenital heart disease, chromosomal or metabolic diseases, requiring resuscitation in the delivery room, and the parents not giving written consent. The mothers of the patients did not have any diseases. There were no patients receiving antibiotic treatment.

Statistical power analysis revealed that there needed to be at least 26 patients in each group.

Within the 4 months that the study was scheduled to take place, there were 62 patients who conformed to the inclusion criteria and whose parents gave consent for participation. Among these, 2 patients were excluded from the study because they were determined to have congenital heart disease, and the family of 1 patient opted out of participation. Twelve patients were excluded from the study due to not being able to produce enough urine within the first 6 hours postnatally. Moreover, 11 patients who were diagnosed with congenital pneumonia and 4 patients who were diagnosed with early neonatal sepsis were also excluded from the study. The remaining 32 patients were included in the study.

Control group

The control group consisted of neonates born to healthy mothers within the same period of time whose postnatal examination was normal and who were housed together with their mothers (during the 24-h postnatal observation), and who had no health problems during the follow-up. After obtaining written consent from the families, a single urine sample was collected within the first 6 hours postnatally. The demographic and neonatal data of the subjects were recorded.

Due to the high birth rate in this hospital, patients from only 1 day of the week were included as controls. Urine samples were collected from neonates that were born on the second day of each week (Tuesday) during the

determined time period to healthy mothers, who were healthy themselves, and whose parents signed informed consent forms. During this time, the parents of 89 patients agreed to participation in the study. Of the patients, 48 failed to produce an adequate amount of urine. In addition, several patients were excluded due to the following reasons: 3 patients developed early neonatal sepsis, 3 patients developed indirect hyperbilirubinemia, 1 patient was not feeding properly, and 1 patient was admitted to the NICU with intestinal obstruction. Hence, 33 patients were included in the control group.

The study was granted ethical approval by the Ethics Committee of Gaziantep University Şahinbey Training and Research Hospital (No:2019/476).

Definition of transient tachypnea of the newborn

The duration of respiratory distress (tachypnea, nasal flaring, subcostal retraction, grunting) is the main determinant for the diagnosis of TTN.[12] TTN is a diagnosis of exclusion. A neonate with respiratory distress should be considered to have TTN after other potential causes have been ruled out. Tests that are used to rule out diagnoses other than TTN include preductal and postductal oxygen saturation, complete blood count (to exclude sepsis), blood culture, C-reactive protein, procalcitonin, blood gases, lactate, and posteroanterior lung radiography.[13] Since TTN is a benign and self-limiting condition, it does not require antibiotic treatment.[14]

In lung radiography, the presence of hyperinflation of pulmonary fissures, prominent perihilar vasculature, and interlobar septa or fluid support the TTN diagnosis.[13,15] If the tachypnea does not improve within 72 hours, TTN diagnosis is ruled out.[11]

Urine collection

Völkel et al.[16] reported that the urinary excretion half-life of BPA may be less than 6 hours, while Pottenger et al.[17] stated that this duration may range from 18 to 72 h, depending on the gender and dosage. In addition, Iribarne-Durán et al.[18] reported that neonates might be exposed to multiple sources of BPA and parabens in NICUs via inhalation, dermal, oral, and intravenous (IV)/parenteral routes. As a result I collected the urine samples from neonates in the first 6 hours postnally.

After obtaining consent, urine samples were collected by placing cotton balls in the diapers of the neonates, and urine was squeezed out of the cotton balls into a glass sample container using nitrile gloves (the cotton balls were not contaminated with feces). After being transferred into glass containers, the urine samples were stored at –20 °C until they were transported to the laboratory. The stored urine samples were transferred collectively on dry ice.

The study aimed to study the BPA levels and creatinine values in spot urine. Urine samples were not collected using invasive methods (bladder probing or suprapubic catheter insertion). For this study no blood was obtained from the patients. The routine follow-ups of the patients remained unchanged. In addition, the urine samples did not come into contact with any plastic derivatives during collection or transport.

Chemicals and reagents

BPA (99+% purity, Aldrich® brand) and D16-BPA (D16-BPA, ≥98% purity, Aldrich® brand) were purchased from Sigma-Aldrich. BPA β-Glucuronidase, reagents and mobile phases were obtained from Jasem Brand. An Agilent 6470 triple quadrupole LC–MS system (Agilent Corporation, MA, USA) equipped with 1290 Binary pump, 1290 multisampler and 1290 TCC therm. column compartments was used for all analyses. The system was controlled by MassHunter software (Agilent Corporation, MA, USA).

A method for measuring total BPA in human urine using LC-MS/MS after incubation and extraction was developed. BPA Glucuronide

was disintegrationed to BPA by breaking it down with enzymes. Thus total BPA result was obtained. The method is simple, requires less LC-MS/MS run time than previous methods, and yields more sensitive results. The presence of BPA Glucuronide indicated successful degradation of Bisphenol A.

Sample preparation

Human urine samples were stored at −20 °C and thawed on the first day of the extraction. Preparation of Glucuronidase Enzyme; Preparated 2mg/ml jasem glucuronidase enzyme with 1ml Jasem buffer reagent. Pipet 200 μL of urine was sampled into a glass centrifuge tube with cap and a 25 μL of internal standard and 50 μL Jasem Glucuronidase Enzyme was added and vortex for 5 seconds and incubated at 37°C for 3 hours. After incubation 250 μL of Jasem Reagent-1 was added and additionally vortex for 5 seconds. Then, centrifuged at 3000 rpm for 5 minutes. The supernatant was decanted into the HPLC vial and injected to the LC-MS/MS system.[19] Standard curves ranging from 1.00 to 100 ng/mL BPA and from 10 to 1000 ng/mL BPAG were run.

Urine analysis

To assess the impact of creatinine adjustment on the total variance of spot urine samples, urine creatinine levels were analyzed using a modified method developed and validated for creatinine analysis by Park et al.[19,20] Urine BPA levels were expressed in 2 forms: uncorrected BPA (μg/L) and corrected BPA/creatinine (μg/g creatinine), which was corrected by adjusting the measured BPA level by dividing it by the measured creatinine level (mg/L).

History concerning plastic exposure

Mothers of the neonates were questioned about the long-term use of plastic materials or their exposure to heat and the possible harms of plastic water bottles.

Results

The demographic data of the 32 TTN patients and the 33 healthy controls are given in Table I. The gender and mode of delivery of patients with TTN and healthy controls were not statistically different (p=0.492 and p=0.478, respectively). Also, the groups were statistically similar in the rate of birth with or without labor among the cesarean deliveries (p=0.515, Table I).

Birth weight, length, and head circumference, and gestational week were generally lower in the study group when compared to the control group, but these findings were not statistically significant (p=0.581, p=0.398, p=0.411, p=0.873, respectively; Table I). There were no abnormal findings in the genitourinary system examinations of all newborns. The 5-min Apgar scores of the TTN group were significantly lower than those of the control group (p=0.001). The groups were statistically similar in terms of the prevalence of low birth weight for gestation (p=0.999, Table I). There were no neonates with fetal placental doppler abnormalities in my study. The median length of NICU stay of the TTN patients was 10 days (interquartile range (IQR): 7–12.8), while the median duration of mechanical ventilation was 2 days (IQR: 1–2). The mortality rate was zero in both the study and control group. Patients in the study group did not require antibiotics during the follow-up period because they were diagnosed with TTN.

The spot urine BPA, creatinine, and BPA/creatinine values of the study and control groups are given in Table II. The median BPA results in the spot urine collected within the first 6 hours postnatally were 2.9 μg/L (IQR: 1.2–7.5) for the TTN group and 1.2 μg/L (IQR: 0.5–1.8) for the control group. The BPA levels were statistically higher in the TTN group (p=0.0001). The spot urine creatinine values of the 2 groups were not significantly different (p=0.091, Table II).

The spot urine BPA/creatinine ratio was significantly higher in the study group when compared to the control group (p=0.001). The

Table I. Demographic characteristics of the subjects.

Characteristics	Study group n=32	Control group n=33	P-value
Gender (male/female)	22/10	20/13	0.492
Mode of delivery (NVSD/CS)	9/23	12/21	0.478
The cesarean deliveries without labor among	11	8	0.515
Gestational age (weeks)*	38 (36.3-39)	38 (36.5-39.5)	0.873
Birth weight (g)*	2925 (2390-3395)	3100 (2400-3550)	0.581
Birth length (cm)*	50 (49-51)	50 (50-51)	0.398
Birth head circumference (cm)*	35 (34-35)	35 (35-35)	0.411
Apgar (5 min)*	8 (8-9)	10 (9-10)	0.001
Low birth weight for gestation	4	4	0.999
Mechanical ventilation (days)*	2 (1-2)	0	-
Length of NICU stay (days)*	10 (7-12.8)	0	-
Discharged/Exitus	32/0	33/0	-

*: Median (interquartile range), CS: Cesarean section, NSVD: normal spontaneous vaginal delivery.

Table II. Spot urine results of study and control groups.

Parameters evaluated in spot urine	Study group, median (IQR)	Control group, median (IQR)	P-value
BPA (μg/L)	2.9 (1.2-7.5)	1.2 (0.5-1.8)	0.0001
Creatinine (mg/L)	381.4 (227.9-576.2)	553.1 (360.3-654.2)	0.091
BPA/creatinine (μg/g)	8.0 (3.6-26.1)	2.0 (1.0–3.9)	0.0001

BPA: bisphenol A, IQR: interquartile range.

median BPA/creatinine ratio was 8.0 μg/g (IQR: 2.6–26.1) in the TTN group and 2.0 μg/g (IQR: 1.0–3.9) in the control group (Table II).

In the study group, 24 patients (75.0%) required noninvasive respiratory support and 6 patients (18.8%) required invasive respiratory support. Two patients (6.2%) were followed-up without requiring a mechanical ventilator. Since there were only 2 patients who did not require a ventilator, these patients were not included in the statistical comparison. The comparison of patients in the study group according to mechanical ventilator requirement (non-invasive and invasive) is given in Table III.

In the study group, patients that required invasive respiratory support were more likely to be male and delivered by cesarean section, but this finding was not statistically confirmed due to the small number of patients (Table III). The mean birth weight of patients who required invasive respiratory support was significantly lower than that of the patients who required non-invasive respiratory support (p=0.029). They also had a lower mean gestational week, but this finding was not statistically significant (p=0.065, Table III).

Urine creatinine levels were not found to be significantly associated with mechanical ventilation requirements (p=0.082). The spot urine BPA and BPA/creatinine values of the TTN patients who required invasive respiratory support were significantly higher than those of the patients who required non-invasive support (p=0.009 and p=0.001, respectively; Table III).

In order to assess whether the difference in the BPA levels between the study and the control groups was caused by the patients requiring invasive respiratory support, the 6 TTN patients who required invasive respiratory support were excluded, and the spot urine results were then re-assessed. These results are given in Table IV.

Table III. Comparison of the TTN patients according to mechanical ventilator requirements.

	Invasive respiratory support n (%)	Non-invasive respiratory support n (%)	P-value
Gender (male/female)	5/1	15/9	-
Mode of delivery (NVSD/CS)	1/5	8/16	-
Gestational age (weeks)*	36 (35-37.8)	38 (37-39)	0.065
Birth weight (g)*	2400 (2023-2533)	3200 (2496-3475)	0.029
BPA (μg/L)*	27.9 (14.6-89.3)	2.1 (1.2-5.3)	0.009
Creatinine (mg/L)*	202.5 (148.3-527.9)	416 (250.4-609.7)	0.082
BPA/creatinine (μg/g)*	98.5 (18.6-442.1)	6.1 (3.1-11.6)	0.001

*: Median (interquartile range).
BPA: bisphenol A .

Table IV. Comparison of the spot urine results after the exclusion of patients that received invasive support.

Parameters evaluated in spot urine	Study group median (IQR)	Control group median (IQR)	P-value
BPA (μg/L)	2.1 (1.2-5.8)	1.2 (0.5-1.8)	0.001
Creatinine (mg/L)	413.5 (246.7-588.6)	553.1 (360.3-654.2)	0.222
BPA/creatinine (μg/g)	6.1 (3.2-12.4)	2.0 (1.0-3.9)	0.0001

BPA: bisphenol A.

Table V. ROC curve parameters of the BPA and BPA/creatinine.

	BPA	BPA/creatinine
AUC	0,778	0,829
95% CI	0,667-0,889	0,727-0,930
p	0,0001	0,0001
Cut-off	1,18	2,65

AUC: area under the curve, BPA: bisphenol A, CI: confidence interval, ROC: receiver operating characteristic.

Table VI. ROC curve parameters of TTN patients who did or did not require invasive respiratory support.

	BPA	BPA/creatinine
AUC	0,837	0,917
95% CI	0,568-1,000	0,797-1,000
p	0,011	0,002
Cut-off	15,64	19,10

AUC: area under the curve, BPA: bisphenol A, CI: confidence interval, ROC: receiver operating characteristic, TTN: transient tachypnea of the newborn.

After the 6 TTN patients who required invasive respiratory support were excluded, the spot urine BPA and BPA/creatinine values of the remaining TTN patients (n = 26) and healthy controls (n = 33) were compared again, and it was observed that the spot urine BPA and BPA/creatinine values of the healthy subjects were still statistically significantly lower (p =0.001 and p=0.0001, respectively; Table IV).

The values of patients whose TTN duration was longer than 48 hours (n=11) and patients whose TTN duration was shorter than 48 hours (n=21) were compared. The median BPA results were 3.1 μg/L (IQR: 1.2–6.8) for the long-term TTN group (n=11) and 2.8 μg/L (IQR: 1.2–9.9) for the short-term TTN group (n=21). Also the median BPA/creatinine ratio was 4.6 μg/g (IQR: 3.2–21.9) in the long-term TTN group and 8.5 μg/g (IQR: 4.7–39.6) in the short-term TTN group. But these findings were not statistically significant (p=0.999, p=0.411, respectively).

The receiver operating characteristic (ROC) analysis results that aimed to determine the cut-off values for the patient (n = 32) and control (n = 33) groups are presented in Table V. The ROC curve is presented in Fig. 1.

The ROC curve analysis determined the cut-off value of BPA to be 1.18 μg/L (95% confidence interval (CI): 0.667–0.889, sensitivity: 78.1%, and specificity: 51.5%). The cut-off value of BPA/creatinine was calculated as 2.65 μg/g (95% CI:

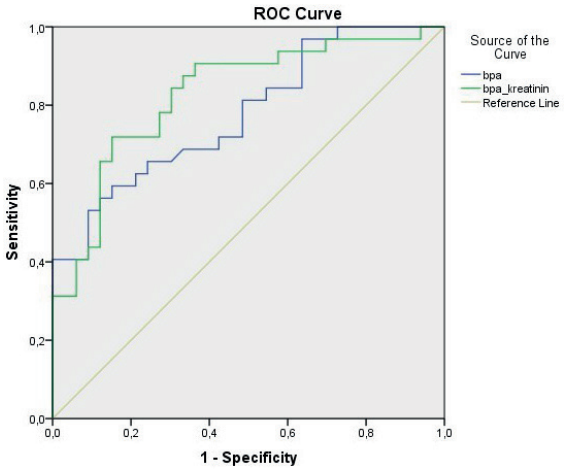

Fig. 1. Receiver operating characteristic curves of the BPA and BPA/creatinine values.

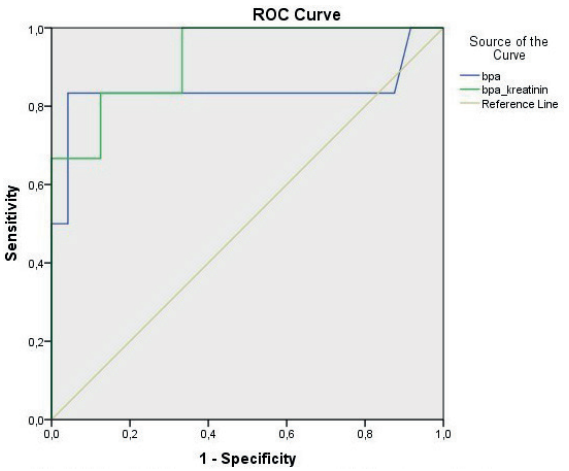

Fig. 2. BPA and BPA/creatinine receiver operating characteristic curves of newborns with transient tachypnea who did or did not require invasive respiratory support.

0.727–0.930, sensitivity: 84.4%, and specificity: 66.7%).

The ROC curve analysis results of TTN patients who did and did not require invasive respiratory support are presented in Table VI and Fig. 2.

For requiring invasive respiratory support, the ROC curve analysis determined the cut-off value of BPA to be 15.64 μg/L (95% CI: 0.568–1.000, sensitivity: 83.3%, and specificity: 96.2%) and the cut-off value for BPA/creatinine was 19.10 μg/g (95% CI: 0.777–1.000, sensitivity: 83.3%, and specificity: 84.6%).

Discussion

BPA exhibits estrogen hormone like properties that mimic endocrine-disrupting chemicals. BPA is mainly used in polycarbonate plastic production and is among the most commonly used materials.[4,21] A comprehensive study from the United States found at least 0.4 μg/L of BPA in 92.6% of human urine samples.[21,22] BPA is a common chemical that people are exposed to in everyday life, and its intrauterine effects are of interest to scientific research. Ranjit et al.[23] found that BPA exposure caused intrauterine growth restriction, and Wolff et al.[24] did not find a correlation between birth weight and BPA. In other words, different studies have obtained variable results.[25] In the current study, the birth weight, length, and head circumference findings of TTN patients (who had higher BPA levels) were not significantly different from those of the control group. There have been many studies investigating the relationship between BPA levels and endocrine disruption in newborns.[25,26] There have also been studies indicating that intrauterine BPA exposure may have adverse effects on fetal brain development.[27]

A study by Hijazi et al.[7,8] demonstrated that BPA has negative effects on fetal pulmonary development and stated that this effect was through pathways affecting the ENaC channel. There are ongoing studies that are investigating the pulmonary effects of BPA.

Even though TTN is a harmless and self-limiting condition, the tachypnea it causes can require oxygen support, non-invasive or (rarely) invasive respiratory support, and care in the neonatal intensive care unit.[14] TTN is a disease commonly observed in NICUs. An increased prevalence of cesarean sections was indicated to be one of the underlying causes of TTN and certain studies have mentioned genetic predispositions as another factor.[11] Reduced ENaC activity or ENaC immaturity on the apical surface of the pulmonary epithelium is one of the causes of TTN.

The BPA levels obtained in this study cannot clearly reflect intrauterine exposure because of the short half-life of BPA. However, there are no studies on the half-life of BPA in neonates. In addition, glucuronidation in the liver must work adequately for the removal of BPA from the body. As well known, neonates are insufficient in glucuronidation.[28] Therefore, urine BPA levels obtained in the early postnatal period raise the question of whether intrauterine reflection may occur. For this reason, there is a need for publications that will comprehensively investigate the relationship between amnion samples obtained from pregnant women in different trimesters and intrauterine exposure to BPA levels and TTN.

The control group was matched for gender, cesarean section, week of pregnancy, birth weights, and low birth weight for gestation.[10] This prevented the need for correcting the data for these variables while investigating the relationship between TTN and BPA.

BPA is also a known endocrine disruptor, but in this study no abnormal findings were observed in the genitourinary system examinations of any of the newborns.

Most of the mothers in this study had no idea about BPA and its possible harms. Although this was not measured, it could be because of the low educational status of the patients served at our hospital. It is necessary to raise societies awareness about this issue.

In lung cell culture studies; BPA is a pro-inflammatory factor via the estrogen receptor, and the BPA acts on the estrogen receptor to activate the Nuclear Factor kappa B signaling pathway. Nuclear Factor kappa B signaling pathway decreases glucocorticoid receptor activity. Therefore, ENaC expression is suppressed in lung epithelial cells. Studies have reported that suppression is temporary (8), but there is no information about its duration. Further studies are needed on this subject.

While there was no statistical difference between the spot urine creatinine values of the newborns admitted to the NICU with TTN and those of the healthy neonates who were housed with their mothers (p=0.091), there was a statistically significant difference between their urine BPA and urine BPA/creatinine values (p<0.005). The ROC curve analysis comparing the TTN and control groups determined the cut-off value of urine BPA for TTN to be 1.18 μg/L (95% CI: 0.667–0.889, sensitivity: 78.1%, and specificity: 51.5%) and the cut-off value of urine BPA/creatinine to be 2.65 μg/g (95% CI: 0.727–0.930, sensitivity: 84.4%, and specificity: 66.7%).

Infants with TTN usually need non-invasive respiratory support.[29] However, those with a severe clinical course may also need invasive respiratory support.[30] The spot urine BPA and BPA/creatinine results of TTN patients who required non-invasive and invasive respiratory support were compared and it was found that these values were significantly higher in patients who required invasive respiratory support (p<0.005). The ROC curve analysis comparing the TTN patients who required invasive and non-invasive respiratory support determined the cut-off value of urine BPA for requiring invasive respiratory support to be 15.64 μg/L (95% CI: 0.568–1.000, sensitivity: 83.3%, and specificity: 96.2%) and the cut-off value for urine BPA/creatinine to be 19.10 μg/g (95% CI: 0.777–1.000, sensitivity: 83.3%, and specificity: 84.6%).

Studies on BPA and newborns have indicated that total or free BPA levels are minimal in urine during the first few postnatal days. Sayıcı et al.[31] determined the median total BPA level in infant (aged >45 days) urine samples to be 0.13 μg/L (range 0.02–0.44). To the best of my knowledge, there are no other studies that have assessed BPA levels in urine samples collected within the first few hours of birth. However, many studies have been conducted with infants. For instance, Völkel et al.[32] investigated total BPA levels in the urine samples of infants and found it to be 17.85 μg/L. Calafat et al.[21] determined this value to be 1.70 μg/L. Herein, the urine BPA levels were found to be 2.9 μg/L (1.2–7.5) in the study group and 1.2 μg/L (0.5–1.8) in the control group.

Nachman et al.[33] determined glucuronide-conjugated BPA levels up to 11.21 μg/L in the urine of 6-day-old newborns. In their study, Lee et al.[5] collected urine samples within 2 days of birth and determined urine BPA levels to be 4.75 μg/L (0.93–14.5). I believe that BPA, whose half-life is known as 6 hours, will be affected by postnatal exposures which could be the reason for different results in different studies. In fact, maternal nutrition may also be effective in breastfeeding mothers, however more extensive studies are needed on this subject.

To the best of my knowledge, this study is the first to investigate the relationship between TTN and BPA; hence, it was not possible to compare these results with similar studies. Studies have shown that the incidence of allergic asthma and wheezing were higher among individuals who developed TTN as newborns.[34,35] Moreover, in their study, Spanier et al.[36] demonstrated the association between neonatal BPA levels and developing asthma later in life. All of these studies have suggested an association between TTN, wheezing/asthma in older ages, and neonatal BPA levels.

The results obtained in the current study may have been associated with the ENaCs located on the apical surface of the pulmonary epithelium. However, the effects of BPA on newborns need to be further investigated by further studies with larger samples that extensively evaluate cell cultures.

Another point to consider is that the urine samples obtained within the first few postnatal hours may be more valuable, but the collection of urine samples from newborns is very difficult with a high likelihood of fecal contamination.

Studying BPA from the pregnant women's urine just before birth or from the cord blood could support the hypothesis of intrauterine BPA exposure. This is one of the shortcomings of this study. Further studies are needed to analyze BPA in cord blood and newborn urine.

In conclusion, BPA and BPA/creatinine values were higher in the urine of newborns diagnosed with TTN than in those of healthy newborns (cut-off values 1.18 μg/L and 2.65 μg/g, respectively), in samples collected within the first 6 hours after birth, which may be a reflection of intrauterine factors. Furthermore, among patients with TTN, the prognosis of the patients with higher urine BPA levels was more severe.

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Ethical approval

The study was granted ethical approval by the Ethics Committee of Gaziantep University Şahinbey Training and Research Hospital (No:2019/476).

Author contribution

The author confirm contribution to the paper as follows: study conception and design: MB; data collection: MB; analysis and interpretation of results: MB; draft manuscript preparation: MB. The author reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The author declares that there is no conflict of interest.

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Original Article

Assessment of aortic elasticity parameters in obese and overweight children

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ABSTRACT

Background. Aortic elasticity is a predictor and recognized factor for future cardiovascular events in children. The aim of the study was to evaluate the aortic stiffness in obese and overweight children compared to healthy ones.

Methods. The study evaluated 98 sex matched children aged 4 to 16 years that were equally distributed in asymptomatic obese or overweight and healthy children groups. All the participants were free of any heart diseases. Arterial stiffness indices were determined using two-dimensional echocardiography.

Results. The mean ages in the obese and healthy children were 10.40±2.50 years and 10.06±1.53 years, respectively. Aortic strain was significantly higher in obese children (20.70±5.04%), compared to healthy (7.06±3.77%) and overweight children (18.59±8.08%, p<0.001). Aortic distensibility (AD) was significantly higher in obese children (0.010±0.005 $cm^2dyn^{-1}x10^{-6}$), compared to healthy (0.0036±0.004 $cm^2dyn^{-1}x10^{-6}$) and overweight children (0.009±0.005 $cm^2dyn^{-1}x10^{-6}$, p<0.001). Aortic strain beta (ASβ) index, was significantly higher in healthy children (9.26±6.17). Pressure-strain elastic modulus (PSEM) was significantly higher in healthy children (7.52±4.76 kPa). Systolic blood pressure increased with body mass index (BMI) significantly (p<0.001) but diastolic blood pressure did not change (p=0.143). BMI had significant effect on arterial stiffness (AS) (β=0.732, p<0.001), AD (β=0.636, p<0.001), ASβ index (β=-0.573, p<0.001) and PSEM (β=-0.578, p<0.001). Age had significant effect on systolic diameter of the aorta (β=0.340, p<0.001) and diastolic diameter of the aorta (β=0.407, p<0.001).

Conclusions. We concluded that aortic strain and aortic distensibility increased in obese children when aortic strain beta index and PSEM decreased. This result suggests that, as atrial stiffness is a predictor for future heart diseases, dietary treatment for children with overweight or obese status is important.

Key words: aortic elasticity, obese, overweight, children.

Arterial stiffness (AS) is a new detectable manifestation of damaging structural and practical adjustments in the vessel wall. It is also often an effect of incorporating cardiovascular hazard elements of the blood vessel.[1,2] Estimating AS offers the possibility to detect the activities of coronary vascular disease (CVD) earlier than the primary signs, which is an essential component of health care.[2] An increase in AS takes place because of arteriosclerosis and aging that is probably tormented by numerous components of hereditary inclination and CVD danger elements which include weight problems or hypertension.[2-4] In childhood, most of the large arteries are very elastic, however stiffen with aging.[3-5] In addition, AS increases in many conditions which include celiac disease, asthma, diabetes mellitus, chronic kidney disease, thalassemia and obesity.[5-10] Obese children are at risk for many persistent diseases which include excessive blood pressure, CVD, dyslipidemia and diabetes and each one of these disorders may have a robust impact on AS.[11] In a study, it has been reported that the prevalence of obesity is 10.1% and 4.79% in children aged 6 and 18 years, respectively, with more

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occurrence in females; and in these children, approximately 70% have one and 39% have two or more risk factors for CVD.[12-14] In addition to association with heart disease in children, these CVD risk factors and obesity are also associated with an increased prevalence in adults.[15] Flynn et al.[16], found that there is a rising prevalence of childhood systolic and diastolic blood pressure in obese children. Ayer et al.[17], observed an increase in total cholesterol (CHO), low-density lipoprotein (LDL), and triglycerides (TG) and lower high-density lipoprotein (HDL) level in obese participants. In this regard, Ayer et al.[17], Dangardt et al.[18] found that AS increased rapidly in obese children; and Charakida et al.[19] found that vascular dysfunction was not observed among prepubertal obese children. AS is recognized as a marker of cardiovascular risk but it has also been reported that it is increased in obese children without overt cardiovascular diseases.[20] Therefore, the aim of this study was to evaluate the effect of obesity on AS in children and compare the results to children of normal weight.

Material and Methods

Study design

This case-control study involved 98 children and adolescents between the ages of 4 and 16 who were evenly divided into two groups: obese or overweight children and controls. Children with a normal BMI served as the controls. The research was carried out in 2020, at Ali Asghar Hospital in Zahedan City, Sistan and Baluchestan province, Iran, in collaboration with the center for specific diseases.

Criteria

For each participant, the following exclusion criteria were taken into consideration: diabetes mellitus, hypertension, dyslipidemia, systemic autoimmune disease, active infection, evidence of liver, renal, or lung disease, smoking exposure, concurrent treatment with antihypertensive medications, lipid-lowering medications, and a positive family history of dyslipidemia are all co-morbid conditions.

Echocardiography measurements

The medical history, physical examination, chest X-ray, and echocardiography were the primary procedures performed on patients. The same pediatric cardiologist used a MyLab 60 Class C, Esaote, with a transducer of 3 to 8 MHz to perform echocardiography on the participants. Measurement was repeated for three cycles and the average was taken into consideration in order to achieve high precision in the results of echocardiography. Participants underwent an echocardiogram without having to hold their breath. M-mode echocardiography revealed the following: diastolic and systolic diameters of the aorta, respectively (AOD and AOS). The M-mode was used to obtain ascending aorta from 3 centimeters above the aortic valve following a routine echocardiographic examination. The distance between the inner edges of the aorta's anterior and posterior walls at systole and diastole was used to calculate aortic diameters. The mean was determined by taking measurements during three consecutive pulses.[9]

Blood pressure measurements

A sphygmomanometer was used to measure blood pressure (BP) from the brachial artery at the level of the heart after at least 5 minutes of rest in the supine position. Three measurements were carried out, at least 2 min. apart, and the average of the two readings that were closest together was recorded. Korotkoff phases I and V were utilized for the systolic and diastolic BP levels, respectively, with a pressure drop rate of approximately 2 mm Hg/second. Pulse pressure (PP) was calculated by subtracting systolic BP from diastolic BP.

Assessment of aortic elasticity

The systolic and diastolic ascending aortic diameters were recorded in M-mode under echocardiographic and electrocardiographic

guidance approximately 3 cm above the aortic valve from parasternal long axis views. The systolic aortic diameter was measured at the time of maximum anterior motion of the aorta while the diastolic diameter was measured at the start of the QRS complex in electrocardiography. The aortic elasticity of the aorta was evaluated using the formulas listed below.[9]

Aortic strain (%) = (aortic SD [systolic diameter] - aortic DD [diastolic diameter]) x100 / aortic DD.

Aortic stiffness beta (ASβ) index = natural logarithm (systolic BP / diastolic BP) / ([aortic SD-aortic DD] / aortic DD).

Aortic distensibility ($cm^2dyn^{-1}x10^{-6}$) = 2 x ([aortic SD - aortic DD] / aortic DD) / (systolic BP-diastolic BP).

Pressure strain elastic modulus (kPa) = (systolic BP–diastolic BP) / ([aortic SD–aortic DD] / aortic DD)

Anthropomorphic measurements

An experienced expert measured the participants' height and weight using standard equipment. Height was measured to the nearest 0.1 cm and weight to the nearest 0.1 kg, wearing light indoor clothes without shoes using an integrated calibrated weight and stadiometer (ADE, Modell MZ10023, Hamburg, Germany). BMI (kg/m^2) was calculated. Those with a BMI between 18-25 were assigned to the control group, those with a BMI between 25.0 to 30 were defined as overweight and those who had a BMI higher than 30 was defined as obese. The overweight and obese participants were assigned to the study group.

Ethical approval

The study, a project proposed to the Children and Adolescent Health Research Center, was approved as by the Ethics Committee of Zahedan University of Medical Sciences, Zahedan, Iran (IR.ZAUMS.REC.1400.095, 2021-5-26). Written informed consent form was obtained from the participants or their guardians.

Statistical analysis

The software of SPSS 20.0 was used to analyze the data (SPSS Inc., Chicago, IL, USA). Kolmogorov-Smirnov test was used to assess distribution of continuous variables where homogeneity was tested. Independent t-test was used to compare two mean values of quantitative variables with normal distribution while Mann-Whitney U test was used to compare quantitative variables with skewed distribution. One-way ANOVA test was used for comparison between three groups, followed by Tukey post-hoc test and Kruskal-Wallis test was used for comparison between three groups, followed by Dunn's post-hoc test. Linear regression was used to determine how certain variables affected the parameters of the heart's stiffness. A significance level of 0.05 was deemed statistically significant.

Results

The mean age of all participants was 10.23±2.07 years; such that in the overweight or obese group it was 10.40±2.50 years and it was 10.06±1.53 years in the healthy children. Of the participants, 41 (41.8%) were female and 57 (58.2%) were male. In the overweight or obese children group 18 (36.7%) were female and 31 (63.3%) were male, in the healthy children 23 (46.9%) were female and 26 (53.1%) were male (p=0.306).

All variables had had normal distribution, except AOS and AOD. Primary analysis showed that atrial strain, AD had increasing trends by increasing BMI when ASβ index and PSEM had declining trends. Atrial strain increased with BMI and was higher in obese children (20.70±5.04 $cm^2dyn^{-1}x10^{-6}$), compared to normal (7.06±3.77 $cm^2dyn^{-1}x10^{-6}$) and overweight children (18.59±8.08 $cm^2dyn^{-1}x10^{-6}$) (p<0.001). AD was significantly higher in obese (0.010±0.005%) children compared to normal weight (0.0036±0.004%) and overweight children (0.009±0.005%) (p<0.001). ASβ index and PSEM had inverse trends compared with atrial strain and AD. The results showed that ASβ index had decreasing trend with BMI such

that normal weight children had higher value (9.26±6.17), compared to overweight (3.48±2.06) and obese children (2.67±1.15; p<0.001). Same trends occurred for PSEM; the highest value was in normal weight children (7.52±4.76 kPa) and then decreased when BMI increased. SBP increased with BMI significantly (p<0.001) but DBP did not change (p=0.143). Both AOS and AOD significantly increased with BMI (Table I).

Table I also shows the Tukey post-hoc test which indicated the significant differences in different pairs of groups. The results showed that all study parameters except DBP and AOD were different in the normal weight children compared to the overweight and obese group.

Table II shows the linear regression analysis which predicted the effect of age, sex and BMI on aortic stiffness parameters in all participants, in obese or overweight and in normal weight children. In the case of obese or overweight children, analyses revealed that age, sex and BMI predicted 2.6%, 5.0%, 6.0%, 7.0%, 17.6%, 8.3%,41.4% and 34.0% of changes in atrial strain, AD, ASB index, PSEM, SBP, DBP, AOS and AOD, respectively. We also observed that age was the only predictor that had a significant effect on PSEM, DBP and AOS in obese or overweight children.

Table I. Aortic stiffness parameters, blood pressure and aortic diameters according to body mass index groups.

Variables	Groups	Mean ± SD	p	Normal vs. overweight	Normal vs. obese	Overweight vs. obese
	Normal	7.06 ± 3.77				
AS (%)	Overweight	18.59 ± 8.08	<0.001	<0.001	<0.001	0.363
	Obese	20.70 ± 5.04				
	Normal	0.0036 ± 0.003				
AD ($cm^2dyn^{-1}x10^{-6}$)	Overweight	0.009 ± 0.005	<0.001	<0.001	<0.001	0.238
	Obese	0.010 ± 0.005				
	Normal	9.26 ± 6.17				
ASβ index	Overweight	3.48 ± 2.06	<0.001	<0.001	<0.001	0.246
	Obese	2.67 ± 1.15				
	Normal	7.52 ± 4.76				
PSEM (kPa)	Overweight	3.01 ± 1.66	<0.001	<0.001	<0.001	0.238
	Obese	2.34 ± 1.01				
	Normal	103.49 ± 5.30				
SBP (mm Hg)	Overweight	111.71 ± 11.74	0.006	0.005	0.015	0.909
	Obese	113.08 ± 14.55				
	Normal	63.80 ± 4.70				
DBP (mm Hg)	Overweight	66.83 ± 5.66	0.143	0.023	0.435	0.975
	Obese	68.40 ± 11.63				
	Normal	20.09 ± 3.01				
AOS (mm)	Overweight	24.05 ± 3.02	<0.001	<0.001	<0.001	0.222
	Obese	25.10 ± 3.04				
	Normal	18.78 ± 2.90				
AOD (mm)	Overweight	20.36 ± 2.94	0.005	0.026	0.003	0.429
	Obese	20.86 ± 3.00				

AD: aortic distensibility, AOD: aortic diameter in diastole, AOS: aortic diameter in systole, AS: aortic strain, ASβ index: aortic stiffness beta index, DBP: diastolic blood pressure, PSEM: pressure strain elastic modulus, SBP: systolic blood pressure.

Table II. The effect of the age, sex and body mass index on aortic stiffness parameters, blood pressure and aortic diameters.

Parameters	Factors	All participants				Obese or overweight children				Normal body mass index			
		Standardized β	t	P	R^2	Standardized β	t	P	R^2	Standardized β	t	P	R^2
	Age	-0.076	-0.937	0.351		-0.68	-0.402	0.690		-0.193	-1.214	0.231	
AS	Sex	0.031	0.407	0.685	50.7%	0.017	0.100	0.921	2.6%	0.026	0.173	0.864	3.5%
	BMI	0.732	9.515	<0.001		0.163	0.082	0.285		0.126	0.773	0.432	
	Age	-0.183	-1.987	0.05		-0.223	-1.399	0.187		-1.171	-1.072	0.289	
AD	Sex	0.04	0.455	0.65	36.2%	0.013	0.081	0.936	5.0%	0.101	0.680	0.500	3.3%
	BMI	0.636	7.269	<0.001		0.106	0.716	0.478		0.047	0.297	0.768	
	Age	0.175	1.808	0.074		0.128	0.773	0.443		0.321	2.072	0.044	
ASB index	Sex	-0.067	-0.73	0.467	29.6%	0.037	0.229	0.820	6.0%	-0.057	-0.400	0.691	8.9%
	BMI	-0.573	-6.236	<0.001		-0.222	-1.500	0.141		-0.121	-0.781	0.437	
	Age	0.18	1.864	0.065		-0.199	1.209	0.233		0.309	1.990	0.053	
PSEM	Sex	-0.061	-0.67	0.504	30.00%	0.027	0.169	0.866	7.0%	-0.049	-0.341	0.735	8.2%
	BMI	-0.578	-6.306	<0.001		-0.204	-1.387	0.172		-0.141	-0.990	0.365	
	Age	0.228	2.296	0.024		0.316	2.038	0.047		-0.096	-0.614	0.543	
SBP	Sex	-0.012	-0.125	0.901	25.60%	0.045	0.296	0.769	17.6%	-0.200	-1.376	0.176	6%
	BMI	0.384	4.063	<0.001		0.195	1.412	0.165		-0.051	-0.377	0.745	
DBP	Age	0.032	0.297	0.767		0.126	0.769	0.446		-0.183	-1.254	0.216	
	Sex	0.114	1.105	0.272	10.20%	0.128	0.796	0.430	8.3%	0.104	0.771	0.445	19.2%
	BMI	0.271	2.608	0.011		0.170	0.166	0.250		-2.299	-2.299	0.026	
	Age	0.34	3.998	<0.001		0.564	4.318	<0.001		0.189	1.183	0.234	
AOS	Sex	0.048	0.596	0.553	45.4%	0.106	0.823	0.415	41.4%	-0.015	-1.000	0.921	3%
	BMI	0.464	5.734	<0.001		0.100	0.854	0.398		0.057	-0.357	0.713	
	Age	0.407	4.076	<0.001		0.537	0.876	<0.001		0.223	1.442	0.156	
AOD	Sex	0.034	0.36	0.72	24.8%	0.085	0.027	0.534	34%	-0.022	-0.148	0.833	4.4%
	BMI	0.163	1.71	0.091		0.616	0.126	0.900		-0.088	-0.558	0.580	

AD: aortic distensibility, AOD: aortic diameter in diastole, AOS: aortic diameter in systole, AS: aortic strain, ASB index: aortic stiffness beta index, DBP: diastolic blood pressure, PSEM: pressure strain elastic modulus, SBP: systolic blood pressure.

Discussion

The results of the present study showed that atrial strain, AD increased by BMI when ASβ index and PSEM decreased. Among the predicted variables, BMI had a significant effect on atrial strain, AD, ASβ index and PSEM in all participants; and age was the only predicted variable that had a significant effect on PSEM, DBP and AOS in obese or overweight children.

Skilton et al.[21] reported that AS was affected by a few parameters such as; sex, hypertension, smoking, dyslipidemia, chronic inflammatory disease, aging, increased weight, a lower height and several studies have shown changes in AS associated with obesity and have found similar findings with the present study.[4,22] AS is evaluated with numerous parameters, such as atrial strain, AD, ASB index, PSEM, pulse wave velocity (PWV) and augmentation index (AIx).[23] The AIx is a measure of systemic AS derived from the ascending aortic pressure wave form and is inversely associated with body height but is not associated with age, sex or weight.[24] Obesity introduces several structural and hemodynamic changes which cause fat deposition around the vessel wall and changes in compliance; in addition some mediators such as endothelin and nitric oxide, may lead to vasodilatation in resistance vessels.[25] Kulsum-Mecci et al.[4] conducted a study to find the effect of obesity and hypertension on AS using PWV. They found higher PWV in obesity and in hypertension, as well as in combination. They also found a "paradoxical" decrease in PWV with obesity when Acree et al.[26] showed an unexpected result in adults consistently that show higher PWV with obesity.[4] Earlier puberty leading to earlier maturation to peak arterial compliance and increased body size in obese children is one of the hypotheses for the paradoxical decrease.[25]

Hudson et al.[27] showed that obese children were more likely to suffer from a significant damage to AS parameters. They demonstrated that there was a positive correlation between arterial pressure and aortic elasticity independent of obesity. In contrast to our findings that AD was more prevalent in overweight or obese children, changes in AS parameters are a complex phenomenon characterized by a decrease in the distensibility of the large arteries.[24] Both Koopman et al.[28] and Sen et al.[29] reported that obese children had an increase of PWV and changes in AS parameters compared with normal BMI children. In this regard, it could be suggested that obesity can have a significant impact on arterial changes and could play an critical role in the pathophysiology of macrovascular disease.

Dangardt et al.[18] found that obesity had substantial changes in AS parameters as early as 14 to 19 years of age, beyond what was observed in the controls. The degree and duration of obesity are suggested to be important factors for determining the cardiovascular changes. This finding might indicate that obesity initially adapted to accommodate the larger blood volume, generated by the marked increase in fat mass, by overall 'vasodilatation' and increased cardiac functions. But, it seems that there is a limit to this physiological adaptive response; when the limit is reached during adolescence, diastolic blood pressure increases, and it results in the loss of the previously augmented cardiac output effect, evidenced by the observed increase of arterial stiffness.[30] Adiposity itself may have a driving influence on vasculature leading to the development of hypertension.[31] It is also known that conventional risk markers are frequently unstable across adolescence. Hudson et al.[27] found no association between AS parameters and stage of puberty but found that AS parameters change by age. Haraguchi et al.[32] found that AS parameters were significantly related to obesity. It is noteworthy that in addition to hypertension a change in AS also may contribute to the development of cardiac hypertrophy in the obese population.[33] Abdominal adiposity that is measured with a simple clinical tool such as abdominal fat, alone or combined with hypertriglyceridemia, remains a good cardiovascular predictor.[33]

The main limitation of the study was the small sample size due to a single center data

collection. But despite this, we believe the study is important as it applied certain measures of AS such as aortic strain, AD, ASβI, and PSEM instead of PWV and AIx.

We conclude that aortic strain and AD increased by BMI increased when ASB index and PSEM decreased. The study also concluded that systolic blood pressure was increased with BMI but diastolic blood pressure did not change. Systolic and diastolic diameters of the aorta increased in obese children. This suggests that AS is a predictor for future heart diseases and children with overweight or obese status must be closely followed to prevent these consequences.

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Ethical approval

The study was approved as a project proposed to the Children and Adolescent Health Research Center by the Ethics Committee of Zahedan University of Medical Sciences, Zahedan, Iran (IR.ZAUMS.REC.1400.095, 2021-5-26).

Author contribution

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

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

What has changed in the last 25 years in osteosarcoma treatment? A single center experience

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ABSTRACT

Background. Osteosarcoma is the most common type of primary malignant bone tumor in the extremities. The main purpose of this study was to determine clinical features, prognostic factors, and treatment results of patients with osteosarcoma at our center.

Methods. We retrospectively analyzed the medical records of children with osteosarcoma between the years 1994-2020.

Results. 79 patients were identified (54.4% male, 45.6% female). The most common primary site was the femur (62%). Twenty-six of them (32.9%) had lung metastasis at diagnosis. The patients were treated between 1995-2013 according to the Mayo Pilot II Study protocol, while the others were treated with the EURAMOS protocol between the years 2013-2020. Sixty-nine patients underwent limb salvage surgery as a local treatment, whereas seven underwent amputation. The median follow-up time was 53 months (2.5-265 months). The event-free survival (EFS) and overall survival (OS) rates at 5 years were 52.1% and 61.5%. The 5-year EFS and OS rates were 69.4% and 80% in females; 37.1% and 45.5% in males (p=0.008/p=0.001). The 5-year EFS and OS rates of the patients without metastasis were 63.2% and 66.3%; with metastasis 28.8% and 51.8% (p=0.002/p=0.05). For good-responders, the 5-year EFS and OS rates were 80.2% and 89.1%; while for poor-responders, 35% and 46.7% (p=0.001). Mifamurtide was used in addition to chemotherapy as of the year 2016 (n=16). The 5-year EFS and OS rates were 78.8% and 91.7%, respectively for the mifamurtide group; 55.1% and 45.9%, respectively for the non-mifamurtide group (p=0.015, p=0.027).

Conclusions. Metastasis at diagnosis and poor response to preoperative chemotherapy were the most important predictors of survival. Females had a better outcome than males. In our study group, the mifamurtide group's survival rates were significantly higher. Further large studies are needed to validate the efficacy of mifamurtide.

Key words: osteosarcoma, childhood, treatment, surgery, chemotherapy.

Osteosarcoma is the most common type of primary malignant bone tumor in children. Osteosarcoma accounts for approximately 3% of all pediatric malignancies. The incidence rises with age and reaches a peak incidence during puberty.[1] The tumor usually arises from the extremities and especially from the long bones' metaphyseal region. The most common site is the distal femur, followed by the proximal tibia. However, axial bones (pelvis, vertebra, head bones) can also be involved. Osteosarcomas are high-grade malignancies, and 15-17% of patients usually have lung metastasis at diagnosis.[2]

Current treatment for this aggressive tumor is neoadjuvant multiagent chemotherapy followed

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by surgery and adjuvant chemotherapy. With this multimodal treatment, 5-year event-free survival (EFS) rate is about 60-70%. Cisplatin, doxorubicin, methotrexate, and in some regimens ifosfamide are the main drugs of this combination chemotherapy.[3-7]

In surgery, resection of the tumor with wide margins is important. With the introduction of neoadjuvant multiagent chemotherapy, limb-sparing surgery rather than amputation is the treatment of choice in most extremity tumors.[8] After resection, patients usually undergo external prosthesis replacement. However, in some cases, extracorporeal irradiation (ECI) and reimplantation have been preferred in recent years.

Adjuvant chemotherapy after surgery usually depends on the histologic response of the patient. Treatment protocols include similar drugs for good responders, but for poor responders, it is controversial. Which drug is effective for the latter group is not well-established. Current prospective trials evaluate whether altering postoperative chemotherapy in poor responders improves outcomes.

There has been little improvement in the survival rates of osteosarcoma patients in more than three decades. Therefore, novel strategies are needed to improve survival. Mifamurtide is a synthetic lipophilic analog of muramyl dipeptide. This molecule acts as an immunostimulant with an anti-tumor effect. In recent years, the addition of immunostimulant mifamurtide after surgery to postoperative chemotherapy has been reported to have a significant effect on the overall survival of non-metastatic patients, however, it is yet to be answered for metastatic patients.[9,10]

The main purposes of this study were to share our treatment experience, and to document demographic characteristics, clinical features, and prognostic factors of non-metastatic and metastatic patients with osteosarcoma of the extremities treated at our center.

Material and Methods

We retrospectively analyzed 79 children with extremity osteosarcoma treated at Ege University Hospital between the years 1994 and 2020. All patients underwent an initial tru-cut biopsy for definitive diagnosis at the Department of Orthopedics. The extent of the disease was evaluated by magnetic resonance imaging of the lesion, computerized tomography of the chest, and a radionucleotide bone scan.

All patients were treated according to the Mayo Pilot II protocol between the years 1995 and 2013 or EURAMOS protocol between 2013 and 2020. As per the Mayo Pilot II study, patients received cisplatin (120 mg/m^2/day; week 10) and doxorubicin (25 mg/m^2/day x 3, week 0, 5), ifosfamide (1.8 g/m^2/day x 5, week 0, 5, 10), and high-dose methotrexate (12 g/m^2 week 3, 4, 8, 9, 13, 14) with leucovorin rescue. Surgery was carried out at around week 15 or earlier if tumor progression was seen based on clinical and radiological findings. The surgery aimed to remove the tumor and achieve wide margins. Limb-sparing surgery was the treatment of choice. Amputation was restricted to those for whom limb-sparing surgery could not yield wide margins or adequate function. The Huvos necrosis grading system was used in histopathological evaluation to assess chemotherapy response.[11] Based on the percentage of tumor necrosis after chemotherapy, patients can be classified as poor or good responders. The patients who achieved at least 90% of tumor necrosis in the resected specimen were categorized as good responders. They continued to receive similar postoperative chemotherapy to complete 42 weeks. Poor responders (less than 90% tumor necrosis) received the same regimen before 1996, but high-dose ifosfamide alone (14 g/m^2/day over 3.5 days, in 21-day intervals) after this year.

In the EURAMOS study protocol, all patients were planned for the same pre-operative therapy for 10 weeks consisting of 120 mg/m^2 of cisplatin and 75 mg/m^2 of doxorubicin (weeks 1 and 6)

followed by 12 g/m^2 of high-dose methotrexate (weeks 4, 5, 9 and 10). Surgery was carried out at around weeks 11-12. Patients with a good histological response (≥ 90% of tumor necrosis) were continued with postoperative therapy for 29 weeks consisting of 120 mg/m^2 of cisplatin (weeks 12 and 17), 75 mg/m^2 of doxorubicin (weeks 12, 17, 22, 26) followed by 12 g/m^2 of high-dose methotrexate (weeks 15, 16, 20, 21, 24, 25, 28 and 29). The patients with a poor histological response (<90% of tumor necrosis) were continued with postoperative therapy for 40 weeks with cisplatin, doxorubicin, and high-dose methotrexate with additional ifosfamide and etoposide.

As an adjuvant therapy, only mifamurtide was used in addition to postoperative chemotherapy treatment after 2016. Mifamurtide was given 2 mg/m^2 twice weekly at least 3 days apart for 12 weeks, followed by once-weekly treatments for an additional 24 weeks for a total of 48 doses in 36 weeks.

The ethical committee of our institution approved the study (Ege University Faculty of Medicine, report number: 22-4T/55).

Statistical analysis

Data were analyzed using SPSS software (version 21 for Windows). Continuous variables are presented as means (ranges) and categorical variables as numbers (percentages). A p-value ≤ 0.05 was considered to indicate statistical significance. Kaplan-Meier survival analysis followed by log-rank tests were used to identify significant relationships among EFS, categorical variables, and overall survival (OS).

Results

Patients

There were 43 male and 36 female (M:F=1.2) patients in the study with a mean age of 13.1 ± 2.8 years (4.5-18 years). Demographic features of the patients are given in Table I. The most common primary tumor site was the femur (62%). According to WHO histologic classification, 49 patients had conventional osteoblastic osteosarcoma. Twenty-six out of 79 patients (32%) had metastasis at diagnosis, of whom 24 (92.3%) had pulmonary metastases, 2 (7.7%) had both pulmonary and bone metastases. Between 1995 and 2013, the patients were treated according to the Mayo Pilot II Study Protocol (n=59), and the EURAMOS treatment protocol between 2013 and 2020 (n=20) (Table II).

Table I. The demographic and clinical characteristics of patients (n=79).

Sex	
Male	43 (54.4%)
Female	36 (45.6%)
Age, years	
Mean ± SD	13.1± 2.8 years
< 10 years	10 (12.7%)
≥ 10 years	69 (87.3%)
Tumor site	
Femur	49 (62%)
Tibia	18 (22.8%)
Humerus	10 (12.7%)
Fibula	2 (2.5%)
WHO histological classification	
Osteoblastic	49 (62%)
Chondroblastic	15 (19%)
Fibroblastic	4 (5.1%)
Telangiectatic	4 (5.1%)
Others	7 (8.9%)
Metastasis	
Lung	24 (30.4%)
Lung + bone	2 (2.5%)
Treatment protocol	
Mayo Pilot II study	59 (74.7%)
EURAMOS study	20 (25.3%)
Histologic response	
Good (≥90%)	32 (40.5%)
Poor (<90%)	42 (53.2%)

Surgical Outcomes

Local control using surgical resection was planned after pre-operative chemotherapy. Upfront surgical resection was performed in

Table II. Demographic and clinical characteristics of patients according to the treatment groups (n=79).

	Mayo Pilot II Study Protocol (n=59)	EURAMOS (n=20)
Sex		
Male	33 (55.9%)	10 (50%)
Female	26 (44.1%)	10 (50%)
Age, years		
< 10 years	7 (11.9%)	3 (15%)
≥ 10 years	52 (88.1%)	17 (85%)
Metastasis		
Lung	19 (32.2%)	5 (25%)
Multifocal metastasis	2 (3.4%)	0
Surgery		
Limb salvage	49 (83.1%)	20 (100%)
Amputation	7 (11.9%)	0
Histologic response		
Good	21 (35.6%)	11 (55%)
Poor	33 (55.9%)	9 (45%)

two patients, and three patients (3.8%) were not operated on because of the tumor progression. The median time for surgery was 19.5 weeks in the patients treated with the Mayo Pilot II study and 14.5 weeks in patients treated with the EURAMOS protocol. Sixty-nine (87.3%) patients underwent limb salvage surgery, whereas seven patients (12.7%) underwent amputation. Out of these 69 patients, 63 patients (79.7%) experienced a prosthesis replacement following tumor resection. Six patients (7.6%) received extracorporeal irradiation and reimplantation of the bone. The number of patients undergoing limb-salvage surgery (n=20) increased in the EURAMOS group (83.1% vs. 100%). By contrast, the number of patients undergoing amputation (n=0) decreased (11.9% vs. 0%), but this was not statistically significant (p=0.18).

Seventy-four out of 79 patients were evaluated for histologic response. Of these patients, 32 (40.5%) had a good response, and 42 (53.2%) had a poor response. Comparison of the treatment responses of the two treatment groups revealed that the number of patients with good responses increased in the EURAMOS group (n=11) (55% in EURAMOS vs. 35.6% in Mayo Pilot II). Consequently, the poor responders (n=9) seemed to be less in the EURAMOS treatment group (45% in EURAMOS vs. 55.9% in Mayo Pilot II), but this was not significant (p=0.21).

Twenty-six patients with poor histologic response were given high-dose ifosfamide alone as a postoperative regimen after 1996. The other patients with a poor response and good responders were treated according to the postoperative chemotherapy regimen as mentioned in the protocols. Among the poor responders, there was no difference in EFS and OS rates with the addition of high-dose ifosfamide (p=0.33).

Survival

Median follow-up time was 53 months (2.5-265 months). The EFS and OS rates for all patients were 52.1% and 61.5% at 5 years, respectively (Fig. 1). The estimated 5-year EFS and OS rates were 49% and 57.3% for the Mayo Pilot II study group. The estimated 5-year EFS and OS rates were 60.9% and 71.8%, respectively for the EURAMOS treatment group (Fig. 2).

The females had significantly better outcomes than the males. The 5-year EFS rate was 69.4% in females versus 37.1% in males (p=0.008). The 5-year OS was 80% in females versus 45.5%

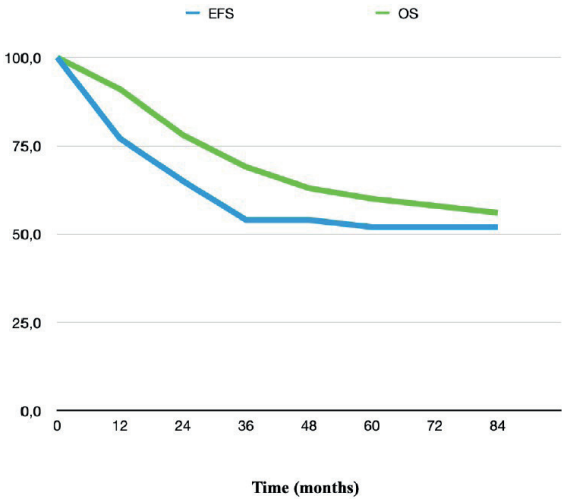

Fig. 1. Survival analysis of all patients.

in males (p=0.001). There was no significant relationship between age groups and survivals. The estimated 5-year OS rate was 42% in patients <10 years of age (n=10), and the estimated 5-year OS rate was 63.8% in patients ≥ 10 years of age (n=69) (p=0.28).

For the non-metastatic group the 5-year EFS rate was 63.2% while for the metastatic disease it was 28.8% (p=0.002). The 5-year OS rates was 66.3% for the non-metastatic group and 51.8% for metastatic patients (p=0.05) (Fig. 3).

Among the non-metastatic patients (n=53), 21 patients (39.6%) were good-responders and 29 patients (60.3%) poor-responders. Three patients did not have a pathological evaluation. Kaplan–Meier analysis showed that good responders have higher EFS at 5 years than poor responders (80.2% vs. 35%, p=0.001). The 5-year OS rates were 94.4% in the non-metastatic good-responders and 50.8% in the non-metastatic poor-responders (p=0.001).

Among the metastatic patients (n=26), 11 patients (42.3%) were good-responders and 13 patients (50%) were poor-responders. Two patients did not have a pathological evaluation. The 5-year EFS rates were higher at good-responders than those for poor-responders (51.1% vs. 15.4%, p=0.008). The 5-year OS rates were 77.9% in metastatic good responders and 38.5% in metastatic poor responders (p=0.04).

Among all patients, the estimated 5-year EFS and OS rates for good-responders were 80.2% and 89.1%, while for poor-responders the same rates were 35% and 46.7 % (p=0.001 vs. p=0.001) (Fig. 4.).

Disease progression occurred in 12 patients and relapse in 25 patients after treatment cessation. The median time to progression or relapse was 13 months (2.5-55 months). Among these 25 patients, 19 patients relapsed only with pulmonary metastases, three had a local plus pulmonary relapse, and three had distant bone plus pulmonary metastasis. Thirty-two patients (40.5%) died from the disease during follow-up. In the poor-response group (n=42), 18 patients relapsed, and 9 patients had progressive disease. In contrast, 6 patients relapsed in the good response group (n=32). We treated most of the poor responders (n=26) with high-dose ifosfamide in our study group. There was

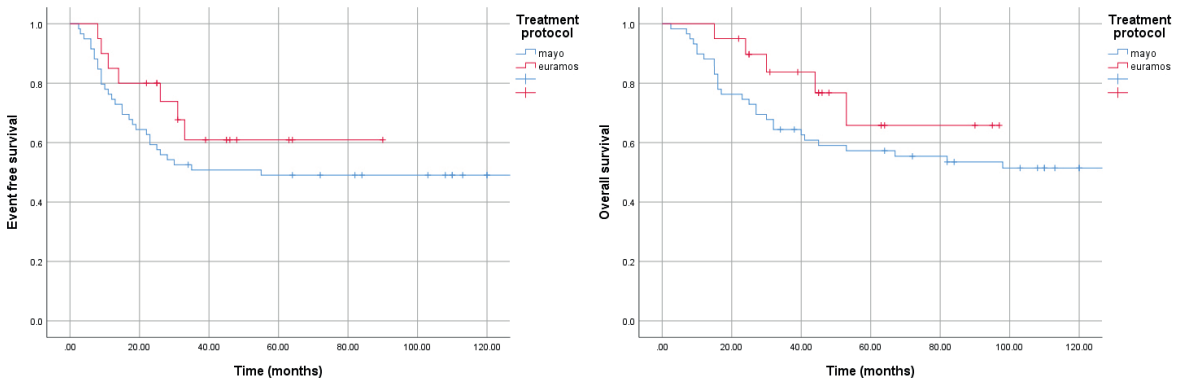

Fig. 2. Survival analysis of patients according to the treatment groups.

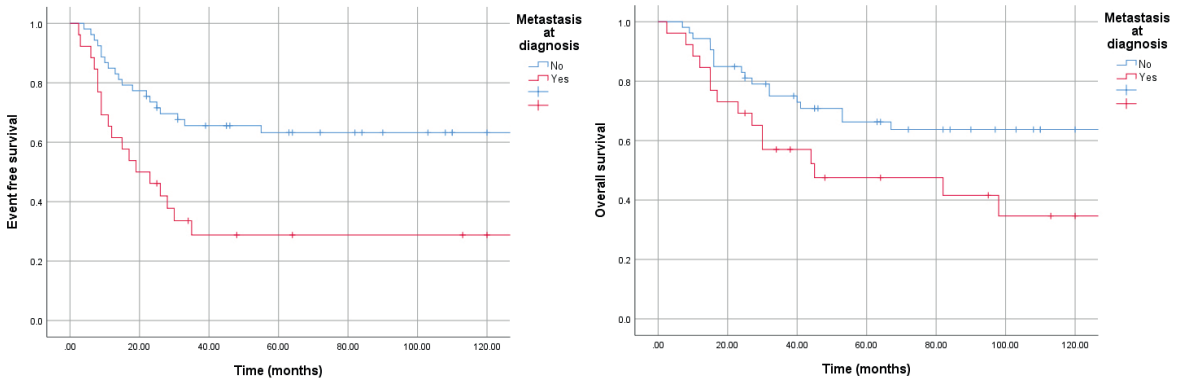

Fig. 3. Survival analysis of all patients according to the metastasis diagnosis.

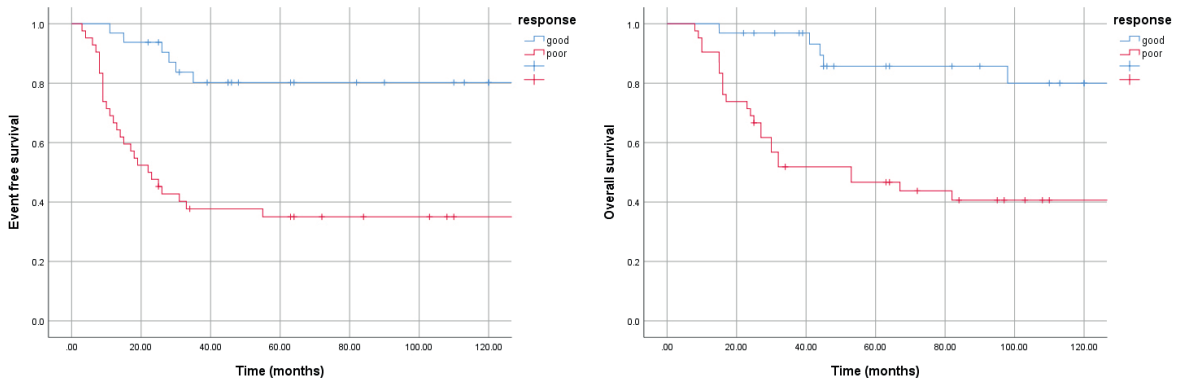

Fig. 4. Survival analysis of all patients according to the histologic response.

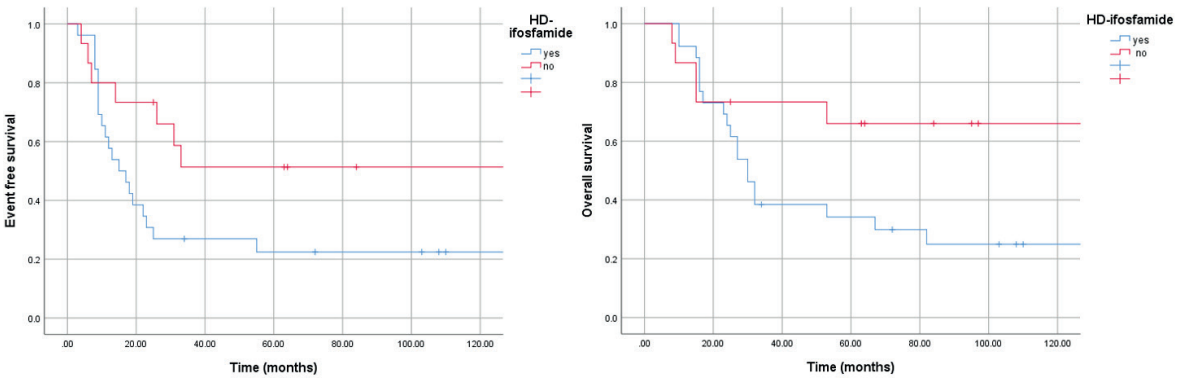

Fig. 5. Survival analysis of patients treated with HD-ifosfamide.

no difference in EFS and OS rates of patients treated with high-dose ifosfamide (Fig. 5).

Mifamurtide was given to 16 patients (10 female, 6 male). Of these, 13 had non-metastatic (8 good response, 5 poor response), and 3 had metastatic disease (all good response). The 5-year EFS and OS rates were 78.8% and 91.7% for the mifamurtide group, and 55.1% and 45.9%, for the non-mifamurtide group (p=0.015 vs. p=0.027), respectively. To evaluate the efficacy of mifamurtide treatment, we evaluated the patients in the EURAMOS treatment protocol because, in the Mayo Pilot II study protocol, only 2/59 patients received mifamurtide. In the EURAMOS treatment group (n=20), 5-year EFS and OS in the mifamurtide group (n=14) were

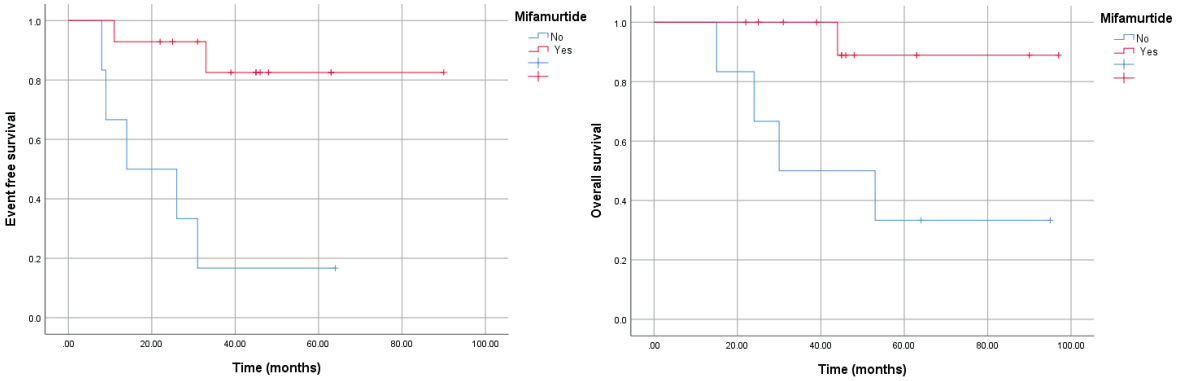

Fig. 6. Effect of mifamurtide in survival analysis of patients in EURAMOS treatment group.

82.5% and 100%, whereas 5-year EFS and OS in the non-mifamurtide group (n=6) were 16.7% and 33.3%, respectively (p=0.001 vs. p=0.003) (Fig. 6.). There were five patients (83.3%) with tumor relapse in the non-mifamurtide group and two patients (14.3%) with tumor relapse in the mifamurtide group (p=0.007). From these patients in the poor response group, 5-year EFS and OS in the mifamurtide group were 50% and 100%, while 5-year EFS and OS in the non-mifamurtide group were 16.7% and 33.3%, respectively. From 16 patients with mifamurtide treatment, 15 patients were alive (3 metastatic at diagnosis), and only one patient died from the poor response group.

Discussion

This study retrospectively reviewed 79 patients diagnosed and treated with osteosarcoma at Ege University Hospital over 25 years. One of the important outcomes of our study was that females had a significantly better outcomes than the males. Most of the studies in the literature reported no significant effect of gender on survival. However, following our results, the EURAMOS-1 study protocol reported a more favorable outcome for females.[12] Two other studies, one of them being a systematic review of 40 studies, reported that females experienced significantly higher overall survival rates than males.[13,14] On the other hand, these two series reported more favorable prognoses for younger patients. Our study did not detect any significant relationship between age groups and survival (<10 yrs vs. ≥10 yrs). The poorer prognosis of male patients might be related to several factors. Firstly, osteosarcoma is more common in males. Furthermore, since females generally reach puberty earlier than males, the peak incidence of osteosarcoma is seen in females at younger ages. As reported in previous studies, the prognosis is better in younger patients. All of these bring to mind the effect of hormonal activity and its effect on skeletal growth. However, the interaction of gender and age has never been formally studied in osteosarcoma patients.

One of the differences in our study was the high rate of lung metastases at diagnosis. According to the literature, 15-17% of the patients were considered to have metastases at diagnosis.[15] In our study population, it was high (32%). This might be due to the late presentation of our patients. However, our patient's event-free and overall survival rates were compatible with the literature.

Between the years 1995 and 2013, we used the Mayo Pilot II Study Protocol. The backbone of chemotherapy was ifosfamide, cisplatin, and high-dose methotrexate. In 2013 we started to use the EURAMOS protocol and added mifamurtide to postoperative chemotherapy after 2016. During this period (between these two protocols), the estimated 5-year EFS and OS rates increased, but this was not statistically significant. Our results were compatible with

previous studies. The EURAMOS-1 trial was a risk-stratified randomized controlled trial investigating treatment based on histological response to preoperative chemotherapy. They reported that 5-year EFS was 54% and 5-year OS 71%.[12] In our patient group with the same treatment protocol after 2013, 5-year EFS was 60.9% and 5-year OS 71.8%. We could not compare our results with the Mayo Pilot II Study Protocol as there was no published data (personal communication with Dr. Carola Arndt).

Increasing the doses of preoperative chemotherapy did not improve good histologic response and survival rates in osteosarcoma of the extremity in the study by Bacci et al.[16], including children and adults. They recommend that preoperative treatment's degree of tumor necrosis reflects an innate sensitivity to chemotherapy, which is not altered by increasing drug doses. On the other hand, intensifying chemotherapy with increased dose intensity resulted in a statistically significant increase in favorable histologic response rate, but not in increased progression-free or overall survival.[5]

In our study population, the histological response is one of the strongest predictors of survival. Patients with a poor response to preoperative chemotherapy have a worse survival rate than those with a good response. The chemotherapy regimen in the postoperative period is controversial, especially for poor responders. Several studies suggest that altering postoperative chemotherapy might improve the outcome for patients with a poor histological response. We treated most of the poor responders (n=26) with high-dose ifosfamide in our study group. Our results showed no difference in EFS and OS rates of patients treated with high-dose ifosfamide.

Similarly, the EURAMOS-1 study results showed that event-free survival did not differ with the addition of ifosfamide-etoposide to postoperative chemotherapy in patients with poorly responding osteosarcoma.[17] Another randomized controlled trial from the Italian Sarcoma Study Group evaluated the addition of ifosfamide to postoperative chemotherapy for poor responders. There was no significant difference in survival rates with the addition of ifosfamide.[18] Also, postoperative intensification with high-dose cyclophosphamide and melphalan in patients with localized osteosarcoma with poor histological response did not improve survival.[19] As a result, intensification of postoperative chemotherapy did not impact survival rates.

Other study groups using different 3- or 4-drug schedules from these same active drugs have reported similar results. Therefore ifosfamide was recommended for patients with a poor histologic response to methotrexate, cisplatin, and doxorubicin.[18-20] In the EURAMOS treatment protocol, preoperative chemotherapy consisted of three drugs (MAP), and the time of surgery was earlier. In our study, the patients in the EURAMOS group had better EFS and OS rates than the Mayo Pilot II Study group; preoperative chemotherapy consisted of four drugs (MAP plus ifosfamide).

Similar conclusions might be drawn from the INT- 0133 study.[9] In this Children's Oncology Group study, patients treated with MAP had a better 5-year EFS of 64% than patients treated with the four-drug combination (MAP plus ifosfamide), who had a 6-year EFS of 58%. They reported that the addition of ifosfamide to cisplatin, doxorubicin, and methotrexate did not enhance EFS or OS for patients with osteosarcoma.

In recent years, the addition of mifamurtide to postoperative chemotherapy after surgery was reported with a statistically significant effect on the overall survival of non-metastatic patients. Meyers et al.[9] reported that the addition of MTP to chemotherapy resulted in a statistically significant improvement in overall survival and a better EFS. Múdry et al.[21] analyzed the treatment results of patients with localized osteosarcoma treated with or without mifamurtide. They reported significantly

better EFS and PFS for mifamurtide group. According to these, mifamurtide addition could be a promising treatment option for localized osteosarcoma. However, it is not clear as to whether it should be added to metastatic patients. Chou et al.[10] reported that the 5-year EFS in the metastatic patients who had received mifamurtide with the chemotherapy regimen was 42% compared to 26% for patients who had received chemotherapy alone; however, this was not statistically significant. Since 2016, we have used mifamurtide with chemotherapy in the postoperative period. As our study was a retrospective study, and we started mifamurtide treatment at the same period as the EURAMOS treatment protocol, we have a limited number of patients to evaluate the effect of mifamurtide.

Nevertheless, survival rates were significantly higher in the mifamurtide group. Again relapse rate was significantly lower in the mifamurtide group with a median 45-month follow-up time. In the EURAMOS treatment group, only three patients with a poor response group had mifamurtide treatment. The number of patients was insufficient to evaluate mifamurtide's effect on EFS and OS rates. Our results were similar to another study from our country. Taçyıldız et al.[22] reported that adding mifamurtide in the high-risk group decreased the probability of distant metastasis, increased the median time to distant metastasis, and improved event-free survival.

In conclusion, we had a broad experience with 79 patients with osteosarcoma. Treatment seems to be more successful in females. Limb salvage surgery rate is greater than amputation in our series. International treatment protocols such as Mayo Pilot II Study Protocol and EURAMOS give similar results for non-metastatic and metastatic patients in collaboration with experienced orthopedic surgeons and pediatric oncologists. The addition of ifosfamide to the postoperative period does not influence the outcome. Our limited experience with mifamurtide addition seems promising, but it should be tested in large randomized studies.

Ethical approval

Ege University Ethics Committee approved the study (report number: 22-4T/55). Informed consent form was obtained from the parents.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: EA, MK; data collection: ŞÖG, HK, İT, BK, MA, BD, DS, ZB; analysis and interpretation of results: EA, ŞÖG, MK; draft manuscript preparation: EA. 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

Risk factors for coronary arterial involvement in Turkish children with Kawasaki disease: a multicenter retrospective study

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ABSTRACT

Background. Coronary arterial lesions (CALs) are the major component of Kawasaki disease (KD), associated with significant morbidity, which affect a substantial proportion of patients despite proper treatment. The aim of this study was to define the risk factors for CALs in Turkish children with KD.

Methods. Medical records of 399 KD patients from five pediatric rheumatology centers in Turkey were reviewed retrospectively. Demographic, clinical (including duration of fever before intravenous immunoglobulin [IVIG] and resistance to IVIG), laboratory and echocardiographic data were noted.

Results. The patients with CALs were younger, had a higher male ratio and a longer duration of fever before IVIG. They also had higher lymphocyte and lower hemoglobin values before the initial treatment. Multiple logistic regression analyses defined the following three criteria as independent risk factors for predicting CALs in Turkish children with KD: age ≤12 months, male gender and duration of fever before IVIG ≥9.5 days. High sensitivity rates of elevated risk of CALs up to 94.5% were calculated despite specificity values falling to 16.5%, depending on which of these three parameters are taken into account.

Conclusions. Based on the demographic and clinical features, we established an easily applicable risk-scoring system for predicting CALs in Turkish children with KD. This may be useful for choosing appropriate treatment and follow-up for KD to prevent coronary artery involvement. Further studies will show whether these risk factors can be used in other Caucasian populations as well.

Key words: coronary arterial involvement, intravenous immunoglobulin, Kawasaki disease, risk factors, Turkish children.

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Kawasaki disease (KD), also known as mucocutaneous lymph node syndrome, is a febrile vasculitis of the infancy period, that affects small and medium sized vessels including the coronary arteries.[1] Cardiovascular involvement may be irreversible and account for morbidity and mortality, while symptoms

such as prolonged fever, cervical adenitis, non-purulent conjunctivitis, oral mucosal changes and enanthems are self-limiting and reversible.[2-4] Nearly one quarter of children (15-25%) with KD suffer from coronary arterial lesions (CALs) if untreated.[5,6] Intravenous immunoglobulin (IVIG) is effective for the control of clinical signs and decrease in the rate of CALs, due to its immunomodulatory effects.[7] Unfortunately, CALs develop in nearly 10% of patients despite appropriate treatment.[1,8]

Previous studies in the literature defined several risk factors such as younger age, male gender, treatment delay, resistance to initial IVIG, high acute phase reactants and low albumin levels regarding increased risk of CALs.[9-14] Recent studies defined more specific parameters including brain natriuretic peptide (pro-BNP), immunoglobulin M, von-Willebrand factor and inflammatory cytokine levels along with sensitive measurement of coronary arteries, for defining increased risk.[14-18] However, clinicians need easily applicable and low-cost risk score systems to identify patients with increased risk of CALs as soon as possible.

On the other hand, different geographical regions of the world such as Europe, the Middle-East, Far-East and America should define their own self-risk-scoring systems, as current risk assessments are incapable of predicting CALs for all populations due to genetic and environmental differences.[5,6,13,19-27]

Recent studies about KD in Turkey reported more frequent CALs rates compared to Far-East populations.[4,6,14,24-27] Similar risk factors including IVIG resistance and treatment delay along with some laboratory parameters such as lower hematocrit with higher white blood cells (WBC) levels were defined for increased development of CALs.[26,27] However, these studies lack a risk scoring system for the development of CALs.

The objective of the present study was to define an easily applicable and cost-effective risk scoring system for the development of CALs in Turkish children with KD.

Material and Methods

Patients and Definition of KD

Medical records of 399 Kawasaki patients [233 boys (58.3%) and 166 girls (41.7%)] from five pediatric rheumatology centers in different regions of Turkey (Dokuz Eylul University from Izmir, Hacettepe University from Ankara, Cerrahpasa and Capa Faculty of Medicine of Istanbul University, and Umraniye Training and Research Hospital from Istanbul) who received IVIG treatment between 1990 and 2020 were reviewed in this study. The diagnosis of KD was made using previously defined clinical criteria including prolonged fever (>5 days), exanthema, mucosal changes of oral cavity, bilateral non-exudative conjunctival injections, changes in the peripheral extremities and acute non-suppurative cervical lymphadenopathy.[28] Patients, who met at least five of the six criteria were diagnosed as complete KD (cKD), while incomplete KD (iKD) was defined as having four or less.[29] Regarding demographical and clinical features, age, sex and duration of fever before initial IVIG treatment were also recorded.

Treatment Regimens and Terms of IVIG Resistance

High dose IVIG (2 g/kg) with high dose acetylsalicylic acid (80-100 mg/kg/day) were administered to all patients as the first line treatment. Resistance to IVIG treatment was defined as persistent fever 48 h after administration of the first dose.[23,30] Patients who were defined as IVIG resistant, received a second dose of IVIG, and high dose steroids (IV methylprednisolone 30 mg/kg dose), where appropriate, as second line treatment. Sixty-one patients (15.3%) were resistant to initial IVIG treatment in this study.

Laboratory Assessment

The following laboratory results were recorded from the laboratory records of all patients regarding both of the periods before and 48 hours after IVIG: (1) complete blood count parameters (white blood cells [WBC], absolute

neutrophil count [Neu], absolute lymphocyte count [Lym], hemoglobin levels [Hb], absolute platelet count [Plt], mean platelet volume-[MPV]) and (2) acute phase reactants (C-reactive protein [CRP] and erythrocyte sedimentation rate [ESR]). (3) Biochemical parameters (serum albumin [Alb] and total bilirubin [T-bil], alanine and aspartate aminotransferase [ALT and AST], and electrolyte levels of sodium [Na], potassium [K] and calcium [Ca]).

Neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratios (PLR) were calculated from the obtained data.

Echocardiographic Assessment and Definition of CALs

Pediatric cardiologists examined all patients, and echocardiographic assessment was performed at least twice; at the time of diagnosis and in the subacute phase (two weeks after initial IVIG treatment). CALs were defined by using echocardiographic measurement criteria, which were established by the Japanese Ministry of Health and Welfare Guidelines.[31] According to echocardiographic findings, patients with perivascular echogenicity, ectasia/dilatation and aneurysm formation were recorded as CALs positive in order of severity of lesions, respectively. Increased echogenicity of pericoronary tissue minus blood pool was defined as perivascular echogenicity. If the internal diameter was up to 1.5 times that of the adjacent segment, it was defined as coronary arterial ectasia. Furthermore, internal diameters over 4 mm or enlargements over 1.5 times the size of the adjacent segment (for ≥5 years old patients) were accepted as coronary arterial aneurism. Coronary artery involvement was determined in 126 patients (31.5%) prior to initial IVIG treatment.

The ethical approval was obtained from Dokuz Eylul University Ethics Committee (number: 2021/12-31).

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 22 software. Kolmogorov-Smirnov test was performed to evaluate the normality of the variables. Normally distributed variables were presented as mean ± standard deviation, while heterogeneously distributed ones were as median and interquartile ranges (IQR 25-75). Chi-square test was used to examine differences between categorical variables. Normally distributed variables were compared by independent t-test, while non-normally distributed variables by Mann-Whitney U test. We used multiple logistic regression with stepwise backward elimination method for finding the independent variables that define risk factors. Variables having statistically significant differences among groups were evaluated by receiver operating characteristic (ROC) curves to determine the optimal cut-off values and area under curves (AUC). Subsequently, independent risk factors for CALs were determined, and the odds ratio (OR) and 95% confidence interval (CI) were calculated by the multiple logistic regression analysis. $p<0.05$ was considered as statistically significant.

Results

The medical records of 233 boys (58.3%) and 166 girls (41.7%) were included in this study. The median age at the time of diagnosis was 32 (17-54) months. Regarding the clinical type of KD, 252 patients (63.2%) were defined as cKD and 147 (36.8%) were iKD. The median duration of fever before the first IVIG treatment was 7 (5-10) days. In 126 patients (31.6%), CALs were determined in the first echocardiographic assessment, and resistance to initial IVIG treatment was observed in 61 patients (15.3%). There were no statistically significant differences between the IVIG responsive and resistant groups in terms of age, sex, frequency of CALs and the clinical type of KD.

Patients from the CALs positive and negative groups were compared regarding demographical and clinical findings. Patients from the CALs positive group had younger age, male predominance and a longer duration of fever before treatment when compared to the CALs negative group ($p<0.05$ for all parameters) (Table I).

In terms of laboratory parameters prior to the first IVIG treatment, the CALs positive group had lower hemoglobin ($p=0.006$) and higher lymphocyte ($p=0.048$) values than the CALs negative group. In further evaluation two days following IVIG treatment, we found higher WBC, Lym and lower Hb, MPV, bilirubin and potassium levels in the CALs positive group ($p<0.05$ for all parameters). Acute phase reactant levels such as ESH and CRP were higher in the CALs positive group both before and after treatment periods; however, differences were not statistically significant (Table II).

Multiple logistic regression analysis was performed with the possible risk factors which were determined in the univariate analysis. Variables such as gender, age, Hb, Lym and duration of fever before IVIG prior to the treatment period and WBC, Lym, Hb, MPV, bilirubin and potassium following the treatment period were evaluated. The variables including gender, age and duration of fever before IVIG were determined as independent risk factors of CALs in the multiple analysis with the backward elimination technique. The predictive value of the variables revealed male gender ($p<0.001$), age ($p=0.002$) and duration of fever prior to the first IVIG treatment ($p=0.036$) as independent predictors of CALs. When we applied a ROC analysis to numerical variables, the best cut-off values were calculated as duration of fever ≥9.5 days and age ≤12 months. Based on these cut-off values, binary logistic regression analysis was applied for each parameter, and odds ratios were calculated as 2.525 for male gender, 3.112 for age ≤12 months and 2.084 for duration of fever before initial IVIG ≥ 9.5 days (Table III). The final models' results were presented including odds ratios, 95% confidence intervals and p values in Table III and the results of the ROC curve analyses including optimal cut-off value, sensitivity, specificity, PPV, NPV and accuracy parameters for each variable and their binary and triple combinations were presented separately in Table IV.

Discussion

Coronary arterial involvement represents the major contributor to morbidity and mortality related to KD, and the main focus of researchers is to predict children at high risk for this complication. However, risk factors are not universal and may vary between populations due to genetic and environmental differences. Despite higher rates of CALs reported in some studies of Turkish children, there was, to the best of our knowledge, no defined risk scoring system.[25-28,32-34] In the current study, we defined an easily applicable risk scoring system prior to treatment for Turkish children, by using multicenter data, including only clinical features such as age, gender and duration of fever.

The current American Heart Association guidelines for KD recommend that younger infants should be evaluated as a risk group for cardiovascular involvement, and

Table I. Comparison of demographic and clinical data between CALs (+) and (-) groups.

Characteristics	CALs (+)	CALs (-)	p value
Gender (male/female)	2.40 (89/37)	1.11 (144/129)	<0.001
Age (months)*	27.5 (12-45)	36 (19.5-56.5)	0.002
Clinical type (complete/incomplete)	80/46	172/101	0.508
IVIG resistance, n (%)	22 (17%)	39 (14%)	0.249
Duration of fever before IVIG treatment (days)**	9.57 ± 6.11	7.89 ± 4.04	0.036

*median (IQR 25-75), ** mean ± SD. CALs: coronary arterial lesions, IVIG: intravenous immunoglobulin.

Table II. Comparison of laboratory parameters between coronary arterial lesions (+) and (-) groups.

Parameters	Before IVIG treatment			2 days after IVIG treatment		
	CALs (+)	CALs (-)	p value	CALs (+)	CALs (-)	p value
WBC (10^3/μL)	15.2 (11.8-17.6)	14.5 (14.4-18.3)	0.455	16.1 ± 7.3	10.4 ± 4.2	0.023
Neu (10^3/μL)	8.1 (6.6-11.9)	8.9 (6.5-13.2)	0.965	5.1 (4.6-5.8)	4.2 (1.8-6.7)	0.907
Lym (10^3/μL)	3.2 (2.3-5.5)	2.9 (1.8-4.5)	0.048	7.8 (3.3-9.1)	4 (2.9-5.8)	0.030
Platelet (10^3/μL)	386 (298-582)	395 (306-560)	0.258	442 (441-444)	492 (334-696)	0.156
Hb (g/dL)	10.3 ± 1.5	11 ± 1.3	0.006	8.45 ± 1.34	10.7 ± 0.92	0.029
NLR (Neu/Lym)	2.07 (1.2-3.97)	2.54 (1.4-4.9)	0.098	1.02 (0.37-1.26)	1.07 (0.31-1.98)	0.138
PLR (Plt/Lym)	120.5 (89-177.5)	139.2 (91-250)	0.313	84.6 (35.7-101)	118 (85-155)	0.091
MPV (fL)	7.3 (6.7-7.9)	7.1 (6.6-7.9)	0.938	7.05 ± 0.63	7.4 ± 0.56	0.010
ESH (mm/h)	68 (42-90.2)	63 (40-80)	0.786	124.5 (110-160)	59 (36-10.5)	0.532
CRP (mg/L)	82 (25-141)	68 (22.8-146.5)	0.550	48.4 (12.5-62)	15.6 (6.55-25)	0.888
ALT (U/L)	22.5 (13-52.2)	36 (16-80)	0.205	27.5 (8-32)	26 (20-37)	0.902
AST (U/L)	31.5 (21-46.5)	34 (25-57)	0.102	38 (19-46)	41 (22-54)	0.583
T. bilirubin (mg/dL)	0.28 (0.21-0.6)	0.41 (0.25-0.75)	0.004	0.27 (0.26-0.29)	0.39 (0.33-0.55)	0.03
Albumin (g/dL)	3.54 ± 0.56	3.6 ± 0.49	0.147	3 (2.8-3.4)	3.5 (3.2-3.6)	0.770
Sodium (mmol/L)	136 (135-137)	135 (133-137)	0.060	134.5 ± 2.1	138 ± 2.4	0.365
Potassium (mmol/L)	4.46 ± 0.66	4.32 ± 0.62	0.275	4.2 ± 0.84	4.7 ± 0.37	0.008
Caicium (mg/dL)	9 (8.6-9.5)	9.1 (8.8-9.6)	0.873	8.86 ± 0.47	9.4 ± 0.55	0.817

Data are presented as median (IQR 25-75) or mean ± standard deviation.
ALT: alanine aminotransferase, AST: aspartate aminotransferase, CALs: coronary arterial lesions, CRP: C-reactive protein, ESH: erythrocyte sedimentation rate, Hb: hemoglobin, Lym: lymphocyte count, MPV: mean platelet volume, Neu: neutrophil count, NLR: neutrophil/lymphocyte ratio, PLR: platelet/lymphocyte ratio, T. bilirubin: total bilirubin, WBC: white blood cells.

Table III. Multiple logistic regression analysis of predicting factors for coronary arterial lesions.

Parameters	Cut-off value	β	SE	Wald	df	Sig	Exp B	%95 Confidence interval	
								Lower	Upper
Gender (male)		0.926	0.243	14.547	1	<0.001	2.525	1.569	4.065
Age	≤12 months	1.135	0.308	13.572	1	<0.001	3.112	1.701	5.693
Duration of fever before IVIG	≥ 9.5 days	0.735	0.241	9.270	1	0.002	2.084	1.299	3.343

IVIG: intravenous immunoglobulin.

echocardiographic assessment should be performed even if diagnostic criteria are not fulfilled.[1] McCrindle et al.[10] reported that younger age at presentation was the most risk-increasing factor in their CALs prediction scoring system. Also, in a study from China[34], age under 12 months was defined as an independent risk factor. In the current study, our results were consistent with the literature, and patients under one-year old were associated with an elevated risk, which had an OR of 3.11. Additionally, male gender had a higher prevalence (58.3%) and nearly two and a half times increased risk of CALs. In a study from the USA, Callinan et al.[35] reported that male patients with KD had a higher risk, accompanied by a younger age, Asian race and delayed treatment. Another multicenter study from Spain[36] also emphasized male gender as an increased risk for developing CALs in the course of KD. Similarly, Qiu et al.[14] defined female sex as an independent protective factor from CALs development.

Table IV. Results of receiver operating characteristic (ROC) analysis of predicting factors for coronary arterial lesions.

Variables and cut-off values	Sensitivity (%)	Specificity (%)	PPV	NPV	Accuracy (%)
• Male gender	47.3	70.6%	0.408	0.777	54.4
• Age ≤12 months	87.3	26.4%	0.478	0.717	67.6
• Duration of fever before IVIG ≥9.5 days	74.4	37.2%	0.381	0.724	62.2
• Age ≤12 months • Male gender	94.5	16.7%	0.583	0.711	69.9
• Age ≤12 months • Duration of fever before IVIG ≥9.5 days	91.9	16.5%	0.476	0.712	68.7
• Male gender • Duration of fever before IVIG ≥9.5 days	71.0	47.1%	0.331	0.577	57.4
• Male gender • Age ≤12 months • Duration of fever before IVIG ≥9.5 days	70.6	29.3%	0.361	0.698	63.2

IVIG: intravenous immunoglobulin, NPV: negative predictive value, PPV: positive predictive value.

The main goal in KD treatment is to control the vascular inflammation in its earliest stages, since a time lag in the administration of IVIG could lead to an increased risk for CALs. The duration of the febrile period prior to therapy in this regard has been the focus of attention. Previously reported studies emphasized the related parameter and reported that the duration of the febrile period before treatment was positively correlated with the risk of coronary artery involvement and resistance to IVIG. This study also found that patients who received initial IVIG treatment later than 9.5 days had a doubled risk of CALs development. Our recent single-center study[27] reported the same critical cut-off value for the duration of fever before IVIG treatment, which was associated with both CALs and IVIG resistance. A similar result by Bal et al.[37] reported this cut-off value as 10 days. On the contrary, the well-known scoring systems from Japan such as Kobayashi et al.[38] and Egami et al.[39] reported a shorter duration of fever (≤4 days) had increased risk for IVIG resistance, leading to CALs development. They speculated that the patients treated in the early period had severe clinic and greater inflammation. We consider that delayed treatment causes a prolonged inflammation of vessel walls and increases risks for CALs.

A strong correlation between the risk of CALs development and IVIG resistance was reported in several studies by many researchers. Kobayashi et al.[38] defined a risk score system for predicting IVIG resistance by using elevated liver function tests, hyponatremia, thrombocytopenia, younger age and higher acute phase reactants. They also defined these risk factors for CALs development, because of the strong correlation between these two complications.[38] Similarly, in a study from the USA, Ghelani et al.[12] reported a higher incidence of CALs in IVIG resistant patients with an OR of 5.27. Our previous study as a single center also showed a positive correlation between these parameters; however, the current multicenter study did not find a statistically significant correlation between these two complications, unlike the literature. Thus, our risk scoring system mainly depends on the risk for CALs development, independent of IVIG resistance. It might be helpful for the early administration of additional treatments, such as steroids and/or biologics to prevent severe coronary arterial involvement.[40,41]

Although there is not a specific biomarker defined for KD and its complications, there has been a great focus on many laboratory markers

and their threshold values for predicting increased CALs development. Higher ESR and CRP values were associated with elevated risks for CALs indicating severe inflammation.[11-15,42,43] Hematological parameters such as lower hemoglobin and hematocrit levels, and elevated leukocyte and platelet counts were reported as positively correlated, as well.[15,34,43,44] In this study we found lower hemoglobin and higher lymphocyte counts, with elevated ESR and CRP levels prior to IVIG treatment in the CALs positive group; however, they were inadequate to be included in the risk scoring system.

In conclusion, based on clinical and demographical data, we presented a new scoring system to predict CALs in Turkish children: younger age (≤12 months), male gender and duration of fever before IVIG (≥ 9.5 days). We suggest that our score is easily applicable and has high sensitivity, although the specificity is low. Further prospective studies are needed to assess its performance in other ethnic groups and the correlation with possible biomarkers.

Ethical approval

The ethical approval was obtained from Dokuz Eylul University Ethics Committee (number 2021/12-31).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: ST, BM, EÜ; data collection: ST, ÜAK, FÇ, FH, FD, TK, NÜ; analysis and interpretation of results: ST, BM, EÜ, SÖ, EK, YB, NAA, BS, ÖK; draft manuscript preparation: ST, UAK, FÇ, FH, FD, EK, TK, NÜ; revising the manuscript critically: BM, YB, EÜ, BS, NAA, ÖK, SÖ, EK. 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

Evaluation of thymopoiesis in healthy Turkish children aged 0-6 years

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ABSTRACT

Background. Early diagnosis and effective treatment serve as life-saving procedures for primary immunodeficiencies (PIDs) which are very common and a major public health problem in Turkey. Severe combined immunodeficiency (SCID) is constitutively a T-cell defect in which naïve T-cell development is defective due to the mutations in genes responsible for the T cell differentiation and insufficient thymopoiesis. So, assessment of thymopoiesis is very important in the diagnosis of SCID and several combined immune deficiencies (CIDs).

Methods. The purpose of this study is to examine thymopoiesis in healthy children via measurement of recent thymic emigrants (RTE); T lymphocytes that express CD4, CD45RA and CD31 to establish the RTE reference values in Turkish children. RTE were measured in the peripheral blood (PB) of 120 healthy infants and children between 0-6 years including cord blood samples, by flow cytometry.

Results. The absolute count of RTE cells and their relative ratios were found to be higher during the first year of life, being highest at the 6th month and tending to decrease significantly by age following birth (p=0.001). In the cord blood group, both values were lower than those in the 6-month-old group. The absolute lymphocyte count (ALC) varying by age, was found to reduce to 1850/mm^3 in 4-years and after.

Conclusions. Here we evaluated normal thymopoiesis and established the normal reference levels of RTE cells in the peripheral blood of healthy children aged between 0-6 years. We believe that the collected data will contribute to early diagnosis and monitoring of immune reconstitution; serving as an additional fast and reliable marker for many PID patients especially for SCID including many other CIDs, especially in nations where newborn screening (NBS) via T cell receptor excision circles (TREC) has not yet become available.

Key words: recent thymic emigrants (RTE), thymopoiesis, flow cytometry.

Primary immune deficiencies (PIDs) are a heterogeneous group of diseases that emerge as a result of mutations in the genes that code the components of the immune system. According to the classification of the International Union of Immunological Societies (IUIS) that was revised in 2020, immunodeficiencies are divided into 10 groups.[1] Due to the higher rates of parental consanguinity in Turkey, combined immunodeficiencies (CID) inherited as an autosomal recessive pattern are seen more frequently.[2,3] Among CID diseases, severe combined immunodeficiencies (SCID) are a pediatric emergency that result in mortality in cases where it is not diagnosed early and treated appropriately. SCID is mainly a

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developmental disorder of T-cells. About 20 genetic defects that lead to this disease have been defined.[4] Screening of newborn patients during their asymptomatic period will provide opportunities for early diagnosis and treatment. Early diagnosis and treatment may be lifesaving in SCID and other CIDs.

Absent or low autologous naïve T-cell count is the hallmark of SCID and in most CID due to abnormal and insufficient thymopoiesis. T cell receptor excition circles (TREC) measurement is the most reliable and appropriate method in the diagnosis of SCID and CID. TREC is an indicator of thymopoiesis, and it has been used in the United States of America since 2008 for screening of SCID and early diagnosis of it from heel blood samples collected from newborns.[5]

Today, thymopoiesis, namely the function of the thymus gland, can be measured by the evaluation of recent thymic emigrants (RTE). RTE can be assessed by two different methods. The first is the enumeration of TREC copy numbers via molecular assay which involve the measurement of DNA residues with circular structures that remain from the recombination of V(D)J genes during the molecular development of T-cell receptors. The second is the measurement of naïve T cell levels expressing CD4, CD45RA and CD31 together on their surfaces by flow cytometry. These cells have high TREC contents.

Since peripheral blood (PB) lymphocyte subtype analysis is a very important and informative tool for the diagnostic immune work-up in most PIDs mainly CID, RTE enumeration should also be included in the basic diagnostic panel of flow cytometric analysis of PIDs.

This study aims to examine thymopoiesis in a cross-sectional manner in healthy children from birth to age 6 years and to obtain RTE reference levels and counts by enumerating CD4, CD45RA, CD31 expressing T cells in PB in healthy children between 0-6 years. We believe that the collected data will contribute to early diagnosis and monitoring of immune reconstitution serving as an additional fast and reliable marker for many PID patients especially for CID including SCID, particularly in nations where NBS via TREC has not become available yet.

Material and Methods

Characteristics of Healthy Subjects

A total of 120 healthy children aged between 0-6 years who had normal developmental features and were free from any sign or symptom of acute infections; 20 in each of 6 subgroups (cord blood, 6 months, 1 year, 2 years, 4 years and 6 years) were included to the study during the period of February 2017 to December 2017.

Those who had a past personal history or family history of PID or other chronic diseases receiving immunosuppressive agents were excluded from the study.

Cord blood samples were collected during normal vaginal delivery of term at gestation newborns who were born to pregnant women without any known chronic disease or infection at the Gynecology and Obstetrics Department. Those with a history of Cesarean section or perinatal asphyxia (5th-minute APGAR score of <7), those with clinical (hypo-hyperthermia, apnea-respiratory disorder, lethargy, color change in the skin) and laboratory (leukopenia <5000/mm^3 or leukocytosis >25000/mm^3) left-shifted peripheral blood smear (immature-to-total neutrophil ratio of >20%) finding indicating infection or those with infection proven by a positive culture, those with Hb values of<12.5 gr/dl and anemia and/or requirement of blood exchange, those with ABO and Rh-Rh incompatibility and infants with congenital anomalies were excluded from the study.

This study was approved by the Ethics Committee of Ankara University on 23.01.2017. Written informed consent was obtained from the parents of all infants and children.

Peripheral Blood (PB) Sampling

2 ml of peripheral blood or cord blood was collected to Ethylenediaminetetraacetic acid (EDTA) containing tubes in all children and given to the laboratory immediately. Whole blood count (leukocytes and absolute lymphocyte counts (ALC) and RTE levels were measured simultaneously in all samples.

Flow Cytometric Analysis of RTE

The RTE measurements were carried out via flow cytometer (NAVIOS, Beckman Coulter, USA) by 4-color staining using the fresh PB samples and whole blood lysis method at the Immunology Research Laboratory of the Department of Pediatric Immunology-Allergy at Ankara University Medical School. In the CD4+ cell population within the CD45/SS gate, CD45RA+CD31+ cell levels were measured. Flow cytometric data were analyzed by Kaluza 1.3 software (Beckman Coulter, USA).

Statistical Analysis

The data were analyzed at the Department of Biostatistics at Ankara University Medical School by using the IBM SPSS Statistics 15.0 (IBM Corp., Armonk, New York, USA) software. The descriptive statistics included frequencies (n), percentages (%), mean ± standard deviation, medians, 5th percentile and 95th percentile values. Pearson's chi-square and Fisher's exact tests were used to analyze the categorical variables. The normal distribution of the numerical variables was analyzed by the Shapiro Wilk normality test and Q-Q plots. Two groups were compared by independent-samples t-test for the normally distributed variables and by Mann Whitney U test for the non-normally distributed variables. $p<0.05$ was accepted to be statistically significant.

Results

The age and sex characteristics of the age-based 6 subgroups are shown in Fig. 1. The median and 5-95% intervals of white blood cell, absolute lymphocyte counts and CD4+ T cell percentages of all children are given in Table I.

The absolute lymphocyte count (ALC) was also found to vary based on age. In the 6-month-old and 1-year-old age groups, ALC was 3132/mm³ and 3056/mm³ at the 5% lower limit

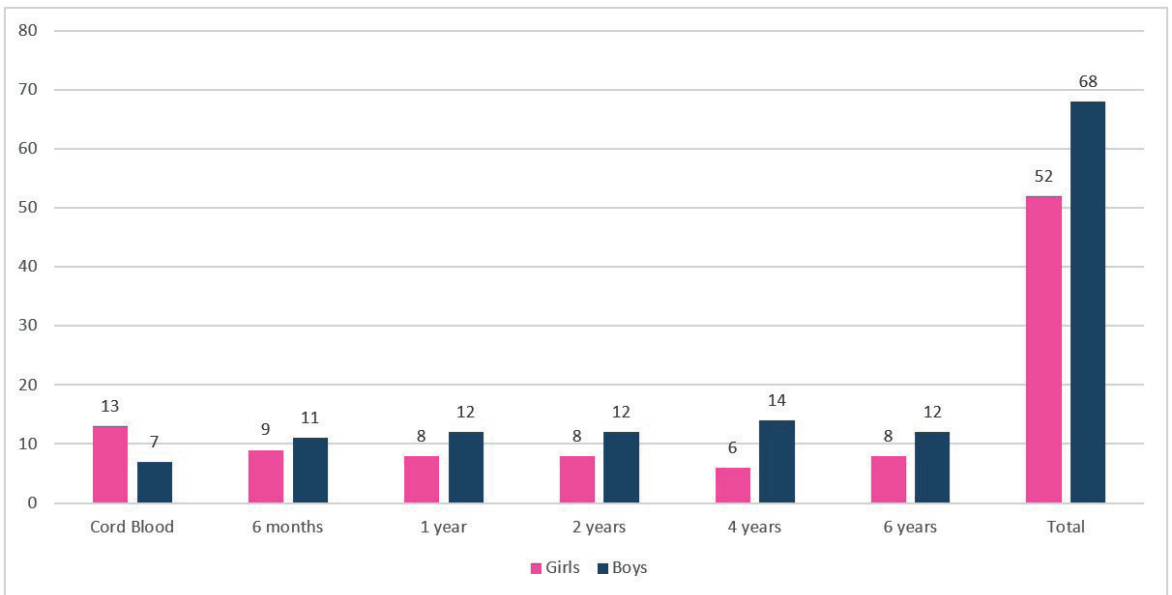

Fig. 1. The age and sex characteristics of 6 subgroups.

Table I. WBC, ALC and CD4+ T cell percentages in peripheral blood based on age.

		CB	6 months	1 year	2 years	4 years	6 years	p
WBC/mm³	Median	12935	8535	9410	8260	8215	7940	p=0.001
			p=0.001	ns	ns	ns	ns	
	5%	5967	5901	5770	6521	4652	4640	
	95%	27545	17071	13356	11555	11922	16508	
ALC/mm³	Median	3930	5260	5385	4865	3395	3105	p=0.001
			p=0.012	ns	ns	p=0.002	ns	
	5%	2484	3132	3056	2870	1850	1881	
	95%	8949	12092	9652	7747	5784	7244	
CD4+ T cells (%)	Median	50.68	51.55	45.60	39.80	39.45	39.83	p=0.001
			ns	ns	ns	ns	ns	
	5%	27.22	33.63	32.43	28.26	28.38	30.59	
	95%	63.07	62.35	58.69	51.23	52.04	51.08	

* p>0,05: ALC: absolute lymphocyte count, CB: cord blood, ns: not significant, WBC: white blood cells.

respectively, decreasing with the increase of age, and reducing to 1850/mm³ in the 4-year-old group and after.

The median and 5-95% intervals of relative rates of CD4+CD45RA+CD31+ cells (RTE) and absolute counts of RTE cells in all children are given in Table II.

The absolute counts of RTE and their relative rates (%) significantly (p=0.001) decreased by age; found to be highest during the first year of life, especially the 6th month (median 77% and 2243/mm³), the decrease gradually reaching median 58% and 732/mm³ (69 - 73% and 1654-406/mm³) at 6 years. The absolute count and percentage value (relative rates) of the RTE cell levels in the cord blood group were found to be lower than those in the 6-month-old group, respectively (p=0.004, p=0.001) (Fig. 2 and Fig. 3)

Table II. Absolute RTE counts and RTE percentages in peripheral blood based on age.

		CB	6 months	1 year	2 years	4 years	6 years	p
RTE (%) CD4+ CD45RA+ CD31+	Median	68.36	76.85	73.00	68.90	63.97	58.05	p=0.001
			p=0.001	ns	ns	ns	ns	
	5%	59.66	63.59	57.15	57.19	55.46	53.73	
	95%	81.09	81.79	82.37	79.36	74.47	69.89	
Absolute RTE/mm³	Median	1319	2043	1829	1377	852	732	p=0.001
			p=0.012	ns	ns	p=0.002	ns	
	5%	896	929	969	646	544	406	
	95%	2299	4981	3832	2284	1516	1654	

* p>0,05: CB: cord blood, ns: not significant, RTE: recent thymic emirgants.
Severe Combined Immunodeficiency (SCID) [Internet]. Available from: https://esid.org/Working-Parties/Clinical/Resources/Severe-Combined-Immunodeficiency-SCID.

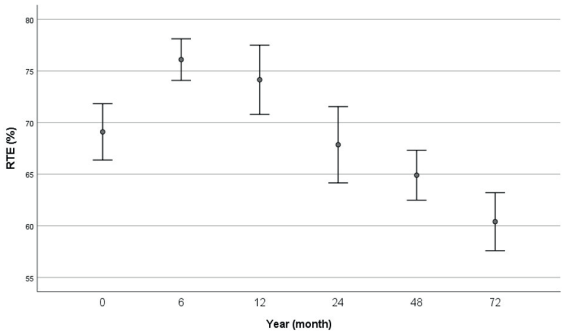

Fig. 2. Age-dependent change in recent thymic emigrant (CD4+CD45RA+CD31+) cell levels.

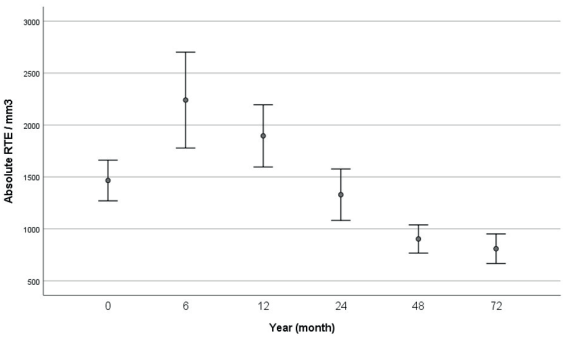

Fig. 3. Age-dependent change in absolute counts of recent thymic emigrant (CD4+CD45RA+CD31+) cells.

Discussion

TREC measurement by RT-PCR or RTE (CD4CD45RA+CD31+) measures by flow cytometry are used to assess thymopoiesis in severe combination immunodeficiency and several other combined immunodeficiencies using DNA extracted from heel blood. In our country, SCID is not yet included in the newborn screening program. Compared to the molecular enumeration of TREC by RT-PCR, the identification of RTE by flow cytometry is much faster and more valuable for the diagnostic workup of PID suspected patients. There is no information on reference values of RTE in healthy Turkish children. The aim of this study was to identify normal RTE levels in children aged 0-6 years.

In the literature, there are limited data measured in healthy infants and children, that would allow for commenting on whether the percent and absolute counts that are obtained as a result of measuring thymopoiesis immunophenotypically are normal or low. NBS of SCID with RTE measurement, was not found to be cost-effective due to the high cost and requirement of more blood samples, flow cytometry devices, and experienced personnel.[6] However, it is well known by clinical immunologists that at the stage of decision, reference values obtained from age-matched healthy children are needed for an accurate diagnostic approach. Thus, we believe that the data obtained in our study will be helpful to us and other clinical immunology staff in this respect and promote early diagnosis in SCID and CID patients.

Another result we obtained in this study was that the ALC values varied by age. In the 6-month-old and 1-year-old age groups, ALC was found to be 3132/mm³ and 3056/mm³ at the 5% lower limit, while it expectedly decreased in older age groups, and it was reduced to 1850/mm³ in the 4-year-old group and after as the 5% lower limit.

It was also shown in this study yet again that the lymphopenia limit in the first year of life is 3000/mm³ and an absolute lymphocyte count of less than 3000 per mm³ in a newborn can be used as the cutoff for consideration of a T-cell disorder.[7] Absolute lymphocyte count is age-dependent and as opposed to the neonatal period, from the age of 1 on, values under 1500/mm³ are considered lymphopenia. Six of 11 screening programs in the United States defined significant T-cell lymphopenia as a T-cell count less than 1500/μL.[8] However, the data in our study showed that ALC decreases gradually by age, and the 5% lower limit of ALC at the age of 4 years and later is 1850/mm³. In the light of these data, it was shown that the lower limit of lymphopenia was higher than 1500/mm³, which is the generally accepted lower limit, and ALC should be carefully evaluated in patients with suspicion of PID.

Schatorjé et al.[9] demonstrated that absolute T lymphocyte numbers increase 1.4-fold during the first months of life, and after 9–15 months,

they decrease threefold to adult values; this is mainly caused by the expansion of recent thymic emigrants and naive cells. Helper and cytotoxic T lymphocytes show the same pattern. In our study, PB RTE absolute counts and percentage values were significantly lower in the cord blood group in comparison to the 6-month-old group. Based on the reviews, it was also reported in the literature that the CD45RA+ naïve T-cell relative and absolute rates peak in the 6th postpartum month, and these levels in the cord blood are lower in comparison to those in 6-month-olds.[10] Not unexpectedly, our data show that RTE follow the pattern of naive helper T lymphocytes after the neonatal period. This difference in the absolute counts and relative rates of RTE cells may have been caused by the unique composition of cord blood. Nevertheless, the lymphocyte levels in cord blood are lower in comparison to newborns and breastfeeding infants.[11]

As we emphasized earlier, RTE cells contain a high level of TREC and there are several studies in the literature. Junge et al.[12] demonstrated that TREC content in CD31+CD45RA+CD4+T cells was 18 times higher than in CD31–CD45RA+CD4+lymphocytes. Garcia-Prat et al.[13] measured TREC levels with the PCR method and reported that these levels tended to decrease with age. In the study where the correlation between CD4+ naive T-cell levels and RTE and TREC was also examined, it was reported that the correlation between the CD4+ naïve T-cells and RTE (r^2 = 0.4160) was more significant than that with TREC (r^2 = 0.3013). In this study, a correlation between TREC and RTE values was detected.

With increasing age, the absolute counts of RTE cells (p=0.001) and their relative ratios (p=0.001) decreased. The main reason for this change is the structural change in the thymus tissue and involution by age. In the cross-sectional study by Schatorjé et al.[9] which examined the stages of major T-cell differentiation in the childhood period, the study emphasized that RTE increased by 1.6 times in the first 5 months of life, and then, it reached adult values by decreasing down to an eightfold. It was reported that the CD4+ T-cells of a newborn included more RTE in comparison to those in adults, and RTEs followed the distribution of naïve T lymphocytes after the newborn period.

It is known that due to the higher rates of parental consanguinity in Turkey, CID inherited as an autosomal recessive pattern are seen more frequently. The study, which was conducted in Turkey in 2019 and evaluated the usability of CD31 in the evaluation of thymic production, included 66 children with suspected immunodeficiency based on clinical and laboratory findings. The most common diagnoses among the patients were humoral immunodeficiencies (34.8%), combined immunodeficiencies (34.8%) and patients with thymus aplasia/hypoplasia or thymectomy due to cardiac surgery (7.6%). The percentages of RTE did not differ statistically between these groups (p=0.231), but the absolute numbers of RTEs were significantly lower in the CIDs group (p=0.007). At least one cardiac pathology was observed in 36.9% (n: 24) of the patients in this study. Eight patients had corrective cardiac surgery before the study. RTE cell relative ratios and absolute counts were lower in the operated group, respectively (p=0.011, p=0.032). This data shows that similar to the decrease in RTE relative ratios and absolute counts due to the development of thymic involution with age, damage to thymic tissue also causes a decrease in RTE relative ratios and absolute counts.[14]

Allogenic hematopoietic stem cell transplantation (HSCT) is mainly a curative treatment in some certain PID. The objective of HSCT in PID is to create a new and normally operating immune system by forming stable and permanent engraftment and immune reconstitution (IR). For exactly this purpose, inspection and monitoring of IR in patients after the transplantation is one of the factors that play a significant role in determining the prognosis. Likewise, longitudinal monitoring of thymopoiesis by measurement of RTE levels

after transplantation contributes greatly to the assessment of the IR after HSCT and the determination of the prognosis. In this case, the normal values obtained in this study from healthy infants and children aged 0-6 years may allow objective assessment of immune constitution after HSCT and thymopoiesis.

Similarly, the RTE cell levels in the peripheral blood of 22 SCID patients who received allogenic HSCT between the years 2008-2014 and were monitored for two years at the Department of Pediatric Immunology-Allergy at the Faculty of Medicine at Ankara University were retrospectively analyzed by examining CD4+CD45RA+CD31+ cells. It was discovered that after HSCT, RTE cell levels increased noticeably in all patients, regardless of donor type or immunophenotype, from the first to the sixth month after transplantation, and remained high and stable from the 6th month to the 2nd year. The study emphasized that measuring RTE levels in peripheral blood by measuring CD4+CD45RA+CD31+ cells in peripheral blood is a valuable indicator in assessing and monitoring immune reconstitution after HSCT.[15]

Consequently, this study in which we assessed the RTE (CD4+CD45RA+CD31+) cell levels of 120 healthy infants and children is in the position of being the first study that has been conducted in Turkey on this topic. The normal values of the RTE cells in the peripheral blood of healthy Turkish children as well as in the cord blood of 0-6 year old age groups were determined. In Turkey, where primary immunodeficiencies constitute a significant health problem, we believe that assessment of the RTE cell levels and counts of patients with pre-diagnosis of PID by comparison to the normal values of these age groups will significantly contribute to the early diagnoses and treatments of patients. Hence, the determination of CD4+CD45RA+CD31+ cells should be included in the peripheral blood lymphocyte sub-group panels at all centers that perform the diagnosis and treatment of PID patients.

Ethical approval

The study was approved by the Ethics Committee of Ankara University (Number: 02-61-17, date: 23.01.2017).

Author contribution

The authors confirm contribution to the paper as follows: Study conception and design: AK, Aİ; data collection: NECI, ST, EO, CI; analysis and interpretation of results: DB, MA, AK, SKB, SH, Aİ; draft manuscript preparation: AK, SH, FD, Aİ. 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

Expanding the clinical and molecular features of tricho-rhino-phalangeal syndrome with a novel variant

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ABSTRACT

Background. Tricho-rhino-phalangeal syndrome (TRPS) is a rare, autosomal dominant disorder characterized by typical craniofacial features, ectodermal and skeletal findings. TRPS type 1 (TRPS1) is caused by pathogenic variations in the *TRPS1* gene, which relates to the vast majority of cases. TRPS type 2 (TRPS2) is a contiguous gene deletion syndrome involving loss of functional copies of the *TRPS1, RAD21,* and *EXT1*. Herein, we reported the clinical and genetic spectrum of seven TRPS patients with a novel variant. We also reviewed the musculoskeletal and radiological findings in the literature.

Methods. Seven Turkish patients (three female, four male) from five unrelated families aged between 7 to 48 years were evaluated. The clinical diagnosis was confirmed by either molecular karyotyping or *TRPS1* sequencing analysis via next-generation sequencing.

Results. Both TRPS1 and TRPS2 patients had some common distinctive facial features and skeletal findings. All patients had a bulbous nose with hypoplastic alae nasi, brachydactyly, short metacarpals and phalanges in variable stages. Low bone mineral density (BMD) was identified in two TRPS2 family members presenting with bone fracture, and growth hormone deficiency was detected in two patients. Skeletal X-ray imaging revealed cone-shaped epiphysis of the phalanges in all, and multiple exostoses were present in three patients. Cerebral hamartoma, menometrorrhagia and long bone cysts were among the new/rare conditions. Three pathogenic variants in TRPS1 were identified in four patients from three families, including a frameshift (c.2445dup, p.Ser816GlufsTer28), one missense (c.2762G>A), and a novel splice site variant (c.2700+3A>G). We also reported a familial inheritance in TRPS2 which is known to be very rare.

Conclusions. Our study contributes to the clinical and genetic spectrum of patients with TRPS while also providing a review by comparing with previous cohort studies.

Key words: Tricho-rhino-phalangeal syndrome, *TRPS1* gene, multiple exostoses, novel mutation, TRPS2.

Tricho-rhino-phalangeal syndrome (TRPS) is a rare autosomal dominant disorder characterized by typical craniofacial features, ectodermal (hair, nail, skin, teeth), and skeletal anomalies. The exact prevalence of TRPS is unknown; the estimated prevalence is 0.2-1/100,000.[1,2] It was first described in 1966 by Giedion, and less than 250 TRPS patients have been reported.[3,4] There are two clinical subtypes of TRPS; one of them is TRPS type 1 (TRPS1) (OMIM #190350) caused by pathogenic variants in the *TRPS1* gene located on chromosome 8q23.3, and the other one is TRPS type 2 (Langer–Giedion syndrome, LGS), (OMIM #150230), a contiguous gene deletion syndrome is caused by submicroscopic deletion of the chromosomal segment 8q23.3-8q24.11, containing *TRPS1, RAD21,* and *EXT1*. Although both subtypes have similar clinical manifestations, TRPS2 is differentiated by multiple cartilaginous exostoses and intellectual

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disability. A different subtype of TRPS has also been described; however, TRPS type 3 is now accepted within the spectrum of TRPS1 with more severe brachydactyly and short stature.[2,5]

TRPS1 gene is a zinc finger transcription factor expressed in cartilage, kidneys, and hair follicles that represses GATA-regulated genes and regulates bone perichondrium mineralization, chondrocyte proliferation, differentiation, and apoptosis.[6,7] TRPS1 has high penetrance and wide phenotypic variability. More than 130 pathogenic variants have been described in the *TRPS1* gene. TRPS2 is usually sporadic, although a few familial cases have been reported.[1,8-10]

In our study, we aimed to reveal the clinical and molecular spectrum of patients with TRPS and provide a review by comparing them with previous cohort studies.

Material and Methods

Ethical compliance

Ethical approval was obtained from the Ethical Committee of Akdeniz University (Project no: 20.07.2022/KAEK-484).

Written informed consent was obtained from patients older than 18 years and the parents of all children.

Patients and clinical evaluation

Seven TRPS (type 1 and 2) patients from five unrelated families were included in this study. Clinical and molecular diagnosis of the patients were performed at two tertiary centers; Akdeniz University and Ankara University, between the years 2017-2021. The patients whose diagnoses were not confirmed via genetic analysis and, who did not have regular follow-ups, were excluded.

Demographic, laboratory, and radiological data were obtained from the hospital records. Prematurity was defined as <37 weeks due to gestational age. Birth weights greater than the 90th percentile for their gestational age were described as large for gestational age (LGA). The anthropometric measurement values were evaluated according to the national reference data for Turkish children by Neyzi et al.[11] Weight of standard deviation score (SDS) < -2 was described as underweight, height SDS < -2 was described as short stature, and head circumference SDS < -2 was described as microcephaly. Ankara Developmental Screening Inventory (AGTE) and Wechsler Intelligence Scale for Children-R (WISC-R) tests were used to evaluate the mental status. Intelligence quotient (IQ) values were described as mild intellectual disability (ID) (IQ score: 55-70), moderate ID (IQ score: 40-54), and severe ID (IQ score: 25-39).

Molecular Analysis

Molecular karyotyping was performed using the DNA samples of patients obtained from peripheral whole blood. Methods used for molecular karyotyping were either single nucleotide polymorphism (SNP) array (Affymetrix, Illumina) or array comparative genomic hybridization (aCGH) (Agilent) depending on the laboratory the sample was sent and the procedures were followed according to the manufacturers' instructions. All the coding exons and the intronic–exonic boundary regions of the *TRPS1* gene were sequenced by the next-generation sequencing (NGS) method (Miseq-Illumina) after extracting genomic DNA from whole blood samples. Variants were described using the Human Genome Variation Society nomenclature guidelines and were checked against those available in 1000 Genomes, dbSNP, ClinVar, and Human Genome Mutation Database. American College of Medical Genetics and Genomics Standards and Guidelines were used to determine variant pathogenicity.[12]

Statistical analyzes were performed with the SPSS 23.0 package program. Descriptive statistics were presented as n (%) and mean±standard deviation and median (min-max) values.

Results

Clinical features, dysmorphic facial features and growth parameters

Our study included seven patients (three females, four males) from five unrelated families. The median age of the patients was 15 years (min 7 - max 48 years). The median age at molecular diagnosis was 12 years (min 7 - max 45 years). Diagnostic delay between initial examination and diagnosis ranged from 2 months to 5 years. A mother had gestational diabetes, while the others' prenatal screening tests were normal. Five patients had term delivery. The median birth weight was 3350 grams (min 2800 - max 4500 grams), and two patients (P2, P5) were LGA. One patient (P2) had hypotonia and feeding problems in infancy. Five patients had microcephaly. Short stature was present in five patients. At the last visit, the median height SDS was -2.68 SDS (min -4.66 - max -0.6 SDS). Height SDS was normal in P1 and P6; P1 had decreased growth velocity (3 cm/year). Anthropometric measurements and clinical features of TRPS patients are summarized in Table I.

All patients had a bulbous nose with hypoplastic alae nasi, and all patients except one (P3) had a large nose. The characteristic facial features of the patients are shown in Fig. 1. Only P3 had normal scalp hair. Three patients (P1, P2, P5) had blond hair. One patient (P3) had synophrys, and laterally thin eyebrows were seen in five patients.

Two patients (P2, P6) had mild intellectual disability. Neuromotor developmental delay was seen only in one patient (P2), and diffuse cerebral atrophy was revealed in her cranial MRI. Millimetric T2, FLAIR hyperintense nodular appearance in the left globus pallidus (hamartoma) was detected in P3.

Skeletal findings

The bone age was delayed in five prepubertal patients. Low BMD was detected in one older TRPS2 patient (P4) and his son (P3) [P4; lumbar z score -2, hip z score -2,9, P3; lumbar z score -4,6, hip z score -3,4 (low z-score for age <-2)]. A history of bone fracture was noted in one patient (P3). Two patients had chronic joint pain.

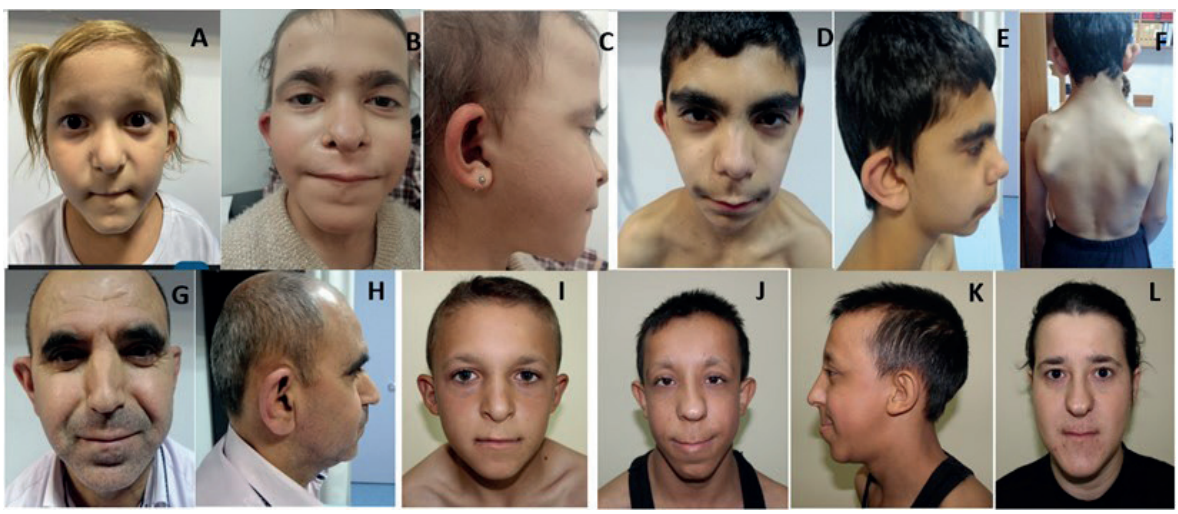

Fig. 1. Clinical photographs of 7 patients with TRPS. All patients had bulbous nasal tip, large nose except for Patient 3, triangular face, low-set anteverted ears, underdeveloped alae nasi, wide columella, long-smooth philtrum, thin upper lip, horizontal groove on chin, sparse hair except for Patient 3, medial/total thick eyebrows. Sparseness of the lateral part of the eyebrows were seen in some patients. Patient 3 had winged scapula (F).

A. Patient 1, 7-year-old girl with TRPS1; B and C. Patient 2, 11-year-old girl with TRPS2; D, E and F. Patient 3, 15-year-old boy with TRPS2; G and H. Patient 4, 48-year-old man with TRPS2; I. Patient 5, 12-year-old boy with TRPS1; J and K. Patient 6, 16-year-old boy with TRPS1; L. Patient 7, 19-year-old girl with TRPS1.

Table I. Anthropometric measurements and clinical features of TRPS patients.

Family	F1	F2	F3		F4	F5		Total (n=7)
Patient	P1	P2	P3	P4	P5	P6	P7	
Gender	Female	Female	Male	Male	Male	Male	Female	4M/3F
Age	7.5y	11y	15y	48y	15y	20y/4m	22y/5m	Mean 19.8±13.3 y
								Median 15 y (7,5-48)*
Clinical type	TRPS 1	TRPS 2	TRPS 2	TRPS 2	TRPS 1	TRPS 1	TRPS 1	
Genetic origin	De novo	De novo	Paternal	De novo	De novo	Paternal	Paternal	
Age at diagnosis	7y	7y	12y	45y	11y/9m	15y/8m	19y	Mean 16.7±13.1 y
								Median 12 y (7-45)*
Birth age	Term	Preterm	Term	Term	Term	Postterm	Term	
Weight (birth)	3300 gr	3400 gr	2800 gr	NA	4500 gr	NA	NA	
Weight (last examination)	21.5 kg	39.9 kg	32 kg	54 kg	30.5 kg	42.6 kg	65 kg	Mean 40.6±14.8 kg
	(-0.38 SDS)	(-0.16 SDS)	(-4.56 SDS)		(-1.64 SDS)	(-2.6 SDS)		Median 39.9 kg (21.5-65)*
Height (last examination)	113.9 cm	129.9 cm	140 cm	144.8 cm	131.5 cm	145 cm	159 cm	Mean 137±14.3 cm
	(-1.45 SDS)	(-2.54 SDS)	(-4.66 SDS)		(-2.68 SDS)	(-3.93 SDS)		Median 131 cm (113.9-159)*
Head circumference	51 cm	53 cm	52 cm	52.5 cm	51 cm	52.5 cm	52 cm	Mean 52±0.7 cm
	(-0.13 SDS)	(-0.36 SDS)	(-2.5 SDS)		(-2.3 SDS)	(-3.09 SDS)		Median 52 cm (51-53)*
Craniofacial features								
Face								
Prominent, long philtrum	+	+	+	+	+	+	+	7/7
Horizontal groove on chin	+	+	+	+	-	+	-	5/7
Ears								
Large, prominent ear	-	-	+	+	-	-	-	2/7
Low set ears	+	+	+	+	+	+	+	7/7
Anteverted ears	+	+	+	+	+	+	+	7/7
Overfolded helices	+	+	-	-	-	-	-	2/7

ASD: atrial septal defect, BMD: bone mineral density, GH: growth hormone, LT4: levothyroxine, MRI: magnetic resonance imaging, NA: not applicable, PS: pulmonary stenosis, SDS: standard deviation score, TRPS1: tricho-rhino-phalangeal syndrome 1, TRPS2: tricho-rhino-phalangeal syndrome 2, VSD: ventricular septal defect, VUR: vesicoureteral reflux. *Median (min-max).

Table I. Continued.

Eyes								
Thin eyebrows (laterally)	+	+	-	-	+	+	+	5/7
Thick eyebrows (medially)	+	+	+	+	+	+	+	7/7
Synophrys	-	-	+	-	-	-	-	1/7
Nose								
Large nose	+	+	-	+	+	+	+	6/7
Bulbous nose tip	+	+	+	+	+	+	+	7/7
Short nose	-	-	+	-	-	-	-	1/7
Anteverted nares	-	-	+	-	-	-	-	1/7
Hypoplastic alae nasi	+	+	+	+	+	+	+	7/7
Mouth								
Narrow palate	+	+	+	+	-	+	-	5/7
Thin upper lip	+	+	+	+	+	+	+	7/7
Teeth								
Small teeth	+	+	-	+	+	-	-	4/7
Carious teeth	+	+	+	+	+	+	+	7/7
Dental malocclusion	+	-	+	+	+	+	+	6/7
Crowded teeth	+	-	+	+	-	+	-	4/7
Skeletal findings								
Chest								
Rib exostoses	-	+	+	+	-	-	-	3/7
Scapulae exostoses	-	+	+	-	-	-	-	2/7
Winged scapulae	-	-	+	-	-	-	-	1/7
Osteoarthritis (late-onset)	-	-	-	+	-	-	-	1/7
Joint pain	-	+	-	+	-	-	-	2/7
Scoliosis/ Lordosis	-	Scoliosis	Scoliosis/ Lordosis	Scoliosis	Scoliosis	-	-	4/7

ASD: atrial septal defect, BMD: bone mineral density, GH: growth hormone, LT4: levothyroxine, MRI: magnetic resonance imaging, NA: not applicable, PS: pulmonary stenosis, SDS: standard deviation score, TRPS1: tricho-rhino-phalangeal syndrome 1, TRPS2: tricho-rhino-phalangeal syndrome 2, VSD: ventricular septal defect, VUR: vesicoureteral reflux. *Median (min-max).

Table I. Continued.

Low BMD	-	-	+	+	-	-	-	2/7
Pelvis								
Hip dysplasia	-	Narrow joint space	Narrow joint space	Narrow joint space	-	Unilateral coxa magna	-	4/7
Limbs								
Multiple exostoses	-	+	+	+	-	-	-	3/7
Hyper extensible joints	+	+	+	-	-	-	-	3/7
Fracture history	-	-	+	-	-	-	-	1/7
Hands								
Short hands	+	+	+	+	+	+	+	7/7
Short metacarpals	+	+	+	+	+	-	+	6/7
Short phalanges	+	+	+	+	+	+	+	7/7
Swelling of proximal interphalangeal joints	+	+	+	+	+	+	+	7/7
Cone-shaped epiphyses	+	+	+	+	+	+	+	7/7
Feet								
Short metatarsals	-	+	+	+	+	+	+	6/7
Short feet	-	+	+	+	+	+	+	6/7
Pes planus	-	+	-	-	-	+	+	3/7

ASD: atrial septal defect, BMD: bone mineral density, GH: growth hormone, LT4: levothyroxine, MRI: magnetic resonance imaging, NA: not applicable, PS: pulmonary stenosis, SDS: standard deviation score, TRPS1: tricho-rhino-phalangeal syndrome 1, TRPS2: tricho-rhino-phalangeal syndrome 2, VSD: ventricular septal defect, VUR: vesicoureteral reflux. *Median (min-max).

Table I. Continued.

Ectodermal findings								
Skin								
Dry skin	+	+	+	+	+	+	+	7/7
Hair								
Sparse, thin hair	+	+	-	+	+	+	+	6/7
Temporal baldness	+	+	-	+	+	+	+	6/7
Fine,blonde scalp hair	+	+	-	-	+	-	-	3/7
Nails								
Thin nails	-	+	+	+	+	+	+	6/7
Brittle nails	-	+	+	+	+	+	-	5/7
Neurological abnormalities								
Hypotonia	-	+	-	-	-	-	-	1/7
Intellectual disability	-	Mild	-	-	-	Mild	-	2/7
Developmental delay	-	+	-	-	-	-	-	1/7
Cranial MRI	-	Diffuse cerebral atrophy	Nodular appearance in the left globus pallidus (hamartoma)	-	-	-	-	2/7
Other findings	-	Hypothyroidism, thyroid hypoplasia, left renal focal caliectasis, Mild hearing loss, GH deficiency, strabismus	VSD, PS, ASD-Myopia		Mild hearing loss, inguinal hernia	Bicuspid aortic valve, bilateral VUR, nephrocalcinosis, Left atrophic kidney, GH deficiency, Mild hearing loss	Menometrorrhagia	
Therapies	-	GH, LT4	-	-	-	-	-	

ASD: atrial septal defect, BMD: bone mineral density, GH: growth hormone, LT4: levothyroxine, MRI: magnetic resonance imaging, NA: not applicable, PS: pulmonary stenosis, SDS: standard deviation score, TRPS1: tricho-rhino-phalangeal syndrome 1, TRPS2: tricho-rhino-phalangeal syndrome 2, VSD: ventricular septal defect, VUR: vesicoureteral reflux. *Median (min-max).

Three patients had hyperextensible joints, and four patients developed scoliosis. Three patients had pes planus, and one patient (P3) had winged scapulae. All patients had brachydactyly, short metacarpals and phalanges in variable stages. Swelling of proximal interphalangeal joints and deviation of the fingers were also detected in all patients. Short feet and short metatarsals were seen in six patients. Hand and feet photography of the patients are shown in Fig. 2.

Skeletal X-ray imaging revealed cone-shaped epiphysis at the phalanges in all patients. Multiple exostoses (at the rib, scapula, knee and ankle joints, bilateral femur, tibia, fibula, humerus, radius, ulna metaphyseal, and diaphyseal segments) were present in three patients. One patient (P6) had unilateral coxa magna, three patients had hip joint space narrowing, and one patient (P3) had a cystic lesion in the femur. P4 had a subluxation in the radiocarpal joint of the left wrist and a fusion of the C2-C3 cervical vertebrae spinous processes. The radiological findings of the patients are shown in Fig. 3 and 4.

Other findings

Endocrinologic evaluation revealed hypothyroidism and hypoplasia of thyroid gland in P2. P7 was followed-up due to menometrorrhagia. Growth hormone deficiency was detected in P2 and P6.

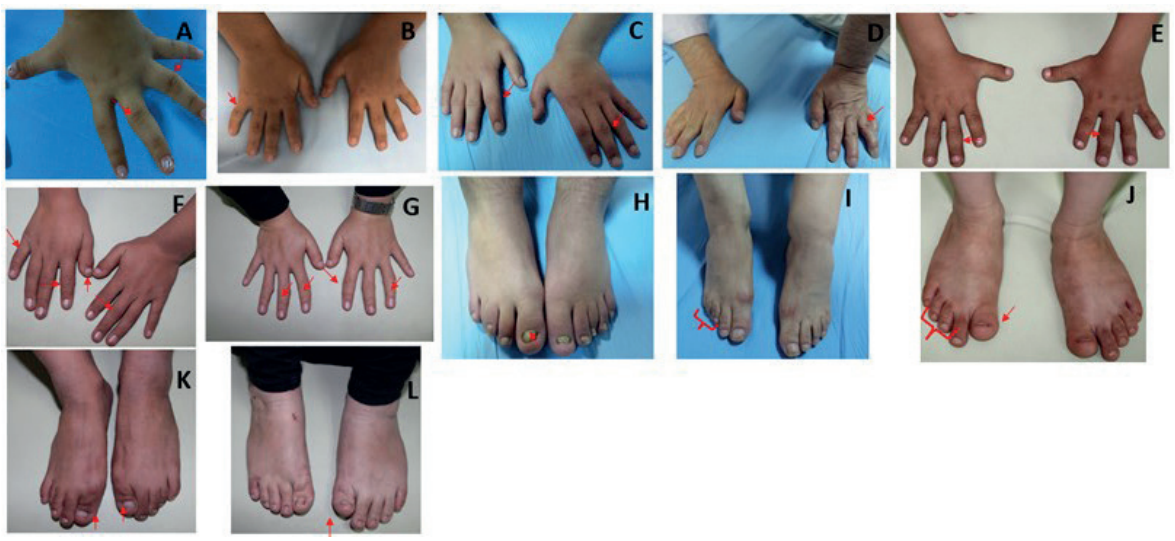

Fig. 2. Photographs of hands and feet of the patients.

A. Patient 1, 7-year-old girl with TRPS1. Note radial and ulnar deviation of the phalanges and swelling of proximal interphalangeal joints.

B. Patient 2, 11-year-old girl with TRPS2. Note brachydactyly and clinodactyly.

C and H. Patient 3, 15-year-old boy with TRPS2. Note brachydactyly, swelling of interphalangeal joints, short feet, metatarsal shortening and dystrophic nails.

D and I. Patient 4, 48-year-old man with TRPS2. Note short left hand, deformities of the phalanges, short feet and metatarsal shortening.

E and J. Patient 5, 12-year-old boy with TRPS1. Note brachydactyly, metacarpal shortening, swelling of interphalangeal joints, metatarsal shortening except for second metatarsal.

F and K. Patient 6, 16-year-old boy with TRPS1. Note the swelling of the interphalangeal joints, deviation of the phalanges, short feet, and shortening of the first metatarsals.

G and L. Patient 7, 19-year-old girl with TRPS1. Note radial and ulnar deviation of the phalanges, metacarpal shortening, short feet and metatarsals.

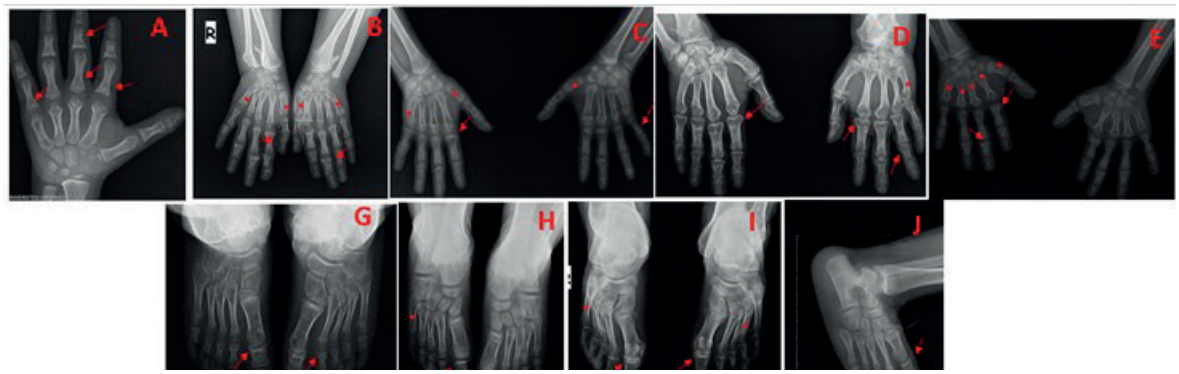

Fig. 3. X-ray imaging of the hands and feet shows cone-shaped epiphyses at the phalanges and metacarpal and metatarsal shortening. Note variably shortening of metatarsals and metacarpals. Patient 5 had a more severe shortening of the metacarpals.

A. Patient 1, 7-year-old girl with TRPS1; B and G. Patient 2, 11-year-old girl with TRPS2; C and H. Patient 3, 15-year-old boy with TRPS2; D and I. Patient 4, 48-year-old man with TRPS2; E and J. Patient 5, 12-year-old boy with TRPS1; F. Patient 6, 16-year-old boy with TRPS1.

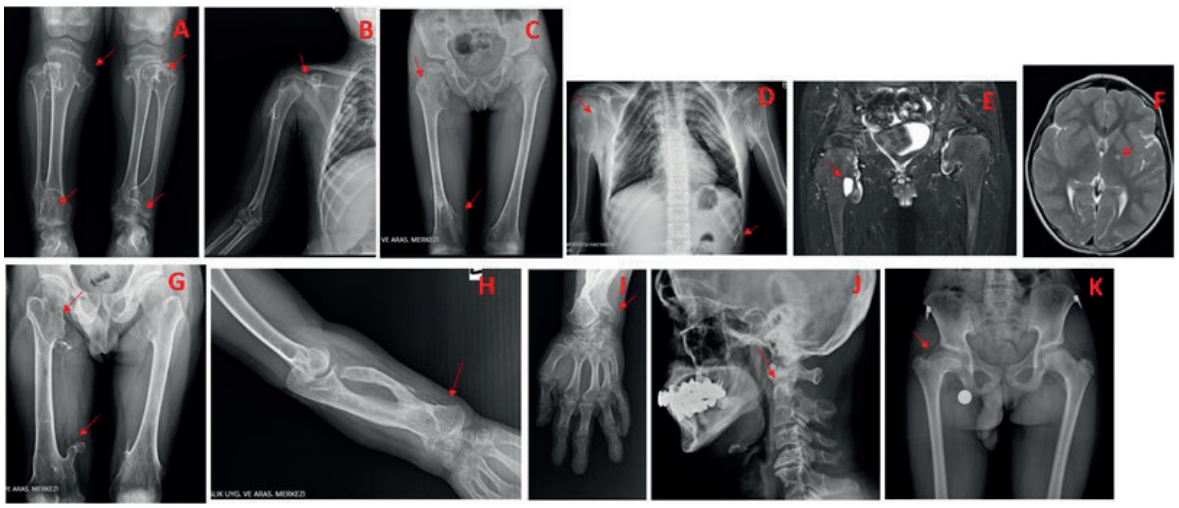

Fig. 4. Radiological findings of the patients

A and B. Patient 2 at the age of 7 years and 2 months, X-ray showed exostoses on the scapulae and around the knees and ankles.

C, D, E, and F. Patient 3 at the age of 12 years, X-ray showed exostoses at long bones, rib, scapula. Pelvic MRI showed a cystic lesion in the femur. Cranial MRI showed millimetric T2 FLAIR hyperintense nodular appearance in the left globus pallidus (hamartoma).

G, H, I and J. Patient 4 at the age of 45 years, skeletal X-Ray showed exostoses at long bones, subluxation in the radiocarpal joint, and fusion of the C2-C3 cervical vertebrae spinous processes, respectively.

K. Patient 6 with TRPS1 at the age of 16 years, pelvic X-Ray shows coxa magna.

Genitourinary anomalies such as unilateral focal caliectasic areas in renal collecting system, bilateral vesicoureteral reflux, nephrocalcinosis, and unilateral atrophic kidney were detected in renal ultrasonography of P2 and P6. One patient (P5) underwent surgery due to unilateral inguinal hernia.

Cardiovascular defects were presented in two patients (P3, P6). Echocardiographic examination revealed ventricular septal defect, pulmonary stenosis, and right arcus aorta, a bicuspid aortic valve. P3 was operated on due to pulmonary stenosis at the age of ten months.

Three patients (P2, P5, P6) had mild conductive hearing loss. One patient (P3) had myopia, and P2 underwent surgery due to strabismus.

Molecular Results

The clinical diagnosis was confirmed by either molecular karyotyping or *TRPS1* sequencing analysis via next-generation sequencing. *De novo* variants were identified in four patients, while three had paternal inheritance. SNP array analysis revealed a deletion in the q23.3q24.11 band region of chromosome 8 in three TRPS2 patients. P2 had the 2.619 kb deleted region, including *TRPS1, EIP3H, RAD21, SCL30AB, MED30*, and *EXT1*. P3 and P4 had a 3.488 kb deleted region, including *EIF3H, RAD21, SLC30A8, MED30, EXT1, TNFRSF11B, COLEC10, MAL2, NOV*, and *ENPP2*.

Three different pathogenic variants in *TRPS1* gene were identified in four patients from three families, including a frameshift (c.2445dup, p.Ser816GlufsTer28) in the exon 5, one missense (c.2762G>A), and a novel splice region variant (c.2700+3A>G). The results of the genetic analysis are presented in Table II.

Discussion

Phenotypical Features and Ectodermal Findings

Tricho-Rhino-Phalangeal Syndrome has a broad phenotypic spectrum. Short stature, sparse hair and prominent nose with bulbous tip represent the clinical hallmarks of the disease. Maas et al.[1] reported similar facial features in TRPS patients either with microdeletion or *TRPS1* variants; besides broad eyebrows are more common in TRPS2. Ectodermal findings including dry skin, sparse hair, dystrophic nails, oligodontia, dental malocclusion, and carious teeth are the other common features of the syndrome.[1,2,8,13] In our study, a large nose with bulbous nasal tip, hypoplastic alae nasi, prominent long philtrum and anteverted ears appear as the most recognizable facial findings both in childhood and adults. Sparse and slow-growing hair was seen in almost all our patients, but P3 had some facial and ectodermal features of Cornelia de Lange syndrome (CdLS) such as a small, upturned nose, thick eyebrows, synophrys,

Table II. The results of the genetic analysis.

	Patient no	Exon/ Intron	Nucleotide change	Protein Change	Coding impact	ACMG classification[12]	Reference
TRPS1 Sequence analysis	1	Exon 5	c.2445dup	p.S816EfsTer28	Nonsense	Likely Pathogenic	Momeni et al.[6]
	5	Exon 6	c.2762G>A	p.R921Q	Missense	Pathogenic	Maas et al.[1]
	6	Intron 5	c.2700+3A>G		Splice site	Likely Pathogenic	NR
	7	Intron 5	c.2700+3A>G		Splice site	Likely Pathogenic	NR
	Patient no	Deletion size	Deleted genes				
Chromosomal microarray analysis	2	2.619 kb	*TRPS 1, EIP3H, RAD21, SCL30AB, MED30, EXT1*				
	3	3.488 kb	*EIF3H, RAD21, SLC30A8, MED30, EXT1, TNFRSF11B, COLEC10, MAL2, NOV, ENPP2*				
	4	3.488 kb	*EIF3H, RAD21, SLC30A8, MED30, EXT1, TNFRSF11B, COLEC10, MAL2, NOV, ENPP2*				

NR: not reported.

and hirsutism. He had a 3.48 kb deletion in chromosome 8q23.3q24.11 containing *RAD21* and *EXT1* genes. Herrero-García A. et al.[14] reported a mixed phenotype of TRPS2 and CdLS in an 8q23.3-q24.1 microdeletion and they hypothesized the *RAD21* gene deletion has a mixed phenotype of TRPS2 and CdLS, similar to P3.

Anthropometric Measurement and Growth

The growth parameters such as weight and head circumference are usually normal in TRPS1; however, microcephaly can be seen in one-third of TRPS2. Recently, Maas et al.[1] reported six patients with microcephaly; three had TRPS2, and two had *TRPS1* missense mutation. Consistent to the report, two of our TRPS patients with microcephaly had TRPS2, one had *TRPS1* missense mutation, and two had *TRPS1* splice site mutation.

Linear growth is reduced in almost all TRPS patients, more marked in TRPS2. Lüdecke et al.[5] reported the average height of 75 TRPS patients was -1.41±1.15 SDS; besides, patients with *TRPS1* missense mutations are shorter than those with nonsense mutations.[1,5,15] Growth hormone (GH) deficiency or insensitivity are described in some TRPS patients, but the results of GH treatment were variable.[16] In our study, the average height SDS was -2.88. Short stature was more significant in TRPS2, and similar to the literature, the patient with missense mutation was shorter than the patient with nonsense mutation. Only one patient (P2) had started to receive GH treatment at the age of four years and for longer duration, resulting in an increase in height SDS from −4.1 SDS to −2.3 SDS. Growth responds consistently to GH therapy; however, further studies and larger cohort of patients are required for the determination of efficiency.

Musculoskeletal and Radiologic Features

Deformities of the hands and feet are seen frequently, like brachydactyly, ulnar or radial deviation of fingers, metacarpal or metatarsal shortening, and short feet. The fourth and fifth metacarpals or metatarsals are the most commonly affected ones.[1,5] Other skeletal findings include winged scapula, scoliosis, pectus carinatum/excavatum, and joint hypermobility.[1,2,17] Low BMD is usually detected in adults, except in two reported children, and it may rarely present in TRPS1, but is more common in TRPS2.[1,18-20] Osteoarthritis-like changes, joint pain, and decreased mobility affect the small/large joints, and hips, which can be related to long-term morbidity. These complaints can be mistakenly interpreted as rheumatoid arthritis.[1,18] Hip dysplasia such as coxa vara, coxa plana, joint space narrowing, and Perthes-like femoral head changes are present in 47-70% of patients.[1,8,17,18] Hemivertebrae, long bone cysts, non-ossifying fibroma, tibial hemimelia, and duplicated thumb have been rarely reported.[1,21-23]

The most typical radiographic feature in TRPS is cone-shaped epiphyses, which are usually present in the second finger's middle phalanx. They are detectable typically after two years of age and can cause ulnar or radial deviation.[1,24] Exostoses usually occur around the elbows, knees, and scapula in TRPS2 from the newborn period, and do not progress after puberty. Malignant transformation of osteochondromas in TRPS2 has not been reported, but the risk cannot be excluded.[1,25-27] The comparison of musculoskeletal and radiological findings of TRPS via literature is presented in Table III.

In our study, all patients had brachydactyly, interphalangeal joint deformity, cone-shaped epiphysis, and limb deformities. Hands were more affected than the feet. Scoliosis, short metacarpals and metatarsals were seen in more than half of TRPS patients. Exostoses were seen at the rib, scapula, long bones, and joints in three patients. The mobility decreased only in a patient due to joint pain and knee exostoses at the age of 11 years. No malignant transformation was observed, and one of them had exostosis excision on his left arm due to pain, limitation of movement, and nerve compression. In TRPS2 family members (P3, P4), low BMD and vitamin D deficiency were detected, and P3 had

osteoporosis presenting with an ulnar fracture at the age of 15 years. In TRPS, low BMD can be a risk in adolescents and we should recommend checks on vitamin D and dual energy X-ray absorptiometry (DEXA) scans at appropriate intervals to assess bone loss and fracture risk.

Table III. Comparison of musculoskeletal and radiological findings of TRPS in literature.

	Maas et al. 2015[1]	Lüdecke et al. 2001[5]	Wang et al. 2020[27]	Schinzel et al. 2013[25]	de Barros et al. 2014[17]	Present study
	n=103 (%)	n=51 (%)	n=5 (%)	n=4 (%)	n=1 (%)	n=7 (%)
Musculoskeletal findings						
Short stature	37/66 (56%)	24/75 (32%)	4/5 (80%)	4/4 (100%)	1/1 (100%)	5/7 (71%)
Brachydactyly	65/99 (66%)	29/45 (64%)	5/5 (100%)	4/4 (100%)	1/1 (100%)	7/7 (100%)
Deformity in interphalangeal joints	NA	NA	NA	NA	1/1 (100%)	7/7 (100%)
Polydactyly	1/99 (1%)	NA	NA	0/4 (0%)	0/1 (0%)	0/7 (0%)
Oligodactly	0/0 (0%)	NA	NA	1/4 (25%)	0/1 (0%)	0/7 (0%)
Syndactyly	NA	NA	NA	1/4 (25%)	0/1 (0%)	0/7 (0%)
Short feet	38/65 (58%)	NA	NA	1/4	NA	6/7 (85%)
Hyperextensible joints	38/63 (60%)	NA	NA	2/4 (50%)	NA	3/7 (43%)
Scoliosis/kyposis	30/93 (32%)	NA	4/5 (80%)	4/4 (100%)	NA	4/7 (57%)
Winged scapulae	17/58 (32%)	NA	NA	2/4 (50%)	-	1/7 (14%)
Chest deformity	NA	NA	NA	2/4 (50%)	NA	0/7 (0%)
Pes planus	NA	NA	NA	NA	NA	3/7 (43%)
Fractures	20/87 (23%)	NA	NA	2/4 (50%)	0/1 (0%)	1/7 (14%)
Joint pains	22/34 (65%)	NA	NA	1/4 (25%)	1/1 (100%)	2/7 (28%)
Limb length discrepancy	10/45 (22%)	NA	NA	2/4 (25%)	0/1 (0%)	1/7 (14%)
Hip surgery	7/65 (11%)	NA	NA	0/4 (0%)	0/1 (0%)	0/7 (0%)
Other ortopedic surgery	18/93 (19%)	NA	NA	2/4 (50%)	0/1 (0%)	1/7 (14%)
Radiological Findings						
Cone shaped epiphysis	58/60 (97%)	NA	5/5 (100%)	4/4 (100%)	1/1 (100%)	7/7 (100%)
Short metacarpals	58/93 (62%)	NA	NA	2/4 (50%)	0/1 (0%)	6/7 (86%)
Short metatarsals	34/58 (59%)	NA	NA	NA	NA	6/7 (86%)
Delayed bone age before puberty	NA	20/21 (%95)	NA	NA	1/1 (100%)	5/5 (100%)
Accelerated bone age after puberty	NA	4/5 (%80)	NA	1/4 (25%)	NA	0/3 (0%)
Coxa plana	8/15 (%53)	NA	NA	NA	1/1 (100%)	0/7 (0%)
Coxa magna	NA	NA	NA	NA	0/1 (0%)	1/7 (14%)
Perthes-like changes/hip dysplasia	18/38 (%47)	NA	NA	1/4 (25%)	1/1 (100%)	0/7 (0%)
Dislocated patellae	9/64 (%14)	NA	NA	NA	NA	0/7 (0%)
Subluxation in other joints	NA	NA	NA	2/4 (50%)	NA	2/7 (28%)
Osteopenia	11/44 (%25)	NA	NA	1/4 (25%)	1/1 (100%)	2/7 (28%)
Osteoarthritis	NA	NA	NA	NA	1/1 (100%)	2/7 (28%)
Exostoses	12/85 (%14)	NA	1/5 (%20)	4/4 (100%)	0/1(0%)	3/7 (43%)

NA: not available, TRPS: tricho-rhino-phalangeal syndrome.

Other findings

Intellectual disability in TRPS1 is similar to the general population; however, mild to moderate intellectual disability occurs in two-thirds of the TRPS2. Language development is better than motor skills, and practical skills are better than IQ scores in some TRPS2 patients. Neuromotor delay may occur secondary to hip dysplasia. Seizures, neurologic abnormalities, and cranial MRI findings such as polymicrogyria, hydrocephalus, and Chiari malformation have also been reported.[1,25,27] In our study, mild intellectual disability was revealed in two patients. One of them was TRPS1 (P6), and the other was TRPS2 (P2), who had a neuromotor delay in infancy and cerebral atrophy. None of patients had seizures. Hamartoma at globus pallidus in P3 has been considered a new condition that may accompany TRPS2, which has not been defined in the literature. Intracranial hamartoma which is located in a different region from the hypothalamus is also extremely rare.[28]

Urogenital anomalies include unilateral kidney, ureter-bladder junction stenosis, vesicoureteral reflux, renal cysts, hydrometrocolpos, vaginal atresia, and persistent cloaca. Endocrinologic abnormalities such as hypothyroidism and idiopathic hypoglycemia have been reported.[1,8] Cardiovascular defects were reported in a minority (15%) of TRPS patients, and usually not severe and life-threatening. Persistent ductus arteriosus, patent foramen ovale, bicuspid aortic valves, mitral valve regurgitation, aortic stenosis, and anomalous venous return have been reported.[2] Hearing impairment was detected in 11% and ocular findings like myopia, hypermetropia, astigmatism, optic disc atrophy, and strabismus have been reported.[1]

In our study, focal caliectasis, bilateral vesicoureteral reflux, nephrocalcinosis, and left atrophic kidney were detected. Recently, a study mentioned menorrhagia and metrorrhagia in TRPS1 females above 16 years.[1] Similarly, our TRPS1 patient (P7) was followed-up long term due to menometrorrhagia. Cardiovascular defects were seen in two patients, and one of them underwent surgery due to pulmonary stenosis. There was a conductive hearing loss in three patients especially secondary to recurrent otitis media. Both TRPS1 and TRPS2 patients should be evaluated in terms of endocrinological, genitourinary, cardiological, audiological, and ophthalmological system. Menstrual irregularities should be assessed at the diagnosis or follow-up in TRPS females.

Genotype

No exact genotype–phenotype correlation in TRPS has yet been identified, except for TRPS2, which was associated with multiple exostoses and more marked intellectual disability. Nonsense and frameshift variants are the most common pathogenic variants in *TRPS1*. Missense variants clustering in exon six and intragenic and whole gene deletions with variable breakpoints have also been reported.[1,29,30] Lüdecke et al.[5] showed that most patients with nonsense *TRPS1* mutations have the less severe phenotype, whereas patients with missense mutations in the GATA-type zinc finger domain have more severe phenotype. Mutations in exon six might have more marked facial characteristics and shortening of hands and feet.[5] Consistent with the literature, P5 who had missense mutation in the GATA-type zinc finger domain had more severe brachydactyly. We observed that marked variability can be seen even in families with the same pathogenic variant. In familial TRPS1 patients (P6, P7) with a novel splice site mutation an intrafamilial clinical variability was observed. While P6 had severe short stature, his sister's (P7) height was normal. In addition, only a few TRPS2 patients without deletion of *TRPS1* gene have been reported, like our patients P3 and P4. It may be related to the proximal breakpoint being close to the *TRPS1* gene and the alteration of gene expression even if undamaged.[31]

In conclusion, a detailed clinical and radiological evaluation should be obtained in patients presenting with short stature, typical facial and skeletal findings, TRPS syndrome

should be considered. Genetic tests are needed to confirm the clinical diagnosis. Imagings of the central nervous system, and follow-ups of skeletal system are also recommended. Further research will help outlining the comorbidities and genotypic spectrum of patients.

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Ethical approval

This study was approved by the Ethics Committee of Akdeniz University (20.07.2022/KAEK-484).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: NÖ, BN; data collection: BN, HM, ÖYB, EM; analysis and interpretation of results: NÖ, BN, HM, GK, GOÇ; draft manuscript preparation: NÖ, BN, HM. 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

Clinically important intracranial abnormalities in children presenting with first focal seizure

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ABSTRACT

Background. Management of pediatric patients presenting with first seizure is challenging, especially with regards to emergent neuroimaging. The rate of abnormal neuroimaging findings is known to be higher in focal seizures than in generalized seizures, but those intracranial abnormalities are not always clinically emergent. In this study, we aimed to determine the rate and indicators for clinically important intracranial abnormalities that change acute management in children presenting with a first focal seizure to the pediatric emergency department (PED).

Methods. This study was conducted retrospectively in the PED at a University Children's Hospital setting. The study population consisted of patients aged between 30 days and 18 years with first focal seizure and who had emergent neuroimaging at the PED between the years 2001 and 2012.

Results. There were 65 eligible patients meeting the study criteria. Clinically important intracranial abnormalities requiring emergent neurosurgical or medical intervention were detected in 18 patients (27.7%) at the PED. Four patients (6.1%) underwent emergent surgical procedures. Seizure recurrence and the need for acute seizure treatment in the PED were significantly associated with clinically important intracranial abnormalities.

Conclusions. Neuroimaging study yielding of 27.7% shows that first focal seizure must be evaluated meticulously. From the emergency department's point of view; we suggest that first focal seizures in children should be evaluated with emergent neuroimaging, if possible with magnetic resonance imaging. Especially patients with recurrent seizures at presentation requires more careful evaluation.

Key words: children, first seizure, first focal seizure, emergent neuroimaging, important intracranial abnormalities, seizure recurrence.

Seizures are the most common neurological problem reported during childhood. It has been shown that 4-6% of children between the ages of 0-16 years have a seizure at least once during their life, and seizures account for a major part of pediatric emergency department (PED) visits (1%).[1]

The management of "first seizure" other than simple febrile seizure (SFS) has been discussed in many prior studies in relation to emergent laboratory investigations, especially neuroimaging.[2-4] In the case of 'first focal seizure' the rate of abnormal neuroimaging results is known to be higher than generalized seizures, and emergent neuroimaging in PED is obtained more in routine practice.[5-7] However, not all abnormal findings lead to an alteration in acute management.[6] In the PED; the main

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priority must be to rule out a life-threatening intracranial condition and determine the need for emergent neurosurgical or medical intervention in order to prevent loss of time and labor, deferring non-urgent evaluation to a later time to be performed electively. With this aim, defining the patients who will benefit most from emergent neuroimaging is an important task. Guidelines published by Hirtz et al.[2] in 2000 recommended emergent neuroimaging for first seizure in pediatric patients who had a persistent post-ictal neurologic deficit and/or that could not return to baseline neurologic status, but the focal seizure was subject to non-emergent neuroimaging. A reassessment was published by Harden et al.[8] in 2007, and focal seizure was considered as an indication for emergent neuroimaging. On the other hand, some authors recommend emergent neuroimaging in the first afebrile focal seizure based on the patient's age.[9,10] Guidelines published from Ontario in 2015 recommend emergent neuroimaging in the first afebrile focal seizure.[11]

In this study, we aimed to determine the rate of clinically important intracranial abnormalities that change acute management in patients presenting with a first focal seizure and to investigate the clinical indicators and risk factors for emergent neuroimaging at the PED in this patient group.

Material and Methods

This study was conducted retrospectively in the PED at a University Children's Hospital setting. The study was approved by the Ethics Committee of Hacettepe University (GO 14/577) and conducted in accordance with the latest version of the Declaration of Helsinki.

In the study, inclusion criteria were defined as; age between 30 days and 18 years, presenting with first focal seizure (no previous seizure other than SFS), receiving emergent neuroimaging (computed tomography-CT and/or magnetic resonance imaging-MRI) at the PED before discharge. Thus; infants and children aged between 30 days and 18 years who presented with first focal seizure (febrile or afebrile) to the PED between January 2001 and February 2012 who had emergent neuroimaging were included. Patient history, physical examination, and clinical follow-up notes were manually written and archived as files on those dates; whereas laboratory tests and radiological results were recorded on the hospital information system. In our center, the first focal seizure is a criterion for emergent neuroimaging even in the absence of any other clinical alarming signs although there is no written local protocol. For obtaining study patients properly; first, the list of all patients who underwent brain CT and/or MRI in the PED for any reason between the study dates were identified. Then, sections of short clinical information for ordering neuroimaging in the hospital information system were searched for the words 'focal seizure' and a preliminary list of patients with the first focal seizure was created. Files of these patients were obtained from the medical archive system, and after a detailed manual evaluation of these files, patients with first focal seizure who had undergone brain CT and/or MRI were included in the study. Exclusion criteria were; age younger than 30 days, incomplete basic information, unclear seizure type, and having a previous seizure other than a SFS. More detailed information for determining study patients is presented in Figure 1. The following information was obtained from the medical records of each patient and recorded on a standardized data collection form: Age, gender, associated symptoms, family history of seizure or epilepsy, developmental history, presence of any chronic systemic or neurological disease, physical and neurological examination findings, laboratory test results, treatment with anticonvulsants in the PED, neuroimaging findings, electroencephalogram (EEG) findings, final diagnosis, and antiseizure medication prescription for maintenance therapy (if present) and results in follow-up (if present).

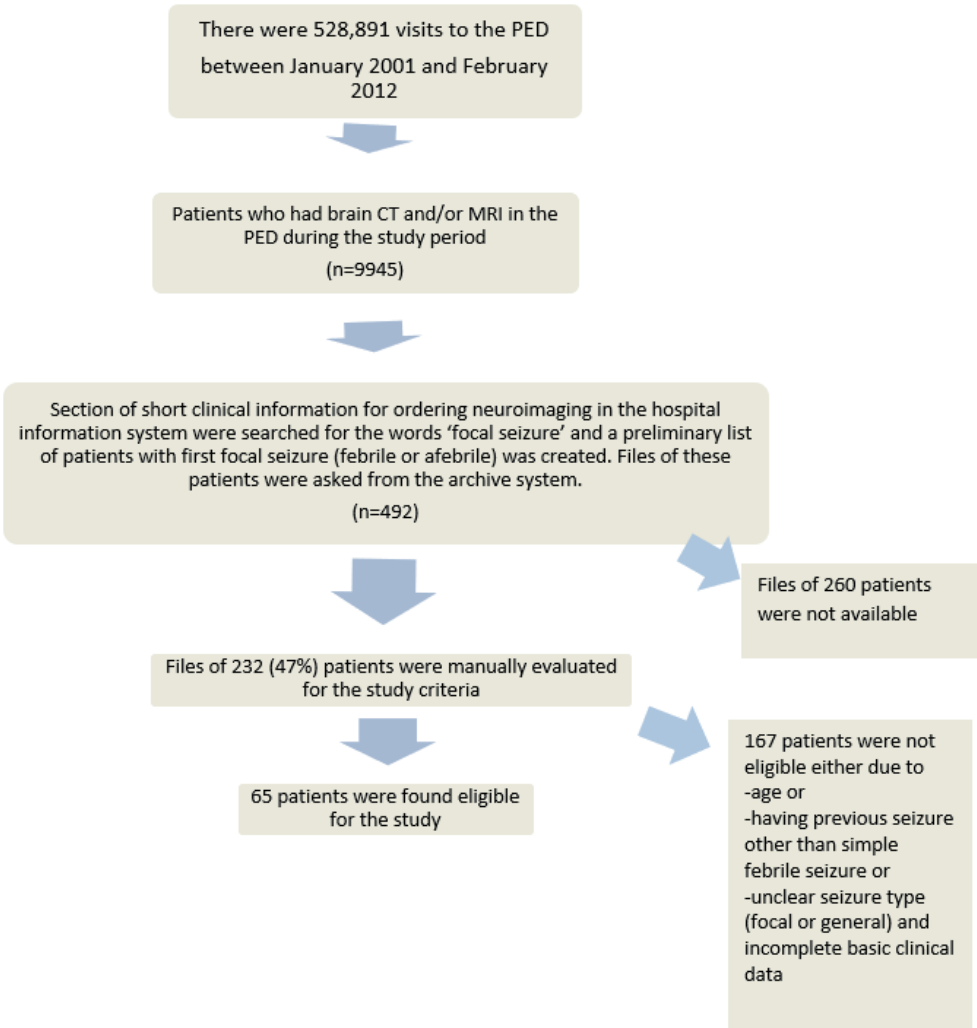

Fig. 1. Identification of study patients.

We defined focal manifestations as a clinical seizure with motor components including head and eye deviation to one side, clonic and/or tonic movements, or loss of muscle tone in only one side of the body, with or without evolution to bilateral tonic clonic seizure in accordance with the International League Against Epilepsy (ILAE) guidelines.[12] Focal seizures that are recorded to be more than one episode either just before coming to PED or during the period in the PED are considered as 'seizure recurrence' within 24 hours. According to International League Against Epilepsy (ILAE) guidelines, when the duration of seizure is ≥5 minutes patients are diagnosed and treated as status epilepticus SE.[13] However, for the scope of this study, status epilepticus (SE) was considered as a single seizure episode or multiple episodes lasting ≥30 minutes without recovery of consciousness/function in between episodes.

A complex febrile seizure was defined as a seizure associated with fever without evidence of intracranial infection or defined cause and has a focal onset and/or lasts longer than 15 minutes and/or occurs more than once in 24 hours in a child between six months and five years of age.[14]

Acute bacterial meningitis was defined as pleocytosis and/or the growth of a pathogen in cerebrospinal fluid (CSF).

Outcome Assessment

The principal outcome of our study was defined as clinically important intracranial abnormalities in brain imaging requiring emergent neurosurgical or medical intervention. We defined this based on the ILAE classification which was established in 2009 and is widely used for the evaluation of intracranial pathologies in terms of a clinical emergency, management, and follow-up (Table I).[15] For our study, we considered clinically important intracranial abnormalities as Class IV and Class V lesions which are described as pathologies having therapeutic implications and those that need more immediate/urgent intervention. In this context, encephalitis/meningitis was classified as Class IV if empiric therapy was started prior to imaging and as Class V if so after imaging.[16]

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS) for Windows 15.0 program was used for all data analyses in this study. Data was expressed as percentage, median, or mean ± standard deviation. The continuous variables were compared using a two-tailed t-test for the parametrically distributed data or the Mann-Whitney U test for the nonparametrically distributed data. The categorical variables were analyzed using a chi-square test. A p value of 0.05 or less was considered to be significant.

Results

Between January 2001 and February 2012, 9945 patients who underwent neuroimaging in the PED were identified. After the evaluation of patient records and files, 65 patients who met the criteria were enrolled in the study (Fig. 1). The mean age of the patients was 56 ± 52.4 months (31 days to 195 months) and 43 (66%) of the patients were male. Although the number of patients with fever was 30 (46%), only 10 of them had a complex febrile seizure. Sixteen patients (24.6%) had an underlying chronic illness, either neurological or systemic. These included; cognitive/motor delays (n= 10), hydrocephalus (n= 1), brain tumor (n= 2), and other chronic systemic diseases (n= 3); hereditary spherocytosis (n= 1), cystinosis (n= 1), Down syndrome and atrioventricular septal defect (n= 1). Demographic characteristics of the study patients are presented in Table II.

Abnormal neurological examination findings consisted of focal symptoms/signs in 27 (41%) of the patients (Table II). These were Todd paralysis in four patients, hemiparesis in one patient, unilateral central facial paralysis in one patient, tongue deviation to one side and asymmetric increased/decreased deep tendon reflexes (DTR) or unilateral pathological reflexes in the remaining patients. Other symptoms/signs such as drowsiness, lethargy, and hypo/hyperactive DTRs were present in 15 patients

Table I. Classification of neuroimaging findings (15).

Categories	Examples
Class I- Nonspecific	Periventricular leukomalacia, atrophy
Class II- Static remote lesions	Porencephaly, malformation of cortical development
Class III- Focal lesion responsible for the seizure that does not require immediate intervention but would be potentially amenable to epilepsy surgery	Focal cortical dysplasia or mesial temporal sclerosis
Class IV- Subacute or chronic process that has therapeutic implications and requires more immediate intervention or that has important diagnostic or prognostic implications	Brain tumor, leukodystrophies, metabolic disorder
Class V- Acute process that requires urgent intervention or need for additional urgent diagnostic evaluation and counseling	Hydrocephalus, acute stroke or hemorrhage, meningoencephalitis, metabolic cytopathy

and neurological examination was normal in 23 (35%) patients.

Sixty-two patients initially underwent brain CT, while the other three patients initially had brain MRI at the PED. A subsequent brain MRI was also performed in 19 patients after brain CT at the PED before discharge/admission. Overall neuroimaging at the PED revealed clinically important intracranial abnormalities requiring emergent neurosurgical or medical intervention in 18 patients (27.7%). The demographic, clinical characteristics and radiological results of these patients are presented in Table III. Brain CT and MRI of three of these patients are shown in Figures 2 and 3. In four patients, brain CT was normal, but MRI revealed clinically important intracranial abnormalities, namely three cases of meningoencephalitis and one cerebrovascular event (arterial occlusion).

Four patients (6.1%) underwent emergent surgical procedures (Table III). In addition to these four patients, the evaluation of a patient with a right focal seizure revealed the presence of a hemorrhagic cavernoma in the left frontal region, although he was not operated on until one month after admission.

In order to identify risk factors; we performed a comparison between the characteristics of the patients with clinically important intracranial abnormalities requiring emergent neurosurgical or medical intervention and other patients (those with normal imaging and nonemergent abnormalities) (Table IV). This comparison revealed that seizure recurrence in PED or within 24 hours, and the need for acute seizure treatment in PED were significantly associated with clinically important intracranial abnormalities (p; 0.004, 0.017, and 0.017 respectively).

Lumbar puncture (L/P) was performed in 23 patients (36.5%). Although the CSF cultures were all negative, other CSF findings were abnormal in four patients. In these patients, the final diagnosis was miliary tuberculosis

Table II. General characteristics of the study patients (n = 65).

Age (months)*	56 ± 52.4
1 - 6 months	9 (14)
6 - 12 months	6 (9)
12 months - 5 years	24 (37)
5 – 18 years	26 (40)
Male gender	43 (66)
Symptoms accompanying seizure	
Fever	30 (46)
Vomiting	26 (40)
Headache	10 (15)
Physical examination findings	
Any abnormal neurological findings	42 (64)
Focal symptoms/signs after seizure	27 (41)
Past medical history and family history	
Underlying illness	16 (24.6)
History of SFS	3 (4.6)
History of SFS in family	5 (7.7)
Family history of epilepsy	6 (9)

Data are presented as number (percentage), unless indicated otherwise. *Mean±standard deviation; SFS: simple febrile seizure.

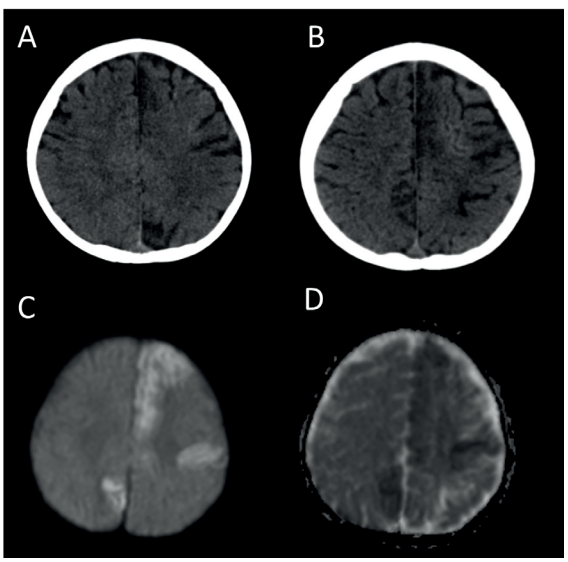

Fig. 2. Images of an 18-month-old patient presenting with a right focal seizure lasting about 2 minutes. Axial non-enhaced CT images (A, B) show cortical hypodense lesions which then were shown as acute cortical infarcts by diffusion restriction of cytotoxic edema on diffusion-weighted imaging (C, D) obtained an hour later. The patient was diagnosed with hereditary spherocytosis on further work-up.

and tuberculous meningitis in one patient, and meningoencephalitis in the other three patients.

Electroencephalogram was performed in 48/65 (73%) patients and there was an abnormality in 35/48 (73%) of them. Among these, epileptiform activity was present in 15 (31%) patients. Other abnormalities were seen in the remaining 23 patients such as irregular, asymmetric or slow background activity. Antiseizure medication was initiated in 41 (63%) patients.

After discharge from the PED, during the long-term follow-up, brain MRI was performed additionally for 14 patients, and none revealed any abnormalities necessitating emergent intervention. Considering 19 patients who underwent MRI at the PED after CT before discharge, a total of 33 patients had cranial MRI in addition to CT. Among these patients, there was a discrepancy between the CT and MRI results in 12 patients (36%) with normal CT findings despite abnormal MRI results. Eight of them were non-emergent abnormalities which could be important in long term follow-up, while four of them (12%) were clinically important intracranial abnormalities: Three cases of meningoencephalitis and one acute cerebrovascular event (arterial occlusion). On the other hand, the abnormality rate in the overall imaging including those performed during follow-up (brain CT and/or MRI) was 55%.

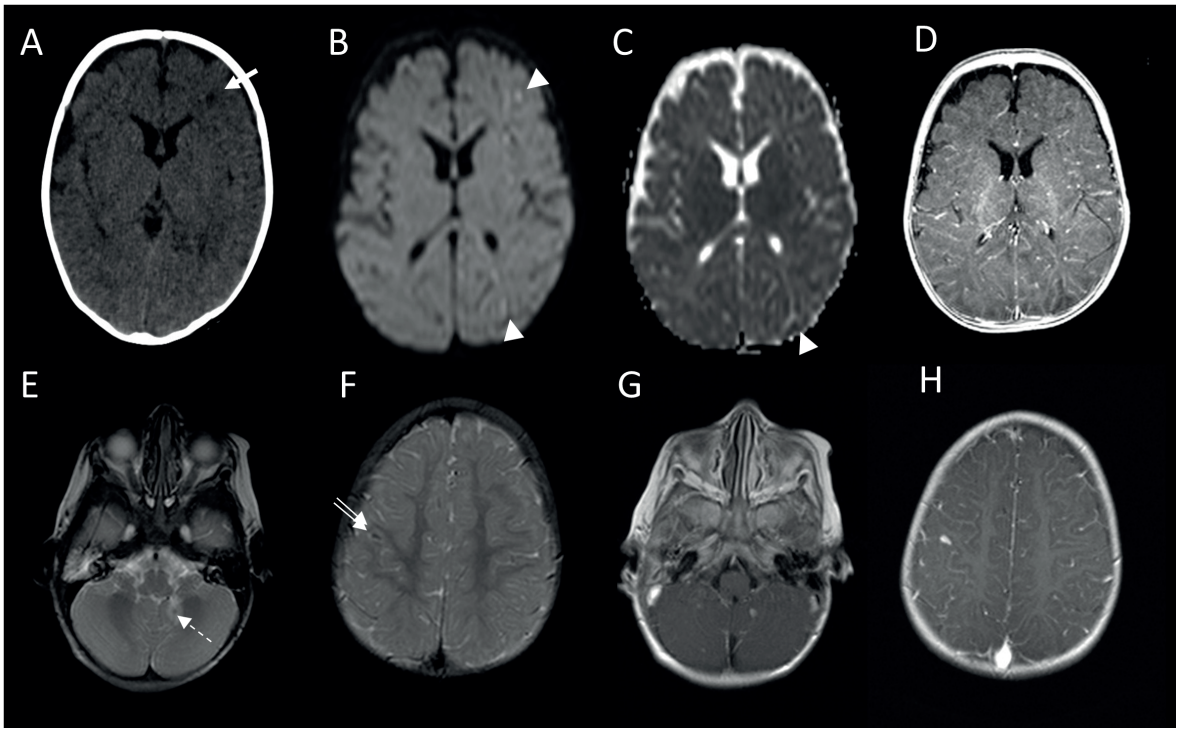

Fig. 3. Images of a child at the age of 5 months (upper row; A to D) and 15 month-old (lower row; E to H) child presenting to Pediatric Emergency Department with focal seizures. Nonenhanced CT of the patient shows nonspecific subtle left frontal periventricular hypodensity (A, arrow) while MRI obtained 6 hours later shows left frontal and parietal cortical tiny foci of diffusion restrictions and diffuse pial enhancement on diffusion-weighted imaging (B, C, arrowheads) and post-Gadolinium T1W image (D). Lower row shows nodular T2 hypointense lesions in the left medial cerebellar hemisphere (E, dashed arrow) and right frontal cortical (F, double arrow) which enhance on post-Gadolinium T1W series (G, H). Please note increased number of lesions are seen on post-contrast imaging. These patients received diagnosis of meningoencephalitis (upper row) and CNS tuberculosis (lower row) lesions.

Table III. Patients with clinically important intracranial abnormalities requiring emergent neurosurgical or medical intervention.

Patient number	Age, gender	Neurological exam findings	Seizure characteristics**	Brain CT	Brain MRI	Final diagnosis
1*	2 years, M	Hypoactive DTRs in lower extremities	LFS, one episode, 20 min.	-	Brain abscess, 4 cm diameter in the right frontoparietotemporal region	Brain abscess. Craniotomy and abscess drainage were performed.
2	11 years, M	Positive meningeal irritation signs, drowsiness	LFS, multiple seizures, focal to bilateral tonic clonic seizure, 15 min.	Normal	Cerebral edema, encephalitis	Encephalitis
3	45 days, F	Positive Babinski sign on the left side, hyperactive DTRs	LFS, one episode, 4 min.	Intraparencyhmal hematoma in right temporal region	-	Late hemorrhagic disease of the newborn
4	16 years, M	Deviation of tongue to the left side	RFS, one episode, 3 min.	Edema and hypodense lesions in left parietal region and cerebellar hemispheres	New lesions in left parietal region and cerebellar hemispheres	Non-Hodgkin lymphoma and secondary cerebellar PNET in follow-up, progression in the mass leading to brain edema
5	15 months, M	Irritability (+), drowsiness (+)	RFS, LFS, multiple seizures, 5 min.	Normal	Diffuse cortical swollen lesions, meningoencephalitis	Meningoencephalitis
6*	4 years, M	Hemiparesis on the right side of the body	RFS	A mass lesion in the left frontal region, 5 cm diameter	Acute hemorrhage into cavernoma in the left frontal lobe, 4.5 cm in diameter	Cavernoma Craniotomy and excision were performed.
7*	4 years, M	Todd paralysis on the right side	RFS, multiple seizures, 60 min.	Chiari Type-1 malformation, chronic compensated hydrocephalus, third ventriculostomy	Chiari Type-1 malformation, acute deterioration of CSF circulation, left parietooccipital polymicrogyria	Acute deterioration of CSF circulation, tap procedure was performed.

CSF: cerebrospinal fluid, CT: computed tomography, DTR: deep tendon reflexes, F: female, M: male, min: minutes, MRI: magnetic resonance imaging, LFS: left focal motor seizure, PNET: Primitive Neuro-Ectodermal Tumor, RFS: right focal motor seizure, *Patients who needed emergent surgical intervention. **Accurate information for seizure duration was not available in some patients.

Table III. Continued.

Patient number	Age, gender	Neurological exam findings	Seizure characteristics**	Brain CT	Brain MRI	Final diagnosis
8	5 months, F	Hyperactive DTRs at the right side	RFS, multiple seizures, 30 min.	Normal	Widespread lesions, dominant in the left cerebral hemisphere, compatible with meningoencephalitis	Meningoencephalitis
9	18 months, F	Central facial paralysis on the right side (newly diagnosed), hemiparesis on the right side (previously present)	RFS, one episode, 2 min.	Acute and early subacute infarction in right parietal and left paramedian regions and chronic ischemic lesions in the left cerebral hemisphere	Ischemic infarct	Hereditary spherocytosis, cerebrovascular event
10	7 months, M	Drowsiness	LFS, multiple seizures	Hemorrhage in right temporal region 22 mm in diameter	Hyperintensity in bilateral temporal lobes consistent with herpes simplex encephalitis	Herpes simplex encephalitis
11	3 years, M	Disturbed cooperation, hyperactive DTRs	RFS, one episode, 1 min.	Normal	Diffuse meningeal enhancement consistent with encephalitis	Encephalitis
12	15 months, F	Decreased spontaneous movements on the left upper and lower extremity	LFS	4 mm lesion in diameter in the right frontal lobe	Infra and supratentorial intense meningeal linear and nodular inflammation	Tuberculous meningitis, miliary tuberculosis
13	4 months, F	Lethargy	RFS, LFS, multiple seizures, 4 min.	Low density area in the left frontal region	Diffuse cortical and subcortical acute ischemic lesions predominantly in the left hemisphere compatible with encephalitis	Encephalitis

CSF: cerebrospinal fluid, CT: computed tomography, DTR: deep tendon reflexes, F: female, M: male, min: minutes, MRI: magnetic resonance imaging, LFS: left focal motor seizure, PNET: Primitive Neuro-Ectodermal Tumor, RFS: right focal motor seizure, *Patients who needed emergent surgical intervention. **Accurate information for seizure duration was not available in some patients.

Table III. Continued.

Patient number	Age, gender	Neurological exam findings	Seizure characteristics**	Brain CT	Brain MRI	Final diagnosis
14	2 years, M	Normal in the acute phase, left hemiplegia was apparent during follow-up	LFS, multiple seizures, 3 min.	Normal	Occlusion in the right middle cerebral artery	Cerebrovascular event, infarction
15	18 months, M	Decreased movements against gravity on the right extremities, Todd paresis	RFS, multiple seizures	A mass lesion with surrounding edema and internal microcalcifications, 38x30 mm in diameter, in the left parietal lobe	A large cavernoma, 43x33 mm in diameter, with hemorrhage and surrounding edema in the left parietal region	Hemorrhagic cavernoma
16	5.5 years, M	No pathologic findings	LFS, multiple seizures, 5 min.	Linear fracture in the right parietal bone and accompanying cerebral edema	-	Cerebral edema and posttraumatic seizure
17*	4 months, M	Irritability	RFS, multiple seizures, 5 min.	Right subdural collection, midline shift, compression in the lateral ventricles	-	Right subdural empyema, craniotomy, and drainage were performed.
18	6 years, F	Disturbed consciousness, bilateral spontaneous positive Babinski sign, clonus at the right lower extremity	LFS, multiple seizures, 3 min.	Diffuse leptomeningeal contrast enhancement and findings attributable to increased intracranial pressure	-	Meningoencephalitis, cochlear partition abnormality

CSF: cerebrospinal fluid, CT: computed tomography, DTR: deep tendon reflexes, F: female, M: male, min: minutes, MRI: magnetic resonance imaging, LFS: left focal motor seizure, PNET: Primitive Neuro-Ectodermal Tumor, RFS: right focal motor seizure, *Patients who needed emergent surgical intervention. **Accurate information for seizure duration was not available in some patients.

Table IV. Comparison of patient characteristics based on presence of clinically important intracranial abnormalities by neuroimaging (brain CT and/or MRI).

	Patients with clinically important intracranial abnormalities on neuroimaging N=18	Other patients N=47	p
Age (months), median (range)	21.5 (1.5-195)	53 (1-188)	0.322
Male gender	12 (66)	31 (66)	0.957
History			
Fever	6 (33)	24 (51)	0.199
Vomiting	8 (44)	18 (38)	0.651
Chronic disease	7 (38.9)	9 (19.1)	0.098
Seizure characteristics			
Seizure duration (min.), median (range)	3 (0.1-60)	5 (0.5-70)	0.211
Number of seizure episodes/day, median (range)	2 (1-7)	1 (1-7)	0.073
Patients with >1 seizure episode/day	11 (68.8)*	20 (42.6)	0.070
Focal symptoms/signs after seizure	10 (55.6)	17 (36.2)	0.156
Acute seizure treatment in PED	12 (66.7)	16 (34)	**0.017**
Seizure recurrence in PED	12 (66.7)	13 (27.7)	**0.004**
Seizure recurrence within 24 hours	13 (72.2)	18 (39.1)	**0.017**

Data are presented as number (percentage), unless indicated otherwise. p values less than 0.05 are printed in bold.
CT: computed tomography, MRI: magnetic resonance imaging, min.: minutes, PED: pediatric emergency department.
*Information regarding number of seizure episodes were missing in two patients in this group.

Discussion

In this study, pediatric patients who presented with a first focal seizure and had neuroimaging in the PED were evaluated, and the requirement for emergent neuroimaging from an emergency department point of view was assessed. The rate of clinically important intracranial abnormalities was 27.7% whereas the total abnormality rate in neuroimaging was 55%. Furthermore, the rate of patients who needed emergent surgical intervention was 6.1%. Seizure recurrence in PED or within 24 hours and the need for acute seizure treatment in PED were significantly associated with the presence of clinically important intracranial abnormalities in neuroimaging.

The need for emergent neuroimaging and its effect on the management of the first seizure in children have been discussed previously.[2-11,17] In general, it is suggested that emergent neuroimaging is not necessary for first seizure patients. However, regarding 'first focal seizure' different approaches have been suggested in the literature. Some authors recommend emergent neuroimaging[8,11] whereas some authors recommend it only if there are any predisposing conditions or if the age of the patient is younger[3,9,10] and some further recommend non-emergent imaging.[2] Sharma et al.[9] reviewed 500 children with new onset afebrile seizure, of whom 167 (33%) presented with documented focal features. After excluding patients with predisposing conditions, they found that 12.7% of the focal seizure patients had clinically significant abnormal imaging findings, mostly at younger ages, and suggested emergent imaging for focal seizure patients if the age is under 33 months.[9] In a recent study, Amagasa et al.[18] found that focal seizure, prolonged seizure, and seizure cluster (similar to ours') were risk factors for CT or MRI abnormalities. The only study in the literature that included solely first afebrile

seizure patients with focal manifestations with quite wide exclusion criteria such as underlying chronic disease, altered mental status, acute trauma, etc., found the rate of clinically urgent intracranial pathology as 4.1%, with hemorrhage and infarction being the most common.[10] In that study, the authors suggested emergent neuroimaging for focal seizure patients under the age of 18 months. Recently Brugman et al.[16] published a study about the necessity of urgent brain CT for the first seizure in which they used the same case classification system as ours'. In that study, predictors for clinically important intracranial pathologies were found as persistent post-ictal abnormal neurological state and a new post-ictal focal finding however focal onset failed to reach statistical significance. Compared with most of these studies, in our study the rates are quite higher which may be due to several factors. Since we aimed to assess general risk factors for clinically important intracranial abnormalities in first focal seizure not only in previously healthy children but for all pediatric patients (30 days-18 years) admitted to the PED, we did not exclude patients with fever or chronic underlying conditions. Our hospital is a tertiary referral hospital localized in the center of the country and an important part of the patients admitted to the PED have underlying chronic conditions. Therefore, our cohort might represent a more severe and complicated patient group than the general population. The rate of 27.7% that we found for clinically important intracranial abnormalities in emergent neuroimaging supports the need for emergent neuroimaging in this patient group. The differences between several previous studies and our study may be related to the differences both in patient inclusion/exclusion criteria and the description of "clinically important abnormalities".

In this study, seizure recurrence in PED or within 24 hours and the need for acute seizure treatment in PED were significantly associated with clinically important intracranial abnormalities. Our comparison also revealed that the median number of seizure episodes/day and rate of patients with multiple seizure episodes/day were higher in the group with clinically important intracranial abnormalities although results were not statistically significant, most likely due to the low number of cases (Table IV). Here we note that seizure recurrence at the PED or multiple focal seizures at presentation should alert the clinician for emergent neuroimaging at the PED. There are a few studies pointing to the number of seizures at the presentation of the first seizure.[3,18] Other than emergent imaging, recurrence of seizure also supports the need for a longer follow-up at the PED or admission to the wards.[19]

In the current study, there was a discrepancy between the yields of CT and MRI results in 12 patients (36%) with normal CT and abnormal MRI results. Of note, four of 12 patients (12%) had clinically important intracranial abnormalities; three meningoencephalitis cases and an arterial ischemic infarct. In the study by Sharma et al.[9], among the patients whose brain CT was normal, 43% underwent brain MRI and clinically significant abnormalities were detected in 3.7% of them. In another study that included patients under two years of age (excluding newborns) presenting with first afebrile seizure, among the patients with normal CT, the rate of abnormality in the MRI was 33%.[19] Most of these abnormalities were not urgent but important for the patient's prognosis and long-term management, however, in one patient MRI revealed a watershed infarct that required acute management similar to our study.[19] As an imaging modality, with superior soft tissue contrast, MRI shows minor developmental malformations, white matter lesions, parenchymal lesions, neurocutaneous disease lesions, and thrombotic cerebrovascular events, and pathologies of posterior fossa better than CT. Requirement for anesthesia, especially in young children, and higher cost represent the key disadvantages of MRI. However, CT offers the advantages of widespread availability, speed, low cost, and superiority in acute bleeding and calcification as well as fractures; yet, with the major disadvantage of radiation

exposure. In the guidelines published by ILAE, it is mentioned that MRI is superior to CT in patients presenting with seizures, although CT can be considered a screening method when MRI is not available or cannot be performed.[15] In the same report, it is also noted that in cases of recurrent seizures, abnormal neurological examination, or for better description of the abnormalities seen in CT, brain MRI can be performed. Similarly, it has been mentioned in other studies that an MRI should be the preferred neuroimaging modality in children presenting with first afebrile seizure although it may not be available or applicable for emergent cases.[7,11] From the view of the management at the PED; we suggest a faster MRI protocol including fluid-attenuated inversion recovery (FLAIR) and diffusion-weighted imaging designed for prompt diagnosis and treatment, especially in the early phase of vascular infarcts.

The present study has some limitations, mainly due to its retrospective nature and small sample size. As shown in Figure 1, we were unable to reach all patient files for manual review, most likely due to the shortcomings in the archive system during those dates. This was the major handicap that led to a small sample size despite the fact that our center is a reference hospital. Other than these factors, although first focal seizure was a strict criterion for performing emergent neuroimaging in our center, our method for patient selection may have created a possible bias and a small portion of patients may have been missed. Also, some seizures with a very short focal onset may be overlooked as general seizures and hence not listed as focal seizures.

In this study, pediatric patients with first focal seizure were evaluated, and the yield of neuroimaging studies for clinically important intracranial abnormality was 27.7%. This shows that focal seizures must be evaluated meticulously. From an emergency department point of view, we suggest that first focal seizures in pediatric patients should be evaluated with emergent neuroimaging, if possible with a dedicated MRI protocol. Especially those with recurrent seizures at presentation should be subject to more careful evaluation. Prospective studies in these patient groups are needed and will contribute more to identifying the risk factors.

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Ethical approval

Ethical approval was obtained from the Ethics Committee of Hacettepe University (GO 14/577).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design; TK, ÖT, GT; data collection; TK, BK; analysis and interpretation of results; TK, ÖT, GT, KKO, GH, DY; draft manuscript preparation; TK, ÖT. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Efficacy of single dose of phenytoin/fosphenytoin in benign convulsions with mild gastroenteritis

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ABSTRACT

Background. This study evaluated the efficacy of a single dose of phenytoin/fosphenytoin (PHT) to control repetitive seizures in children with benign convulsions with mild gastroenteritis (CwG).

Methods. Children aged between 3 months and 5 years with CwG were retrospectively enrolled. Convulsions with mild gastroenteritis were defined as (a) seizures with acute gastroenteritis without fever or dehydration; (b) normal blood laboratory results; and (c) normal electroencephalography and brain imaging findings. Patients were divided into two groups according to whether or not intravenous PHT (10 mg/kg of phenytoin or phenytoin equivalents) was administered. Clinical manifestations and treatment efficacy were evaluated and compared.

Results. Ten of 41 children eligible for inclusion received PHT. Compared to children in the non-PHT group, those in the PHT group had a higher number of seizures (5.2 ± 2.3 vs. 1.6 ± 1.0, $P < 0.001$) and a lower serum sodium level (133.5 ± 3.2 mmol/L vs. 137.2 ± 2.6 mmol/L, $P = 0.001$). Initial serum sodium levels were negatively correlated with seizure frequency ($r = -0.438$, $P = 0.004$). In all patients, seizures were completely resolved with a single dose of PHT. There were no significant adverse effects from PHT.

Conclusions. A single dose of PHT can effectively treat CwG with repetitive seizures. The serum sodium channel may play a role in seizure severity.

Key words: phenytoin; fosphenytoin; sodium channel; benign convulsions; mild gastroenteritis.

Benign convulsions with mild gastroenteritis (CwG) are clinically diagnosed by afebrile seizures with mild gastroenteritis symptoms that are not accompanied by clinical signs of dehydration, electrolyte imbalance, or hypoglycemia, in previously healthy infants and young children aged 1 month to 6 years.[1,2] Although CwG has predominantly been reported in children of East Asian countries, cases in Europe and the United States of America have also been described.[3-9] In almost half of the affected patients, the seizures tend to occur in clusters over 1-2 days, but there is no long-term risk of recurrent seizures.[2,10] Benzodiazepines are commonly used as first-line antiseizure medications in the emergency department; however, these medications are not effective in children with repetitive seizures during acute CwG.[3] Although clustering seizures in CwG have shown a good prognosis without persistent neurologic complications, they can cause parental anxiety, and clinicians may repeat or administer unnecessarily high doses of antiseizure medications to control repetitive seizures.

Previous studies have reported the effectiveness of carbamazepine, phenobarbital, lidocaine or fosphenytoin in controlling repetitive seizures in CwG.[3,11-13] We hypothesized that voltage-gated sodium channels play a role in initiating

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seizures in CwG. In the present study, we evaluated the effectiveness of phenytoin/fosphenytoin (PHT), which has inhibitory effects on sodium channels, in controlling repetitive seizures in CwG. Patients with more than two seizures during the acute illness were treated with a lower dose of PHT than that used in previous studies, to minimize the risk of side effects. Patient's clinical characteristics and treatment responses were assessed.

Material and Methods

Patients

This was a retrospective study to evaluate the efficacy of PHT in controlling repetitive seizures in CwG patients aged between three months and five years who were admitted to Busan Paik Hospital in South Korea from January 2013 to December 2018. All patient records were reviewed to collect data on clinical manifestations, laboratory studies, electroencephalography (EEG), and brain magnetic resonance imaging (MRI) findings. Ethical approval for this study was provided by the institutional review board of Busan Paik Hospital (approval number: 180179). Written informed consent by the patients was waived due to the retrospective nature of our study.

In this study population, CwG was defined as follows: 1) seizures accompanied by acute gastroenteritis symptoms in previously healthy infants and children; 2) normal neurodevelopment and neurological examination findings; 3) normal laboratory findings without hyponatremia (serum sodium < 130 mg/dl), hypoglycemia (serum glucose ≤ 50 mg/dL), and cerebrospinal fluid (CSF) abnormalities; 4) normal EEG and brain imaging findings.[2] We excluded patients who were diagnosed with meningitis, encephalitis, or epilepsy and had seizures associated with fever during acute gastroenteritis.[2] Patients were divided into two groups according to whether they received intravenous PHT or not. A single dose of intravenous PHT was slowly administered over 30 minutes at a dose of 10 mg/kg for phenytoin from 2013 to 2016. Fosphenytoin was introduced at our institution in 2017 and children treated from 2017 to 2018 received 10 mg phenytoin equivalents (PE)/kg using the same rate of administration. We administered low doses of intravenous PHT, 10 mg/kg, because previous studies reported good efficacy with fewer adverse effects for low doses of antiseizure medications such as phenobarbital at a dose of 5–10 mg/kg[3,4,13], carbamazepine at a dose of 5 mg/kg[11], and phenytoin at a dose of 5–10 mg/kg[3] in controlling repetitive seizures.

The efficacy of PHT was evaluated in the acute phase of CwG. Vital signs, including body temperature, blood pressure, pulse rate, respiratory rate, and percutaneous oxygen saturation were monitored during and after seizures, and when administering antiseizure medications. All patients were hospitalized and discharged when they had been seizure-free for more than 24 hours.

Clinical data including age, sex, preceding gastroenteritis symptoms, the interval from gastroenteritis symptoms to seizure onset, seizure characteristics (semiology, duration, frequency, and interval between the first seizure and the last seizure), benzodiazepine usage during the acute seizure period, length of hospital stay, previous history of seizures and antiseizure medications, were collected for each patient.

Laboratory tests

The following laboratory tests were performed in all patients at the time of presentation to the emergency department: complete blood cell count, electrolyte panel, serum glucose, C-reactive protein, and liver enzymes. The positive rate of stool rotavirus or norovirus, EEG features, CSF analysis and brain MRI findings were also reviewed. We conducted an immunochromatographic assay using the SD BIOLINE Rotavirus Rapid kit (Standard Diagnostics, Inc., Korea) for the detection of group A rotavirus in fecal specimens. For

the detection of norovirus, a QuickNavi®-Norovirus 2 kit was used according to the manufacturer's instructions. The rotavirus and norovirus antigen tests were performed until 2014. A stool multiplex real-time RT-PCR with Allplex GI-Virus Assay (Seegene, Seoul, Korea), detecting norovirus, rotavirus, adenovirus, and astrovirus was introduced in our hospital when it became available in 2015.

Statistical analyses

All statistical analyses were performed using SPSS for Windows, version 26.0 (SPSS Inc., an IBM Company, Chicago, Illinois, USA). Numerical variables were assessed for the assumption of normality of variables by the Shapiro Wilk test. Continuous variables are presented as mean ± standard deviation or median (interquartile range) and qualitative variables are expressed as percentages. The comparisons were performed through the Student's t-test when the continuous variable was distributed normally or Mann-Whitney U test when the variable was not distributed normally. Pearson's chi-squared test or Fisher's exact test were used for categorical variables. Fisher's exact test was used for cases where the expected frequency is less than 5. Two-tailed null hypotheses of no difference were rejected if *P* values were less than 0.05. The association between serum sodium level and frequency of seizures in all patients was evaluated using the Pearson correlation coefficients.

Results

Patients' clinical findings

Forty-six children with CwG were enrolled during the study period, of whom 41 were included in the analysis (Fig. 1). The average age was 18.1 ± 10.4 (range, 3–56) months and 18 (44%) were male (Table I). Gastroenteritis symptoms of vomiting and diarrhea were observed in 33 (81%) and 36 (88%) patients, respectively, and they occurred within three days before the onset of seizures. Three patients had previously experienced febrile seizures. The most common semiology of seizure was a generalized motor seizure (85%), and the majority of seizures (98%) were shorter than five minutes. Repetitive seizures were observed in 23 patients (56%) and 13 patients (32%) had more than three seizures. None of the participants experienced prolonged seizures that lasted >15 minutes. Brain MRI was performed in 40 patients and interictal EEG in 39 patients, both of which showed no abnormalities. Six of 32 patients returned positive tests for rotavirus and nine of 20 patients returned positive tests for norovirus. Cerebrospinal fluid tests were performed on 13 patients and were normal.

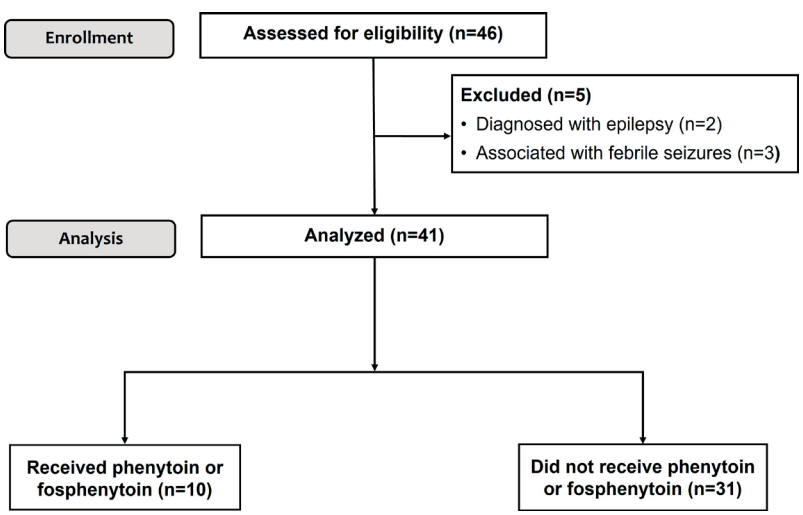

Fig. 1. Study flow chart describing the study population.

Table I. Comparison of clinical findings between groups.*

Variables	PHT group (n = 10)	Non-PHT group (n = 31)	p
Age at diagnosis, mean±SD	17.2 ± 5.3	18.4 ± 11.6	0.76[a]
Male sex	4 (40)	14 (45)	1.0[b]
Vomiting	8 (80)	25 (81)	1.0[b]
Diarrhea	8 (80)	28 (90)	0.58[b]
Latency to seizure onset**			0.43[b]
< 24 hours	2 (20)	7 (23)	
24–48 hours	8 (80)	18 (58)	
48–72 hours	0 (0)	6 (19)	
Two or more seizures	10 (100)	13 (42)	0.002[b]
Three or more seizures	10 (100)	3 (42)	< 0.001[b]
Number of seizures, mean±SD	5.2 ± 2.3	1.6 ± 1.0	< 0.001[a]
Interval from 1st seizure to last (hours), median (IQR)	8 (6–11)	6 (5–11)	0.47[c]
Seizure semiology			1.0[b]
Focal to bilateral tonic-clonic	1 (10.0)	5 (16)	
Apparently generalized	9 (90.0)	26 (84)	
Seizure duration			1.0[b]
< 1 min	1 (10)	5 (16)	
1–5 min	9 (90)	25 (81)	
> 5 min	0 (0)	1 (3)	
Use of initial lorazepam	10 (100)	7 (23)	< 0.001[b]
Length of hospital stay (days), median (IQR)	3 (3–5)	3 (2–4)	0.13[c]

P values refer to comparisons across the two groups. *Data are indicated as number (percentage) unless indicated otherwise.
**Latency to seizure onset was defined as the interval between the onset of gastroenteritis and the onset of seizures.
Abbreviations: IQR, interquartile range; PHT, phenytoin or fosphenytoin.; SD, standard deviation.
[a]Student's t-test, [b]Fisher's exact test, [c]Mann Whitney-U test

Clinical and laboratory characteristics in the PHT group

Ten children were administered a single dose of PHT to control repetitive seizures; phenytoin was administered to six patients and fosphenytoin to four (Table II). The clinical characteristics and laboratory findings were compared between the two groups according to whether (PHT group) or not (non-PHT group) they received phenytoin or fosphenytoin (Tables I and III). There was no significant difference in clinical characteristics such as age, sex, gastroenteritis symptoms, the interval between the onset of gastroenteritis and seizures, seizure semiology and duration between the groups (Table I).

Patients in the PHT group had more seizures before treatment than the non-PHT group had in total (5.2 ± 2.3 vs. 1.6 ± 1.0, $P < 0.001$) and all patients who received PHT had three or more seizures during the acute period, although the median interval from the onset of the first seizure to the last seizure was not significantly different between groups. All patients in the PHT group experienced recurrent seizures despite receiving an initial dose of intravenous lorazepam; therefore, a single dose of PHT was administered, which resolved the seizures for all 10 patients. No other antiseizure medications, aside from lorazepam, were administered to patients in the non-PHT group. Seizures were controlled in seven patients in the non-PHT group who received an initial dose of intravenous lorazepam. The seizures ceased spontaneously in the remaining 24 patients of the non-PHT group, and these patients did not require lorazepam.

Table II. Clinical manifestations of 10 patients who received phenytoin or fosphenytoin.

Patient no.	Age (Months)	Sex	Number of seizures before PHT	Interval from the first to last seizure (h)	Administered antiseizure medications	Causative virus	Initial serum sodium level (mmol/L)	Follow up serum sodium level (mmol/L)
1	22	Male	5	8	Lorazepam, phenytoin	Unknown	130	136
2	13	Female	3	3	Lorazepam, phenytoin	Rotavirus	130	137
3	23	Male	4	9	Lorazepam, phenytoin	Rotavirus	140	142
4	6	Male	10	10	Lorazepam, phenytoin	Unknown	132	134
5	15	Female	7	8.5	Lorazepam, phenytoin	Norovirus	133	138
6	24	Female	3	7	Lorazepam, phenytoin	Unknown	136	139
7	17	Female	7	6.5	Lorazepam, fosphenytoin	Norovirus	132	135
8	18	Female	6	16	Lorazepam, fosphenytoin	Norovirus	136	139
9	17	Female	4	13	Lorazepam, fosphenytoin	Unknown	135	136
10	17	Male	3	4	Lorazepam, fosphenytoin	Norovirus	131	139

PHT: phenytoin or fosphenytoin.

Table III. Comparison of laboratory parameters between the two groups.

Variables	PHT group (n=10)	Non-PHT group (n=31)	p
Peripheral WBC (/mm^3)	10738 ± 3155	9294 ± 4674	0.37[a]
Hemoglobin (g/dL)	12.0 ± 0.8	12.2 ± 0.9	0.60[a]
C-reactive protein (mg/dL)	0.12 ± 0.18	0.42 ± 0.71	0.19[a]
Sodium on admission (mmol/L)	133.5 ± 3.2	137.2 ± 2.6	0.001[a]
Hyponatremia on admission*	7 (70)	7 (23)	0.02[b]
Sodium after 1–2 days of admission (mmol/L)	137.5 ± 2.4	138.9 ± 1.7	0.06[a]
Calcium (mg/dL)	9.9 ± 0.6	9.7 ± 0.6	0.57[a]
Ionized calcium (mmol/L)	1.09 ± 0.15	0.94 ± 0.27	0.09[a]
Venous glucose (mg/dL)	86.5 ± 21.2	87.2 ± 18.6	0.92[a]
Positive test for rotavirus	2/9 (22)	4/23 (17)	1.00[b]
Positive test for norovirus	4/5 (80)	5/15 (33)	0.13[b]

Note: Values are expressed as mean ± standard deviation or number of patients (%). The denominator is the number of patients for which data is available, and the numerator is the number of patients corresponding to the variable. *Number of patients with hyponatremia, defined as a serum sodium level of 130–135 mmol/L. Abbreviations: PHT, phenytoin or fosphenytoin; WBC, white blood cell.
[a]Student's t-test, [b]Fisher's exact test

No significant differences in laboratory findings including complete blood cell count, C-reactive protein, serum calcium, glucose level, or positive rates of rotavirus or norovirus were found between the two groups. However, the average initial sodium level was lower in the PHT group (133.5 ± 3.2 mmol/L vs. 137.2 ± 2.6 mmol/L, *P* = 0.001). The follow-up mean sodium level one or two days after PHT administration was 137.5 ± 2.4 mmol/L, with no significant difference compared to the non-PHT group. No patients required treatment for hyponatremia. In this cohort, low serum sodium levels correlated with more frequent seizures, although the size of the correlation was low (r = -0.438, *P* = 0.004). There were no meaningful adverse effects associated with the administration of phenytoin or fosphenytoin.

Discussion

In the present cohort study of children with CwG, PHT demonstrated good efficacy and safety for controlling repetitive seizures. After administration of 10 mg/kg of phenytoin or 10 mg PE/kg of fosphenytoin, all children showed complete cessation of seizures that were not controlled by initial lorazepam therapy. The initial serum sodium level was lower in children who received PHT than in those who did not, and this improved after one or two days. The sodium level negatively correlated with the frequency of seizures although the size of the correlation was low. The results of this study suggest that PHT is an effective drug for controlling repetitive seizures and that sodium channel abnormalities may be the cause of repetitive seizures in CwG.

In this study, about half of the patients (n = 23, 56%) had two or more seizures with the same semiology within 24 h. Lorazepam was administered to 17 patients (41%) with two or more seizures during the acute phase, but it was not effective in 10 patients (59%); however, the administration of PHT was effective in controlling repetitive seizures for these patients. Seizures spontaneously resolved in the remaining six patients who experienced two or more seizures without the administration of any additional antiseizure medications. These findings are consistent with those of the prior studies, which demonstrated that repetitive seizures could occur in CwG and that these may be refractory to benzodiazepine.[3,7,14-16] Previous studies reported that benzodiazepine therapy as a first-line treatment is effective at preventing further seizures in 25–59% of CwG patients, indicating the necessity of identifying other treatment strategies.[3,7,15,17]

Our study demonstrated the superior efficacy of PHT to control repetitive seizures compared to lorazepam, supporting prior studies that reported that antiseizure medications with inhibitory effects on voltage-dependent neuronal sodium channels seem to be effective for CwG.[3,4,11,12,15,16,18,19] Nakazawa et al.[12] reported the efficacy and tolerance of fosphenytoin in children with CwG. Fourteen of 16 patients (88%) experienced no further seizures after administration of fosphenytoin (median dose, 22.5 mg/kg) and no side effects from the medication. Our patients received only a half loading dose of PHT (10 mg/kg) to avoid side effects, but this was effective at controlling repetitive seizures. No patients experienced side effects of PHT such as hypotension, cardiac arrhythmia, or local skin reactions because low doses of PHT were slowly injected over 30 minutes and continuous blood pressure and cardiac rhythm monitoring were conducted by attending physicians and nurses. Some previous studies have reported that low-dose carbamazepine (5 mg/kg/day) or lidocaine that blocks voltage-gated sodium channels also showed good efficacy in controlling repetitive seizures in CwG.[3,11,16,19] However, patients with repetitive seizures usually cannot take carbamazepine orally because they are generally drowsy after seizures. Furthermore, it is not easy to insert a nasogastric tube as these patients may also experience recurrent vomiting with CwG. Lidocaine infusion has not been approved for controlling seizures and cannot be administered as an antiseizure

medication. A recent Japanese small cohort study demonstrated the efficacy of intravenous phenobarbital to prevent further seizures in CwG.[13] Their randomized, placebo-controlled trial showed that a single dose of 10 mg/kg of phenobarbital was effective in all seven patients in the phenobarbital group, and five of six patients in the placebo group had recurrent seizures after administration of normal saline. Phenobarbital not only stimulates GABA-A receptor subunits to induce hyperpolarization, but also selectively blocks the inactive form of closed sodium channels.[16,20] However, excessive somnolence, drowsiness, and unsteadiness can be problematic side effects of phenobarbital usage.[19]

It is not clear why antiseizure medications that block sodium channels are more effective than benzodiazepines for CwG. Weng et al.[21] tried to identify pathogenic variants of neuronal sodium channel alpha 1 subunits (*SCN1A*) in 12 patients with CwG. However, no pathogenic variants in the *SCN1A* gene were identified in their study.[21] Our study showed that serum sodium was lower in patients who received PHT than in those who did not, and this was significantly associated with the number of seizures patients experienced, although the changes in sodium levels were within the normal range. These results suggest that modulators of sodium channels or single nucleotide polymorphism of sodium channels may be responsible for provoking repetitive seizures in CwG. Motoyama et al.[16] demonstrated that serum sodium and chloride levels in patients with seizures were lower than those without seizures in rotavirus gastroenteritis, indicating the possible relevance of sodium channels in CwG. Additionally, other studies also reported similar results of lower serum sodium levels associated with CwG.[22,23] However, no studies have defined the pathomechanism of CwG so far. The implication of decreased sodium levels in the PHT group and some negative correlation between sodium levels and the number of seizures in the setting of CwG are uncertain and merit future research.

Our study has some limitations. First, this was a single center study with a small sample size, and the study design was a retrospective observational study. Prospective randomized controlled studies from larger cohorts are required to define the efficacy and safety of PHT for the control of repetitive seizures in CwG. Second, the efficacy and safety of PHT could not be compared with other antiseizure medications such as phenobarbital. Further comparative studies with other antiseizure medications will be necessary to confirm the effectiveness of PHT in controlling repetitive seizures. Third, we did not evaluate long-term follow-up data. However, the results of this study are valuable because they demonstrate that a single low dose of PHT was effective at resolving seizures during admission, and there were no cases of recurrent seizures after discharge. Several previous studies have also demonstrated the excellent prognosis of CwG.[2,14,23-25] Finally, we did not evaluate the mechanism by which sodium channels contribute to CwG pathogenesis.

In conclusion, the present study suggests that a single dose of PHT in CwG effectively and safely controlled repetitive seizures. Further studies should be conducted to demonstrate the efficacy of PHT compared with other antiseizure medications in CwG and the possible mechanism of action associated with sodium channels.

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Ethical approval

This work was approved by the institutional review board of Busan Paik Hospital (approval number: 180179). Written informed consent by the patients was waived due to a retrospective nature of our study.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: BLL; data collection: KSL, BLL; analysis and interpretation: BLL; draft manuscript: KSL, BLL. 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

Implications of serial magnetic resonance imaging in the management of a newborn with vein of Galen aneurysmal malformation and a review of the relevant literature

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ABSTRACT

Background. Despite advanced endovascular methods and comprehensive intensive care in the neonatal vein of Galen aneurysmal malformation, overall mortality ranges between 37-63% in treated patients with 37-50% of survivors possessing poor neurologic outcomes. These findings stress the need for more accurate and timely recognition of the patients who may and may not benefit from aggressive intervention.

Case. This case report presents a newborn with a vein of Galen aneurysmal malformation whom antenatal and postnatal follow-up included serial magnetic resonance imaging (MRI) including diffusion-weighted series.

Conclusions. Given the experience from our current case and in light of the relevant literature, it is plausible that diffusion-weighted imaging studies may widen our perspective on dynamic ischemia and progressive injury occurring within the developing central nervous system of such patients. Meticulous identification of patients may favorably influence the clinical and parental decision on early delivery and prompt endovascular treatment versus aiding avoidance of further futile interventions both antenatally and postnatally.

Key words: vein of Galen aneurysmal malformation, magnetic resonance imaging, diffusion-weighted imaging, newborn.

Vein of Galen aneurysmal malformation (VGAM) is a relatively rare (1:25000 live births) congenital arteriovenous malformation (AVM) of the central nervous system (CNS) associated with high mortality rates in the neonatal period.[1,2] Endovascular treatment (ET) is the therapy of choice in symptomatic newborns and aims to relieve life threatening high-flow heart failure.[1,2] Despite recent improvements and comprehensive follow-up, survival rates as well as overall clinical outcomes are relatively poor, with neurodevelopmental disorders frequently seen among survivors, raising questions regarding our understanding of the disease.[1-4] This case report highlights the importance of individualized antenatal and postnatal magnetic resonance imaging (MRI) in particular, diffusion-weighted imaging (DWI) in newborns with VGAM, while addressing its possible contribution to clinical and parental decision making both antenatally and postnatally.

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Case Report

A singleton male neonate weighing 2530 grams (50-75th percentile) with a prenatally diagnosed VGAM was born to a 29-year-old mother by cesarean section at 34+5 weeks of gestation. The neonate was admitted to the neonatal intensive care unit (NICU) for further evaluation immediately after endotracheal intubation and stabilization of vital signs.

Antenatal diagnosis of VGAM was made incidentally during routine antenatal ultrasound screening at 23 weeks of gestation at another institution, where early termination of pregnancy was not considered. The patient was further evaluated at a later stage during pregnancy at 31 weeks of gestation in our institution with fetal MRI (Fig. 1), which revealed a significantly dilated median prosencephalic vein in continuum with a large choroidal type VGAM. DWI studies showed restricted diffusion with corresponding low signal areas on apparent diffusion coefficient (ADC) maps consistent with acute ischemia of the left occipital and the left posterior parietal regions.

The first examination showed a lethargic appearing baby with general hypotonicity and hypoactive newborn reflexes, an occipitofrontal circumference of 33 cm (75-90th percentile), a tense anterior fontanelle with a continuous murmur and hyperactive precordium with 2/6 systolic murmur on auscultation. Tachycardia (heart rate: 180/minute) and tachypnea (respiratory rate: 70/minute) with sub- and intercostal retractions were remarkable. Chest X-ray revealed cardiomegaly and ruled out concomitant severe respiratory parenchymal disease. Bedside echocardiography demonstrated supra-systemic pulmonary hypertension (right ventricle systolic pressure: 75 mmHg) in concurrence with dilated right-sided cavities of the heart, a wide patent ductus arteriosus (PDA) with right-to-left shunt and a retrograde aortic flow. Bedside cranial Doppler ultrasound imaging confirmed the diagnosis of VGAM.

Time-of-Flight MRI performed on the 2nd day of life showed the choroidal type VGAM located to the left of the midline, with a venous pouch of 4.4 cm in craniocaudal direction and multiple arterial feeders predominantly from the left posterior cerebral artery (PCA) and left middle cerebral artery (MCA) (Fig. 2A and 2B). When compared to fetal MRI findings, the venous pouch and feeding vessels had increased in

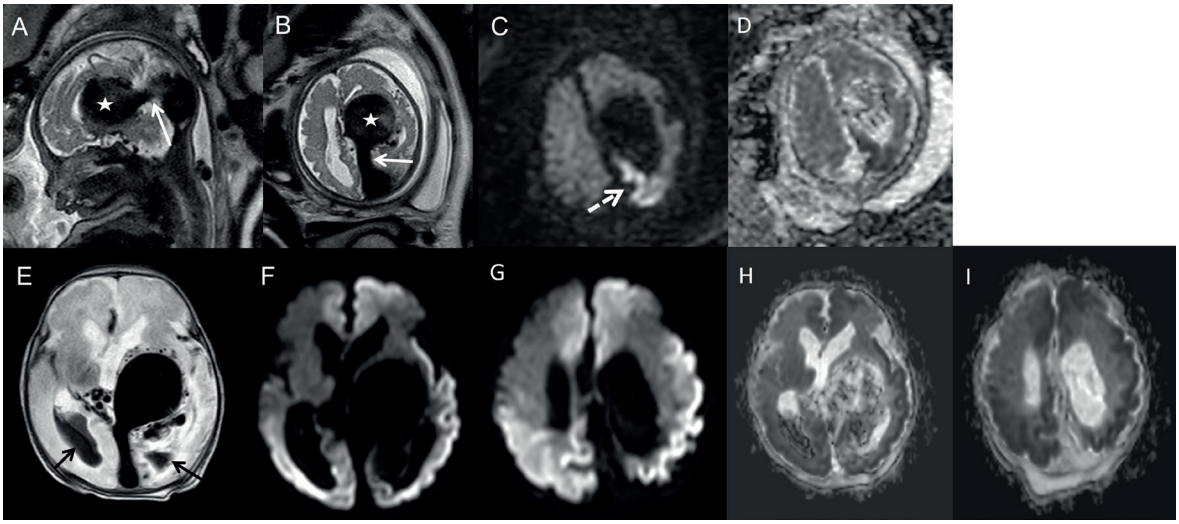

Fig. 1. Cranial MRI studies of the patient performed in-utero **(A-D)** and following endovascular treatment at 7 days of age **(E-I)**. Enormously enlarged Torcula and median prosencephalic vein (arrow) ending in a venous aneurysm (asterix) are displayed on sagittal **(A)** and axial **(B)** T2-weighted images. Restricted diffusion on average diffusion-weighted image **(C)** and corresponding low signal on ADC map **(D)** suggestive of cytotoxic edema due to acute ischemia of the left medial parietal cortex (dashed arrow) is observed. Following endovascular treatment, there is an intraventricular hemorrhage layering in the occipital horns of the lateral ventricles (black arrows) and accompanying hydrocephalus are shown on axial T2-weighted image **(E)** as well as bilateral extensive hemispheric cortical diffusion restriction **(F-I)** on both average diffusion-weighted image and ADC maps this time.

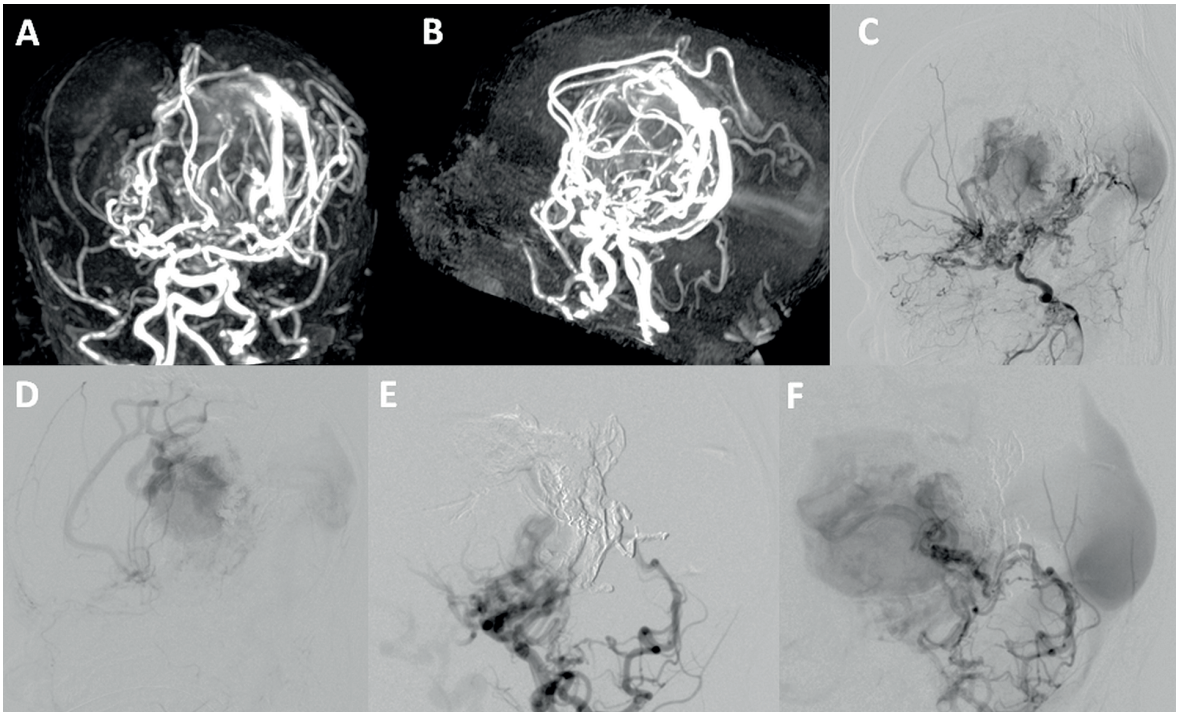

Fig. 2. Pre-embolization reconstructions (maximum intensity projection) of time-of-flight cranial MR angiogram in anteroposterior **(A)** and lateral **(B)** projections show a diffuse conglomerate of arteries and drastic enlargement of a venous pouch. Both anterior cerebral, posterior cerebral arteries and all choroidal arteries supply the arteriovenous malformation. On DSA obtained after the third session of embolization, on the lateral views of the left **(C)** and right **(D)** internal carotid artery injections as well as early **(E)** and late **(F)** phases of left vertebral arteriograms, there is still a significantly large residual arteriovenous malformation.

size, resulting in compression of the left lateral ventricle and left mesencephalon with a 1-cm subfalcine shift from the midline to the right. Both lateral ventricles were dilated.

The child continued to deteriorate and developed signs of circulatory collapse despite the administration of maximum cardiopulmonary support including dopamine, adrenaline and high frequency oscillatory ventilation. Given the inefficiency of medical treatment, a multidisciplinary consensus was reached to commence with ET. On the 3rd, 7th and 10th days of life, three sessions of ET were performed under general anesthesia and fluoroscopic guidance with full cardiorespiratory support were given by both the neonatology and anesthesia teams. In all sessions, the umbilical artery was used for arterial vascular access. Arterial feeders arising from the left PCA, left internal carotid artery (ICA) and left anterior cerebral artery (ACA) were super-selectively catheterized in the 1st, 2nd and 3rd procedures, respectively (Fig. 2). Embolization was achieved with coils, n-butyl cyanoacrylate/lipiodol mixture (Glue) and Squid (Emboflu, Switzerland). Angiograms were performed with a water-soluble nonionic iodine-based contrast material with a maximum dose limited to 7 ml per kilogram for each session.

Cranial MRI was performed after the 2nd session of ET for post-treatment follow-up imaging and for guidance regarding further endovascular intervention. It showed the decreased maximum size of the venous pouch with a caudocranial length of 3.2 cm, a small intraventricular extravasation and persistence of ventricular dilatation as well as midline shift. DWI studies and ADC maps showed increased extension of diffusion restriction of the hemispheric cortices bilaterally (Fig. 1) suggesting the

futility of the 2 previous treatments. As DWI restrictions symmetrically involved the main cortical areas, and as these findings were in part present in the prenatal imaging and finally since such restrictions were also present in the right anterior circulation which had not been accessed during any of the interventions, it was concluded that the MRI findings represented a severe and progressive arterial insufficiency involving the cerebral hemispheres. Although neuroimaging findings suggested undesirable neurologic outcomes, the 3rd session of ET was pursued upon request of the family who understood the poor prognosis of the child and also in accordance with the current legislation demanding that all that could be done unless brain death was officially announced.

The third neuroradiological intervention and meticulous neonatal intensive care failed an improvement in neurological findings. Serial echocardiographic examinations after each ET session showed only partial but no significant improvement in heart failure. Respiratory distress followed a refractory course, precluding extubation. The neonate was lost due to cardiorespiratory failure on the 14th day of life.

Discussion

Vein of Galen aneurysmal malformation (VGAM) is the most commonly seen congenital AVM in the neonatal period with morbidity and mortality reaching almost 100% in the absence of accurate management.[1,2,5] Despite advanced endovascular methods and thorough intensive care; overall mortality ranges between 37-63% (13-35% with successful ET).[1-7] Furthermore, 37-50% of survivors possess poor neurologic outcomes, while ones with presumed good outcome experience some neurocognitive or functional deficits in the later stages of their lives.[1-4,6,7] These findings indicate the need for a more accurate identification of the VGAM patients who will and will not benefit from aggressive intervention. While further comparative research is needed, it is plausible that antenatal and postnatal conventional MRI combined with DWI may potentially allow us to ascertain the dynamic progression and foresee the prognosis in patients with VGAM.

Despite being the first line imaging modality, antenatal ultrasound imaging may be insufficient to detect ischemic injury due to VGAM of the fetal brain, while this can be unequivocally demonstrated by antenatal conventional MRI.[4,8,9] Antenatal DWI studies in particular may stand out to have a potential diagnostic and prognostic importance in the evaluation of VGAM to detect impending or recently established brain ischemia of the fetal brain, where even the conventional MRI is still not yet informative.[9] Fetal or neonatal MRI lesions among patients with VGAM are reported to be predominantly supratentorial in location and usually bilateral.[10] Presence of diffusion restriction is most commonly but not exclusively due to underlying ischemia, neuronal migration disorders are also included in the differential diagnosis.[11] Venous congestion and venous hypertension seem to be the main underlying pathogenesis for the development of cerebral ischemia in patients with VGAM. In some cases, venous hypertension and subsequent ischemic changes can be so severe that widespread white matter destruction and cerebral atrophy ensue.[12] Several other factors related to ET can play role in the progression of cerebral ischemic lesions: Connection between the deep venous system and the venous pouch, inadvertent embolization of the venous pouch with resultant venous hypertension and both venous ischemic and hemorrhagic complications or rapid subsequent expansion of the thrombosis through the venous pouch after successful total embolization of the fistula especially when performed in a single session.[13,14]

Since the findings of a severe brain injury is a predictor of poor neurological outcome in the later stages of life, antenatal conventional MRI in addition to DWI studies may serve to understand the gravity of acutely presenting, progressively worsening or already irreversibly pronounced ischemic fetal brain damage.[4,7-9] This may allow a more precise and timely

interpretation of the prognosis and possible overall outcome of the developing brain, thereby favorably influencing the clinical and parental decision on early delivery and prompt intervention to prevent further injury versus the termination of the pregnancy which may be applicable to prevent unnecessary aggressive invasive interventions.[4,7-9]

Many referral centers do not offer ET in patients with widespread and irreversible brain damage detected by postnatal conventional MRI (massive brain infarct, diffuse loss of parenchymal volume, encephalomalacia) due to expectation of a poor neurologic outcome, although possible future modulating effects of neuroplasticity is taken into consideration intensively.[3,4,6-9] On the other hand, withholding further interventions may not be an option in cases without concrete radiologic evidence of progressive worsening unresponsive to therapy, given the fact that legislations of many countries (including Turkey) demand not to withdraw medical care in such patients as long as the family prefers the continuation of treatment. Serial postnatal DWI studies suggesting progressively worsening and expanding brain ischemia beyond a level that cannot be ameliorated by neuroplasticity may add weight to findings of conventional MRI, providing convenience in decision making and aiding avoidance of further futile interventions.[6]

In conclusion, given the experience from our current case and in the light of literature, conventional MRI strengthened with DWI studies may widen our perspective in the understanding of the dynamic processes involved in the developing CNS of patients with VGAM while aiding clinical and parental decision antenatally and postnatally. Further reports and studies in the field are also required for accurate and safe guidance.

Ethical approval

Informed consent has been obtained from the mother of the baby.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: AA, AA, ŞY; data collection: AA, SB, HKKO; analysis and interpretation of results: HKKO, AA, ŞY; draft manuscript preparation: AA, SB, HKKO, AA, ŞY. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

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Case Report

A rare case of Klippel-Trenaunay syndrome presenting with chronic myeloid leukemia

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ABSTRACT

Background. Klippel-Trenaunay syndrome (KTS) is an overgrowth syndrome associated with capillary/venous/lymphatic malformations with limb hypertrophy and cancer risk. Various cancers, mostly Wilms tumor, have been reported in patients with KTS, but not leukemia. Chronic myeloid leukemia (CML) is also a rare disease in children, where there is no known disease or syndrome to predispose to CML.

Case. We report a case of CML incidentally diagnosed in a child with KTS when he was bleeding from surgery of the left groin for vascular malformation.

Conclusions. This case reflects the variety of cancer types that may accompany KTS and provides information about CML prognosis in such patients.

Key words: Klippel-Trenaunay syndrome, chronic myeloid leukemia, overgrowth syndrome, PIK3CA gene, vascular malformation, CVLM syndrome.

Klippel-Trenaunay syndrome (KTS) is a rare congenital vascular disorder characterized by cutaneous capillary malformations (port-wine stain), varicosities, and hypertrophy of soft tissues and long bones. KTS is also defined as an overgrowth syndrome and has recently been reclassified as capillary/venous/lymphatic malformation (CVLM) syndrome; whether it predisposes to malignancy is not clear.[1] Wilms tumor, rhabdomyosarcoma, osteoblastoma, basal cell carcinoma, squamous cell carcinoma of the skin, and angiosarcoma have been noted with CVLM, but not leukemia.[1] Chronic myeloid leukemia (CML) in children and adolescents is a rare malignancy of the hematopoietic system. Its incidence increases with age, and the incidence of CML in childhood is 0.6-1.2/million children/year.[2,3] It is characterized by hyperleukocytosis with myeloid and erythroid precursors, and increased platelets in peripheral blood.[2] Here we describe a case of CML diagnosed in a male pediatric patient with CVLM who presented with bleeding from the surgical region.

Case Report

A 14-year-old boy born of non-consanguineous parents presented with a painless mass on his left groin extending to his knee. This mass occurred initially at the age of 3 years and had been growing since. Physical examination revealed splenomegaly, limb length discrepancy, left lower extremity hypertrophy, and capillary hemangiomas over the left thigh's posterolateral skin. CVLM was suspected and confirmed with heterozygous mutation (c1634A>C/p.Glu545Ala) at the *PIK3CA* gene. He had multiple surgeries including left inguinal lymph node dissection, lymphatic lesion excision, and hematoma drainage from the wound site regarding deformities of the left limb. The patient was consulted to hematology due to hemorrhage complications occurring in the last surgery. Complete blood counts showed a hemoglobin level of 7.3 g/dL, white blood cells as $164x10^9$/L, neutrophil $76.4x10^9$/L,

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and thrombocytes 104x10^9/L. The differential was 20% metamyelocytes, 4% bands, 70% neutrophils, 4% eosinophils, 2% lymphocytes and 4% normoblasts, except for circulating blasts. Serum lactate dehydrogenase was 483 U/L (normal range: <248) and uric acid was 4 mg/dL (normal range: 3.5-7.2). Coagulation parameters were within normal limits. Bone marrow aspiration showed normocellular myeloid/erythroid ratio of 23:1, granulopoiesis with left shift, increased megakaryocytes were seen with normal maturation, and blasts were lower than 5%. Conventional karyotyping revealed 46 XY, t(9,22) (q34;q11.2) (20/20, 100%) without any additional cytogenetic abnormalities. The Breakpoint cluster region protein- Tyrosine-protein kinase ABL1 (BCR-ABL1) (p210) transcripts were detected as 57.7 international scale (IS) in a quantification limit of 0.0063%. Chronic phase CML (CML-CP) was diagnosed, and imatinib was initiated with a 300 mg/m^2 dose daily.

Nevertheless, the patient had inadequate drug compliance and follow-up visits delayed. Imatinib treatment was discontinued due to severe leg pain. Splenomegaly persisted and the complete molecular response had not yet been achieved on the 12th month of therapy, with a BCR/ABL (p210) transcript 2.68 IS. Mutation analysis for tyrosine kinase inhibitor (TKI) resistance was performed, and no BCR/ABL gene mutation was determined. The patient was switched to dasatinib therapy due to a lack of molecular response and an inability to tolerate imatinib. He used dasatinib treatment regularly and achieved a molecular response. At 18 months after diagnosis, the BCR/ABL (p210) transcript was 0.09 IS. The patient was considered to have an optimal response to dasatinib, and the therapy was continued. He is currently receiving dasatinib, now for 23 months. The BCR/ABL (p210) transcript at 32 months of treatment was negative and complete molecular response was achieved. We obtained informed consent from his family.

Discussion

Capillary venous lymphatic malformation is a rare syndrome characterized by the triad of capillary malformations, vascular anomalies, hypertrophy of bony and soft tissues.[4,5] In 1900, CVLM was defined and named by the two French doctors who described the syndrome. The estimated incidence of CVLM is 1 in 30,000 live births and it affects males and females in equal numbers.[6]

The exact pathophysiology and genetic etiology of CVLM are unknown with a sporadic occurrence, although a paradominant inheritance pattern has been suggested. A paradominant theory of inheritance tries to explain CVLM by the occurrence of a lethal mutation in a gene. Accordingly, homozygous embryos are lost, but heterozygous embryos remain alive and are normal. Familial cases are very uncommonly reported.[7,8] Chronic venous hypertension secondary to deep vein abnormality or occlusion has been suggested to play a role in the pathogenesis of CVLM. In addition, the persistence of a part of the embryological vascular system has been proposed as another underlying cause in the pathogenesis of CVLM. Mesodermal anomaly affecting angiogenesis may explain the features of CVLM syndrome.[9,10] The affected limbs tend to exhibit increased blood flow. This has been thought to be associated with vascular endothelial growth factor (VEGF) mediated angiogenesis, but there is insufficient evidence.[11] Haploinsufficiency of *AGGF1* was attributed to CVLM development through inhibition of angiogenesis by inactivating phosphatidylinositol 3-kinase (PI3K) and AKT serine/threonine kinase 1 (AKT).[4,11,12] Additionally, the *PIK3CA* gene has also been reported to be responsible from the genotype of some of the patients with CVLM.[1,13] Somatic mutations in *PIK3CA* cause many overgrowth syndromes (particularly Cloves, fibro adipose hyperplasia and megalencephaly-capillary

malformation) that have recently been identified as the *PIK3CA*-related overgrowth syndromes.[14,15]

Many types of cancer have been reported in individuals with CVLM. However, the relationship between cancer and underlying syndromes is not clear. The risk of embryonal cancer in children with CVLM does not appear to be higher than in the general population. Overgrowth syndromes such as Beckwith Wiedemann Syndrome and PTEN hamartoma tumor syndrome have increased cancer risk. Both of these syndromes are associated with genetic mutations in tumor suppressor genes. No patient with CVLM has been reported to have structural mutations in tumor suppressor genes or oncogenes. Isolated hemihypertrophy without other features of known syndromes has been recognized as an indication for childhood cancer surveillance. Therefore, it may be predicted that CVLM carries a risk of cancer on the basis of hemihypertrophy alone. Wilms tumor, rhabdomyosarcoma, basal cell and squamous cell carcinoma of the skin were identified with CVLM, except CML has not yet been associated in the literature.[1]

Chronic myeloid leukemia is also rare in children and accounts for <5% of all leukemias in children less than 15 years of age, the incidence being higher among adolescents.[16] Compared to adults, children, and adolescents with CML tend to present with higher white blood cell count, larger spleen size in proportion to body size, and higher frequency of advanced phases at diagnosis.[17] Although the molecular basis of chronic myeloid leukemia is known, an etiological cause cannot be revealed in most children. Ionizing radiation is the only environmental factor involved in CML etiology. CML develops after a long latent period of exposure to radiation. There is no ethnic or genetic predisposition. However, CML is rarely observed as a secondary malignancy in some adult and pediatric cases following irradiation and chemotherapy, mostly in Hodgkin and non-Hodgkin lymphoma treatments.[3]

The therapeutic approach to CML has changed drastically since introducing the TKI, imatinib, followed by the second-generation TKI, dasatinib, and nilotinib. Treatment with imatinib has also improved outcomes in children. The second-generation TKIs (2G-TKIs), dasatinib, and nilotinib were approved recently as first-line treatment in children, and they have expanded treatment options and made allogeneic stem cell transplantation a third-line treatment.[5,18-20]

The PI3K-AKT-mTOR pathway is one of the critical pathways that control cell growth and proliferation during development. Postzygotic somatic activating mutations in *PIK3CA* are responsible for the clinical spectrum of overgrowth associated with *PIK3CA*.[15] Somatic mutations in the *PIK3CA* gene are found in many other types of cancer, including ovarian, breast, lung, brain, and stomach cancer. These mutations also play a role in colorectal cancer.[21] *PIK3CA* encodes p110a, a critical component of the PI3-kinase enzyme, which activates signaling pathways involved in cellular proliferation, survival, and growth. These mutations change single amino acids in the p110α protein. Activated in response to tyrosine kinase receptor-ligand binding, PI3K converts phosphatidylinositol (4,5)-diphosphate into phosphatidylinositol (3,4,5)-triphosphate and leads to AKT activation. This activation of AKT leads to increased cellular proliferation via mTOR.[15,21] Continuous activation of this pathway has been shown to cause venous malformations due to reduced apoptosis of endothelial cells and improper assembly of vascular smooth muscle.[21,22]

As a conclusion, it has been shown that CML may accompany CVLM. The reporting of the associated malignancies in this rare disorder may have a further impact on understanding the cancer mechanisms.

Ethical approval

We obtained informed consent from the patient's family.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: ÇC, TA, FG, ŞÜ; data collection: ÇC, TA, FG, ŞÜ; analysis and interpretation of results: ÇC, TA, FG, ŞÜ; draft manuscript preparation: ÇC, TA, FG, ŞÜ. All authors reviewed the results and approved the final version of the manuscript.

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Case Report

Meningococcemia in a vaccinated child receiving eculizumab and review of the literature

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ABSTRACT

Background. Atypical hemolytic uremic syndrome (aHUS) is a rare and severe disease characterized by uncontrolled activation and dysregulation of the alternative complement pathway and development of thrombotic microangiopathy. Eculizumab, which is used as a first-line therapy in aHUS, blocks the formation of C5 convertase and inhibits the formation of the terminal membrane attack complex. It is known that treatment with eculizumab increases the risk of meningococcal disease by 1000-2000-fold. Meningococcal vaccines should be administered to all eculizumab recipients.

Case. We describe a girl with aHUS who was receiving eculizumab treatment and experienced meningococcemia with non-groupable meningococcal strains which rarely cause disease in healthy people. She recovered with antibiotic treatment and we discontinued eculizumab.

Conclusions. In this case report and review, we discussed similar pediatric case reports in terms of meningococcal serotypes, vaccination history, antibiotic prophylaxis and prognosis of patients who experienced meningococcemia under eculizumab treatment. This case report highlights the importance of a high index of suspicion for invasive meningococcal disease.

Key words: meningococcemia, eculizumab, atypical hemolytic uremic syndrome, child, vaccine.

Atypical hemolytic uremic syndrome (aHUS) is a rare and severe disease that develops due to dysregulation in the alternative complement pathway by way of mutations in the complement gene or acquired autoantibodies against complement regulatory proteins.[1] This thrombotic microangiopathy is characterized by non-immune hemolytic anemia, thrombocytopenia and acute kidney injury.[2] Uncontrolled activation of the alternative complement system causes overproduction of the membrane attack complex in children with aHUS. Eculizumab (Soliris®; Alexion Pharmaceuticals, New Haven, CT, USA), which is used as a first-line therapy in aHUS, is a humanized, chimeric monoclonal antibody.[3] Eculizumab prevents the formation of the membrane attack complex that is important for meningococcal serum bactericidal activity. Therefore, eculizumab treatment is a well-known risk factor for meningococcal disease.[1,4,5] Meningococcal vaccines must be administered at least 14 days before initiating treatment or antibiotic prophylaxis should be given if eculizumab is to be administered earlier due to the urgent condition of the patient.[4] However, meningococcal disease can occur in eculizumab recipients despite vaccinations and antibiotic prophylaxis.[5-7] In this case report and review, we present a 19-month-old girl who developed meningococcemia under treatment with eculizumab and discuss related *Neisseria meningitidis* serotypes and vaccination history

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and prognosis of similar cases presented in the literature.

Case Report

A 19-month-old girl, who was receiving eculizumab for aHUS, was admitted to our pediatric emergency department with fever and low appetite; but no vomiting, rash, or neck stiffness. She was diagnosed as having aHUS when she was eight months old, after presenting with anemia, thrombocytopenia, and acute kidney injury. After obtaining consent from the family and performing meningococcal ACWY (Menveo; GSK Biologicals) and B serotype (Bexsero; GSK Biologicals) vaccinations, eculizumab was initiated with antibiotic prophylaxis (40 mg/kg/day amoxicillin and clavulanic acid). After two weeks, antibiotic prophylaxis was stopped. Hematological and renal improvement was achieved and eculizumab 300 mg was given every two weeks during the follow-up. She had two doses of meningococcal ACWY and three doses of B serotype vaccine. Family history revealed consanguineous marriage. Genetic analysis revealed complement factor I homozygous missense mutation on exon 11; c.1421G>A (p.R474Q).

On admission, her physical examination was unremarkable except a body temperature of 38°C and hyperemia of the right tympanic membrane on otoscopic examination. She had received the 22nd dose of eculizumab six days prior. Laboratory examination showed leukocyte count 20,200/mm^3 (<10,000/mm^3), CRP 2.9 mg/dl (<0.5mg/dl), and procalcitonin level 15.9 ng/ml (<0.5 ng/ml, >10ng/ml: septic shock). Treatment with empirical ceftriaxone at a dose of 75 mg/kg was started after obtaining blood culture. On follow up, vital signs were normal and she was free of fever after the first 12 hours. *N. meningitidis* was detected in the blood culture and serotyping revealed non-groupable *N. meningitidis.* The patient completed 10 days of intravenous ceftriaxone treatment. Eculizumab was stopped and fresh frozen plasma (FFP) in two-week intervals was scheduled as a replacement.

Primary immunodeficiency was also investigated, due to the presence of consanguinity, and meningococcemia with a meningococcal strain which is unusual to be pathogenic in healthy individuals. Laboratory evaluations showed normal complete blood count and immunoglobulin levels. Extended lymphocyte phenotyping also revealed normal percentages of T and B lymphocyte subsets and natural killer cells. Immune dysregulation clinical exome sequencing analysis was conducted and no pathogenic or possibly pathogenic variant was detected, consistent with the patient's clinical findings and inheritance pattern. In the 'variant of unknown significance (VUS)' variant list, a homozygous missense variant, causing a mutation of *TET2*, c.4832 with a C>A amino acid substitution was found which is associated with 'Immunodeficiency-75 (IMF75), OMIM #619126' phenotype. Sanger sequencing analysis confirmed the variant.

She was followed up closely by the pediatric nephrology unit with intermittent FFP infusions and eculizumab was not reintroduced. At the fifteenth month of follow-up, she was free of aHUS attack with normal hemoglobin values, renal functions, and without proteinuria.

Written informed consent was received from the parents for publication.

Discussion

Herein, we present a patient with aHUS due to complement factor I mutation; she was receiving eculizumab treatment and developed meningococcemia with non-groupable *N. meningitidis* strains despite meningococcal vaccinations. Interestingly, she had very mild symptoms on presentation. This observation highlights the importance of having high clinical suspicions for invasive meningococcal disease in children receiving eculizumab treatment even if vaccinations have been administered appropriately. *N. meningitidis*

serotype was non-groupable in our case and the vaccines administered to the patient provided protection only for meningococcus ACWY and B serotypes. In Turkey, antibiotic chemoprophylaxis is officially in use in the first two weeks of eculizumab treatment; chemoprophylaxis can be stopped thereafter. However, many countries including the USA, France and the United Kingdom recommend prolonged chemoprophylaxis during eculizumab treatment.[7-9] Chemoprophylaxis may help to reduce the risk of meningococcemia in eculizumab recipients. Our case had received antibiotic prophylaxis only for 2 weeks and this may have facilitated the development of meningococcemia.

Our patient also had a VUS in *TET2* gene shown in the immunodeficiency genetic panel. Individuals with *TET2* mutation reported so far in the literature usually presented with severe and recurrent infections in infancy with lymphoproliferation and various types of hypo- or hypergammaglobulinemia and T- or B-cell deficiencies.[10] Sanger sequencing analysis results confirmed the variant; however, the clinical findings, family history, and laboratory results were not found to be clinically related to the patient's condition.

Cases of meningococcemia in eculizumab recipients have been reported in the literature.[5-7] In a study published by the Centers for Disease Control and Prevention in 2017, meningococcal disease was reported in 16 patients (age interval 16-83 years) who used eculizumab between 2008 and 2016. Findings on admission were nonspecific and included fever, chills, diarrhea, vomiting, muscle pain and joint pain. Fourteen patients were vaccinated against MenACWY serotypes and 3 were vaccinated against MenB before the onset of the disease. The majority of cases were caused by non-groupable *N. meningitidis* (n= 11). The others included serogroup Y in four patients (three were vaccinated for ACWY) and undetermined in one patient. Antimicrobial susceptibility test showed that one patient was resistant to penicillin. One patient died.[5] The reason for meningococcal disease in vaccinated children could be the fact that eculizumab therapy inhibits development of meningococcal antibodies against vaccine serotypes and antibodies developing against vaccine serotypes do not provide cross-protection against non-groupable serotypes.

Socié et al.[11] showed that the overall meningococcal infection rate was 0.25 per 100 patients per year for eculizumab recipients. They presented 76 cases of meningococcemia between March 2007 and October 2016, and eight patients were under the age of 16 years. The majority of the patients were aged between 16 and 44 years and most common serotypes were non-groupable and B. They reported eight fatal cases. Gäckler et al.[12] evaluated antibody titers after meningococcal vaccination in 25 patients diagnosed with aHUS who received eculizumab treatment. Only 20% of the patients had antibody responses to all serotypes, and 28% did not develop an antibody response to any serotype. An increase in bactericidal antibody titer was observed against all serotypes with repeated vaccinations. The authors advised evaluation of antibody titers and booster doses. Also, even if booster doses were performed, vaccinations were not 100% protective against meningococcemia and the authors advised antibiotic prophylaxis to all eculizumab recipients.

Review of the literature reveals a limited number of pediatric case reports that describe patients who were on eculizumab treatment and experienced meningococcemia despite vaccinations or chemoprophylaxis plus vaccinations (Table I). Findings on admission were nonspecific and included fever, chills, headache, macular rash and/or myalgia. Fatal cases were also reported. Polat et al.[6] described an 11-year-old boy who developed meningococcemia due to *N. meningitidis* serotype Y. He had two doses of MenACWY-D (*Neisseria meningitidis* serogroup A, C, W, Y vaccine, Menactra; Sanofi Pasteur, Inc, Swiftwater, PA, USA) vaccine and antibiotic prophylaxis. He died within hours in pediatric intensive care unit despite antibiotic treatment,

Table I. Pediatric patients who experienced meningococcemia under eculizumab treatment.

Study	Age at admission for meningococcemia/ Gender	Diagnosis/ Genetic defect	Microbiology-Antibiotic susceptibility	Eculizumab duration before meningococcemia	Antibiotic prophylaxis	Men B vaccination	Men ACWY vaccination	Prognosis	Eculizumab continued/ discontinued
Polat M et al. 2018[6]	11 years, boy	aHUS N/A	Serogroup Y-intermediate penicillin susceptibility, with minimal inhibitory concentration of 0.19 mg/L *	16 months	Yes	No	Yes (2 doses)	Fatal	-
Cullinan N et al. 2015[13]	4 years, girl	aHUS Hybrid CFH/ CFHR3 mutation	Serogroup W135-intermediate penicillin sensitivity, with minimal inhibitory concentration of 0.13 mg/L *	30 months	Yes	No	Yes (1 dose)	Survived	Revaccinated, eculizumab continued
Menne J et al. 2019[14]	13-19 age category, N/A	aHUS C3 mutation	Blood culture is negative	N/A	Yes	No	Yes	Survived	Eculizumab continued
Rondaeu E et al. 2019[15]	15 years, N/A	aHUS N/A	Serogroup B	21 months	No	Yes	No	Survived	Eculizumab continued
Nolfi-Donegan D et al. 2018[7]	16 years, girl	PNH	ST-2578 and non-groupable, penicillin susceptible	<1 month	N/A	Yes	Yes	Fatal	-

N/A: not available, aHUShus: atypical hemolytic uremic syndrome, CFH: complement factor H, CFHR3: complement factor H receptor 3, PNH: paroxysmal nocturnal hemoglobinuria, * sensitive, 0.06 mg/L, resistant 0.25 mg/L

aggressive fluid resuscitation and inotropic and ventilatory support. Nolfi-Donegan et al.[7] reported a 16-year-old-girl who had fatal meningococcemia only twenty-four hours after the second eculizumab dose. They showed that neither Men B-4C vaccination (*Neisseria meningitidis* serogroup B vaccine; Bexsero; Glaxo Smith Kline, Bellaria Rosia, Sovicille, Italy) which matched 2 antigens in the strain, nor the high serum antibody titers prevented the rapidly fatal disease.

Annual measurement of meningococcal vaccine responses was advised in eculizumab recipients.[13] Cullinan et al.[13] presented a patient with meningococcemia due to *N. meningitidis* W135 after 30 months of treatment. They showed that vaccine responses were suboptimal and they performed revaccination. After successful treatment of meningococcemia, they continued eculizumab treatment and they experienced neither relapse of aHUS, nor meningococcemia.

Some clinicians did not interrupt eculizumab treatment after meningococcemia. Menne et al.[14] described a boy with C3 mutation and renal transplantation and clinically diagnosed meningococcemia. Blood cultures were negative and they continued eculizumab treatment. Rondeau et al.[15] released a five-year safety report of eculizumab. They presented a pediatric patient who experienced meningococcemia with meningococcus B serotype despite vaccination for that serotype. They did not discontinue eculizumab treatment.

In conclusion, eculizumab treatment increases the risk of meningococcemia, and neither vaccination nor antibiotic prophylaxis provide 100% protection from meningococcemia. Vaccination should be performed against both A, C, W, Y and B serotypes to reduce the risk, and antibiotic prophylaxis should be considered due to possible fatal course of the disease. Repeated doses of vaccines may be needed for adequate antibody titers. The presentation of meningococcal disease under eculizumab treatment might be vague. Initial symptoms are often mild and nonspecific. However, the disease can progress to shock and death within hours. In patients who present with fever under eculizumab treatment, the risk of meningococcemia should be carefully evaluated and early treatment should be performed regardless of prophylaxis and vaccination status.

Ethical approval

Written informed consent was received from the parents for publication.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: DÜ, NG, EK, NK, MA, CC; data collection: DÜ, NG, CC; analysis and interpretation of results: DÜ, NG, CC; draft manuscript preparation; DÜ, NG, NK, EK, MA, CC. All authors reviewed the results and approved the final version of the manuscript.

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Case Report

A family with interleukin-17 receptor A deficiency: a case report and review of the literature

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ABSTRACT

Background. Chronic mucocutaneous candidiasis (CMC) is characterized by recurrent or persistent infections of the skin, nail, oral, and genital mucosa with Candida species, mainly *Candida albicans*. In a single patient, the first genetic etiology of isolated CMC autosomal recessive interleukin-17 receptor A (IL-17RA) deficiency was reported in 2011.

Case. We report four patients with CMC who displayed autosomal recessive IL-17RA deficiency. The patients were from the same family, and their ages were 11, 13, 36, and 37 years. They all had their first CMC episode by six months of age. All patients manifested staphylococcal skin disease. We documented high IgG levels in the patients. In addition, we found the coexistence of hiatal hernia, hyperthyroidism, and asthma in our patients.

Conclusions. Recent studies have provided new information on the heredity, clinical course, and prognosis of IL-17RA deficiency. However, further studies are needed to reveal the full picture of this congenital disorder.

Key words: chronic mucocutaneous candidiasis, interleukin-17 receptor A (IL-17RA), staphylococcal infection, family.

In human beings, fungi of the genus Candida are found in the normal flora of the skin and mucous membranes of the gastrointestinal, genitourinary, and respiratory systems. *Candida albicans* (*C. albicans*) does not cause chronic disease in healthy individuals. Chronic mucocutaneous candidiasis (CMC) is a selective cellular immune deficiency characterized by persistent candida infection of the mucosa, skin, scalp, and nails. CMC usually does not have a systemic spread and is a heterogeneous disorder. It is thought that the insufficiency causes the primary defect in the proliferative response of T lymphocytes against the candida antigen and the production of cytokines.[1]

Chronic mucocutaneous candidiasis is divided into two groups; syndromic and isolated CMC. Patients with a predisposition to infectious agents other than Candida and staphylococcus or autoimmune clinical signs are defined as syndromic CMC. Syndromic CMC manifestations include STAT1 gain-of-function (GOF), STAT3 loss-of-function (LOF), AIRE, DOCK8, CARD9, IL-12p40, IL-12Rβ1, ROR-γ/γT, and Dectin-1 mutations.[2-6] Interleukin-17 receptor A (IL-17RA), IL-17RC, IL-17F and ACT1 (TRAF3IP2) mutations cause isolated CMC disease.[2,7-9] In isolated CMC patients, symptoms usually begin early in infancy, and staphylococcal skin infections are also seen in some patients. In addition, patients with primary immunodeficiencies affecting T cells, including severe combined immunodeficiency and combined immunodeficiencies, are also prone to CMC.[10]

IL-17RA deficiency shows autosomal recessive inheritance and causes isolated CMC disease. IL-17RA deficiency is the second most common cause of CMC after STAT1 GOF.[5,11] Among clinical findings of IL-17RA deficiency,

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candidiasis in the anogenital area, skin, scalp, nails, oral mucosa, pustules, folliculitis, furunculosis, and skin abscess formation, seborrheic dermatitis, scaly pustule formation on the scalp can be counted. Other clinical findings of IL-17RA deficiency are; conjunctivitis, development of tuberculosis (pulmonary tuberculosis, tuberculous meningitis), eczema, sinusitis, otitis, lobar pneumonia, and bronchitis.[11]

In this study, in light of the literature, we reviewed IL-17RA deficiency in a family (father, two daughters, and father's sister) with a history of recurrent mucocutaneous candidiasis and diagnosed with homozygous IL 17 RA mutation in the genetic analysis.

Case Report

Case 1

A thirteen-year-old female patient was admitted to our clinic at three years of age. The patient had complaints of dandruff on the scalp and white plaque in the mouth that started from birth and recurred. In addition, the patient had a history of acne-like rashes that started at the age of eight months and was more common on the anterior and posterior parts of the chest. Although the patient was intermittently using oral nystatin drops and oral antibiotic treatment, her complaints were constantly recurring. There was no pathological finding in her prenatal and natal history. It was learned that the patient's father and aunt had similar recurrent complaints and that the frequency and severity of the complaints decreased when they reached adulthood. The pedigree of the patient's family is given in Fig. 1.

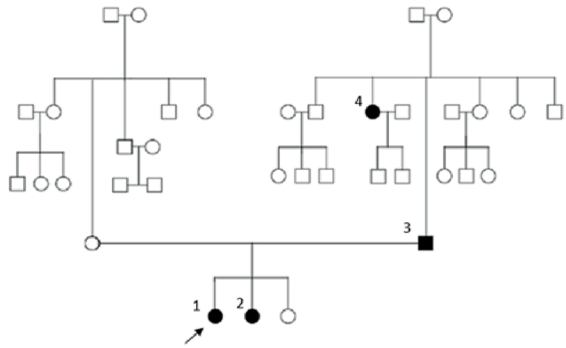

Fig. 1. Pedigree of the patients diagnosed with IL-17RA deficiency (the index patient is shown with arrow).

The patient's vital signs were normal. There were widespread papular rashes all over her body (Fig. 2a). There were white plaques on the tongue and in the mouth (Fig. 2b). Microbiological examination of the white plaque lesions was performed, and yeast and pseudohyphae

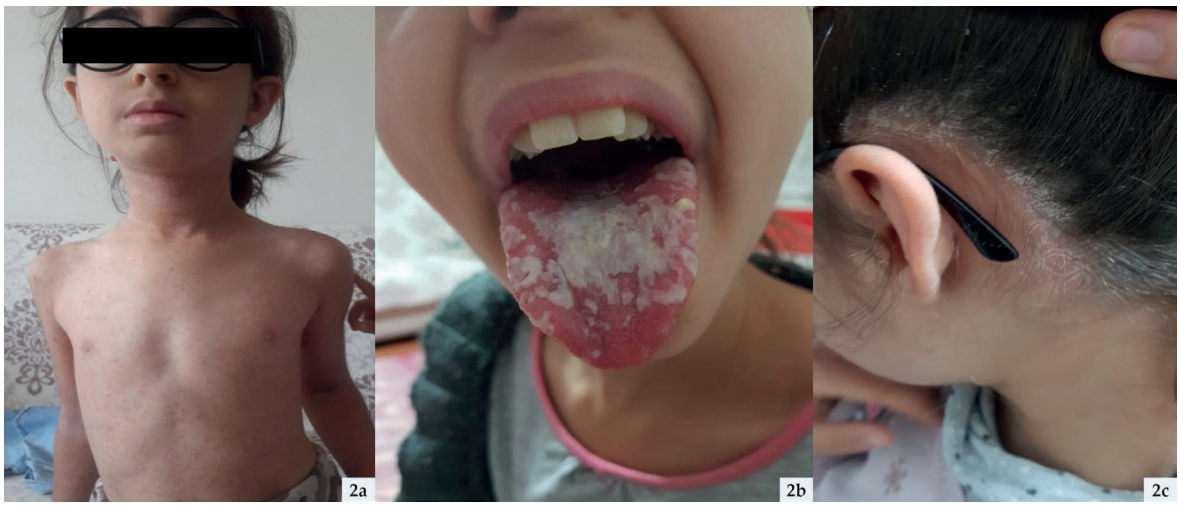

Fig. 2. 2a: Diffuse erythematous papular rashes on the patient's chest, 2b: Diffuse candida plaques on the patient's tongue, 2c: White scalings detected on the patient's scalp.

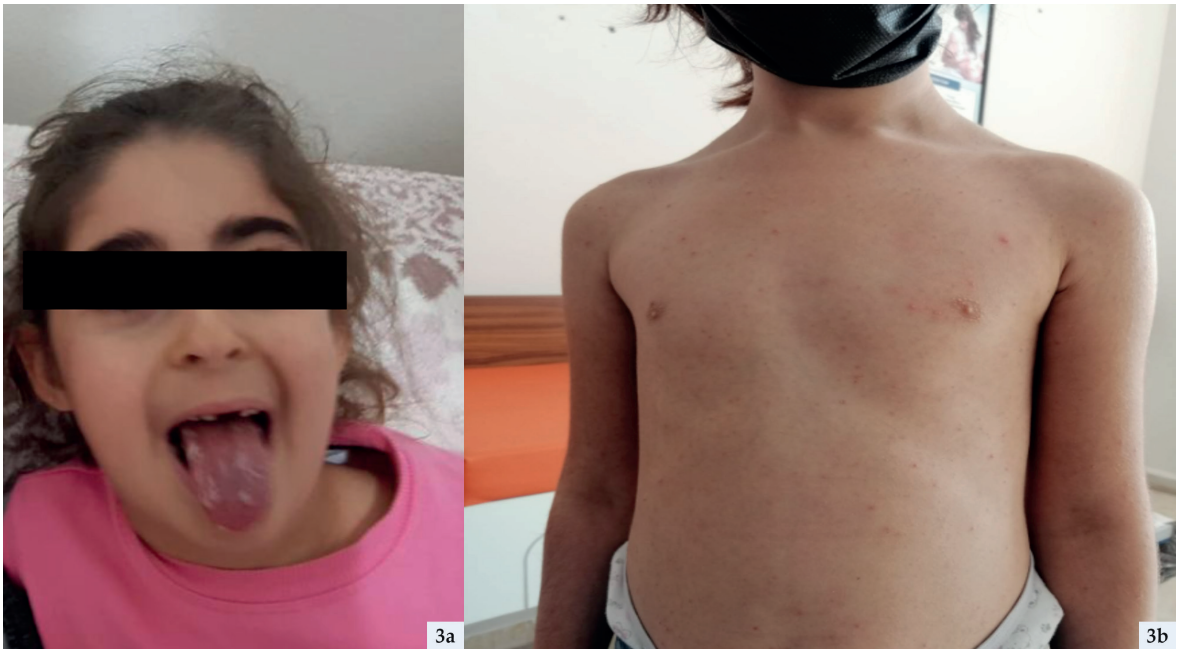

Fig. 3. 3a: Candida plaques on the patient's tongue, 3b: Diffuse erythematous papular rashes on the patient's chest.

were seen on Gram staining. Gram-positive cocci were grown in the wound swab culture taken from the skin lesions. In addition, there were white scales on the occipitotemporal region of the scalp (Fig. 2c). In the laboratory examination, the results of the biochemical tests were within normal limits. Other laboratory findings of the patient are given in Table I. We used an investigation based on whole-exome sequencing for CMC in the patients.[11] Genetic analysis revealed a homozygous mutation of c.1159G>A (p.Asp387Asn) in the *IL17RA* gene. With the current clinical and laboratory findings, we considered the diagnosis of CMC due to *IL17RA* mutation. Therefore, oral prophylactic fluconazole and trimethoprim-sulfamethoxazole treatment was started.

Case 2

An eleven-year-old female patient applied to our clinic when she was six months old. The patient's history revealed the presence of white plaque in the mouth that started when she was two months old. She also had acne-like rashes on her trunk that started when she was five months old. Oral nystatin treatment was given to the patient to treat her oral lesions, but the patient's complaints did not regress. There was no pathological finding in her prenatal and natal history. The patient's older sister, father, and aunt had similar complaints in the family history.

Her vital signs were within normal limits. In her physical examination, there were white plaques on the tongue and in the mouth (Fig. 3a) and diffuse papular eruptions, which were more intense on the anterior and posterior parts of the chest, and all over the body. (Fig. 3b). Microbiological examination of the samples taken from the white plaque lesions was performed, and yeast and pseudohyphae were seen on Gram staining. Gram-positive cocci were grown in the wound swab culture taken from the skin lesions. In the laboratory examination, biochemical test results were within normal ranges. Other laboratory findings of the patient are given in Table I. In the patient follow-up, complaints of recurrent cough, shortness of breath, and wheezing emerged. At the age of seven, prolonged expiration, wheezing, and rhonchi were detected on physical examination.

Table I. Laboratory findings of the cases.

Laboratory findings	Case 1	Case 2	Case 3	Case 4
Hb (g/dL)	13.8	13.2	14.4	12.4
Hct (%)	43.8	40.2	44.4	40.5
MCV (fL)	86.4	79.8	87.2	79.3
MCHC (g/dL)	31.5	32.8	32.5	30.6
RDW (%)	12.1	12.7	13.1	14
RBC (10^6 /mm^3)	5.08	5.04	5.09	5.11
WBC (/mm^3)	7700	8050	7090	7420
PLT (/mm^3)	283000	409000	291.000	337.000
ANC (/mm^3)	3620	3440	3860	4490
ALC (/mm^3)	3270	3740	2560	2330
Eosinophils (/mm^3)	200	350	120	90
CRP (mg/dL)	3.4	4.2	0.14	8.2
ESR (mm/h)	11	14	8	41
C3 (g/dL)	1.15 (0.9-1.8)	1.43 (0.9-1.8)	1.08 (0.9-1.8)	1.45 (0.9-1.8)
C4 (g/dL)	0.15 (0.1-0.4)	0.25(0.1- 0.4)	0.21 (0.1- 0.4)	0.23 (0.1- 0.4)
CH50 (%)	88.8 (51-150)	86.9 (51-150)	85.7 (51-150)	93 (51-150)
IgG (mg/dL)	2140 (441-1135)	821 (215-704)	1590 (700-1600)	1460 (700-1600)
IgA (mg/dL)	66.5 (22-159)	34 (8.1-68)	203 (70-400)	408 (70-400)
IgM (mg/dL)	90.5 (47-200)	60 (35-102)	91 (40-230)	240 (40-230)
IgE (IU/mL)	16.4 (0.19-16.9)	14.0 (0.44-16.3)	121 (1.53-114)	46.4 (1.53-114)
IgG1 (mg/dL)	1432 (280-1120)	705.5 (230-710)	1380 (280-1020)	917 (280-1020)
IgG2 (mg/dL)	334.6 (30-630)	204.4 (30-170)	340 (60-790)	480 (60-790)
IgG3 (mg/dL)	74.4 (40-250)	55.4 (11-98)	119 (14-240)	49 (14-240)
IgG4 (mg/dL)	50.5 (11-620)	30.3 (4-43)	70 (11-330)	32 (11-330)
CD45RO+ (%)	32 (14-44)	30 (14-44)	32 (14-44)	34 (14-44)
CD3+ (%)	64.1 (43-76)	57.8 (50-75)	59 (52-78)	61 (52-78)
CD4+ (%)	35.5 (23-48)	33.8 (33-58)	36 (28-57)	33 (28-57)
CD8+ (%)	27.5 (12-30)	26.1 (13-26)	28 (10-39)	29 (10-39)
CD19+ (%)	21.6 (11-31)	23 (14-41)	23 (10-30)	21 (10-30)
CD20+ (%)	21 (11-29)	22 (13-40)	22 (9-28)	19 (9-28)
CD16+/56+ (%)	14 (5-28)	15 (5-23)	15 (8-30)	15 (8-30)
Blood group	0 Rh (+)	0 Rh (+)	0 Rh (+)	B Rh (+)
Anti-A titer	1/32	1/32	1/16	1/16
Anti-B titer	1/32	1/32	1/16	1/16
Anti rubella IgG	+	+	-	+
Anti mumps IgG	+	-	-	+
Anti measles IgG	+	+	-	+
Anti-HBs IgG	-	+	-	-
Anti tetanus IgG	-	-	-	-
Genetic result	c.1159G>A (p.Asp387Asn)	c.1159G>A (p.Asp387Asn)	c.1159G>A (p.Asp387Asn)	c.1159G>A (p.Asp387Asn)

ALC: absolute lymphocyte count, ANC: absolute neutrophil count, C: complement, CD: cluster of differentiation, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, fL: femtoliter, Hb: hemoglobin, Hct: hematocrit, Ig: Immunoglobulin, MCHC : mean corpuscular hemoglobin concentration, MCV: mean corpuscular volume, PLT: platelet count, RBC: red blood cell, RDW: red cell distribution width , WBC: white blood cell count.

Increased aeration in the patient's chest X-rays and early reversibility in the pulmonary function tests were detected. No sensitization was detected in the skin prick test performed with inhaled allergens. The results of a child food panel-specific IgE (fx5) and phadiatop test were negative. The patient was diagnosed with asthma based on the current history, physical examination, laboratory test results, and treatment with 5 mg oral montelukast and inhaled fluticasone propionate in two doses of 250 mcg/day was started. To prevent developing oral candidiasis due to fluticasone propionate treatment, we recommended that the patient administer the drug through the chamber spacer and gargled with plenty of water after each application.

We had previously diagnosed CMC in the patient's sister due to *IL17RA* mutation, and documented a homozygous mutation of c.1159G>A (p.Asp387Asn) in the *IL17RA* gene So, this sibling also got the diagnosis of IL-17RA deficiency. In addition to asthma treatment, oral prophylactic fluconazole and trimethoprim-sulfamethoxazole were started.

Case 3

It was learned that a 36-year-old male patient had complaints of white plaques in the mouth and acne-like rashes on the body, which started in childhood. During this period, although the doctors prescribed the patient with a bicarbonate mouthwash and topical skin creams containing fusidic acid, the patient's complaints constantly recurred. However, the frequency and severity of the patient's complaints decreased after adolescence. Later, at the age of 23, the patient complained of a sour or bitter taste in his mouth, belching, and abdominal pain that aroused him from sleep. Because of these complaints, the patient was diagnosed with a hiatal hernia and gastroesophageal reflux. The case was operated on twice for the hiatal hernia. Additionally, endoscopic findings consistent with esophageal candidiasis were detected. Furthermore, when the patient was 32 years old, complaints of diarrhea that continued intermittently for six months emerged. Abundant Candida was detected in stool microscopy. After using oral fluconazole treatment, the patient's diarrhea resolved.

The patient's vital signs were within normal limits. His physical examination was unremarkable except for scars due to previous operations on the abdomen. In the laboratory examination, the results of biochemical tests were within normal limits. Other laboratory findings of the patient are given in Table I. A homozygous mutation of c.1159G>A (p.Asp387Asn) in the *IL17RA* gene was detected, and a diagnosis of IL-17RA deficiency was made.

Case 4

It was learned that a 37-year-old female patient developed complaints of white plaque in the mouth and acne-like rashes on the body, which started in childhood. During this period, although the doctors gave the patient oral care with bicarbonate mouthwash and topical skin creams containing fusidic acid, the patient's complaints were constantly recurring. However, the frequency and severity of these complaints decreased after adolescence. Later, the patient was diagnosed with hyperthyroidism at the age of 33 because of irritability, palpitations, sweating, and weight loss, and propylthiouracil treatment was started.

The patient was admitted to our clinic because of the detection of *IL17RA* mutation in his brother and two nieces. The patient's vital signs were normal. There was no pathology in the physical examination of the patient. Her biochemical test results were within normal limits. Other laboratory findings of the patient are given in Table I. A homozygous mutation of c.1159G>A (p.Asp387Asn) in the *IL17RA* gene was detected, and a diagnosis of IL-17RA deficiency was made. We obtained consent from his family to publish this report and to include their photographs.

Discussion

Approximately 50 years ago, CMC was defined as a primary immunodeficiency.[12] In recent years, many genetic mutations that cause CMC have been described. Most of these mutations are directly or indirectly related to defects in IL-17. IL-17 is mainly produced by Th17 cells. IL-17 belongs to the six-membered cytokine family (IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, IL-17F).[13-15] Unlike IL-17, the IL-17 receptor (IL-17R) is expressed in all body cells. Therefore, many different cells are the target of IL-17.[14,16,17] Five receptors for IL-17 have been identified so far; IL-17RA, IL-17RB, IL-17RC, IL-17RD, and IL-17RE. IL-17RA and IL-17RC have been shown to bind IL-17A and IL-17F. However, IL-17RA primarily binds to IL-17A with high affinity, while it has a lower binding affinity for IL-17F.[16-18] The binding of IL-17A and IL-17F to their receptors induces various signaling pathways and causes the secretion of some cytokines and chemokines. These cytokines and chemokines recruit and activate polymorphonuclear neutrophils in that area and provide protection against various infectious agents in the environment.[19] In IL-17RA deficiency, the functions of epithelial cells, fibroblasts, mononuclear cells, and phagocytes are affected. The IL-17RA signaling pathway is essential for mucocutaneous immunity against *C. albicans* and *Staphylococcus aureus* (*S. aureus*). It has also been reported that the IL-17RA-dependent signaling pathway is important for protective immunity against various bacteria in the respiratory tract.[11]

IL-17RA deficiency was first described in a Moroccan boy. The patient developed recurrent CMC infection resistant to local antifungal therapy since the first month of his life. In this case, *C. albicans* dermatitis developed in the newborn period and *S. aureus* dermatitis at five months. In addition, the development of recurrent folliculitis and abscess in his hip caused by *S. aureus* was also reported.[7] In the literature, in addition to mucocutaneous candidiasis and skin infections caused by *S. aureus* in CMC patients, concomitant recurrent lower and upper respiratory tract bacterial infections, tuberculosis, conjunctivitis, and eczema have been reported.[11] The pathogenesis of staphylococcal disease in CMC patients has not been clarified yet. Staphylococcal skin disease is frequently seen in patients with ACT1 and IL-17RA deficiencies but not reported in patients with IL-17F and IL-17RC deficiencies. This observation suggests that staphylococcal disease may be due to a disorder in the cytokine responses of IL-17E (IL-25), which requires ACT1 and IL-17RA.[11] In general, clinical findings of IL-17RA deficiency include candidiasis on the anogenital region, scalp, nails, and oral mucosa, skin pustules, folliculitis, furunculosis, skin abscess, seborrheic dermatitis, and crusty pustular lesions on the scalp. In all our cases, there was a history of oral candidiasis that started in early childhood and skin infection caused by S. *aureus*. However, it was learned that the frequency and severity of these infections decreased after adolescence in the children's father and aunt. This improvement in symptoms may be due to the development of the adaptive immune system with age. In addition, when the immunological examinations of the patients were evaluated, we found elevated IgG in 3/4 of the patients. IgG elevation may be due to chronic *C. albicans* and *S. aureus* infections. In chronic mucocutaneous candidiasis, there is a predisposition to many microorganisms together with a candida infection. Therefore, in patients with CMC as a major finding, the differential diagnosis should be primarily made between isolated (IL-17RA, IL-17RC, IL-17F, ACT1) and syndromic CMC (STAT1 GOF, STAT3 LOF, AIRE, CARD9, IL-12p40, IL-12Rβ1, ROR-γ) /γT).[2-9] IL-17RA, ACT1, and IL-17RC deficiencies are autosomal recessive, while IL-17F deficiencies are autosomal dominant disorders. It has been reported that patients with IL-17F or IL-17RC deficiency have a relatively mild, while patients with ACT1 or IL-17RA deficiency have a more severe phenotype.[7-9,20] In contrast to IL-17RA and ACT1 deficiencies, recurrent staphylococcal infections and severe or recurrent bacterial infections are

not seen in patients with IL-17RC deficiency.[8] In ACT1 deficiency, attacks of recurrent folliculitis caused by *S. aureus* and bilateral blepharitis are seen together with CMC.[9]

CARD9 deficiency is associated with chronic candidiasis, deep dermatophytosis, invasive exophiala dermatitis, subcutaneous phaeohyphomycosis, candida meningoencephalitis, and colitis.[2,21] In patients with STAT3 LOF deficiency characteristic facial features, high palate, retention of primary teeth, chronic candidiasis, scoliosis, osteoporosis and hyperextensibility of joints, eczema, eosinophilia, IgE elevation, severe skin, and pulmonary infections due to staphylococcus have been reported.[3] Also, in APECED (APS-1) syndrome signs of autoimmune polyendocrinopathy such as Addison's disease, hypoparathyroidism, and hypogonadism are seen.[2,4] In addition, fungal infections other than candidiasis, viral infections, mycobacterial infections, autoimmune symptoms (hypothyroidism, type 1 diabetes, and cytopenias), and IPEX-like clinical picture are seen in STAT1 GOF deficiency.[5] In IL-12Rβ1 and IL-12p40 deficiencies, Mendelian susceptibility to mycobacterial disease and recurrent salmonella infections[2,6], and RORγT deficiency, severe mycobacterial infections are observed.[2] In our cases, there was no history of infection due to microorganisms other than mucocutaneous candida infection and folliculitis caused by *S. aureus*. The 36-year-old male patient (Case 3) had a hiatal hernia and gastro-oesophageal reflux, the 37-year-old female patient (Case 4) had hyperthyroidism, and we diagnosed the eleven-year-old girl (Case 2) with asthma.

Most cases of CMC are treated with topical or systemic antifungal agents. As a topical treatment, nystatin can be used, but cases are often refractory to this treatment. Fluconazole is the first choice for oral therapy, followed by itraconazole, posaconazole, or voriconazole.[2] Flucytosine is also among the treatment options. These treatments can be used continuously or intermittently. In cases where oral antifungal therapy is ineffective, intravenous fluconazole or miconazole may be administered. As a second alternative, amphotericin B can be used. Surgical removal of the nails is also recommended along with, oral antifungals for onychomycosis.[2,7] In severe cases, candida-specific transfer factors may be effective when given antifungal treatments. In addition, leukocyte transfusions provide temporary relief of symptoms. Staphylococcal infections develop in addition to CMC in IL-17RA and ACT1 deficiency patients. Antibiotic prophylaxis with trimethoprim-sulfamethoxazole may effectively treat these patients.[7,9] It has been reported that an H2 receptor antagonist (cimetidine) can stimulate the cellular immune system in some cases with CMC. Hematopoietic stem cell transplantation appears to be an effective treatment for CMC. However, large case studies are needed to implement hematopoietic stem cell transplantation in all individuals with CMC.[22] In our patients, we started oral prophylactic fluconazole and trimethoprim-sulfamethoxazole treatment for both of the female patients (Cases 1 and 2). After starting the treatments, we were able to control the complaints of folliculitis and mucocutaneous candidiasis.

In conclusion, the genetic etiologies of syndromic and isolated CMC cases have been defined recently, revealing that IL-17 has an important role in mucocutaneous immunity against *Candida spp.* and *S. aureus*. These discoveries have provided new information about the inheritance, clinical course, and prognosis of CMC manifestations, including IL-17RA deficiency. However, further studies are needed to reveal the full picture of this congenital disorder.

Ethical approval

We obtained consent from the family to publish this report and to include their photographs.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: MK, ET, MHÖ; data collection: MK, MHÖ; analysis and interpretation of results: MK, ET, MHÖ, AŞ; draft manuscript preparation: MK, ET, MHÖ, AŞ. 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

Celiac disease and catatonia: more than a coincidence?

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ABSTRACT

Background. Catatonia is a complex neuropsychiatric disorder involving stupor, waxy flexibility, and mutism lasting more than 1 hour. It has arisen mostly from mental and neurologic disorders. Organic causes are more prominent in children.

Case. A 15-year-old female who had refused to eat and drink for 3 days, had not talked, and had stood in a fixed position for long periods was admitted to the inpatient clinic, and she was diagnosed with catatonia. Her maximum score on the Bush-Francis Catatonia Rating Scale (BFCRS) was 15/69 on day 2 of her stay. On neurologic examination, the patient's cooperation was limited, and she was apathetic to her surroundings and stimuli and inactive. Other neurologic examination findings were normal. To investigate catatonia etiology, her biochemical parameters, thyroid hormone panel, and toxicology screening were conducted but all parameters were normal. Cerebrospinal fluid examination and autoimmune antibodies were negative. Sleep electroencephalography showed diffuse slow background activity, and brain magnetic resonance imaging was normal. As a first-line treatment for catatonia, diazepam was started. With her poor response to diazepam, we continued to evaluate the cause and found the transglutaminase levels were 153 U/mL (normal values, <10 U/mL). The patient's duodenal biopsies showed changes consistent with Celiac disease (CD). Catatonic symptoms did not benefit from a gluten-free diet or oral diazepam for 3 weeks. Then, diazepam was replaced with amantadine. With amantadine, the patient recovered within 48 hours, and her BFCRS retreated to 8/69.

Conclusions. Even without gastrointestinal manifestations, CD may present with neuropsychiatric symptoms. According to this case report, CD should be investigated in patients with unexplained catatonia, and that CD may only present with neuropsychiatric symptoms.

Key words: amantadine, catatonia, Celiac disease, child, gluten-free diet.

Celiac disease (CD), an immune-mediated enteropathy, is precipitated by dietary gluten consumption in genetically susceptible individuals.[1] The prevalence is approximately 0.3% to 2.9% in children, with increasing rates in recent years.[2]

CD affects the small intestine, but it is accepted as a systemic disease. There is a wide range of extraintestinal manifestations in CD, such as neurologic and psychiatric disorders, including headache, seizure, ataxia and neuropathy, mood disorders, anxiety, and attention deficit hyperactivity.[3-5] Previous studies showed a 1.4-fold increased risk of developing a future psychiatric disorder in children and adolescents with CD compared with the general population.[5]

Catatonia is a complex neuropsychiatric disorder involving stupor, waxy flexibility, and mutism. It can arise from a variety of medical and psychiatric conditions.[6] Diagnostic and Statistical Manual of Mental Disorders 5th edition (DSM-5) requires three of the following 12 symptoms for diagnosis: stupor, catalepsy, waxy flexibility, mutism, negativism, posturing,

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mannerisms, stereotypy, agitation, grimacing, echolalia, and echopraxia.[6] Twenty percent of catatonia has a general medical cause.[7] However, little is known about the etiology and physiopathology of catatonia in children and adolescents. Here, we present the case of a 15-year-old female patient who presented with catatonia as the first symptom and was diagnosed with CD. According to this case report, CD should be included in the etiology of catatonia.

Case Report

A 15-year-old female patient was brought to the outpatient clinic. She had refused to eat and drink for 3 days, had not talked, and had stood in a fixed position for long periods. Her family history was unknown because she was an adopted child. Her foster parents did not notice any food allergens. The patient's vital signs and physical examination were normal. Before this presentation, she maintained weight and height above the 25th centile. In her neurological examination, the patient's cooperation was limited, she was apathetic to the surroundings and stimuli, and she was inactive. Other neurologic examination findings were normal, but sense and cerebellar examinations could not be performed. The symptoms of mutism, negativism, and posturing were detected in the psychiatric examination, and she was diagnosed with "catatonia associated with other mental disorders" and "schizophrenia" according to DSM-5. We performed a diazepam challenge test, which was positive, confirming the catatonia diagnosis. Her maximum score on the Bush-Francis Catatonia Rating Scale (BFCRS) was 15 on day 2 of her hospitalization. She was accepted by the inpatient clinic to investigate the organic causes of catatonia. Her vital follow-ups were normal. A nasogastric tube was placed. Complete blood counts, serum electrolytes, liver, and renal function tests, and thyroid hormone panels were performed, and no pathology was detected. Biochemical parameters and toxicology screening to exclude drug-related catatonia were normal. The cerebrospinal fluid (CSF) revealed normal protein and glucose levels. The CSF culture and serology for viruses were negative. The tests for Epstein bar virus, cytomegalovirus, herpes simplex virus, and respiratory tract viruses were negative. Antinuclear antibodies, antiphospholipid antibodies, anti-myelin oligodendrocyte glycoprotein antibodies, paraneoplastic encephalitis antibodies (anti-NMDA, anti-LGI), and anti-thyroid antibodies were also negative. Sleep electroencephalography (EEG) showed diffuse slow background activity, and brain magnetic resonance imaging (MRI) was normal.

As a first-line treatment for catatonia, we started diazepam, and the dose was gradually increased. After getting a poor response from her to diazepam treatment, we continued to search for catatonia's etiology, and the deamidated gliadin protein antibody (AGA) level was 33 U/mL (normal values, <10 U/mL), and the transglutaminase level was 153 U/mL (normal values, <10 U/mL). The patient's duodenal biopsies showed changes consistent with gluten-sensitive enteropathy, including subtotal villous atrophy, intraepithelial lymphocyte infiltration, and crypt hyperplasia (Marsh-Oberhuber 3c). The histological findings and positive tissue IgA anti-tissue transglutaminase antibody testing supported the presumptive diagnosis of CD. After CD was diagnosed, the patient began a gluten-free diet with oral diazepam. This dual treatment continued for 3 weeks, but her BFCRS did not improve (15/69). Ultimately, amantadine was added and titrated over 2 days to a maximum (200 mg, twice a day), and oral diazepam was weaned. The patient's response to amantadine was dramatic; within 48 hours, she was alert and communicative. At that time, she scored 8/69 on the BFCRS. Throughout the subsequent 4 months of amantadine treatment and a gluten-free diet, her situation continued to improve, and showed progress in speech, and her EEG was normal. Informed consent was received from the family.

Discussion

Celiac disease is a systemic immune-mediated enteropathy characterized by injury to mostly the small intestinal mucosa. This autoimmune response to gliadin leads to an inflammatory reaction and causes many extraintestinal manifestations, such as neuropsychiatric disorders, including mood disorders, eating disorders, anxiety, and attention deficit hyperactivity.[4,5]

Catatonia is a group of symptoms that involve a lack of movement as well as a lack of communication. It coexisted with several mental disorders, yet it has also presented with other medical conditions.[8] Although previously it was often categorized as schizophrenia, with the new changes in DSM-5, catatonia is not just associated with psychiatric disorders.[8] Recognizing catatonia and determining its etiology protects the patient from possible complications.

Twenty percent of catatonia has a general medical cause, of which central nervous system inflammation, including infective and immune causes, accounts for 29%.[7] In a systematic review, catatonia caused by an autoimmune disorder was reported. Most cases were observed with NMDAR encephalitis, systemic lupus erythematosus, autoimmune thyroid disorders, or demyelinating disorders.[6,7] The exact reason why some medical conditions lead to catatonia is not understood well; however, direct neurotoxic effects, the patient's psychological reaction to the insult, or mediation by acute phase reactants have all been suggested as potential causes.[9] Benarous et al.[6] reported that autoimmune investigations should be conducted for young patients with catatonia. Because of the higher morbidity and mortality rates in patients with catatonia, detection of the underlying cause of catatonia becomes critical.[10]

In a recent study, CD and some other autoimmune disorders (hypothyroidism, alopecia areata) were reported in 3 of 7 patients with Down syndrome diagnosed with catatonia. The authors underlined a high prevalence of autoimmune disorders (57%) in their patients.[11] Given that the current evidence on pediatric catatonia that advice autoimmune investigations should be conducted[6], it may be a reasonable strategy to screen for celiac disease in children with unexplained catatonia[11] The most recent hypothesis suggests that different manifestations of CD depend on the role of transglutaminase antibodies in the humoral immune response. Transglutaminase 6 seems to be important in brain damage.[12] With the support of functional neuroimaging studies, previous studies have shown that the γ-aminobutyric acid (GABA)-ergic inhibition response decrease in the cortical regions plays a crucial role.[6] This also explains the quick response to GABA-A agonists during treatment. Hypothetically, in CD, immune-mediated dysregulation of inhibitory GABAergic interneurons may cause catatonia.[13] In the catatonic brain, there is a neural excitatory/inhibitory imbalance, and we hypothesize that CD may have an effect similar to the mechanism proposed in anti-NMDAR encephalitis.[13] Further studies will help improve the understanding of immune-mediated triggers in catatonia.

Even though the specific pathophysiologic mechanisms underlying catatonia has yet to be defined, they are thought to primarily involve dysfunction in dopamine, GABA, glutamate, and acetylcholine circuits. GABA-A agonists such as benzodiazepines are frequently used for the treatment of catatonia because dopamine antagonists induce catatonia-like symptoms.[14] Previous studies showed that almost all patients initiated first-line treatment with oral lorazepam as soon as catatonia was diagnosed.[15] Deficiencies in GABA, dysfunction of the GABA receptors, and dysfunction and/or hyperactivity of NMDA receptors are believed to contribute to the motor symptoms and inhibition seen in patients with catatonia. The imbalance in the GABAergic inhibitory and glutamatergic excitatory pathways is thought to impair motor planning and execution, leading to motor symptoms, such as stupor

and ambitendency.[16] The NMDA antagonists amantadine and memantine are currently being studied in the treatment of catatonia.[17]

To the best of our knowledge, the patient is one of the few cases in the literature under the age of 18 who used amantadine for catatonia and who presented with catatonia as the first symptom of CD. To conclude, this case underlines the rare but possible associations between CD and catatonia as the onset symptom, even in the absence of gastrointestinal manifestations. Autoimmune investigations and early detection of the etiology of catatonia are crucial and may decrease further complications. In addition, the patient is important as one of the rare pediatric cases in the literature in which amantadine was used in the treatment of catatonia.

Ethical approval

Written informed consent was obtained from the patient's relatives as institutional review board accepted.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: PÖ, KG; data collection: PÖ, AEK, DKM, HÖ; analysis and interpretation of results: PÖ, AEK, DKM, HÖ, KG; draft manuscript preparation: PÖ, KG. 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 entity in a pediatric patient: coexistence of emphysematous cystitis and emphysematous pyelonephritis

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ABSTRACT

Background. Emphysematous cystitis (EC) and emphysematous pyelonephritis (EPN) are rare urinary tract infections. They have a wide spectrum of clinical manifestations; ranging from asymptomatic to septic shock at presentation. In children, EC and EPN are rare complications of urinary tract infections (UTIs). Their diagnosis is based on clinical manifestations, laboratory results and characteristic radiological findings of gas within the collecting system, renal parenchyma and/or perinephric tissue. Computed tomography is the best radiological option in the diagnosis of EC and EPN. Despite the availability of various treatment modalities including medical and/or surgical treatment alternatives, these life-threatening conditions have high mortality rates reaching up to 70 percent.

Case. Urinary tract infection was detected in the examinations of an 11-year-old female patient suffering from lower abdominal pain, vomiting and dysuria for two days. Air was detected in the bladder wall on X-ray. EC was detected in the abdominal ultrasonography. Air formations in the bladder lumen and calyces of both kidneys in abdominal computed tomography confirmed the presence of EPN.

Conclusions. Individualized treatment should be instituted according to the severity of EC and EPN, and the overall health condition of the patient.

Key words: emphysematous pyelonephritis, emphysematous cystitis, Enterobacter aerogenes, child.

Emphysematous cystitis (EC) is a rare, life-threatening clinical condition caused by gas-producing microorganisms in the bladder. The presence of gas in the urinary tract was first described in 1671 in a patient who stated that air came from the urethra. Eisenlohr first detected intramural gas in the bladder in an autopsy in the late 1800s,.[1] EC was defined as Bailey's disease in 1961.[2] EC is common in diabetic elderly women. In a study of 153 adult patients with EC, 63.4% of cases were female and 66.7% were diabetic.[3]

Emphysematous pyelonephritis (EPN) is an uncommon but severe necrotizing infection of the kidneys, characterized by gas formation in the renal parenchyma, perinephric tissue or collecting system after a urinary tract infections (UTI).[4] It is most commonly caused by gas-producing bacteria in the kidney. This condition was first described by Kelly and MacCullum[5] in 1898 in an adult patient. The first pediatric case reported in 1985 was a 10-year-old girl.[6] More than 80% of reported adult cases were predominantly diabetic female patients. However, not all pediatric cases were diabetic.[6,7]

In children, EC and EPN are rare conditions that occur as complications of UTIs. We did

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not find any reports in the English literature on children with both EPN and EC. It is more common in diabetic adult women. Diabetes mellitus, neurogenic bladder and advanced age are important risk factors for the development of EC and EPN.[8] Early diagnosis and treatment of EC and EPN is extremely important for the prevention of serious morbidities such as bladder necrosis and sepsis. Since it does not have typical clinical findings, the diagnosis can be made by imaging studies rather than physical examination.

In this case report, we present a rare case in children with recurrent EC associated with EPN.

Case Report

An 11-year-old girl presented with complaints of lower abdominal pain, vomiting and dysuria for two days. Her past medical records were uneventful and vital signs were stable. The patient was not febrile. Body temperature was 36.5°C. The patient had no history of change in urine color, dysuria, or abdominal pain in her background. There was no urgency, nocturia, day or night urinary incontinence, difficulty in initiating voiding, intermittent voiding, post-void dripping, but there were complaints of urinary retention, decreased frequency of voiding (≤ 3/day) and urination. The patient had no constipation.

On physical examination, suprapubic and bilateral costovertebral angle tenderness was detected. The patient was evaluated according to the Dysfunctional Voiding Scoring System (DVSS).[9] No symptoms were detected, except for a decrease in the frequency of voiding and urinary retention. Laboratory test results were as follows: white blood cells 11,240/mm^3, hemoglobin 11 g/dL, and platelets 212,000/mm^3; blood glucose 100 mg/dL, urea 22 mg/dL, creatinine 0.44 mg/dL, potassium 4.5 mEq/L, and sodium 140 mEq/L; and C-reactive protein (CRP) 77.2 mg/L (0-5 mg/L) and procalcitonin 0.21 mg/L (0-0.12 mg/L). In the urinalysis, the leukocyte count was 21 cells/HPF, leukocyte esterase (+++), erythrocyte negative, nitrite negative, and urine protein negative. The urine sample for urine culture was taken from the patient's midstream urine. Culture of urine yielded 100,000 colonies of *Enterobacter aerogenes* per ml. The air level in the bladder lumen under the anterior wall was detected in the standing abdominal X-ray (Fig. 1). Both air and echogenicity in the bladder lumen and an increase in bladder wall thickness (6.5 mm) detected in the abdominal ultrasonography (US) were evaluated as EC. In addition, the diameter of the renal pelvises was 7 mm, and there was 20 mm of residual urine in the bladder after voiding. Computed tomography (CT) imaging was performed after ultrasonographic evaluation of the patient with abdominal pain at her first admission. CT was performed to exclude conditions such as trauma, colovesical and vesicovaginal fistula, gas gangrene of the uterus and vagina, which are included in the differential diagnosis of EC. On the CT scan, air formations were present in the bladder lumen

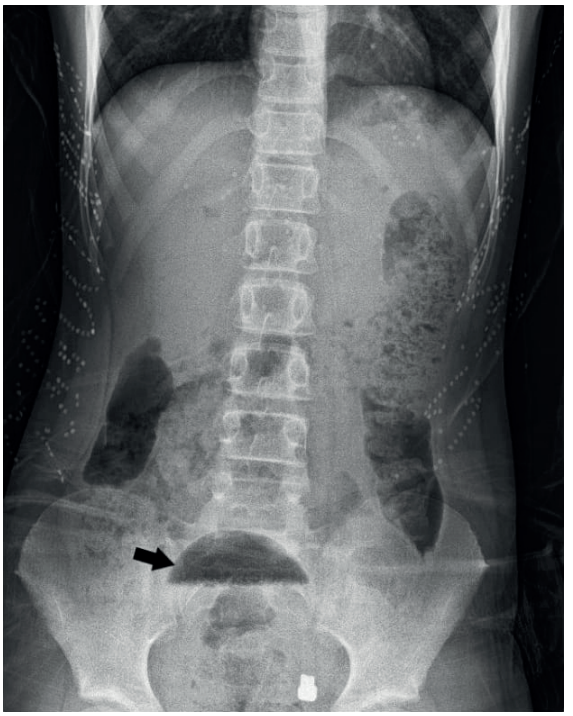

Fig. 1. Level of gas formation in the bladder on the standing abdominal X-ray (black arrow).

and the calyces of both kidneys, and the bladder wall was slightly edematous (Fig. 2).

The patient was hospitalized and intravenous fluids and ceftriaxone treatment were started. Then the patient was trained to correct her voiding habit. On the fifth day of antibiotic therapy, control urine analysis revealed no leukocyte and its culture yielded no bacterial growth. No vesicoureteral reflux was detected in voiding cystourethrography (VCUG). In the follow-up, control CRP and procalcitonin were normal. On the 10th day of treatment, Tc99m dimercaptosuccinic acid (DMSA) scintigraphy showed that the left kidney was smaller than the right kidney, and inhomogeneous activity uptake and hypoactive notches in the upper and middle outer parts of the kidneys were observed. Hypoactive areas in the upper pole of the left kidney were compatible with cortical defect. Contributions of right and left kidneys to total kidney functions were determined as 34% and 66%, respectively. The patient, whose intravenous antibiotic treatment continued for 14 days, was discharged from the hospital with antibiotic prophylaxis. Trimethoprim-sulphamethoxazole was started as antibiotic prophylaxis. The patient was not symptomatic when she applied for the follow-up visit one month later. In the urinalysis the leukocyte count was 7 cells/hpf, leukocyte esterase (+++), erythrocyte negative, nitrite negative and protein negative. Catalase positive *Staphylococcus aureus* was recovered from the urine culture. In the urinary US, the post-voiding bladder wall thickness was measured as 7 mm and several millimetric-sized air formations were observed under the anterior bladder wall. Repeated urinalysis after oral antibiotic was normal and urine culture was negative. Control urinary US was also unremarkable. The patient was given voiding training because of her hypoactive bladder. In the control DMSA repeated at the sixth month, it was observed that the notching, suggesting scar formation, continued in the upper pole and lateral of the middle part of the right kidney, as in the previous image. Contributions of left and right kidneys to total renal DMSA uptake were calculated as 65.3% and 34.7%, respectively. Informed consent was obtained from the family to publish this report.

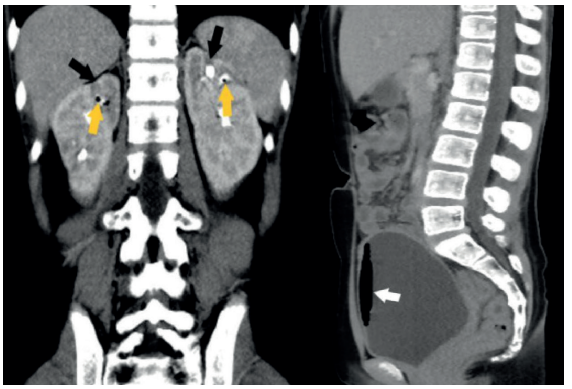

Fig. 2. In the coronal contrast -enhanced abdominal computed tomography (CT) image, the size of the right kidney was smaller than the contralateral one. More prominent on the right, there were hypodense areas in both renal cortices, suggesting a scar formation, causing focal recessions (black arrows). There were gas appearances within the bilateral renal calyces (yellow arrows). Leveled gas formation in the bladder lumen seen in the sagittal contrast-enhanced CT image of the abdomen (arrow).

Discussion

Immunosuppressive conditions other than diabetes mellitus, neurogenic bladder, urethral catheterization, vesicorectal fistula, end-stage kidney disease, bladder outlet obstruction are among risk factors for the development of EC. EC has nonspecific clinical manifestations ranging from asymptomatic or mildly symptomatic cases to cases with severe peritonitis and septic shock. Dysuria, hematuria, pollakiuria, fever, suprapubic tenderness, and pneumaturia are frequent symptoms of EC. Its more specific symptom of pneumaturia is very rarely observed. Thomas et al.[2] reported that 7% of their cases were asymptomatic and these cases were detected incidentally by abdominal imaging. In pediatric cases with EPN, vomiting, flank pain, nausea, fever, and dysuria are frequently observed. EPN should be considered in a patient with unresolved UTIs.[4-6]

Escherichia coli is the most common pathogen isolated in cases with EC and EPN. Additionally,

Klebsiella pneumoniae, *Enterobacter aerogenes*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, Citrobacter, *Staphylococcus aureus*, *Clostridium perfringens*, streptococci and Candida species have been reported as etiological agents.[4,7,10] Similarly, *Enterobacter aerogenes* was detected in the urine culture of our patient.

The pathogenesis of EPN is still unclear. Increased glucose levels in tissues, urinary tract obstruction, presence of gas-forming organisms, decreased host immunity, and impaired tissue perfusion may have a role in the pathogenesis of EPN.[11] Increased glucose levels in tissues, along with decreased blood flow to the kidneys, contribute to the anaerobic metabolism of glucose and lactate by gas-forming organisms resulting in the production of carbon dioxide, hydrogen, nitrogen, oxygen, and methane.[4,12]

To date, very few pediatric cases of EPN have been cited in the literature.[7,13] These patients have risk factors such as sepsis, end-stage kidney disease, HIV infection, kidney transplantation and urinary system obstruction.[13] No underlying risk factors were found in the present case.

CT is the best radiological option in the diagnosis of EC and EPN. Characteristic finding of EC is the appearance of small, multiple gas-filled vesicles under the bladder mucosa. Grupper et al.[11] reported that air vesicles could be seen in the bladder wall and bladder lumen in 94.4% and 3.7% of the cases, respectively. Air formation was detected in the bladder lumen and both renal calyces in our patient. CT is also useful in the differential diagnosis of EC: vesicovaginal and colovesical fistula, trauma, gas gangrene of uterus, and vagina. Ultrasound has a lower diagnostic sensitivity than CT, but it helps to measure bladder wall thickness and to determine possible echogenicities. In EPN, plain abdominal X-rays may show gas shadows in the affected kidney and a crescent-shaped gas around it. Abdominal radiograms are sensitive in detecting air in the renal collecting system, but they lack diagnostic specificity due to the superposition of intestinal gas.[7,12] Since previous studies have shown that US has a diagnostic accuracy between 50 to 86% in cases of EPN, CT is the recommended diagnostic tool in these cases.[12,14,15]

Treatment of EC includes intravenously administered broad-spectrum antibiotics, bladder drainage, glycemic control, and correction of the underlying predisposing conditions. Hence, treatment with ceftriaxone, fluoroquinolones, aminoglycosides or carbapenems should be initiated until the causative agent is isolated. However, there is no consensus on the duration of antibiotherapy. Surgical interventions including partial or total cystectomy are rarely necessary. In our case, since the isolated pathogen was sensitive to ceftriaxone, we preferred this drug as the empirical antibiotic treatment, and maintained the treatment for 14 days. If left untreated, EC may affect the kidneys or ureter and even result in the development of fatal complications.[16] Although the mortality rate in EC is approximately 7%, this rate rises to 14% if the upper urinary tract is affected.

Management of EPN necessitates delivery of supplemental oxygen, intravenous fluids, correction of acid-base imbalance, intravenous antibiotics and inotropes, and even renal replacement therapy in some patients. Clear-cut guidelines for the surgical treatment of these patients have not been established yet. The surgical treatment proposed by Huang and Tseng[17] is based on radiological classification of EPN.

Positional instillation of contrast (PIC) cystography can be considered if recurrent urinary tract infections continue in the follow-up of this case with pathological findings in DMSA screening. In studies, it has been reported that occult reflux can be detected with PIC cystography in patients with recurrent febrile urinary tract infections and no reflux with VCUG.[18] Although PIC cystography has the disadvantage of being applied under anesthesia, it also has advantages such as detecting occult

reflux, facilitating treatment with endoscopic injection if necessary, and being both diagnostic and therapeutic in the same anesthesia session.

In conclusion, although EC and EPN are generally seen in adult diabetic women, the present case shows that these conditions can also be seen in children, and EC can be also very rarely associated with EPN. Early diagnosis and appropriate treatment are vital for the successful treatment of these patients.

Ethical approval

Informed consent was obtained from the family to publish this report.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: BEA, AK, MKG; data collection: BEA, AK, MKG; analysis and interpretation of results: BEA, AK, MKG, YE; draft manuscript preparation: BEA, AK, MKG. All authors reviewed the results and approved the final version of the manuscript.

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Case Report

Chronic inflammatory demyelinating polyradiculoneuropathy associated with Sjögren's syndrome in a child

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ABSTRACT

Background. Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is a peripheral nervous system disease associated with polyautoimmunity.

Case. We report a previously healthy 13-year old boy who was referred to our outpatient clinic with gait disturbance and distal lower limb weakness that had been increasing for six months. The patient had decreased deep tendon reflexes in the upper extremities and absence in the lower extremities, reduced muscle strength in the distal and proximal lower extremities, muscle atrophy, drop foot, and normal pinprick sensations. The patient was diagnosed with CIDP as a result of clinical findings and electrophysiological studies. Autoimmune diseases and infectious agents were investigated in terms of triggering CIDP. Although there was no clinical sign other than polyneuropathy, he was also diagnosed with Sjögren's syndrome due to positive antinuclear antibodies and antibodies against Ro52, and with autoimmune sialadenitis. After six months of monthly intravenous immunoglobulin and oral methylprednisolone treatments, the patient was able to dorsiflex his left foot and walk without support.

Conclusions. To our knowledge, our case is the first pediatric case with the coexistence of Sjögren's syndrome and CIDP. Therefore, we suggest investigating children with CIDP in terms of underlying autoimmune diseases such as Sjögren's syndrome.

Key words: Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), neuropathy, polyautoimmunity, Sjögren's syndrome.

Chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) is a peripheral nervous system disease associated with an immune-mediated response.[1] The prevalence of CIDP in children is 0.22 per 100,000 and is rarer than in adults.[2,3] Environmental and genetic factors may contribute to the autoimmunity of CIDP.[4] It is characterized by symmetric weakness and sensory dysfunction in limbs, lasting at least eight weeks. Moreover, autonomic nervous system involvement, cranial nerve palsy and neuropathic pain may occur less frequently.[5] Invaluable findings for the diagnosis of CIDP include the absence or decreased deep tendon reflexes (DTR) in neurological examination, and demyelination in electrophysiological studies. Diagnostic criteria including clinical, laboratory, electrophysiological, and laboratory findings for all age groups were updated in 2021 by the European Academy of Neurology/Peripheral Nerve Society.[6] However, diagnosis of pediatric CIDP is difficult due to the inadequacy or misinterpretation of electrophysiological studies.[3]

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According to the literature, CIDP may be associated with some autoimmune diseases such as systemic lupus erythematosus (SLE), myasthenia gravis (MG), multiple sclerosis (MS), Sjögren's syndrome (SS), Hashimoto's thyroiditis, rheumatoid arthritis (RA), type 1 diabetes mellitus (T1D), vitiligo, Graves' disease, primary biliary cholangitis, and autoimmune hepatitis.[4] However, only SLE, MG, MS, T1D, Graves' disease and autoimmune hepatitis have been reported to be associated with CIDP in childhood.[7-15] We aim to present the first pediatric case with the coexistence of CIDP and Sjögren's syndrome.

Case Report

A 13-year-old boy presented with progressive gait disturbance and distal lower extremity weakness for six months. The medical history was uneventful except for a history of 30-week prematurity. He was born after an uneventful pregnancy and delivery, to non-consanguineous parents. His developmental milestones were reported as normal. The patient had a cousin with a history of Guillain–Barré syndrome.

On neurological examination at admission, DTRs were found as decreased in the upper extremities and absent in the lower extremities. Moderate muscle atrophy and reduced muscle strength were observed in the bilateral distal more than proximal lower extremities. He had bilateral drop-foot and no dorsiflexion of either feet. He could walk with support. He had normal pinprick sensations in his limbs and trunk.

Cranial and spinal magnetic resonance imaging (MRI), and genetic analysis for Charcot Marie Tooth variants were normal. Electromyography

Table I. The results of nerve conduction study, needle electromyography findings, and F waves of the patient with CIDP associated with Sjögren's syndrome.

Nerve	Left			Right		
Motor Nerve	MCV (m/s)	CMAP (mV)	Distal latency (ms)	MCV (m/s)	CMAP (mV)	Distal latency (ms)
Median nerve	36.5	6.22	4.7	30.2	4.79	5.3
Ulnar nerve				43	11.6	3.1
Peroneal nerve	No response	No response	No response	No response	No response	No response
Sensory nerve	SCV (m/s)	SNAP (μV)	Distal latency (ms)	SCV (m/s)	SNAP (μV)	Distal latency (ms)
Median nerve	39.2	9.30	3.32	45.2	10.8	3.1
Ulnar nerve				41	17	3.8
Sural nerve	No response	No response	No response	No response	No response	No response
F wave conduction velocity						
Ulnar nerve				35		

Muscle	Left				Right			
	Interference	Fibrillation	Positive sharp waves	MUAP	Interference	Fibrillation	Positive sharp waves	MUAP
Biceps brachii muscle	Normal	Negative	Negative	Normal	Normal	Negative	Negative	Normal
Extensor digitorum communis muscle	Normal	Negative	Negative	Normal	Normal	Negative	Negative	Normal
Tibialis anterior muscle	No action	++	Negative	No action	No action	++	Negative	No action
Gastrocnemius medialis muscle	Reduced	Negative	Negative	High amplitude	Reduced	Negative	Negative	High amplitude

CMAP: compound motor action potential, MCV: motor conduction velocity, MUAP: motor unit action potential, SCV: sensory conduction velocity, SNAP: sensory nerve action potential.

and nerve conduction studies were performed (Table I). In the upper extremity, distal latencies were detected as prolonged, motor conduction velocities were found as decreased, and there was also a conduction block. Despite using supramaximal stimulation, motor and sensory nerve conductions could not be obtained in the lower extremity. Neurogenic motor unit action potentials (MUAPs) were observed in the lower extremity muscles. These electromyoneurography (EMNG) findings were consistent with sensory and motor demyelinating polyneuropathy with predominant demyelination, severe in the lower extremities, mild in the upper extremities, and accompanied by loss of axons in the lower extremities.

The cerebrospinal fluid (CSF) evaluation revealed increased protein levels (97 mg/dl, normal reference: 15-45 mg/dl). The IgG index (CSF/serum ratio for IgG) was found as 0.7 (normal reference: 0-0.77). Infectious markers, erythrocyte sedimentation rate (11 mm/h, normal reference limit 0-15 mm/h), anti-ganglioside antibodies, neurofascin 155 and 186 were all unremarkable. Based on these clinical and neurophysiological findings, he was diagnosed with CIDP and was treated with monthly intravenous immunoglobulin (IVIg, 2 g/kg in 5 days). Serum autoantibodies were analyzed in terms of underlying autoimmune diseases. Antinuclear antibody (ANA; 1:80 arbitrary units (AU), normal reference limit <1:40 AU), thyroid autoantibodies [thyroid peroxidase antibody (anti-TPO):153 IU/ml, normal reference limit 0-34 IU/ml; thyroglobulin antibody (anti-TG):155 IU/ml, normal reference limit 0-115 IU/ml], and antibodies against Ro52 were found to be positive. However, rheumatoid factor (RF), anti-double-stranded DNA (anti-dsDNA), anti-Ro/SSA, and anti-La/SSB antibodies were found negative. Unfortunately, antibodies against Ro60 could not be tested. These autoantibody tests were repeated and confirmed two more times within three months. The thyroid ultrasonography (USG) and thyroid function tests were unremarkable. The Schirmer's test was considered abnormal because of the result of 35 mm/5 minutes. The salivary gland biopsy showed an inflammatory focus exceeding 50 lymphocytes (>1 focus/4mm^2), consistent with autoimmune sialadenitis and supportive of Sjögren's syndrome. He was diagnosed with definitive Sjögren's syndrome, according to the Japan Pediatric Sjögren's syndrome clinical practice guideline, by getting seven points serologically, two points from the exocrine gland score and 0 points from the lacrimal gland score.[16]

Based on the clinical and electrophysiological findings, we suggested that the patient had coexistence of CIDP and Sjögren's syndrome. We administered pulse intravenous methylprednisolone (1g/day for 3 days) and then continued with oral methylprednisolone (1 mg/kg/day) with monthly IVIg (total 1-2 g/kg in 2-5 days). The patient was able to dorsiflex his left foot and walk without support at the 6th month of his first admission.

Written informed consent was obtained from both the parents and the participant of the study after the treatment for the publication of this case report.

Discussion

Based on the current clinical features and electrophysiological findings, the patient was diagnosed with CIDP according to the latest European Academy of Neurology/Peripheral Nerve Society guidelines.[6] Demonstration of increased protein in CSF and partial recovery with IVIg therapy are supportive findings for the diagnosis of CIDP in our patient. Other supportive findings, according to the latest guidelines, are the use of USG and MRI, and nerve biopsy. Median nerve segments and brachial plexus imaging with USG are not recommended in the pediatric population, and are used in possible CIDP diagnostic criteria in adults.[6] Magnetic resonance imaging may detect enlargement or hyperintensity of the nerve roots, but it was normal in our patient.

Since nerve biopsy is not recommended in cases with a definite diagnosis of CIDP, it was not performed on our patient.

The patient was evaluated for possible underlying infectious, autoimmune, and genetic etiologies. The findings which support Sjögren's syndrome such as high autoantibody levels including ANA, anti-Ro-52, anti-TPO, anti-TG, and the autoimmune sialadenitis which was demonstrated by salivary gland biopsy were defined. A single anti-Ro test combining anti-Ro-60 and anti-Ro-52 antibodies in solid-phase immunoassays was found to be negative in our patient. However, separately performed assays for anti-Ro-60 and anti-Ro-52 are more useful.[17] Accordingly, these findings fulfilled the definitive diagnostic criteria for Sjögren's syndrome according to the Japan Pediatric Sjögren syndrome clinical practice guidelines.[16]

As is known, neurological involvement, such as polyneuropathy, often occurs much earlier than classical Sjögren's syndrome findings, such as dry eyes, dry mouth.[18] Seeliger et al.[19] suggested that this association can be called neuro-Sjögren, and when they evaluated their patients with neuro-Sjögren retrospectively in their study, they noticed that most of the patients met the diagnostic criteria for atypical CIDP, which shows that the association of CIDP and Sjögren's syndrome is significant.

The association between CIDP and Sjögren's syndrome was first reported in an 83-year-old woman in Taiwan.[20] Subsequent studies also showed that female gender predominance was higher than male gender in patients with pure CIDP.[3,21-23] In a comparative study in the adult population, Seeliger et al.[24] suggested that female gender predominance and cranial nerve involvement may be red flags for an additional Sjögren's syndrome in patients with CIDP. On the other hand, a significant difference between CIDP with and without Sjögren's syndrome in terms of clinical, electrophysiological and CSF findings was not observed.[24] However, the validity of these findings need to be confirmed in further studies.

The patient is currently receiving monthly IVIg and oral methylprednisolone therapy, and clinically moderate improvement in muscle strength has been observed. In addition, some clinicians recommend adding rituximab to treatment in the coexistence of CIDP and Sjögren's syndrome or the presence of IVIg and corticosteroid resistance.[25]

To our knowledge, our case is the first pediatric case with the coexistence of Sjögren's syndrome and CIDP. This report expands on the comorbidities of CIDP and suggests that CIDP may present with Sjögren's syndrome in childhood. Therefore, we suggest investigating children with CIDP in terms of underlying autoimmune diseases such as Sjögren's syndrome. Further case series are needed to identify a potential correlation between CIDP and Sjögren's syndrome.

Ethical approval

Ethical approval was waived by the local Ethics Committee of Ankara University in view of the retrospective nature of the study and all the procedures being performed were part of the routine care. The study was conducted in accordance with the declaration of Helsinki. Written informed consent was obtained from both the parents and the participant of the study.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: NYS, ATK, MY, ŞE; interpretation of results: FA, ZBÖ, ÖB, ST; draft manuscript preparation: NYS, MY. All authors reviewed the results and approved the final version of the manuscript.

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Case Report

Spontaneous hyphema in juvenile idiopathic arthritis uveitis

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ABSTRACT

Background. Juvenile idiopathic arthritis (JIA) is a rheumatic disease that may be associated with ocular involvement in childhood. Classical findings of JIA uveitis are cells and flare; hyphema, bleeding in the anterior chamber of the eye, is a rare finding.

Case. An 8-year-old girl presented with 3+ cells and a flare in the anterior chamber. Topical corticosteroids were started. A follow-up examination 2 days later revealed hyphema in the affected eye. There was no history of trauma or drug use, and the laboratory test results did not suggest any hematological disease. Systemic evaluation resulted in the diagnosis of JIA by the rheumatology department. The findings regressed with systemic and topical treatment.

Conclusions. The most common cause of hyphema in childhood is trauma, but it can rarely be seen with anterior uveitis. This case highlights the importance of recognizing JIA-related uveitis in the differential diagnosis of hyphema in childhood.

Key words: hyphema, Juvenile idiopathic arthritis, uveitis.

Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease in childhood. Among the extra-articular complaints of JIA, uveitis is a frequent finding. JIA-related uveitis often presents as silent chronic anterior uveitis in oligoarticular or rheumatoid factor (RF) (-) polyarticular forms.[1] This type of uveitis is generally asymptomatic and can be detected on slit-lamp examination but can lead to complications such as cataracts, glaucoma, band keratopathy and cystoid macular edema which can cause visual impairment and blindness.[2] Hyphema, which is bleeding in the anterior chamber of the eye, is often caused by trauma in childhood. Iris anomalies, inflammatory/infectious factors, post-surgical conditions and some systemic diseases should also be considered in the etiology.[3,4] There are very few reports of hyphema related to uveitis and it is an even less common condition in JIA uveitis.[3]

This paper presents a case of unilateral spontaneous hyphema with JIA, highlighting the need to consider JIA-related uveitis in the differential diagnosis of spontaneous hyphema in children.

Case Report

An eight-year-old girl presented with decreased vision in her left eye for the last two days. On ophthalmic examination, her best corrected visual acuity was 20/20 in the right eye and counting fingers from 3 meters in the left eye. Anterior segment examination of the right eye was within normal limits, but in the left eye the conjunctiva was slightly hyperemic and 3+ cells and flare were observed. Intraocular pressure

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was measured as 12 mmHg in the right eye and 14 mmHg in the left eye. A dilated examination revealed normal bilateral fundi. The history taken from the parents revealed that she had experienced swelling and pain attacks in her left ankle, left heel and left wrist lasting 4-5 days in the previous year and uveitis attacks twice but did not receive any systemic treatment. Topical dexamethasone 0.1% hourly and cyclopentolate 1% drops 3 times per day were prescribed for anterior uveitis. At the follow-up examination two days later, there was no regression in inflammatory activity and approximately 1 mm hyphema was observed in the left anterior chamber (Fig. 1). Neither rubeosis iridis nor any iris anomalies were detected. Trauma history and drug use such as aspirin were excluded.

A consultation to the Department of Pediatric Rheumatology confirmed that she had arthritis at the age of seven and had been treated for arthritis. She was diagnosed with oligoarticular JIA. Further evaluation of complete blood count, peripheral blood smear and coagulation parameters were within normal limits. Chest X-ray and PPD tests were reported to be normal. Brucella, Salmonella typhi and paratyphi and HLA B5/ B51/ B27 antigen tests were negative, RF value, anti-nuclear antibody and anti- ds DNA antibody test were within normal limits.

The Pediatric Rheumatology department prescribed subcutaneous methotrexate (MTX) 10 mg weekly with oral prednisolone 0.5 mg/kg per day. Two weeks later, visual acuity increased to 20/40 in the left eye, reaction decreased to 1+ cells, hyphema disappeared and iris pigments became evident on the lens. By the end of the first month, visual acuity improved to 20/20 in the left eye. Oral corticosteroids were tapered over a period of two months. Two years later, MTX had to be discontinued due to abnormal liver function tests. As the patient experienced recurrent uveitis and arthritis, adalimumab was initiated. Despite systemic treatment, the patient had 1-2 anterior uveitis attacks per year during the four-year follow-up period, there was no recurrence of hyphema in any of the other attacks.

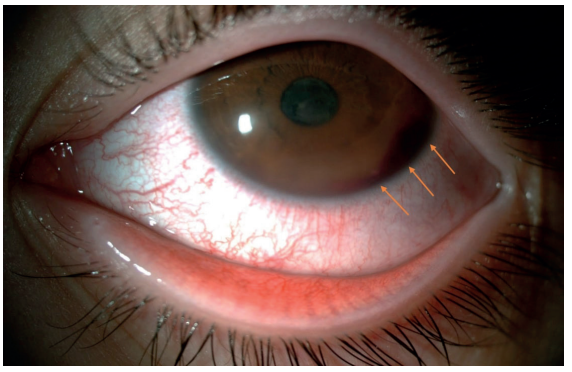

Fig. 1. Hyphema in the anterior chamber

The family was informed about the case presentation and written consent was obtained.

Discussion

In this report, we present a female patient with JIA uveitis and unilateral spontaneous hyphema. Trauma is the most important cause of hyphema in childhood.[5] Post-traumatic hyphema pathophysiology can be classified as rupture of vessels in the iris and/or ciliary body after stress caused by antero-posterior equatorial expansion of the globe in blunt trauma, deterioration of fragile vascular structures in the pupillary sphincter or angle due to a sudden increase in intraocular pressure, and direct damage to vessels and hypotonia in lacerating traumas.[6] Spontaneous hyphema may be seen in hematological diseases with impaired blood parameters and a tendency to bleed, such as sickle cell anemia, leukemia, haemophilia and immune thrombocytopenia in childhood.[7,8] Furthermore, the use of anti-coagulants[9] or non-steroidal anti-inflammatory drugs (NSAID) (anti-platelet activity)[3,10], may contribute to hyphema formation. In addition, infiltrating iris anomalies such as histiocytosis, retinoblastoma or juvenile xanthogranulomatosis involving the iris and ciliary body may cause hyphema.[7]

Hyphema due to non-traumatic reasons such as uveitis is a rare condition in children. Duke- Elder and Perkins[11] described petechial

hemorrhage and hemorrhagic iritis in severe inflammation of uveitis and explained hyphema in uveitis with 3 mechanisms; vessel damage secondary to vasculitis, leakage from sensitive rubeotic vessels, and increased diapedesis of inflammatory cells.[11] Rubeosis iridis is usually a sign of proliferative diabetic retinopathy, retinal vein occlusion, or anterior segment ischemia and appears as small, fine, disorganized vessels on the anterior surface of the iris. During anterior segment inflammation, prominent dilated iris vessels can be observed and can be distinguished from rubeosis iridis with stromal and radial location. It is thought that hyphema may occur in severe inflammation from prominent dilated and damaged vascular structure, increased capillary permeability due to the breakdown in the blood-aqueous barrier then subsequent diapedesis and leakage from vessels.[12] Fong and Raizman[3] reported five different cases with hyphema accompanying anterior uveitis. The diagnoses were Reiter's syndrome, juvenile chronic arthritis, ankylosing spondylitis (AS), idiopathic anterior uveitis, and herpes simplex. Rubeotic vessel formation was detected in 3 of 5 cases, one case was taking NSAID, and no suspicious condition was reported in the remaining case. In the current patient, no neovascularization or a prominent vascular structure were detected on the iris, and there was no history of systemic drug use.

There is only one previous case report of spontaneous hyphema related to JIA in the literature, reported by Shimada et al.[4] This was a 5-year-old girl with bilateral hyphema as a result of anterior uveitis. Her arthritis, ocular findings and her age suggested this disease. If rheumatological signs and uveitis are seen together in childhood, JIA, Behçet's disease and juvenile sarcoidosis/Blau syndrome should be considered first.[1] In uveitis due to JIA, an inflammatory reaction is seen in the anterior chamber, but the development of hyphema is rare. Increased permeability and diapedesis after inflammation may be the cause of hyphema. In the current case, the reason for spontaneous hyphema was not clearly identified. Spontaneous hyphema has also been reported in one case of syphilitic uveitis.[13]

In childhood, after excluding trauma and bleeding diatheses, it should be kept in mind that hyphema may occur in other conditions including JIA-related uveitis without a prominent iris vascular structure.

Ethical approval

The family was informed about the case presentation and consent form was obtained.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: FBA, SK; data collection: FBA; analysis and interpretation of results: FBA, SK; draft manuscript preparation: FBA, SK, SÖ. All authors reviewed the results and approved the final version of the manuscript.

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The authors declare that there is no conflict of interest.

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Case Report

Chronic inflammatory demyelinating neuropathy after etanercept therapy in the course of juvenile idiopathic arthritis

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ABSTRACT

Background. Chronic inflammatory demyelinating neuropathy has been reported after the use of tumor necrosis factor inhibitors. The mechanisms of nerve injury caused by tumor necrosis factor inhibitors are not yet well understood.

Case. In this paper, we report a 12 year and nine month old girl who developed chronic inflammatory demyelinating neuropathy in the course of juvenile idiopathic arthritis after etanercept withdrawal. She became non-ambulant with four-limb involvement. She received intravenous immunoglobulins, steroids, and plasma exchange, but had a limited response. Finally, rituximab was given and a slow, but progressive clinical improvement was seen. She was ambulant four months after rituximab treatment. We considered chronic inflammatory demyelinating neuropathy as a probable adverse effect of etanercept.

Conclusions. Tumor necrosis factor inhibitors could elicit the demyelinating process, and chronic inflammatory demyelinating neuropathy might persist despite treatment discontinuation. First-line immunotherapy may be inefficient as in our case, and aggressive treatment may be necessary.

Key words: tumor necrosis factor inhibitors, etanercept, chronic inflammatory demyelinating neuropathy, adverse drug reaction.

Juvenile idiopathic arthritis (JIA) is an autoimmune disease affecting joints. Treatment is directed at suppressing inflammation that causes joint damage. First-line agents for the treatment of JIA are non-steroidal anti-inflammatory drugs and non-biologic disease-modifying anti-rheumatic drugs, such as methotrexate. In severe cases, biological agents are used as second-line drugs, with the most common being tumor necrosis factor (TNF) inhibitors, including infliximab, etanercept, and adalimumab.[1] However, demyelinating inflammatory disorders have been reported after the use of anti-TNF agents in post-marketing surveillance and case reports.[2] Demyelinating central nervous system disorders, including optic neuritis and multiple sclerosis, are the major reported events elicited by anti-TNF agents. Relatively less frequently, anti-TNF-associated neuropathies such as Guillain-Barré syndrome (GBS) and its variants, chronic inflammatory demyelinating neuropathy (CIDP) and its variants, multifocal motor neuropathy, and axonopathies have also been described.[3-6]

In this paper, we report a child, who developed CIDP in the course of JIA after etanercept

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withdrawal and discuss the causal relationship of TNF inhibitors with CIDP in view of the literature.

Case Report

A 12-year-and-nine-month old girl presented to our clinic with difficulty walking for the previous two weeks. She had a five-year history of arthritis and was followed up at our pediatric rheumatology department for the last year with a diagnosis of rheumatoid factor negative polyarticular JIA. The main affected joints at admission were the temporomandibular joints, wrists, elbows, knees ankles, and neck. She had joint contractures in the bilateral proximal interphalangeal joints. Her initial laboratory results showed a slightly elevated erythrocyte sedimentation rate (36 mm/hr). Antinuclear antibodies and rheumatoid factor were negative. The radiography of the affected joints showed chronic arthritic changes, periarticular osteoporosis, and narrowing of joint spaces. Initially, she was treated with a short course of prednisone (1 mg/kg/day methyl-prednisolone, followed by 0.2 mg/kg/day for two months) in addition to methotrexate (15 mg/week) and etanercept (0.8 mg/kg/week). She showed a good clinical response, and prednisone was stopped early in the course. She received etanercept for 10 months. However, one month after the withdrawal of etanercept, she presented with difficulty walking and gait instability. She had no history of a recent infection or vaccination. Her family history was also unremarkable. The physical examination revealed active synovitis in both knees and ankles. She had a wide-based waddling gait and weakness in the proximal and distal lower limbs [4/5 on the Medical Research Council (MRC) scale]. The patellar and achilles deep tendon reflexes were absent. Her upper extremity muscle strength was normal. According to the cerebrospinal fluid (CSF) analysis, her protein level was 205 mg/dl, glucose level was 66 mg/dl, white blood cell count was 0/ul, and red blood cell count was 0/ul. There was no oligoclonal band in the CSF analysis. The lumbosacral magnetic resonance imaging showed an increased thickness in the fibers of the cauda equina and diffuse contrast enhancement in the nerve roots. The electromyography (EMG) findings were compatible with acute-subacute sensorimotor demyelinating polyneuropathy. In the nerve conduction studies, there was no sensory nerve action potential in the upper and lower extremity. Other findings included absent F waves, prolonged distal latency of the median and ulnar motor nerves, temporal dispersion, slower motor conduction velocity, and absence of tibial and peroneal motor nerve action potentials. The patient received daily intravenous immunoglobulin (IVIG) (0.4 g/kg) for five days with the diagnosis of GBS. Clinical improvement was observed after the IVIG treatment. Three weeks later, her neurological symptoms worsened, she was unable to walk and the upper extremities were also involved. She was treated with plasma exchange. Although her weakness improved after the plasma exchange, she had relapses that gradually worsened during the follow-up, and she was eventually diagnosed with CIDP. EMG was repeated and showed chronic demyelinating sensorimotor polyneuropathy. In the third month, when she had a maximum disability, her MRC scale score was 3/5 for the upper extremity and 1/5 for the lower extremity. She had mild exotropia and diplopia, but no bulbar involvement. She was treated with monthly IVIG, steroid treatment (1 g methylprednisolone for 5 days, followed by 1 mg/kg methylprednisolone for 3 months, and monthly pulse steroid for 6 months) with minimal response, and plasma exchange with partial response. She also had findings of active arthritis of the wrists and small joints of her hands under methotrexate and hydroxychloroquine treatment. For both refractory CIDP and JIA, rituximab treatment was started at a dose of 375 mg/m^2 weekly for two weeks at the sixth month of symptom onset. She started to improve after three months and began to walk independently after four months of rituximab therapy. For 9 months she was completely immobile. Rituximab was repeated six months after the first dose, followed by monthly IVIG for one year. At the last follow-up, when she was 14

years old, she was walking independently and her muscle strength was normal, except for slight weakness in the bilateral knee extensor and foot dorsiflexor muscles (MRC scale score +4/5). Her biceps reflexes were hypoactive, and patellar and Achilles reflexes were absent. She had no arthralgia or arthritis. She was receiving methotrexate and hydroxychloroquine for JIA.

Informed consent was obtained from the family.

Discussion

Peripheral neuropathies may be seen in the course of autoimmune diseases due to vasculitis, nerve entrapment, nutritional imbalances, or drug toxicity.[4] Autoimmune diseases may coexist in a patient; however, JIA and rheumatoid arthritis are not typically associated with peripheral neuropathy. In the literature, all reported CIDP cases among patients with rheumatoid arthritis or JIA are associated with the use of TNF inhibitors. When we applied the Naranjo algorithm, also known as the Adverse Drug Reaction Probability Scale (definite, over 9; probable, 5-8; possible, 1-4; and doubtful, below 0) to investigate the relationship between CIDP and etanercept treatment in our patient, we determined the score as 7, indicating probable adverse drug reaction.[7]

TNF-α inhibitors are used in several advanced inflammatory diseases, including JIA, rheumatoid arthritis, psoriasis, and inflammatory bowel diseases.[8] TNF inhibitors are known to cause demyelinating disorders both in the central nervous system and peripheral nervous system. In a French national survey, 33 patients developed demyelinating disorders in a period of three years after a median of 10.2 months of treatment, and 22 patients had central nervous system involvement and 11 had peripheral nervous system involvement. In that series, there was only one child, a 13-year old girl diagnosed with JIA, who developed optic neuritis after etanercept treatment.[9] In Switzerland, of the 2,017 patients treated with TNF inhibitors, 12 developed associated neuropathy, with the prevalence being calculated as 0.60%.[10] Peripheral neuropathies reported secondary to the use of TNF inhibitors include GBS, Miller Fisher syndrome, multifocal motor neuropathy, CIDP, Lewis-Sumner syndrome (a variant of CIDP), and axonopathies.[3,9,10] Yagita et al.[3] reviewed 60 patients with peripheral neuropathies, who were receiving biological therapy. The duration of therapy prior to the onset of neuropathy ranged from eight hours to five years. The majority of the patients (47/60) had demyelinating neuropathy. The outcome was favorable in most patients, and the discontinuation of the agent resulted in spontaneous resolution in most reported patients. In the literature, nearly all the published data belong to adult patients. We found only one case report in the pediatric age group, in which Alqurashi et al.[11] described an eight-year-old girl with polyarticular JIA, who developed demyelinating peripheral neuropathy a few months after etanercept withdrawal. She was treated with IVIG and abatacept. This is similar to our patient whose symptoms also started after the withdrawal of etanercept. To our knowledge, this is the second pediatric case that developed CIDP after TNF inhibitor treatment.

The underlying mechanism of demyelinating neurologic diseases in patients treated with TNF inhibitors needs to be elucidated. Both humoral and cellular immune mechanisms are implicated in the pathogenesis. TNF-α is a cytokine with both pro-inflammatory and immunoregulatory properties. In the peripheral immune system, TNF-α plays important roles as antigen-presenting cells and in the regulation of the apoptosis of auto-reactive T cells. Paradoxically, TNF-α is also involved in the pathogenesis of inflammatory demyelinating neuropathies. Serum TNF- α levels are increased in a subgroup of GBS and CIDP cases during the active disease and decreased after immunotherapy with clinical recovery.[8,12] Bosch et al.[2] reported that chronic TNF inhibition might increase the anti-myelin

immune response through the activated T lymphocytes. Nerve ischemia and inhibition of signaling support for axons have also been proposed as mechanisms for secondary axonal loss.[8] Demyelinating disorders may persist even after the withdrawal of the offending agent, suggesting that after TNF inhibitors trigger the demyelinating process, the disease progresses independently of the eliciting agent.[9]

In our case, the treatment plan was supposed to cover not only CIDP but also JIA. We preferred rituximab, a monoclonal anti-CD20 antibody, for refractory CIDP in our patient. Rituximab is an alternative treatment for both JIA and refractory CIDP disease.[13,14] It is shown to be effective in CIDP patients with hematological or autoimmune diseases.[15] Rituximab was effective for both CIDP and JIA in our patient, indicating that humoral mechanisms may be important in the pathogenesis of TNF inhibitor-associated neuropathy.

CIDP may be a rare adverse event in children treated with TNF inhibitors. Neuropathy symptoms may start after the withdrawal of the offending agent, and patients may be unresponsive to first-line therapy, including steroids and IVIG, necessitating further immunosuppressive treatment.

Ethical approval

Informed consent was obtained from the family.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: HMG, BK, BS; data collection: HMG, BK, RS, HGS, BS; analysis and interpretation of results: HMG, BK, BS; draft manuscript preparation: HMG, BK, RS, HGS, BS. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

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Case Report

Mercury exposure mimicking systemic lupus erythematosus in a thirteen-year-old girl

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ABSTRACT

Background. The clinical presentation of mercury (Hg) intoxication may mimic rheumatic diseases. Hg exposure is associated with systemic lupus erythematosus (SLE)-like disease in genetically susceptible rodents and Hg is among the environmental factors in the development of SLE in humans. Herein, we presented a case with clinical and immunological features suggestive of SLE but diagnosed with Hg intoxication.

Case. A thirteen-year-old female with myalgia, weight loss, hypertension and proteinuria was referred to our clinic for the evaluation of possible SLE. Physical examination of the patient was unremarkable except for a cachectic appearance and hypertension, laboratory investigation revealed positive anti-nuclear antibody, dsDNA antibody and hypocomplementemia with nephrotic range proteinuria. Inquiry for toxic exposures revealed a continuous exposure to an unknown silverly shiny liquid for a month which was thought to be Hg. Due to the fulfillment of Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE, a percutaneous kidney biopsy was performed whether proteinuria resulted because of the Hg exposure or flare of lupus nephritis. Blood and 24-hour urine Hg levels were high, and no findings associated with SLE were observed in the examination of the kidney biopsy. The patient was diagnosed with Hg intoxication and, clinical and laboratory findings, including hypocomplementemia, positive ANA and anti-dsDNA antibody, improved with chelation therapy. Also, no findings associated with SLE were observed in the follow-up of the patient.

Conclusions. In addition to the toxic effects, Hg exposure may cause autoimmune features. As far as we know, this is the first-time Hg exposure was associated with hypocomplementemia and anti-dsDNA antibody in a patient. Also, this case highlights the inconvenience of the use of classification criteria for diagnostic purposes.

Key words: autoimmunity, mercury, nephritis, systemic lupus erythematosus.

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder characterized by autoantibody development with marked heterogeneity between presentations. SLE develops through the effects of epigenetic, immunomodulatory, hormonal and environmental factors in genetically susceptible individuals.[1] Air pollution, ultraviolet light exposure, infections, vaccinations, pesticides, and heavy metals are among the several possible environmental factors.[2]

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Mercury (Hg) is a heavy metal with a shiny white-silver appearance which has toxic properties affecting both humans and the environment. Gold mining, dental amalgams, thermometers, and other measuring devices are sources of elemental (metallic) Hg exposure and the primary route of exposure is through inhalation. Elemental Hg has toxic effects on the nervous system, lungs, kidneys, and skin.[3]

Besides toxic effects, Hg may induce autoimmunity. It has been shown that Hg exposure is associated with the development of anti-nucleolar antibodies and lupus-like autoimmune disease in genetically susceptible rodents. Also, Hg triggers the production of

pro-inflammatory mediators in humans. It might have a role in development of several autoimmune diseases like scleroderma, multiple sclerosis, membranous nephropathy and SLE.[4]

Herein, we report a pediatric patient with Hg poisoning, referred to our department with a possible diagnosis of SLE. The relationship between Hg and autoimmunity has also been briefly reviewed.

Case Report

A thirteen-year-old female with myalgia, weight loss, hypertension and proteinuria was referred to our clinic for the evaluation of a possible rheumatic disease. She had developed myalgia about a month ago, which got worse over time and had lost five kilograms in that process. She had neither arthritis nor morning stiffness, and her pain was not responsive to non-steroid anti-inflammatory drugs or gabapentin. She suffered from a rash on her fingers at the time of the occurrence of myalgia but this resolved spontaneously in two days. She had no other complaints, including fever, photosensitivity, malar rash, oral/nasal ulcers, alopecia, and Raynaud's phenomenon. Her family history was negative for rheumatic diseases. Her weight was 35 kg (< 3rd centile) with a height of 162 cm (50th-75th centiles). Her blood pressure was high (150/100 mmHg) with mild tachycardia (110/min). Respiratory rate, body temperature and oxygen saturation were within normal limits. Her physical examination was unremarkable except for a cachexic appearance and generalized myalgia. Laboratory findings, including hemoglobin, lymphocyte, leukocyte, and thrombocyte indices, were within normal ranges and acute phase reactants were not elevated. Urinalysis revealed a proteinuria of 100 mg/dL with a density of 1.010 without hematuria. Despite having nephrotic range glomerular proteinuria (44 mg/m^2/hr), serum albumin (38 g/L) and creatinine (0.38 mg/dL) levels were within normal limits. Serum level of complement C3 was low (0.64 g/L) with a normal complement C4 (0,1 g/L) level. Anti-nuclear antibody (ANA) was positive, with a titer of 1/320 and stained as nucleolar pattern and anti-double stranded DNA (dsDNA) antibody levels were high (260 IU/mL, normal range < 100 IU/mL) with a normal screening result for extractable nuclear antigen antibodies. She fulfilled the Systemic Lupus Erythematosus International Collaborating Clinics (SLICC) classification criteria for SLE[5] with proteinuria as a clinical criterion and hypocomplementemia, positive ANA and anti-dsDNA antibody as laboratory criteria. A calcium channel blocker and angiotensin converting enzyme inhibitor were required to control her hypertension. She was started on prednisolone (40 mg/day) and a percutaneous kidney biopsy was planned for possible lupus nephritis. On the second day of admission, we found out that her 16 year old brother was also admitted to the hospital for myalgia in his legs. Due to the presence of myalgia in two individuals from the same household, a more detailed inquiry for toxic exposures was made. It was found out that she had been playing with an unknown silvery shiny liquid which was thought to be Hg and kept it under her pillow and had slept with it for over a month. Hg analysis in blood and urine were studied for suspected Hg intoxication. Percutaneous kidney biopsy was performed to identify whether nephrotic range proteinuria resulted from Hg exposure or a flare of lupus nephritis. Light microscopic changes were consistent with acute tubular necrosis and membranous nephropathy, but no immune complex deposition was observed in immunofluorescence microscopy. Hg levels in 24-hour urine were high (520 μg/L, normal range < 10 μg/L) and all the findings in the renal biopsy were attributed to Hg intoxication. Chelation therapy with dimercaptosuccinic acid (DMSA) was initiated with the tapering of prednisolone therapy. DMSA was given at a dose of 30 mg/kg per day for five days followed by a dose of 20 mg/kg/day for 14 days. Due to the high urinary Hg levels (242 μg/L) two weeks after the

first course of treatment, chelation therapy was continued with two cycles of treatment each lasting 7 days at a dose of 20 mg/kg/day. Her brother was also admitted to another institution with a diagnosis of Hg poisoning with mild clinical findings. Her mother, who shared the same bed with our patient, also presented with myalgia and proteinuria (1.9 gr/day). She was also diagnosed with Hg poisoning with elevated urinary Hg level (215 μg/L) and her biopsy findings were consistent with membranous glomerulonephritis. Myalgia in our patient recovered in two weeks, hypertension resolved in the first month of treatment, and she started to gain weight at the end of the first month. Complement levels also normalized with negative ANA and anti-dsDNA antibody in a month after chelation therapy. It took two months of chelation therapy to achieve an acceptable range of urinary Hg level (<20 μg/L). Despite the resolution of hypertension, angiotensin converting enzyme inhibitor therapy was continued for its anti-proteinuric and renoprotective effects. Proteinuria gradually decreased and resolved completely at the sixth month follow up and no complaints or findings associated with SLE were observed.

The patient and her legal guardian gave verbal and written consent for publishing this case report.

Discussion

Diagnosis of SLE should be kept in mind, especially in patients with multisystemic autoimmune disease involvement, but no gold standard test exists for its diagnosis. Constitutional symptoms, myalgia, and proteinuria along with positive ANA, anti-dsDNA antibody and hypocomplementemia, in our patient were consistent with SLE. SLICC 2012 criteria could aid referral or consultation to pediatric rheumatology but it is important to remember that these criteria were developed for classification and not for diagnosis.[6] As seen in our case, non-rheumatic diseases may mimic SLE and even fulfill SLICC criteria.

Even though our case was not SLE, Hg exposure is regarded as an environmental factor in the development of the disease.[2] In a study with 265 SLE patients, self-reported Hg exposure was associated with SLE development with an odds ratio of 3.6.[7] Additionally, Dahlgren et al.[8] reported a 20 time increased risk for SLE in a population living in an oil waste site, which had higher ambient air Hg levels.[8] In an experimental study, methyl-Hg exposure of peripheral blood monocytes resulted in an increased pro-inflammatory response in patients with SLE when compared with healthy controls.[9] But, in a study with 53 SLE patients, Hg exposure was not associated with increased disease activity or damage.[10] Besides being an environmental factor for SLE development, Hg exposure was linked with an increased prevalence of ANA and anti-nucleolar antibodies in selected populations.[11] Also in a study with gold miners, exposure to Hg was associated with increased concentrations of pro-inflammatory cytokines besides the increased prevalence of ANA.[12] Our case had low levels of C3 and positive anti-dsDNA besides positive ANA. As far as we know, there is no report of hypocomplementemia and positive anti-dsDNA antibody in association with Hg exposure in the literature. Owing to the recovery of hypocomplementemia and disappearance of dsDNA antibody on chelation therapy, concomitant SLE was ruled out. Because of the immune effects of Hg exposure, it was thought that these findings might be secondary to Hg exposure.

In animals expressing H-2^s haplotype, Hg exposure is associated with systemic autoimmunity. These animals are susceptible to autoimmunity only with an environmental factor. Anti-nuclear, anti-glomerular basement membrane, anti-dsDNA and anti-fibrillarin antibodies were among the autoantibodies observed in animal models of Hg exposure and these antibodies are also known to be associated with human diseases.[10] Hg exposure causes oxidative stress and induces alterations in mitochondrial function resulting in reactive oxygen species production and apoptotic

signal activation in human T lymphocytes.[13] Mitochondrial dysfunction and associated aberrant lymphocyte behavior, increased apoptosis and redox imbalance might also contribute to the immunopathology of SLE.[14]

Elemental Hg is highly lipophilic and rapidly distributed throughout the body, and exposure may result from several reasons. However, elemental Hg exposure is rare in most developed countries and occurs accidentally.[3] Our patient found a bottle that was filled with Hg in a schoolyard. Thus, we thought that exposure was welded by laboratory equipment. Proper storage and disposal of such laboratory equipment are important to avoid accidental exposures.

Proximal tubules are the most sensitive part of the kidney to the toxic effects of Hg exposure. With higher concentration of exposure, more distal parts of the nephrons might also be affected. Accumulation of Hg in proximal tubular cells induces oxidative stress, which might result in acute tubular necrosis.[15] Hg exposure is also associated with glomerular changes. It was suggested that high concentrations of Hg directly damage the podocytes and causes minimal change disease while long-standing exposure to low concentration of Hg may cause membranous glomerulonephritis through an immune mechanism.[16] A summary of the toxic and immunologic effects of Hg exposure is shown in Fig. 1. In most of the in-vitro studies investigating the effects of Hg exposure on the immune system, higher Hg concentrations than expected in the general population were used.[10] Therefore, the results of these studies may not reflect the true effects of Hg exposure.

Symptoms and findings of Hg exposure like pain, weight loss, hypertension and proteinuria could be seen in association with systemic rheumatic diseases and the occurrence of these findings may cause the referral to pediatric rheumatology like our case. Two case series from Turkey reported a total of 12 pediatric cases with Hg poisoning, referred to pediatric rheumatology. In those studies, weight loss and extremity pain were seen in eight and nine out of 12 patients, respectively. Interestingly, none of those cases had positive ANA.[17,18] Our case had hypocomplementemia and positive dsDNA antibody together with ANA positivity. Continuous long-term exposure to Hg over a month could explain those findings, which was the major difference in our patient than the reported cases above.

In conclusion, symptoms and findings of Hg poisoning may mimic rheumatic diseases. Hg may induce autoimmunity and cause autoantibody production; thus misdiagnosis is possible, especially when classification criteria

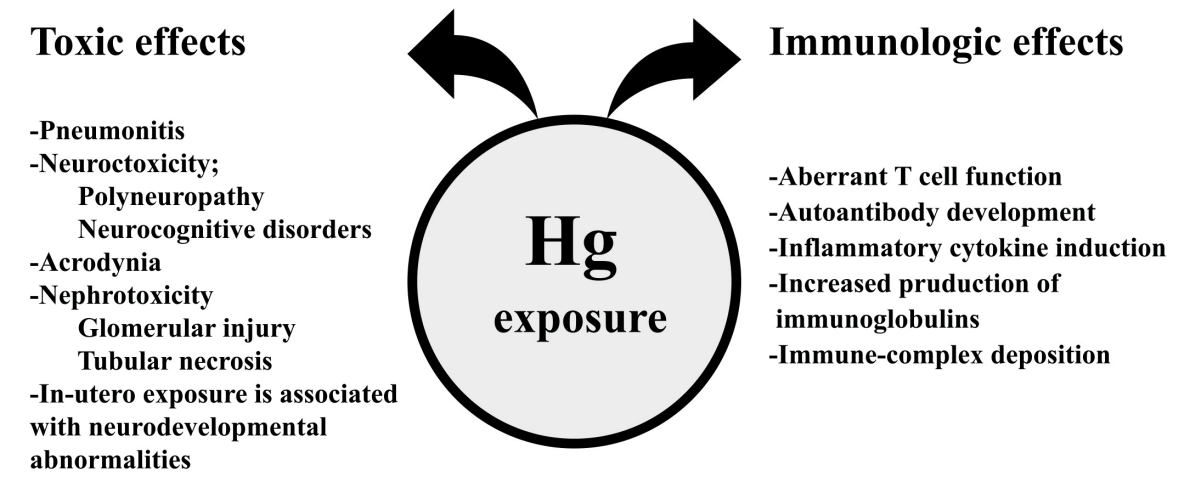

Fig. 1. Summary of toxic and immunologic effects of mercury (Hg) exposure.[3,10]

are used for diagnostic purposes. To the best of our knowledge, this is the first case of Hg intoxication mimicking SLE that even fulfilled the SLICC classification criteria. This case also highlights the importance of investigation for toxic exposures, when more than one person in the same household present with similar symptoms.

Ethical approval

The patient and her legal guardian gave verbal and written consent for publishing of this case report.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: HK, OB; data collection: HK; analysis and interpretation of results: HK, OB, MK; draft manuscript preparation: HK. 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

Self-inflicted intravesical insertion of 83 magnetic balls in a 10-year-old boy: a case report and literature review

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ABSTRACT

Background. A magnetic ball is a toy for children that can cause physical injury when used improperly. The injury of urethra and bladder caused by magnetic ball is rarely reported.

Case. Here we present a case of self-inflicted intravesical insertion of 83 magnetic balls by a 10-year-old boy. Preliminary diagnosis was made by a plain radiograph of the pelvis and ultrasonic examination of bladder and all the magnetic balls were removed under cystoscopy successfully.

Conclusions. For children with recurrent bladder irritation, the possibility of bladder foreign body should be considered. Surgery is an effective method. For patients without serious complications, cystoscopy is the gold standard for diagnosis and treatment.

Key words: self-inflicted, intravesical, magnetic balls, children.

A magnetic ball is a common toy for children that can cause physical injury when used improperly. The most common clinical injury is accidental ingestion of magnetic balls, but the injury of urethra and bladder is rarely reported.[1,2] Here we present a case of self-inflicted intravesical insertion of 83 magnetic balls by a 10-year-old boy. Through the diagnosis and treatment of this case, we hope to provide experience for diagnosis and treatment of self-inflicted intravesical magnetic balls in children.

Case Report

A 10-year-old boy was admitted to the pediatric surgery clinic for recurrent urinary tract infection for one month. The patient was a student and appeared mentally sound who denied any history of surgery or trauma. The family history was negative for urinary tumor and malformation.

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Physical examination showed no abnormalities. The urine routine showed elevated red blood cells (+++) and white cells (++). The laboratory testing showed elevated white blood cells (white blood cell count 14.7×10^9/L ↑, neutrophil count 9.8×10^9/L ↑, hemoglobin 12.5 g/dL, platelet count 255×10^9/L). Liver function, kidney function, cardiac enzymes and electrolytes were normal. Bladder color ultrasound showed a foreign body in the bladder, but no abnormalities such as perforation or diverticulum. The foreign body was irregular in shape, about 4 x 3 cm in size, and could move with the change of patient's posture. The plain radiograph of the pelvis showed a metallic dense foreign body that was composed of many small balls in the pelvic region (Fig. 1).

Cystoscopy was performed under general anesthesia and dozens of magnetic balls were found in the bladder (Fig. 2). All 83 magnetic balls were successfully removed with foreign body forceps under cystoscopy (Fig. 2). Mostly, a magnetic ball could be removed one at a time, yet occasionally several or more than ten magnetic balls could be removed at a time due to the balls arranging in a line. Because the

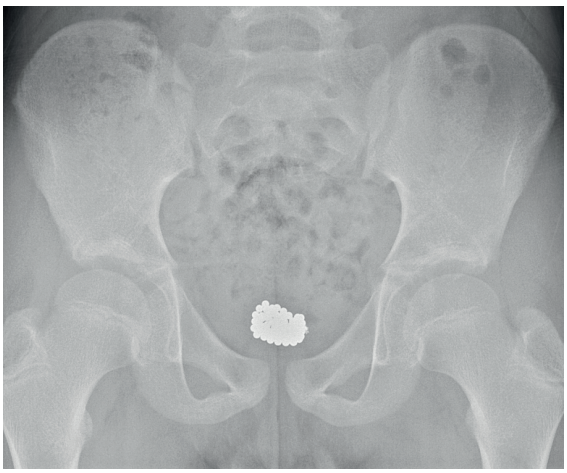

Fig. 1. Image of Pelvic X-ray. Pelvic X-ray showed a metallic dense foreign body that was composed of many small balls in the pelvic region.

cystoscope was used repeatedly, edema and bleeding occurred in the urethra, and a catheter was inserted after the cystoscopy.

The patient was discharged smoothly after 3 days. No complications occurred after 1-year follow-up. Informed consent was obtained from his parents for publication and photographs.

Discussion

Multiple magnetic balls in the bladder of children is rarely reported.[3] We searched previous literature with "bladder", "magnetic", "foreign body" and "children" as keywords, and a total of 5 cases were retrieved (Table I).[2-5] In one patient alone reported in the literature had the magnetic balls successfully removed under cystoscopy. Cystotomy was performed in 2 cases due to difficulty in removing foreign bodies under cystoscope and open cystolihtotomy was planned in 1 case because of the size of the foreign body. Transurethral surgery was performed in 1 case because of urethrocutaneous fistula. We successfully removed all magnetic balls under cystoscope in this case, and our case had the largest number of magnetic balls among all reported cases.

Most likely due to a feeling of shame or fear, patients often do not dare to tell the truth until complications occur. The case we report denied self-inflicting the foreign bodies at the initial diagnosis of bladder foreign body. As children may insert foreign bodies into the urethra

Table I. Case reports of magnetic balls in urethra or bladder in children.

Authors	Time	Age	Sex	Symptoms	Number	Location	Treatment
Ellimoottil C, et al.[2]	2013	11 y	male	acute onset of gross hematuria and difficulty voiding	24	bladder and urethra	offset pediatric cystourethroscope
Özdemir T, et al.[3]	2017	3 y	male	intermittent hematuria and dysuria	4	bladder	open cystolihtotomy
Kinjo T, et al.[4]	2019	14 y	male	fever, left scrotal pain and urinary incontinence	24	bladder	transurethral surgery
Gibson E, et al.[5]	2018	11 y	male	hematuria	16	bladder	cystoscope and cystotomy
Gibson E, et al.[5]	2018	18 y	male	dysuria and gross hematuria	about 40 to 50	bladder and urethra	cystoscope and cystotomy
Chao L, et al. (this study)	2023	10 y	male	recurrent urinary tract infection	83	bladder	cystoscope

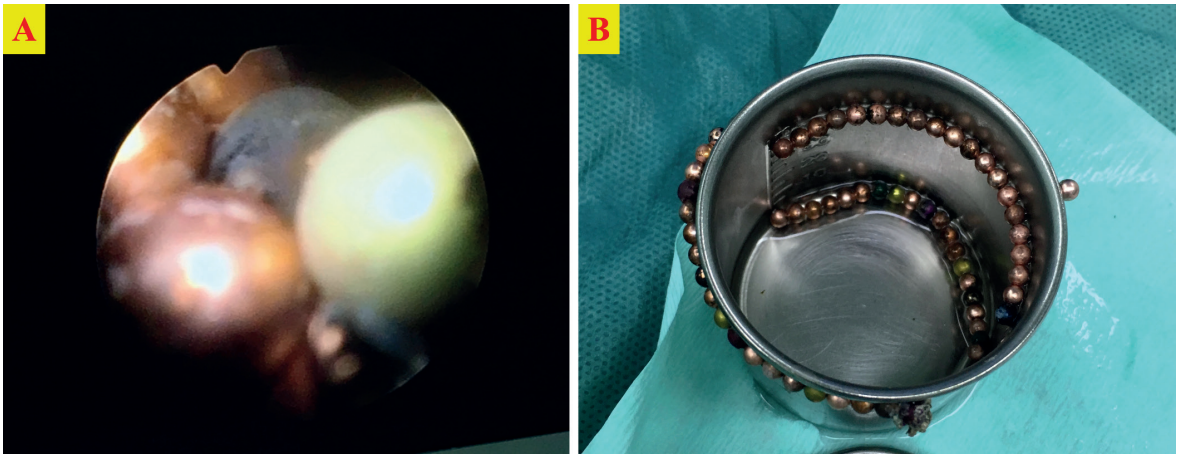

Fig. 2. Images of magnetic balls. (A) Cystoscopy showed dozens of magnetic balls in the bladder. (B) All the magnetic balls were removed successfully.

due to sexual curiosity or psychiatric illness, postoperative psychological consultation is necessary.[5]

Foreign bodies in the bladder are characterized by urinary retention, urinary tract infection, urethral or abdominal pain, hematuria.[2] The plain radiograph and color ultrasound of the pelvis are mostly used for diagnosis. While considering bladder diverticulum or perforation, computed tomography is also necessary.

Treatment of a foreign body in the bladder depends on the shape, size, sharpness and complications such as bladder perforation. The purpose of the operation is to remove the foreign body with minimal morbidity and damage to the urethra and bladder. Vesicotomy is the safest way in the case of perforation of bladder or difficulty in removal of foreign body through the urethra.[6] For patients without serious complications, cystoscopy is the first choice for diagnosis and treatment.

There may be some difficulties in the treatment of magnetic balls in pediatric bladders under cystoscopy. Firstly, the urethra is too narrow to operate smoothly. Secondly, it is difficult to separate one from a pile of magnetic balls because of the magnetic force.[7] Thirdly, metal foreign body forceps can also be pulled by the magnetic balls, making it difficult to grasp the magnetic ball accurately. Lastly, most of the time we can only take out one ball at a time. In the case of multiple magnetic balls, cystoscopy is required repeatedly which may cause injury of the urethra. In the case we reported, urethral edema and bleeding occurred, but no complications such as urethral stricture occurred.

The possibility of bladder foreign bodies should be considered in children with recurrent bladder irritation. Surgery is an effective method. Cystoscopy is the gold standard for diagnosis and treatment in patients without serious complications.

Ethical approval

This study was approved by the Ethics Committee of Shandong University Qilu Hospital (Qingdao) [KYLL-KS-2021031].

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: LC, LX; data collection: LC, LX, ZQ; analysis and interpretation of results: LC, ZL; draft manuscript preparation: LC. 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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NOTICE TO AUTHORS

Instruction for authors

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Covering Letter: The covering letter is important to help the Editor in his/her preliminary evaluation, please indicate why you think the paper is suitable for publication. A statement that each author listed on the manuscript has seen and approved the submission of this version of the manuscript and takes full responsibility for the manuscript must be provided. The covering letter will be uploaded as a separate file.

Title Page: A separate title page should include authors' names and academic degrees; departmental and institutional affiliations of each author, and sources of financial assistance, if any. Designate one author as the correspondent, and provide an address as well as business and home telephone numbers. Galley proofs and order forms for reprints will be sent to the corresponding author, at a later date. On the title page please add a short running title, word count (excluding abstract and references), number of tables and figures, ethical approval, funding, conflict of interest, authors contribution.

Types of Articles:

Original Article: Original articles should report scientific findings within the pediatric field of interest. They must include a structured abstract of less than 300 words, with the following headings: background, methods, results and conclusions to appear after the title page. The manuscript should be structured with the following headings: introduction, materials and methods, results, and conclusions. The introduction should put the study in context with the current literature and reflect the purpose of the study. The materials and methods should include the study methodology, the setting for the study (although affiliation must be covered), the subjects (number and type), the treatment or intervention, principal outcomes measured, and the type of statistical analysis. The results section should include the outcome of the study and statistical significance, if appropriate. The conclusion(s) states the significance of the results and limitations of the study. The manuscript should be no longer than 3,500 words.

Case Reports: Case reports should contain accounts of rare syndromes, new diagnostic tools and methods, new kinds of treatment and laboratory research with foreseeable practical application. Case reports should consist of a structured abstract with the following headings: background, case and conclusions that summarizes the case(s), a brief introduction, a section that details patient presentation, initial diagnosis and outcome, as well as a discussion that includes a brief review of the relevant literature and describes how this case brings new understanding to the disease process. The number of authors is limited to 6 for case reports. If more than 6 authors have had a significant contribution defined by the International Committee of Medical Journal Editors (ICMJE) then the authors may contact the editorial office to request an increase.

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Structured manuscript for systematic reviews are recommended. Headings should include: Structured abstract with the following headings: background and objectives, material and methods results and conclusions, Introduction, Data Sources, Study Selection, Data Extraction, Results, Limitations, and Conclusions. The general instructions regarding submission (including cover letter, title page requirements, journal style guidance, and conflict of interest statements) also apply to Review Articles.

Short communication: Short communications are opinion pieces consisting of a main point and supporting discussion. They may address general issues or controversies in the field of pediatrics. No abstract is needed but the general submission instructions (including cover letter, title page, journal style guidance, and conflict of interest statements) also apply to short communications.

Clinicopathological exercises: Authors can submit for consideration an clinicopathological exercises that is interesting, instructive, and visually attractive, along with a few lines of explanatory text and references. No abstract, discussion or conclusion are required but the general submission instructions (including cover letter, title page, journal style guidance, and conflict of interest statements) also apply to clinicopathological exercises.

Image of the month: Authors can submit for consideration a striking image that is interesting, instructive, and visually attractive, along with a few lines of explanatory text and references. Images can be either clinical, pathologic (gross or microscopic), endoscopic, or radiographic. They must be of high quality (300 PPI resolution) and illustrate the diagnosis well. No abstract, discussion or conclusion are required but the general submission instructions (including cover letter, title page, journal style guidance, and conflict of interest statements) also apply to the Image of the month.

Letters to the Editor: Letters to the Editor should pertain to papers published in The Turkish Journal of Pediatrics within the past year. Provide a unique title for the Letter on the title page with complete contact information for the author(s). References, including reference to the pertinent article(s) in The Journal, should conform to style for manuscripts. No abstract is needed for a letter to editor. Letters to the Editor may be sent to the authors of the original article to which the Letter was written for review and/or Reply. If the Editors choose to publish the Reply, it will be published in the same issue as the Letter to the Editor.

Summary / Abstract and key words: An informative structured abstract not exceeding 300 words must accompany each manuscript, Unexplained abbreviations and references should not be given. The abstract should state the objective (the purpose of the study and hypothesis) and summarize the rationale for the study. It is critically important that all significant findings should be presented in the abstract. The abstracts should be structured with the following headings for an original article: Background, Methods, Results and Conclusions and Background, Case Report, Conclusion for a case report.

The authors should list three to five key words or phrases taken from Index Medicus

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