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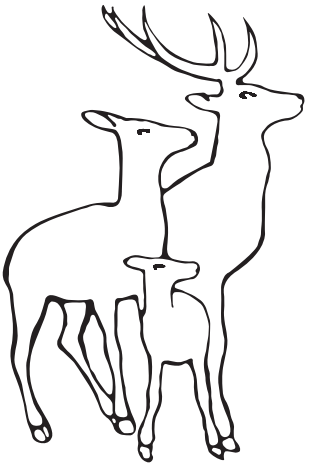

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Original Article

Association between serum vitamin A, D and E status and respiratory distress syndrome in preterm infants – a propensity score matching analysis

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ABSTRACT

Background. This study aimed to assess whether the serum levels of vitamin A, vitamin D and vitamin E are associated with respiratory distress syndrome (RDS) in preterm infants.

Methods. This retrospective research included 179 neonates born before 35 weeks of gestation in Jiaxing Maternity and Child Health Care Hospital from January 2020 to December 2020. Depending on whether or not they had RDS, participants were classified into the RDS group (59 neonates) and the control group (120 neonates). The 1:1 propensity score matching (PSM) analysis was performed to balance the baseline confounding factors and then the groups were compared in terms of serum vitamin levels and RDS morbidity.

Results. A total of 34 pairs of preterm infants were involved after PSM. There were significant differences in vitamin D level (12.13 (8.44-17.85) ng/mL vs. 16.84 (10.75-25.83) ng/mL), vitamin D deficiency rate (85.3% vs. 55.9%), as well as vitamin A level (134.91 (105.01-156.74) ng/mL vs. 152.46 (120.06-200.00) ng/mL) in the two groups. However, the vitamin A deficiency rate, vitamin E status, as well as vitamin E deficiency rate did not differ significantly between the two groups. Logistic analysis showed that a low level of vitamin D was an independent risk factor for RDS in preterm neonates (*OR 0.917, 95%CI 0.851-0.989*).

Conclusions. Low serum vitamin D levels may contribute to the development of RDS in preterm infants, but no significant effect of serum vitamin A and vitamin E levels was found.

Key words: vitamin A, vitamin D, vitamin E, respiratory distress syndrome, propensity score matching.

Respiratory distress syndrome (RDS) is known as a major complication in preterm infants and also one of the important causes of neonatal death, with structural immaturity of lung development and deficiency of pulmonary surfactant (PS) being key factors in its pathogenesis.[1] Vitamin A (Vit A), vitamin D (Vit D) and vitamin E (Vit E) are all fat-soluble vitamins whose metabolites are important ligands for several transcription factors and are involved in various biological processes in the body.[2,3] It has been shown that Vit A, Vit D and Vit E can play essential roles in the growth and development, metabolism, and immune regulation of the body.[4] Some previous studies have shown a positive effect of Vit D on neonatal respiratory diseases[5-7], but there are few systematic studies that focus on the relationship between fat-soluble vitamins and RDS in preterm neonates. In this study, we investigated the relationship between serum Vit A, Vit D and Vit E status and RDS in preterm infants by using the propensity score matching (PSM) method to balance the confounding factors between subgroups, with the aim of providing a basis for early prevention and treatment of RDS in preterm neonates.

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Material and Methods

Group setting and data collection

According to the inclusion and exclusion criteria, preterm infants hospitalized in the neonatal intensive care unit of Jiaxing Maternity and Child Health Care Hospital from January 2020 to December 2020 were selected, in which those diagnosed with RDS were admitted to the RDS group while others without RDS were admitted to the control group.

This research was approved by the Medical Ethics Committee of Jiaxing Maternity and Child Health Care Hospital (No. 2020-3).

Inclusion criteria: ① gestational age at birth ≤ 35 weeks; ② admission within 24 hours after birth and complete serum Vit A, 25-hydroxyvitamin D (25(OH)Vit D) and Vit E determination; ③ informed consent from guardians; ④ complete clinical case data.

Exclusion criteria: ① congenital genetic metabolic diseases or malformations; ② combined with liver and kidney diseases or thyroid function abnormalities; ③ maternal presence of liver and kidney function abnormalities, thyroid function abnormalities, bone metabolic diseases or anticonvulsant, epilepsy, tuberculosis drugs during pregnancy.

Maternal and neonatal characteristics were collected from the medical record database, including single or twin gestation, sex, birth weight, gestational age (GA), season of birth, mode of delivery, Apgar scores at 1 and 5 min, maternal age, maternal body and mass index (BMI), antenatal steroid use, presence of premature rupture of membranes (PROM), gestational hypertension, as well as gestational diabetes mellitus (GDM). Serum concentrations of Vit A, 25(OH)Vit D and Vit E of preterm infants were determined from peripheral blood samples taken within 24 hours of life and measured by mass spectrometry.

Diagnostic criteria

Neonatal RDS was diagnosed using the 2017 edition of the Montreux definition.[8] Vit A <200 ng/mL was defined as Vit A deficiency, 25(OH) Vit D <20 ng/mL as Vit D deficiency, and Vit E <5 μg/mL as Vit E deficiency in accordance with the relevant association standards.[9,10]

Statistical analysis

All the data were analyzed by SPSS software (SPSS Inc., Chicago, IL) version 22.0.

Logistic regression was used for propensity score calculation from baseline patient characteristics. Matching based on propensity scores incorporating different sets of covariates was performed using a 1:1 nearest-neighbor algorithm, with a caliper width of 0.1. For continuous variables, data that matched normal distribution were expressed as mean ± standard deviation and compared by using the Student's t-test, while non-normally-distributed data were presented as medians (interquartile range) and compared using Wilcoxon test. Categorical data were expressed as number (percentage) and the chi-square test was used for comparison. It should be noted that paired t-test and McNemar's test were applied for comparison of continuous and categorical variables respectively after PSM. Variables with a p-value < 0.05 in the univariate analysis were selected for the multivariable analysis. Binary logistic regression analysis was used to investigate whether vitamin levels were independently associated with the occurrence of RDS. A p-value of <0.05 was considered statistically significant.

Results

Comparison of maternal and neonatal characteristics between the two groups before and after PSM

A total of 179 preterm infants who met the selection criteria were enrolled in this study.

Of them, 59 were admitted to the RDS group, whereas 120 were admitted to the control group. After PSM in a 1:1 ratio, 34 pairs of neonates were successfully matched and included for analysis. The baseline characteristics of the patients before and after PSM were summarized in Table I.

Before PSM, the between-group differences in single fetus (*P=0.001*), sex (*P=0.006*), birth weight (*P=0.000*), gestational age (*P=0.000*), Apgar score at 1 min (*P=0.000*) and 5 min (*P=0.000*), as well as the incidence of PROM (*P=0.048*) and gestational hypertension (*P=0.029*) were significant. After PSM, all p-values between groups were greater than 0.1 for the comparison of the listed maternal and neonatal characteristics.

Comparison of serum Vit A, 25(OH)Vit D, and Vit E concentrations in the two groups after PSM

The serum concentrations of Vit A and Vit D in the RDS group were 134.91 (105.01-156.74) ng/mL and 12.13 (8.44-17.85) ng/mL respectively, which were significantly lower than those in the control group. Moreover, the Vit D deficiency rate in the RDS group was observed as 85.3%, which was higher than that in the control group, with statistically significant differences (*P<0.05*). However, no between-group significant differences were observed in the comparison of Vit A deficiency rate, serum Vit E level, and Vit E deficiency rate (*P>0.05*). For details, see Table II.

Table I. Comparison of baseline characteristics between the two groups before and after PSM.

baseline characteristics	before PSM				after PSM			
	RDS group (n=59)	control group (n=120)	$t/Z/\chi 2$	*P*	RDS group (n=34)	control group (n=34)	*t/Z*	*P*
single fetus, n (%)	43 (72.9%)	56 (46.7%)	10.996	0.001	24 (70.6%)	29 (85.3%)	-	0.227
male, n (%)	38 (64.4%)	51 (42.5%)	7.593	0.006	17 (50.0%)	17 (50.0%)	-	1.000
birth weight (g), $\bar{x}\pm s$	1556±398	1801±375	-4.035	0.000	1675±363	1686±388	-0.126	0.900
GA at birth (week), $\bar{x}\pm s$	30.71±1.81	32.57±1.53	-7.197	0.000	31.53±1.56	31.76±1.84	-0.619	0.540
season of birth, n (%)								
spring (March – May)	19 (32.2%)	36 (30.0%)			10 (29.4%)	8 (23.5%)		
summer (June – August)	15 (25.4%)	35 (29.2%)			8 (23.5%)	9 (26.5%)		
autumn (September – Novenber)	7 (11.9%)	21 (17.5%)	1.856	0.603	2 (5.9%)	6 (17.6%)	4.510	0.608
winter (December – February)	18 (30.5%)	28 (23.3%)			14 (41.2%)	11 (32.4%)		
cesarean, n (%)	47 (79.7%)	85 (70.8%)	1.592	0.207	28 (82.4%)	23 (67.6%)	-	0.267
Apgar score at 1 min, *M (IQR)*	7 (6-8)	8 (8-8)	-5.787	0.000	8 (7-8)	7 (7-8)	-1.054	0.292
Apgar score at 5 min, *M (IQR)*	8 (7-8)	8 (8-8)	-5.562	0.000	8 (7-8)	8 (7.75-8)	-0.832	0.405
maternal age (year), $\bar{x}\pm s$	30.80±5.13	29.71±4.87	1.382	0.169	31.24±5.39	30.59±4.34	0.551	0.585
matemal BMI, $\bar{x}\pm s$	25.37±2.44	26.37±3.51	-1.971	0.050	25.63±2.33	25.39±3.77	0.317	0.753
antenatal steroid use, n (%)	49 (83.1%)	102 (85.0%)	0.114	0.736	29 (85.3%)	28 (82.4%)	-	1.000
PROM, n (%)	17 (28.8%)	53 (44.2%)	3.915	0.048	13 (38.2%)	13 (38.2%)	-	1.000
gestational hypertension, n (%)	13 (22.0%)	12 (10.0%)	4.767	0.029	4 (11.8%)	6 (17.6%)	-	0.687
GDM, n (%)	11 (18.6%)	22 (18.3%)	0.003	0.960	9 (26.5%)	6 (17.6%)	-	0.607

- McNemar's test, no statistics

PSM: propensity score matching, IQR: interquartile range, GA: gestational age, BMI: body and mass index, PROM: premature rupture of membranes, GDM: gestational diabetes mellitus

Table II. Comparison of vitamin levels and deficiency rate between the two groups after PSM.

vitamin levels and deficiency rate	RDS group (n=34)	control group (n=34)	*Z*	*P*
Vit A (ng/mL), *M (IQR)*	134.91(105.01-156.74)	152.46 (120.06-200.00)	-2.317	0.021*
Vit A deficiency rate, n (%)	32 (94.1%)	26 (76.5%)	-	0.109
25(OH)Vit D (ng/mL), *M (IQR)*	12.13 (8.44-17.85)	16.84 (10.75-25.83)	-2.445	0.014*
Vit D deficiency rate, n (%)	29 (85.3%)	19 (55.9%)	-	0.006**
Vit E (μg/mL), *M (IQR)*	2.74 (2.24-3.54)	3.00 (2.67-3.60)	-1.086	0.278
Vit E deficiency rate, n (%)	32 (94.1%)	30 (88.2%)	-	0.687

**P<0.05, **P<0.01*, - McNemar's test, no statistics
PSM: propensity score matching, IQR: interquartile range, Vit A: vitamin A, 25(OH)Vit D: 25-hydroxyvitamin D, Vit D: vitamin D, Vit E: vitamin E

Logistic regression analysis of the effect of vitamin levels on RDS in preterm infants

When the univariate analysis was conducted, the serum levels of Vit A and Vit D were considered as possible risk factors for neonatal RDS. Thus, we took Vit A and Vit D levels as covariates and the occurrence of RDS as the dependent variable. Multivariate analysis showed that low serum Vit D level was an independent risk factor for the development of RDS in preterm infants (*OR 0.917, 95% CI 0.851-0.989*), as detailed in Table III.

Discussion

RDS is a common complication of prematurity and presents as progressive dyspnea, cyanosis and respiratory failure within hours of birth. Related studies have shown that combined RDS can increase neonatal mortality by more than three times.[11] Therefore, our study investigates the relationship between different fat-soluble vitamins and RDS, providing new strategies for the prevention and treatment of RDS in preterm infants from a vitamin perspective.

In our study, the comparison between the groups was adjusted for confounding variables with PSM to minimize selection bias, and thus the baseline characteristics of the RDS and control groups were equalized and comparable.

Vit A is involved in the growth and differentiation of airway epithelial cells, maintaining their structural and functional integrity to act as a barrier.[12] Also, Vit A acts on the lung's retinoic acid receptors to upregulate the transcription and expression of the surfactant protein-B gene and thus promotes the synthesis of pulmonary surfactant.[13] In addition, Vit A has demonstrated an effect on the proliferation and differentiation of T cells to perform immunoregulatory functions.[14] Studies in animal models have confirmed that Vit A plays a key role in lung injury prevention.[15] Chen et al.[16] also reported that Vit A deficiency is associated with an increased risk of adverse lung outcomes in newborns. The results of our study showed that after PSM, although serum Vit A levels were significantly lower in preterm infants in the RDS group than that in the control group (*P<0.05*), logistic regression analysis did not suggest a significant effect of low serum Vit A levels on the development of RDS in preterm infants. It might be related to the fact that serum Vit A levels do not fully reflect the local Vit A concentration within the lung epithelial tissue. Therefore, the correlation between serum Vit A levels and the development of RDS in preterm infants still requires further study.

Table III. Logistic regression analysis

Covariates	*B*	*S.E.*	*Wald*	*P*	*OR*	*95%CI*
Vit A	-0.008	0.006	2.076	0.150	0.992	0.980-1.003
Vit D	-0.086	0.038	5.074	0.024	0.917	0.851-0.989

Vit A: vitamin A, Vit D: vitamin D

Vit D is a steroid hormone, and recent studies have identified its more extraosseous influence, especially positive effects on the regulation of lung maturation and development, such as the impact on alveolar type II cells, fibroblast proliferation, surfactant synthesis, alveolarization, and upregulation of vitamin D receptor in the lungs.[17] The research conducted by Dogan[18] and Treiber et al.[19] also confirmed that preterm infants with RDS are born with lower Vit D levels. The results of our study showed that after PSM, serum Vit D levels were lower in the RDS group of preterm infants than that in the control group, with a significant difference. ($P<0.05$). Furthermore, logistic regression analysis suggested that Vit D deficiency was an independent risk factor for the development of RDS in preterm infants. Therefore, Vit D supplementation may have a positive effect on preventing the occurrence and development of RDS in preterm infants.

Vit E, also known as tocopherol, is a potent antioxidant capable of neutralizing free radicals and reactive oxygen species by providing hydrogen ions through its chromogranin ring.[20,21] Oxidative stress-mediated cellular damage is thought to underlie the pathophysiology of respiratory distress syndrome, and antioxidant vitamins are thought to inhibit the harmful effects of free radicals and have protective potential in the therapy of acute respiratory distress syndrome.[22] However, our present study did not find significant differences either in Vit E levels or deficiency rates between the RDS and control preterm infants after PSM, which may be explained by the fact that low antioxidant plasma levels may not necessarily indicate low total body stores as the critical illness itself may induce redistribution of antioxidants.[23] Although animal experiments have shown that nebulized inhaled Vit E improves ventilation parameters and has a potential benefit on lung disease, it has not been confirmed in human studies.[24] Therefore, the correlation between serum Vit E levels and the development of RDS in preterm infants is not definitive and further studies are warranted to clarify this.

In conclusion, the present study concluded that Vit A, Vit D and Vit E deficiencies are more common in preterm infants and that low serum Vit D levels are an independent risk factor for the development of RDS, but no significant effect of serum Vit A and Vit E on RDS in preterm infants was found, which needs to be further confirmed by studies with larger sample sizes. Adequate Vit D supplementation during pregnancy is expected to reduce the incidence of RDS in preterm infants, but the amount of supplementation needs to be further studied.

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Ethical approval

This study was approved by the Medical Ethics Committee of Jiaxing Maternity and Child Health Care Hospital (No. 2020-3).

Author contribution

The author confirms contribution to the paper as follows: study conception and design: YYZ; data collection: YYZ; analysis and interpretation of results: YYZ; draft manuscript preparation YYZ. 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 ciliary functions and ciliary beat frequency via cell culture method in patients with primary ciliary dyskinesia

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ABSTRACT

Background. Cell culture increases both diagnostic specificity and sensitivity of primary ciliary dyskinesia (PCD) and the most important reason to use cell culture for definitive diagnosis in PCD is to exclude secondary ciliary defects. Here we aimed to evaluate the cilia functions and cilia ultrastructural abnormalities after ciliogenesis of cell culture in patients with definitive diagnosis of PCD. We also aimed to compare high speed videomicroscopy (HSVM) results of patients before and after ciliogenesis and to compare them with electron microscopy, genetic and immunofluorescence results in patients with positive diagnosis of PCD.

Methods. This study was conducted as a cross-sectional study in patients with PCD. HSVM, transmission electron microscopy (TEM) and immunofluorescence staining results of the nasal biopsy samples taken from patients with the definitive diagnosis of PCD were evaluated and HSVM findings before and after cell culture were described.

Results. Ciliogenesis and regrowth in the cell culture occurred in the nasal biopsy sample of eight patients with PCD. The mean age of the patients was 15.5±4.2 years (8.5-18 years). Mean beat frequency was found to be 7.54±1.01 hz (6.53-9.45 hz) before cell culture, and 7.36±0.86 hz (6.02-7.99 hz) after cell culture in the nasal biopsy of patients. There was no significant difference in the beat frequency of PCD patients before and after cell culture. Ciliary function analysis showed the similar beating pattern before and after cell culture in patients with PCD.

Conclusions. This study showed us that there was no difference between cilia beat frequency and beat pattern before and after cell culture in patients with definitive diagnosis of PCD and repeated HSVM would be a useful diagnostic approach in patients who have no possibility to reach other diagnostic methods.

Key words: primary ciliary dyskinesia, cell culture, high speed videomicroscopy, cilia function analysis.

Primary ciliary dyskinesia (PCD) is a rare genetic disease caused by congenital abnormalities in both structure and function of the motile cilia characterized with recurrent upper and lower airway infections. Ciliary dysfunction leads to impairment of the mucociliary transport and this is the leading cause of chronic respiratory infections and progressive lung disease since the first years of life. Early diagnosis and treatment of the disease can prevent the development of bronchiectasis.[1-3]

There is no "gold-standard" test for PCD and guidelines recommend to use the combination of different methods for early diagnosis of these

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patients. According to European Respiratory Society (ERS) taskforce, in patients with strong clinical suspicion, it is necessary to demonstrate the hallmark of ciliary ultrastructural defects on transmission electron microscopy (TEM), or pathogenic biallelic mutations in PCD causing genes for positive diagnosis of PCD. Otherwise, low nasal nitric oxide (nNO) results plus abnormal high-speed video microscopy analysis (HSVM) findings on three occasions or following cell culture even with normal TEM make PCD 'highly likely'.[4-6] In a subset of patients, the features of ciliary dysmotility only became apparent after ciliogenesis in cell culture. Thus, cell culture increases both diagnostic specificity and sensitivity of PCD and this method may help to reduce false-positive diagnosis in patients with secondary ciliary dysfunction.[7-9] The use of cell culture for PCD diagnosis has been developed by Jorissen et al. using a submerged cell culture system.[10-12]

The most important reason to use cell culture for definitive diagnosis in PCD is to exclude secondary ciliary defects. Here, we aimed to evaluate the cilia functions and cilia ultrastructural abnormalities after ciliogenesis of cell culture in patients with definitive diagnosis of PCD. We also aimed to compare HSVM results of patients before and after ciliogenesis and to compare them with electron microscopy results in patients with a positive diagnosis of PCD.

Material and Methods

This study was conducted as a cross-sectional study in patients with PCD. In clinical practice, nasal NO measurement, ciliary functional analysis with HSVM and genetic tests are being used in the evaluation of patients with PCD in our Department of Pediatric Chest Diseases. This study was approved by the local institutional review board and supported in part of the University Scientific Research Committee Project with number THD-2016-9044. Informed consents were obtained from the children and parents.

Children between the ages of 6-18 years who had definitive PCD diagnosis based on clinical, radiological findings, nasal NO, HSVM and genetic analysis according to ERS guidelines from September 2016 to December 2017 were included to study. Children with other chronic lung diseases and who were highly likely to be diagnosed with PCD according to ERS guidelines were excluded.

At the same time, two different nasal biopsies and nasal brushing samples were obtained from the inferior nasal concha into the isotonic saline and gluteraldehyde solution by punch biopsy method in the Ear Nose and Throat Department from children who were symptom free for two weeks before the date of nasal biopsy.

In this study, HSVM, TEM and Immunofluorescence staining results of the nasal biopsy samples taken from patients who were followed up with the definitive diagnosis of PCD were evaluated and obtained results were analyzed through Matlab software. Additionally, HSVM findings before and after cell culture were described and these findings were compared with TEM and genetic or Immunofluorescence staining results.

Cell culture method

After the nasal biopsies were obtained, the tissues were washed with saline in a petri dish, to remove debris and blood. Cells were first grown in a monolayer to expand the basal cell population without cilia (dedifferentiation). After three weeks the cells reached confluency and ciliated cells disappeared, cells were then transferred to a suspension medium to induce redifferentiation into ciliated epithelial cells (ciliogenesis). After two weeks of suspension culture functional cilia reappear on the spheroids and these ciliated aggregates can be kept in culture for more than several months. Jorissen et al.[13] developed this method and this process was performed according these rules.

Cilia Function Analysis with High Speed Videomicroscopy

Ciliary functions including cilia beating pattern and beat frequency (CBF) were analyzed by HSVM before the cell culture process started. After six weeks of cell culture, cilia beating pattern was assessed with HSVM and cilia beat frequency was measured. An inverted microscope was used and images were acquired by a high speed camera, connected to the microscope. For every sample at least three or four regions with a colony of ciliated cells were included. The CBF value was computed using Matlab software. The CBF value was expressed as a histogram and the mean CBF value of this histogram was used as the result for one CBF measurement (reference values obtained in our laboratory were CBF 12 Hz, SD 0.8 at 37°C). Cilia beat pattern was categorized as hypokinetic cilia, hyperkinetic cilia, stiff pattern, and abnormal circular movement according to HSVM motion analysis.

Electron Microscopy Analysis

The biological samples from patients (both cultured cells and biopsy samples) were fixed in 2.5% phosphate buffered glutaraldehyde solution for 1 hour at room temperature. Samples were postfixed with 1% osmium tetroxide in the dark for 30 minutes. After washing and centrifuging, pellets of cells were embedded in 37°C warm agar. Eventually, both agar-embedded cells and tissue samples were dehydrated in graded alcohols. The samples were cleared using propylene oxide and embedded into araldite. The samples were polymerized at 60°C for 48 hours. Semi-thin and thin sections were obtained from the plastic blocks. Sections stained with uranyl acetate and lead citrate were analyzed under transmission electron microscopy (TEM) (JEOL, JEM 1400 attached with a Gatan Orius SC 1000 CCD camera). Defects in the outer dynein arms, outer and inner dynein arms, inner dynein arms with microtubule disorganization, radial spokes, or central apparatus provided confirmation of PCD diagnosis according to ERS guidelines.[14]

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 23.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were presented as a mean ± standard deviation (SD) for normal distribution or median (min-max) for non-normal distribution.

Results

A total of 43 patients were included in the study. The cell culture process was terminated in seven patients due to infected cells during the procedure. Cilia regrowth did not occurr in 28 patients during the cell culture procedure at the sixth week.

Ciliogenesis and regrowth in the cell culture occurred in the nasal biopsy sample of eight patients with PCD. Clinical characteristics of these patients are shown in Table I. The mean age of the patients was 15.5 ± 4.2 years (8.5-18 years). Mean beat frequency was found to be 7.54 ± 1.01 hz (6.53-9.45 hz) before cell culture, and 7.36 ± 0.86 hz (6.02-7.99 hz) after cell culture in the nasal biopsy of eight patients with proliferation in cell culture. There was no significant difference in the beat frequency of PCD patients before and after cell culture. Ciliary function analysis showed a similar beating pattern before and after cell culture in patients with PCD. Table II and Figure 1 show the distribution of ciliary beat frequency in these patients.

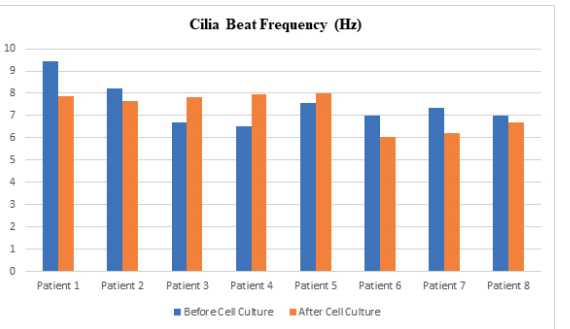

Fig. 1. Distribution of cilia beat frequency in patients with PCD.

Table I. Clinical characteristics of patients with PCD.

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8
Age at diagnosis (years)	12	14	8	12	11	8	13	12
Gender	Female	Male	Female	Female	Female	Female	Female	Female
Neonatal respiratory distress	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive
Chronic rhinitis	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive
Recurrent sinusitis	Positive	Positive	Positive	Negative	Negative	Negative	Positive	Positive
Recurrent otitis	Positive	Negative	Negative	Positive	Negative	Negative	Negative	Positive
Situs inversus totalis	Positive	Negative	Negative	Positive	Negative	Negative	Positive	Positive
Bronchiectasis	Positive	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Nasal NO (ppb)	12 ppb	10 ppb	8 ppb	6 ppb	15 ppb	15 ppb	5 ppb	5 ppb

Table II. HSVM and electron microscopy results of PCD patients grown in cell culture.

	Electron Microscopy				HSVM			Genetic and IF findings
Case	Central complex defect	Microtubular disorganization	Inner dynein arm defect	Outer dynein arm defect	CBF before cell culture (Hz)	CBF after cell culture (Hz)	Cilia beat pattern	
1	Negative	Negative	Negative	Positive	9.45±1.22	7.85±1.86	Hypokinetic	DNAH5
2	Positive	Positive	Positive	Positive	8.2±1.02	7.64±0.79	Hypokinetic, stiff pattern	New mutation
3	Negative	Negative	Positive	Positive	6.69±0.81	7.83±0.73	Hypokinetic	DNAI1
4	Negative	Negative	Negative	Positive	6.53±0.68	7.96±0.29	Hypokinetic	DNAH5
5	Positive	Positive	Negative	Negative	7.58±0.56	7.99±0.21	Hypokinetic, stiff pattern	RSPH4A
6	Positive	Positive	Negative	Negative	7.01±0.51	6.02±0.61	Hypokinetic	RSPH4A
7	Negative	Negative	Negative	Positive	7.33±1.03	6.22±0.91	Hypokinetic	DNAH5
8	Negative	Positive	Positive	Negative	7.01±1.02	6.68±0.84	Hypokinetic	CCDC40

HSVM: high speed videomicroscopy, IF: immunofluorescence

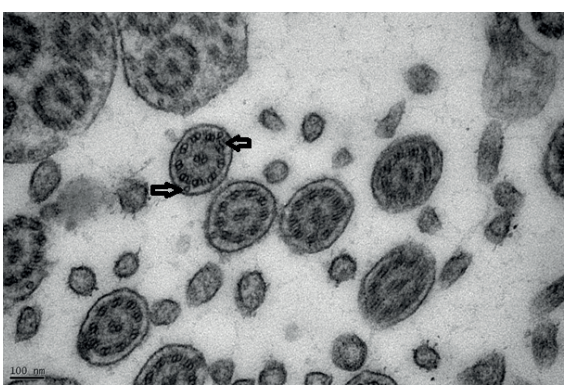

Fig. 2. In the sample of a patient, cilia ultrastructure was observed: The central and peripheral microtubule structures (9 + 2) were not in the normal structure with extratubules (marked by arrow) located in the periphery; electron micrograph (X100000 magnification; Uranyl acetate & Lead citrate).

In TEM, while some of the ciliated cells had no inner or outer dynein protein arms or were deficient, in others there was no microtubule placement (9 + 2) in the periphery and central. It was observed that some of them had an extra tubule structure or the missing tubules (Fig. 2).

TEM findings and HSVM findings in nasal biopsy of cell culture compared with genetic results and Immunofluorescence staining are also indicated in Table II.

Discussion

Cell culture method of nasal biopsy specimens may help to reduce false-positive diagnoses in patients with secondary ciliary dysfunction

(SCD) and confirm the diagnosis of PCD. Recent data demonstrate that cell culture method has almost 100% sensitivity and specificity in differentiating PCD and acquired ciliary dyskinesia.[12,15] This study showed that there was no difference between cilia beat frequency and beat pattern before and after cell culture in patients with definitive diagnosis of PCD. In all cases, dyskinesia associated with PCD was unchanged or became more prominent. This is the first study in our country using a highly specialized and time-consuming method for evaluating the cilia functions and electron microscopy results of PCD patients after the ciliogenesis of cell culture.

Previous reports which used the cell culture method introduced by Jorissen et al.[16] had different success rates.[7,13,16] In this method, monolayer culture and suspension culture procedures were used which takes a longer time of almost six weeks. Abnormalities secondary to respiratory infection and toxic agents disappear and SCD and PCD could be clearly distinguished. Boon et al.[9] found that the success rate of the culture developed by Jorissen et al.[16] was 75%, which was higher compared with other cell culture methods. However, using a monolayer culture technique, Pifferi et al.[17] could not culture sufficient ciliated tissue for a PCD diagnosis. Because of these reasons, alternative methods for growing ciliated cells in culture by exposing cells to an air-liquid interface have been developed and used. The air-liquid interface culture of nasal samples yields more cilia than the suspension culture technique that enables the cilia growth for definitive diagnosis. Hirst et al.[12] found 54% of success rate in their investigation with an air liquid interface culture method. Hirst et al.[12] also reported 43% of successful cilia regeneration in their patients with PCD and 26% success rate in their non-PCD samples on exposure to an air-liquid interface. The success rate of our study was 28% in patients with PCD which may be related with the vast majority of patients referred for diagnostic testing of PCD have chronic nasal symptoms and this increases the chance of losing the culture growth to a secondary infection and ciliary cell shedding.

It was also shown that similar beating patterns were revealed in patients with PCD before and after ciliogenesis. Hirst et al.[12] and Pifferi et al.[18] reported that ciliary function was shown as abnormal before and after cilia cell culture in all subjects with PCD similar to our study. However Boon et al.[9] reported that initial evaluation of the ciliary coordination and ciliary beat frequency in the biopsy was normal in 10.2% of patients with a final diagnosis of PCD. In our study all of the patients with PCD had abnormal beating patterns before the cell culture. Jorissen et al.[16] also found beat frequency results similar to our study before and after ciliogenesis. They found that the mean CBF was 8.4±1.6 Hz in the nasal biopsy materials before cell culture; after the suspension culture the mean CBF was 8.6±0.9 Hz.[16] There was no difference within the cilia beat frequency before and after cell culture. Our results also support these analysis which we found that the mean CBF was 7.54 ± 1.01 Hz before cilia cell culture, and 7.36 ± 0.86 Hz after cilia cell culture. However, Hirst et al.[19] found that CBF decreased in patients with PCD after the cell culture which was different from the previous studies.

TEM of cilia is a time-consuming method and needs experienced people in this area and also cilia ultrastructural analysis requires expensive equipment; but it is not available in all centers. This method is highly contributive to diagnosis of PCD although ultrastructure of cilia is normal in 21% of PCD patients.[20] This study showed that cilia functions were compatible with cilia ultrastructural defects in patients with PCD, here we also confirmed the results with genetic analysis of these patients. Despite the small number of patients in this study, these results showed that repeated HSVM would be a useful diagnostic approach in patients who have no possibility to reach other diagnostic methods. However, when HSVM is performed in specialized centers, in conjunction with

TEM evaluation, it will increase the diagnostic accuracy. Also we compared these results with the genetic and Immunofluorescence staining findings which is one of the strengths of our study.

A combination of functional and ultrastructural evaluation of the cilia before and after ciliogenesis seems to be the best approach for a PCD diagnosis.[8] The most important limitation of this study was the termination of cell culture procedure in some of the nasal biopsy samples due to infection-related problems. Because of recurrent infections in patients and low proliferation of ciliated cells in the cell culture, the success of this procedure was lower compared with previous reports. Furthermore during the ciliary deterioration and ciliogenesis, the most important thing is the regrowth of cilia again. Also, this technique is time consuming, invasive, requires significant expertise in cell culture, and as such is unlikely to be widely available outside of specialist diagnostic centers.[12] During TEM analysis, among the evaluated patients with cytoplasmic cilia like extensions in small microscope magnification, these findings were consistent as metaplasia in the respiratory epithelium and thickening of the stromal layer in these patients, which was another limitation of this study.

In conclusion, this study has provided an important diagnostic method for patients who are positive to be definitive PCD if ciliary function analysis is available and suggestive for the disease, but cannot be diagnosed with the causes of difficulties in diagnostic methods. Also, successful ciliated cell culture from the nasal biopsy samples will reduce the need to perform repeated biopsies in a number of patients. The results obtained from this study will, then, be used for early diagnosis of patients with PCD to prevent progression of disease complications such as bronchiectasis and respiratory failure, increasing the life span and quality of life of the patients, decreasing hospitalization and drug use costs.

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Ethical approval

This study was approved by Hacettepe University local institutional review board with number GO 15/638-14.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: NE, RH, UÖ, ÖG; .data collection: BK, EB, PA, GDT, SEP, MGH; analysis and interpretation of results: NE, UÖ, DD, EY, NK, ÖG; draft manuscript preparation: NE; RH, UÖ, DD, EY, NK, PA. 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

Computed tomography findings of COVID-19 in pediatric patients

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ABSTRACT

Background. In this study, we aimed to evaluate the thorax Computed Tomography (CT) findings of pediatric patients diagnosed with coronavirus disease-19 (COVID-19) and to discuss these findings in light of the results of adult patients from the literature.

Methods. The CT scans of pediatric patients (1-18 years old) with a diagnosis of COVID-19 by reverse transcriptase-polymerase chain reaction (RT-PCR) in our hospital between March 2020 and January 2021 were retrospectively reviewed. The scans were interpreted regarding the distribution and localization features, and involvement patterns including ground-glass opacity, consolidation, halo/reversed halo sign, interlobular septal thickening, air bronchograms and bronchiectasis. The frequencies of these findings in pediatric cases in our study were recorded.

Results. A total of 95 patients with a mean age of 13±4.6 years were included in this study. Among them, 34 (36%) had lesions associated with COVID-19 on CT scans. Bilateral involvement was detected in 15 (44%) while unilateral in 19 (56%) patients. Eighteen (53%) patients had single lobe involvement. In 16 (47%) patients a solitary lesion was detected and in 18 (53%) multiple lesions were present. Ground-glass opacity appearance was observed in 28 (82%), consolidation in 9 (26%), and ground-glass opacity with consolidation in 8 (24%), halo sign in 9 (26%), reversed halo sign in 2 (6%), interlobular septal thickening (interstitial thickening) in 1 (3%) patients.

Conclusions. As symptoms are relatively milder in children with COVID-19, CT findings are less extensive than in adults. It is essential to know the thorax CT findings that aid in the diagnosis and follow-up of the disease.

Key words: COVID-19, children, computed tomography, chest imaging, pneumonia.

The number of pediatric COVID-19 patients is growing significantly. Although most children with COVID-19 have had mild clinical symptoms, cases with severe signs of disease or even death have been reported.[1,2] Early diagnosis of the infection in children is important, as they also increase the transmission risk. Thus, the Computed Tomography (CT) scans of children with a suspicion of pneumonia must be carefully evaluated to both protect children and prevent spreading.[1]

The main diagnostic and screening tool for COVID-19 pneumonia is reverse transcriptase polymerase chain reaction (RT-PCR). However, the accuracy of the test depends on the quality of the throat swab and the viral load. Radiological features are very important for the diagnosis of COVID-19, and thorax CT can also detect pulmonary findings suggestive of COVID-19 infection even in patients who have initial negative RT-PCR results.[3]

While knowledge about the clinical and epidemiological features of COVID-19 in

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children is rapidly increasing, large studies of radiological findings are still lacking. In this study, we aimed to present the radiological features of COVID-19 in children.

Material and Methods

Thorax CT scans of pediatric patients who were admitted to our hospital between March 2020-January 2021 and diagnosed with COVID-19 by RT-PCR were retrospectively reviewed using the hospital database. CT examinations were performed with a 16-slice CT device (Toshiba Alexion 16) in a 3mm slice thickness in the supine position and the appropriate thoracic protocol (kVp: 100-120, mAs: 50-100). An intravenous or oral contrast agent was not administered to the patients. The images were sent to a workstation (Syngovia Siemens Medical System, Siemens/Germany) and evaluated in both the mediastinal and parenchymal windows in all three planes (axial, sagittal, and coronal). All the images were evaluated independently by two radiologists with 19 and 9 years of experience in thoracic imaging who were blinded to the each others interpretations. The final decisions were reached with consensus in cases of conflict.

The demographic characteristics of the patients including age, gender, and CT features of the lesions were examined. The location of the lesions was classified as unilateral-bilateral, anterior-posterior, central (parenchymal areas adjacent to the hilus)-peripheral (parenchymal areas close to the pleura) according to parenchymal involvement. The distribution of the lesions was classified as the right upper, middle, lower and left upper and lower lobe. The number of lobes affected was also examined. The morphological characteristics of the ground-glass opacities were classified as patchy or nodular forms. The morphological structure of the consolidation areas was described as round, linear or irregular. Interlobular septal thickening ("crazy paving"), tree-in-bud appearance, air bronchograms, bronchial wall thickening, bronchiectasis, air bubble, reversed halo sign, halo sign, nodule, linear atelectasis, pleural thickening, pleural effusion, pericardial effusion, and mediastinal lymphadenopathy (lymph node short axis dimension >10mm) were identified as present or absent.[4-6]

Statistical analysis was performed using the SPSS program (IBM SPSS Statistics for Windows Version 21.0. Armonk, NY: IBM Corp, USA). The demographic variables were expressed as mean±standard deviation. Other variables were presented as number (N) and percentage (%). A Cohen's kappa coefficient (κ) was calculated to assess the interobserver agreement between the two radiologists who interpreted the CT scans.

This study was approved by Kırşehir Ahi Evran University Ethics Committee on 09/02/2021 with the number of 2021-03 / 28.

Results

A total of 95 pediatric patients were included in this study. Among them, 61 (64%) had no pathology on thorax CT. Of the 34 (36%) patients with positive CT findings, 19 (56%) were male and 15 (44%) were female. The mean age was 13 ± 4.6 (1-18) years. The interobserver agreement between the two radiologists was substantial (κ: 0.7925).

Bilateral involvement was detected in 15 (44%) patients. Among 19 (56%) patients with unilateral involvement, 10 (53%) had lesions on the right and 9 (47%) on the left lobe. Although the lower lobes were mostly affected, the upper lobes were also involved with a similar rate (Right upper lobe in 13 (38%), right middle lobe in 5 (15%), right lower lobe in 16 (47%), left upper lobe in 12 (35%), left lower lobe in 18 (53%) patients). Parenchymal lesions were present in one lobe in 18 (53%) patients, in two lobes in 8 (24%), in three lobes in 3 (9%), in four lobes in 3 (9%), and in five lobes in 2 (6%) patients (Table I).

Eighteen patients had single lobe involvement. Involvement rates were similar in all lobes except the right middle lobe (right upper lobe:

Table I. Distribution of lesions in lung areas.

Findings		N	(%)
Pathology	Absent	61	64
	Present	34	36
Affected lung side	Bilateral	15	44
	Unilateral	19	56
	Right	10	53
	Left	9	47
Affected lung lobe	Right upper lobe	13	38
	Right middle lobe	5	15
	Right lower lobe	16	47
	Left upper lobe	12	35
	Left lower lobe	18	53
Number of lobes affected	1	18	53
	2	8	24
	3	3	9
	4	3	9
	5	2	6
Single lobe involvement (n:18)*	Right upper lobe	4	22
	Right middle lobe	1	6
	Right lower lobe	4	22
	Left upper lobe	5	28
	Left lower lobe	4	22
Affected lung field	Anterior	4	12
	Posterior	26	76
	Anterior-Posterior	4	12
	Peripheral	29	85
	Central	0	0
	Both Central and Peripheral	5	15
Number of lesions	Single	16	47
	Multiple	18	53

*Lober distribution percentages in this heading referred the ratio of involvement number of each lobes to the patient number with single lobe involvement (n:18)

4 (22%), right middle lobe: 1 (6%), right lower lobe: 4 (22%), left upper lobe 5 (28%), left lower lobe: 4 (22%)). In 26 (76%) patients, the lesions were located only in the posterior (posterior to the hilus), while 4 (12%) were located in the anterior (anterior to hilus) and 4 (12%) were both anterior and posterior parenchymal areas.

The lesions were located only in the peripheral regions of the lungs in 29 (85%) and both in the peripheral and in the central regions in 5 (15%) patients. There was no patient with only a central lesion in this study. A single lesion was detected in 16 (47%) and multiple lesions in 18 (53%) patients.

Ground-glass opacity appearance was seen in 28 (82%), consolidation in 9 (26%), and ground-glass opacity with consolidation in 8 (24%) patients. These ground-glass opacities were nodular type in 15 (44%) patients, and patchy type in 8 (24%) patients (Fig. 1,2). In 5 (15%) patients, nodular and patchy ground-glass opacities were seen together (Table II).

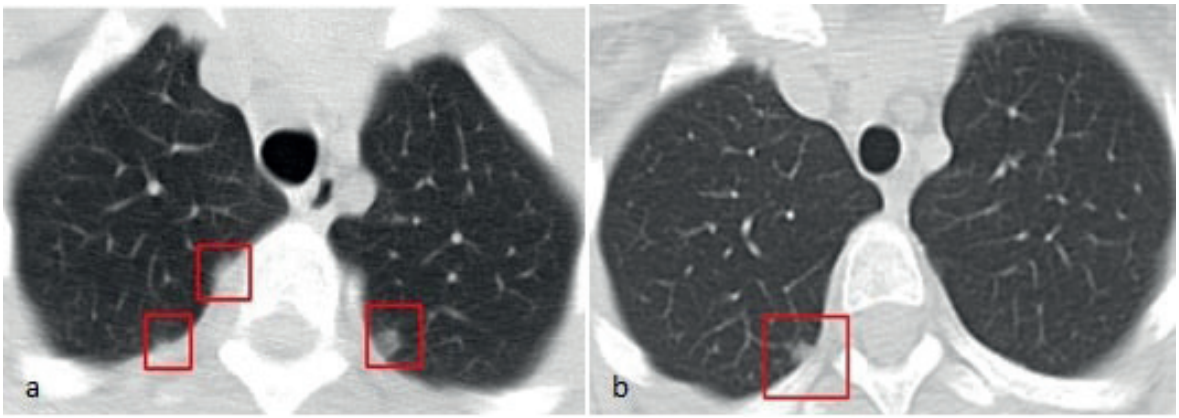

Fig. 1a-b. Peripheral nodular type ground-glass opacities in the bilateral upper lobes (a) in a 16 year old male (a) and a peripheral nodular type ground-glass opacity in a 17 year old male (b).

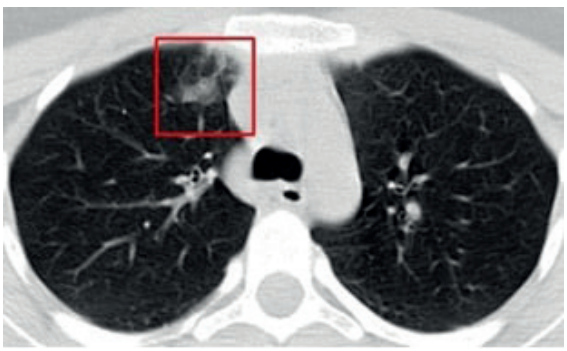

Fig. 2. A patchy peripheral ground-glass opacity in the right upper lobe in a 5 year old female.

Consolidation areas were only round-shape in 13 (38%), round+linear in 1 (3%), round+irregular in 2 (6%) and round+lineer+irregular in one patient (3%) (Fig. 3). All of the irregular and linear-shaped consolidation areas were accompanied by the round consolidation areas.

Nine (26%) patients had a halo (Fig. 4) and 2 (6%) had a reversed halo sign (Fig. 5). Seven (21%) patients had air bronchograms within areas of consolidation. Bronchiectasis was detected in 2 (6%) patients. Bronchial wall thickening was present in 3 (9%) patients (Fig. 6). Air bubble sign appearance was detected in 1 (3%) patient (Fig. 7). Vascular enlargement was present in 2 (6%) patients (Fig. 8). Interlobular septal thickening (interstitial thickening) was present in 1 (3%) patient (Fig. 9). Tree-in-bud appearance was detected in 3 (9%) patients (Fig. 10,11). Nine (26%) patients had subpleural and perivascular nodules with a mean diameter of 4.6 mm ± 1.3 (3-7 mm) (Fig. 12). Parenchymal band structure (atelectasis) was observed in 2 (6%) patients (Figure 13). Pericardial fluid was observed in 1 (3%) patient (Fig. 14). Lymphadenopathy and pleural fluid were not found in the patients.

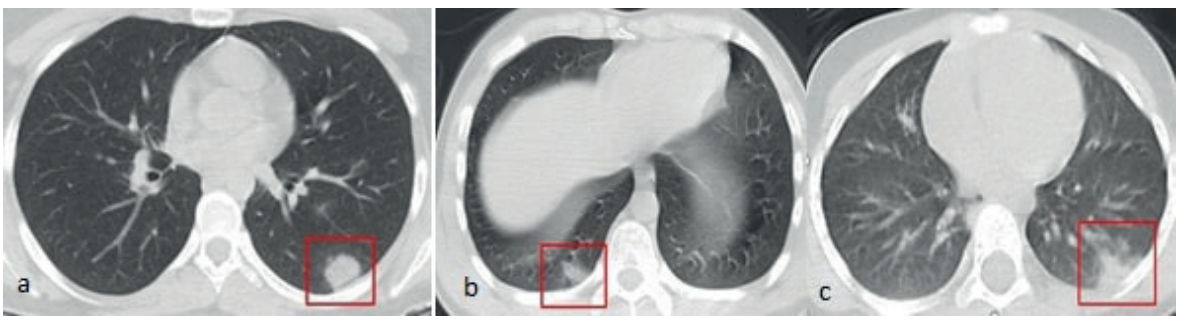

Fig. 3a-c. A peripheral round consolidation in the left lower lobe in a 15 year old female (a).A peripheral lineer consolidation in right lower lobe in a 15 year old male (b) and a peripheral irregular consolidation in the left lower lobe in a 5 year old female (c).

Table II. Frequency of parenchymal lesions.

Findings		N	%
Ground-glass opacity		28	82
Consolidation		9	26
Ground-glass opacity with consolidation		8	24
	Nodular	15	44
Ground-glass opacity morphology	Patchy	8	24
	Nodular + Patchy	5	15
	Round	17	50
Consolidation morphology (n:17)*	Linear	2	6
	Irregular	3	9
Air bronchogram		7	21
Bronchiectasis		2	6
Bronchial wall thickening		3	9
Air bubble sign		1	3
Vascular enlargement		2	6
Interlobular septal thickening		1	3
Halo sign		9	26
Reversed halo sign		2	6
Tree-in-bud sign		3	9
Nodule		9	26
Linear Atelectasis		2	6
Pleural effusion		0	0
Pericardial Effusion		1	3
Lymphadenopathy		0	0

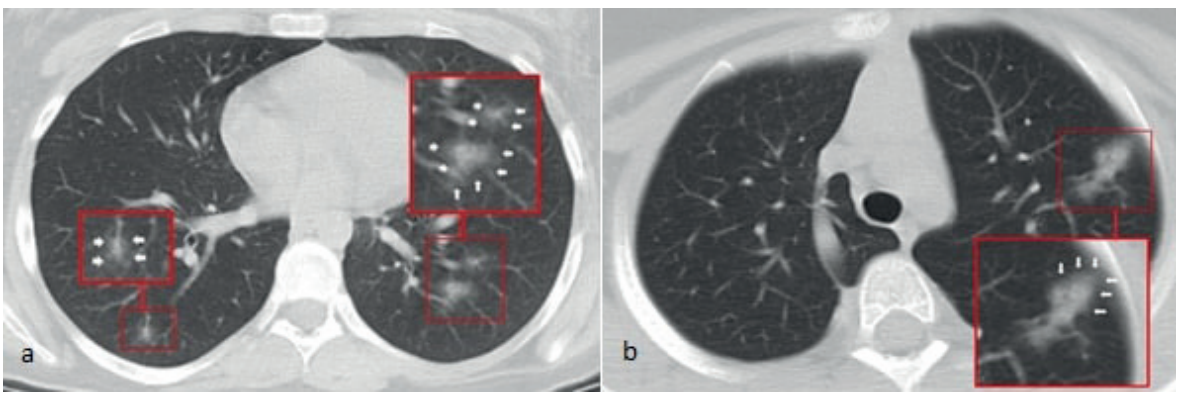

Fig. 4a-b. Multiple halo signs (white arrows) surrounding nodules in bilateral lungs in a 15 year old female (a) and in a halo sign (white arrows) surrounding nodules in the left upper lobe in a 14 year old female (b).

We identified 8 (24%) patients with multiple Ground-glass opacity appearances and multiple consolidation areas (Fig. 15). Among 3 patients with a control CT scan (7-15 days after the first scan) lesions completely resolved in 2 (Fig. 16) while they remained in one patient, the lesions progressed and new lesions appeared (Fig. 17).

Among the 95 patients with CT scans, 42 had a prior chest X-ray. The X-rays were interpreted

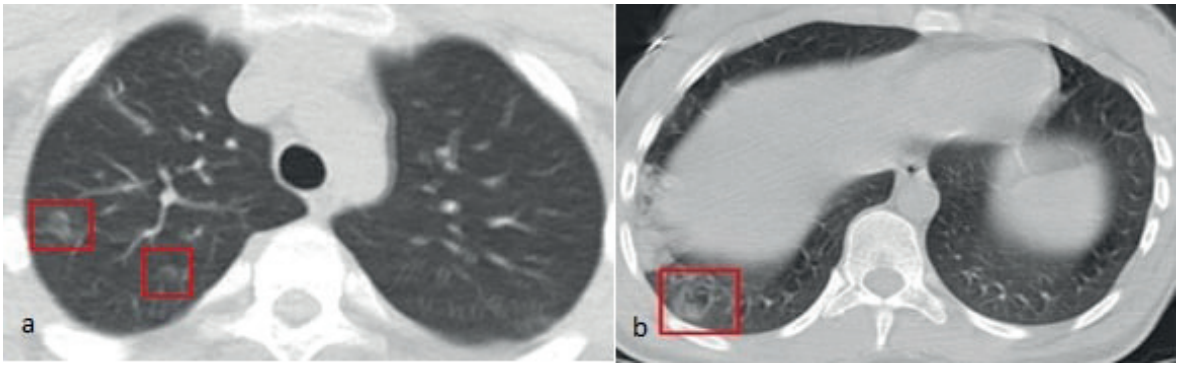

Fig. 5a-b. Reversed halo sign in right upper lobe in a 15 year old male (a) and in right the lower lobe in a 3 year old male (b).

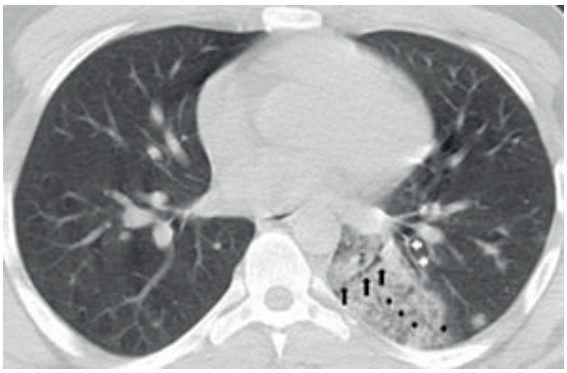

Fig. 6. Bronchiectasis (long black arrows), bronchial wall thickening (white arrows) and air bronchograms (short black arrows) within a central-peripheral consolidation in the left lower lobe in a 16 year old male.

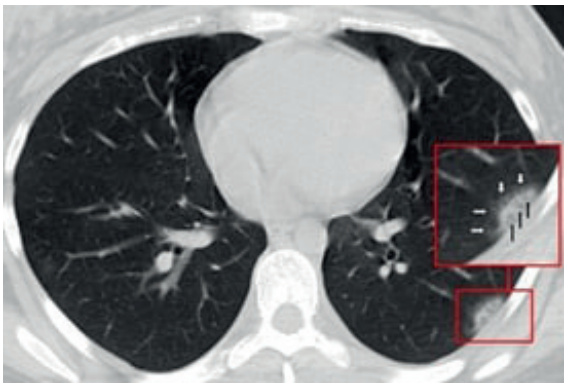

Fig. 7. An air bubble sign (black arrows) within a peripheral round consolidation surounding by a halo sign (white arrows) in the left lower lobe in a 10 year old female.

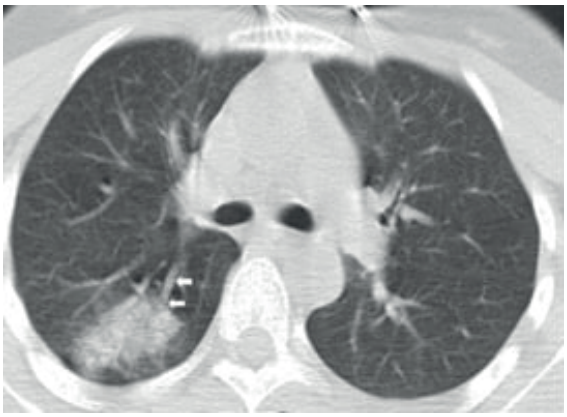

Fig. 8. Vascular enlargement (white arrows) adjacent to a nodular consolidation in right lower lobe in a 17 year old female.

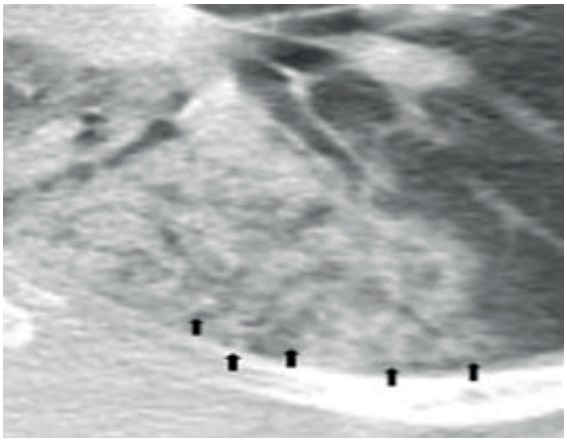

Fig. 9. Interlobular septal thickening (black arrows) within a consolidation (crazy paving) in the left lower lobe in a 16 year old male.

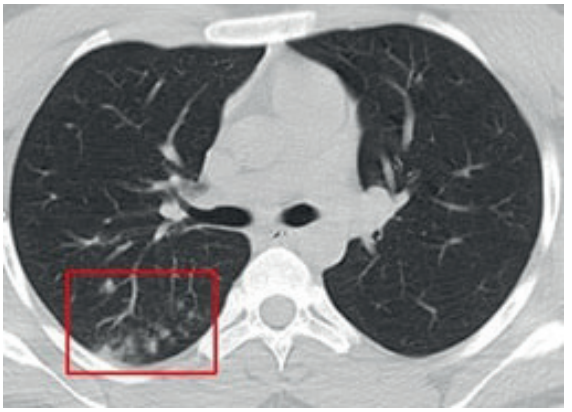

Fig. 10. Tree-in-bud appearance in right lower lobe in 7 year old male.

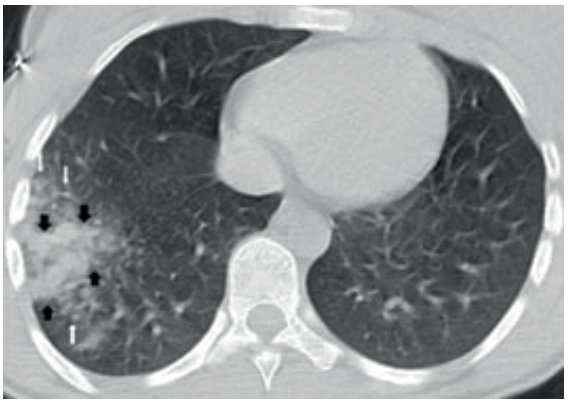

Fig. 11. An irregular consolidation (black arrows) and tree-in-bud appearance (white arrows) in a 3 year old male.

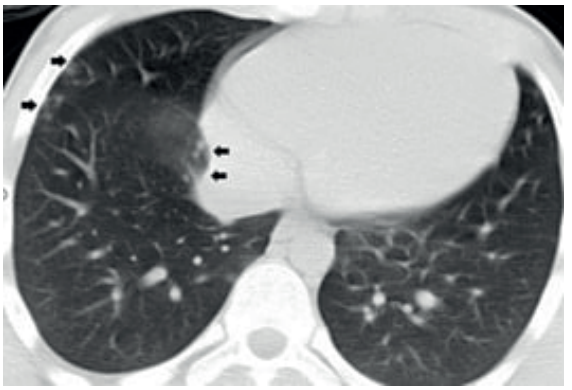

Fig. 12. Subpleural nodules (black arrows) in right lower lobe in a 17 year old male.

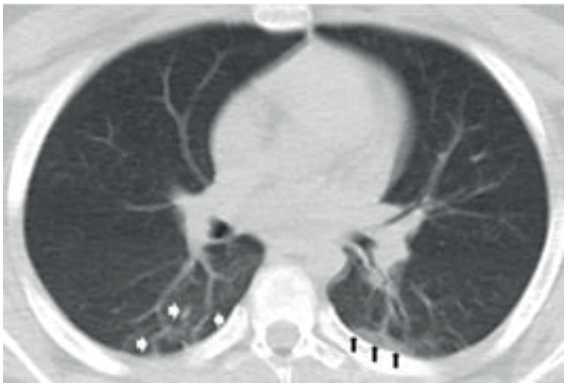

Fig. 13. A round consolidation (black arrows) in left lower and linear atelectasis (white arrows) in the right lower lobe in a 12 year old male.

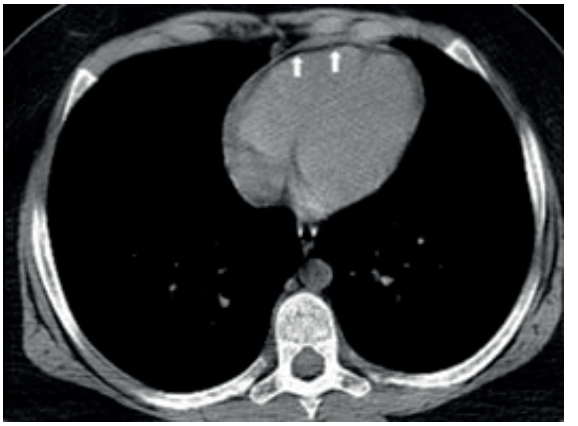

Fig. 14. A thin pericardial effusion (white arrows) in a 12 year old male.

as normal in 38 of these 42 patients. Focal radiopacities compatible with consolidation were observed in 4 patients (Fig. 18). As a result, focal consolidation was the only pathological finding detected on X-ray of the patients. No other pathological signs were detected on chest X-rays. Pathological findings were also seen on CT scans in all these 4 patients while there was no patient with signs on X-ray who did not have a pathological finding on CT.

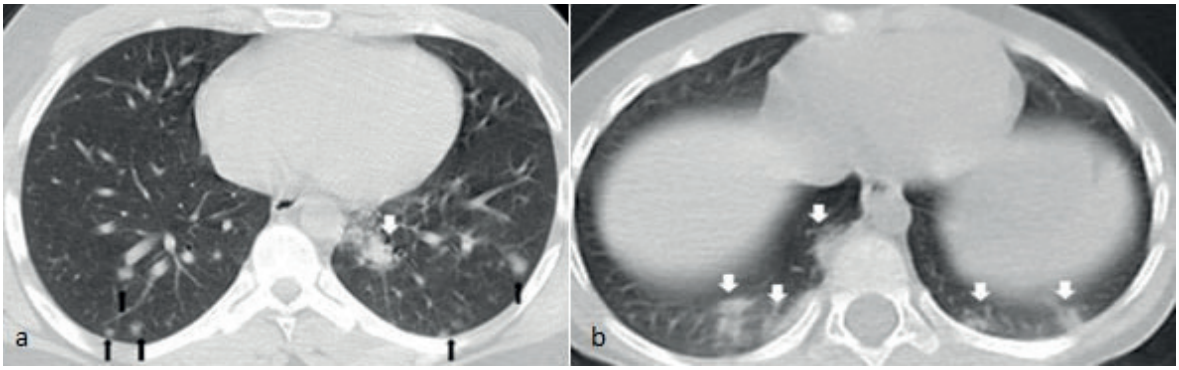

Fig. 15a-b. Ground-glass opacities (black arrows) in both lower lobes and consolidation (white arrow) in left lower lobe in a 17 year old male (a). Consolidations (white arrows) in both lower lobes in a 3 year old male (b).

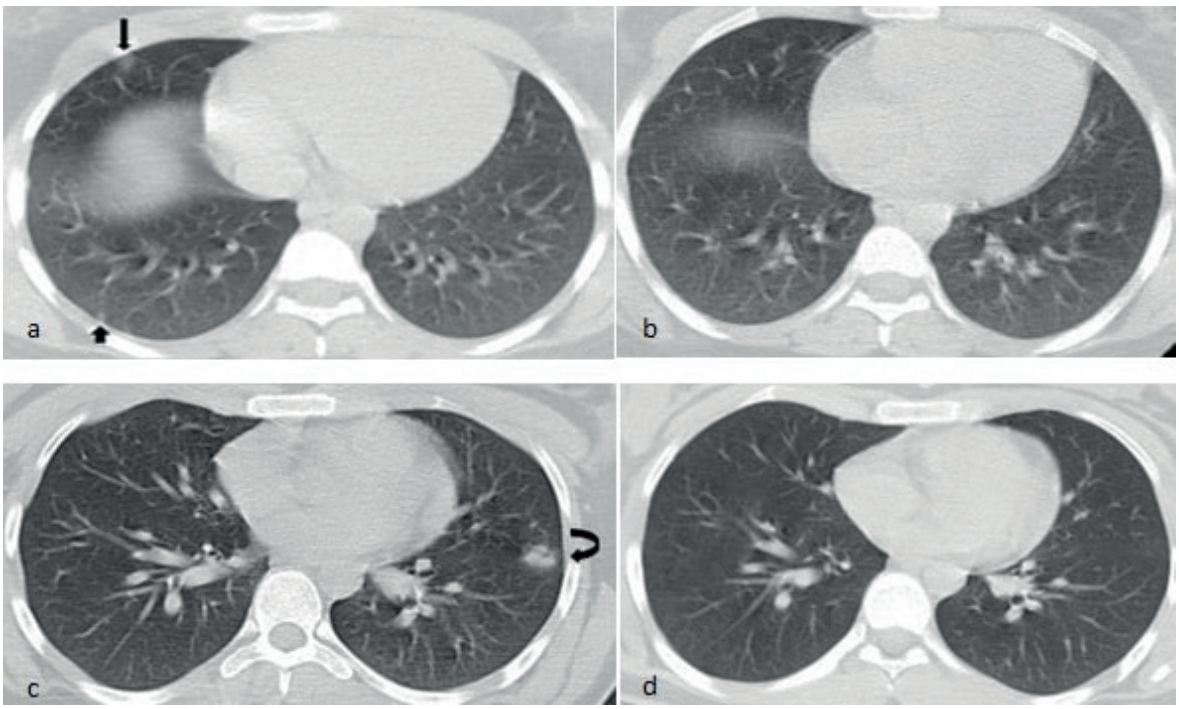

Fig. 16a-d. Ground-glass opacities (long black arrow) and a nodule (short black arrow) in a 16 year old male on initial scan (a). Lesions were not seen 7 days after first scan (b). A round consolidation (curved black arrow) in a 8 year old on first scan (c). Lesion disappeared after 7 days (d).

Discussion

COVID-19 disease can cause a highly contagious acute infectious pneumonia caused by SARS-CoV-2 that can be transmitted by an infected patient or an asymptomatic carrier. Since most pediatric patients are asymptomatic, they have a critical role in the spread of the disease. In pediatric cases with mild clinical symptoms, usually, a plain chest X-ray does not provide sufficient information, which leads to misdiagnosis. In some children with negative COVID-19 RT-PCR tests and clinical findings, especially in the initial stages of the disease, a thorax CT examination may be very useful for the diagnosis.[7]

When compared with adult COVID-19 patients, unilateral involvement is higher in pediatric patients.[7,8] Involvement rates of the

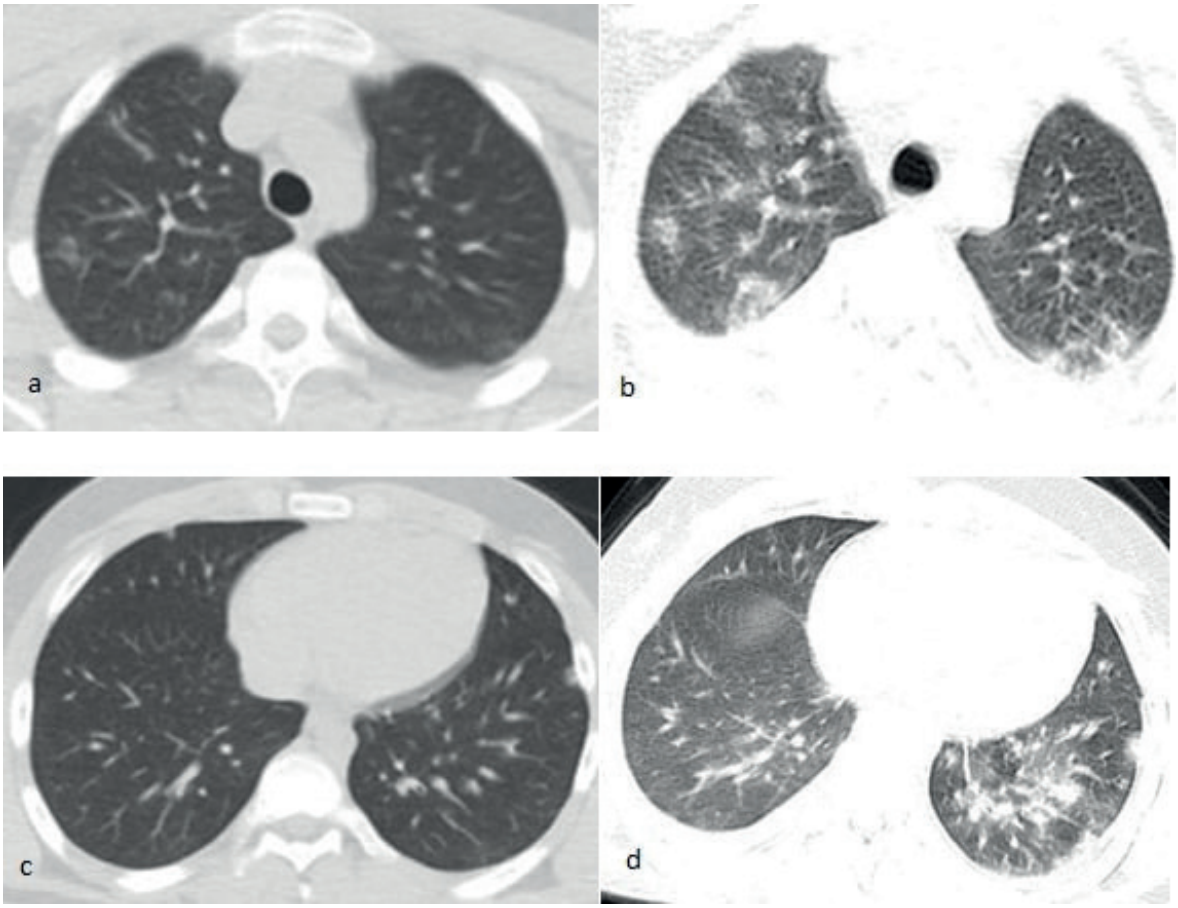

Fig. 17a-d. Lesions on first scan of 16 year old male (a-c). Progression of the lesions on the second scan after 15 days (b-d).

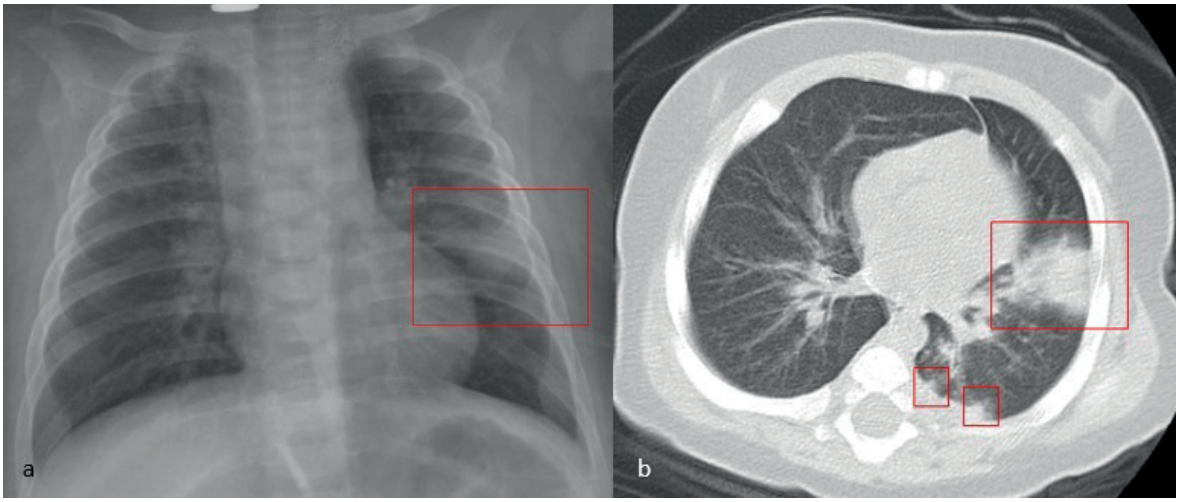

Fig. 18a-b. Radiopacity at middle left lung zone on prior X-ray (a) and consistent consolidations on CT (b) of a 5 year old male.

lower lobes are high in pediatric cases.[8,9] In our study, consistent with the literature, lower lobe dominance was present while left lower lobe involvement was the most common. Chen et al.[10] stated that pediatric patients tended to have less extensive involvement than adult patients in their study. Sharon et al.[11] suggested that lesions in pediatric patients predominantly involved 1 or 2 lobes. In a study by Palabiyik et al.[4] single lobe involvement was dominant in pediatric patients as well as unilateral lung involvement. Similar to our data, the number of patients with multiple lesions was found to be higher than with a single lesion.[12] However, our relatively high rate of single lesions may be due to less severity of the involvement or to the early phase of the disease at the time of the CT scan. While parenchymal lung lesions are often located peripherally, both peripheral and central lesions may be seen in the same patient. However, only central involvement is very rare[13,14] as in our study.

In a meta-analysis by Katal et al.[1] isolated ground-glass opacity, consolidation, and the concomitance of ground-glass opacity with consolidation were suggested as the most common findings in children with COVID-19. The most common radiological finding detected in COVID-19 pneumonia is ground-glass opacity. In our study, consistent with the literature, ground-glass opacities were smaller in size with a lower density and they were less diffuse in pediatric patients when compared to adults. Also, interlobular septal thickening less frequently accompanies ground-glass opacities in children.[15,16] Consolidation is common in COVID-19 pneumonia as it can be seen as an isolated finding but concurrence of consolidation and ground-glass opacity may be seen frequently. They become prominent in the peak period of the disease, especially in the posterior and peripheral aspects of the lower lobes. It can be morphologically round, linear, and irregular in shape, and may be accompanied by air bronchograms.[6,7,17,18] In our study, round-shaped consolidation areas were predominant while linear and irregular consolidations frequently accompanied round consolidations.

Halo sign is termed as the presence of a surrounding ground-glass opacity around a nodule or mass.[19] The halo sign is more common in pediatric COVID-19 cases compared to adult patients.[20] In a study by Xia et al.[7] they stated that the halo sign surrounding the consolidations was observed at a rate of 50% and this finding could be considered specific for pediatric patients. In our study, the frequency of halo sign associated with consolidation areas and nodules was found as 9%. Interlobular septal thickening refers to the collection of inflammatory cells in the interstitium. In COVID-19 pneumonia, it can be isolated or accompanied by ground-glass opacities and consolidation areas ("crazy paving"). This is one of the common findings of COVID-19 pneumonia in adults and it was less frequent in children in our study when compared with studies on adults in the literature.[21-23] Reversed halo sign is one of the atypical findings of COVID-19 pneumonia and is rare in children.[11] Bronchial wall thickening due to airway changes is a finding reflecting the severity of the disease.[24] In a study with 10 pediatric COVID-19 cases, Tan et al.[25] found bronchial wall thickening in 1 patient.

Bronchiectasis may occur due to volume loss during the organization of consolidation areas.[17] The air bubble sign can refer to the pathological expansion of physiological air space in the parenchyma of the lung or the rounded appearance of existing bronchiectasis or the air gaps formed during the resorption of the collapsed structures.[26]

Vascular enlargement is described as an increase in the size of subsegmental pulmonary vessels (> 3 mm), especially in lung areas where parenchymal involvement is more prominent. In patients with COVID-19 pneumonia, these findings may be related to the damage and thickening of the vascular wall structures caused by inflammatory processes.[27] We could

not find detailed data on bronchiectasis, air bubbles, and vascular enlargement in pediatric COVID-19 patients. In our study, we detected bronchiectases in 2 (6%), air bubble finding in one (3%), and vascular enlargement in 2 (6%) patients.

Nodules are round or irregular opacities less than 3 cm in diameter, well or poorly circumscribed, and are often associated with viral pneumonia. Its' incidence in children with COVID-19, is slightly higher than in adult patients.[7,19,28] In our study, 9 (26%) patients had mostly well-circumscribed subpleural and perivascular nodules with a mean diameter of 4.6 mm ± 1.3 (3-7 mm). The tree-in-bud finding, which is usually an indicator of small airway disease, is one of the atypical findings of COVID-19. These lesions should raise a suspicion of the presence of bacterial or viral co-infection and patients should be evaluated in this respect.[26] In the study of Xia et al.[7] they concluded that co-infection is seen frequently in pediatric COVID-19 patients (40%). In our study, 3 (9%) patients had nodular infiltration areas. However, we did not have additional sufficient evidence to support the presence of co-infection in these patients.

The incidence of pericardial effusion of adult COVID-19 cases on CT images is approximately 5%[20,29], but we could not find data about the frequency in pediatric COVID-19 cases. In our study, one patient had pericardial fluid. Pleural fluid and lymphadenopathy in pediatric COVID-19 patients were rarely reported in previous studies.[30] Also, pleural fluid and lymphadenopathy were not found in our study.

The clinicians and radiologists should be in consensus on the evaluation of pediatric patients regarding the sensitivity and specificity of CT, the accuracy of RT-PCR tests, and radiation exposure. It should be kept in mind that CT findings in COVID-19 are not specific and may occur in various diseases such as other viral or atypical pneumonia, hypersensitivity pneumonia, eosinophilic lung diseases. Also, a relatively lower positive predictive value is another potential limitation of CT especially in some regions with a low COVID-19 prevalence.[5,31] In the new guidelines by the North American Radiology Association (RSNA), the RT-PCR test is suggested as the first method to be used for the diagnosis of COVID-19 in children. They noted that imaging is not indicated unless the patient has potential risk factors or a progression in clinical symptoms.[32]

In the study by Ma et al.[23] they stated that a significant improvement of the lesions was observed in most of the pediatric cases on follow-up images. Therefore, control CT scans should be obtained when they are only necessary, considering the clinical changes in the patients. A guideline by the North American Radiology Association (RSNA)[32], recommends bidirectional (posterior-anterior and lateral) chest radiography for follow-up pediatric patients with COVID-19.

The primary limitations of the study are its retrospective nature and the relatively small patient number. Also, we could not exclude the presence of a bacterial or viral co-infection in some patients with suspicious CT findings such as nodular infiltrations.

In conlusion the number of pediatric COVID-19 cases is gradually increasing. There are some differences in the thoracic CT features of COVID-19 in children compared to adults. Awareness of CT findings of COVID-19 in children is important for both rapid isolation and control of the disease. The use of CT for the follow-up the pediatric COVID-19 patients must be limited because of the high radiation dose.

Ethical approval

This study was approved by Kırşehir Ahi Evran University Ethics Committee on 09/02/2021 with the number of 2021-03 / 28.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: YD, SÖ; data collection: YD, SÖ, EÜ, RA, MA; analysis and interpretation of results: YD, SÖ, EÜ; draft manuscript preparation: YD, SÖ. 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

Imaging manifestations of neonatal necrotizing enterocolitis to predict timing of surgery

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ABSTRACT

Background. To find the predictor of optimal surgical timing for neonatal necrotizing enterocolitis (NEC) patients by analyzing the risk factors of conservative treatment and surgical therapy.

Methods. Data were collected from 184 NEC patients (Surgery, n=41; conservative treatment, n=143) between the years 2015 and 2019. Data were analyzed by univariate analysis, and multivariate binary logistic regression analysis.

Results. Univariate analysis showed that statistically significant differences between the surgery and conservative treatment groups. The results of multivariate Logistic regression analysis indicated intestinal wall thickening by B-ultrasound and gestational age were independent factors to predict early surgical indications of NEC (p<0.05). The true positive rate, false positive rate, true negative rate and false negative rate in the diagnosis of necrotic bowel perforation guided by DAAS (Duke abdominal X-ray score) ≥7 and MD7 (seven clinical metrics of metabolic derangement) ≥3 were 12.8%, 0.0%, 100.0% and 87.2%, respectively.

Conclusions. In summary, the ultrasound examination in NEC children showing thickening intestinal wall and poor intestinal peristalsis indicated for early operation.

Key words: necrotizing enterocolitis, abdominal X-ray, abdominal ultrasound, operation timing.

Necrotizing enterocolitis (NEC) is a severe intestinal disease, mainly manifested by abdominal distension. NEC is an acquired disease resulting in intestinal mucosal damage, mucosal ischemia and hypoxia, local or diffuse necrosis of the colon and small intestine.[1,2] In traditional NEC treatment, taking the intestinal perforation as the absolute indication of surgery will miss the opportunity for treatment and increase the risk of postoperative death. Previous studies have shown that early surgical intervention before intestinal perforation or full-thickness necrosis of intestinal wall significantly improves therapeutic efficacy and reduces the probability of mortality and complications.[3] Meanwhile, early surgical intervention also effectively controls systemic metabolic disorders, and reduces postoperative complications such as intestinal stenosis.[4,5] However, the evidence of pneumoperitoneum on plain radiography is the only absolute criteria for operative intervention[6], and the single surgical indication may lead to limitations in clinical application. In this study, we provide an approach for clinicians to predict the best surgical timing by comparing X-ray, ultrasound, and the related factors such as abdominal X-ray score (DAAS score) and metabolic disorder score (MD7 score), and finally guide NEC children to be transferred to surgical treatment before intestinal perforation due to inadequate conservative treatment.

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Material and Methods

Research object

All cases were collected from 273 children with NEC who were hospitalized in the Children's Hospital of the Capital Institute of Pediatrics in China from January 2015 to December 2019. Among them, 184 cases met the inclusion criteria, including 41 cases in the surgical group and 143 cases in the non-surgical group.

Inclusion criteria

1) Meet the revised Bell's NEC II or III clinical diagnostic criteria[7], and the X-ray features suggests of NEC, including pneumatosis, portal venous gas, gasless abdomen, or fixed dilated loops.[8]

2) Complete clinical case records were necessary which included onset time, weight, metabolic disorder indicators, abdominal x-rays, abdominal ultrasound, and surgical records.

Exclusion criteria

1) Children with severe complications who died within 48 hours after admission.

2) Child who needed surgery but had been refused by their guardian.

3) Newborns with gastrointestinal malformations.

Grouping Standard

1) Surgery group: 41 children who met the NEC diagnostic criteria and underwent surgery were included in the surgery group, consisting of 23 cases with intestinal perforation and 18 cases without perforation.

2) Conservative treatment group: 143 children who met the NEC diagnostic criteria without surgery and received conservative treatment.

Research methods

Research content

Imaging of ultrasonography, and metabolic disorder scores of children with NEC were recorded. Duke abdominal X-ray score (Duke abdominal assessment scale, DAAS) was calculated; seven clinical metrics of metabolic derangement (MD7) were combined to evaluate the best time for NEC surgery.

1) Basic information: gender, age of onset, weight of onset.

2) Indicators of metabolic disorders: blood culture, blood pressure, platelets, pH, blood sodium, rod-shaped granulocytes, and neutrophils.

3) Abdominal X-ray: intestinal dilatation, gas accumulation, thickening of the intestinal wall, gas accumulation in the intestinal wall, gas accumulation in the portal vein, and pneumoperitoneum signs.

4) Abdominal ultrasound: intestinal dilatation, thickening of the intestinal wall, intestinal wall ischemia, poor intestinal motility, gas accumulation in the intestine wall, and gas accumulation in the portal vein. The representative images of abdominal ultrasound are shown in Figure 1.

5) DAAS scoring method: Based on the abdominal X-ray examination of children with NEC, the 184 children with NEC included in the study were scored using the DAAS scoring standard developed by Coursey et al.[9] The specific criteria are as follows: 0 point, the intestinal cavity inflation is normal; 1 point, the intestinal cavity inflation is mildly dilated; 2 points, the intestinal cavity inflation is moderately dilated, or the intestinal cavity inflation is normal but with stool-like penetrating shadow; 3 points, the intestinal cavity is inflated with local and moderate expansion of the intestinal loop; 4 points, there is intestinal loop separation or local thickening of the intestinal wall; 5 points, there are multiple separated bowel loops; 6 points, intestinal wall gas accumulation, and there is suspected of abnormal abdominal clinical signs; 7 points, fixed or persistent dilatation of bowel loops; 8 points, clinically diagnosed or with intestinal wall gas; 9 points, there is portal vein gas; 10 points, there is pneumoperitoneum.

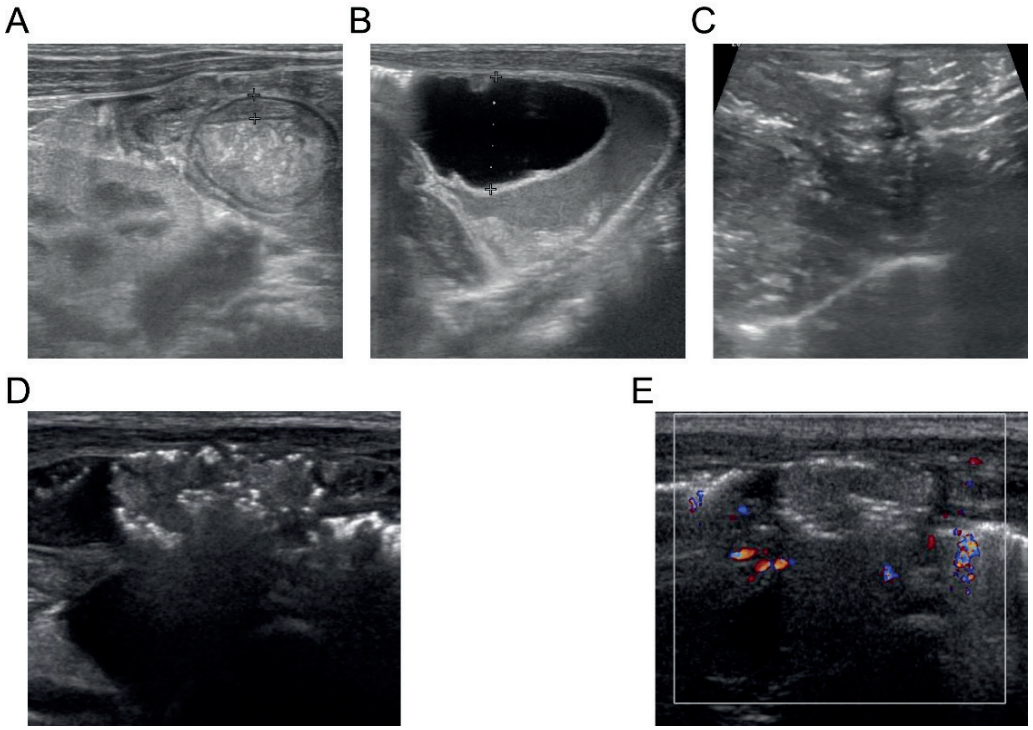

Fig. 1. The representative ultrasound images. A. Intestinal wall edema; B. Dilated bowel with seroperitoneum; C. Hepatic portal venous gas; D. Pneumatosis intestinalis; E. Intestinal ischemia.

6) MD7 assessment method: NEC children included in the study were scored by the frequency of occurrence of seven metabolic disorders[10], including positive blood culture, hypotension, thrombocytopenia, acidosis and hyponatremia that can't be corrected over 48 hours, increased band neutrophils, and neutropenia. The occurrence of one disorder was scored as 1 point, and the MD7 score ranged from 0 to 7.

Procedure

Univariate analysis of all indicators in the surgical group and non-surgical group was performed at first. After that, multivariate logistic regression analysis was performed on those with statistical significance to find out the relevant factors of NEC early surgical treatment and predict the best time for early surgery.

Statistical analysis

Data was analyzed by SPSS22.0 software and expressed as the number of cases and percentages. The data which conformed to the normal distribution or approximately was expressed by the mean ± standard deviation (x ± S); the data comparison between groups was performed by X^2 test, and t test was used for normal distribution. Binary logistic stepwise regression analysis was used for multivariate analysis, and the results were shown by modified odds ratios (*OR*), and 95% confidence intervals (95% confidence intervals, *95% CI*). $p<0.05$ was considered statistically significant.

Result

Research object status

184 cases of NEC were eventually incorporated into the diagnosis, of which 89 cases were ruled out. Finally, there were 41 cases in the surgical group and 143 cases in the conservative treatment group.

The single factor analysis

Research object analysis

There was no significant difference in gender and age of onset between the surgical group and the conservative treatment group ($p>0.05$). The difference between the number of weeks of pregnancy and weight at onset was statistically significant ($p<0.05$) (Table I).

X-ray results

X-ray analysis showed that there were statistically significant differences between the surgical group and conservative treatment group in thickening of the intestinal wall, pneumoperitoneum ($p<0.05$). But there were no statistically significant differences in intestinal dilatation, intestinal wall gas accumulation and portal gas accumulation ($p>0.05$), as shown in Table II.

Abdominal ultrasound results

B-scan ultrasound analysis showed that there were statistically significant differences between the surgical group and conservative treatment group in intestinal wall thickening and intestinal peristalsis weakening ($p<0.05$). There was also no statistical significance in intestinal flatulence, intestinal wall gas accumulation ($p>0.05$), as shown in Table III.

Multivariable Logistic regression analysis

As shown above, nine factors related to NEC surgery were found by the single factor analysis: gestational age, body weight at onset, X-ray seperated bowel loops, X-ray pneumoperitoneum, ultrasound intestinal wall thickening, ultrasound intestinal peristalsis weakening. The above indicators were included in the multivariate stepwise Logistic regression analysis and the results are shown in Table IV.

Table I. Comparison of general conditions between the surgery and the conservative treatment group.

Survey items	Factors	Surgery, *n*(%)	Conservative treatment, *n*(%)	χ^2	*p* value
Gender	Male	21 (51.22)	80 (55.94)	0.287	0.592
	Female	20 (48.78)	63 (44.06)		
Gestational age	<32 week	13 (31.71)	19 (13.29)	14.358	0.001
	32 week~	19 (46.34)	48 (33.57)		
	≥37 week	9 (21.95)	76 (53.15)		
The weight of onset	<1500 g	7 (17.07)	12 (8.39)	12.901	0.002
	1500 g~	24 (58.54)	51 (35.66)		
	≥2500 g	10 (24.39)	80 (55.94)		
Days of onset	<7 day	15 (36.59)	26 (18.18)	0.096	0.953
	7 day~	11 (26.83)	36 (25.17)		
	≥14	15 (36.59)	51 (35.66)		

Table II. Comparison of physical examination between surgery and conservative treatment group.

Clinical feature	Surgery, *n*(%)	Conservative treatment, *n*(%)	χ^2	*p* value
Intestinal dilatation	11 (100)	58 (56.64)	2.563	0.109
Intestinal wall thickening	5 (58.54)	55 (12.59)	10.004	0.002
Intestinal wall gas accumulation	7 (82.93)	18 (32.87)	0.546	0.460
Portal gas accumulation	3 (12.20)	3 (11.89)	2.752	0.097
Pneumoperitoneum	12 (4.90)	0 (0)	44.774	0.000

Table III. Comparison of abdominal ultrasound results between surgery and conservative treatment group.

Clinical feature	Surgery, *n*(%)	Conservative treatment, *n*(%)	χ^2	*p* value
Intestinal flatulence	4 (9.76)	20 (10.99)	0.503	0.478
Intestinal wall thickening	14 (34.15)	15 (10.49)	13.431	0.000
Intestinal wall gas accumulation	7 (17.07)	22 (15.38)	0.068	0.794
Intestinal peristalsis weakening	12 (29.27)	15 (10.49)	8.974	0.003

Table IV. Multivariable logistic regression analysis of factors related to NEC surgery.

Factor	*B* value	SE value	Wald value	*p* value	*OR* value	*OR* value *95%CI*	
						Lower limit	Upper limit
B-ultrasound intestinal wall thickening	5.119	1.960	6.819	0.009	167.1	3.585	7788.758
Gestational age	-1.592	0.694	4.857	0.028	0.217	0.056	0.844
Constant	2.543	2.345	1.176	0.027	12.715		

Table V. DAAS combined with MD7 score results.

Evaluation index		Number of cases	Conservative treatment	Surgery	
				Intestinal perforation	Intestinal necrosis
DAAS≥7					
MD7	≥3	5	0	5	0
	<3	21	7	9	5
DAAS<7					
MD7	≥3	17	8	3	6
	<3	136	125	5	6

Table VI. DAAS combined with MD7 to guide surgical timing (%).

	True positive rate	False positive rate	True negative rate	False negative rate
DSSA≥7	48.7	5.0	95.0	51.3
MD7≥3	53.8	16.9	83.1	46.2
DSSA≥7 and MD7≥3	12.8	0.0	100.0	87.2

B-scan ultrasound indicated intestinal wall thickening (*OR*=167.1, *95%CI*: 3.585-7788.758) and gestational age (*OR*=0.217, *95%CI*: 0.056-0.844) were independent factors to predict early surgical indications of NEC ($p<0.05$).

DAAS and MD7 evaluation results

DAAS and MD7 scores were calculated in 184 NEC children. After surgical treatment, intestinal perforation was confirmed in 5 cases with DAAS ≥7 and MD7 ≥3. There were 136 cases with DAAS <7 and MD7 <3, including 125 cases with conservative treatment and 11 cases with surgery, as shown in Table V. The true positive rate, false positive rate, true negative rate and false negative rate in the diagnose of necrotic bowel perforation guided by DAAS ≥7 and MD7 ≥3 were 12.8%(5/39), 0.0%(0/140), 100.0%(140/140) and 87.2%(34/39), respectively, as shown in Table VI. These data suggest the sensitivity of DAAS combined with MD7 score in predicting the timing of NEC surgery was poor.

Discussion

NEC is a common digestive disease worldwide. Although previous studies have tried to reveal the pathogenesis of NEC, the NEC process remains unclear. Scholars believe that many risk factors are involved in the pathogenesis of NEC.[4] The main purpose of surgical treatment for NEC children is to remove the necrotic intestinal tissue as completely as possible to reduce the abdominal inflammatory condition and preserve the intestinal tissue to avoid the occurrence of short bowel syndrome.[11] However, this indication is difficult to grasp in clinical work. Pneumoperitoneum, abdominal wall redness, abdominal mass, and intestinal obstruction are considered as indications for early surgical operation.[12,13] However, traditionally, the pneumoperitoneum on plain radiography is the only absolute criteria for operative intervention, and this makes some NEC children without intestinal perforation miss the therapeutic opportunity, leading to an increased risk of death. In recent years, studies have showed that early surgical intervention before intestinal perforation significantly raises therapeutic efficacy, as well as reduce the probability of mortality and complications.[5,8]

In the present study, we found that the presence of pneumoperitoneum on abdominal x-rays indicates intestinal perforation, suggesting surgical indications. Meanwhile, our present study suggested that intestinal perforation already existed before pneumoperitoneum was detected, which led to a loss of the predicted power. A previous study showed that the transit time of the entire intestine is prolonged in NEC condition, thus, the continuous X-ray examination describes food transportation throughout the intestine and records the regional dependent effects of NEC damage on intestinal transport in premature infants.[14]

In this study, we found that the predictive ability of surgery reached 90.24% (37/41) in NEC children who received surgical intervention according to the abnormal ultrasound results, including the thickened intestinal wall and poor intestinal wall ischemia not the intestinal gas accumulation, ascites, and portal gas accumulation. Additionally, we found that the ultrasound examination also revealed the thickening of the intestinal wall in the early stage of NEC lesions, and indicated intestinal wall ischemia and poor intestinal motility in the middle stage, as well as the deterioration of the intestinal wall in the late stage.[15] In recent years, abdominal ultrasound examination has been widely used and become more standardized in the diagnosis and treatment of NEC diseases.[16] Although the ultrasound is important in the early diagnosis of NEC intestinal wall, the examinational power is strongly influenced by the subjectivity of a sonologist, especially, a sonologist with insufficient experience.[17]

It was reported that abdominal ultrasound is a non-invasive imaging examination that is increasingly being used in the evaluation of NEC intestinal perforation.[18] Abdominal ultrasound is equipped to measure intestinal wall thickness and evaluate the characteristics of ascites. Cuna et al.[18] reported that the sensitivity of ultrasound mentioned is not high, but the sensitivity of ultrasound for intestinal wall thickening is high. Abdominal ultrasound has a high specificity in bowel lesion severity. For example, the severity of intestinal lesions revealed by ultrasound was earlier than that by X-ray imaging. However, ultrasonic testing is dependent on the capacity of the ultrasound operator. Therefore, it is necessary to improve the skills of sonologist in diagnosing NEC. What's more, Lok et al. reported that sensitivities below 70% and specificities largely above 80% for diagnosing definite NEC, but the timing of the ultrasound is not specified.[19] Most of the ultrasonic testing was performed 4 to 6 hours after the X-ray testing, which is a limit for ultrasound application. Actually, we can increase the frequency of ultrasound examinations and make good use of the non-invasive characteristics of ultrasound examinations to dynamically monitor the degree of intestinal infection in children, so as to find the possibility of intestinal necrosis for the first time.

In our present study, we transferred the subjective abdominal X-ray results into the half quantitative analysis using the DAAS evaluation system. Meanwhile, the incidence frequency of seven metabolic disorders (MD7), which include positive blood culture, hypotension, thrombocytopenia, uncorrected acidosis and hyponatremia over 48 hours, rod nucleocytosis and neutropenia, were selected as the joint evaluation system of the DAAS.[10] The results suggested that the DAAS combined with MD7 evaluation system made a high clinical diagnostic specificity for guiding surgical treatment in NEC children but with low sensitivity. This indicates that it is likely to lead to misdiagnosis and delay the best timing of surgery using the DAAS evaluation system in NEC children, which is mainly due to the rapid progress of NEC and the subjectivity of radiologists in reading the film. Thus, the DAAS combined with MD7 evaluation system were not suitable for the indicator of surgical timing selection in NEC children.

The development of NEC is affected by many factors, the early prevention and detection of high-risk factors are particularly important in the diagnosis and therapy of NEC. Our present study suggests that B-scan ultrasound showing thickening intestinal wall and poor intestinal peristalsis can be used as early surgical indications for children with NEC. The sensitivity of DAAS combined with MD7 score in predicting the timing of NEC surgery was poor. Accurate ultrasound is a better predictor of the timing of NEC surgery than X-ray. Finally, the timing of surgery should be selected according to the actual situation of children with NEC.

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Ethical approval

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Author contribution

The authors confirm contribution to the paper as follows: study conception and design: LY, LM; data collection: CL; analysis and interpretation of results: LY, YC, LX; draft manuscript preparation: CL, LM. 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 the predictability of clinical and radiological findings in the diagnosis of malrotation

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ABSTRACT

Background. To evaluate the predictability of clinical and radiological findings in the diagnosis of malrotation.

Methods. Between 2010 and 2020, children with presumptive diagnosis of malrotation were included. The demographic features, clinical and radiological findings, operative findings and outcome were recorded. The upper gastrointestinal series (UGIS) were evaluated by two radiologists. All parameters were correlated with surgical findings to evaluate the predictability.

Results. Seventy patients were included. The presenting symptom was bilious vomiting in 29 cases (41.4%), and atypical symptoms (non-bilious vomiting, food refusal, etc.) in 40 cases (57%). One of the cases (1.6%) was asymptomatic and diagnosed incidentally during UGIS. 52 cases had abdominal X-ray and 14 (26.9%) of them were normal. Doppler ultrasonography (US) (n=20) revealed evidence of malrotation in 13 cases (65%). The location of duodenojejunal junction (DJJ) in UGIS was compatible with malrotation in 33 cases. 48 (61%) cases underwent surgical exploration; 35 cases had malrotation and seven cases had midgut volvulus. Median follow-up time was one year (0.5-7 years). Volvulus has recurred in one case and another case operated for volvulus died because of short bowel syndrome. The statistical analysis for predictability revealed that bilious vomiting (sensitivity: 57.1%, specificity: 82.1%), Doppler US (sensitivity: 92.3%, specificity: 75%) and right-sided DJJ in UGIS (sensitivity: 96.8%, specificity: 75%) have highest predictability.

Conclusions. The bilious vomiting, Doppler US findings and right-sided DJJ have the highest predictability to confirm the diagnosis. However, presenting with atypical symptoms and having atypical or normal findings in UGIS do not rule out malrotation.

Key words: malrotation, bilious vomiting, treitz ligament, duodenojejunal junction.

Malrotation represents a spectrum of disorders occurring due to abnormal bowel rotation and fixation during embryogenesis. This abnormal bowel development leads to a narrow mesenteric root lying between medially-located duodenojejunal junction (DJJ) and cecum on left side, and peritoneal bands crossing over duodenum.[1] The most catastrophic scenario of this pathologic condition is twisting of midgut around the narrow mesenteric root causing bowel ischemia, necrosis and eventually sepsis and death. On the other hand, a small number of cases do not become symptomatic throughout their life.[1] Moreover, an important minority of patients experience late-presenting atypical symptoms such as gastroesophageal reflux, intermittent vomiting, chronic and intermittent abdominal pain, and failure to thrive.[1-3]

Malrotation is seen in 1 in 500 live births and 75% of the cases become symptomatic during neonatal period and up to 90% of the cases are diagnosed during infancy.[4] The most common

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symptom of these early-diagnosed cases is sudden onset of bilious vomiting. The diagnosis and treatment are obvious in these patients.[4,5] However, asymptomatic cases and cases with atypical symptoms require radiological examinations for diagnosis. Eventually, a controversy exists regarding the management of incidentally diagnosed malrotations. Although there is still an ongoing disagreement regarding management of asymptomatic cases, most surgeons prefer to perform prophylactic surgery to prevent the risk of emergency situations including volvulus.[5] Therefore, the predictability of radiologic investigations that may become symptomatic have paramount importance in deciding prophylactic surgery in asymptomatic cases. The most frequently used radiological sign for the diagnosis of malrotation is the evaluation of DJJ with upper gastrointestinal contrast series (UGIS).[2,6,7] The evaluation and interpretation of the findings in UGIS has a crucial role in the diagnosis since several clinical scenarios such as splenomegaly, gastric distention, and retroperitoneal tumors may mimic malrotation. Furthermore, the variations of DJJ positioning should be kept in mind to prevent false positive or false negative results.[7,8] In recent decade, Doppler ultrasonography (US) has becoming the first diagnostic tool choice in the diagnosis of malrotation with an advantage of no radiation risk.[6,9,10] Although there are well-defined sonographic signs of malrotation in the literature, it was also stated that the normally located superior mesenteric vein does not exclude malrotation.[6] Therefore, we conducted a study reviewing the historical medical records of our cases to evaluate the predictability of clinical and radiological findings in the diagnosis of malrotation.

Material and Methods

We carried out a case series analysis on all cases that were evaluated for suspicion of malrotation between 2010 and 2020. At first, we performed a keyword search of radiology reports including the keywords 'malrotation, DJJ position, Treitz ligament position'. Then, the extracted reports were re-identified for the patients' characteristics and the patients evaluated in the pediatric surgery department were included. The excluded cases were adult ones, the ones that were not admitted to the department of pediatric surgery, and ones in which images of radiological examinations could not be obtained from the radiology picture archiving and communication system.

After approved by the local ethical committee and the local hospital management, we exported 357 records containing the keywords 'malrotation, DJJ position, Treitz ligament position' via the keyword search of radiology reports. We had 184 records after the repeated examinations were excluded. The cases of these records were reviewed from the hospital patient recording system and 80 cases were found eligible for investigation according to the pre-defined inclusion criteria. The images of the radiological examinations could not be found in the hospital system for 10 cases. Therefore, we included a total of 70 cases in the final review of the study.

The medical records of the included cases were evaluated by using the hospital patient recording system. The demographic features, clinical findings, Doppler US results, operative findings and outcome were recorded from the hospital patient recording system. The radiological examinations were re-evaluated by two blind radiologists from the radiology

picture archiving and communication system. Since only the records of abdominal X-ray and UGIS exist in the database, these images of the included cases could be re-evaluated.

For the measurement of predictability, we defined each parameter of the admission symptoms, abdominal X-ray findings, Doppler US findings and UGIS findings separately. The admission symptoms were evaluated in three groups as asymptomatic, symptomatic (bilious vomiting), and atypical symptoms (non-bilious vomiting, intermittent abdominal pain, food refusal, failure to thrive). Abdominal X-ray findings were evaluated as normal if normal bowel gas distribution is seen and pathologic if gasless abdomen, air-fluid levels or bowel gas collected only on one side are seen. Doppler US findings were evaluated as normal if superior mesenteric artery (SMA) and vein (SMV) positions are normal and pathologic if there is a whirlpool sign or SMV is located anterior or on the left side of SMA. Each finding in UGIS was considered as a different parameter. These findings were defined according to the position of DJJ; (a) malrotation, the duodenum and jejunum remain to the right of the spine, (b) malrotation with a corkscrew duodenum and jejunum, (c) malrotation with low position of the DJJ at midline and inferior of duodenal bulb, (d) partial rotation of the duodenum with the DJJ over the right pedicle, (e) normal location of the DJJ in the left upper quadrant. The location of cecum was considered as mobile cecum or located at left side of the abdomen. Then we correlated all parameters with surgical outcome to evaluate predictability separately.

Statistical analysis was performed by using The Statistical Package for the Social Sciences version 23.0 for Windows (SPSS, Inc., Chicago, IL, USA). The descriptive statistics for quantitative data are given in arithmetic mean, standard deviation, median, and minimum-maximum values. Qualitative data were summarized using frequency and percentages. Pearson Chi-square test or Fisher's Exact test were used to compare categorical variables. Sensitivity and specificity was obtained for each diagnostic parameter. A p value of less than 0.05 was considered to indicate a statistically significant difference. The study was performed under adherence to the Declaration of Helsinki and by approval of the Local Ethical Committee (GO19/400-2019/10-38).

Results

We included a total of 70 cases into the final review of the study. 60% of cases (n=42) were less than 1 year of age at the time of admission, and 15 of them (21.4%) were newborn at admission. The remaining 40% of cases (n=28) were older than 1 year of age at admission. The presenting symptom was bilious vomiting in 29 cases (41.4%) and atypical symptoms (non-bilious vomiting, food refusal, etc.) in 40 cases (57%). Only one case was asymptomatic and Abdominal US was performed for cystic fibrosis work-up and nephrocalcinosis. The US surprisingly revealed that SMV was located anterior to SMA and the patient was diagnosed as malrotation. The diagnosis was confirmed with UGIS.

Abdominal X-ray was performed in 52 cases and revealed normal in 26.9% (n=14). The pathologic findings in abdominal X-ray were abnormal gas distribution in 35 cases (67.4%), air-fluid level in 2 cases (3.8%) and gasless abdomen sign in 1 case (1.9%).

Doppler US was performed in 20 cases and revealed evidence of malrotation in 13 (65%). Among these 13 cases, the whirlpool sign was present in 4 cases (20%) and SMV was located at left side of SMA in 9 cases (45%).

Table I. The summary regarding the management of cases in comparison with admission symptoms and UGIS findings.

	Management			
	Medical follow-up (n=22)	Surgery (Ladd procedure) (n=42)	Surgery (Other pathologies) (n=6)	Total
Admission symptom				
No symptom	1	0	0	1
Atypical symptom	19	18 (V:1)	3	40
Symptomatic (bilious vomiting)	2	24 (V:6)	3	29
UGIS findings				
a. Malrotation with right sided DJJ	3	22	0	25
b. Malrotation with corkscrew sign	0	8 (V:5)	0	8
c. Malrotation with low position of the DJJ at midline	5	5 (V:1)	0	10
d. Partial rotation of the duodenum with the DJJ over the right pedicle	9	6 (V:1)	2	17
e. Normal location of the DJJ	5	1	4	10

UGIS: Upper gastrointestinal series, DJJ: Duodenojejunal junction

The UGIS examination revealed that the location of DJJ was compatible with malrotation in 33 cases (47%). Among these 33 cases, DJJ was located at the right side of the vertebra in 25 cases and at the right side of the vertebra with corkscrew sign in 8 cases. The UGIS revealed malrotation with low position of the DJJ at midline in 10 cases (14.3%) and revealed partial rotation of the duodenum with the DJJ over the right pedicle in 17 cases (24.3%). The location of cecum could be examined in 28 cases revealing left sided cecum in 3 cases (10.7%) and mobile cecum in 13 cases (46.4%).

Forty-eight (61%) cases underwent surgical exploration and 35 of them had malrotation, 7 of them had midgut volvulus and 6 of them had other surgical pathologies (duodenal web, adhesive band, etc.) (Table I). The median follow-up time was 1 year (0.5-7 years). Volvulus has recurred in one of the surgically managed cases and one case operated for volvulus died because of short bowel syndrome.

The parameters including admission age, admission symptom, and pathological findings in abdominal X-ray, Doppler US and UGIS were correlated with surgical findings to measure the predictability of each parameter. The detailed results of the comparison are given in Table II revealing that statistically significant results are found for the parameters including bilious vomiting, Doppler US findings, and positioning of DJJ at right side of vertebra in UGIS. The low position of DJJ at midline and partial rotation of the duodenum with the DJJ over the right pedicle had a non-significant effect on surgical outcome. The normal position of the DJJ in UGIS (n=10) did not exclude malrotation in one case. The statistical analysis for predictability revealed that bilious vomiting (sensitivity: 57.1%, specificity: 82.1%), Doppler US (sensitivity: 92.3%, specificity: 75%) and positioning of DJJ at right side of vertebra in UGIS (sensitivity: 96.8%, specificity: 75%) had the highest sensitivity and specificity values. The sensitivity and specificity of the parameters are given in Table III.

Table II. The comparison of each parameter with surgical findings.

	Malrotation/Volvulus *		
	Present	Absent	Total
Admission age			
<1 age	27	15	42 (60%)
>1 age	13	15	28 (40%)
			p=0.139
Admission symptom			
None or atypical symptom	18	23	41 (58.5%)
Bilious vomiting	24	5	29 (41.5%)
			p=0.001
Abdominal X-ray finding (n=52)			
Normal	6	8	14 (26.9%)
Pathological	26	12	38 (73.1%)
			p=0.093
Abdominal USG finding (n=20)			
Normal	1	6	7 (35%)
Pathological	12	1	13 (65%)
			p=0.001
UGIS finding (n=70)			
a. Malrotation with right sided DJJ	22	3	25 (35.7%)
b. Malrotation with corkscrew sign	8	0	8 (11.3%)
c. Malrotation with low position of the DJJ at midline	5	5	10 (14.3%)
d. Partial rotation of the duodenum with the DJJ over the right pedicle	6	11	17 (24.4%)
e. Normal location of the DJJ	1	9	10 (14.3%)
			p<0.001

*: **present**: malrotation/volvulus was found in surgical exploration, **absent**: cases that did not surgically managed or did not have malrotation/volvulus in surgical exploration. USG: Ultrasonography, UGIS: Upper gastrointestinal series, DJJ: Duodenojejunal junction

Table III. The summary of statistical analysis showing sensitivity and specifity values for each parameter.

	Sensitivity	Specificity
Bilious vomiting	57.1%	82.1%
Abdominal X-ray	81.3%	40%
Doppler US	92.3%	85.7%
UGIS Parameters		
Malrotation with right sided DJJ with/without corkscrew sign	96.8%	75%
Malrotation with low position of the DJJ at midline	83.3%	64.3%
Partial rotation of the duodenum with the DJJ over the right pedicle	85.7%	45%
UGIS revealing mobile cecum	62.5%	66.%
UGIS revealing cecum located at left side	25%	85.7%

US: Ultrasonography, UGIS: Upper gastrointestinal series, DJJ: Duodenojejunal junction

Discussion

The life-threatening consequences of malrotation make clinicians alert for immediate diagnosis of malrotation especially in neonates complaining of sudden onset bilious vomiting. Previous studies showed that the increase in awareness of malrotation and its consequences have caused an increased number of UGIS examinations but did not change the detection rate of malrotation.[11] It was reported that malrotation was found in 9% of UGIS of infants having bilious vomiting.[11] This challenge occurred not only because of the possibility of other etiologies of bilious vomiting, but also the possibility of atypical and asymptomatic presentation of malrotation. Therefore, radiological examinations become critical in precise diagnosis and consequently early management of malrotation. On the other hand, as Goldman-Yassen et al. stated, the yield of UGIS should be considered while we are performing in cases with non-specific symptoms in order to decrease radiation exposure.[12] In the present study, malrotation was confirmed via surgical exploration in 82% of cases having bilious vomiting. Whereas, malrotation was confirmed in 44% of cases having atypical symptoms. This significant difference also shows an important point that atypical symptoms do not rule out the malrotation.

Since malrotation is the abnormal positioning of intestines due to an embryological defect in intestinal rotation and fixation, the main target of radiological examinations is to show this abnormal positioning of the bowels. The UGIS is still accepted as the gold standard examination method in diagnosis of malrotation. The important landmark that should be examined via UGIS is mainly the location of DJJ and secondly the location of cecum. The findings that are seen in malrotation are; DJJ located at the right side of the left pedicle of vertebra, DJJ located inferior to duodenal bulb, anteriorly located DJJ on lateral images, gathering of proximal jejunal loops at the right side of abdomen.[7] Although location of cecum can also be used as a landmark in diagnosis of malrotation, it should be kept in mind that normal location of cecum does not exclude malrotation.[7] Although these landmarks are well-defined in the literature, there could be subtle findings or several variations that make the diagnosis challenging.

The sensitivity of UGIS is reported as 93 to 100% in the literature. However, false positive results up to 15% or false negative results up to 6% can be seen.[2,6,7] False positive results can be seen in several clinical situations including splenomegaly, retroperitoneal tumors, gastric over distention, small bowel obstruction and scoliosis that could mimic malrotation in UGIS images.[2] In the present study, none of these conditions were present in the patients. Nevertheless, malrotation was not confirmed in 12% of cases having right-sided DJJ in UGIS. Several variations of duodenal anatomy should be kept in mind while examining the UGIS such as wandering duodenum, duodenum inversum and mobile duodenum to prevent false positive results.[7,8] Although the location of DJJ is the gold standard landmark in diagnosis of malrotation, sensitivity and specificity for each separate location of DJJ has not been reported before. In the present study, we calculated sensitivity and specificity for each separate location of DJJ, and found that the highest scores were seen for right-sided DJJ. The DJJ located at midline or inferior to duodenal bulb has lower sensitivity and specificity values with a statistically non-significant difference.

In recent decades, Doppler US has becoming the first choice diagnostic tool in diagnosis of malrotation since it has less radiation risk.[6,9,10] The diagnostic findings in Doppler US are twisting of SMV around SMA, namely whirlpool sign, and inversion of SMA and SMV course.[10,13] Although it is dependent on the experience of radiologist, it can also be performed as a fast

diagnostic method called point-of-care US especially in emergency conditions.[13] However, normal SMA and SMV course does not exclude malrotation[14] as we found in our study. In the present study, we performed Doppler US in 20 cases with high sensitivity (92.3%) but relatively lower specificity (85.7%). These results are not compatible with the ones in some reports revealing 93.8% sensitivity and 100% specificity for Doppler US.[9] This difference may be due to the small number of cases having non-specific symptoms in that study9 in contrast to our study.

The management of clinically and radiologically suspected malrotation cases is obviously surgical exploration especially in emergency conditions. However, elective surgery or watchful waiting is controversial in these cases since elective surgery is not totally innocent and watchful waiting leaves the patient susceptible to catastrophic consequences such as volvulus and intestinal necrosis. In the present study, the long-term complications including recurrent volvulus and short bowel syndrome and death were seen in urgently operated cases. This observation confirms the reports revealing that emergency operation has higher complication rates.[5] Until further studies will show which patients will become symptomatic and undergo volvulus, we suggest operating all suspected cases to prevent catastrophic consequences of malrotation.

This study has several limitations because of its retrospective nature and small sample size. Despite its limitations, it has some strong points such as examining each separate parameter for its predictability in diagnosis of malrotation. Nevertheless, further prospective studies are needed to confirm our results.

In conclusion, the bilious vomiting, Doppler US findings consistent with malrotation and right-sided DJJ have the highest predictability to confirm the diagnosis. However, presenting with atypical symptoms and having atypical or normal findings in UGIS do not rule out malrotation.

Ethical approval

The study was performed under adherence to the Declaration of Helsinki and by approval of the Local Ethical Committee (GO19/400-2019/10-38).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: TS, FCT, MH; data collection: OBT, BRU, HNO; analysis and interpretation of results: UEA, MH, TS, FCT; draft manuscript preparation: OBT, HNO. All authors reviewed the results and approved the final version of the manuscript.

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Original Article

Early predictors of high-flow nasal cannula oxygen treatment failure in patients with respiratory distress admitted to the pediatric emergency department

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ABSTRACT

Background. High-flow nasal cannula (HFNC) therapy is a relatively new method used in patients with respiratory distress. The aim of the study was to evaluate the outcomes and to determine the baseline predictors of HFNC treatment failure in children with acute respiratory distress/failure in the pediatric emergency department.

Methods. Children with respiratory distress/failure aged 1 month to 18 years who underwent HFNC therapy with the pre-established protocol were retrospectively analyzed. HFNC therapy was used in respiratory and non-respiratory pathologies. HFNC failure was defined as the need for escalation to non-invasive ventilation or invasive mechanical ventilation. HFNC responders and non-responders were compared based on baseline clinical data.

Results. Of the 524 cases (median age:13 months; 292 males / 232 females), 484 (92.4%) had respiratory tract and 40 (7.6%) had non-respiratory tract pathologies. HFNC therapy was unsuccessful in 62 (11.8%) patients. The success rates were 81% and 55% in respiratory and non-respiratory diseases, respectively. In children with respiratory system pathologies, the pre-treatment venous pCO_2 level (p: 0.045; OR: 0.958; 95%CI: 0.821-0.990) and the clinically important radiological finding on chest X-ray (lobar infiltration, atelectasis, pleural effusion) (p: 0.045; OR: 3.262; 95%CI: 1.178-9.034) were the most significant parameters in predicting HFNC failure. In children with non-respiratory pathologies, the pre-treatment venous lactate level (p: 0.008; OR: 1.558; 95%CI: 1.125-2.158) was a significant predictor of HFNC failure. There were no cases of pneumothorax or any other reported adverse effects related to HFNC therapy.

Conclusions. HFNC treatment is a safe oxygen therapy in children with respiratory distress/failure due to various etiologies in the emergency department. The lower venous pCO_2 level increases and the clinically important radiological finding on chest radiograph decreases the success of HFNC treatment in respiratory pathologies. The higher venous lactate level is a predictor of HFNC treatment failure in non-respiratory pathologies.

Key words: pediatric emergency, oxygen therapy, respiratory distress, high-flow nasal cannula.

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High-flow nasal cannula (HFNC) oxygen therapy is one of the non-invasive methods of respiratory support therapy that is well tolerated by patients and has been used in neonates, infants, children, and adults in recent years.[1-3] The device consists of an air/oxygen

mixer, an active humidifier, a heated circuit, and a nasal cannula. Application and patient follow-up are extremely easy for healthcare providers. Reduction of anatomical dead space, positive end expiratory pressure effect, and the constant fraction of inspired oxygen are the physiological effects of HFNC. HFNC therapy decreases the work of breathing, improves oxygenation, and provides a continuous positive airway pressure (CPAP) effect for a range of respiratory distress pathologies.[1,2,4,5] However, non-invasive (NIV) and invasive mechanical ventilator (MV) support may be delayed if treatment is persisted even though no positive clinical effect is observed. This may adversely affect the patient's clinical outcomes. It is therefore important to predict the failure of HFNC oxygen therapy before and during treatment. However, there are very few studies to predict treatment failure, especially in the emergency department.[2,5-7]

Most of the studies in HFNC were performed on respiratory system diseases, especially bronchiolitis.[3,8-10] There are a limited number of studies in the literature on non-respiratory pathologies that may cause respiratory distress such as sepsis, heart failure, and metabolic diseases. Finally, the clinical studies were generally conducted in intensive care units.[2,5-7]

The aim of the study is to evaluate the clinical outcomes and to determine the baseline predictors of HFNC treatment failure for children with respiratory distress/failure in the pediatric emergency department.

Material and Methods

The patients aged 1 month to 18 years who underwent HFNC oxygen treatment with a pre-established protocol between the dates of 01.01.2016 and 01.04.2018 in the pediatric emergency department of Tepecik Teaching and Research Hospital were retrospectively analyzed. Children transferred to another hospital for any reason were excluded from the study. The study was approved by the Ethics Committee of Tepecik Training and Research Hospital (2018/6-1).

Tepecik Teaching and Research Hospital is a tertiary pediatric referral hospital, which is located in a populous and low socio-economic district of Izmir. The pediatric emergency department has a pediatric emergency sub-specialty fellowship program and approximately 165.000 pediatric cases visit per year.

Respiratory distress is typically characterized by signs of increased work of breathing, such as tachypnea, use of accessory muscles, nasal flaring, and/or retractions. The diagnosis of respiratory failure requires at least two clinical signs of respiratory distress and one laboratory criterion (arterial $PaCO_2$> 50 mmHg and PaO_2 < 50 mm Hg in room air; $PaCO_2$>50 mm Hg and pH<7.30; arterial $PaCO_2$ > 60 mmHg and PaO_2 < 60 mm Hg when FiO_2 0.60 in patients without cyanotic heart disease; oxygen saturation <90% when FiO_2 0.60). Oxygenation of the patients was monitored by pulse oximetry and lower than 94% was accepted as hypoxemia.[2]

In our retrospective study group, oxygen support was started with a simple mask, nonrebreathing mask or HFNC according to the pathology that causes respiratory distress, clinical severity, and clinician decision. Treatment failure is defined if three following criteria are met and an escalation of treatment or level of care is required: Heart rate remains unchanged or increased compared to admission, respiratory rate remains unchanged or increased compared to admission, oxygen requirement in HFNC support exceeds FiO2 ≥ 50 % to maintain a targeted oxygen-saturation level. If the patient is not hemodynamically (heart rate, capillary refill time, quality of central and peripheral pulses, level of consciousness, etc.) stable at the end of the first hour of HFNC support, it is considered as a treatment failure criterion alone. We have used a pre-established HFNC protocol since 2013. According to this protocol, the initial flow rate is 5-10 L/min in infants and 15-20 L/min in children. If the oxygen saturation is <94%

initial FiO2 level is set as 1.00; if the oxygen saturation is >93%, FiO_2 is set as 0.30. When the target oxygen saturation level is reached, FiO_2 is reduced by 0.05-0.10 every 3-5 minutes. The flow rate is reduced by 1 L/min in infants, 5 L/min in young children and 5-10 L/min in older children at one-hour intervals when the FiO_2 level is below 0.50. According to our HFNC protocol, the patient is escalated to NIV or MV, if there is any clinical deterioration (increase in oxygen requirement, work of breathing, respiratory rate, or heart rate) in the first 30 min. If there is no change in clinical findings in the first 30 min, the patients are followed for at least an additional 30 min.[2] If the patient does not improve clinically, the maximum follow-up time is 2 hours under HFNC support.[2,5-7]

The demographic, clinical and laboratory findings of the patients were recorded by the same person on a standard form. Venous blood gas analysis is routinely used in our department. Arterial blood sample is taken in patients considered to have respiratory failure. Three pediatric emergency specialists evaluated chest X-rays retrospectively. The presence of at least one of the clinical manifestations of lobar infiltration, atelectasis, traumatic pulmonary contusion, pleural effusion, and pulmonary edema was accepted as a clinical important radiographic finding. The duration of HFNC therapy was recorded as days. In the hospital medical registration system, HFNC treatments lasting less than one day are recorded as one day. The patients were divided into two main groups as patients with respiratory system pathologies and non-respiratory system pathologies.

The patients were classified into five sub-groups according to the pathophysiology of the underlying disease in terms of diagnostic groups: Respiratory system (bronchiolitis, pneumonia, asthma attack, croup syndrome, foreign body aspiration, whooping cough, chest trauma, carbon monoxide intoxication), cardiovascular system (heart failure), central nervous system (head trauma, status epilepticus), gastrointestinal system (acute pancreatitis), hematologic/oncologic diseases, and metabolic/endocrine diseases (inherited metabolic disease, sepsis, diabetic ketoacidosis). In each main group, patients were divided into two groups: HFNC responders (success) and non-responders (unsuccessful, failure). HFNC failure was defined as the need for escalation to NIV or MV.

The HFNC system (Fisher & Paykel Healthcare Airvo 2) comprises a humidifier (MR290) and a continuous-flow circuit (900PT531 for infants, 900PT501 for children). We selected the nasal prong size that best fit each patient's nostrils (Optiflow, OPT318, OPT842, OPT844, OPT846).

Statistical Analysis

In the statistical analysis, the numerical data were expressed as the median and interquartile range (IQR) when they were not normally distributed. Normally distributed data was expressed as mean ± standard deviation (SD) and minimum and maximum values. The categorical data were expressed in numbers (n) and percentages (%). Mann-Whitney U test or Student's t-test was used to compare the numerical data in two independent groups, and Chi-square or Fischer's Exact Test was used to compare the categorical data, whichever was appropriate. Multivariable logistic regression analysis was used to identify risk factors for HFNC failure. The odds ratio (OR) and 95% confidence interval (CI) were calculated.

Statistical analyzes were performed using SPSS for Mac (IBM Statistical Packages for the Social Sciences; Armonk, NY, USA) 20.0 and $p<0.05$ was considered statistically significant.

Results

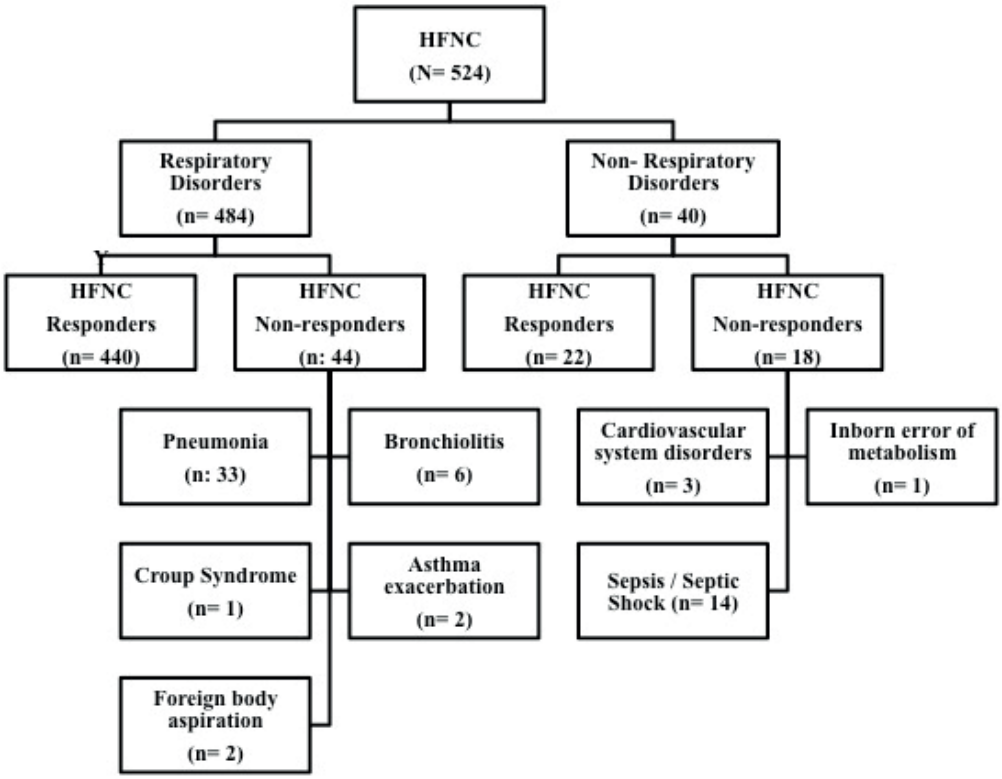
In the present study, the medical records of 529 patients were analyzed. Five cases were excluded from the study because they were transferred to another hospital. Finally, 524 patients (median age: 13 months; IQR: 6-30 months; minimum: 1 month, maximum: 231 months; 292 boys/232 girls) were included

Table I. Diagnosis and treatment indications of patients receiving high-flow nasal cannula oxygen therapy.

Diagnosis, n, (%)	Indications, n, (%)
Pneumonia, 206 (39,3)	
Bronchiolitis, 171 (32,6)	Respiratory system pathology, 484 (92,4)
Asthma exacerbation, 76 (14,5)	Non-Respiratory system pathology, 40 (7,6)
Sepsis, 23 (4,4)	Metabolic/endocrine disorders, 24 (4,6)
Croup Syndrome, 20 (3,8)	Cardiovascular, 8 (1,5)
Heart failure, 6 (1,1)	Central nervous system, 5 (1)
Foreign body aspiration, 4 (0,8)	Hematologic/oncologic disorders, 2 (0,4)
Trauma, 4 (0,8)	Gastrointestinal system, 1 (0,2)
Poisoning, 4 (0,8)	
Inborn errors of metabolism, 3 (0,6)	
Pertussis, 3 (0,6)	
Status epilepticus, 2 (0,4)	
Pulmonary edema, 1 (0,2)	
Acute pancreatitis 1 (0,2)	

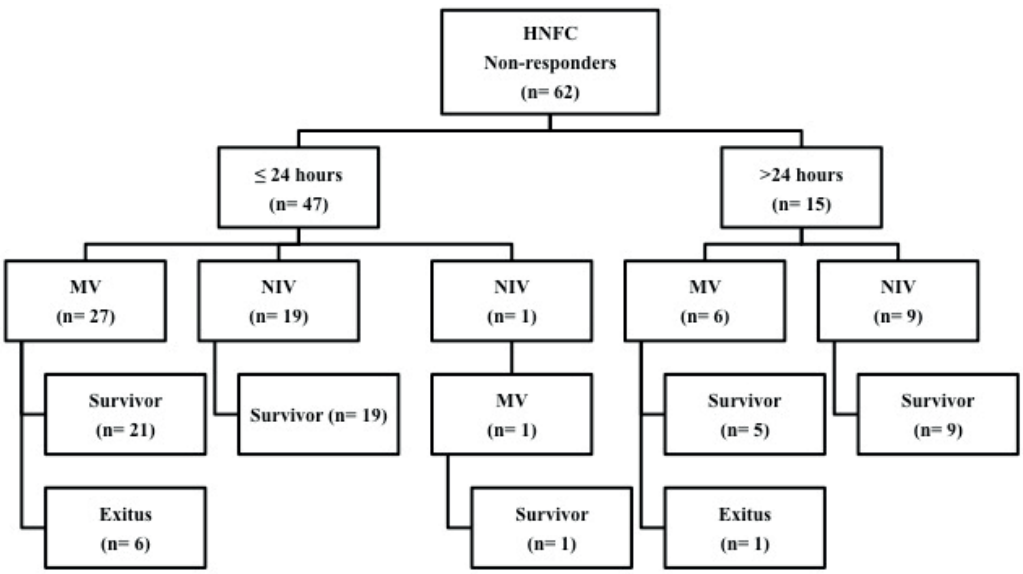
in the study. A total of 210 patients (40.1%) had a chronic disease. Respiratory failure was determined in 96 patients (18.3%). In general, HFNC was used in 484 (92.4%) patients with respiratory pathologies and in 40 (7.6%) patients with non-respiratory pathologies (Table I). One patient died in the emergency department (6-month-old girl, heart failure, hypertrophic cardiomyopathy), 102 patients (19.5%) were admitted to the pediatric intensive care unit and 376 patients (71.8%) were admitted to pediatric wards. A total of 45 patients (8.6%) were discharged from the emergency department at the end of the follow-up. In the follow-up, three patients were transferred from the wards to the pediatric intensive care unit. Thus, a total of 105 (20%) patients were followed in the pediatric intensive care unit. HFNC treatment was unsuccessful in 62 patients (11.8% of 524 patients: 60% in sepsis, 50% in cardiovascular disease; 16% in pneumonia, 3.5% in bronchiolitis, 2.6% in asthma) (Fig. 1). Among 62 patients in HFNC non-responder group, 33 patients were escalated to MV, and 28 were escalated to NIV (Fig. 2). A total of 7 patients (1.3%) died (3 sepsis, 3 heart failure and 1 inborn error of metabolism (Table II). The clinically significant radiologic findings on chest X-ray were determined in 54 (10.3%) patients (28 lobar infiltration, 24 atelectasis, 1 lobar infiltration and pleural effusion,1 pulmonary edema). The median duration of HFNC was 2 days (IQR:1-4 days; minimum: 1 day, maximum: 30 days).

The HFNC failure rate was 44/484 (9%) in children with respiratory pathologies, 18/40 (45%) in children with non-respiratory pathologies. We compared HFNC responders and non-responders in children with respiratory pathologies. Age of patient, initial venous pH and pCO_2, leukocyte and neutrophil counts in peripheral blood, and existence of clinically important finding on chest X-ray were significantly different between the two groups ($p<0.05$). According to the logistic regression analysis, existence of a clinically important finding on chest X-ray (p: 0.045; OR: 3.262; 95%CI: 1.178-9.034) was found to predict HFNC failure. Conversely, lower initial venous PCO_2 level (p: 0.045; OR: 0.958; 95%CI: 0.821-0.990) decreased the HFNC failure risk (Table III). Among patients with non-respiratory pathologies, initial venous lactate and C-reactive protein levels were significantly higher in non-responder group ($p<0.05$) (Table IV). Higher venous lactate level (p: 0.008; OR: 1.558; 95%CI: 1.125-2.158) was determined as a predictor of HFNC failure in logistic regression analysis. During the study, none of the identified adverse effects were related to HFNC therapy.



HNFC: High-flow nasal cannula oxygen therapy, n: number of patients

Fig. 1. Etiological distribution of patients on high-flow nasal cannula oxygen therapy.



HNFC: High-flow nasal cannula oxygen therapy; MV: Mechanical ventilation; NIV: Non-invasive ventilation; n: number of patients

Fig. 2. Clinical outcomes of patients who high-flow nasal cannula oxygen therapy failed.

Table II. Clinical features of deceased patients.

Diagnosis	Age	Gender	Chronic disease, pathology, congenital anomaly	Results
Heart Failure	5 Months	Female	Ventricular Septal Defect, Congenital Mitral Insufficiency	Intubation (Day 7) Exitus (Postoperative day 8)
Heart Failure	6 Months	Female	Hypertrophic Cardiomyopathy	Intubation (Day 1) Exitus (Day 1)
Heart Failure	4 Months	Male	Ventricular Septal Defect,	Intubation (Day 1) Exitus (Day 32)
Sepsis	2 Months	Female	Truncus Arteriosus	Intubation (Day 1) Exitus (Day 7)
Sepsis	16 Months	Male	Cerebral Palsy	Intubation (Day 1) Exitus (Day 5)
Sepsis	2 Months	Female	None	Intubation (Day 1) Exitus (Day 13)
Metabolic Disorders	2 Months	Female	Inborn error of metabolism	Intubation (Day 1) Exitus (Day 14)

Table III. Comparison of responders and non-responders receiving HFNC treatment for respiratory pathology.

Parameter	HFNC Responder (N= 440)	HFNC Non-responder (N= 44)	P value	Logistic Regression	
				P value	OR (%95 CI)
Age, (month), median (IQR)	14 (7-30)	10 (2-30)	0.027*	>0.05	-
Male Gender, n (%)	249 (56,6)	30 (68,2)	0.138**	-	-
Chronic disease, n (%)	171 (38,9)	17 (38,6)	0.976**	-	-
Clinically important findings on chest x-ray, n (%)	37 (8,4)	13 (29,5)	<0.001**	0.045	3,262 (1,178-9,034)
Oxygen saturation (%), n (%)	89 (87-91)	88 (82-90)	0.377*	-	-
Body temperature (°C)	37.3±0.8	37±0.6	0.103***	-	-
Median±SD (Min-Max)	(36-38.5)	(36-38.2)			
Leucocyte (/mm^3), median (IQR)	13700 (10200-18600)	10600 (8100-14300)	0.002*	>0.05	-
Neutrophil (/mm^3), median (IQR)	8300 (5100-12700)	5900 (3100-9000)	0.001*	>0.05	-
Hemoglobin (gr/dL), median (IQR)	11 (10.3-12.1)	10.4 (9.7-11.8)	0.115*	-	-
C-reactive protein (mg/ml), median (IQR)	11.7 (4-30.2)	16.9 (7.1-36.7)	0.070*	-	-
pH (mmHg), median (IQR)	7.37 (7.32-7.41)	7.34 (7.27-7.40)	0.044*	>0.05	-
HCO3- (mmol/L), median (IQR)	22.1 (20.7-24)	21.6 (19.8-24.3)	0.498*	-	-
pCO2(mmHg), median (IQR)	39.2 (34.2-44.5)	42.7 (35.5-49.3)	0.033*	0.045	0,958 (0,821-0,990)
Lactate (mmol/L), median (IQR)	1.9 (1.4-2.7)	1.9 (1.3-2.7)	0.974*	-	-

HFNC: High-flow nasal cannula therapy, IQR: Interquartile range, SD.: Standard deviation, HCO_3-: Bicarbonate; pCO_2: Carbon dioxide, *:Mann-Whitney U Testi; **: Chi Square Test, ***: Student's t Test, OR: Odds Ratio, CI: Confidence Interval, Min: minimum, Max: maximum

Table IV. Comparison of responders and non-responders receiving HFNC treatment for non-respiratory pathology.

Parameter	HFNC Responder (N= 22)	HFNC Non-responder (N= 18)	P value	Logistic Regression	
				P value	OR (%95 CI)
Age (month), median (IQR)	13.5 (6-45)	8.5 (2-36)	0.270*	-	-
Male Gender, n (%)	9 (40.9)	4 (22.2)	0.209**	-	-
Chronic disease, n (%)	11 (50)	10 (55.6)	0.726**	-	-
Clinically important findings on chest x-ray, n (%)	1 (4.5)	3 (16.7)	0.310**	-	-
Oxygen saturation (%), n (%)	89 (86-94)	93 (87-97)	0.235*	-	-
Body temperature (°C), median (IQR	36.7 (36-37)	36.7 (36-38)	>0.999*	-	-
Leucocyte (/mm^3), median (IQR)	14800 (7800-22200)	12400 (3900-29100)	0.463*	-	-
Neutrophil (/mm^3), median (IQR)	7000 (5000-14100)	7000 (2200-18900)	0.723*	-	-
Hemoglobin (gr/dL), median (IQR)	10.4 (9.2-10.8)	10.6 (8.8-11.8)	0.770*	-	-
C-reactive protein (mg/ml), median (IQR)	1.2 (0.2-3.2)	41.4 (4.3-90.7)	0.007*	>0.05	-
pH (mmHg), median (IQR)	7.29 (7.17-7.36)	7.19 (7.06-7.37)	0.211*	-	-
HCO3- (mmol/L), median (IQR)	17.6 (10-22.2)	19 (9-27)	0.221*	-	-
pCO2(mmHg), median (IQR)	36 (25-42)	31 (25-56)	0.924*	-	-
Lactate (mmol/L), median (IQR)	2.2 (1.2-4.8)	4.5 (2.2-8.5)	0.001*	0.008	1,558 (1,125-2,158)

HFNC: High-flow nasal cannula therapy, IQR: Interquartile range, SD: Standard deviation, *:Mann-Whitney U Test, **: Chi Square Test, OR: Odds Ratio, CI: Confidence Interval

Discussion

HFNC oxygen treatment with the pre-established protocol was used in 524 patients aged 1 month to 18 years with various etiologies of respiratory distress/failure in the pediatric emergency department. The overall success rate was 88.2%. The rate of HFNC failure was 9% in children with respiratory pathologies and 45% in children with non-respiratory pathologies. No adverse effects related to HFNC treatment were observed. In children with respiratory pathologies, existence of a clinically important finding on chest X-ray increased and lower initial venous PCO_2 level decreased the risk of HFNC treatment failure. Higher venous lactate level was a risk factor for HFNC treatment failure in children with non-respiratory pathologies.

Most of the clinical studies on HFNC oxygen treatment were conducted in children with bronchiolitis and lower respiratory tract infections. High-flow nasal cannula oxygen therapy is a more effective treatment option than standard nasal oxygen therapy.[3,6,8,9,11] In a prospective study from Australia, Keproetes et al.[10] showed that the therapy failure rates were 14% in HFNC group and 33% in the standard nasal oxygen group among patients aged lower than 24 months with moderate bronchiolitis. Additionally, more than half of the children who experienced treatment failure on standard therapy were rescued with HFNC. They concluded that HFNC is safe and effective as a rescue therapy to reduce the pediatric intensive care unit admission in children with moderate bronchiolitis.[10] In a multicenter randomized

controlled study, Franklin et al.[3] evaluated the effectiveness of early HFNC therapy during hospital admission among infants with bronchiolitis if they had a need for supplemental oxygen therapy to keep the oxygen-saturation level in an acceptable range. The therapy failure rates were 12% in HFNC group and 23% in the standard nasal oxygen group.[3] In the limited number of studies, HFNC was used in children with non-respiratory diseases, most of them conducted in pediatric intensive care unit.[2,5-7] The risk of HFNC failure was increased in children with cardiovascular pathology, sepsis, extrapulmonary pediatric acute respiratory distress syndrome, trauma-induced lung contusion, neuromuscular disease.[2] The success of HFNC depends on its operating principle, such as reducing respiratory workload and inspiratory resistance, eliminating anatomic dead space in the nasopharynx, increasing mucociliary activity due to heated and highly humidified air, and a limited increase in positive end-expiratory pressure. As expected, the positive effect in the respiratory distress related to a circulatory pathology is more limited.[12,13] In our study, we assessed all children receiving HFNC oxygen treatment. Most of the children had respiratory tract pathologies and 7.6% of them had non-respiratory tract pathologies. The HFNC oxygen treatment was successful in 81% of children with respiratory disease and 55% of children with non-respiratory disease. The HFNC failure risk was higher in pneumonia among respiratory diseases and in sepsis among non-respiratory diseases. Similar to the literature, the success rate of HFNC in bronchiolitis is high. Although there is very limited information in the literature, we observed that HFNC is a reliable option in croup syndrome and pediatric asthma exacerbation in our daily practice. Nevertheless, we believe that HFNC is a reliable oxygen therapy option for children in pediatric emergency departments.

Inability to identify patients who will fail NIV early may cause a delay in intubation, which can lead to clinical deterioration and increased morbidity and mortality.[14] Therefore, it is important to predict the HFNC treatment failure to prevent morbidity and mortality caused by receiving HFNC treatment longer than necessary.[2,8,11] According to studies conducted in pediatric intensive care units, respiratory distress caused by congenital heart disease[7,11], oxygen need caused by non-respiratory diseases[5], previous endotracheal intubation[11], age greater than 10 years, high mortality and respiratory scores, and low SpO_2/FiO_2 ratio have been reported as predictors for HFNC failure.[7,11] There are limited studies on this subject in pediatric emergency departments. Significant tachypnea, hypercapnia and acidosis were reported as predictors of HFNC treatment failure in patients younger than two years with respiratory disease.[6] In adults, HFNC has been found to be less successful in patients with severe parenchymal damage such as bacterial pneumonia.[12,13] The main limitation of the oxygen treatment via HFNC is its inability to create sufficient pressure in patients older than one month of age. Although it cannot be measured routinely, HFNC generates some flow dependent positive end-expiratory pressure. However, these pressure levels are probably not sufficient for some patients with parenchymal damage or hypercarbia. In pathologies such as bronchiolitis, delivering heated and humidified oxygen via HFNC device is often sufficient to correct pathology.[3,6,8-13] The mechanism of respiratory distress is different in non-respiratory pathologies. In circulatory failure, blood lactate level is an indicator of tissue perfusion. In these situations, HFNC provides the only safe and comfortable oxygen supply.[15,16] Unlike other studies in the literature, we investigated the predictors of HFNC failure in children with respiratory and non-respiratory pathologies, separately. In children with respiratory disease, existence of significant radiological finding on chest X-ray increased the likelihood of HFNC therapy failure. We determined that venous $PaCO_2$ was significantly higher in HFNC non-responders than HFNC-responders. Statistically, lower venous $PaCO_2$ level decreased the risk of HFNC therapy failure. In children with non-respiratory

pathologies, higher blood lactate level increased the risk of HFNC treatment failure. When the results are evaluated in general, both $PaCO_2$ and lactate levels were high in HFNC non-responders. This result suggests that HFNC success is lower in later stages of the diseases. Our results are consistent with HFNC device working principle.

In the literature, HFNC is generally described as a safe oxygen delivery method. Few studies have reported air leakage syndromes.[9,17] In our pediatric emergency department, we used HFNC with a pre-established protocol. We did not see any adverse effects related to HFNC therapy in the study period.

This study has several limitations. Firstly, this is a single center study giving the results of special pediatric emergency settings in a special region with distinctive necessities and also limited by the retrospective data. Secondly, there was no control group to compare the efficacy. Thirdly, we did not record the vital parameters, blood gas analysis on the follow-up. Fourthly, the exact time interval between initiation of HFNC and initiation of further respiratory support for respiratory failure in patients could not be obtained from hospital records as the study design was retrospective. According to the protocol used in the pediatric emergency department, the maximum follow-up period of patients whose clinical picture does not improve under HFNC support is two hours. However, the study has got some positive contributions to the literature. The total number of patients in the study is quite high when compared to other single-center studies. We evaluated the outcomes of HFNC treatment with pre-established protocol in children with respiratory distress/failure due to various etiologies. In the literature, there are very few studies evaluating HFNC outcomes in non-bronchiolitis diseases in pediatric patients older than one month. Lastly, there are limited studies to evaluate the predictors of HFNC failure in pediatric emergency setting.

In conclusion, HFNC is a reliable oxygen delivery method in children with respiratory distress due to many different etiologies in the pediatric emergency department. The efficacy of HFNC is higher in children with respiratory pathologies than in children with non-respiratory pathologies. The presence of clinically important radiological findings on chest X-ray decreases and lower $PaCO_2$ increases HFNC success in children with respiratory pathologies. The higher blood lactate level increases the risk of HFNC failure in children with non-respiratory pathologies.

Ethical approval

The study has been approved by the Ethics Committee of Tepecik Training and Research Hospital (2018/6-1).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: MA; data collection: ŞD, GY, SBY, EB, GG; analysis and interpretation of results: ABA, MA, FK, ŞB, GD; draft manuscript preparation: ŞD, ŞB, GD. 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

Assessment of auditory functions in patients with hepatic glycogen storage diseases

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ABSTRACT

Background. Hepatic glycogen storage diseases are a group of diseases manifesting mainly with hypoglycemia and hepatomegaly. The patients require frequent daytime and nocturnal feedings. Hypoglycemia may cause sensorineural hearing loss and nocturnal feeding is a risk factor for the development of gastroesophageal reflux that may cause chronic otitis media and hearing loss consequently. We aimed to determine the prevalence and characteristics of hearing loss in hepatic glycogen storage diseases.

Methods. A total of 24 patients with hepatic glycogen storage disease (15 glycogen storage disease type I and 9 non type I) and 24 age/sex matched healthy controls were enrolled in the study. Pure tone audiometer, immitansmetry, acoustic reflex measurement, otoacoustic emission test (OAE) and auditory brainstem response (ABR) tests were applied to all participants.

Results. Hearing loss was determined in 17/24 patients (12 glycogen storage disease type I and 5 non type I) with pure tone audiometer. Interpretation of all the findings revealed a total of 8 patients had conductive and 9 had mixed hearing loss. All parameters were significantly different than the control group.

Conclusions. This is the first study to comprehensively assess the auditory functions of patients with hepatic glycogen storage disease. Audiological findings determined a significantly increased prevalence of conductive/mixed type hearing loss in the patient group which is a new finding in the literature. Further studies with extended patient numbers are required to enlighten the underlying pathophysiology.

Key words: glycogen storage disease, hearing loss, auditory function, nocturnal feeding, hypoglycemia.

Glycogen is the storage form of glucose in mammalian cells, and it is mostly stored in muscle and liver. Glycogen is utilized as a glucose source to maintain blood glucose levels within the normal ranges between meals. In the muscle, glycogen provides glucose for glycolysis and ATP production and this energy is utilized during active contraction. Glycogen storage diseases (GSD) are a group of inherited metabolic diseases accompanied by abnormal glycogen storage or utilization resulting from variable genetic deficiency of enzymes in glycogen degradation or synthesis or mutations of regulatory proteins in glycogen metabolism. It is classified based on the enzyme deficiencies or affected tissues. Hypoglycemia and hepatomegaly are cardinal presenting manifestations in hepatic glycogenosis.[1,2]

Hepatic GSDs are type 0, I, III, VI and IX. In type I, either glucose 6- phosphatase (G6Pase) enzyme (type Ia) or glucose 6-phosphate (G6P) transporter (type Ib) is deficient, and hypoglycemia is earlier and more severe due to both gluconeogenesis and glycogenolysis impairment.[1,3] Glycogen synthase enzyme is deficient in type 0 resulting in impairment of glycogen synthesis and symptoms are seen after weaning and are less severe. Abnormal glycogen

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accumulates in type III due to the debrancher enzyme deficiency. Glycogen phosphorylase and phosphorylase kinase enzymes are utilized in glycogenolysis, and deficiencies cause type VI and IX respectively.[1,4,5]

The severe forms of GSDs in childhood are associated with very short fasting intervals of less than 4 hours, overnight continuous gastric high-carbohydrate feedings; frequent daytime feedings with supplementing of uncooked cornstarch are quite a typical requirement of type I but can also be required in other types.[6]

Hypoglycemia may cause complications in the central nervous system involving vision loss, seizures, unconsciousness and auditory dysfunction. Hearing loss (HL) is seen in many kinds of metabolic disorders involving biotinidase deficiency, mitochondrial, peroxisomal and lysosomal diseases.[7-14] The primary defects of hearing loss in these diseases are lack of energy, disruption of inner ear cells due to substrate accumulation or vascular damage.[15,16] In the literature there are many reports indicating the association between hypoglycemia and auditory dysfunction. Hyperinsulinemia, hypocortisolemia, type II diabetes may cause hearing loss due to hypoglycemia.[17-19]

However, the data related to auditory functions in hepatic GSD is limited. Hearing loss was shown in many studies conducted with GSD type II (Pompe disease) patients; however, it is a lysosomal storage disease, and the pathophysiology is different. There is limited data suggesting that sensorineural hearing loss might be seen in type I patients. Melis et al.[20] and Aydemir et al.[21] evaluated the auditory functions of the type I patients with auditory brainstem response (ABR) only and found abnormalities. Since hepatic GSD are hypoglycemia associated and require overnight feeding that may cause gastroesophageal reflux (GER); an association between hepatic GSDs and HL could be hypothesized. In the present study we aimed to obtain a comprehensive assessment of auditory functions and determine the prevalence and characteristics of HL in hepatic GSD patients compared with healthy controls.

Material and Methods

Subjects

Patients with hepatic GSD and followed up in our clinic were evaluated. All patients were diagnosed with either presence of biallelic mutation in the concerning gene and/or low tissue enzyme activity. Those who had head trauma, otologic surgery, idiopathic urgent HL, acute acoustic trauma, exposed long term noise, frequent otitis media, familial HL, diabetes mellitus and exposure to ototoxic medicine were excluded. The study was conducted with hepatic GSD patients and age/sex matched healthy controls. Informed oral and written consent were obtained from all subjects and their parents before enrollment. The study was approved by Gazi University Ethical Comittee. (613/21.09.2020)

Hearing Assessments

Pure tone audiometer, immitansmetry, acoustic reflex measurement, otoacoustic emission test (OAE) and auditory brainstem response (ABR) tests were applied to all participants.

Pure-tone thresholds were analyzed for both air and bone conduction using TDH 39 supraaural headphones and GSI audiometry.[22] Degree of clinical hearing loss was classified according to the normative hearing data derived from WHO and based on a four-frequency pure-tone average (500, 1000, 2000 and 4000 Hz). Slight HL ranged between 16-24 dB; mild HL 25-39, moderate HL 40-69 dB, severe HL 70-95 dB, and profound HL >95 dB.[23]

To describe middle ear functions, immitansmetrical evaluation with middle ear pressures, static compliance and ear canal volume and ipsilateral reflex thresholds at 500, 1000, 2000 and 4000 Hz were measured.

Transient otoacoustic emission (TEOAE) test was applied to determine the cochlear functions using Interacoustics Eclipse 15 device and the responses in which a signal to noise ratio exceeding 3 dB in at least three of the five frequencies were recorded as "present" and other conditions as "absent". Present TEOAE shows that cochlear and middle ear functions are normal, and absence of TEOAE indicates cochlear and/or middle ear functions are abnormal.[24]

ABR was performed using Interacoustic Eclipse 15 device and ER-3A insert headphones to identify retrocochlear dysfunction. Click stimulus were presented in both ears and I, III, V wave latencies and amplitudes at 85 dB nHL were detected. In ABR test, I, III and V are the basic waves and can predict the type and degree of HL according to latency-amplitude values. Wave I originates from the distal region of the 8th cranial nerve, wave III from the cochlear nucleus and superior olivary complex, and wave V originates from the superior olivary complex and lateral lemniscus.[25] Prolonged I-III and I-V interpeak and interaural latencies and the absolute wave latencies show cochlear/ retrocochlear or conductive type pathology.

The origin of the hearing deficit was estimated by the combined interpretation of the ABR, otoacoustic emissions, and impedance audiometry.

Statistical Analysis

Mean, standard deviation, median, minimum, maximum value frequency and percentage were used for descriptive statistics. The distribution of variables was checked with Kolmogorov-Smirnov test. Mann-Whitney U test were used for the comparison of quantitative data. Wilcoxon test was used for the repeated measurement analysis. The chi-square test was used for the comparison of qualitative data. ROC analysis was used to show the effect level. SPSS 27.0 was used for statistical analyses.

Results

Fifteen GSD type I and 9 non type I patients were included in the study. The age of patients ranged from 3 to 26 years (mean 8.8 ± 4.54). The mean age of the patient group was 11.08 ± 6.52 years. The female/male ratio was 12/12. The control group consisted of 24 healthy individuals (13 female and 12 male, mean age 10.88 ± 5.53 years, range 2–23 years).

No risk factors for hearing loss were identified by questionnaire and neither neurologic, nor intellectual complications were present in the patients. The results of age, gender distribution, pure tone audiometer, middle ear pressure, acoustic reflex threshold, click threshold, ABR I-III, III-V interpeak latencies, otoacoustic latencies are shown in Table I.

There was no significant difference between age and gender distribution amongst patient and control groups ($p > 0.05$). In the patient group, right, left and right-left mean values of pure tone audiometer, acoustic reflex threshold, click threshold, ABR I-V and III-V interpeak latencies, prolonged I-V interpeak latency rate and OAE rate were significantly higher and middle ear pressures were significantly lower than the control group ($p < 0.05$) (Table I).

The pure tone audiometer determined HL in a total of 17 (70.8%) patients which were slight in 9 (37%) and mild in 8 (33%) patients. HL was not described in 7 (29.1%) patients. Findings were significantly different than the control group ($p < 0.001$) (Table II, Fig. 1).

Immitansmetrical evaluation showed middle ear pressures were significantly decreased in the patient group (Table II). According to the Jegger classification, 14 (58.3%) patients (11 type I, 3 non type I) had Type C tympanogram, however all of the individuals in the control group had type A (24) (Fig. 2). Acoustic reflex couldn't be measured or measured at high decibels (approximately 100 dB) compatible with type C tympanogram, whereas acoustic

Table I. Summary of clinical features and audiologic results of patients.

Patient No/Disease type/Gender/ Age (y)/ metabolic control	Weight/ height percentiles	Right Ear					Left ear				
		Estimated Hearing Threshold	Tymp	OAE	I-V interpeak lantencies	III-V interpeak lantencies	Estimated Hearing Threshold	Tymp	OAE	I-V interpeak lantencies	III-V interpeak lantencies
1/type Ia/F/12/good	94/36	25	Abnormal	Absent	Normal	Normal	27	Abnormal	Absent	Normal	Normal
2/type Ia F/12/good	36/42	20	Abnormal	Absent	Normal	Normal	18	Abnormal	Absent	Normal	Normal
3/type Ia /F/8/good	98/84	32	Abnormal	Absent	Prolonged	Normal	30	Abnormal	Absent	Prolonged	Normal
4/type Ia /F/12/poor	18/<3	25	Abnormal	Absent	Normal	Normal	23	Abnormal	Absent	Normal	Normal
5/type Ia M/11/poor	<3/<3	27	Abnormal	Absent	Prolonged	Normal	25	Abnormal	Absent	Prolonged	Normal
6/type Ia /F/7/poor	5/<3	30	Abnormal	Absent	Prolonged	4.33	28	Abnormal	Absent	Prolonged	4.35
7/type Ia/F/4/poor	<3/7	33	Abnormal	Absent	Prolonged	Normal	28	Abnormal	Absent	Prolonged	Normal
8/type Ia /F/5/poor	99/56	23	Abnormal	Absent	Prolonged	Normal	20	Abnormal	Absent	Prolonged	Normal
9/type Ia F/9/poor	72/28	22	Abnormal	Absent	Prolonged	3.65	18	Abnormal	Absent	Prolonged	3.67
10/type Ia /M/7/poor	59/32	15	Normal	Present	Normal	Normal	13	Normal	Present	Normal	Normal
11/type VI/F/5	61/12	18	Normal	Present	Normal	Normal	13	Normal	Present	Normal	Normal
12/type Ib/M/4/good	88/6	25	Abnormal	Absent	Prolonged	Normal	21	Abnormal	Absent	Prolonged	Normal
13/type 0/M/6	75/25	15	Normal	Present	Normal	Normal	15	Normal	Present	Normal	Normal
14/type VI/M/7	53/8	12	Normal	Present	Normal	Normal	10	Normal	Present	Normal	Normal
15/type III/M/10	30/25	18	Abnormal	Absent	Prolonged	Normal	15	Abnormal	Absent	Prolonged	Normal
16/type III/F/3	91/7	25	Abnormal	Absent	Prolonged	Normal	22	Abnormal	Absent	Prolonged	Normal
17/type III/F/7	82/24	22	Abnormal	Absent	Normal	Normal	25	Abnormal	Absent	Normal	Normal
18/type Ia /F/22/poor	>18 year old	16	Normal	Present	Normal	Normal	14	Normal	Present	Normal	Normal
19/type VI/M/14	58/9	17	Normal	Present	Normal	Normal	17	Normal	Present	Normal	Normal
20/type III/M/16	44/<3	15	Normal	Present	Normal	Normal	14	Normal	Present	Normal	Normal
21/type I/M/16/poor	4/<3	21	Abnormal	Absent	Normal	Normal	25	Abnormal	Absent	Normal	Normal
22/type VI/M/24	>18 year old	13	Normal	Present	Normal	Normal	14	Normal	Present	Normal	Normal
23/type Ia /M/26/poor	>18 year old	10	Normal	Present	Normal	Normal	12	Normal	Present	Normal	Normal
24/type Ia /M/19/poor	>18 year old	15	Normal	Present	Normal	Normal	15	Normal	Present	Normal	Normal

Tym.: tympanometry, OAE: otoacustic emission

reflex thresholds were at normal ranges in controls (Fig. 3).

In the TEOAE test, no response was present in 11 type I and 4 non type I patients (58.3%) which was compatible with the immitansmetric findings (Table II). However, in the control group, TEOAE response was present in all the individuals (Fig. 4).

Table II. Audiologic findings of control and patient groups.

		Control Group		Patient Group		p
		Mean±sd/n-%	Median	Mean±sd/n-%	Median	
Age		10.9 ± 5.5	9.5	11.1 ± 6.5	9.5	0.877 [m]
Gender	Girl	13 54.2%		12 50.0%		0.773 [X²]
	Boy	11 45.8%		12 50.0%		
Pure Tone Audiometer						
Right Ear		7.8 ± 3.1	8.0	20.6 ± 6.3	20.5	0.000 [m]
Left Ear		8.5 ± 2.6	8.0	19.4 ± 6.4	18.0	0.000 [m]
R-L Mean		8.1 ± 2.4	8.8	20.0 ± 6.2	19.5	0.000 [m]
Middle Ear Pressure						
Right Ear		-6.2 ± 18.3	-7.5	-119.7 ± 81.6	-123.0	0.000 [m]
Left Ear		-5.2 ± 12.8	-4.5	-102.6 ± 64.3	-116.0	0.000 [m]
R-L Mean		-5.7 ± 10.5	-5.3	-111.2 ± 70.2	-120.3	0.000 [m]
Acustic Reflex Threshold						
Right Ear		85.0 ± 0.0	85.0	94.8 ± 8.5	95.0	0.000 [m]
Left Ear		85.0 ± 0.0	85.0	95.0 ± 6.4	95.0	0.000 [m]
R-L Mean		85.0 ± 0.0	85.0	94.6 ± 7.8	96.3	0.000 [m]
Click Threshold						
Right Ear		20.0 ± 0.0	20.0	29.4 ± 7.7	30.0	0.000 [m]
Left Ear		20.0 ± 0.0	20.0	29.6 ± 7.4	30.0	0.000 [m]
R-L Mean		20.0 ± 0.0	20.0	29.5 ± 7.3	28.8	0.000 [m]
ABR I-V Interpeak Latencies						
Right Ear		3.8 ± 0.4	3.8	5.2 ± 1.0	5.1	0.000 [m]
Left Ear		3.8 ± 0.4	3.8	5.1 ± 0.9	5.2	0.000 [m]
R-L Mean		3.8 ± 0.4	3.8	5.2 ± 0.9	5.2	0.000 [m]
ABR III-V Interpeak Latencies						
Right Ear		2.2 ± 0.1	2.2	3.2 ± 0.8	3.2	0.000 [m]
Left Ear		2.2 ± 0.1	2.2	3.1 ± 0.8	3.3	0.000 [m]
R-L Mean		2.2 ± 0.1	2.2	3.2 ± 0.8	3.3	0.000 [m]
Prolonged	Prolonged	0 0.0%		15 62.5%		0.000 [X²]
	Normal	24 100.0%		9 37.5%		
Otoacustic Emission (OAE)						
Right Otoacustic	(-)	0 0.0%		15 62.5%		0.000 [X²]
Emission(OAE)	(+)	24 100.0%		9 37.5%		
Left Otoacustic	(-)	0 0.0%		15 62.5%		0.000 [X²]
Emission(OAE)	(+)	24 100.0%		9 37.5%		
R or L Otoacustic	(-)	0 0.0%		15 62.5%		0.000 [X²]
Emission(OAE)	(+)	24 100.0%		9 37.5%		

[m] Mann-whitney u test [X²] Chi-square test

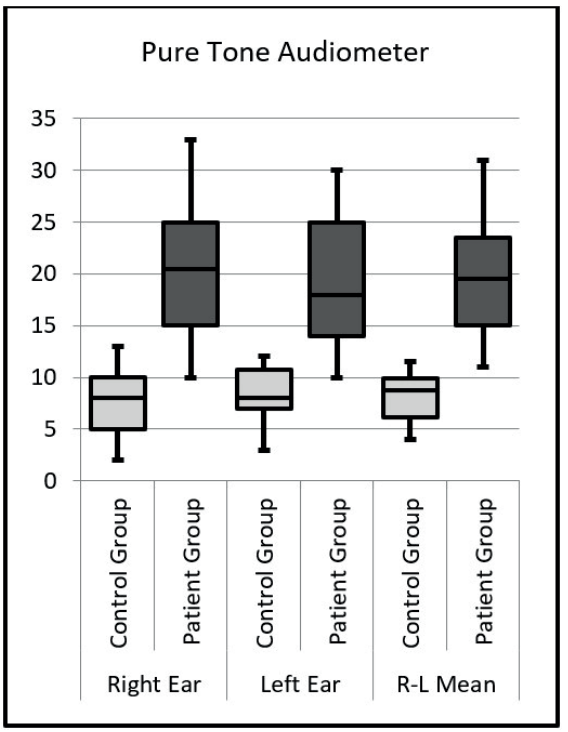

Fig. 1. Estimated hearing thresholds (dB) of patient and control group.

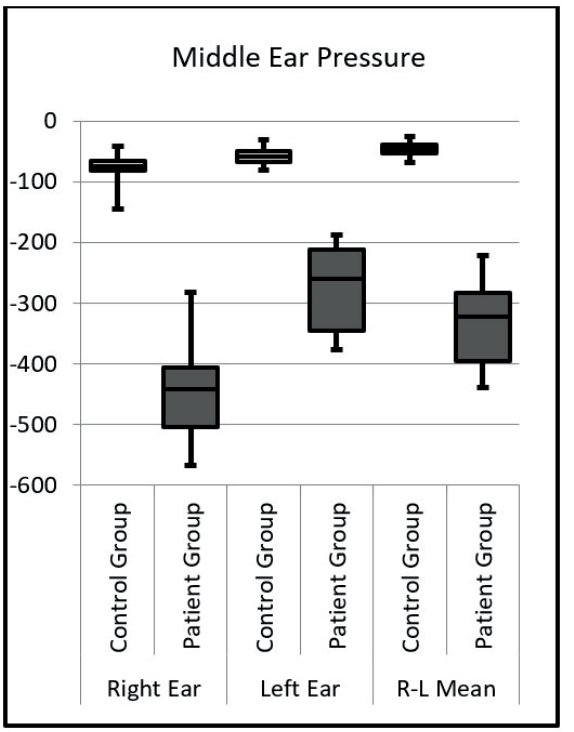

Fig. 2. Middle ear pressures of control and patient groups.

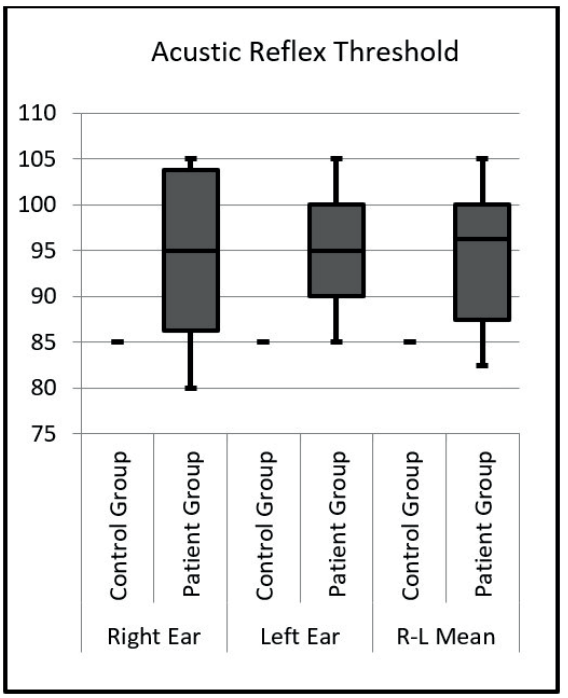

Fig. 3. Acustic reflex thresholds of control and patient groups.

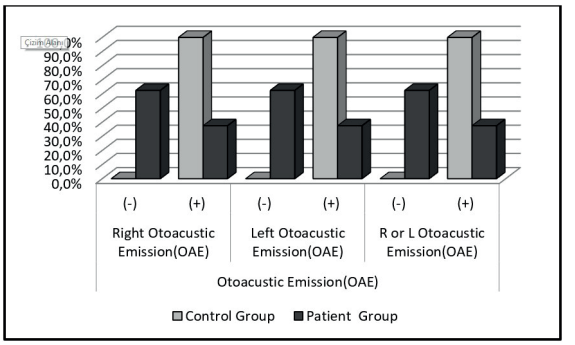

Fig. 4. Percentages of presence (+) and absence (-) of otoacustic emission in control and patient groups.

In click ABR test, thresholds of the patients were significantly higher ($p < 0.001$) (Table II, Fig. 5). Moreover, type I patients' thresholds were significantly higher than non-type I patients ($p = 0.031$). I-V and III-V interpeak latencies were significantly higher in the patient group ($p < 0.001$) (Fig. 6). However, no significant difference was detected between type I and non-type I patients. I-V interpeak latencies were

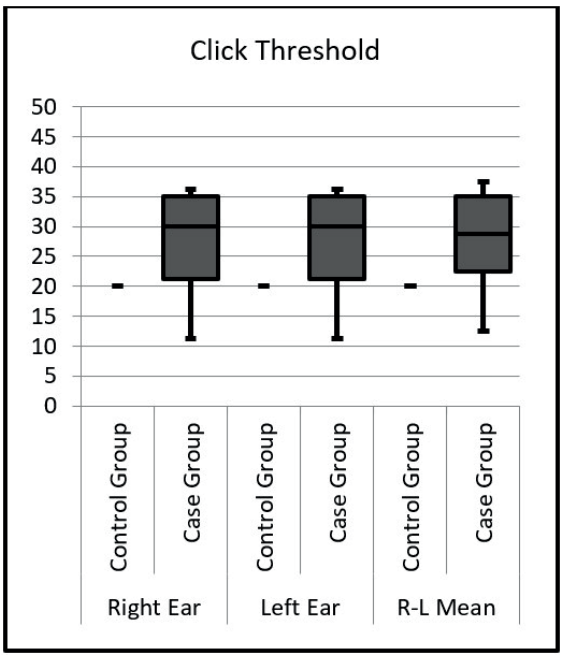

Fig. 5. Click thresholds of control and patient groups.

prolonged in 9 (37.5%) patients. Since, these patients also had type C tympanograms, they were diagnosed with mixed HL.

Interpretation of all the audiologic findings determined that 9 patients had a mixed type and 8 had conductive type hearing loss (Table I). Furthermore, all audiological findings showed a significant difference between GSD I patients and controls.

Type I patients were divided into two subgroups: those with good and poor metabolic control according to the criteria defined by European Study on Glycogen Storage Disease Type I.[21] Patients were assigned as good metabolic control if blood glucose was > 72 mg/dl, triglycerides < 531 mg/dl, uric acid < 6 mg/ dl, and lactate < 22.5 mg/dl. No correlation could be established between metabolic control and hearing assessment values in type I patients (Table II).

Because older patients are more likely to have fewer episodes of hypoglycemia and need less frequent nocturnal feeding, patients were also classified into two groups, those who were >12 and those who were <12-year-old. Comparing of two groups revealed that pure tone audiometer,

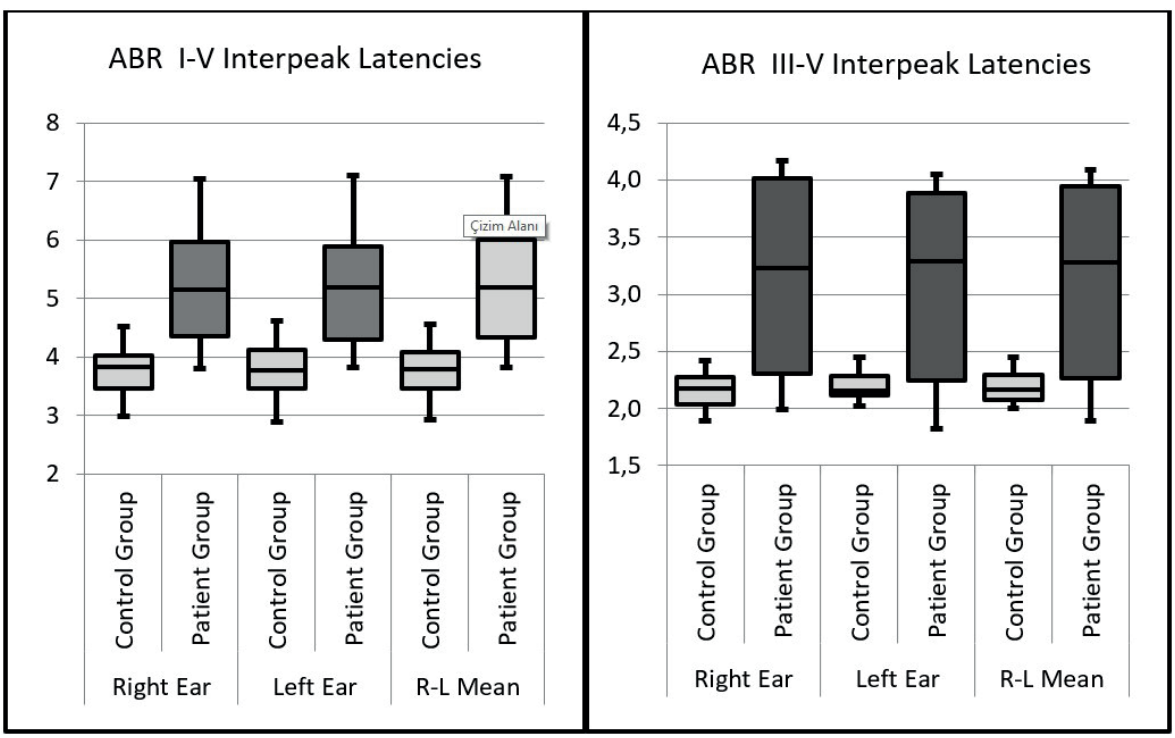

Fig. 6. ABR I-V and III-V interpeak latencies of control and patient groups.

middle ear pressure, acoustic reflex threshold, click threshold, ABR I-V and III-V interpeak latencies values were higher and hearing loss was more prevalent in patients < 12-year-old. In addition, otoacoustic emission responses were absent in 82.4% of patients <12 and 14.3% of >12-year-old.

Discussion

The results of the conducted study were consistent with the hypothesis. HL was detected in the individuals with GSD, particularly in type I. In GSD patients HL prevalence was significantly higher than in the controls. Slight/mild levels of conductive auditory thresholds in pure tone audiometer, type C tympanogram findings and prolonged I., III., V. interpeak latencies despite normal I-V interpeak latencies in ABR suggested conductive type HL in 8 patients.[26] In 9 patients, presence of prolonged III-V and I-V interpeak latencies suggest dysfunction of cochlear and retrocochlear structures that is consistent with mixed hearing loss. Conductive/mixed hearing loss suggests chronic middle ear dysfunction.[27] Infections, allergy, immunologic factors, and GER are the main causes of chronic otitis media.[28-30] Thus, we aimed to determine the etiologic factors of conductive/mixed hearing loss in the patients.

In GSD Ib patients, recurrent infections such as otitis, upper respiratory infections, gingivitis and mouth ulcers, abscesses and pneumonia are seen frequently due to neutropenia and neutrophil dysfunction.[31] However, these infections are much less frequent in GSD Ia. Nevertheless, we detected different reports in the literature relevant to recurrent otitis media, adenoid/tonsillar hypertrophy in GSD Ia patients. Bevan[32] reported a child diagnosed with GSD Ia who exhibited adenoid hypertrophy preventing nasogastric tube feeding and required a gastrostomy. In van Creveld et al.'s[33] report, a GSD Ia patient developed mouth thrush infection and recurrent otitis media which resolved with adenoidectomy. Bustamente et al.[34] reported a patient with GSD Ia who underwent adenoidectomy, tonsillectomy, and myringotomy tube placement due to recurrent suppurative otitis media and obstructive sleep apnea at 4 years of age. Farrington et al.[35] identified a GSD Ib patient with recurrent otitis media and oral thrush beginning at the age of 8 months explained with the possible result of partial obstruction introduced by the nasogastric tube. She had undergone bilateral myringotomy at age of 25 months followed by tonsillectomy and adenoidectomy at age of 51 months. In addition, recurrent otitis media in GSD III patients was shown in the studies of Assiri et al.[36] and Williams et al.[37]

Although adenoid or tonsillar hypertrophy and otitis are not common features of hepatic GSDs, the fact that they have been seen in many patients supports a predisposition to this condition in some way. This possible predisposition might be due to unknown factors affecting immune dysfunction. In addition, patients are fed with lactose, fructose and sucrose are restricted except for fruits, vegetables and small amounts of milk products which might cause consumption of insufficient essential nutrients, vitamins and minerals and could result in secondary immune dysfunction. There are some scarce data relevant to immune dysfunction in GSD Ia. Kim et al.[38] showed that impaired glucose homeostasis resulted in myeloid dysfunction in GSD Ia and Ib.

They demonstrated an elevated progenitor cell frequency in the bone marrow and spleen and increased serum levels of granulocyte colony stimulating factor and cytokine-induced neutrophil chemoattractant in mice with GSD Ia and Ib. These changes were more prominent in GSD Ib mice which was consistent with myeloid dysfunction. Weston et al.[39] also identified four patients with GSD Ia. They were homozygous for G188R mutation and presented with hypoglycemia, recurrent infections, and neutropenia. Bilateral ventilation tubes were placed in one patient because of recurrent otitis media. Neutrophil function analysis revealed neutrophil dysfunction like GSD Ib patients. They suggested that G6Pase might play a role

in the microsomal membrane transport of G6P, however because G6Pase gene is not highly expressed in human PMN, it is often difficult to interpret.

On the other hand, hepatic GSD patients are subjected to being fed during the night continuously or every 3-4 hours. During the feeding they are likely to be in a supine position which is a risk factor for gastroesophageal and extraesophageal reflux. The main side effect of this kind of nutrition is gastro-esophageal reflux disease (GERD), in 25-60% of cases.[40-42] Another possible mechanism for GERD may be an increase in antral volume during nutrition. Scott et al.[43] identified increased reflux episodes in children with cystic fibrosis who were under continuous nighttime nasogastric feeding due to poor nutrition status compared with their asymptomatic siblings. In a mouse model, it was shown that GERD causes the middle ear to be exposed to gastric enzymes which cause Eustachian tube dysfunction, impaired clearance of middle ear contents, and hearing impairment consequently.[44-47] Recent studies established the presence of gastric contents in the middle ear effusions of children with recurrent otitis media.[48,49]

Due to the angle and immaturity of the Eustachian tubes in children and the supine position in which infants are usually placed for prolonged periods, the possibility of reflux of stomach contents from the nasopharynx to the middle ear is considered especially in the pediatric age group.[44,50] When the stomach contents reach the middle ear, pepsin is present in active or inactive form depending on the pH of the environment. The pepsin/pepsinogen protein concentrations measured in middle ear effusions were 1000 times higher than the levels in serum.[51] Scott et al.[43] demonstrated that GER, when it becomes laryngopharyngeal reflux, could reach the middle ear, indicating a possible reflux passage through the Eustachian tube into the middle ear, could lead to otitis media. Crapko et al.[52] obtained middle ear effusion samples and analyzed pepsin presence in patients with otitis media with effusion and detected pepsin in 60% of patients confirming that extraesophageal reflux occurs in the middle ear in those children.

The patients with a history of frequent otitis media associated with any known causes were excluded in this study and because of this we have excluded 4 patients with known recurrent otitis media or ventilation tube placement or hearing loss. Therefore, the cause of conductive component of hearing loss might be associated with fluid collection in the middle ear as a result of adenoid and/or tonsil hypertrophy, otitis media due to the presence of possible immune dysfunction or GER due to nocturnal feeding, feeding in supine position and presence of more horizontal Eustachian tube in children.[16] The Eustachian tube reaches its physiologically normal position in older children. Prevalence of abnormal middle ear pressures and hearing loss were lower in patients >12-year-old who were on less frequent nocturnal feeding than type I patients <12-year-old. These findings also suggest that possible association. In addition, it might also be indirectly suggested with lacking correlation between metabolic control and hearing loss in type I patients.

Despite pure tone audiometer and particularly ABR values did not define pure sensorineural dysfunction in the patients, considering the interpeak latencies, cochlear and retrocochlear components of the HL might be suggested. In the study of Aydemir et al.[21] and Melis et al.[20], sensorineural hearing loss was detected in ABR in 20% and 15.7% of type I patients respectively. In these previous studies auditory functions were assessed with ABR only, however in the present study, it was comprehensively evaluated both in type I and non-type I patients. Moreover, in this study, tympanogram findings defined conductive hearing loss in the patients that elucidates a new complication of the disease group. Hypoglycemia might lead to sensorineural hearing loss as shown in patients with hyperinsulinism and diabetes. Previous studies on GSD I patients also suggest the association between hypoglycemia and sensorineural hearing loss, however conductive

hearing loss in hepatic GSD patients is a new finding.

Additionally, Iwanicka-Pronicka et al.[53] recently reported a study that is closest to the hypothesis of the present study. In this study ,10% of patients had bilateral sensorineural hearing loss that decreased towards high frequencies. Contrarily, in our current study, hearing loss was diagnosed in 17 patients with an accompanying conduction component in 9 patients. Especially considering the patients with mixed-type hearing loss in the current study, as Iwanicka-Pronicka et al.[53] suggested, pathophysiological changes related to the inner ear may also explain the sensorineural hearing loss. Accordingly, the specific mechanism of hearing impairment in GSD is unknown to date. The possible ototoxic effect of recurrent hypoglycemia, dyslipidemia or hypertension can also lead to inner ear damage. Therefore, the influence of the inner ears of patients with mixed hearing loss in our current study might be also explained by these theories. Unlike the current study, Iwanicka-Pronicka et al.[53] added molecular histopathological analysis, which enabled them to better explain the pathophysiological changes associated with cochlea and its components. The otoacoustic emission, ABR test findings and accordingly inner ear effect mechanisms are compatible with our current study findings. Additionally, our study included immitansmetric tests to further investigate the findings for conductive hearing loss. Thus, it brought a new perspective to the relevant literature and suggested that alertness should be given to pathologies originating from the outer ear/middle ear.[53]

To the best of our knowledge, this is the first study to comprehensively assess auditory functions in hepatic GSD patients by comparing type I and non-type I patients. Our study suggests that hearing loss might be seen in patients with hepatic GSDs, particularly in type I. Even in slight/mild HL, decreased academic performance, social and speech development might be seen. We suggest that in addition to the risk factors seen in the normal population such as recurrent otitis media, eustachian tube dysfunction and adenoid hypertrophy; the risk factors of hearing loss in GSD might be gastroesophageal reflux, immune deficiency and hypoglycemia. However, in terms of pathogenesis, further detailed studies in a larger number of patients are required to be enlightened.

Ethical approval

The study was approved by Gazi University Ethical Comittee (613/21.09.2020).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: MEŞ, İO, FE, HT, LT; data collection: NYG, MEŞ, EÖ, AK, Aİ; analysis and interpretation of results: NYG, BG, HT, MEŞ, LT; draft manuscript preparation: MEŞ, HT, LT. 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

Identifying the effects of excess weight, metabolic syndrome and insulin resistance on liver stiffness using ultrasound elastography in children

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ABSTRACT

Background. Metabolic syndrome (MetS) and insulin resistance (IR) are known predictors of nonalcoholic fatty liver disease (NAFLD) which is one of the significant comorbidities of obesity. Obese children with MetS and IR are reported to be more likely to have advanced liver fibrosis compared to those without MetS or IR. The aim of this study is to determine the effects of excess weight, MetS and IR on liver fibrosis assessing liver stiffness in children using ultrasound elastography and compare gray scale ultrasonographic findings of hepatic steatosis (HS) with liver fibrosis.

Methods. The study group involved 131 overweight/obese children. The control group involved 50 healthy lean children. Groups were adjusted according to body mass index (BMI) and BMI-standard deviation scores (SDS). Liver stiffness measurements which are expressed by shear wave velocity (SWV) were performed for each individual. The study group was further subgrouped as children with MetS and without MetS, with IR and without IR.

Results. The mean SWV of liver was 1,07 ± 0,12 m/s in the control group and 1,15 ± 0,51 m/s in the study group. The difference was significant (p=0,047). SWV of liver was weakly correlated with age, BMI, BMI-SDS, Homeostatic Model Assessment-Insulin Resistance and high-density lipoprotein cholesterol. The mean SWV of the liver in the study group for children without MetS was 1,1 ± 0,44 m/s, with MetS was 1,23 ± 0,70 m/s. The difference was not significant (p=0,719). The mean SWV of the liver in the study group for children without IR was 1,02 ± 0,29 m/s, with IR was 1,24 ± 0,61 m/s. The difference was not significant (p=0,101). In multivariate regression analysis, the only independent factor affecting liver stiffness was BMI-SDS (OR:2,584, 95% CI: 1,255-5,318, p=0,010).

Conclusions. Obesity itself, regardless of MetS or IR seems to be the major problem affecting liver stiffness in this study. However, large scale longitudinal studies might clarify this issue.

Key words: childhood obesity, metabolic syndrome, insulin resistance, shear wave elastography, liver stiffness.

Childhood obesity is a significant health problem in industrialized countries.[1] The prevalence rates for obesity and being overweight were found at 9.8% and 23.2%, respectively in the Turkish population.[2] The prevalence of metabolic syndrome (MetS) and insulin resistance (IR) is increasing in parallel with the rise of the proportion of the obese population. The prevalences are at 33% and 43% for MetS and IR, respectively in obese Turkish children.[3,4] Abnormal glucose metabolism, dyslipidemia, and hypertension are the main features of MetS.[5]

The increase of nonalcoholic fatty liver disease (NAFLD) in obese children is worrisome.[6] NAFLD is a general name of a

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spectrum of diseases varying from hepatic steatosis to nonalcoholic steatohepatitis (NASH). Accumulation of fat in hepatocytes progressively can cause inflammation and fibrosis, which is called NASH.[7] NASH may cause cirrhosis, malignancy, and organ failure in children. Also, after liver transplantation, survival is shorter in NASH compared with the general population. Children with NASH, have increased liver-related mortality compared with children of the same age in the general population.[8-12] Obese children with MetS and IR are reported to be more likely to have advanced liver fibrosis compared to those without MetS or IR.[13,14]

Liver biopsy is the trademark for the assessment and classification of NAFLD. However, it has some risks associated with its invasiveness. Technical difficulties, anesthesia requirements, small tissue sample size, and nonsuitability for patient follow-up are limitations of this procedure.[15] Thus, a non-invasive imaging modality is needed to assess liver fibrosis. Conventional Ultrasound (US) is the preferred noninvasive imaging modality for assessing liver steatosis with its safety, low cost, and easy access. However, detecting mild steatosis or differentiating steatosis from fibrosis are the limitations of this modality.[16] Lately, ultrasound based elastography has emerged as non-invasive imaging modality in the assessment of liver tissue stiffness. The working principle of elastography is based on the tissue stiffness. There are two types of ultrasound-based elastography techniques; static and dynamic. Strain elastography (SE) is the static method. Shearwave Elastography (SWE), Acoustic Radiation Force Impulse Elastography (ARFI) and Transient elastography (TE) are the dynamic types. SWE is an elastography technique that forms in vivo shear waves in the interested organ by generating transient tissue deformation using US forces. The square root of tissue elasticity is proportional to shear wave velocity (SWV).[17,18] After initiating the pulse, the measurement is displayed either in m/s or kPa.[19] Under pathological conditions such as fibrosis, as parenchymal tissue gets stiffer, the shear wave velocity (SWV) increases.[7] In point (p)-SWE, using real-time B-mode ultrasound imaging, an ROI is placed on the parenchymal tissue to perform the measurements. Real-time ultrasound imaging identifies the large vessels and masses which is avoided in the parenchymal measurements.[19] SWE is a promising technique for the non-invasive staging of liver fibrosis in children.[20-25]

The SWV measurements (m/s) of liver were classified in three categories in the literature; the values <1.20 m/s regarded as normal, ≥1.20 m/s -<1.60 m/s regarded as insignificant fibrosis, ≥ 1.60 m/s regarded as significant fibrosis.[6,18]

In the present work, our goals were to assess the effects of obesity on liver stiffness and to compare the liver stiffness of overweight/obese children with MetS and IR with those without MetS and IR using p-SWE, and also to compare gray scale ultrasonographic findings of hepatic steatosis (HS) with fibrosis categories.

Material and Methods

This study was approved by the Erciyes University, Medical School, Ethics Committee (approved number 07.03.2018-134). Signed informed consent was obtained from the children's parents according to the World Medical Association Declaration of Helsinki, revised in 2000, Edinburgh.

Study population

Children included in the study were between the ages of 6 and 18 years. One hundred thirty-one overweight/obese children were included in the study group, and 50 lean children were included in the control group. The participitants of the study and control groups were mainly adjusted according to the body mass index (BMI), BMI- standard deviation scores (SDS).

Almost all of the overweight/obese children included in the study were referred from the Division of Pediatric Endocrinology or

Division of Pediatric Nutrition and Metabolism. Medical and laboratory histories were carefully investigated. Children with chronic inflammatory or autoimmune diseases, acute or chronic viral hepatitis, and using drugs known to cause steatosis were not included in the study group.

The control group was formed by lean children without any signs of liver disease. The majority of them had visited the pediatric radiology division with urinary incontinence, urinary infection, nephrolithiasis or chronic abdominal pain. Medical histories and laboratory findings were investigated for the presence of any systemic diseases before sonographic examination. Children with any abnormality in liver echo structure on ultrasonography were excluded from the control group.

Anthropometric and clinical characteristics

At the onset, BMI of all individuals was obtained. BMI is calculated by dividing weight by the square of the height. In addition to BMI, BMI-SDS were calculated according to growth charts using age and sex.[26] Individuals with more than 1 SDS above the median were grouped into the study group. Individuals with 1 SDS above the median to 2 SDS below the median were grouped as the control. Systolic and diastolic blood pressure of all individuals were measured. Laboratory tests for all individuals included were as follows: fasting blood glucose, fasting insulin, triglycerides, high-density lipoprotein (HDL) cholesterol, aspartate aminotransferase (AST), and alanine aminotransferase (ALT). The time interval between laboratory measurements and p-SWE measurements was around 0-5 days.

Individuals were evaluated for MetS and IR. International Diabetes Federation (IDF) criteria were used to evaluate MetS.[27] The MetS diagnosis was established if the patient had altered abdominal circumference and two or more of the following criteria; fasting blood glucose ≥100 mg/dl, triglycerides ≥ 150 mg/dl, HDL≤ 40 mg/dl, taking a lipid-lowering drug, systolic blood pressure ≥ 130 mmHg, diastolic blood pressure ≥85 mmHg, and taking an antihypertensive drug. There are no established cut–off values for glucose metabolism, dyslipidemia, and arterial hypertension for children below 10 years. Pelin et al.[28] defining pediatric MS, used adapted IDF criteria. They added the criteria, TG ≥ 95th percentile, and BP ≥ 95th percentile for age and sex, in addition to the above criteria. Therefore, children younger than 10 years old, fulfilling the above criteria (only 3 children) were classified in MetS, in this study. Homeostatic Model Assessment-Insulin Resistance (HOMA-IR) levels were used to evaluate IR. The following formula is used for calculating HOMA-IR: fasting plasma glucose (mg/dL) × fasting plasma insulin (IU/mL)/405.[29] HOMA-IR is a good indicator of insulin resistance. As the value gets higher, the severity of insulin resistance gets higher. Cut-off values for HOMA-IR were regarded as 2.67 in boys and 2.22 in girls for the prepubertal period and 3,16 in both genders for the pubertal period.[30,31]

The study group was divided into subgroups as children with MetS and without MetS, with IR and without IR.

US Measurements

Ultrasound examinations were performed with Siemens Acuson S3000 using a 6C1 transducer. P-SWE was used for elastography measurements. SWV is measured in an ROI. A single radiologist with an experience of 15 years in abdominal ultrasonography and two years in SWE, who was blinded to the children's laboratory findings performed the examinations.

Patients were laid in supine position for the examination. Gray scale ultrasonography was performed to examine the liver echotexture. Hepatic steatosis (HS) was scored as grade 0, 1, 2, 3 according to liver echotexture, clarity of blood vessels and distinguishability of the diaphragm and liver parenchyma in echo amplitude.[32] SWE measurements were obtained

during the patient's free breathing, using intercostal approach, from the liver's right lobe, approximately from the same location for all individuals. Measurements were obtained at a depth of 4-5 cm from the skin. The ROI was placed about 3 cm beneath the liver capsule in an area of homogeneous parenchyma, free of visible vessels. Ten valid measurements were performed for all individuals, and the mean value is recorded (Figs. 1 and 2). The entire exam duration time took approximately 10 minutes.

The SWV measurements (m/s) of liver ≥ 1.20 m/s were regarded as meaningful for fibrosis.[6,18]

Statistical Analysis

Statistical analysis was conducted with SPSS IBM Statistics Version 22.0. Values were expressed as the mean ± SD and range (minimum to maximum). The Shapiro Wilk test was used to confirm the normality of distribution for continuous variables. The Student's t-test or Mann-Whitney U test was used to compare continuous variables between two groups according to the normality of distribution. Pearson correlation was used for correlation analysis. To compare the categoric variables the χ^2 test was used. To determine the variables affecting liver stiffness, univariate

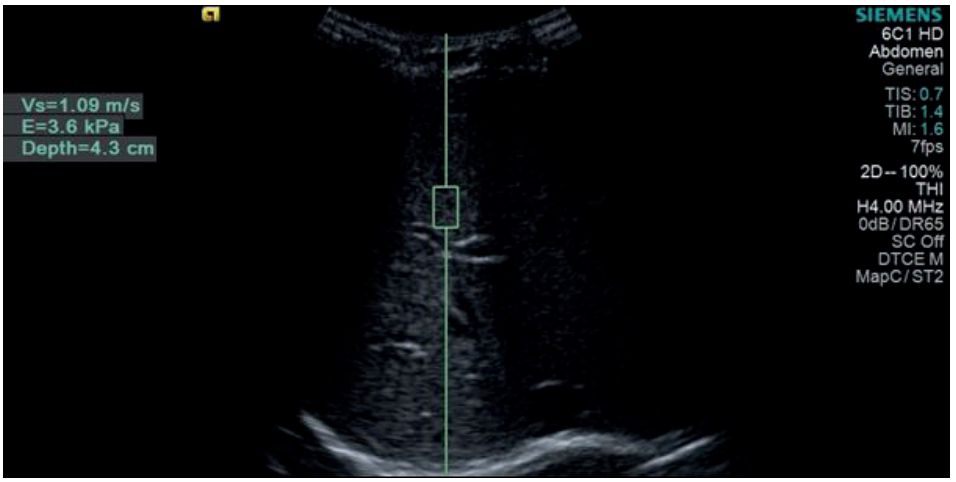

Fig. 1. SWE measurement of an 11 year old girl in control group.

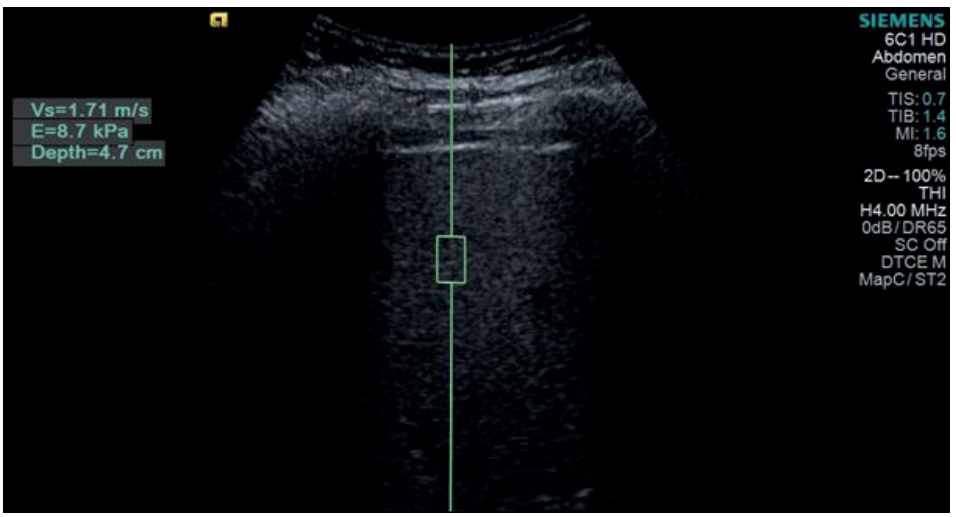

Fig. 2. SWE measurement of a 14 year old boy in study group.

logistic regression analysis was performed. Multivariate logistic regression analysis was performed to identify the independent factor for liver stiffness after adjusting for age. Interclass correlation coefficient was used for reliability measurements of p-SWE. Differences were regarded as significant at $p<0.05$.

Results

Demographic, anthropometric, metabolic, and laboratory parameters of the study and the control group are compared in Table I.

No statistically significant differences were found between the study and control group for

Table I. Comparison of demographic, anthropometric, metabolic and laboratory parameters of the study and control group.

	Control group (n=50)	Study group (n=131)	
	mean ± SD (range)	mean ± SD (range)	*p* value
Males (n (%))	14 (28%)	58 (44.3%)	0.067
Age (years)	12.5 ± 3.5 (6.2-18.9)	13 ± 2.7 (6.4-18)	0.92
Weight (kg)	42.7 ± 15.3 (15-83)	70.3 ± 20.4 (25.3-124.3)	<0.001
Height (cm)	149.1 ± 18.5 (104-183.5)	153.7 ± 14.2 (117.3-188)	0.166
BMI (kg/m2)	18.6 ± 2.9 (13.9-26.2)	29.1 ± 4.6 (18.3-42.8)	<0.001
BMI-SDS	-0.26 ± 0.84 (-1.69- 1.00)	2.4 ±0.6 (1.1-4.2)	<0.001
SBP(mmHg)	101 ±7.7 (90-120)	110.8 ± 14.4 (90-150)	0.04
DBP (mmHg)	63.1 ± 9.5 (50-80)	69.3 ± 8.8 (50-90)	0.013
Fasting blood glucose(mg/dl)	86.8 ± 9.4 (61-107)	86.9 ± 7.8 (65-121)	0.905
Fasting insulin (microıu/ml)	10 ± 7.8 (4.4- 15.8)	18.8 ± 12.3 (1.4-76.4)	0.021
Triglycerides (mg/dl)	83.8 ± 33.9 (28-147)	128.1 ± 75.9 (41-524)	0.004
HDL cholesterol (mg/dl)	55.6 ± 10.5 (40-80)	45.4 ± 10.4 (28.4-86)	<0.001
AST (IU/L)	23.1 ± 5.6 (15-40)	26.1 ± 13 (10-100)	0.173
ALT (IU/L)	14.9 ± 4.8 (9-31)	25.5 ± 20.4 (6-163)	<0.001
HOMA-IR	3.2 ± 3.5 (0.91-12.4)	4.10± 2.9 (0.30-17)	0.101
SWV of liver (m/s)	1.07 ± 0.12 (0.78-1.35)	1.15 ± 0.51 (0.63- 3.36)	0.047

BMI: body mass index, BMI-SDS: body mass index-standard deviation score, SBP: systolic blood pressure, DBP: diastolic blood pressure, HDL cholesterol: high-density lipoprotein cholesterol, AST: aspartate aminotransferase, ALT: alanine aminotransferase, HOMA-IR: homeostatic model assessment-insulin resistance, SWV: Shear wave velocity

gender, age, height, and blood glucose. In the study group, the mean SWV was 1.20 ± 0.56 m/s in girls and 1.08 ± 0.43 m/s in boys. The difference was not significant (p = 0.18). In control subjects, the mean SWV was 1.07 ± 0.13 m/s in girls and 1.07 ± 0.11 m/s in boys. The difference was not significant (p = 0.91). The mean SWV of the study group was significantly higher than the control group. The mean SWV was 1.07 ± 0.12 m/s and 1.15 ± 0.51 m/s for control and study group, respectively (p= 0.047).

Correlation analysis was performed between SWV and anthropometric, metabolic parameters for all individuals (Table II). SWV showed a weak positive correlation with age, BMI, BMI-SDS and HOMA-IR and a weak negative correlation with HDL.

The relation between hepatic steatosis (HS) and fibrosis categories was analyzed with χ^2 test Although no statistically significant difference was found between HS and fibrosis categories ($\chi 2$ =2.423, p=0.65), 15 overweight-obese children with no or mild steatosis had SWV values over 1,60 m/s.

All of the individuals were evaluated for criterias of MetS and IR. None of the subjects in the control group met any of the criteria for MetS or IR. Twenty-nine out of 131 overweight-obese children were diagnosed with MetS. Seventy-five of them was diagnosed with IR. The study group was subgrouped as with MetS, without MetS and with IR, without IR. The relationship between subgroups of MetS and IR was also analyzed using χ^2 test. A statistically significant difference was found between these subgroups. (χ^2 =5.271, p=0.022) (Table III).

While 41% of overweight-obese children who were not grouped in MetS had IR, 5% of overweight-obese children who did not have IR was grouped in MetS. Therefore, subsequent analyses were performed for MetS and IR subgroups, separately.

SWV measurements, anthropometric, metabolic, and laboratory parameters were compared in subgroups, with MetS and without MetS (Table IV). No statistically significant difference was found between the two subgroups for gender and age, height and diastolic blood pressure, blood glucose, AST, and ALT. Statistically significant differences were found for most of the MetS parameters as expected. The mean SWV was 1.1 ± 0.44 (m/s) and 1.23 ± 0.70 (m/s) for the subjects without MetS and with MetS, respectively. The difference was not significant (p=0.719).

Table II. The results of correlation analysis between SWV and anthropometric, metabolic parameters.

	Shear-wave velocity (m/s)
Age	0.154
	0.038
BMI	0.319
	<0.001
BMI-SDS	0.185
	0.018
Fasting glucose	-0.085
	0.278
AST	-0.007
	0.932
ALT	0.051
	0.525
HOMA-IR	0.199
	0.020
Triglycerides	0.020
	0.808
HDL cholesterol	-0.202
	0.014

The first line is r value, the second line is p value for all parameters. BMI: body mass index, BMI-SD: body mass index-standard deviation, HDL cholesterol: high density lipoprotein cholesterol, HOMA-IR: homeostatic model assessment-insulin resistance, AST: aspartate aminotransferase, ALT: alanine aminotransferase, SWV: Shear wave velocity

Table III. The relationship between MetS and IR.

	Without IR	With IR
Without MetS	37.4 %	40.5 %
With MetS	5.3 %	16.8 %

(χ^2 =5.271, p=0.022)

SWV measurements, anthropometric, metabolic, and laboratory parameters were compared in subgroups, without IR and with IR (Table IV). No statistically significant difference was found for age. Anthropometric, metabolic and laboratory parameters were statistically different between these subgroups except for systolic, diastolic blood pressure, AST, and ALT. The mean SWV was 1.02±0.29 m/s and 1.24±0.6 m/s for overweight-/obese children without IR and with IR, respectively. The difference was not significant (p=0.101).

Univariate regression analysis was used for analyzing the relationship between the parameters and liver fibrosis (SWV≥1.20). Age, gender, BMI-SDS, MetS and IR were chosen as independent variables. Multivariate regression analysis was used for assessing the parameters that are independently associated with liver fibrosis after adjusting for age. The only significant independent predictor of liver stiffness was BMI-SDS (OR: 2.584, %95 CI:1.255-5.318, *p*=0.010) (Table V).

Table IV. Comparison of SWV measurements, anthropometric, metabolic and laboratory parameters were compared in study group between children without MetS and with MetS, without IR and with IR.

	Without MetS	With MetS	*p*	Without IR	With IR	*p*
	N=102	N=29		N=56	N=75	
	mean ± SD (range)	mean ± SD (range)		mean ± SD (range)	mean ± SD (range)	
Males (%)	44(43.1 %)	14 (48.3%)	0.62	34(60.7 %)	24(32.0 %)	<0.001
Age (years)	12.3±2.7 (6.4-18)	13.4±2.6 (7-17.2)	0.069	12.1±2.9 (6.4-18)	12.9±2.4 (6.9-17.2)	0.70
BMI (kg/m^2)	28.4±4.5 (18.3-42.8)	31.4±4.4 (23.8-40)	0.002	27.3 ± 4.5 (18.3-38.8)	30.4 ± 4.3 (23.7-42.8)	<0.001
SDS	2.4±0.6 (1.1-4.2)	2.7±0.6 (1.2-3.8)	0.029	2.2±0.6 (1.1-3.8)	2.6 ± 0.6 (1.2-4.2)	0.001
Systolic blood pressure (mmHg)	108.2±12.6 (90-140)	117.8±16.7 (90-140)	0.023	101±7.7 (90-140)	112.1±13.9 (90-150)	0.323
Diastolic blood pressure (mmHg)	68.3±7.1 (50-80)	71.8±12.1 (50-90)	0.364	67.9±8.9 (50-85)	70.1 ± 8.7 (60-90)	0.462
Triglycerides (mg/dl)	107.3±53.4 (41-367)	200.8±96.5 (63-524)	<0.001	112.7±67.7 (41-391)	139.4±80.1 (41-524)	0.008
HDL cholesterol (mg/dl)	47.8±10.1 (28.4-86)	37.3±6.7 (30-58)	<0.001	48.4±11.4 (30-86)	43.3 ± 9.2 (28.4-71)	0.004
AST (IU/L)	25.46±11.90 (10-100)	28.40±16.31 (13-88)	0.625	27.3±13.9 (10-100)	25.2±12.3 (11-88)	0.207
ALT (IU/L)	24 ±16.3 (6-95)	31.5±31.6 (10-163)	0.212	24.3±17.6 (6-88)	26.5±2.5 (9-163)	0.338
HOMA-IR	3.7±2.4 (0.3-12.4)	5.6±3.9 (0.9-17)	0.012	1.8±0.8 (0.30-3.12)	5.7±2.8 (2.81-16.98)	<0.001
SWV of liver (m/sn)	1.1±0.44 (0.70-3.18)	1.23±0.70 (0.63-3.36)	0.719	1.02±0.29 (0.63-2.14)	1.24 ± 0.61 (0.63-3.36)	0.101

BMI: body mass index, BMI-SDS: body mass index-standard deviation, SBP: systolic blood pressure, DBP: diastolic blood pressure, HDL cholesterol: high-density lipoprotein cholesterol, AST: aspartate aminotransferase, ALT: alanine aminotransferase, HOMA-IR: homeostatic model assessment-insulin resistance, SWV: Shear wave velocity, MetS: metabolic syndrome, IR: insulin resistance

Table V. The results of univariate and multivariate regression analysis assessing the parameters that are independently associated with liver fibrosis.

	Liver stiffness (SWV ≥1.20m/sn)					
	Univariate analysis			Multivariate analysis adjusted for age		
Parameters	OR	%95 CI	*p* value	OR	%95 CI	*p* value
Age	1.203	1.055-1.371	0.006			
Gender	0.579	0.272-1.1230	0.155			
BMI-SDS	1.321	0.954-1.830	0.094	2.584	1.255-5.318	0.010
MetS	0.967	0.368-2.540	0.946			
IR	0.304	0.120-0.768	0.012			

BMI-SDS: body mass index- standard devaiation score, MetS: metabolic syndrome, IR: insulin resistance

The intraobserver agreement which is expressed as interclass correlation coefficient was 0.83 (95 % CI, 0.79-0.87; p <0.001). The results demonstrated that the SWV measurements had good agreement reproducibility.

Discussion

This study was mainly interested in the effects of excess weight and metabolic parameters on liver stiffness using SWE in children. In the first step, the relation between excess weight and liver stiffness was assessed. Although p value (0.047) was close to 0.05, the mean SWV of the overweight/obese children was statistically higher than the control group. With more control and study subjects the difference might be more obvious. In a study by Bailey et al.[33], SWV measurements were significantly lower in the normal group than the obese group concordant with the current study. The mean SWV was 1.08±0.14 m/s and 1.44±0.39 m/s in order of the healthy and obese group. The value for healthy group was in close agreement with the current findings. However, the value for the obese group was relatively higher than the results of the current study, indicating more liver stiffness. This difference may be related to their study population consisting mostly of Hispanic children who have a predisposition for obesity-based abnormalities, such as liver diseases and diabetes.[34] On the other hand, in a study using TE.[35] no significant difference was found for liver stiffness measurements between overweight, obese, and healthy children. Berná-Serna et al.[6] observed a weak positive correlation between SWV and BMI. In concordance with their research, there was a weak positive correlation between SWV and BMI, BMI-SDS in the present study. Age was positively associated with SWV. This result contributed to the studies, stating that liver stiffness measurements have an age-dependent increase.[33,36]

In the second step, qualitative assessments of conventional US was compared with p-SWE measurements. No statistically significant difference was found between fibrosis categories and HS categories. Bailey et al.[33] reported that SWV is significantly higher in abnormally hyperechoic livers than livers with normal echoes on conventional US. The present results were compatible with their study. Berná-Serna et al.[6] found significant differences between fibrosis categories and HS grades. Nine obese or overweight children out of 148 with normal liver echotexture or mild steatosis on the grayscale showed significant fibrosis in SWE measurements of their study. Unlike Berná-Serna et al.[6] the difference between fibrosis categories and HS grades in the current study was not significant. However, it was intriguing that 15 overweight or obese children out of 116 with normal echotexture or mild steatosis, showed significant fibrosis on SWE measurements, similar with their study.

Although MetS and IR are closely related metabolic factors, they are actually different entities.[37] Kurtoglu et al.[37] reported that IR was

prominent in obese patients not only with MetS but also without MetS. Also, they stated that IDF criteria of MetS were not sufficient to discover patients with IR. Based on this study, the effects of IR are analyzed separately in this study. Contributing to their study, 41% of overweight-obese children who were not grouped in MetS had IR, 5% of overweight-obese children who did not have IR were grouped in MetS.

The association of liver stiffness and MetS, IR was studied in the following steps. No statistically significant difference was found between the mean SWV of the patients with MetS and without MetS. Also, there was no statistically significant difference for SWV between the patients with IR and without IR. Due to the coexistence of visceral obesity, IR, dyslipidemia, NAFLD is considered to be the hepatic manifestation of MetS.[38,39] Some studies in adults state that MetS is associated with a higher liver fibrosis degree in subjects with NAFLD.[40-42] A study assessing the effect of MetS on liver stiffness in children using TE stated that, fibrosis is three times more likely to occur in the presence of the MetS.[13] In the current study, although the patients with MetS have higher SWV measurements than patients without MetS, the difference was not statistically significant. The association of IR and liver stiffness was reported in some studies.[43-47] A few pediatric studies[46,47] were encountered in the literature, determining the effects of IR on liver stiffness. Kwon et al.[46] utilizing TE, Stepanov et al.[47] using SWE, reported that liver stiffness measurements were correlated with HOMA-IR. In the current study, SWV measurements were weakly correlated with HOMA-IR (p=0,020). However, there was no significant difference for SWV between patients with or without IR. On the other hand, in a higher age group, the effects of these factors on liver stiffness may be more evident as the duration of MetS and IR increase.

In the last step of the study, regression analysis was performed for determining the most significant factors affecting liver stiffness. The only independent factor affecting liver stiffness was BMI-SDS after adjusting for age in the multivariate analysis. Huh et al.[48], in a large study cohort in adults, categorized their subjects into four groups according to metabolic health status and obesity: metabolically unhealthy obese, metabolically healthy obese, metabolically healthy non-obese, metabolically unhealthy non-obese. They reported that obese patients were at a higher risk for liver fibrosis than non-obese patients regardless of metabolic parameters. Obesity and metabolic abnormalities are regarded as the two underlying mechanisms of NAFLD. A commonly accepted hypothesis on NAFLD pathogenesis is the 'two hits thesis'. Obesity is the 'first hit' increasing the sensitivity of the liver to injury. Metabolic abnormalities are the 'second hit' injuring the liver by oxidative and inflammatory cytokines leading to liver fibrosis.[49-51] In the current study, multivariate analysis revealed that liver stiffness was 2,6 times more likely to occur with the increase of BMI-SDS per one unit (OR:2.584, 95% CI: 1.255-5.318, p=0.010). Similar with Huh et al.[48], the results of the current study point out that, obesity itself has a direct impact on liver fibrosis and join to the formation of liver fibrosis without the 'second hit' step. Obesity was the only significant factor affecting liver stiffness. On the other hand, the duration of MetS and IR was short in this study. In advancing ages with longer duration of these factors, the results would be more accurate.

One of the important limitations of this study was the absence of liver biopsy for histologic confirmation. However, liver biopsy is an invasive, impractical procedure that is not preferred especially in children. The lack of other reference standards such as Magnetic Resonance Elastography is another limitation of the study. This study was a cross-sectional study and is not adequate to see cause and effect relationship. Longitudinal follow-up studies are needed to reveal the factors causing liver fibrosis, accurately.

To our knowledge, the current study is the first to analyze and compare the effects of excess weight, MetS and IR on liver stiffness in children utilizing p-SWE technique.

According to the results of this study obesity itself, rather than MetS or IR, seems to be the major problem affecting liver stiffness. However further, large scale longitudinal studies following children in advancing ages might clarify this issue.

Ethical approval

This study was approved by the Erciyes University Medical School, Ethics Committee (approved number 07.03.2018-134).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: ZFK, FK, MK, AC; data collection: ZFK, SS, GD; analysis and interpretation of results: ZFK, NH; draft manuscript preparation: ZFK, NH. 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

Development of preschool refugee children living under temporary protection status

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ABSTRACT

Background. The conflict in Syria following the anti-regime demonstrations that started in March 2011 created one of the greatest humanitarian crises. The United Nations High Commissioner for Refugees (UNHCR) reports that refugee and resettlement experiences can influence the critical stages of intellectual, social, emotional and physical development of children. There is a lack of sufficient information about the prevalence of developmental delay in forcibly displaced children. In this study, we aimed to describe the impact of the Syrian crisis on the development of children after resettlement, factors that are associated with developmental problems and domains in which developmental delays are more likely to occur.

Methods. Refugee children (n=60) between the ages of 18-72 months admitted to the Yenimahalle Community Health Center Immigrant Health Unit to receive primary health care services between 1 November 2018- 1 March 2019 were included in this study. The control group included 60 Turkish children between 18-72 months admitted to the İsmail Ulucan Family Health Center which is in the same building. Developmental assessments were conducted by the researchers using the Denver II Developmental Screening Test (DDST-II). Sociodemographic characteristics of the child, family and caregivers as well as risk factors related to development were collected using a questionnaire. The interviews with refugee families were conducted with an interpreter.

Results. Developmental delay was more frequent in refugee children compared to Turkish children. The DDST-II were normal in 82.1%, questionable in 10.7% and abnormal in 7.1% of Turkish children; in the study group, 22.2% of the patients were found to be normal, 33.3% were questionable and 44.4% were abnormal. The differences were statistically significant ($p<0.05$). Multiple logistic regression analysis revealed that, being a forcibly displaced refugee was the single significant risk factor for developmental delay alone. In the DDST II subdomain analysis, it was seen that high monthly income reduces the risk of caution-delay in personal-social domain. It was found that birth weight below 2500 g increased the risk of caution-delay in the fine-motor and gross-motor domain and being a forcibly displaced refugee and consanguinity increased the risk of caution -delay in the language domain.

Conclusions. This study showed that being a forcibly displaced refugee was the most important risk factor for developmental delay. We emphasized the importance of surveillance and screening development in these high-risk children as well as early intervention services.

Key words: refugee, war, child, child development, developmental delay, forced displacement.

Children are extremely vulnerable to forced displacement and humanitarian emergencies. The Syrian conflict has raised these numbers to an unprecedented level. Currently, Syria's conflict has spawned 4.8 million refugees in neighbouring countries, hundreds of thousands in Europe, and 6.6 million people displaced inside Syria against a pre-war population of over 20 million.[1] Turkey hosts the highest refugee population in the world (3,671,761 million).[2]

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During the process of migration; care of newborns and children with chronic diseases is interrupted, exposure to infectious diseases increases, immunization processes are disrupted; furthermore, proclivity towards criminality in later life stages and susceptibility to abuse tend to rise. At the same time, asylum-seekers have unique development and learning needs. Infections, nutritional deficiencies and traumatic experiences in the early stages of life have a significant effect on the development of the child.[3] Experiencing traumatic events, including violence, abuse or neglect leads to cognitive, emotional, and behavioral changes, affecting learning and academic performance.[4-6] Exposure to violence is a risk factor for a range of mental health problems and such effects are dose-dependent; ie, more excessive and repeated exposures lead to a greater effect.[7] Early childhood is a period in which environmental factors have significant effects on the development of the brain and central nervous system and includes children's skills in physical, mental, social and emotional fields. During early childhood nutrition, health and the environment in which the child grows, are determinants of development.[8,9] The United Nations High Commissioner for Refugees (UNHCR) reports that refugee and resettlement experiences may affect critical stages of intellectual, social, emotional and physical development of children.[10] Socioeconomic distress, exposure to violence in the countries of origin, followed by migration and ultimately resettlement in a new environment exposes them to several cumulative risks for their physical, emotional and social development.[11] There is insufficient data in the literature on the prevalence of developmental delay in forcibly displaced children or the optimal approach to detection or screening in this group. Developmental delay in these children may not be detected by families, and when diagnosed, interventions may be delayed due to cultural barriers, creating unfavorable academic and psychosocial consequences that may also affect later life.[12,13] Given the evident positive effects of early intervention, it is important to identify the most appropriate approach to effectively screen refugee children.

The aim of this study was to evaluate the developmental levels of refugee children aged 18-72 months living under temporary protection status in Ankara; to determine the associated risk factors that may affect child development and compare with Turkish children of the same age range as a control group. The secondary aim of the study is to emphasize the importance of developmental screening tests in primary care and to contribute to further studies on the subject.

Material and Methods

Study Design

This is a case-control study conducted to evaluate the developmental level of the children of families who came to Turkey after the Syrian conflict and who are living under temporary protection status and to compare their developmental level with Turkish children.

Population and Sample

A pilot study that included 15 patients from both groups was conducted to calculate the sample size to establish a statistically significant relationship between the two groups. Using the PASS 11 program, the sample size was calculated to be at least 28 to attain 90% power at 95% confidence level. The study included 60 patients from each group and 120 patients in total. Refugee children (study group) (n=60) aged between 18-72 months admitted to the Yenimahalle Community Health Center No. 2 Migrant Health Unit (MHU) for primary health care between November 1, 2018 and March 1, 2019 were included in this study. MHUs have been devised in order to provide basic and preventive health services to Syrians in Turkey in a more effective and efficient way. The control group consisted of Turkish children (n=60) aged 18-72 months who applied to the İsmail Ulucan Family Health Center located in the same building. Exclusion criteria were the presence

of any visual, auditory or physical disabilities and health problems that would impair their general condition.

Ethical approval of the study was obtained from Hacettepe University Non- interventional Clinical Research Ethics Board (decision number: 18/450-27, date: 2018/03). Permits were procured from the Department of Migration Policies which is a branch of the Ministry of Internal Affairs' Directorate General of Migration Management (decision number: 621003649-604.02.02-E.42356, date: 2018/09), along with the General Directorate of Public Health of the Ministry of Health (decision number: 49654233-604.02, date: 2018/07).

In this study, developmental level of the children were evaluated by Denver II Developmental Screening Test (DDST II). Height, body weight, and head circumference were measured. Written informed consent was received from families who agreed to participate in the study. The interviews with refugee families were conducted with an interpreter. Sociodemographic characteristics of the child, family and caregivers as well as risk factors related to development were collected using a questionnaire. In addition to these questions, factors related to war (hearing war sounds, witnessing death) and migration were also questioned in the study group. Afterward, DDST II was applied to evaluate developmental status. Growth was evaluated using the National Center for Health Statistics percentile curves.

The DDST II was conducted by a researcher who was certified to apply the test with the assistance of a medical interpreter. After the assessment, recommendations to support development were given to every family. The test was repeated 3 months later for children in both groups who were categorized as "questionable", to increase the reliability of this result. Out of the 27 "questionable" cases in the study group, 21 were available for a second evaluation. In the control group, 16 of the 20 "questionable" cases were available for a second evaluation. The outcome of the second evaluation was used in the final results. The necessary referrals were made for refugee and Turkish children who were questionable as a result of repeated DDST II. The flowchart of the study is shown in Figure 1.

Denver II Developmental Screening Test (DDST II)

DDST II was adapted and standardized for the Turkish society. The test assesses development in children aged 0-6 in four domains: personal-social, fine motor, gross motor and language. It consists of 125 items, and the development of a child is measured based on these 125 items. Each test item is scored as pass, fail or refused. Delay is defined as a child failing a test item which 90% of his or her age mates pass, and caution is defined as a child failing a test item which 75% to 90% of his or her age mates pass. The test usually takes 10–20 min to perform, and the child is classified as normal, questionable, or delayed based on the test results. "Normal" is no delay in any domain and no more than 1 caution; "questionable" is one delay or more than 2 cautions; "abnormal" is 2 or more delays.[14,15]

The Turkish version of DDST II was used in this study.[16] Screening results are given as normal, questionable and abnormal. In each domain the results are defined as normal, caution or delay for age.

Statistical Analysis

Chi-square test was used to investigate the relationships between qualitative data of the children included in the study. For continuous variables, t-test was used in paired groups and ANOVA test was used to determine the differences between parameters with more than 2 groups. $P < 0.05$ was considered statistically significant. A multiple logistic regression model was developed using the variables which rendered $p < 0.05$ in pairwise comparisons and variables that would directly affect development. All statistical analysis were

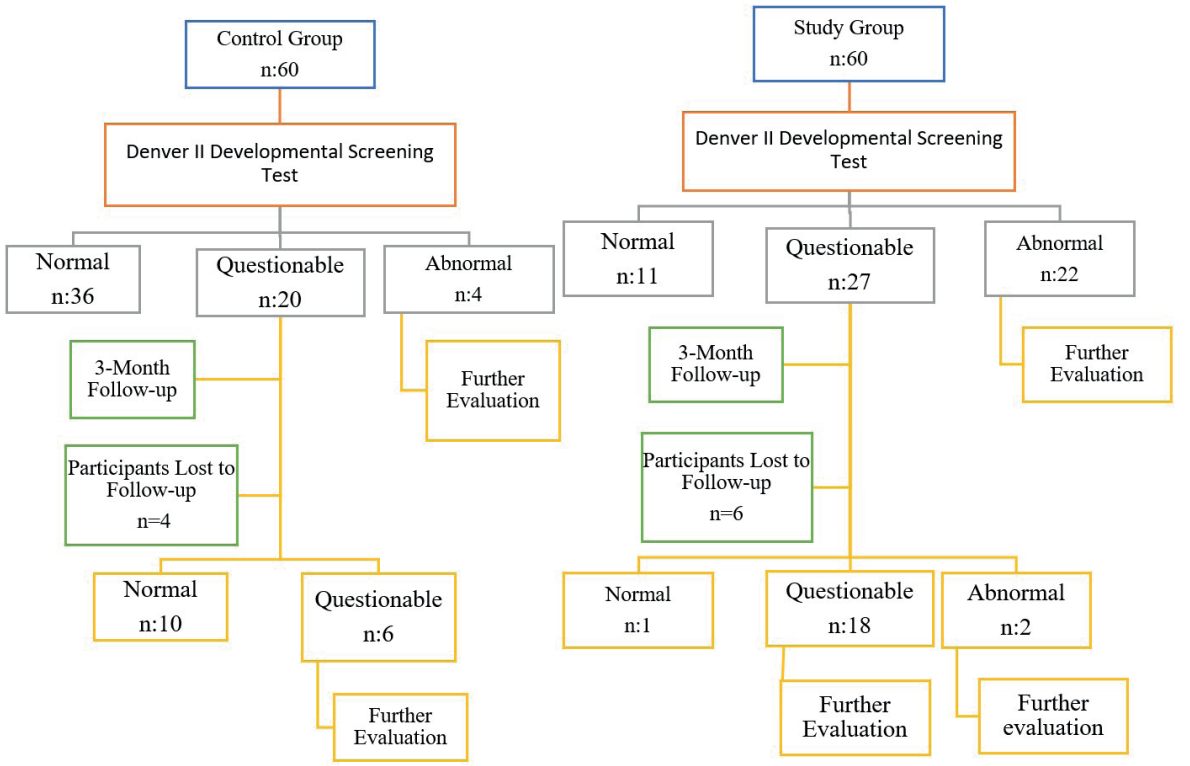

Fig. 1. Flow chart of the study.

performed using SPSS version 22.0 (Statistical Package for the Social Sciences).

Results

In the study, the control and study groups consisted of 60 children in each group and there was no difference in age and sex between the groups. Comparison of descriptive characteristics showed that in the study group the number of siblings, percentage of unemployed mothers, the number of people living in shanties and the number of people living per house, gravida of the mothers and consanguineous marriage rates were higher than in the control group. Also in the study group; mothers were younger, mothers' education level, income levels and the number of rooms per house, the duration of breastfeeding and the mother's age at pregnancy were lower than the control group and these differences were statistically significant ($p<0.05$). (Supplementary table)

When the height and body weight were compared according to the pathological percentile values (<3 percentile and >95 percentile), no significant difference was found, but between the basis of <10, 10-50p and >50p, there was a statistically significant difference ($p <0.05$) between the height and body weight percentile values of the study and control groups.

The characteristics of the study group related to war and migration revealed that 43.3% of the children were in Syria during the war and heard war sounds; 5% had witnessed death during the war. The children who moved 2 times and/or more was 81.7% after they left their country. In the study group, 96.7% of the mothers stated that they were not exposed to discrimination. Among refugees, 56.7% had been in Turkey longer than 24 months; 85% had been in Ankara for longer than 12 months and 68.3% had never stayed in a camp (Table I).

According to the DDST II, 82.1% (n=46) of the children in the control group were normal, 10.7% (n=6) were questionable and 7.1% (n=4) were abnormal with respect to their age. In the

Table I. Descriptive characteristics of the study group related to war and migration.

		Study Group n :60
		n (%)
Turkish-speaking People in the Household	Yes	48 (80)
	No	12 (20)
Presence of Turkish Literate in the Household	Yes	21 (35)
	No	39 (65)
Exposure to War (Child)	No	31(51.7)
	Hearing War Sounds	26 (43.3)
	Witnessing Death	3 (5)
Exposure to Discrimination (Mother)	Yes	2 (3.3)
	No	58 (96.7)
Number of Relocations (Child)	1	11 (18.3)
	2	25 (41.7)
	3	21 (35)
	4	3 (5)
Length of Stay in Turkey (Children) (Months)	≤12	6 (10)
	13-24	20 (33.3)
	25-36	9 (15)
	36-48	17 (28.3)
	≥49	8 (13.3)
Length of Stay in Camp (Child) (Month)	0	41 (68.3)
	≤12	12 (20)
	13-24	4 (6.7)
	25-36	3 (5)
Length of stay in Ankara (Child) (Month)	≤12	9 (15)
	13-24	33 (55)
	25-36	15 (25)
	37-48	3 (5)

study group, 22.2% (n=12) cases were normal, 33.3% (n=18) were questionable and 44.4% (n=24) were abnormal with respect to their age (p = 0.000) (Fig. 2).

Table II shows the comparison of the results of children in the study and control groups according to the developmental domains of DDST II. There was a statistically significant difference between the groups in the language, fine motor and gross motor domains (p <0.05).

When the DDST II results of refugee children born in Syria and Turkey were compared it was shown that 13% (n=7) of Syrian born were scored questionable and 31.5% (n=17) were abnormal with respect to their age. However, 20.4% (n=11) of Turkey born were questionable and 13% (n=7) were abnormal with respect to their age. There was no statistically significant difference between the groups (p>0.05).

When the subjects exposed to war and those who were not exposed to war were compared in terms of DDST II results, it was seen that exposure to war increased the frequency of abnormal DDST II (p = 0.050) in the study group (Table III).

Within the scope of the research, the risk factors that could affect child development were questioned. Among these variables, 17 variables,

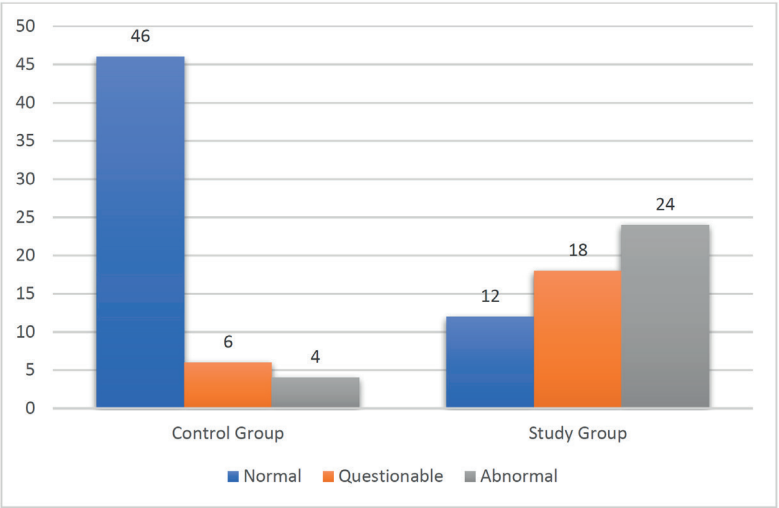

Fig. 2. Distribution of Denver II Developmental Screening Test Results of Children in the Study and Control Groups.

Table II. The comparison of the results of children in the study and control groups according to the Developmental Domains of DDST II*.

	Control Group DDST II Results (n:56)			Study Group DDST II Results (n:54)			p
	Normal n (%)	Caution n (%)	Delay n (%)	Normal n (%)	Caution n (%)	Delay n (%)	
Personal-Social	47 (83.9)	5 (8.9)	4 (7.1)	39 (72.2)	6 (11.1)	9 (16.7)	0.256
Fine Motor	49 (87.5)	5 (8.9)	2 (3.6)	28 (51.9)	9 (16.7)	17 (31.5)	0.000
Language	45 (80.4)	8 (14.3)	3 (5.4)	16 (29.6)	14 (25.9)	24 (44.4)	0.000
Gross Motor	42 (75)	10 (17.9)	4 (7.1)	22 (40.7)	12 (22.2)	20 (37)	0.000

*Denver II Developmental Screening Test

Table III. Relation of war and migration-related characteristics of the study group to DDST II results.

		Study group DDST II results (n:54)		p
		Normal (n:12) n (%)	Questionable (n:42) n (%)	
Exposure to War (Hearing war sounds, witnessing death) (Child)	Yes	3 (11.1)	24 (88.9)	0.050
	No	9 (33.3)	18 (66.7)	

*Denver II Developmental Screening Test

which could directly affect development and displayed statistically significant differences between the two groups, were included in multiple regression analysis. These variables are as follows: number of siblings, mother's and father's education level, age of the mother at birth, monthly income status, consanguinity between parents, gestational week at birth, birth weight, duration of breastfeeding, whether parents read to the child, smoking status of

Table IV. Developmental risk and protective factors determined by logistic regression analysis.

	Variables	Odds Ratio (OR)	%95 Confidence Interval		p
			Lower Limit	Upper Limit	
The Denver Developmental Screening Test II Questionable-Abnormal	Being in Study Group	16,100	6,304	41,120	0.000
Personal-Social Caution-Delay	Monthly Income Over 2000 TL	0,461	0,239	0,889	0.021
Fine Motor Caution-Delay	Being in Study Group	5,247	1,962	14,033	0.001
	Birth Weight Below 2500 g	2,182	0,999	7,916	0.050
Language Caution-Delay	Being in Study Group	5,023	1,674	15,072	0.004
	Consanguineous Marriage	4,013	1,206	13,350	0.023
Gross-Motor Caution-Delay	Being in the Study Group	5,011	1,579	15,903	0.006
	Birth Weight Below 2500 g	5,751	1,605	20,605	0.007

parents, preschool education status, height, body weight, duration of background television exposure and being a refugee (*being forcibly displaced*). The abnormal-questionable cases for DDST II test results and delay/caution in each domain were evaluated together.

Logistic regression analysis revealed that the single significant factor that increased the risk of developmental delay by 16.1 times according to the DDST II assessment was being a forcibly displaced refugee. A monthly income of over 2000 TL reduced the risk of having caution-delay in the personal-social domain of DDST II 0.461-fold; being a forcibly displaced refugee increased the risk of caution-delay in the DDST II fine motor domain by 5,247 times and having a birth weight below 2500 grams increased the risk of caution -delay in this domain by 2,181-fold. Being a forcibly displaced refugee increased the risk of caution-delay in the DDST II language domain 5.023-fold, and consanguineous marriage increased the risk of caution-delay in the language domain 4,013 times. It was seen that being a forcibly displaced refugee increased the risk of caution-delay in the gross-motor area of DDST II by 5.011 times, having a birth weight less than 2500 grams increased the same risk by 5.751 times, and these were all statistically significant ($p < 0.05$) (Table IV).

Discussion

The present study delineates how the Syrian crisis, the extent to which the UNHCR called the greatest humanitarian crisis of the present, affects the developmental aspects of children, the most vulnerable group, after resettlement.

In accordance with the data reported by the Disaster and Emergency Management Presidency, reports by Celik et al.[17] and previous research done in countries with a high Syrian refugee population, such as Jordan and Lebanon; the socioeconomic and housing conditions of the refugee population have been shown to be worse and their education levels lower than the local population.[18,19]

In this study, the breastfeeding duration of Turkish mothers was significantly higher than that of refugee mothers. This difference may be a result of the stress caused by the negative life-experiences of asylum-seekers that prevents breastfeeding their babies. Their high fertility rate may also shorten their breastfeeding duration. In a meta-analysis that included data from 11 studies including a total of 322 immigrant women with children under five years of age, participants agreed that breastfeeding was the best for the baby, although it was difficult to continue breastfeeding while struggling with difficult living conditions in a new country.[20]

Likewise, in a 2013 study conducted in Lebanon, Jordan and Iraq to assess the health and nutrition of Syrian refugees affected by the conflict in the camps, the breastfeeding status of 1452 children between 0-23 months was evaluated. It was reported that the highest breastfeeding rates were in the 0-5 month period and there was a significant decrease in breastfeeding after 1 year of age.[21] The 2018 Turkish Demographic and Health Survey (TDHS) findings also support shorter median breastfeeding in Syrian families (16.7 mo vs 13.7 mo).[22]

In the present study, it was shown that the height and bodyweight of Syrian children were lower than Turkish subjects. In the 2014 study at the Za'atari camp in Jordan, the prevalence of chronic malnutrition was 17% for children staying at the camp, and 9% for children outside the camps.[23] The 2018 TDHS findings revealed a higher prevalance of stunting among Syrian under 5 children (17.4% vs 6%) and obesity (10.4% vs 8.1%) compared to Turkish children. However wasting frequencies were similar (1.9% vs 1.7 %).[22]

In this study, evaluation using DDST II revealed that developmental delay were more common in forcibly displaced refugee children compared to Turkish children. Of the Turkish children, 82.1% were normal, 10.7% were questionable, 7.1% were abnormal for their ages; while in the study group, 22.2% of the cases were normal, 33.3% were questionable and 44.4% were abnormal for their ages.

When multiple logistic regression analysis was used to evaluate the variables which were thought to have a direct effect on development; being a forcibly displaced refugee was found to be the single statistically significant risk factor for developmental delay. Also, when results of DDST II sub-domains were examined, high monthly income tended to reduce the risk of delay in the *personal-social* area, whereas being a forcibly displaced refugee and having low birth weight increased the risk of caution-delay in *fine-motor* and *gross-motor* domains, and being a forcibly displaced refugee and consanguinity increased the risk of caution-delay in the *language* domain. Replacement experiences, deterioration of the family structure, interruption of education, exposure to many traumatic events that may jeopardize development during refugee travel, the difficulty in adaptation to a new language, education system and new social-cultural norms can be said to have a cumulative effect to result in developmental delay. Also, Turkish language acquisition is very important for social integration. The Ministry of National Education has special Turkish courses for Syrian children to overcome the difficulty of adapting to a new language.

The frequency of developmental delay and disability are not well known in the child refugee population. In the study of Martin et al.[24] which aimed to review the literature on developmental screening of migrant and refugee children in 2009, interviews with migrant and refugee families were conducted and obstacles to developmental screening were identified. The obstacles were reported to be language and communication problems, difficulty in accessing health services, not recognizing developmental delays by families and lack of comparison with peers due to being confined to isolated environments during the time they spend with their children which stems from economic difficulties, disruption of family structure, living in the countryside, cultural beliefs, difficulty in comprehension and acceptance of information. Likewise, regression analysis showed high monthly income to be a protective factor against developmental delay in the *personal-social* area.

In this study, it was displayed that encountering war in Syrian children significantly increased the risk of a questionable-abnormal DDST II outcome. Also, the assessment using DDST II revealed that forcibly displaced refugee children born in Syria are 2.42 times more likely to experience developmental delays than Syrian refugee children born in Turkey, but the difference was not significant which may be due to the limited sample size. The difference

may be related to more adverse living, birth and migration conditions of the Syrian born children. When other studies on the effects of war encounter on children in Syria are evaluated, it is noted that these studies are mostly on mental health; no studies on the effects of war exposure on child development has been found. In the Bahçeşehir survey, almost half of the Syrian refugee children experienced post traumatic stress disorder (PTSD) symptoms more than ten times the rate observed in other children and 44% had symptoms of depression.[25] In a study conducted by Save The Children and colleagues in Syria, the number one cause of psychological stress in the daily lives of 84% of adults and nearly all children was the ongoing bombing and shelling; 89% of the children were fearful and tense, 71% of children suffered from bed wetting and incontinence which are common signs of PTSD and toxic stress, and 51% of adolescents used substances to cope with problems.[26] In a more recent study conducted in Turkey, it was found that 33% of Syrian forcibly displaced refugees of all ages met the diagnostic criteria for PTSD.[27] In the study conducted in a camp in Osmaniye by Derman et al.[28], adolescents between the ages of 16-20 underwent a brief symptom inventory; it was found that psychosomatization and hostility to their country of residence were common, they thought their freedom was limited but they struggled to integrate into the society. The results of this study support the view that psychosocial risk factors are strong predictors of current and future developmental and mental health problems and are of particular concern as these negative experiences in early childhood can have a permanent effect.[29]

Although the questionnaire, interviews and the DDST-II assessment have been conducted with experienced interpreters who have long been employed by the immigrant health center, this may be considered a limitation to the study. In future research, it is necessary to implement appropriately validated screening tools and health personnel with language proficiency and training. In addition, a significant relationship could not be established between DDST II results and some factors that may affect the development of the child; this may be attributed to the limited sample size as was previously mentioned and this necessitates further studies with larger sample sizes. However, the sample size of the study is reliable to demonstrate the developmental differences between refugees and Turkish children. The lack of studies in the literature that assess the factors affecting the developmental level of forcibly displaced refugee children has limited the comparison and discussion of the data of this research. On the other hand, this fact once again emphasizes the significance of the present study. Lack of consent for access to laboratory data also limited the present study. Another limitation of the study is that the Turkish and forcibly displaced refugees included in the study consisted of children and families who applied to the immigrant health center for health care so results can not be generalized to the whole population. However, having a control group can be considered as a strength of the study compared with the limited number and sample size of other studies mentioned above.

In light of these results, it was found that refugee children displayed a higher rate of developmental delay. In the light of these results, developmental screening should be expanded as a priority in primary care in order to minimize the negative impact of forced displacement processes that hinder the development of refugee children. And also intervention programs should be started, families should be educated about the developmental process of the child, warning signs should be determined, interventions that support development and awareness should be created, this training should be supported with visual tools and they should be directed to more equipped facilities when developmental concerns arise. For the integration of Syrian children into the Turkish community, language is very important to overcome disadvantages. Considering that encountering war is a risk factor for developmental delay, in order to minimize the

negative effects of this situation and to increase resilience, it is recommended to develop family support, a positive relationship with the parent and to develop support programs for teachers, peers, relatives and health service providers to provide environmental support.

Ethical approval

Ethical approval of the study was obtained from Hacettepe University Non-interventional Clinical Research Ethics Board (decision number: 18/450-27, date: 2018/03).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: ENÖ, MTA; data collection: MTA; analysis and interpretation of results: ENÖ, MTA, DAB, EK, MC; draft manuscript preparation: ENÖ, MTA. 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

The role of self-discontentment and impulsivity for youth smoking behavior, nicotine dependence and future smoking intention in a clinical sample of Turkish adolescents

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ABSTRACT

Background. Smoking is one of the most important public health problems among young people. Potential risk factors that may cause vulnerability to smoke in youth should be well known and investigated. The aim of the present study was to evaluate the associations of current smoking behavior and future smoking intention with high-risk personality traits for substance abuse in a clinical sample of Turkish adolescents, and also evaluate nicotine dependence and smoking characteristics with the personality traits in a subsample of regular smokers.

Methods. A cross-sectional study was adopted in which 196 participants took part (aged 14-18 years with a mean of 16.7 years). The assessment consisted of a sociodemographic questionnaire that also questions current smoking behavior and future smoking intention; and additionally, two self-administered instruments including the Substance Use Risk Profile Scale (SURPS) for all participants, and Fagerström Test for Nicotine Dependence (FTND) for only regular smokers.

Results. Regular smokers scored higher than never smokers on the lack of self-contentment subscale of SURPS ($F(2)=3.30$, $p=.039$). Future smoking intention was found to be associated with nicotine dependence ($F(3)=6.67$, $p=.001$). Regular smokers with high levels of nicotine dependence had higher levels of impulsivity and smoked more cigarettes per day than those with low levels of nicotine dependence ($t=2.489$, $p=.017$; and $t=3.530$, $p=.001$, respectively). The structural equation models (SEM) were created based on these results and the personality theory for substance abuse. The SEM results showed that the first evidence that lack of self-contentment positively influences regularly smoking behavior and impulsivity positively influences future smoking intention through nicotine dependence.

Conclusions. Lack of self-contentment and impulsivity may mediate the transition from current smoking behavior to future tobacco use disorders in Turkish adolescents. The assessment and intervention of self-discontentment and impulsivity can be beneficial in reducing the current smoking behavior in Turkish adolescents.

Key words: smoking, adolescent, personality, nicotine.

Smoking of any tobacco product is one of the most important public health problems among young people all over the world, although the hazards of smoking have been entirely demonstrated and governments have made extensive tobacco control efforts. As tobacco use rapidly turns into addiction; this disorder which causes early death, serious illness and disability; affects brain development, cardiovascular and

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respiratory systems, physical maturation and health; burdens adolescents, their families and national health systems.[1,2] In order to develop effective prevention strategies, potential factors that may cause vulnerability to tobacco use disorder in youth should be well known.

Even rare smoking of any tobacco product during adolescence is associated with the emergence of tobacco use disorder.[3] Older age (upper class), low socioeconomic status and poor academic performance increase the risk of smoking in adolescents.[4] Also, individual characteristics like sensation seeking and rebelliousness, susceptibility to smoking and intention to smoke in the future are potential predictors of tobacco use reported in a systematic review of longitudinal population-based youth studies.[5] Among environmental factors, having friends and family members who smoke, and watching commercials about tobacco promotion are likely to facilitate adolescents to start smoking.[6,7]

Individual factors such as personality traits and their relationships to smoking behavior and intention are the focus of researchers' attention who aim to develop personalized prevention interventions in adolescents.[8,9] Conrod et al. showed that higher levels of anxiety sensitivity, hopelessness (lack of self-contentment), sensation seeking, and impulsivity personality traits predict a higher risk for future alcohol and substance use. According to Conrod's theory of personality, the effects of alcohol and substance in reducing anxiety and pain play a negative reinforcement role, while their stimulant and pleasurable effects serve as positive reinforcement in the reward system through learning with operant conditioning.[10] In addition, Conrod developed the prevention program for alcohol and substance use including cognitive-behavioral interventions that target four at-risk personality traits.[11] However, current findings on smoking and personality traits are contradictory and await clarification. Previous studies identified specific personality traits that predispose adolescents to current smoking behavior and future smoking intention, such as impulsivity, sensation seeking and hopelessness, with the exception of anxiety sensitivity.[8,12,13] Unlike adolescents, several studies in adults reported a significant association between anxiety sensitivity and tobacco use, in addition to other personality traits.[14,15] Moreover, adolescent smoking is linked with impulsivity traits according to the meta-analyses including fifty-one studies.[16] On the contrary, Malmberg et al.[17] reported that impulsivity and anxiety sensitivity did not affect smoking behavior during adolescence, while sensation seeking and hopelessness were related to tobacco use. Smoking is thought to be a self-medication behavior to reduce negative affect related to hopelessness, not to anxiety sensitivity.[18] The peripheral effects of smoking on the human body may increase somatic complaints associated with anxiety such as tachycardia, hypertension, sweating, trembling, and increased respiration rather than treating them.[19] Sensation seeking and impulsivity were found to be associated with the anatomical structure of the cognitive control circuitry including anterior cingulate and medial frontal gyrus, so these traits may render adolescents vulnerable to smoking via impaired cognitive control.[20] Therefore, understanding the role of these personality traits in the transition from smoking behavior to nicotine addiction and tobacco use disorder in adolescents will be useful in planning interventions such as the prevention of tobacco product usage.

We hypothesized that high-risk personality traits might be associated with current smoking behavior and future smoking intention in a clinical sample of Turkish adolescents. There is limited research on these personality traits being associated with the emergence of nicotine dependence among regular smokers. This is the first study known to investigate the relationship between risky personality traits for substance abuse and nicotine dependence levels in a clinical subsample of Turkish adolescents who smoke regularly. Additionally, we hypothesized that those with high levels of impulsivity, sensation seeking, and hopelessness might have increased nicotine dependence as well as

current smoking behavior and future smoking intention. The aim of the present study was to evaluate current smoking behavior, future smoking intention, high-risk personality traits for substance abuse, the associations of current smoking behavior and future smoking intention with these personality traits in a clinical sample of adolescents, and levels of nicotine dependence and the associations of nicotine dependence and smoking characteristics with the personality traits in a subsample of regular smokers.

Material and Methods

Ethical approval was obtained from the Clinical Research Ethics Committee of the University of Health Sciences Ankara City Hospital (Decision No: E1/20/1003). The cross-sectional study was carried out in the Adolescent Health Unit of Dr. Sami Ulus Maternity and Children's Health and Diseases Training and Research Hospital between August 2020 and November 2020.

The sample consisted of 196 adolescents aged 14-18 years who applied to the Adolescent Health Unit outpatient clinic for any reason and agreed to participate in the study voluntarily. Illiterate adolescents, those with an intellectual disability or scales that were incompletely filled in were not included in the study. Participants with any mental or physical disorders were not excluded from the study. Written consents were obtained after detailed information was given to them about the subject and the purpose of the study. They completed the sociodemographic and clinical information forms, as well as self-report scales, including the Substance Use Risk Profile Scale (SURPS) and Fagerström Test for Nicotine Dependence (FTND).

Measures

The Sociodemographic and Clinical Information Form

The sociodemographic and clinical information form was prepared by the researchers to evaluate the sociodemographic and clinical characteristics of the participants. The form consists of questions about age, sex, educational status (grade), family characteristics (age of mother and father, education and employment status, number of siblings), current smoking behavior (never, occasionally/rarely, and regularly smoking), characteristics of smoking behavior (first smoking age, number of cigarettes smoked, total smoking duration) and future smoking intention (definitely smoking, probably smoking, probably not smoking, and definitely not smoking in the future).

The Substance Use Risk Profile Scale

The SURPS is a 23-item self-report questionnaire developed by Woicik et al. in 2009.[12] The scale evaluates four personality traits including anxiety sensitivity, hopelessness, sensation seeking, and impulsivity, which predict future substance use risk in adolescents. The SURPS is widely used for screening in youth due to its good psychometric properties. Each item of the Likert-type scale, which consists of four subscales representing the risky personality traits mentioned above, is scored between 1 and 4. The Turkish adaptation of SURPS was confirmed in a sample of high school students by Uygun et al. in 2019, showing that the Turkish version of the scale was valid and reliable.[21] Cronbach alpha coefficient for the Turkish version of SURPS was .73, and Cronbach alpha coefficients for lack of self-contentment (as it is called hopelessness in the original study), sensation seeking, impulsivity and anxiety sensitivity subscales were calculated as .76, .71, .67 and .66, respectively.[22]

The Fagerström Test for Nicotine Dependence

The FTND, which is the self-report scale developed by Fagerström et al.[23], consists of 6 items in only one sub-dimension. Each question has specific scores based on its answer. The scores obtained from the test are classified as follows: 1) Very low (0-2 points); 2) Low (3-4 points); 3) Medium (5-6 points); 4) High (7-8 points); and 5) Very high (9-10 points). Uysal et al.[24] showed that the Turkish version of

the Fagerström test was valid and reliable to evaluate nicotine dependence. Cronbach alpha coefficient for the Turkish version of FTND was .56.

Statistical Analysis

Statistical analyses were performed using the SPSS Statistics for Windows, version 22.0.[25] The variables were investigated using the Kolmogorov-Smirnov test to determine whether or not they were normally distributed. Descriptive analyses were presented using means and standard deviations for normally distributed variables, and medians and minimum-maximum values for non-normally distributed variables. Categorical variables were presented using frequencies (n) and percentages (%). While investigating the associations between non-normally distributed variables, the correlation coefficients and their significance were calculated using Spearman test. Since the substance use risk profiles and nicotine dependence were normally distributed, these parameters were compared using one-way ANOVA among the current smoking status (never, occasionally/rarely, and regularly smoking) and future intention status (definitely smoking, probably smoking, probably not smoking, and definitely not smoking in the future) groups. Levene test was used to assess the homogeneity of the variances. An overall p-value of less than .05 was considered to show a statistically significant result. When an overall significance was observed, pairwise post-hoc tests were performed using Tukey's test. Since nicotine dependence level was a 5-level categorical variable, those with very low and low levels of nicotine dependence among regular smokers were classified as low nicotine dependence group, and those with medium, high and very high levels were classified as high nicotine dependence group.[24] Since the substance use risk profiles and the number of cigarettes smoked per day showed normal distributions, these parameters were compared using Student's t test between the groups with high and low nicotine dependence. Since the first smoking age and total smoking duration were not normally distributed, these parameters were compared using the Mann-Whitney U test between the groups with high and low nicotine dependence. Pearson-$\chi 2$ and Fisher's exact tests were used for categorical variables. To test models based on Conrod's theory of personality for youth's current smoking behavior, nicotine dependence, and future smoking intention the structural equation modelling (SEM) was performed using the "lavaan" package in the R Project for Statistical Computing (version 4.0.0) program.[26-28] The path coefficients were estimated using diagonally weighted least squares (DWLS) method and hypothesizes were tested. The chi-square/degree of freedom ($\chi 2$/sd), Root Mean Square of Error Approximation (RMSEA), Goodness of Fit Index (GFI), Normed Fit Index (NFI), Comparative Fit Index (CFI) fit indices were used to evaluate the fitness of the SEM model. A 5% type-I error level was used to infer statistical significance.

Results

Of the participants, 161 (82.1%) were male. The mean age of the sample was 16.7 (±1.0) years. Details about the descriptive characteristics of the participants are shown in Table I. The participants who reported never smoking, occasionally or rarely smoking, and regularly smoking were 63% (n=123), 10.8% (n=21), and 26.2% (n=51), respectively. When asked about the future, the percentages of participants that responded as "definitely not smoking", "probably not smoking", "probably smoking", and "definitely smoking" were 53.1% (n=103), 21.6% (n=42), 17.5% (n=34), and 7.7% (n=15), respectively. The median age of first smoking in adolescents who smoked at least once was 13 (5-17) years. The mean number of cigarettes smoked per day by regular smokers was 15.3 (±8.1). The median total smoking duration of regular smokers was 4 (0.4-9) years.

Regular smokers scored higher than never smokers on lack of self-contentment subscale of SURPS (See Table II; F(2)=3.30, p=.039).

Table I. Sociodemographic characteristics, future smoking intention, current smoking behavior, characteristics of smoking behavior, levels of nicotine dependence and substance use risk profiles.

Variables	Outcome
Age (years)[a]	16.7 (1.0)
Sex, n (%)	
Males	161 (82.1)
Females	35 (17.9)
Grades, n (%)	
9	31 (16.1)
10	24 (12.4)
11	49 (25.4)
12	89 (46.1)
Maternal age (years)[a]	41.0 (5.9)
Maternal education status, n (%)	
Primary school	93 (48.0)
Middle school	73 (37.6)
High school	26 (13.4)
University	2 (1.0)
Maternal employment status, n (%)	
Not working	172 (91.0)
Working	16 (8.5)
Retired	1 (0.5)
Paternal age (years)[a]	45.4 (6.1)
Paternal education status, n (%)	
Primary school	79 (41.0)
Middle school	74 (38.3)
High school	33 (17.1)
University	7 (3.6)
Paternal employment status, n (%)	
Not working	10 (5.3)
Working	167 (88.4)
Retired	12 (6.3)
Number of siblings[b]	3 (1-6)
Future smoking intention, n (%)	
Definitely smoking	15 (7.7)
Probably smoking	34 (17.5)
Probably not smoking	42 (21.6)
Definitely not smoking	103 (53.1)
Current smoking behavior, n (%)	
Regularly smoking	51 (26.2)
Occasionally or rarely smoking	21 (10.8)
Never smoking	123 (63)
First smoking age (years)[b]	13 (5-17)*
Number of cigarettes smoked (per day)[a]	15.3 (8.1)**
Total smoking duration (years)[b]	4 (0.4-9)**
Nicotine Dependence by FTND (n, %)[a]	51 (100.0); 5.0 (2.4)**
Very Low	8 (15.7)
Low	15 (29.4)
Medium	6 (11.8)
High	12 (23.5)
Very High	10 (19.6)
Substance Use Risk Profile by SURPS[a]	
Lack of self-contentment (Hopelessness)	13.9 (4.7)
Sensation seeking	16.0 (4.0)
Impulsivity	10.6 (3.1)
Anxiety sensitivity	11.6 (3.2)

[a]: Mean (standard deviation), [b]: Median (minimum-maximum), SURPS: Substance Use Risk Profile Scale, FTND: Fagerström Test for Nicotine Dependence, *: in adolescents smoking at least once, **: in adolescents smoking regularly.

Apart from this finding, current smoking behavior and future smoking intention for all participants; nicotine dependence and characteristics of smoking behavior for regular smokers were not associated with the risky personality dimensions (Table II and III; for all variables p>.05). Adolescents who definitely want to smoke in the future have higher levels of nicotine dependence than those who probably or definitely don't want to smoke in the future (Table IV; F(3)=6.67, p=.001).). Regular smokers with high levels of nicotine dependence had higher levels of impulsivity and smoked more cigarettes per day than those with low levels of nicotine dependence (Table V; t=2.489, p=.017; and t=3.530, p=.001, respectively).

Table II. Comparison of substance use risk profiles in all adolescents (n=195) and regular smokers (n=51) according to future smoking intention and current smoking behavior.

	Substance Use Risk Profile Scale (SURPS) dimensions[a]			
	Lack of Self-contentment	Impulsivity	Sensation Seeking	Anxiety Sensitivity
Future smoking intention				
Definitely smoking	16.1 (6.1)	11.4 (3.0)	15.9 (5.4)	10.3 (3.3)
Probably smoking	15.4 (4.2)	11.1 (2.9)	16.9 (4.2)	11.7 (3.2)
Probably not smoking	13.8 (4.4)	10.5 (2.8)	15.7 (4.0)	11.8 (2.8)
Definitely not smoking	13.3 (4.7)	10.4 (3.3)	15.8 (3.8)	11.7 (3.3)
ANOVA	F(3) = 2.33	F(3) = 0.59	F(3) = 0.57	F(3) = 0.66
p value	.076	.617	.632	.575
Current smoking behavior				
Regularly smoking	15.4 (4.6)	10.6 (3.0)	16.1 (4.3)	11.4 (3.3)
Occasionally/rarely smoking	14.5 (5.6)	10.4 (2.7)	16.3 (3.8)	10.8 (2.7)
Never smoking	13.3 (4.5)	10.6 (3.2)	15.9 (3.9)	11.9 (3.2)
ANOVA	F(2) = 3.30	F(2) = 0.03	F(2) = 0.15	F(2) = 0.93
p value	**.039**	.964	.853	.397
Regularly smoking - Never smoking; Tukey **p = .035**				
Future smoking intention*				
Definitely smoking	15.0 (6.2)	11.3 (3.3)	15.5 (6.0)	10.1 (3.7)
Probably smoking	15.7 (4.2)	11.0 (2.9)	16.6 (4.2)	11.9 (3.3)
Probably not smoking	15.0 (4.9)	9.2 (2.8)	16.0 (3.7)	11.1 (3.0)
Definitely not smoking	16.0 (0)	10.0 (0)	13.0 (0)	14.0 (0)
ANOVA	F(3) = 0.07	F(3) = 1.09	F(3) = 0.31	F(3) = 0.79
p value	.972	.364	.813	.502

a: Mean (standard deviation), *: in adolescents smoking regularly (n=51).

Table III. Correlation analysis of substance use risk profiles with nicotine dependence level and characteristics of smoking behavior.

			Substance Use Risk Profile Scale (SURPS) dimensions			
	n		Lack of Self-contentment	Impulsivity	Sensation Seeking	Anxiety Sensitivity
Cigarettes' number smoked (per day)	44	rho	-.143	.000	.119	-.007
		p	.354	.998	.440	.962
Total smoking (years)	50	rho	-.008	-.084	-.009	.076
		p	.955	.563	.951	.599
First smoking age (years)	62	rho	-.179	-.049	.099	-.073
		p	.164	.703	.443	.573
Nicotine dependence	43	rho	-.076	.160	.021	.013
		p	.630	.305	.895	.933

rho: Spearman correlation coefficient

Table IV. The relationship between nicotine dependence level and future smoking intention in adolescents smoking regularly (n=51).

	Future smoking intention a			
	Definitely smoking	Probably smoking	Probably not smoking	Definitely not smoking
Nicotine dependence[a]	6.8 (1.9)	5.0 (2.2)	3.3 (1.8)	2.0 (0)
ANOVA	F(3) = 6.67, **p = .001**			
Definitely smoking - Probably not smoking; Tukey **p = .002**				
Definitely smoking - Definitely not smoking; Tukey **p = .007**				

a: Mean (standard deviation).

Table V. Comparison of substance use risk profiles, characteristics of smoking behavior and future smoking intention in regular smokers (n=51) according to the levels of nicotine dependence.

	High Nicotine Dependence Group (≥5)	Low Nicotine Dependence Group (<5)	t or z value	p value
SURPS Lack of Self-contentment[a]	15.71 (4.86)	15.09 (4.57)	.434	.667
SURPS Sensation Seeking[a]	16.05 (4.87)	16.32 (3.99)	-.200	.843
SURPS Impulsivity[a]	11.76 (3.18)	9.59 (2.52)	2.489	**.017**
SURPS Sensation Seeking[a]	11.95 (3.58)	11.00 (3.12)	.931	.357
Number of cigarettes smoked (per day)[a]	18.79 (7.69)	11.41 (6.86)	3.530	**.001**
First smoking age (years)[b]	12 (5-17)	12 (9-16)	-.200	.841
Total smoking duration (years)[b]	4 (0.4-9)	4 (1-8)	-.790	.430

a: Mean (standard deviation), b: Median (minimum-maximum), SURPS: Substance use risk profile scale. Since nicotine dependence level was a 5-level categorical variable, those with very low and low levels of nicotine dependence among regular smokers were classified as low nicotine dependence group, and those with medium, high and very high levels were classified as high nicotine dependence group.

Based on Conrod's theory of personality and our analysis results, two separate SEM models were created.[26] As seen in Figures 1 and 2, the structural models and hypotheses were examined. The SEM models based on polychoric correlations were performed using the R Project for Statistical Computing (ver. 4.0.0) program.[27,28] Diagonally weighted least squares (DWLS) method was used to estimate path coefficients. The estimated path coefficients with both p values and their associated z values and the results of the hypothesis for Model 1 were displayed in Table VI. Hypothesis 1a claims that "lack of self-contentment" positively influences "regularly smoking behavior" (Fig. 1). As seen in Table VI, the path from "lack of self-contentment" to "regularly smoking behavior" was positive and significant. Therefore, hypothesis 1a was supported. Other path coefficients were not found to be significant and other hypotheses were not supported. The fit indices were obtained to assess the fitness of the SEM for Model 1. The value of $\chi 2/sd$ was found to be 1.69 ($p<.001$), indicating that the model fit was good (i.e. $\chi 2/sd$ between 1-3). The RMSEA value was .062, indicating a fair fitness (i.e. RMSEA between .05-.08). The GFI value was obtained as .965, showing that the model had a very good degree of fitness (>.95 = very good). The CFI value was .907 (>.95 = good). According to these results, the model demonstrated a fair fitness.[29,30] The estimated path coefficients with both p values and their associated z values and the results of the hypothesis for Model 2 were displayed in Table VII. Hypothesis 1 claims that "impulsivity" positively influences "high nicotine dependence" (Fig. 2). As seen in Table VII, the path from "impulsivity" to

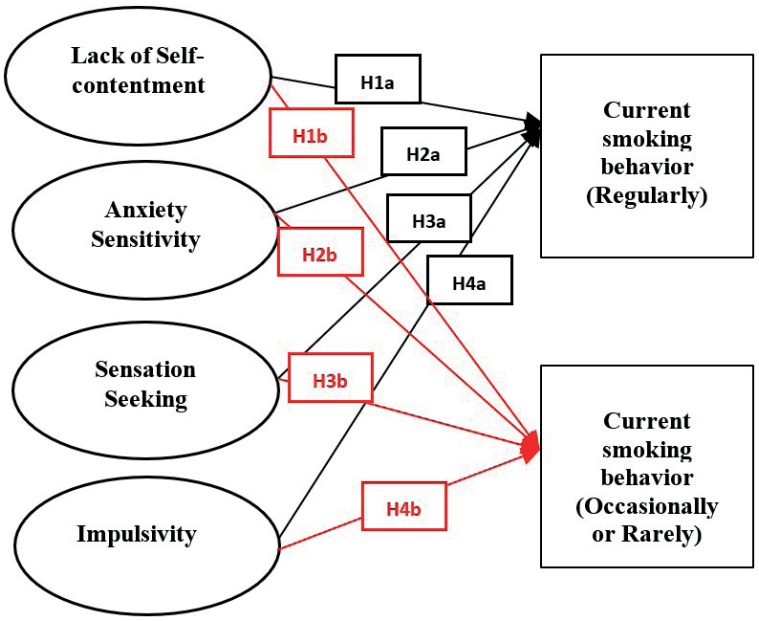

Fig. 1. Theoretical research framework for Model 1.

Since current smoking behavior was a 3-level categorical variable, 2 dummy variables were created by taking non-smokers as the reference category. The hypotheses required to investigate the effect of each latent variable on dummy variables are given below.

H1a: Lack of Self-contentment positively influence regularly smoking behavior.
H1b: Lack of Self-contentment positively influence occasionally or rarely smoking behavior.
H2a: Anxiety Sensitivity negatively influence regularly smoking behavior.
H2b: Anxiety Sensitivity negatively influence occasionally or rarely smoking behavior.
H3a: Sensation Seeking positively influence regularly smoking behavior.
H3b: Sensation Seeking positively influence occasionally or rarely smoking behavior.
H4a: Impulsivity positively influence regularly smoking behavior.
H4b: Impulsivity positively influence occasionally or rarely smoking behavior.

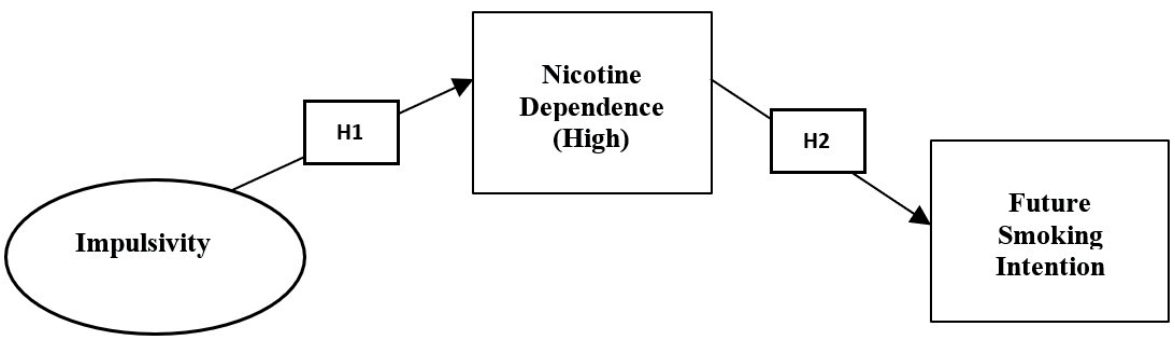

Fig. 2. Theoretical research framework for Model 2.

This model was created for regular smokers. Since nicotine dependence level was a 5-level categorical variable, very low and low levels were grouped as low nicotine dependence, medium, high and very high levels were grouped as high nicotine dependence, and low nicotine dependence was taken as the reference category. Since future smoking intention was a 4-level categorical variable, 2 dummy variables were created as smoking and not smoking in the future by taking not smoking in the future as the reference category. The hypotheses to be investigated for Model 2 are given below.

H1: Impulsivity positively influence high nicotine dependence.
H2: High nicotine dependence positively influence future smoking intention.

Table VI. The structural equation modelling results for Model 1.

Scale Items	Path Coefficients	z value	p value	Results
(I) Impact of Lack of Self-contentment				
H1a: Lack of Self-contentment → Regularly smoking behavior	.098	2.132	**.033**	Supported
H1b: Lack of Self-contentment → Rarely smoking behavior	.003	.078	.938	Not Supported
(II) Impact of Anxiety Sensitivity				
H2a: Anxiety Sensitivity → Regularly smoking behavior	-.014	-.296	.767	Not Supported
H2a: Anxiety Sensitivity → Rarely smoking behavior	-.039	-1.122	.262	Not Supported
(III) Impact of Sensation Seeking				
H3a: Sensation Seeking → Regularly smoking behavior	.028	.807	.420	Not Supported
H3b: Sensation Seeking → Rarely smoking behavior	.001	.034	.973	Not Supported
(IV) Impact of Impulsivity				
H4a: Impulsivity → Regularly smoking behavior	-.031	-.516	.606	Not Supported
H4b: Impulsivity → Rarely smoking behavior	.008	.185	.853	Not Supported

Table VII. The structural equation modelling results for Model 2.

Scale Items	Path Coefficients	z value	p value	Results
(I) Impact of Impulsivity				
H1a: Impulsivity → High nicotine dependence	.243	3.995	**<.001**	Supported
(II) Impact of High Nicotine Dependence				
H2a: High nicotine dependence → Future intention	.488	4.887	**<.001**	Supported

"high nicotine dependence" was positive and significant. Therefore, hypothesis 1 was supported. Hypothesis 2 claims that "high nicotine dependence" positively influences "future smoking intention" (Fig.2). As seen in Table VII, the path from "high nicotine dependence" to "future smoking intention" was positive and significant. Therefore, hypothesis 2 was supported. The fit indices were obtained to assess the fitness of the SEM for Model 2. The value of $\chi 2/sd$ was found to be .63 (p=.838), indicating that the model fit was good (i.e. $\chi 2/sd$ between 1-3). The RMSEA value was .001, indicating a good fitness (≤.06). The GFI value was obtained as .998, showing that the model had a very good degree of fitness (>.95 = very good). The CFI value was 1.000 (>.95 = good). According to these results, the model demonstrated a good fitness.[29,30]

Discussion

We examined the effect of high-risk personality traits on smoking behavior and intention in a clinical sample of adolescents, as well as the effect of these personality traits on nicotine dependence in regular smokers. Preliminary evidence showed that self-discontentment might be playing a role in current smoking behavior, while impulsivity might influence future smoking intention through nicotine dependence.

We found that the rate of adolescents who stated that they had never smoked was 63%. Memetovic et al.[8] determined that 91.8% of 8th and 9th grade students from a community-based cohort of adolescents had never tried smoking. Öztekin et al.[31] also found the rate of smoking at least once as 41.2% in Turkish high school students. When the prevalence

of smoking among adolescents in Canada, England and the United States was examined in 2017 and 2018, it was shown that 31.9-40.4% of adolescents smoked at least once, similar to our rate of 37%.[1] Moreover, 26.2% of our sample reported smoking regularly. According to the Center for Disease Control's 2018 National Youth Tobacco Survey, 27.1% of high school students reported using any tobacco product in the past 30 days.[32] The rate of regular smokers among Turkish high school students varied between 12.2% and 34%.[31,33] It is estimated that adolescent tobacco consumption especially in developing countries has increased over the years.[32] We observed that 7.7% of the adolescents definitely intended to smoke in the future, while 53.1% of them definitely did not intend to smoke. In the study of Memetovic et al.[8], there were no participants who stated that they would definitely smoke in the future, and 71% of the participants had no intention to smoke. Differences in smoking behavior and intention rates between previous studies and our study may be due to demographic characteristics of samples, such as age, sex, and ethnicity; or types of samples, such as population-based or clinical. Considering a cross-sectional study identifying demographic variables such as older age (15 and 16 years old) and male sex to predict smoking status and smoking risk in Malaysian adolescents, the high rates of current smoking behavior and future smoking intention in our clinical sample are not surprising.[34] Because the majority of our sample, whose mean age was 16.7 (±1.0), were male (82.1%), about half of them were 12th grade students (46.1%), and their parental education level and employment status were low. The median age of first smoking in our sample was found to be similar to the mean age of 13-14 years in Polish peers.[35] The mean nicotine dependence score (by FTND) of regular smokers was 5.0 (±2.4) and 45.1% of our sample belonged to the low dependence group, similar to the findings (4.2 ±2.4 and 45.4%, respectively) of a community-based study conducted among 1354 adolescent tobacco users from West Bengal.[36]

In line with our results, Uygun et al.[21] also found that the mean scores for lack of self-contentment, sensation seeking, impulsivity and anxiety sensitivity subscales of SURPS were 13.96 (±4.34), 16.28 (±4.36), 10.57 (±3.46), and 11.00 (±3.50) in a population-based sample of Turkish adolescents, respectively. Spillane et al.[37] evaluated impulsivity-like traits and smoking behavior in 359 college students. Different personality tendencies associated with impulsivity were identified: negative urgency (acting impulsively in response to negative mood), positive urgency (acting impulsively in response to positive mood), sensation seeking (seeking out new and exciting experiences), lack of perseverance (the inability to focus on a task), and lack of planning (acting rashly). They found that all personality tendencies predicted smoker status separately, but when the scores were entered into a logistic regression equation, controlling for age and gender, only "sensation seeking" had significant incremental validity over the others. On the other hand, only "positive urgency" was related to the level of nicotine dependence.[37] Crawford et al.[38] evaluated two adolescent samples for substance use according to their sensation-seeking behavior and found that sensation-seeking had a strong predictive value for coexisting and future marijuana and alcohol use; but only in one sample the initial level of sensation-seeking predicted initial level of tobacco use during high school. In this study, the effect of sensation seeking on smoking was less than expected. This was attributed to the different levels of perceived risk associated with these substances. They argued that high sensation seeking was more predictive of marijuana use, because cigarettes and alcohol were perceived to be more acceptable.[38] Spillane et al.[39] showed that greater sensation-seeking scores at baseline predicted daily smoking for females, but not for males in American-Indian high school students. They explained this gender difference with two mechanisms; they suggested that sensation seeking may affect boys at an earlier age than girls (which is not involved in this study), or tobacco use may be normative in boys and they don't need a specific

personality trait to trigger regular smoking. Consequently, high levels of impulsivity, hopelessness (lack of self-contentment), and sensation seeking were expected to predict tobacco use in Turkish adolescents. Contrary to expectations, we could not find any association between certain personality traits, such as sensation seeking, and smoking-related variables. Current literature has reported that there are sex- and age-related differences in the relationship between personality and smoking during adolescence.[40,41] Kelly et al.[41] found that sensation seeking at age 13 was associated with starting smoking at age 14, while impulsivity at age 13 was associated with starting smoking at age 15 and hopelessness at age 13 was associated with starting smoking at age 16. Also, personality traits may vary differently between girls and boys during adolescence. Mathijssen et al.[40] reported that hopelessness was found to be increased in only girls; impulsivity, sensation seeking and smoking behavior which was related to all personality traits were found to be increased in both girls and boys during early adolescence and the increase in sensation seeking and impulsivity was higher for girls than for boys. In this study, the relationships between smoking behavior and personality traits were evaluated in the entire sample, but sex- and age-specific relationships were not examined. Given the current findings, our results may reflect some characteristics such as sex and age for the majority of the sample.

We gathered the first evidence that impulsivity positively influences future smoking intention through nicotine dependence in Turkish adolescents. Although meta-analyses showed that impulsive traits might play an important role in adolescent cigarette consumption, there were only two studies on the association between adolescent nicotine dependence and impulsive traits, and the strength of the associations was weak in a small range.[16] Novelty seeking as an indirect measure of impulsivity may influence nicotine dependence via a decrease in the ability to inhibit the desire to engage in the behavior as well as a decrease in self-efficacy.[42] The relationship between impulsivity and nicotine addiction may be related not only to the amount and frequency of tobacco use, but also to tobacco use disorder etiologically.[43] The strength of our study comes from including measures of nicotine dependence and future smoking intention while examining the relationship between impulsivity and adolescent smoking. Our preliminary findings suggest that impulsivity may contribute to the transition from casual cigarette consumption to more problematic tobacco use disorder. Therefore, identifying and intervening against impulsivity may be protective for future nicotine dependence and tobacco use disorder. We also showed that self-discontentment (hopelessness) positively influences regularly smoking behavior in Turkish adolescents, similar to the current literature.[40,41] As a result, evaluation of self-discontentment (hopelessness) and the development of personalized cognitive behavioral approaches specific to self-discontentment may be beneficial in reducing the current smoking behaviors of Turkish adolescents.

The results of our study should be evaluated considering some limitations. The sample of the cross-sectional study consisted of a very small clinical-based population. The vast majority of the participants were male and in late adolescence. This dominance in the sample may explain the high prevalence of current smoking behavior and future smoking intention in our clinical-based sample. Our models should be longitudinally retested in a large population-based sample, and sex- and age-specific associations between personality and smoking should be demonstrated.

Ethical approval

Clinical Research Ethics Committee of the University of Health Sciences Ankara City Hospital (Decision No: E1/20/1003).

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: SDU, DAA, SÇ; data collection: SDU, DAA; analysis and interpretation of results: SDU, HKÜ; draft manuscript preparation: SDÜ, DAA, HKÜ, SÇ. 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

Is vasoactive-inotropic score associated with early lactate clearance a predictive outcome of children with septic shock?

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ABSTRACT

Background. The main goal of septic shock therapy is to keep hemodynamic parameters in the normal range for adequate tissue perfusion. Persistent lactic acidemia has increased mortality. We evaluate the association between vasoactive-inotropic score (VIS) and lactate clearance (LC) to predict mortality of septic shock in children.

Methods. This is a retrospective study of consecutive septic shock in children admitted to the pediatric intensive care unit. Vital signs, laboratory values, and VIS were obtained at admission and the 6th hour of hospitalization. LC was calculated at the 6th hour. The associations between LC and VIS were evaluated using univariate and multivariate analysis. Receiver operating characteristic analysis was used to describe the cutoff values of LC and VIS.

Results. Eighty-two children, age 82.3 ± 59.8 months, were included, with an overall lactate clearance of 29 ± 26%, and a mortality rate of 25.6%. The optimal cutoff value of LC was 20%. Children with LC ≥ 20% compared with LC < 20% had a lower VIS [(21.41 ± 8.36) vs. (27.48 ± 10.11) (p: 0.009)]. In multivariate comparison, PELOD score and VIS were significantly associated with 6-hour lactate clearance < 20% but VIS at 6 hours had a significant inverse relationship with LC < 20%. The cutoff for VIS was ≥ 16.2 for prognosticating the 6-hour LC and the high VIS group had a significantly lower LC and higher mortality ratio than the low VIS group.

Conclusions. High VIS was associated with lower lactate clearance and has been described as a predictor of greater mortality among septic shock in children.

Key words: lactate clearance, vasoactive-inotropic score, septic shock, mortality, pediatric.

Septic shock is characterized as a subset of sepsis in which especially severe cardiovascular collapse and abnormalities of cellular metabolism are related to increased mortality risk.[1] Early identification of septic shock and effective resuscitation within the first few hours may prevent the progression of shock and organ failure.[2] Elevated serum lactic acid level is an indicator of anaerobic metabolism and is accepted to be a useful biomarker of reduced tissue perfusion in septic shock patients.[3] Although serum lactate is commonly used to evaluate tissue perfusion in the intensive care unit, lactate clearance (LC) may be more beneficial than the baseline lactate level for assessing patient outcomes.[4,5] For adult patients with septic shock, maintaining mean arterial pressure (MAP) > 65mm Hg is recommended.[6] In the pediatric age group, a certain MAP value has not been determined, however, the goal of MAP is suggested between the 5th and 50th percentile or higher than the 50th percentile for age.[7] It was reported that hypoperfusion was associated with increased mortality risk regardless of the concomitant hypotension.[8] In pediatric septic shock, vasoactive-inotropic scores (VIS) are utilized to improve cardiovascular dysfunction and hypotension, however, increasing VIS is

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associated with the severity of cardiovascular dysfunction and mortality risk.[9,10] The mortality rate of hypotensive children requiring vasoactive agent with lactic acid greater than 2 mmol/L was higher than children with lactate less than 2 mmol/L.[11] Multiple variables such as high serum lactate, low LC, presence of multiorgan dysfunction syndrome, and high inotropic score have been determined as mortality prognosticator factors for septic shock.[4,10] According to our knowledge, the association between VIS and early LC in septic shock children has not been researched yet. This research was performed to evaluate whether VIS might be used to predict LC of patients after sufficient resuscitation therapy in septic shock children.

Material and Methods

Patient population

This is a retrospective, observational single-center research that was performed to analyze the relationship between LC and VIS in septic shock patients who were admitted to the tertiary level pediatric intensive care unit (PICU) from March 2020 to January 2022. This study was approved by the Afyonkarahisar Health Science University Faculty Ethics Committee (date: 07.01.2022, no: 2022/1). Children aged 1 month to 18 years old who were diagnosed with septic shock enrollment. Septic shock was described based on International Consensus Conference on Pediatric Sepsis.[12] All children needed central venous catheterization for continuous inotropic infusion, medical therapy, and oxygenation monitoring. Hemodynamic support was managed according to the prescribed guidelines.[9] Restoration of tissue perfusion was defined by the following targeted resuscitation endpoints[9]:

1) Normal mental status
2) Blood pressure (systolic pressure at least fifth percentile for age): Younger than 1 month of age: 60 mmHg, children with 1 month to 10 years of age: 70 mmHg + [2 x age in years], 10 years of age or older: 90 mmHg
3) Quality of central and peripheral pulses (strong, distal pulses equal to central pulses)
4) Sufficient skin perfusion (warm, and capillary refill <2 seconds)
5) Urine output >1 mL/kg/h (after efficient circulating volume is restored)

Broad-spectrum antibiotics were administered after blood cultures were taken. Children were supported by mechanical ventilation as needed.

Exclusion criteria

The study exclusion criteria were: 1) patients aged > 18 years or < 1 month 2) patients with serious hepatic disease 3) disease of inborn errors of metabolism 4) missing lactate data 5) staying for <24 hours in the Pediatric Intensive Care Unit 6) patients requiring renal replacement therapy within the first 6 hours of PICU admission.

Collection of blood samples

The baseline clinical and demographic information including age, sex, initial vital signs (heart rate, mean arterial pressure, and temperature), peripheral oxygen saturation, and the need for mechanical ventilation, the length of intensive care unit, outcome, and laboratory values at the time of admission were noted. The Pediatric Logistic Organ Dysfunction Score (PELOD) and Pediatric Risk of Mortality III score (PRISM III score) were calculated to determine the severity of the disease on admission. Lactate level in the blood was measured on admission to PICU and was accepted as the initial (0th) lactate level. More than two serum lactate values may be obtained within 6 hours, but only the initial and lactate at 6-hour values were analyzed. VIS contains dopamine, dobutamine, epinephrine, norepinephrine, vasopressin, and milrinone defines the amount of cardiovascular support required by patients (Fig. 1).[13] Data were acquired from patient files and an electronic hospital data management system.

VIS = dopamine dose (μg/kg/min) +
dobutamine dose (μg/kg/min) +
100 x epinephrine dose (μg/kg/min) +
100 x norepinephrine dose (μg/kg/min) +
10,000 x vasopressin dose (unit/kg/min) +
10 x milrinone dose (μg/kg/min)

Fig. 1. Formula of Vasoactive-Inotropic Score (VIS)

Biochemical analysis

Lactate measurements were done by Radiometer Copenhagen ABL 555 blood gas analyzer. Lactate clearance (%) was calculated as follows: lactate at admission minus lactate at 6 hours, divided by lactate at admission, and then multiplied by 100. A positive result shows a reduction of LC, whereas a negative result shows an increase of LC after 6 hours.

Statistical analysis

In the univariable analysis, continuous parameters are described as mean ± standard deviation (SD) for normal distribution, median and interquartile range (IQR) for skewed distribution, and nonparametric data. Categorical parameters were evaluated by using the Chi-square test. Mann Whitney-U test was used for non-parametric parameters and the Student t-test was performed for normally distributed data. The significant parameters in the univariate comparison were then included in a multivariate logistic regression model. Cutoff levels of the VIS and LC were calculated by using the receiver operating characteristic curves (ROC curves). For all results, a p-value < 0.05 was regarded as the significance level. SPSS Statistics 22 software was used for the analysis of data.

Results

During the study period, a total of eighty-six children with septic shock were recorded. According to the exclusion criteria, four patients were excluded. Baseline characteristics, vital signs of the patients, and initial laboratory data are demonstrated in Table I. Mean patient age was 82.3 ± 59.8 months, and 29 (35%) were

Table I. Baseline characteristics and clinical data of the patients.

Total number	82
Age, months	82.3 ± 59.8
Gender (female/male)	29/53
Length of PICU stay (days)	8.3 ± 3.3
Mechanical ventilation (days)*	5 (4)
VIS	26.6 ± 9.5
Source of infection (%)	
Pneumonia	44 (53.7%)
Urosepsis	15 (18.3%)
Intra-abdominal	14 (17.1%)
Others	9 (11%)
Vital signs and hemodynamic parameters	
Heart rate (beats per minute)*	138 (32)
Respiratory rate (beats per minute)	34.6 ± 6.2
Temperature (C°)	37.6 ± 1.4
MAP (mmHg)	38.9 ± 6.2
Peripheral oxygen saturation, %*	94 (6)
Laboratories data	
Hemoglobin (g/dl)*	11.3 (1.7)
White blood cells (x10³ per mm³)	16.07 ± 5.9
Thrombocyte count (x10³ per mm³)	194 ± 105
Creatinine (mg/dl)*	0.37 (0.4)
Albumin (g/dl)*	3 (0.4)
Total bilirubin (mmol/L)	1.6 ± 0.84
D-dimer (ug/mL)	4 ± 1.9
Prothrombin Time (seconds)*	26.2 (23.4)
pH*	7.32 (0.09)
Base deficit (mmol/L)*	-2.8 (6.6)
Lactate (mmol/L)*	3.6 (2.1)
Lactate clearance (%)	29 ± 26
Organ dysfunction / Mortality risk score	
PELOD score	8.3 ± 3.3
PRISM III score	16.6 ± 3.5
Outcome (%)	
28-day mortality rate (%)	21 (25.6%)

*Median (IQR)
PICU: pediatric intensive care unit, PRISM III score: pediatric risk of mortality III score, PELOD: pediatric logistic organ dysfunction score, IQR: interquartile range, VIS: Vasoactive-inotropic score, MAP: Mean Arterial Pressure

male. The total PICU length of stay was 8.3 ± 3.3 days. The baseline hemodynamic variables included mean arterial pressure of 38.9 ± 6.2 mm Hg, heart rate of 138 per min (IQR, 32), and peripheral oxygen saturation of 94 % (IQR, 6). The patient's total mechanical ventilation day was 5 days (IQR, 4). The predominant admission diagnoses were respiratory infection 53.7%. The mean VIS was 26.6 ± 9.5 for admission. Patients had a mean baseline PELOD score of 8.3 ± 3.3, and a PRISM III score of 16.6 ± 3.5. Overall, 61 patients survived and 21 patients died, a primary outcome of the 28-day mortality rate was 25.6%. The median admission serum lactate level was 3.6 mmol/L (IQR, 2.1), and the mean LC was 29 ± 26% at the 6th hour. After 6 hours of resuscitation, an optimal cutoff value of LC was determined as the LC ≥ 20% with the highest specificity of 38.1% and sensitivity of 75.4% for prediction of mortality. Based on the LC cutoff value patients were divided into two groups: LC ≥ 20% (high clearance group), and LC < 20% (low clearance group) (Fig. 2). Comparisons of both the LC groups in terms of age, gender, vital signs, laboratory data, metabolic factors, fluid volume received in the PICU, VIS, PRISM III, and PELOD score were performed (Table II). There was no significant difference between the low clearance group and the high clearance group for initial vital signs, white blood cells, hemoglobin, pH, base deficit, fluid therapy, length of mechanical ventilation, PRISM III score, and duration of hospital stay. Although the low clearance group had significantly higher VIS, PELOD score, and prothrombin time, the high clearance group had lower baseline and 6th-hour lactate values (all p < 0.05). Variables were added to the multivariate logistic regression model via a forward stepwise technique that was statistically significant in the univariate model. In multivariate comparison, PELOD score and VIS scores at baseline was significantly associated with 6 hour LC < 20% but VIS at 6 hour had a significant inverse relationship with LC < 20% (95% CI: 1.10-1.53, p: 0.002; 95% CI: 1.34-2.93, p:0.001; 95% CI: 0.27-0.66, p: <0.001, respectively) (Table III). The cutoff value of VIS was ≥ 16.2 (sensitivity: 85.7%; 55% specificity) for prognosticating LC after adequate intravenous fluid resuscitation and application of vasoactive medications. When used ROC area under the curve (AUC), the estimation of LC was 0.75 (Fig. 3). Based on the VIS score cutoff value patients were divided into two groups: VIS ≥ 16.2 (high VIS group) and VIS < 16 (low VIS group). Although there was no significant differentiation between initial and 6th-hour lactate values, the high VIS group had significantly lower LC than the low VIS group (p: 0.01). Thrombocyte count was higher in the low VIS group (p: 0.01). The base deficit at the 6th hour was more negative in the high VIS group after resuscitation (p: 0.02). The high VIS score group had a significantly higher 28-day mortality rate (p: 0.02) (Table IV).

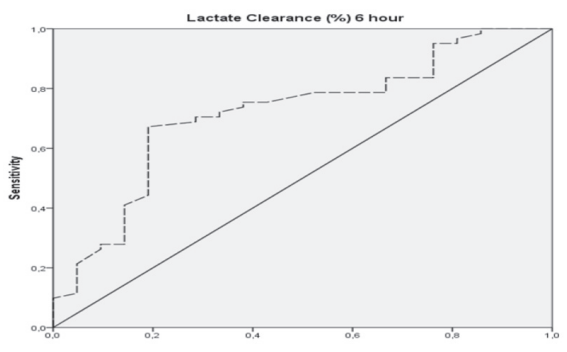

Fig. 2. ROC curve of Lactate Clearance (%) 6 hour

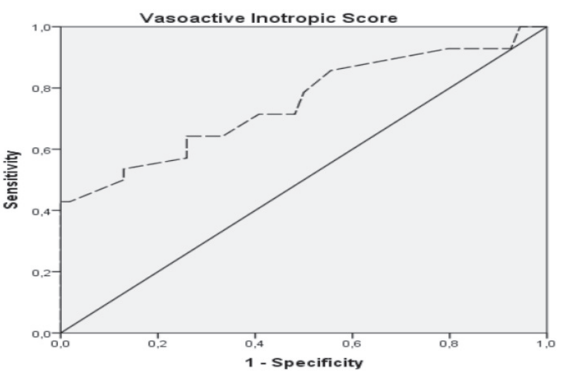

Fig. 3. ROC curve of VIS 6 hour for predicting 6nd Lactate Clearance

Table II. Baseline characteristics, therapy, and outcome comparisons between LC < 20% and LC ≥ 20% groups.

Variables	LC < 20% (n:28)	LC ≥ 20% (n:54)	p value
Age, months	105.86 ± 57.17	79.81 ± 58.73	0.06
Length of PICU stay (days)	7.32 ± 3.59	8.62 ± 2.74	0.07
Mechanical ventilation (days)*	6 (5)	5 (5)	0.09
Fluid therapy ml/kg during first 6 hour	65 ± 22.02	65.18 ± 19.4	0.97
Heart rate (rate/minute) 0 hour	135.46 ± 18.88	139.96 ±19.51	0.31
Heart rate (rate/minute) 6 hour	133.52 ± 17.63	135.81 ±18.43	0.38
Respiratory rate (rate/minute) 0 hour	33.64 ± 5.08	34.31 ± 6.29	0.62
Respiratory rate (rate/minute) 6 hour	33.52 ± 4.76	34.26 ± 5.74	0.56
Temperature (C°) 0 hour	37.75 ± 1.41	37.61 ± 1.45	0.67
Temperature (C°) 6 hour	36.86 ± 1.28	36.70 ± 1.29	0.73
MAP (mmHg) 0 hour	37.54 ± 5.82	39.2 ± 6.31	0.24
MAP (mmHg) 6 hour	44.71 ± 7.23	47.35 ± 8.33	0.16
Peripheral oxygen saturation, % 0 hour*	94.5 (5)	96 (3)	0.08
Peripheral oxygen saturation, % 6 hour*	96 (4)	97 (5)	0.12
Hemoglobin (g/dl) 0 hour*	10.95 (2.35)	11.45 (1.7)	0.35
Hemoglobin (g/dl) 6 hour*	9.82 (2.51)	10.25 (1.3)	0.42
White blood cells (x10^3 per mm^3)	15.94 ± 7.06	15.77 ± 5.5	0.90
Thrombocyte count (x10^3 per mm^3)	190 ± 119	193 ± 97	0.89
Creatinine (mg/dl)*	0.42 (0.52)	0.36 (0.34)	0.11
Albumin (g/dl)*	3.01 (0.51)	3.01 (0.42)	0.79
ALT (U/L)	130.71 ± 119.06	108.84 ± 208.3	0.16
Total bilirubin (mmol/L)	1.8 ± 0.83	1.5 ± 0.76	0.08
D-dimer (ug/mL)	3.93 ± 2.23	3.70 ± 2.04	0.63
Prothrombin Time (seconds)*	25.15 (23.17)	26.25 (23.1)	0.03
pH 0 hour*	7.34 (0.08)	7.35 (0.07)	0.34
pH 6 hour	7.34 ± 0.13	7.36 ± 0.04	0.42
Base deficit (mmol/L) 0 hour*	-2.8 (4.23)	-1.2 (6.60)	0.28
Base deficit (mmol/L) 6 hour	-1.6 ± 3.3	-1.37 ± 3.19	0.21
Lactate (mmol/L) 0 hour*	3.8 (2)	3.4 (2.1)	0.006
Lactate (mmol/L) 6 hour*	3.75 (2.2)	1.7 (1.25)	0.003
Lactate Clearance (%) 6 hour	2.6 ± 13.14	45.41 ± 13.86	>0.001
VIS 0 hour	27.48 ± 10.11	21.41 ± 8.36	0.009
VIS 6 hour	29.07 ± 9.55	20.94 ± 7.71	>0.001
PRISM III score	16.54 ± 3.86	15.24 ± 3.8	0.15
PELOD score	22.17 ± 4.52	27.31 ± 6.95	0.001
28-day mortality rate %	8 (46.4%)	13 (14.8%)	0.002

*Median (IQR)

LC: Lactate clearance, PICU: pediatric intensive care unit, PRISM III score: pediatric risk of mortality III score, PELOD: pediatric logistic organ dysfunction score, IQR: interquartile range, VIS: Vasoactive-inotropic score, MAP: Mean Arterial Pressure, ALT: alanine aminotransferase

(The Formula of Lactate clearance (%) was calculated as follows: lactate at admission minus lactate at 6 hours, divided by lactate at admission and then multiplied by 100)

Table III. Statistically significant univariate variables associated with 6 h Lactate Clearance <20% were analyzed using multivariate logistic regression modeling.

Variables	Odds Ratio	Confidence Interval	p value
PELOD score	1.3	1.10-1.53	0.002
VIS 0 hour	1.98	1.34-2.93	0.001
VIS 6 hour	0.42	0.27-0.66	>0.001
Prothrombin Time	1.01	0.95-1.07	0.67

PELOD: pediatric logistic organ dysfunction score, VIS: Vasoactive-inotropic score, CI: Confidence Interval

Table IV. Baseline characteristics, therapy, and outcome comparisons between VIS < 16.2 and VIS ≥ 16.2 groups.

Variables	VIS < 16.2 (n:28)	VIS ≥ 16.2 (n:54)	p value
Age, months	82.54 ± 56.63	91.91 ± 60.71	0.49
Length of PICU stay (days)	8.71 ± 2.66	7.90 ± 3.29	0.26
Mechanical ventilation (days)*	7 (6)	5 (5)	0.11
Fluid therapy ml/kg during first 6 hour	67.5 ± 18.58	63.88 ± 21.04	0.42
Heart rate (rate/minute) 0 hour	139.11 ± 16.51	138.07 ±20.74	0.82
Heart rate (rate/minute) 6 hour	138.25 ± 15.67	137.92 ±19.66	0.76
Respiratory rate (rate/minute) 0 hour	34.0 ± 6.11	34.13 ± 5.82	0.35
Respiratory rate (rate/minute) 6 hour	33.25 ± 5.36	33.67 ± 4.76	0.43
Temperature (C°) 0 hour	37.86 ± 1.35	37.55 ± 1.46	0.67
Temperature (C°) 6 hour	36.74 ± 1.28	36.43 ± 1.37	0.71
MAP (mmHg) 0 hour	38.75 ± 5.11	38.57 ± 6.69	0.89
MAP (mmHg) 6 hour	46.79 ± 7.05	46.28 ± 8.54	0.78
Peripheral oxygen saturation, % 0 hour*	95 (3)	94 (5)	0.23
Peripheral oxygen saturation, % 6 hour*	96 (4)	95(5)	0.27
Hemoglobin (g/dl) 0 hour*	11.6 (1.0)	11.0 (2.7)	0.14
Hemoglobin (g/dl) 6 hour*	10.8 (1.2)	10.7 (2.2)	0.22
White blood cells (x10^3 per mm^3)	14.45 ± 5.37	16.54 ± 6.28	0.12
Thrombocyte count (x10^3 per mm^3)	230 ± 111	172 ± 96	0.01
Creatinine (mg/dl)*	0.32 (0.32)	0.38 (0.46)	0.81
Albumin (g/dl)*	2.99 (0.42)	3.01 (0.5)	0.39
ALT (U/L)	139.42 ± 279.61	130.53 ± 209.22	0.99
Total bilirubin (mmol/L)	1.57 ± 0.71	1.71 ± 0.84	0.45
D-dimer (ug/mL)	3.35 ± 1.53	4.0 ± 2.32	0.13
Prothrombin Time (seconds)*	26.2 (18.90)	23.30 (19)	0.20
pH 0 hour*	7.34 (0.05)	7.32 (0.06)	0.09
pH 6 hour	7.37 ± 0.04	7.33 ± 0.06	0.11
Base deficit (mmol/L) 0 hour*	-0.34 (3.27)	-2.5 (3.64)	0.09
Base deficit (mmol/L) 6 hour	0.26 ± 2.75	-1.7 ± 3.17	0.02
Lactate (mmol/L) 0 hour*	3.8 (2.4)	3.6 (2)	0.98
Lactate (mmol/L) 6 hour*	2.4 (2)	2.5 (2.3)	0.20
Lactate Clearance (%) 6 hour	39.6 ± 18.77	26.22 ± 26.0	0.01
VIS 0 hour	13.35 ± 1.79	28.74 ± 7.13	>0.001
VIS 6 hour	13.51 ± 1.76	29.0 ± 6.63	>0.001
PRISM III score	14.61 ± 4.04	16.24 ± 3.66	0.06
PELOD score	25.82 ± 5.46	25.42 ± 7.26	0.80
28-day mortality rate %	3 (10.7%)	18(33.3%)	0.02

*Median (IQR)

PICU: pediatric intensive care unit, PRISM III score: pediatric risk of mortality III score, PELOD: pediatric logistic organ dysfunction score, IQR: interquartile range, VIS: Vasoactive-inotropic score, MAP: Mean Arterial Pressure, ALT: alanine aminotransferase

Discussion

The present research is a retrospective clinical research that analyzes the relationship between vasoactive-inotropic score and lactate clearance of septic shock children after resuscitation. The main results of this research are as follows. Firstly, some septic shock patients had high lactate levels and poor lactate clearances after resuscitation of intravenous fluid therapy and vasoactive medications. Mean arterial pressure, hemoglobin concentration, peripheral oxygen saturation, and temperature, which are essential for perceiving of production and consumption of lactate were not sufficient to accurately predict LC after resuscitation. Secondly, VIS was associated with LC closely and had high sensitivity and specificity for the prediction of 6th hour LC after resuscitation in pediatric patients with septic shock. Finally, LC was found as inversely associated with hospital mortality in pediatric septic shock and patients with higher VIS had an increased mortality rate.

In critical illness, the benefit of evaluation of the lactate level has demonstrated that measurement of serum lactate values has an important role in the understanding of mortality risk.[14] During the early stage of resuscitation, values of lactate appear to be more nearly interested in mortality than hemodynamic variables.[15] Several types of research have emphasized that increased lactate values are a powerful sign to predict a high probability of mortality and morbidity ratio in critically ill patients, furthermore increasing LC decreases the odds of mortality at 24 hour.[16] Numerous septic shock patients who still have hyper lactic acidemia after completion of effective fluid resuscitation have a poor prognosis.[17] The significance of monitoring of lactate trend from high lactate value to normal lactate levels has been highlighted in Surviving Sepsis Campaign Guideline in 2016.[2] In this present study has a convenient outcome to previous research such as mortality was significantly higher among patients with lower LC.

Inotropic agents are routinely used to ensure cardiovascular support for adequate delivery of oxygen and tissue perfusion in septic shock children.[10] VIS is a clinical instrument that is used to understand the inotropic requirement of the cardiovascular system and can be utilized to predict morbidity and mortality.[18] VIS is independently associated with outcomes including ventilator days, length of hospital stay, and mortality in patients with sepsis.[19] Nguyen et al.[20] showed that hemodynamic parameters were similar between groups in patients with septic shock, while vasopressor dosage was significantly higher in the low LC group. Nazir et al.[16] presented that mean arterial pressure, central venous pressure, and VIS in the first 72 hours were not significantly different between high and low LC groups. Another previous study demonstrated that hemodynamic variables including mean arterial pressure, central venous pressure, central venous oxygen saturation, and central venous-to-arterial CO_2 difference were not useful to predict LC after resuscitation, but norepinephrine dose was better for prediction of LC.[21] We found that hemodynamic measurements were not useful to predict the early LC in this study, whereas VIS > 16.2 can predict early LC with high sensitivity and specificity after 6-hour resuscitation. Additionally, VIS was positively associated with increased mortality.

Walker et al.[19] reported that an optimal cutoff level of 6th hour LC was found at 36% which was independently associated with hospital mortality and also greater than the previously reported value. In another study, the Cutoff value of LC was calculated at 10% which predicted hospital mortality with maximum sensitivity and specificity.[5] In this research, we found an optimal cutoff of LC < 20% that is consistent with other research related to LC in septic shock patients, and also LC was inversely associated with risk of mortality.

Our research has some limitations. Firstly, this study was a retrospective clinical investigation

and there was a risk of bias due to the limited number of cases. Secondly, it was a single-center study and institution-specific parameters may have affected the outcomes. Furthermore, one could discuss that all patients did not receive a similar antibiotic regimen that might affect lactate production.

As a conclusion, this is the first study to assess the association between LC and VIS to predict mortality among pediatric patients with septic shock. We perceived that some children with septic shock still had poor tissue oxygen metabolism after ensuring normal hemodynamic parameters. A high VIS may be associated with deterioration of tissue oxygen metabolism. After sufficient resuscitation, vasoactive inotropic drug doses should be withdrawn without causing permanent impairment in tissue oxygen metabolism in septic shock. Further prospective research is fundamental to better understand the relationship between lactate clearance and vasoactive-inotropic score in pediatric septic shock.

Ethical approval

Approval was obtained from the ethics committee (date: 07.01.2022, no: 2022/1).

Author contribution

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

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The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

Comparison of pediatric antibiotic prescribing practice between low and high prescribers for children in primary care

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ABSTRACT

Background. Antibiotic prescribing is more prevalent in children. Many factors influence this practice, including the burden of outpatient visits. We aimed to compare antibiotic prescribing for children by low prescribers (LP) and high prescribers (HP) in primary care.

Methods. We analyzed pediatric prescriptions in primary care in Istanbul. Among the physicians randomly selected by systematic sampling, those generating ≥1 pediatric prescription/day (n=1218) were defined as LP or HP when they belonged to the lowest (n=305) or highest (n=304) quartile of prescribing, respectively. The antibiotic prescribing characteristics of these groups were compared.

Results. We identified that 38.5% of the prescriptions written by physicians included antibiotics, significantly higher in HPs (38.8%) than in LPs (37.2%), (p=0.04). Among antibiotic-containing prescriptions, the mean number of drugs and boxes and the percentage of prescriptions containing injectable drugs/antibiotics were significantly higher in HPs compared to that in LPs. We detected that co-amoxiclav was the most frequently prescribed antibiotic in the LP and HP groups (61.1% and 48.3%, respectively). Stratification of antibiotics by their spectra showed that 11.2% were narrow, 79.8% were broad and 0.5% were ultra-broad-spectrum drugs. LPs were significantly more likely to prescribe broad-spectrum antibiotics (82.5%) than do HPs (78.9%,p<0.001).

Conclusions. Antibiotic prescribing remains excessive in pediatric primary care, slightly more marked in HPs. While HPs also tend to prescribe a higher number of overall and injectable drugs/antibiotics, broad-spectrum anti-biotherapy seems to be more practiced by LPs surprisingly. Both physician groups appeared to prefer either narrow- or broad-spectrum drugs without paying enough attention to their pharmacodynamic properties.

Key words: antibiotics, children, co-amoxiclav, pharmacotherapy, primary care.

Despite improvements in antimicrobial treatments, infectious diseases still cause a significant health burden in the pediatric population.[1] The burden of disease is undertaken by general practitioners or pediatricians at different levels of the healthcare system. In this context, more than half of the pediatric prescriptions generated by primary care physicians have been reported to contain antibiotics.[2] However, irrational use of these drugs continues to pose a serious global public health problem.[3,4] The multinational studies showed that antibiotic consumption in Turkey is at the top compared to other countries across Europe, especially for broad-spectrum

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antibiotics.[5,6] Moreover, it has been reported that this consumption is greater in the pediatric population.[7]

Apart from clinical suitability, many parameters influence antibiotic prescribing behavior.[8,9] This includes patient- and physician-related factors like demographic characteristics and/or knowledge level, clinical interaction between the patient and physician, the socio-economic characteristics of the area where the healthcare institution is located, and the availability of healthcare professionals in the region.[5,8,10] In addition, it was reported that physicians who encountered more patients prescribed antibiotics at a higher rate.[11]

Huge evidence of base indicates that half of the antibiotics prescribed to children in primary care are for suspicious/unnecessary indications.[4] For antibiotic use, children require special attention not only in terms of posology but also in terms of indication and safety.[12] It is important to evaluate the consumption of antibiotics from a large perspective in a group like children in which drug use should be more careful. On the other hand, to the best of our knowledge no comprehensive study has been encountered in the literature, which examines pediatric antibiotic prescriptions in primary care and correlates the prescribing pattern with the number of patients served by the physician. We aimed to compare antibiotic prescribing for children between the physicians with either low vs. high pediatric practice in primary care.

Material and Methods

Study design and population

We performed a cross-sectional study that included electronic prescriptions of primary care physicians in Istanbul. Among all these (n=4293) working in Istanbul in 2016, we selected 1431 physicians by systematic sampling. We analyzed all prescriptions generated for the pediatric population (<18 years of age), excluding those of 35 physicians who did not prescribe for children during the study period, with a final sample of prescriptions by 1396 physicians. To compare prescribing performances of the physician groups, 1218 physicians who wrote an average of at least 1 prescription per day for the pediatric population in 2016 were ranked from high to low according to the total number of prescriptions. Physicians in the lowest quartile of the ranking (n = 305) were defined as low prescribers (LP), and in the highest quartile (n = 304) as high prescribers (HP), (Fig. 1). The data set was accessed after the institutional approval. The study was approved by the Ethics Committee for Non-interventional Studies of Istanbul Medipol University (Approval No: 25.06.2020-527).

Study parameters

Prescriptions were examined in terms of descriptive characteristics such as age group (0-2 years, 3-11 years, 12-17 years), gender, and calendar year quartiles. Prescriptions were also analyzed in terms of rational use of medicine indicators such as total number of drugs/boxes/diagnoses, number of drugs/boxes/diagnoses per prescription and percentage of prescriptions containing antibiotics and injectable drugs. The "Anatomical Therapeutic Chemical (ATC)" classification system was used for the examination of the drugs in the prescriptions, and the diagnosis codes "International Statistical Classification of Diseases (ICD)" were used for the analysis of the diagnoses. The distribution of all antibiotics (ATC-3 and ATC-5 level) in the prescriptions as well as injectable antibiotics at ATC-5 level was examined.

The spectrum of antibiotics was evaluated in three groups as narrow (J01CA-penicillins with extended spectrum, J01CE01-benzyl penicillin, J01DB-first-generation cephalosporins, and J01DC-second generation cephalosporins), broad (J01CR-penicillins with beta-lactamase inhibitors, J01DD-third generation cephalosporins, J01EE01-sulfamet

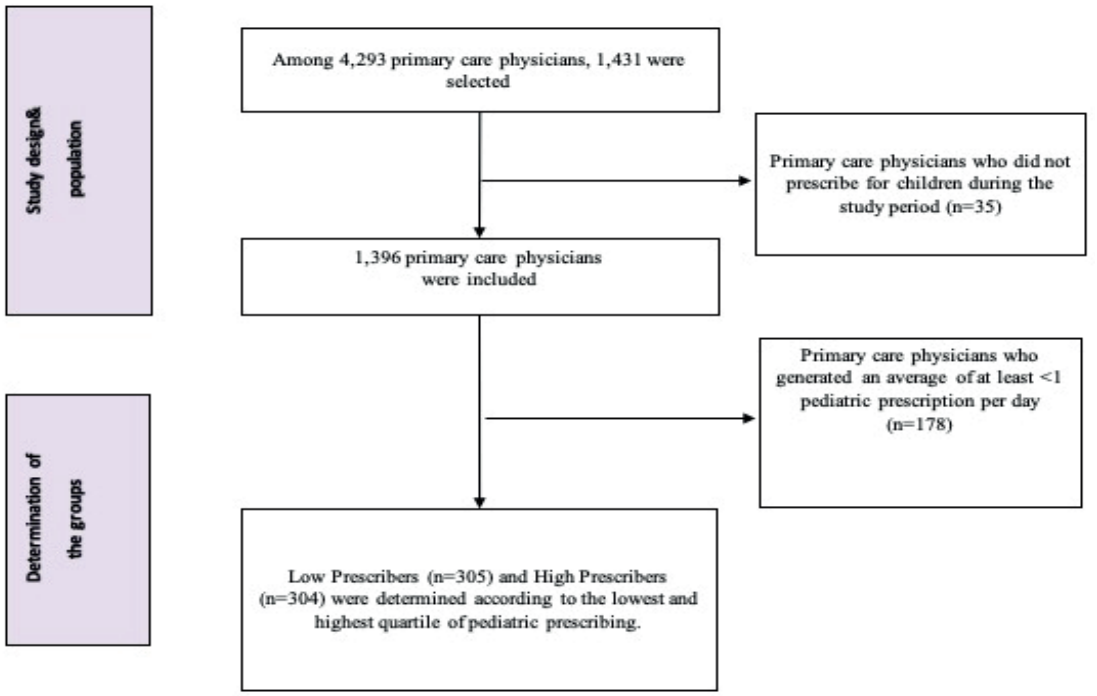

Fig. 1. Selection flowchart of the prescriber groups; high and low prescribers represented at the highest and lowest quartile of primary care physicians according to total number of prescriptions.

trimethoprim, J01FA-macrolides, and J01FF-lincosamides) and ultra-broad (J01AA-tetracyclines, J01BA01-chloramphenicol, J01DH-carbapenems, J01M-quinolones, J01XX01-fosfomycin, J01XX08-linezolid)13 Distribution of the most frequent 10 diagnoses for which antibiotics in these three spectrums was also analyzed.

Statistical analysis

Statistical analysis was performed via SPSS 24.0 program. Descriptive data were presented as frequencies and percentages for categorical variables and mean and standard deviation for continuous variables. Independent samples t -test was used for comparison of continuous variables, and the chi-square test was used for comparison of categorical variables. Type 1 error level below 5% was accepted as statistical significance.

Results

We found that 52.1% of the pediatric prescriptions were written to boys and the most prescribed age group was 3-11 (52.8%). In prescriptions containing antibiotics, the same age group had 60.2% of the prescriptions. These prescriptions were mostly prescribed to children in autumn (36.3%) and least in summer (16.2%).

We determined that 38.5% of the prescriptions contained antibiotics, which was significantly higher in HPs (38.8%) than LPs (37.2%) (p=0.04, Table I). In these antibiotic-containing prescriptions, the number of drugs and boxes per prescription and the percentage of prescriptions containing injectable drugs and antibiotics were significantly higher in HPs compared to LPs (p<0.05 for all comparisons, Table II).

Table I. Comparison of descriptive characteristics of all prescriptions generated by low prescribers (n= 305) vs. high prescribers (n=304).

All prescriptions	Prescriptions of all physicians (n=1,258,688)	Prescriptions of low prescribers (n=63,469)	Prescriptions of high prescribers (n=635,798)	p-value
Number of drugs per prescription	2.36±0.90	2.26±0.80	2.38±0.92	0.01
Number of boxes per prescription	3.57±2.52	3.78±2.78	3.49±1.71	<0.001
Total number of drugs	2,972,351	143,632	1,511,224	-
Total number of boxes	4,489,753	239,713	2,216,575	-
Number of diagnoses per prescription	1.39±0.33	1.36±0.28	1.38±0.45	0.05
Total number of diagnoses	1,748,032	86,356	878,109	-
Percentage of prescriptions containing injectable drug	4.1	4.0	4.1	0.23
Percentage of prescriptions containing antibiotic	38.5	37.2	38.8	<0.001

Table II. Comparison of descriptive characteristics of antibiotic-containing prescriptions generated by low prescribers (n= 305) vs. high prescribers (n=304).

All prescriptions	Prescriptions of all physicians (n=480,136)	Prescriptions of low prescribers (n=23,614)	Prescriptions of high prescribers (n=246,373)	p-value
Number of drugs per prescription	2.91±1.01	2.80±0.72	2.92±0.98	0.02
Number of boxes per prescription	3.35±2.10	3.23±1.25	3.36±2.03	<0.001
Total number of drugs	1,395,829	66,147	718,856	-
Total number of boxes	1,608,034	76,369	828,242	-
Number of diagnoses per prescription	1.38±0.24	1.34±0.52	1.37±0.54	0.04
Total number of diagnoses	662,421	31,563	338,056	-
Percentage of prescriptions containing injectable drug	5.5	4.2	6.1	<0.001
Percentage of prescriptions containing injectable antibiotic	4.1	2.9	4.6	<0.001
Percentage of prescriptions containing only antibiotic	11.1	13.6	10.5	<0.001
Percentage of prescriptions containing single diagnoses	71.5	74.8	71.9	<0.001

Three of the most frequently prescribed ten drug groups were antibiotics: "J01C-penicillins" (21.7%), "J01D-other beta-lactam antibiotics" (6.5%) and "J01F- macrolides, lincosamides and streptogramine" (5.3%), (Fig. 2). This ranking was also similar in HP and LP groups. The most frequently prescribed ten antibiotics in prescriptions consisted of 89.1% of all antibiotics. Four of them were cephalosporin (14.4%) and two of them were macrolide (14.8%). It was determined that amoxicillin+clavulanate was the most frequently prescribed antibiotic in the both HP and LP groups (48.3% and 61.1%, respectively), as well as the prescriptions of

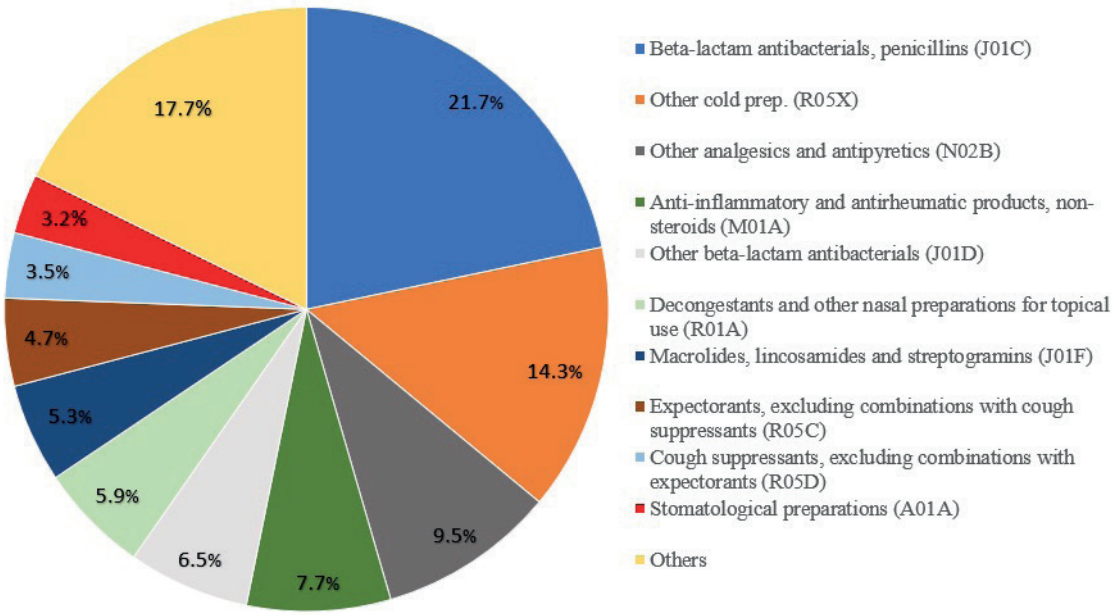

Fig. 2. Distribution of the antibiotics at ATC-3 level.

Table III. Distribution of the top ten frequently prescribed antibiotics in LP and HP groups.

Name of drugs (ATC-5)	All physicians		Low prescriber (LP)		High prescriber (HP)	
	n (%)	%*	n (%)	%*	n (%)	%*
Amoxicillin + clavulanic acid (J01CR02)	250,064 (50.9)	17.9	14,770 (61.1)	22.3	121,963 (48.3)	17.0
Clarithromycin (J01FA09)	56,855 (11.6)	4.1	2,401 (9.9)	3.6	30,614 (12.1)	4.3
Cefixime (J01DD08)	27,439 (5.6)	2.0	839 (3.5)	1.3	15,730 (6.2)	2.2
Amoxicillin (J01CA04)	20,028 (4.1)	1.4	755 (3.1)	1.1	10,377 (4.1)	1.4
Cefuroxime (J01DC02)	18,179 (3.7)	1.3	960 (4.0)	1.5	8,937 (3.5)	1.2
Azithromycin (J01FA10)	15,681 (3.2)	1.1	731 (3.0)	1.1	7,727 (3.1)	1.1
Benzathine phenoxymethylpenicillin (J01CE10)	15,141 (3.1)	1.1	929 (3.8)	1.4	7,395 (2.9)	1.0
Cefpodoxime (J01DD13)	12,867 (2.6)	0.9	221 (0.9)	0.3	6,972 (2.8)	1.0
Cefalexin (J01DB01)	12,232 (2.5)	0.9	180 (0.7)	0.3	7,822 (3.1)	1.1
Metronidazole (J01XD01)	9,467 (1.9)	0.7	264 (1.1)	0.4	5,341 (2.1)	0.7
Subtotal	437,953 (89.1)	31.4	22,050 (91.2)	33.3	222,878 (88.3)	31.0
Others	53,509 (10.9)	3.8	2,134 (8.8)	3.2	29,499 (11.7)	4.1
Total	491,462 (100.0)	35.2	24,184 (100.0)	36.6	252,377 (100.0)	35.1

* Percentage of drugs in prescriptions containing antibiotics.

all physicians for children (50.9%), (Table III). Tetracycline was found in 0.2% of prescriptions and quinolones were found in 0.1%. Overall, the most frequently prescribed drugs after amoxicillin+clavulanate were "R05X-Others cold comb." (14.3%), paracetamol (9.0%), and ibuprofen (7.0%).

Injectable antibiotics were prescribed in 4.1% of the prescriptions. Ceftriaxone was the most frequently prescribed injectable preparation

by all physicians in the HP and LP groups (39.1%, 33.9%, and 34.6%, respectively), (Fig. 3). Besides, although HP and LP groups were similar (2.4% and 2.2%, p=0.057), 2.3% of all prescriptions were found to consist of multiple antibiotics (97.8% of combinations included dual antibiotherapy).

When antibiotics were examined in terms of their spectrum, it was found that 11.2% of them were narrow-spectrum, 79.8% of them were broad-spectrum, and 0.5% of them were ultra-broad-spectrum. This distribution was also preserved overall in HP and LP groups for narrow- (12.4% and 9.1%, respectively) and broad-spectrum (78.9% and 82.5%, respectively) with a significant difference (p<0.001).

Most of the diagnoses in prescriptions containing antibiotics belonged to infectious diseases (74.3%), especially upper respiratory tract infections (RTI). Noninfectious indications among the ten most frequent diagnoses were "vasomotor and allergic rhinitis" (3.1%) and "general examination" (2.4%). "Acute upper respiratory tract infections" was the diagnosis in which both narrow- (16.4%) and broad-spectrum (17.5%) antibiotics were most frequently prescribed. We detected that ultra-spectrum antibiotics were most frequently prescribed for diagnoses related to the urinary system ("Other disorders of the urinary system" 30.1% and "cystitis" 26.9%), (Table IV). In the narrow-spectrum group, amoxicillin (35.9%); in the broad-spectrum groups, amoxicillin+clavulanate (66.1%); and in the ultra-spectrum groups; fosfomycin (52.3%) were the most frequently prescribed antibiotics. The distribution of narrow and broad-spectrum antibiotics in single-diagnosis prescriptions was also similar in the HP and LP groups.

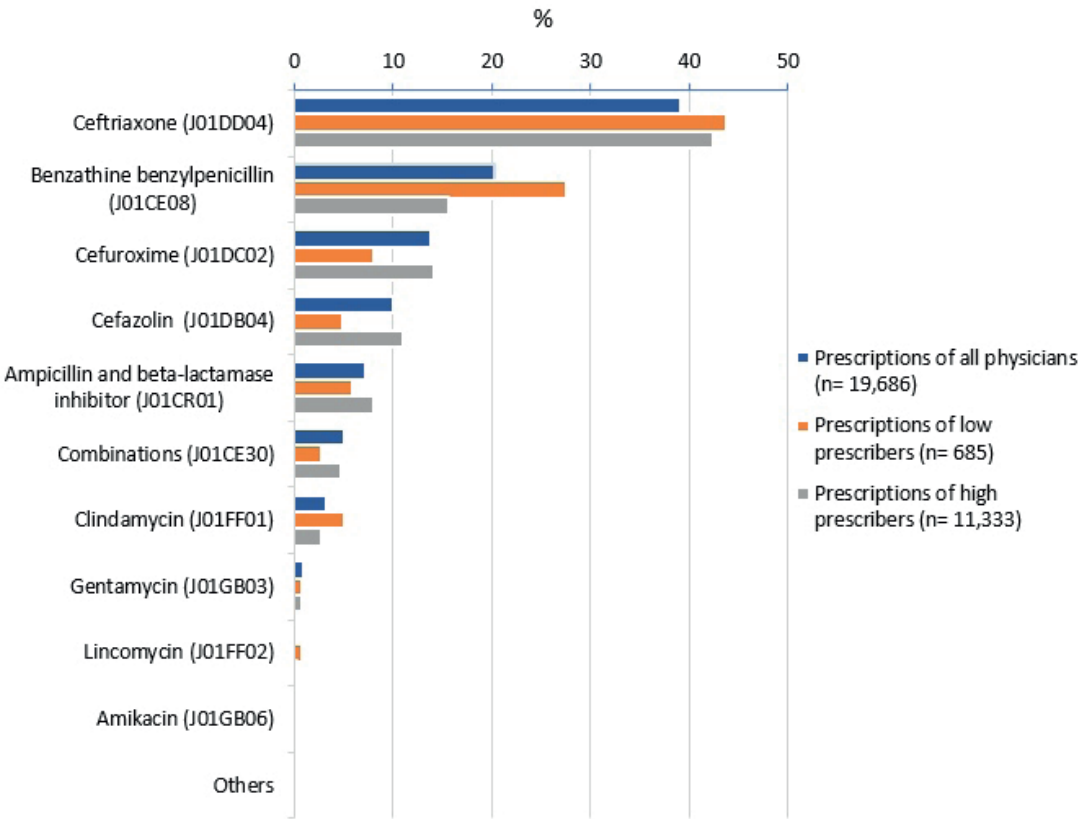

Fig. 3. Distribution of injectable antibiotics in injectable antibiotic-containing prescriptions.

Table IV. Distribution of diagnoses in single-diagnosis prescriptions by antibiotic spectrum.

Narrow			Broad			Ultra-broad		
Diagnoses (ICD)	n	%	Diagnoses (ICD)	n	%	Diagnoses (ICD)	n	%
Acute upper respiratory infections of multiple and unspecified sites (J06)	6.872	16.4	Acute upper respiratory infections of multiple and unspecified sites (J06)	49,302	17.5	Other disorders of urinary system (N39)	490	30.1
Acute tonsillitis (J03)	6,540	15.6	Acute tonsillitis (J03)	39,158	13.9	Cystitis (N30)	438	26.9
Other diseases of upper respiratory tract (J39)	5,216	12.5	Other diseases of upper respiratory tract (J39)	30,905	11.0	Acne (L70)	263	16.2
Acute sinusitis (J01)	4,228	10.1	Acute bronchitis (J20)	28,884	10.3	Other and unspecified noninfective gastroenteritis and colitis (K52)	41	2.5
Acute pharyngitis (J02)	4,120	9.8	Acute sinusitis (J01)	25,742	9.2	Other and unspecified dermatitis (L30)	27	1.7
Acute bronchitis (J20)	3,017	7.2	Acute pharyngitis (J02)	24,674	8.8	Acute upper respiratory infections of multiple and unspecified sites (J06)	22	1.4
Unspecified acute lower respiratory infection (J22)	1,692	4.0	Unspecified acute lower respiratory infection (J22)	16,883	6.0	Acute nasopharyngitis [common cold] (J00)	20	1.2
Suppurative and unspecified otitis media (H66)	1,403	3.4	Suppurative and unspecified otitis media (H66)	9,534	3.4	Cutaneous abscess, furuncle and carbuncle (L02)	17	1.1
Acute nasopharyngitis [common cold] (J00)	958	2.3	Acute nasopharyngitis [common cold] (J00)	4,875	1.7	Other local infections of skin and subcutaneous tissue (L08)	16	1.0
Other disorders of urinary system (N39)	631	1.5	Otitis externa (H60)	4,463	1.6	Encounter for general examination without complaint, suspected or reported diagnosis (Z00)	14	0.9
Subtotal	34,677	82.8		234,420	83.4		1,348	82.9
Others	7,194	17.2		47,400	16.6		276	17.1
Total	41,871	100.0		281,020	100.0		1,626	100.0

Discussion

Inappropriate use of antibiotics is a common negative practice in primary care.[2] This irrational pharmacotherapy becomes more crucial in the pediatric population, which needs extra attention to promote the safety of medicines in children.[12] We showed that near 40% of pediatric prescriptions contain antibiotics, more marked by those physicians who were in the upper quartile of pediatric visits. While these physicians also tend to prescribe a higher number of overall or injectable drugs and antibiotics, broad-spectrum antibiotherapy seems to be more practiced by the physicians in the lower quartile.

The number of drugs per prescription and the percentage of antibiotic-containing prescriptions are well-established indicators of the World Health Organization to evaluate the rational use of medicine.[3] In primary care, 29.5% of all prescriptions were reported to contain antibiotic(s) in Turkey in 2016.[14] We observed a near 10% upward deviation of antibiotic prescribing prevalence in children (38.5%), which may not be unexpected considering the frequent use of antibiotics in children and positive correlation of antibiotic utilization with children density in a given population.[4,7,15] Nevertheless, it can still be considered higher than antibiotic prescribing reported from the large primary care databases in the Netherlands (18%), the US (28%), and the UK (36%).[16,17] In addition, it is known that many factors change the prescribing attitudes of physicians.[7,9,11] For instance, a study performed in Italy with 1164 pediatricians caring for almost 425,000 children reported that the quality of antibiotic prescribing was 4-fold worse among high antibiotic prescribers.[18] While we focused on primary care physicians, those who were visited more by pediatric patients, i.e., HPs, were more likely to escalate already-high antibiotic use (38.8%) compared to LPs (37.2%). The clinical implication of such difference needs designation of further studies beyond poor antibiotic-prescribing performances of both physician groups. In fact, general practitioners with higher consultation rates were also reported to have increased antibiotic prescribing, especially for respiratory tract infections.[19] In addition, the presence of common cold combinations, paracetamol, and ibuprofen in prescriptions containing antibiotics suggests that physicians necessarily add an additional symptomatic agent to prescriptions containing antibiotics, increasing the number of drugs per prescription. This was also higher in HPs and further increased among the antibiotic-containing prescriptions, re-appraising the role of pediatric primary care a potential area for promoting rational use of antibiotics. The last decade has witnessed substantial efforts to address evidence-based targets and reduce pediatric antibiotic prescribing, from clinician-directed strategies to community-based campaigns.[20-24] A meta-analysis in 2013 reported a 6-21% reduction in pediatric antibiotic prescribing with combined parent education and clinician behavior modification with no benefit with passive waiting room education materials.[20] On the other hand, a recent randomized study reported no effect of electronically delivered prescribing feedback in reducing antibiotic prescribing in <15-year-old children, unlike adults.[24] These suggest the need for developing and implementing well-structured and multi-faceted interventions to improve rational antibiotic prescribing in children. Even modest improvements may have a higher impact in terms of rational antibiotic use in such countries like Turkey, with a comparably younger population and excessive antibiotic use.[5,14] Narrow-spectrum antibiotics are the first-line treatment option for many indications that often require antibiotics for children.[25] However, previous studies have shown that broad-spectrum antibiotics, especially co-amoxiclav, are frequently used in children.[9,26-28] In our study, 80% of the antibiotics were of broad spectrum and one of every two antibiotics prescribed by physicians was co-amoxiclav. The predominance of co-amoxiclav in our practice was previously reported in the literature[7], which suggested the persistence of this inappropriate prescribing also in primary care compared to

that reported in the Netherlands (10%), the UK (4%), and in Italy (23%).[17,29] In fact, these figures were significantly higher in LPs in our study, including the preference for broad-spectrum drugs (83% vs. 79%). A US study evaluating almost 400,000 acute primary care visits in children showed the broad-spectrum antibiotic utilization as 42%[16], nearly halving our findings. Another study performed in three European countries reported the ratio of broad-to-narrow-spectrum antibiotics as 0.3 for the UK, 3.2 for the Netherlands, and 74.7 for Italy.[17] Our findings indicated this ratio to be around 7, with a higher level in LPs, emphasizing the need for improving the quality of antibiotic prescribing. Our sample did not include pediatricians or specialists working in secondary/tertiary care, among which the preference for broad-spectrum antibiotics could be regarded as rather more reasonable. In fact, the literature showed that physicians with a low level of knowledge were more likely to prescribe broad-spectrum antibiotics.[9] This indicates that physicians' level of knowledge may be more determinant in rational prescribing, apart from the burden of prescribing. From this aspect, the training to be given by the authorities can improve the rational drug prescribing practice of physicians, focusing on primary care.

One-third of the prescriptions containing antibiotics for children indicated the diagnoses as upper respiratory tract infections.[25] In our study, we also observed a predominance of upper respiratory tract infections with similar frequency for both narrow- and broad-spectrum antibiotic prescriptions. This overall high rate of antibiotic prescribing might be regarded as unnecessary considering that the vast majority of these conditions have viral etiology.[30] Furthermore, a similar distribution of the same diagnoses for narrow- and broad-spectrum drugs may suggest an arbitrary selection of antibiotics, irrespective of HP or LP status. In fact, the preference of broad- over narrow-spectrum antibiotherapy was reported to be associated with higher rates of adverse events and decreased quality of life in children with upper respiratory tract infections.[31] Though our study design did not allow us to follow patients, our findings could likely represent an inappropriate practice. On the other hand, the predominance of urinary system disorders and cystitis for ultra-broad-spectrum antibiotics may suggest that physicians tend to reserve this group for urinary tract infections. Within this group, tetracyclines and quinolones antibiotics are unfavorable antibiotics because of their safety issues in children.[32,33] Previous studies showed that quinolone use had a higher trend in Turkey compared to that of European countries.[5] Relatively low prescribing (0.3%) of tetracyclines and quinolones might indicate a rational behavior of the physicians in terms of antibiotics that have critical safety issues in children in our study.

Limited use of injectable forms of drugs is another indicator of rational use of medicines.[3] Overall injectable drug prescribing (4.1%) in our study appeared lower than that of the general population in Turkey (8.1%), indicating a lower preference for injectable drugs in children.[14] While this was similar between HPs and LPs, it is noteworthy that the rate increased by almost 50% (6.1%) among HPs compared to %5 increase (4.2%) in LPs for the antibiotic-containing prescriptions. This might be attributed to the difference between the use of injectable antibiotics in HPs (4.6%) vs. LPs (2.9%). It was reported that the prescription of antibiotics raised the likelihood of injectable medicine use[34], consistent with our findings, especially for HPs. In addition, primary care physicians with a high practice activity were reported to be more liberal in antibiotic prescribing.[19] This may partly explain the higher use of injectable forms in HPs in our study as they represented the upper quartile of pediatric practice. This is further supported by a primary care study reporting the association of overuse of injections in younger populations.[35] Another remarkable finding of the study is that ceftriaxone, a broad-spectrum antibiotic, is the most frequently used injectable antibiotic in prescriptions. A previous study in primary care

showed the most common injectable form was ceftriaxone in infants, which was replaced by benzathine benzylpenicillin in older age groups of children.[36] Considering the relationship between the use of broad-spectrum antibiotics and antibiotic resistance[37], the fact that almost 40% of injectable antibiotics are ceftriaxone can be considered an irrational choice.

Our findings need to be interpreted with the consideration of the study's limitations. Since we had no data for regional distribution of HPs and LPs at the districts level, we could not control for confounding factors that may influence their prescribing behaviors such as demographic or socioeconomic factors. As we also had no data on their age, gender, duration of occupation etc. that may have affected their practice, we could not overcome such limitation by using a generalized estimating equation or mixed models for correlated data. Besides, stratification of high or low prescribing status was based on the percentage of pediatric visits. The volume of adult visits and the experience with this population could have had an impact on their antibiotic prescribing in children. In addition, our data was based on the solo indications and their drugs on prescriptions. The diagnoses established by the physicians were accepted as correct and possible diagnostic errors could not be taken into account. Nevertheless, extensive coverage of all diagnoses with no particular focus on groups of indications (e.g., respiratory tract infections), or particular infections (e.g., sinusitis or acute otitis media) helped us to eliminate potential coding bias for a better evaluation of antibiotic prescribing practice.[38] Finally, as the study protocol and permissions only allowed for retrieval and collection of 2016 data, we did not have data after the year 2016. While it would be interesting to compare recent data (at latest 2019 because of the very likely confounding effect of the healthcare access-related issues of COVID-19 pandemic), we would not expect any major change between these years (i.e., 2016-2019) regarding pediatric antibiotic use. Nevertheless, temporal change may be expected due to short- and mid-term impact of the restrictions on public access to nonprescribed antibiotic use (applicable as of the year 2013), whose outcomes indicate the need for designation of further quantitative and qualitative studies.

In conclusion, we showed that almost 40% of the prescriptions generated for children contained antibiotics, and 80% were of broad-spectrum. Physicians who had a higher population of pediatric cases were also more likely to prescribe a higher number of any drugs, injectable forms, and antibiotics whereas those who were visited by a lower number of children were more likely to prefer broad-spectrum antibiotics. Both physician groups appeared to prefer either narrow- or broad-spectrum drugs regardless of their antibiotherapy spectrums, mostly for respiratory tract infections. Available findings suggest that irrational antibiotic prescribing for children is still a problem in primary care, which warrants further large-sized studies that would probe clinicians' characteristics and contribute to the development of well-structured interventions to improve antibiotic use in children.

Ethical approval

The study was approved by Ethics Committee for Non-interventional Studies of Istanbul Medipol University (Approval No: 25.06.2020-527). This retrospective database study did not require informed consent either from child and family.

Author contribution

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

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Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

Autosomal chromosome microdeletions in three adolescent girls with premature ovarian insufficiency: a case report

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ABSTRACT

Background. Premature ovarian insufficiency (POI) in the pediatric age group is most commonly related to X chromosome abnormalities such as Turner syndrome. Autosomal chromosome microdeletions in ovarian failure are relatively rare. The present study identified new autosomal deletions in three girls with POI.

Case. We present three adolescent girls aged 14–15 years who had not attained menarche. Upon physical examination, there was a lack of breast tissue and no prominent secondary sexual characteristics. Clinical evaluation, hormonal tests, abdominal ultrasonography, and chromosome karyotyping were performed. Chromosome microarray analysis (CMA) was also performed to detect DNA copy number changes. Luteinizing hormone level was significantly increased, while follicular stimulating hormone level was >25 IU/L with low estradiol levels. Autosomal deletions were detected in all three cases by CMA. The first patient had 0.454 Mb deletion on 15q25.2, the second patient had 1.337 Mb deletion on 19p13.3, and the third patient had 0.163 Mb deletion on 16p11.2.

Conclusions. POI is rare in children and is most commonly associated with X chromosome abnormalities. However, normal karyotype does not exclude the presence of chromosomal abnormality. CMA should be considered in cases with POI to detect microdeletions in autosomal chromosomes.

Key words: premature ovarian insufficiency, chromosome microarray analysis, chromosome karyotype, chromosomal abnormalities, autosomal.

Premature ovarian insufficiency (POI) refers to the development of ovarian dysfunction prior to the age of 40 years. The main manifestations of POI are abnormal menstruation (amenorrhea, oligomenorrhea, or polymenorrhea), elevated gonadotropin levels (FSH >25 IU/L), and a general decrease in the levels of female hormones. POI is further classified into primary POI and secondary POI, according to whether there had been spontaneous menstruation or not.[1,2] In children, POI is usually associated with short stature and delayed puberty. Genetic, iatrogenic, immune, and environmental factors have been implicated in the causation of POI. Nonetheless, the underlying cause remains unclear in more than half of all patients with POI (idiopathic POI). Approximately 20–30% of cases of POI are due to genetic aberrations, including chromosomal abnormalities and genetic variations. Among these, chromosomal abnormalities account for approximately 10-13% of cases, and 94% of aberrations involve the X chromosome. Other genes and autosomal abnormalities account for only 2% of patients with POI.[3-9] Besides, in the majority of POI cases with autosomal abnormalities, the autosomal abnormalities are considered coincidental. The onset of POI is commonly seen amongst older adults; however, cases of POI presenting in adolescence are rare.

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Current guidelines recommend the use of chromosome analysis to rule out potential chromosomal abnormalities in patients with POI.[10] In our previous work, we found that microdeletion may lead to POI, and we further discussed the genes affected by microdeletion. Our research indicated that POI may be related to genes, such as *BNC1*.[11] In the subsequent research, we found that new microdeletions may still lead to POI, indicating the need for further research on the correlation between chromosome microdeletions and POI. The present paper is a corollary to our previous work. In this study, we describe three adolescent girls with POI who had normal karyotype. Chromosomal microarray analysis (CMA) revealed three different microdeletions in autosomal chromosomes.

Case Report

Case 1 was a 14-year-old girl admitted to our clinic with growth retardation for 12 years. Her body weight was 33 kg (-2.2 standard deviation (SD)) and her height was 147 cm. She was admitted to our clinic due to a lag in sexual development and a lack of secondary sexual characteristics. She was diagnosed with intellectual disability shortly after birth. During her early kindergarten education, she was only able to say a few sentences and had a poor academic performance. According to the Wechsler Intelligence Scale for Children, her language IQ was 50, active IQ was 57, and total IQ was 44. Physical examination revealed no peripheral lymphadenopathy, tonsil enlargement, or hepatosplenomegaly. She had not achieved menarche. She was the first child of Han Chinese non-consanguineous parents. Her birth bodyweight (BBW) was 3.3 kg. Her father's height was 170 cm, and her mother's height was 157 cm. She showed poor development of the mammary glands, with widely spaced nipples.

On laboratory investigations, her sex hormone profile was: luteinizing hormone (LH), 35.05 IU/L (normal reference range: 50–9.30 IU/L); follicle stimulating hormone (FSH), 105.6 IU/L (1.4–18.1 IU/L); estradiol, 17.3 pg/mL (0–44.5 pg/mL); testosterone, 19.6 ng/dL (10.83–56.94 ng/dL); prolactin, 14.1 ng/mL (2.1–17.7 ng/mL); progesterone, 0.55 ng/mL (0.28–1.22 ng/mL); dehydroepiandrosterone sulfate (DHEAS), 144.6 μg/dL (34.5–568.9 μg/dL); and androstenedione 1.1 ng/mL (0.6–3.1 ng/mL). Her chromosome karyotype was 46, XX. The *FMR1* gene showed no variation, which excluded fragile X syndrome. On abdominal ultrasound, the uterine size was 1.9×0.7×1.2 cm^3 with no signs of thickening. The cervix was approximately 1.5 cm long, and the cervical intima was thin.

Case 2 was a 15-year-old girl admitted to our clinic for growth delay for 1 year. She also had a lag in prominent secondary sexual characteristics. She was yet to achieve menarche. There was no previous history of surgery or major disease.

She was the third child of Han Chinese non-consanguineous parents. She was born from a full-term spontaneous normal delivery with no complications; her BBW was 2.4 kg. Her father's height was 168 cm, and her mother's height was 155 cm. Her breast tissue was poorly developed. Further examination of the mammary gland showed no abnormal or web neck phenomenon.

Her sex hormone profile was: LH, 24.29 IU/L; FSH, 87.1 IU/L; estradiol, 18.2 pg/mL; testosterone, 34.7 ng/dL; prolactin, 17.9 ng/mL; progesterone, < 0.21 ng/mL; DHEAS, 97.1 μg/L; and androstenedione 0.6 ng/mL. The chromosome karyotype was 46, XX. On abdominal ultrasound, the uterus was 1.4×0.5×1.3 cm^3 in size, and the uterine wall was not thickened. The cervix was approximately 1.7 cm long and the intima was thin.

Case 3 was a 14-year-old girl brought to our outpatient clinic by her parents with complaints of growth retardation for 11 years. She had not yet achieved menarche. Her past history was unremarkable. She was borne of a full-term normal delivery with no complications;

her BBW was 2.4 kg. There was no family history of immunodeficiency or recurrent infections. Physical examination showed good mental development with optimal speech communication for her age. However, she had a short stature (height 136.4 cm, -4.0 SD) for her age, short neck, and underdeveloped breast tissue.

Her sex hormone profile was: LH, 17.02 IU/L; FSH, 61.3 IU/L; estradiol, 16.3 pg/mL; testosterone, 11.9 ng/dL; prolactin, 11.3 ng/mL; and progesterone < 0.21 ng/mL. The chromosome karyotype was 46, XX. Her uterus size was 1.4×1.4×0.6 cm^3, and the uterine wall was not thickened. The cervix was approximately 1.9 cm long and had a thin intima.

Prior to genetic testing, written informed consent was obtained from the probands and their parents (reference number of the research ethics committee of the First Affiliated Hospital, College of Medicine, Zhejiang University: 2018-727). Genomic DNA of the three children was collected, and copy number variance was analyzed using the Phalanx Biotech's Cyto-One-Array Chromosome Chip. The chip contains a total of 33,255 57-63mer oligonucleotide probes covering a total of 331 specific diseases and all the sub telomeric regions designed according to the UCSC hg19 human gene bank (NCBI build 37). The probe resolution was 10–30 kb in disease-specific regions and 1.5–2 Mb resolution in non-disease-specific backbone areas. The three patients were sporadic cases. The family history of the patients revealed no similar case.

All three cases presented in this study are typical cases of ovarian dysfunction developing before the age of 40 years. None of the patients showed signs of secondary sexual development. Notably, the FSH levels of the patients far exceeded the POI standards. Moreover, their estrogen levels were significantly lower. Thus, a diagnosis of POI was established based on the clinical manifestations and sex hormone profile. CMA analysis revealed deletion aberrations in all three cases.

Case 1 had a DNA loss change on 15q25.2 with deletion size of about 0.454 Mb starting from position 83,581,573 to position 84,035,357 (Fig.1). Case 2 had 1.337 Mb (1,043,392-2,380,865) deletion on 19p13.3 (Fig.2). Of note, there are a total of 67 reported genes in this area. However, there is no previously reported association of

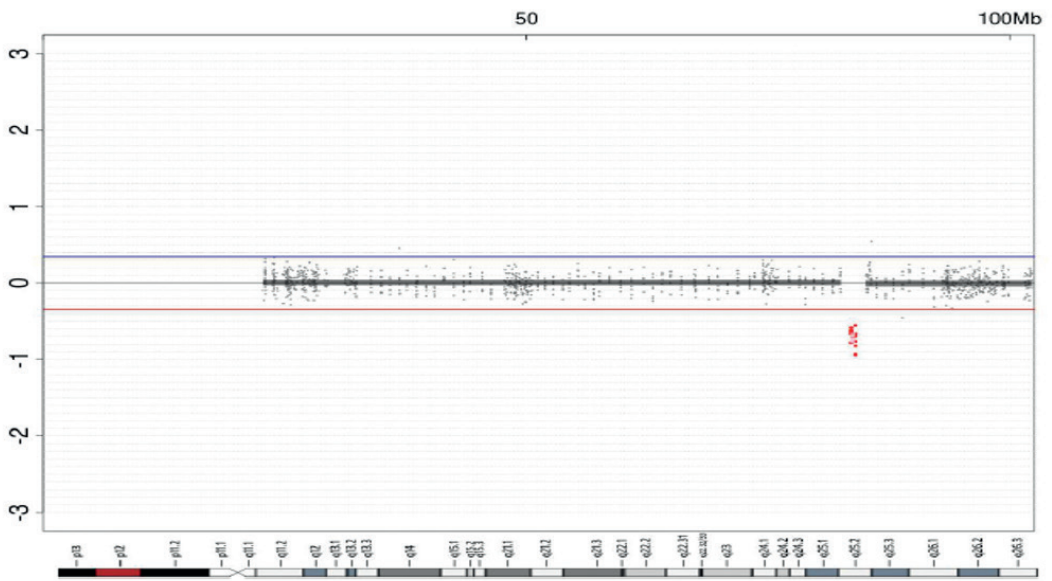

Fig. 1. Case 1 had a DNA loss change on 15q25.2 with deletion size of about 0.454 Mb starting from position 83,581,573 to position 84,035,357.

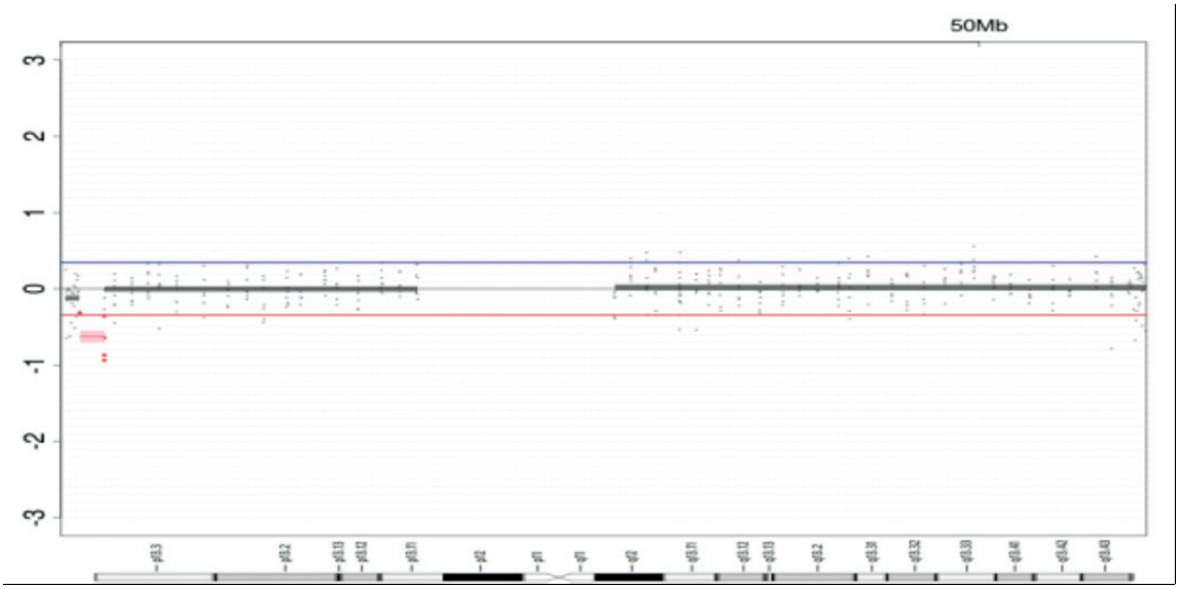

Fig. 2. Case 2 had 1.337 Mb (1,043,392 - 2,380,865) deletion on 19p13.3.

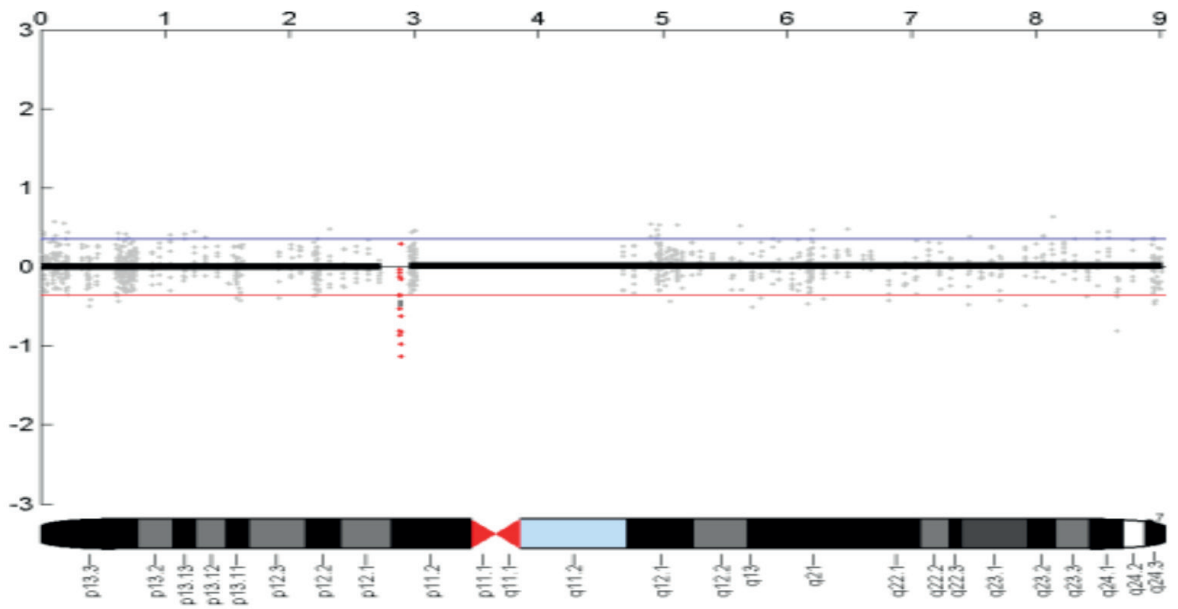

Fig. 3. Case 3 had 16p11.2 deletion with size of about 0.163 Mb (28,824,793 -28,987,798).

these genes with early ovarian insufficiency. However, case 3 had 16p11.2 deletion with size of about 0.163 Mb (28,824,793-28,987,798) (Fig.3). There are a total of 12 reported genes in this area; nonetheless, none of the genes have been reported to be associated with early ovarian insufficiency (Table I).

All 3 patients were treated with estrogen and progesterone for the establishment of artificial menstruation. All achieved menarche and established regular menstruation after treatment. The clinical course has been uneventful to date (as of 3-year follow-up).

Table I. Features of patients with premature ovarian insufficiency.

	Case 1	Case 2	Case 3
Age	14 y/o	15 y/o	14 y/o
Genotype	Del 15q25.2	Del 19p13.3	Del 16p11.2
Position	83,581,573-84,035,357	1,043,392-2,380,865	28,824,793-28,987,798
Size (Mb)	0.454	1.337	0.163
Secondary sexual characteristics	none	none	none
LH (IU/L)	35.05	24.29	17.02
FSH (IU/L)	105.6	87.1	61.3
E2 (pg/mL)	17.3	18.2	16.3
Ovary volume (mL)	0.798	0.455	0.588

LH: luteinizing hormone, FSH: follicle stimulating hormone, E2: estradiol

Discussion

As previously reported, POIs are usually associated with X chromosome abnormalities, and only a few POIs are associated with autosomal abnormalities. The pathophysiology of POIs falls into two categories: follicular dysfunction and accelerated depletion of the primordial follicles. There are several X chromosomal genes related to ovarian follicular dysfunction and apoptosis, such as *USP9X*, *ZFX*, *BMP15*, *SHOX*, *XIST*, *POF1B*, *DIAPH2*, *XPNPEP2*, and *FMR1*. However, there are other genes controlling the apoptosis and functions of autosomal follicles such as *BAX*, *BCL2*, *CDKN1B*, *CYP19A1*, *ESR1*, *FOXL2*, *CASP2*, and *CASP3*.[7,12,13]

The clinical manifestations of Case 1 in this case-series were very similar to those of fragile X syndrome. Fragile X syndrome is the most common cause of hereditary dementia and is the most prevalent genetic cause of autism and intellectual disability. The clinical manifestations may range from minor learning and mental disorders, and autism, to severe intellectual disability. Based on these characteristic features, the first case was suspected to be that of fragile X syndrome. However, after detecting the *FMR1* gene, fragile X syndrome was ruled out.[14] Previous studies suggested that the deletion of the *CPEB1* gene on 15q25.2 may cause POI or stunting. However, 15q25.2 (0.454 Mb) microdeletion in our case did not include the *CPEB1* gene, which, therefore, shows that POIs may not be directly affected by the *CPEB1* gene deletion.[15-18]

To the best of our knowledge, our case series is the first to show that the deletion on 15q25.2 may result in the simultaneous occurrence of POI and stunting.[15,16] Nonetheless, further studies should investigate the pathogenetic mechanism by which 15q25.2 deletions cause POI.

Microdeletion of 16p11.2 was detected in the third case. Aboura et al.[19] had reported a case of a female Caucasian with POI, a microdeletion of 16p11.2, and having a total of 21 genes. *CD19* gene may be associated with the development of POI. Nonetheless, the deletion in the third case was smaller when compared to that reported by Aboura et al.[19], but, however, included the *CD19* gene. Thus, the *CD19* gene deletion might be involved in the pathogenesis of POI.

Microdeletion on 19p13.3 detected in the second case might be the first reported to be associated with POI. Previous reports usually focused on signs such as developmental delays, deformity of the eye, extraocular distance, low set of ear lobes, retinopathy, and erythrocytes abnormalities with the deletion of the 19p13.3 region.[20-22] Our clinical findings suggest that there is a new microdeletion-19p13.3 chromosome phenotype.

Karyotype analysis of adolescents with POI was often used to exclude sex chromosomes or autosome related diseases. Karyotype analysis can quickly find a large deletion of chromosome fragments; however, it has a much lower sensitivity for the detection of chromosome microdeletions compared with CMA. Thus, CMA is the optimal method for genetic testing in order to identify chromosomal aberrations in clinically diagnosed POI patients.

CMA is widely used for prenatal diagnosis and genetic analysis of children with congenital heart disease and growth retardation.[23,24] Our series of studies have demonstrated the feasibility of CMA for the diagnosis of POI in children and adolescents, and reaffirmed the importance of CMA in the genetic diagnosis of childhood diseases.

In conclusion, although autosomal microdeletions are known to be a rare cause of POI, the application of CMA may unravel the involvement of more autosomal microvariations in the causation of POI. CMA may help identify the various causes of POIs and play a vital role in genetic counseling.

Ethical statement

Part of the manuscript was reported and discussed at the 2018 European Society for Pediatric Endocrinology Annual Meeting. The relevant information of case 1 was reported by the members of our research group in September 2020 with a 15q25.2 microdeletion phenotype for premature failure in a Chinese girl: a case report and review of literature.[11] The manuscript mainly discusses the possible effects of the microdeletion site on BNC1 gene, which is different from the research direction of this manuscript.

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Ethical approval

The Research Ethics Committee of First Affiliated Hospital, College of Medicine, Zhejiang University approved the study. Written informed consent was obtained from the parents. Reference number of the research ethics committee of the First Affiliated Hospital, College of Medicine, Zhejiang University: 2018-727.

Author contribution

The authors confirm contribution to the paper as follows: designed the research and drafted the manuscript: KY, LL, CW; interpreted the data: KY, MH, YF; performed the literature search and scientific overview of our case: KY, JZ, CW. All authors critically read and reviewed the final 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 novel *Mecom* gene mutation associated with amegakaryocytic thrombocytopenia in a premature infant

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ABSTRACT

Background. Hereditary bone marrow failure syndromes are a category of biologically different syndromes that can cause cytopenia in at least one hematopoietic cell lineage.

Case. We present a 29-week-old male infant who had a low Apgar Score, advanced delivery room resuscitation, widespread petechial rash, and ecchymoses at birth, without any dysmorphic features. Initial laboratory tests revealed bicytopenia (platelet count 7x10 3 /uL, hemoglobin of 3.9 g/dL, neutrophil 2.0x10^3 /uL) with findings of disseminated intravasculer coagulation (DIC). Imaging studies demonstrated accompanying left-sided congenital pulmonary airway malformation. On the second postnatal week pancytopenia occurred and the bone marrow findings were consistent with congenital amegakaryocytic thrombocytopenia. Further evaluations for differential diagnosis of pancitopenia were performed and the results of congenital viral infections, metabolic and immunologic tests were negative. While supportive treatments were in progress, haploidentical bone marrow transplantation (BMT) was performed from the father at 84th day due to unavailability of HLA-matched relative or nonrelative donor. Whole exome sequencing revealed a novel heterozygous frameshift variation (c.1242dupT [p. Thr538fs]) in exon 8 of the MECOM gene and validated by Sanger sequencing. No variation was detected in the parents genetic analysis.

Conclusions. In this report, we present a patient with congenital bone marrow failure successfully treated with haploidentic BMT and describe a novel, de novo pathogenic variant in MECOM gene.

Key words: MECOM gene, congenital amegakaryocytic thrombocytopenia, neonate.

Inherited bone marrow failure syndromes (IBMFS) are rare disorders and usually caused by a genetic condition represented as single or multilinear cytopenia and physical malformations. Hematological and dysmorphic findings of IBMFS rarely present during neonatal period. The diagnosis of IBMFS should be kept in mind in newborns with cytopenia and / or congenital abnormalities since early diagnosis of an IBMFS is important to optimize clinical management. Congenital amegacaryocytic thrombocytopenia (CAMT) is one of the IBMFS in neonates characterized with severe thrombocytopenia and hypocellular bone marrow.[1-4] In recent years, genetic analysis has added many opportunities to diagnose IBMFS. Whole-exome sequencing (WES) gained a specific interest in terms of genetic diagnostic approach among patients with IBMFS.[5,6] Herein, we report a de novo heterozygote mutation of *MECOM* gene diagnosed by WES in a preterm neonate with CAMT.

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Case Report

A 1471 g male neonate was born at 29 gestational weeks to a 37-year-old G2P1A1 healthy mother by cesarean section, with Apgar scores 5/5 at 1st and 5th minute. A distant consanguinity was defined between parents (3rd degree cousin marriage). The patient required resuscitation and was intubated in the delivery room. First examination in neonatal intensive care unit (NICU) revealed pallor, ecchymosis on four extremities and widespread petechial rashes on truncal part of the body. The patient developed pulmonary hemorrhage in the first hour of life.

The initial blood count showed Hb 3.9 g/dL, Htc 11.6 %, MCV 118 fL, MCH 39.7 pg, MCHC 33.6 g/dL, WBC 2200/mm^3, ANC: 2000/mm^3, ALC 100/mm^3, PLT: 7.000/mm^3, MPV: 11 fL. Peripheral smear revealed leukopenia, myeloid and erythroid precursor cells, few platelets, anisocytosis, polychromasia and several schistocytes. Corrected reticulocyte count was decreased (1.04%) and direct coombs test was negative. Coagulation tests revealed prolonged PT and PTT according to his gestational age (aPTT: 79 s [27.5-78.4], PT: 17 s [10.6-16.2], D.dimer: 10.7 mg/L [0-0.5], Fibrinogen 2.2 g/L [1.5-3.7]. The patient was diagnosed as disseminated intravascular coagulation (DIC) secondary to intrauterine hypoxia, and suspected early onset neonatal sepsis (EONS). In addition to wide spectrum antibiotics for EOS, repeated packed red blood cell (PRBC), platelet and fresh frozen plasma (FFP) transfusions were performed. On the third postnatal day results of whole blood count and coagulation tests were in normal values.

A chest radiograph for the prenatally diagnosed congenital pulmonary airway malformation (CPAM) showed left upper lobe consolidation (Fig. 1). Chest computed tomography (CT) with angiography revealed 32x28 mm large solid intrathoracic mass involving left superior lobe unrelated to bronchial system and normal thoracic aorta branching, that indicates type 3 CPAM. Partial pulmonary venous return anomaly was also detected in the CT scan. Echocardiography, cranial and abdominal ultrasonography showed no congenital anomalies.

On the 14th postnatal day, pancytopenia was detected with PLT: 5.000/mm^3, Hb :9 g/dL, and ANC: 800/mm^3 while the patient had no sign of infection and was stabilized hemodynamically. The patient was consulted with pediatric hematology department for pancytopenia. Bone marrow aspiration revealed profoundly hypocellular bone marrow with very rare myeloid and erythroid precurosrs and total absence of megacaryocytes. There was no dysplasia, malign infiltration or hemaphagocytosis. Considering radioulnar synostosis with amegakaryocytic thrombocytopenia (RUSAT), syndrome an upper extremity radiography was taken and proximal radioulnar synostosis was ruled out.

Tests for inborn error of metabolism and immune system (flow cytometric evaluation of lymphocyte subgroups immunoglobulin levels) were in the normal range. Ophthalmological examination showed pale optical disk, peripapillary hyperpigmentation. Cytogenetic evaluation of patient's peripheral blood revealed 46,XY in 5 metaphases.

From the hematological point of view, pancytopenia persisted and the patient needed recurrent platelet and erythrocyte transfusions. The patient did not respond to high dose G-CSF treatment (up to 20 mcg/kg/day), had persistent severe pancytopenia and required occasional granulocyte transfusions duw to sepsis in the follow-up.

In terms of IBMFS, next- generation sequencing analysis was performed for WES analysis and revealed a novel heterozygous frameshift variation c.1242dupT (p. Thr538fs) in exon 8 of the *MECOM* gene. The mutation is detected in the proband, but not in the parents, is confirmed by Sanger sequencing (Fig. 2). The clinical significance of the variant was examined using the standards and guidelines for the interpretation of sequence variants

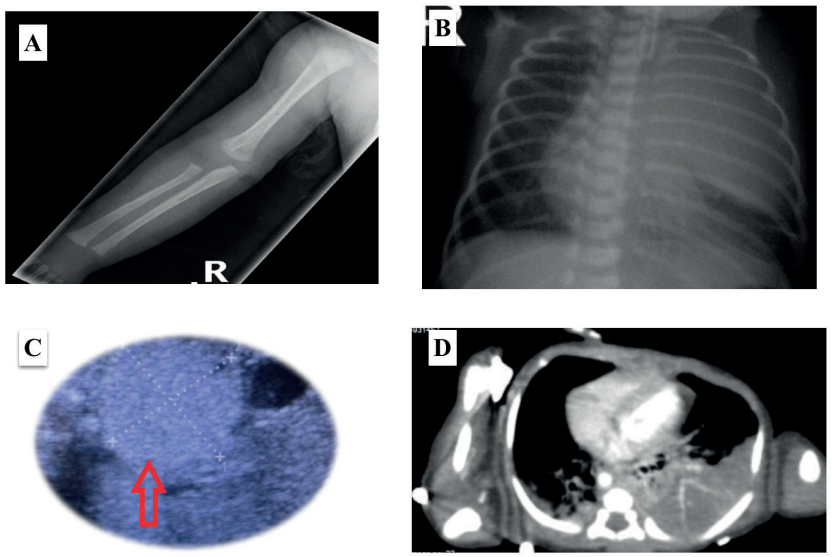

Fig. 1. A, Upper extremity graph ruling out radioulnar synostosis. B, Anterio-posterior chest graph with a suspected congenital pulmonary airway malformation. C, Ultrasound imaging of airway malformation showing hyperechogenic solid thoracic lesion with sharp border (arrow) . D, Chest CT angiography of the patient demonstrating congenital pulmonary airway malformation filling the left hemithorax, pulmonary hypoplasia and pulmonary venous return abnormality.

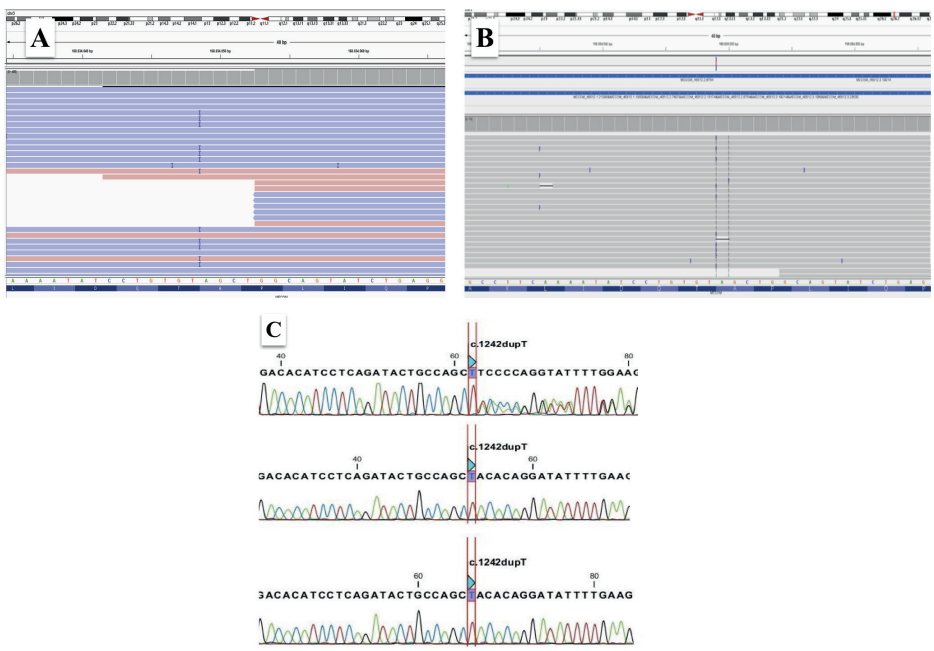

Fig. 2. A, Next generation sequencing analysis of the proband showing heterozygous c.1242dupT (p. Thr538fs) mutation in MECOM gene. B, Validation and segregation analysis of the proband and parents by Sanger sequencing revealing c.1242dupT (p. Thr538fs). C, (Line 1: Index, Line 2: Mother, Line 3: Father)

recommended by the American College of Medical Genetics and Genomics (ACMG Laboratory Quality Assurance Committee) and the Association for Molecular Pathology (AMP).[7]

Due to unavailability of HLA-matched related or unrelated donor, HLA-haploidentical hematopoietic stem cell transplantation from the father was successfully performed on the 84th day of life (postconceptional 41st gestational week). Neutrophil engrafment was achieved on day 13 and thrombocyte engrafment was achieved on day 14. No severe complication was observed in the post-transplant period. The patient experienced a grade II acute GVHD, immune suppressive treatment was tapered and discontinued on post-transplant 9th month. Full chimerism was achieved in the post-transplant first month and persisted in the follow-up.

Informed parental written consent was taken for case publication.

Discussion

In this case report, a 29-week-old premature infant diagnosed as inherited congenital bone marrow failure syndrome with *MECOM* gene mutation is presented.

True incident of IBMFS is not well known, but in a national base survey, over 40 years in Israel revealed 127 patients, only 6% of them had been diagnosed as CAMT.[8] Mucosal bleeding, petechia or ecchymosis usually present as initial symptoms of CAMT in early neonatal period secondary to thrombocytopenia.[9] In our case, the patient presented with petechial rashes and ecchymosis overall the body and pulmonary hemorrhage right after birth. Persistent pancytopenia after clinical stabilization period directed us for further investigations. Presence of decreased megakaryocytes in the bone marrow was diagnostic in addition to clinical suspect.

Skeletal and somatic abnormalities are common in IBMFS, but CAMT usually present without physical malformations in previous reported cases. In a case series of 21 CAMT patients, seven patients had mental and motor retardation, growth retardation, cardiac defects, strabismus, and nystagmus.[10] Additionally, our patient had congenital pulmonary airway malformation and partial pulmonary venous return anomaly.

Thrombopoetin (TPO) is an important regulator for hematopoietic stem cells into proliferation and survival. CAMT is mostly seen with autosomal recessive genetic mutation in the myeloproliferative leukemia virus oncogene (MPL) that encodes TPO receptor. Genetic methods particularly WES bring new insights for understanding underlying pathophysiology and accurate diagnosis. Although some patients phenotypically present as congenital amegakaryocytic thrombocytopenia, associated genetic mutations provide more detailed linkage to other IBMFS such as *DKC1* mutation in dyskeratosis congenita, *HOXA11* and *MECOM* in radioulnar synostosis with amegakaryocytic thrombocytopenia.[11-13]

MECOM gene is located on chromosome band 3q26.2 and has a crucial role in cell differentiation, growth and migration. Clinical spectrum due to *MECOM* gene mutations varies from isolated radioulnar synostosis to severe bone marrow failure. Germeshausen et al.[13] reported 12 patients with CAMT caused by *MECOM* mutations, and beside bone marrow failure additional phenotypes were observed such as clinodactyly, congenital deafness, renal abnormalities and cardiac malformations. A case report by Kjeldsen et al.[14] identified a de novo *MECOM* gene mutation in a neonate with congenital hypoplastic bone marrow and the patient has no dysmorphological findings. In our case skeletal and renal abnormalities were not identified and result of brainstem evoked response audiometry test was in normal limits.

To conclude, we present a CAMT case in a very preterm with several physical abnormalities with a novel de novo *MECOM* gene mutation, who was treated succesfully with stem cell tranplantation. Consideration of bone marrow transplantation on time due to congenital cytopenia was life-saving for our patient. In these patients, genetic diagnosis and genetic counseling of the family are quite critical due to the possibility of re-occurance of the disease in future generations.

Ethical approval

Informed parental written consent was taken for case publication.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: BD, FT, ÖT, SY, AA; data collection: BD, EIC, AD; analysis and interpretation of results: AA, ND, HÖ, HÖ; draft manuscript preparation: BD, FT, HÖ. 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

Rare cause of ketolysis: Monocarboxylate transporter 1 deficiency

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ABSTRACT

Background. Monocarboxylate transporter 1 (MCT1) deficiency (MIM #616095) is a relatively new identified cause of recurrent ketoacidosis triggered by fasting or infections. MCT1 was first described in 2014 by van Hasselt et al. to result from both homozygous and heterozygous mutations in the SLC16A1 gene. Patients with homozygous mutations are known to have a more severe phenotype with developmental delay and epilepsy. Thirteen patients with MCT1 deficiency with ketoacidosis have been reported in the literature to date.

Case. We describe a developmentally normal male patient with heterozygous missense variation in the SLC16A1 gene. Our patient who presented with cyclic vomiting and ketoacidosis episodes was found to have a heterozygous c.303T>G (p.Ile101Met) missense mutation.

Conclusions. It is crucial to take early preventive measures and to minimize the harmful effects of ketoacidotic episodes. MCT1 deficiency should be considered in the differential diagnosis of ketoacidosis in patients with normal SCOT and ACAT1 activities.

Key words: MCT1, ketoacidosis, ketone metabolism, vomiting.

Ketone metabolism provides an important energy source for many tissues during fasting.[1] Succinyl-CoA:3-oxoacid CoA transferase (SCOT), mitochondrial acetoacetyl-CoA thiolase (also known as β-ketothiolase or T2) and monocarboxylate transporter 1 (MCT1) deficiencies are ketone body utilisation and transporter defects and associated with severe intermittent ketoacidosis episodes.[1] Ketone bodies cross cell membranes by diffusion or the facilitator MCT1. The role of MCT1 in ketone metabolism is important, especially in catabolic stress.[2] The clinical findings occur especially in metabolic stress such as fasting and infections, from the first days of life to childhood.[1,3] The clinical features of the diseases are vomiting, dehydration, Kussmaul breathing and decreased consciousness. In general, specific organic acid excretion is detected in T2 deficiency, whereas there is no specific excretion in SCOT and MCT1 homozygous and heterozygous mutations in the *SLC16A1* gene encoding MCT1 in ketoacidotic patients with profound ketoacidosis.[3,4] The clinical features of monocarboxylate transporter-1 deficiency (MCT1D) are vomiting, severe ketoacidosis and ketotic hypoglycemia.[3] To our knowledge, a total of 13 patients (MCT1 deficiency with ketoacidosis) have been reported in the literature to date.[3,5-7] Here we report an infant with ketoacidosis and vomiting caused by a heterozygous mutation in the *SLC16A1* gene.

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Case Report

A two month and 20 day old male patient born to second degree consanguineous parents was referred to the pediatric metabolism department

due to repetitive vomiting and hypotonia. Prenatal and natal history was uneventful, with normal antenatal follow up. The patient began vomiting soon after birth. The first physical examination at the age of two months and 20 days revealed mild axial hypotonia and he was unable to hold his head. He only had esotropia of the right eye as a congenital malformation. No other dysmorphic features were noted. Laboratory findings of the patient were; venous blood pH 7.31, bicarbonate 18.6 mmol/L, BE -6.3 mmol/L, lactate 4.1 mmol/L. Blood ketone was measured as 5.2 mmol/l with a ketone strip. Plasma glucose was 89 mg/dl. Ammonia level was 179.68 μmol/l and 163.83 μmol/l. Significant urinary excretion of ketones was demonstrated. Liver transaminases and creatine kinase remained normal. Serum B12 level was determined as 262 pg/ml (RR: 197-771). The patient was not hypoglycemic. Serum lipid profile (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides) was normal. He was treated with high dextrose concentrations (continuous infusion of dextrose at 8 mg/kg/min) and sodium benzoate. Within the next few hours, the patient's venous blood findings were; pH 7.35, bicarbonate 22.3 mmol/L, BE -2.4, lactate 1.9 mmol/L. Post-treatment ammonia level was measured as 49.32 μmol/l. His quantitative blood aminoacid analysis was normal (glutamine and glycine were normal). Tandem-MS analysis revealed slightly elevated C3 carnitine (C3:3.94 mol/L RR: 0.08-1.77). Homocysteine was 4.32 μmol/L. In the urine organic acid analysis, 3 hydroxy butyric acid excretion had increased 24 times, methylmalonic acid excretion increased 4.6 times, succinic acid excretion increased 3.4 times more than the normal range. The patient was followed up and treated with a preliminary diagnosis of organic acidemia due to metabolic acidosis with ketosis, mild hyperamonemia, slightly elevated C3 and methylmalonic acid excretion. Molecular analysis was performed for methylmalonic aciduria (*MUT, MMAA, MMAB, MMACHC, MCEE, SUCLA, SUCLG1*) and found to be normal. The patient presented three times with vomiting and ketoacidosis episodes. Initial blood ketone levels measured by ketone strip were 5.4, 6.2, and 5.0 mmol/l in each episode, respectively. Serum ammonia levels were detected between the normal range except the first admission. Each acute episode of illness was managed with intravenous dextrose and sodium bicarbonate. Sodium benzoate treatment was not continued. The patient didn't have resistant ketoacidosis episodes and did not need dialysis. Echocardiographic findings were normal. During the follow-up, the developmental milestones gradually improved. When he was nine months old, his weight was 8 kg (-1.18 SDS) and height was 65 cm (-2.75 SDS). The most recent physical examination (including the neurological examination) was normal, except for esotropia and short stature (Fig. 2). Neuroimaging was not performed. He had three ketoacidosis episodes in five months and no symptoms between the acidosis episodes. His current age is one and half years and he has had no ketoacidosis episodes in the last ten months.

Whole Exome Sequencing (WES) analysis was performed. We found a heterozygous c.303T>G (p.Ile101Met) missense variant in exon 3 of the *SLC16A1* (NM_001166496.1) gene. IGV images of the mutation are shown in Figure 1. It was evaluated as a variant of unsignificant (VUS) in insilico predictive tools for the *SLC16A1* gene. We considered this change to be disease-causing in our patient based on the population data (low frequency in GnomAD population database), pathogenic computational predictions (DANN, M-CAP, MutationAccessor, MutationTaster, SIFT) and the clinical finding of ketoasidosis episodes. Segregation analysis was performed on the mother and father of the patient. The same mutation was found to be heterozygous in his father. It was learned that his father had a history of hospitalisation with three metabolic acidosis episodes in his childhood, and he did not have any problems after the age of seven. However, ketone levels during the episodes were unknown. A fasting test was not performed due to a lack of the patient's approval. MCT transport activity could not be performed due to technical incompetence.

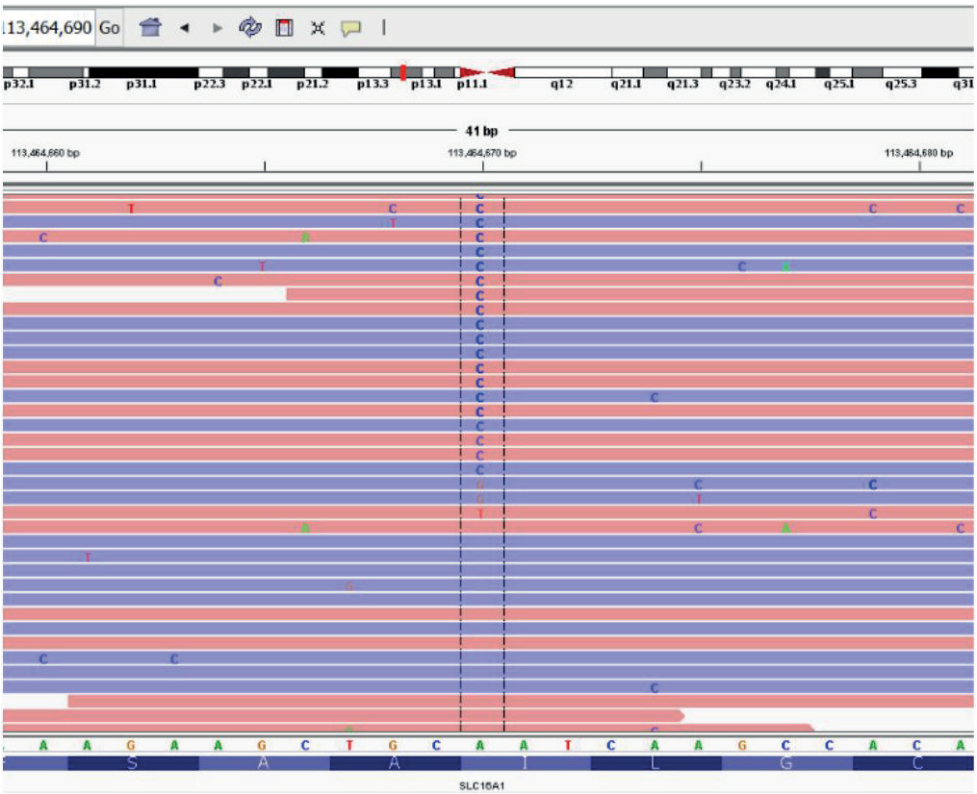

Fig. 1. IGV images of the SLC16A1 gene. c.303T>G (p.Ile101Met) missense variation detected by Whole Exome Sequencing.

Fig. 2. The nine months old male patient with MCT1 deficiency.

The primary aim of the patient's treatment was to prevent decompensation. The parents were informed about high glucose intake during states of metabolic stress such as infections and were warned about the avoidance of fasting.

Discussion

Mutations in the *SLC16A1* gene are associated with erythrocyte lactate transporter defect and monocarboxylate transporter 1 deficiency.[4,8] The MCT1 protein, is a proton-linked monocarboxylate transporter that catalyzes the rapid transport of many monocarboxylates, such as lactate, pyruvate, branched chain oxo acids derived from leucine, valine and isoleucine and the ketone bodies acetoacetate, beta-hydroxybutyrate and acetate across the plasma membrane.[9] Gain-of-function MCT1 promoter mutations have been shown to be associated with hyperinsulinism due to inducing SLC16A1

expression in beta cells.[10] It occurs due to the failure of cell-specific transcriptional silencing. Inactivating mutations in the SLC16A1 gene are associated with ketoacidosis episodes. Neurological problems such as developmental delay and epilepsy, and severe ketoacidosis episodes are more common in homozygous patients. In heterozygotes patients, normal development and normal blood pH and ketone values between episodes have been reported.[3,7]

MCT1 deficiency was first reported by van Hasselt et al. in 2014 as a cause of recurrent severe ketoacidosis induced by fasting or infections starting during infancy.[4] After identifying a homozygous frameshift mutation in a patient by targeted homozygous-region exome sequencing, they sequenced *SLC16A1* gene in 96 patients with ketoacidosis who were known to be normal for SCOT and ACAT1 enzymatic activities. They identified a total of nine patients with mutations in the MCT1, three homozygous and six heterozygous, including two siblings. They studied the effect of mutations in MCT1 on monocarboxylate transport. They found that the mutational status was correlated with MCT1 protein levels, transport capacity, and ketoacidosis severity. The mean lactate transport activity from heterozygous carriers, both symptomatic and asymptomatic, was significantly reduced. They reported that all patients (both homozygous and heterozygous) presented with bouts of ketoacidosis in their first years of life. Patients with homozygous mutations had a more severe phenotype with earlier onset of disease, more profound ketoacidosis, developmental delay and an increased prevalence of epilepsy. The frequency of the ketoacidosis episodes decreased over time with complete resolution after the age of seven years except ketonuria associated with mild infections in some of the patients.[4] van Hasselt et al.[4] reported that in the individuals with inactivating homozygous or heterozygous pathogenic variants in the SLC16A1 gene, hypoglycemia was seen infrequently. Heterozygous c.303T>G (p.Ile101Met) mutation in the *SLC16A1* gene was detected in our patient who presented with vomiting and ketoacidosis without hypoglycemia. Consistent with the literature our patient's blood pH values were within the normal range between attacks and hypoglycemia did not occur.

Balasubramaniam et al. described 2 half-siblings with MCT1D who had heterozygous nonsense mutations in the *SLC16A1* gene.[5] The asymptomatic heterozygous mother showed that additional triggers are needed for the development of ketoacidosis episodes. Segregation analysis was performed on our patient. A heterozygous mutation was detected in his father. We learned that he had acidosis attacks in his childhood, but we could not reach medical records.

Al-Khawaga et al.[6] described 28-month-old female patient with recurrent ketoacidosis and hypoglycemia who was found to have a homozygous pathogenic variant in the *SLC16A1* gene. The patient had seizures, and this was the first report of neuroimaging findings in MCT1D. The cranial MRI of the patient showed subependymal heterotopia and a specific signal alteration. The following and the last report of MCT1D was also about neuroimaging findings and was published by Nicolas-Jilwan et al.[7] MCT1, especially expressed in oligodendroglia, provides lactate delivery to neuronal cells. Lee et al.[11] reported that disruption of this transporter leads to axon damage and neuron loss in animal and cell culture models. It has also been shown that the MCT1 transporter is reduced in amyotrophic lateral sclerosis patients and mouse models.[11] This suggests a role for oligodendroglial MCT1 in the pathogenesis. In many studies, MCT defects are associated with neurodegeneration, epilepsy, cognitive impairment and metabolic disorders. This may explain the neurological deterioration in patients. In the literature, corpus callosum agenesis, T2 hyperintense signal abnormalities in basal ganglia, thalamus, dentate nucleus and hyperintense signal abnormalities in

cortical/subcortical white matter, U-fibers were reported.[6,7] We did not perform neuroimaging in our patient. However, further research is needed to elucidate this.

To the best of our knowledge, 13 cases (MCT1 deficiency with ketoacidosis) have been described in the literature. Hyperammonemia was not reported in any of the patients with MCT 1 deficiency. In our case, ammonia levels were normal without ammonia scavenger treatment in the follow-up. This may be clarified in further studies.

Our patient had short stature which has also been reported in the literature in a 10-year old patient with a heterozygous mutation of the MCT1 gene by van Hasselt et al.[4] We believe the cause of short stature in our patient to be familial, since his parents were also short. A longer follow-up period is required to assess short stature.

The c.303T>G (p.Ile101Met) variant in exon 3 of the *SLC16A1* gene is expected to cause the disease because it has a low frequency in GnomAD population database, has pathogenic computational predictions (DANN, M-CAP, MutationAccessor, MutationTaster, SIFT) and recurrent ketoacidosis finding. However, further functional studies are required to validate the genotype-phenotype correlation. Our case describes another heterozygous patient with MCT1 deficiency who had recurrent ketoacidosis and normal developmental milestones.

MCT1 deficiency should be considered in the differential diagnosis of ketoacidosis in patients with normal SCOT and ACAT1 activities. It is crucial to take early preventive measures and minimize the harmful effects of the ketoacidotic episodes.

Ethical approval

Written informed consent was obtained from the parents to publish this case report.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: AEB, ATÜ; data collection: AEB; analysis and interpretation of results: AEB, ATÜ; draft manuscript preparation: AEB. 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 hypomyelinating leukodystrophy-14 benefiting from ketogenic diet therapy

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ABSTRACT

Background. Hypomyelinating leukodystrophy-14 (HLD14) is a rarely seen neurodevelopmental disease caused by homozygous pathogenic ubiquitin-fold modifier 1 gene variants. The disease has an autosomal recessive inheritance. All patients with this condition reported to date have drug-resistant epilepsy. The post-translational modification of proteins with ubiquitin fold modifier 1 is defective in these patients and is thought to be responsible for severe neurodevelopmental problems. There is no previous report on the effectiveness of the ketogenic diet in the treatment of drug-resistant epileptic seizures in this disease. Therefore, we present a pediatric case diagnosed with HLD14 and whose drug-resistant epileptic seizures were controlled by ketogenic diet therapy.

Case. The patient was a three-year-old male with drug-resistant epilepsy and developmental delay. His brain magnetic resonance imaging revealed cerebellar atrophy, periventricular white matter hypomyelination, and ventricular enlargement. Whole-exome sequencing analysis identified a homozygous pathogenic variant in the ubiquitin-fold modifier 1 gene on chromosome 13q13. Ketogenic diet therapy was initiated for his drug-resistant seizures and subsequently reduced seizure frequency by more than 75%. The patient is still on ketogenic diet therapy.

Conclusions. Ketogenic diet therapy may be beneficial for seizure control in HLD14 patients with drug-resistant seizures.

Key words: drug-resistant epilepsy, hypomyelinating leukodystrophy-14, ketogenic diet therapy, children.

Hypomyelinating leukodystrophy-14 (HLD14, OMIM:610553) is a rare neurodevelopmental disorder that occurs due to homozygous pathogenic *UFM1* gene variants on chromosome 13q13. The disease starts in early infancy and is characterized by microcephaly, hypotonia, and cognitive and motor delay. In addition, most patients have spasticity, extrapyramidal movements, drug- resistant seizures, hearing loss, and vision loss. Patients often require gastric tube feeding and ventilator support, and most die within the first year of life. Brain imaging shows hypomyelination with cerebral and cerebellar atrophy.[1]

Protein modification enables the regulation and expansion of genetic information. Ubiquitination is a protein modification system in which single or multiple ubiquitin molecules are attached to a protein and act as a signal transmitter that controls various cellular functions.[2] The ubiquitin-like modifier (UFM-1) has structural similarities to ubiquitin. UFM-1 can be linked to substrate proteins as a monomer or a lysine-linked polymer. UFMylation is a specialized ribosomal modification to facilitate metazoan-specific protein biogenesis in the endoplasmic

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reticulum and is essential for nervous system development and function. The post-translational modification of proteins on lysine residues has an essential role in several cellular processes.[3,4] Defective UFMylation is thought to be responsible for severe neurodevelopmental problems in HLD14. Ketogenic diet therapy (KDT), which is frequently used for drug-resistant epilepsy, may also be beneficial for myelination disorders.[5]

Here we report a pediatric patient with a rare leukodystrophy, HLD14, who had drug-resistant seizures and benefited from KDT.

Case Report

The patient, a three-year-old boy, was delivered by cesarean section in the 38th week of gestation. This was the mother's third pregnancy. He was born to consanguineous parents, after an uneventful pregnancy. He had two healthy older siblings. He had a history of admission for 16 days in the neonatal period due to respiratory distress. A tracheostomy and mechanical ventilation were required at the age of six months, after which feedings were initiated with a nasogastric tube. The patient was using phenobarbital, levetiracetam, vigabatrin, and vitamin B6 for his seizures. On physical examination, the patient weighed 28 kg (+3 SDS), had dysmorphic facial features including sloping forehead, micrognathia, low ears and plump cheeks, microcephaly, high palate, simian line, fusiform fingers, axial hypotonia with increased muscle tone on extremities, and dystonia. Edematous appearance in the limbs was noted. Deep tendon reflexes were normoactive with no pathological reflexes and limited dorsiflexion of the feet. He could not hold his head up and had no eye-tracking. He had bilateral sensorineural hearing loss. Complete blood count, serum biochemistry, thyroid functions tests, vitamin B12 level and creatine kinase level were within normal limits. Electroneuromyography was normal. Echocardiography revealed a patent foramen ovale. Electroencephalogram (EEG) showed generalized spikes and polyspike and slow waves creating burst activities with suppression periods and was interpreted as modified hypsarrhythmia (Fig. 1a). He had frequent seizures as spasms which did not respond to multiple antiepileptic drugs. Brain magnetic resonance imaging (MRI) showed cerebellar atrophy, hypoplastic corpus callosum, and periventricular white matter hypomyelination (Fig. 2a-b). Magnetic resonance spectroscopy did not reveal any specific metabolite concentration. Metabolic tests (serum ammonia, lactate, pyruvate, biotinidase activity, tandem mass spectrometry with carnitine and acylcarnitine profile, urine organic acids, plasma and urine amino acids, levels of lysosomal enzymes) were unremarkable. He was admitted to the inpatient ward due to having seizures while on multiple

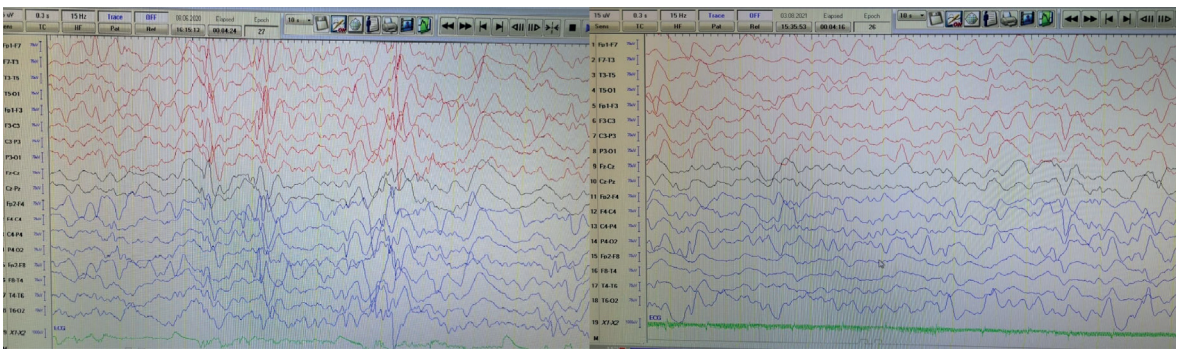

Fig. 1a. Electroencephalogram before ketogenic diet therapy showed generalized spikes and polyspike and slow waves creating bursts with suppression periods and was interpreted as modified hypsarrhythmia. **1b.** Electroencephalogram after ketogenic diet therapy demonstrated mild epileptiform activity in the parietooccipital and temporooccipital regions of the right hemisphere.

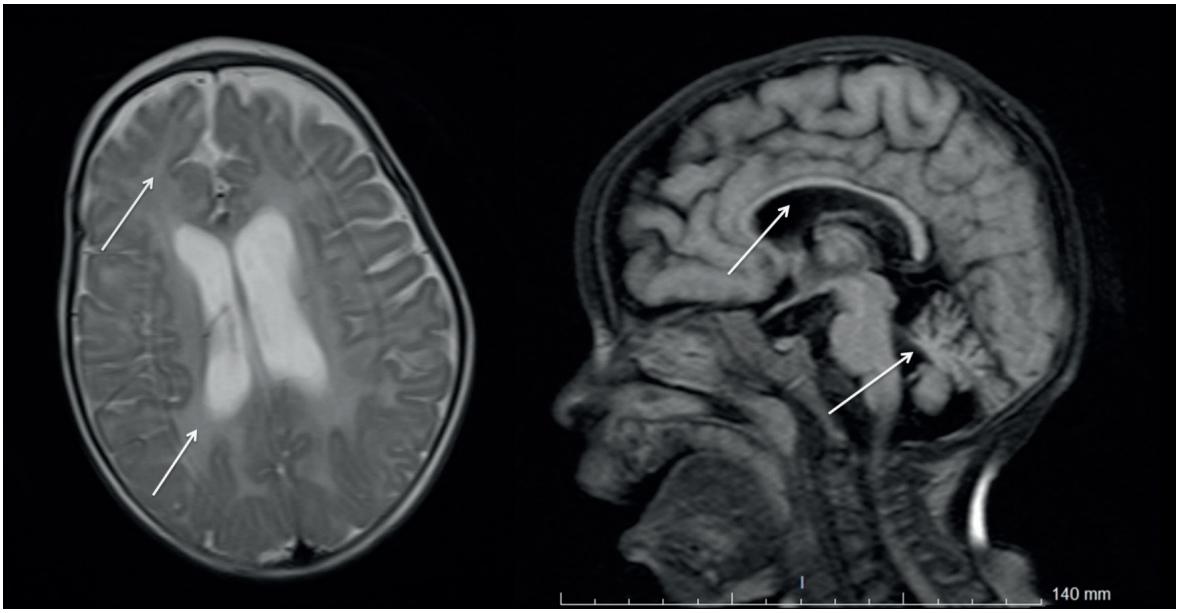

Fig. 2a. Brain MRI axial series showing white matter hypomyelination (white arrows). **2b.** Brain MRI sagittal T1W series showing cerebellar atrophy and hypoplastic corpus callosum (black arrows).

antiepileptic drugs. The patient's seizures were in the form of apnea, focal spasms, and generalized tonic seizures. A classic KDT with a 3.5:1 ratio (ratio of grams of fat to grams of carbohydrate plus protein) was introduced with -one meal per day when the patient was two years old. This was gradually increased, and multivitamins were added.

Using the hydrolyzed milk formulae (Pepti-Junior®), and a 4:1 ketogenic ratio formula (KetoCal®), meals of 50 kcal/day were prepared for the patient via a nasogastric tube. The patient's beta-hydroxybutyrate levels in the blood were measured and ketone levels were monitored. Values between 4-6 mmol/L were accepted as normal, but the patient was considered to have hyperketonemia because his values were above 6 mmol/L. On the 10th day of KDT, the ratio was decreased to 2.5:1 due to this state of hyperketonemia.

The patient's seizure frequency was reduced by more than 75% with KD. Before KDT, the patient had an average of two seizures per day with a duration of 3-5 minutes. After the third month of KDT, his seizures decreased to two times per week with a duration of 2-3 minutes. Figure 1b shows improved epileptic discharges on EEG after KDT. The patient's seizures decreased and vigabatrin treatment was tapered and discontinued in the follow-up. Whole-exome sequencing analysis revealed a single homozygous deletion in the promoter region of *UFM1*, which is c.-273-271 delTCA. The variant is classified as "pathogenic" based on American College of Medical Genetics and Genomics (ACMG) recommendations.[6] Via segregation analysis, both the patient's parents and his healthy siblings were found to be heterozygous carriers. The patient was diagnosed with HLD14.

No significant side effects of KD developed during follow-up. The patient's quality of life and that of his family increased with the control of his seizures. It was observed that the patient started to smile, make sounds, and had increased movements in his head, arms, and legs. The family stated that the care of the patient improved as hisspasticity and dystonia decreased on KD.

A written consent form was obtained from the parents for publication purposes.

Discussion

HLD14 is a severe neurodevelopmental disorder with autosomal recessive inheritance in which most patients develop drug-resistant epilepsy. Impairment of post-translational protein UFMylation is thought to be responsible for severe neurodevelopmental problems.[1]

Hamilton et al.[1] identified 16 pediatric patients with brain MRI findings suggesting hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC) who have a severe clinical phenotype with epileptic encephalopathy and developmental delay. Similar to our patient, they found homozygous pathogenic variants of the *UFM1* gene in these patients. All of their patients had severe clinical phenotype consisting of epileptic encephalopathy with early death.[1]

Nahorski et al.[3] reported four children with a homozygous missense mutation in the *UFM1* gene from two related families with HLD14. Three of the patients died before the age of two. The patients presented with hyper or hypotonia, malnutrition, and severe global developmental delay in infancy. They developed resistant seizures in the first weeks or months of life, and EEG showed hypsarrhythmia in two siblings. The patients had poor overall growth with secondary progressive microcephaly and peripheral edema. Similarly, our patient had cerebellar atrophy, hypoplastic corpus callosum, and extensive periventricular white matter hypomyelination on brain imaging.

A new post-translational protein modification known as ubiquitination is a modification system in which single or multiple ubiquitin molecules are attached to a protein, acting as a signal transmitter that controls various cellular functions.[7] The ubiquitin-like modifier (UFM1) is a ubiquitin-like protein regulator and is required for embryonic development. Ubiquitin and UBL pathways play a role in controlling multiple functions, including signal transduction, transcriptional regulation, and stress response.[2] Various studies show that there is a relationship between both neurodevelopment and neurodegeneration via UFM1.[8] Most evidence points to the role of UFM1 in endoplasmic reticulum homeostasis and protection against apoptosis.[9]

Muona et al.[10] found that reducing the gene expression of *UFM1* in the mouse brain resulted in death on the first day of life. Postmortem examination revealed microcephaly and increased neuronal apoptosis markers in specific brain regions. They suggested that infiltration could be spatially regulated and cell type-specific; only selective neural cell lines are vulnerable to promoter mutation.[10] Available data show that the *UFM1* system is vital for neuronal development and function.[2,11]

Epilepsy is characterized by recurrent unprovoked seizures and is associated with the involvement of neurons in the gray matter. Early or resistant seizures can occur but are an unusual feature of disorders mainly affecting brain white matter.[12] While the focal onset and bilateral tonic-clonic seizures are more common in some cohorts, generalized tonic-clonic, infantile spasm, and myoclonic seizures have also been reported.[13-15]

In a study conducted on patients with leukodystrophy, the incidence of epilepsy was reported to be 49%. The incidence of epilepsy varies among different types of leukodystrophies and was found to occur more frequently in leukodystrophies classified as astrocytopathy, compared to myelin diseases. This finding is likely due to the role that astrocytes play in the pathogenesis of epilepsy. When astrocyte dysfunction occurs, it impairs its extracellular glutamic acid and K^+ elimination ability leading to extracellular accumulation of glutamic acid and K^+, thereby increasing the excitability of neurons and inducing seizures.[16] Zang et al.[17] also found leukodystrophies with early subcortical involvement of white matter were more prone to epilepsy than other types of leukodystrophy. This may suggest subcortical white matter involvement is likely to affect neighboring neurons in the cerebral cortex and can lead to epilepsy.

Since our patient had epileptic encephalopathy and his seizures could not be controlled despite the use of three antiepileptic drugs, he was considered to have drug-resistant epilepsy. Thus, the decision was made to pursue KDT with a ketogenic formula. The patient and his family were extremely compliant with the diet. As the patient had swallowing dysfunction due to generalized muscle weakness, he was fed with a nasogastric tube. Gastrostomy was not found to be appropriate due to his high weight. Since it was stated that subcutaneous adipose tissue increased due to obesity and percutaneous endoscopic gastrostomy could not be done, his calorie intake was reduced to 40 kcal/day in the follow-up.

Lipids have an essential role in the normal functioning of neurons and the structural development of the brain. Neurodegenerative diseases involve dysregulated lipid metabolism. In the central nervous system (CNS), most of the myelin's lipids are synthesized by oligodendrocytes. Pelizaeus-Merzbacher disease (PMD) is a fatal, untreatable, hypomyelinating leukodystrophy. In PMD, brain lipid metabolism is often disrupted as a result of the X-linked myelin gene *PLP1* (proteolipid protein 1) duplication. Overexpression in the PLP gene induces endoplasmic reticulum stress, damaging the oligodendroglial trophic support on axons. Chronic injury leads to demyelination.[7] Unmyelinated axons need more energy to deliver the transmission, causing cells to increase mitochondrial activity to produce this required energy. Demyelination stimulates the inflammatory response, leading to increased reactive oxygen and nitrogen production.[18] It has been shown in vitro and in vivo that lipid supplementation increases myelination in hypomyelinating pathologies and thus supports repair.[19]

Consumption of a high-fat / low-carbohydrate KD causes the liver to form ketone bodies. In the brain, ketone bodies such as beta-hydroxybutyrate facilitate sterol synthesis, which is essential for myelin membrane growth.[19] Therefore, it has been questioned whether a KD that supports CNS lipid metabolism may be useful in hypomyelinating disease.[20,21] Unlike glucose, ketone bodies are directly metabolized by the mitochondria, entering the tricarboxylic acid and oxidative phosphorylation pathways. It was thought that feeding using the KD would provide direct support for demyelinating axons by providing mitochondrial integrity.[5] Bypassing the need for oligodendroglial support can correct axonal mitochondrial functions (and morphology) and correct energy deficits in the axons. It may even contribute to improved survival of mutant oligodendrocytes and improvement of PMD pathology. The KD may be considered a future treatment for myelin diseases. Its two critical therapeutic goals are to provide cholesterol for support of remyelinating oligodendrocytes and to provide ketone bodies for metabolic support of axons, and future clinical trials will reveal its feasibility.[5]

Since our patient also had leukodystrophy, our decision to use classic KD could have been more appropriate because this option creates more ketosis than the medium-chain triglyceride (MCT) KD, which is important for both seizure control and mitochondrial integrity in myelin diseases. As mentioned above, the effect of KDT in hypomyelinating diseases is not directly caused by an increase in lipids, but indirectly by an increase in ketosis and an improvement in mitochondrial functions.

In conclusion, we emphasize that KDT should be considered as a treatment option in this rare leukodystrophy for drug resistant seizures.

Ethical approval

A written consent form was obtained from the parents for publication purposes.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: AÜ,

PK, YG; data collection: AÜ, MK, ÜY; analysis and interpretation of results: AÜ, YG, PK; draft manuscript preparation: AÜ, YG. All authors reviewed the results and approved the final version of the manuscript.

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Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

A pediatric bithalamic high grade glioma with concomitant H3K27M and EGFR mutations

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ABSTRACT

Background. Despite many treatment approaches, survival rates in high grade glial tumors are still not at the desired level. One of the cause of this failure might be that although having similar histologic features, they may display different biological behaviors depending on molecular heterogeneity.

Case. A 10-year-old girl presented with sudden onset left sided hemiparesis, headache, and ataxia. Physical examination was normal except for left sided hemiparesis and ataxia. A hyperintense mass lesion involving the bilateral thalamus was detected in the axial T2-weighted and coronal FLAIR sequences on brain MRI. There was no enhancement in axial T1-weighted contrast-enhanced sequences. Due to the size and location of the tumor, the patient was considered inoperable. Intensity modulated radiotherapy was intended for curative treatment to the patient because the radiological findings suggested a low-grade glial tumor. Tumor was unresponsive to radiotherapy but biopsy could be performed. The histopathological examination revealed a diffuse glial tumor with increased cellularity, mild nuclear atypia and rare mitosis. Due to the infiltrative pattern of the tumor, it was accepted as a high grade diffuse glial tumor. A chemotherapy protocol including cisplatin and etoposide in the first cycle, vincristine and cyclophosphamide in the second cycle, and carboplatin and vincristine in the third cycle were instituted to the patient. After the third cycle of chemotherapy, the tumor progressed radiologically. H3.1 K27M c.83A>T (HIST1H3C p.Lys28Met), ATRX c.2169_2170del (p.Glu723AspfsTer9), TP53 c.338T>C (p.Phe113Ser), and EGFR c.2300_2308dup (p.Ala767_va1769dup) were detected in the genetic assessment of tumor tissue. The patient's treatment was changed to vincristine, temozolomide, and irinotecan. Unfortunately, MRI showed progression after three cycles of second-line chemotherapy. The patient's family refused any further treatment, and the patient died with progressive disease in a short time.

Conclusions. EGFR mutation along with H3.1 K27M mutation is extremely rare in children to our knowledge. It should be kept in mind that if there is a possibility of targeted therapy, there may be a treatment option in this malignant disease with a poor prognosis.

Key words: children, bithalamic high grade glioma, H3K27M mutation, EGFR mutation.

Astrocytomas constitute an important part of childhood central nervous system tumors. High grade glial (HGG) tumors (Grades III and IV) constitute approximately 44.5 % of astrocytomas and 10% of all childhood CNS cancers.[1-5] Despite many treatment approaches, survival rates in HGG are still not at the desired level. One of the possible causes of this failure is that although having similar histologic features, they may display different biological behaviors depending on molecular heterogeneity. In recent years, the biological behavior of these tumors has been better understood with the advances in molecular biology.[6,7]

In adult patients with HGG telomerase reverse transcriptase promotor (TERTp) mutation, epidermal growth factor receptor (*EGFR*) amplification, chromosome7q gain, and 10q

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loss can be seen, while platelet-derived growth factor receptor alpha (*PDGFRA*) amplification and activation of the PI3-kinase/Akt/mTOR pathway are more common in children.[8-12] The mutations such as in the *H3F3A*/*ATRX*/*DAXX* pathway, *H3.3K27M, H3.3 G34R/V* can be detected in children. PDGFRA, MYCN or EGFR amplifications, although extremely rare, can be seen.[13-16]

Herein, we present EGFR c.787A>c mutation in a child with H3K27M-mutant diffuse midline glioma.

Case Report

A ten-year-old girl presented to the pediatric emergency department with sudden onset left sided hemiparesis, headache, and ataxia. The patient's medical and family histories were unremarkable. Physical examination was normal except for left sided hemiparesis and ataxia. A hyperintense mass lesion involving bilateral thalamus was detected in the axial T2-weighted and coronal FLAIR sequences on brain MRI. There was no enhancement in axial T1-weighted contrast-enhanced sequences (Fig. 1 a-c).

Due to the size and location of the tumor, the patient was considered inoperable. Intensity modulated radiotherapy was intended for curative treatment to the patient because the radiological findings suggested a low-grade glial tumor. The performed dose for radiotherapy was 50 Gy, 25 fractions, 5 fractions a week. After radiotherapy, only biopsy could be performed because of the unresponsive disease. The microscopic examination revealed a diffuse glial tumor with increased cellularity mild nuclear atypia and rare mitosis (Fig. 2). No vascular endothelial proliferation or necrosis were identified. Due to the infiltrative pattern of the tumor, it was accepted as a high grade diffuse glial tumor. A chemotherapy protocol including cisplatin (20 mg/m^2/day, days 1-5) and etoposide (50 mg/m^2/day, days 1-5) in the first cycle, vincristine (1.5 mg/m^2/day, days 1) and cyclophosphamide (900 mg/m^2/day, days 1-2) in the second cycle, and carboplatin (150 mg/m^2/day, days 1 and 15) and vincristine (1.5 mg/m^2/day, days 1 and 15) in the third cycle were given to the patient. After the third cycle chemotherapy, the mass was found to have progressed radiologically.

Thereafter, the tumor was screened for molecular alterations by targeted next generation deep DNA and RNA sequencing using Acibadem Molecular Pathology Customized Brain Tumors Panel (Archerdx Fusionplex and Variantplex via Miniseq Sequencing System, Illumina).

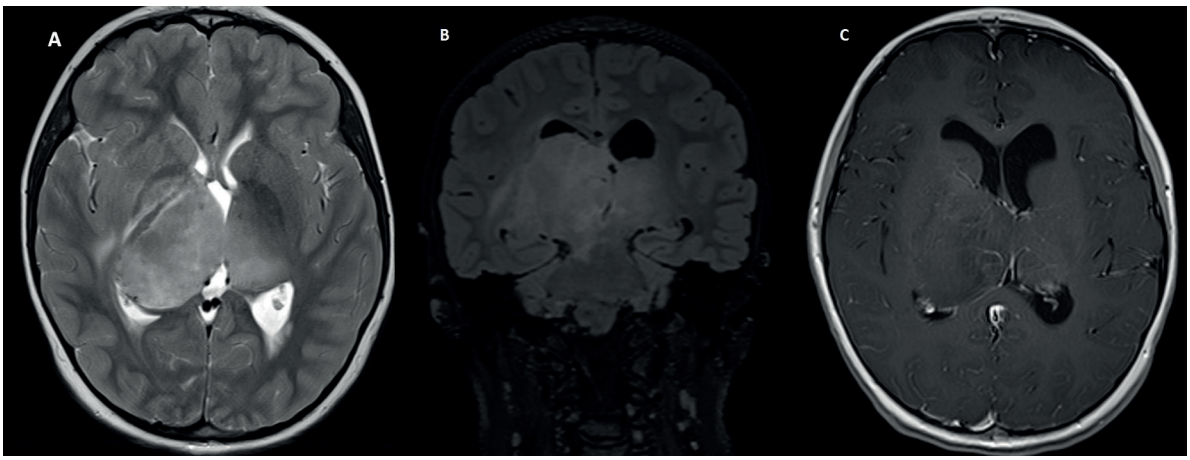

Fig. 1 a-c. Axial T2-weighted and coronal FLAIR image shows hyperintensity and diffuse enlargement of the bilateral thalamus (A, B). Axial T1-weighted gadolinium enhanced image showed low signal intensity in both thalamus and no associated contrast enhancement (C).

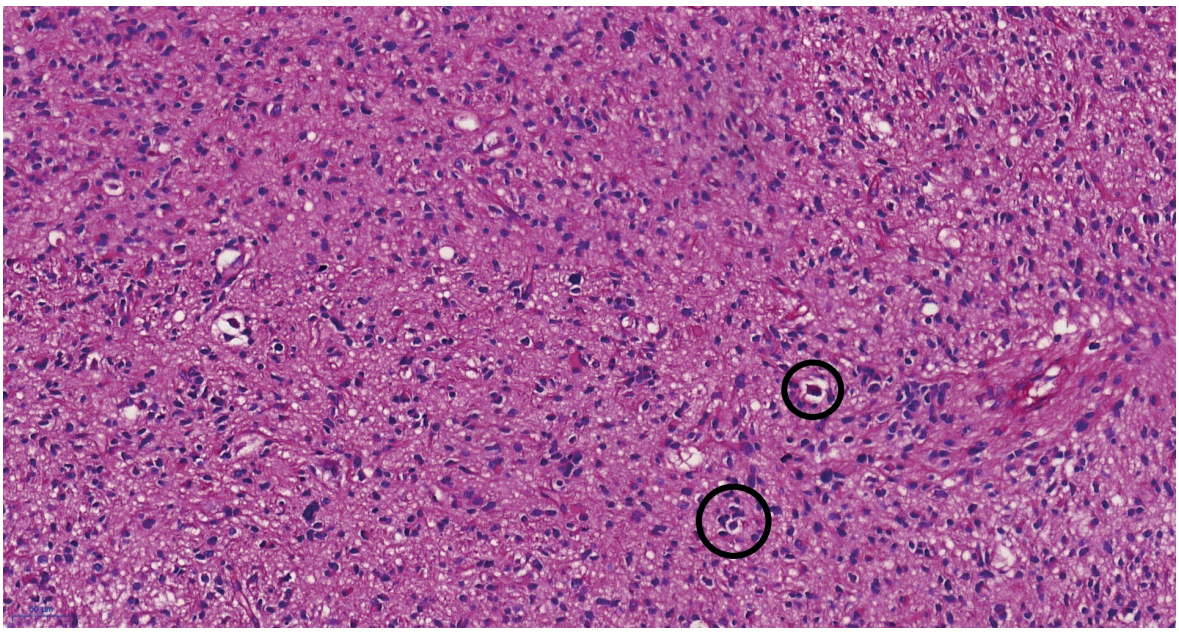

Fig 2. The tumor was composed of glial cells with mild atypia. The entrapped non-neoplastic neurons are seen (circle) which is an evidence of the diffuse infiltrative pattern. (20X, H&E)

H3.1 K27M c.83A>T (HIST1H3C p.Lys28Met), ATRX c.2169_2170del (p.Glu723AspfsTer9), TP53 c.338T>C (p.Phe113Ser), and EGFR c.2300_2308dup (p.Ala767_va1769dup) were detected in the genetic assessment of tumor tissue.

The patient's treatment was changed to vincristine (1.5 mg/m^2, on day 1), temozolomide (150 mg/m^2/day, on days 1-5), and irinotecan (50 mg/m^2/day, on days 1-5). Unfortunately, MRI showed progression after three cycles of second-line chemotherapy. Due to progressive disease the patient's family refused treatment and the patient died in a short time.

Written consent for publication of this case report and accompanying images were obtained from the parents of the patient.

Discussion

High grade glial tumors constitute approximately 10% of all childhood CNS cancers.[4] The biology of childhood HGG tumors has not been exactly explained. However, our knowledge about the biology of childhood HGG tumors is increasing, especially in line with the developments in molecular biology in recent years.[1-4,15] Another problem in childhood HGG tumors is that the desired treatment outcomes have not been achieved yet. Poor outcome and failure to achieve the desired success in treatment increases the interest in investigating the biological behavior of childhood HGG tumors.[15,17,18] Herein, our aims are to present a case of *EGFR c.787A>c* mutation in a child with *H3K27M*-mutant diffuse midline glioma and discuss in light of the literature.

In children with HGG tumors, the failure of treatment success is thought to be related with the heterogeneity in biological behavior of the tumor. The well-known genetic alterations seen in these children are *PDGFRA* amplification, +1q, and the mutations in the *H3F3A*/*ATRX*/*DAXX* pathway.[6-12] It is known that *H3.3K27M* or *H3.1K27M* mutations in patients with midline and pons HGG tumors, and *H3.3 G34R/V*, *IDH* genes or *BRAF^{V600E}* mutations in patients with hemispheric HGG tumors are more common.[13-18] Also, *PDGFRA*, *MYCN* or *EGFR* amplifications can be detected extremely rarely in patients with hemispheric HGG.[13-18] *H3K27M*,

ATRX, *IDH1*, *BRAF*^{V600E}, and *p53* genes were investigated in children diagnosed with HGG tumors with *H3K27M* in Turkey.[16] Overlapping mutations *ATRX* loss and *p53* were detected. Rarely *BRAF*^{V600E} mutation, but no *IDH1* mutation has been detected. In this study, no statistical difference was found between HGG tumor patients, *H3K27M*-mutated patients and wild-type *H3K27M* tumor patients in terms of overall survival rates.[16]

Until the study of Mondal et al.[14], no mutations in EGFR exon 20 were reported in children.[19] In this study, 13 children with bithalamic HGG tumors were re-investigated. Among these patients, *EGFR* mutation was detected in 11 cases. Nine of the patients with *EGFR* mutations were on exon 20, which is the area associated with intracellular tyrosine kinase. In the other two patients, mutation was detected on exon 7. Two cases in their series (15%) harbored H3 K27M mutations along with EGFR alteration. Sievers et al.[13] also investigated pediatric thalamic gliomas for EGFR mutations. Twenty of the genetically evaluated 30 patients demonstrated EGFR alterations, with 15 showing missense mutations and 5 showing in frame insertions. Eight of the 30 tumors (27%) harbored an H3.1 or H3.3 K27M mutation (6 of them with a concomitant EGFR alteration).

Our case has similar features with the cases published in two above mentioned studies. Our patient is a pediatric case, with a bithalamic glioma, harboring EGFR mutation which is an in frame insertion in exon 20. Yet the rare feature seen in both studies; a concomitant H3 K27M mutation was also present in our case.

In the cell-line portion of the study by Mondal et al.[14], it was found that tyrosine kinase inhibition decreased the viability of the astrocytes expressing mutant *EGFR* isoforms. In the light of this data, the authors administered different tyrosine kinase inhibitors to four of their patients and found a slowdown in tumor growth in their patients. However, the important finding is that the median survival time (21.5 months) of the patients in whom tyrosine kinase inhibitor was added to their treatment was higher than the patients whose treatment did not contain tyrosine kinase inhibitors (13 months).

In our patient, both platinum-based chemotherapy scheme and vincristine + irinotecan + temozolomide treatment did not prevent progression. The addition of a tyrosine kinase inhibitor was considered as reported in the study of Mondal et al.[14], but could not be given because the family refused the treatment.

In conclusion, the recent studies by Mondal et al.[14] and Sievers et al.[13] demonstrated that pediatric tumors located in thalamus, mainly bilaterally, frequently harbor EGFR mutations and reveal a distinct methylation profile which makes them a distinct subtype of pediatric thalamic HGG. We believe our case also belong to these rare pediatric thalamic HGGs with EGFR mutation and also has a concomitant H3.1 K27M mutation, which was rarely shown in this distinct tumor group. It is important to note that molecular biological studies should be performed in children with HGG tumors, especially with bithalamic locations. In the light of these findings, it should be kept in mind that if there is a possibility of targeted therapy, there may be a treatment option in this malignant disease with a poor prognosis.

Ethical approval

Written consent for publication of this case report and accompanying images were obtained from the parents of the patient.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: YK, BK, AED, MÖ; data collection: BK, AED, KE; analysis and interpretation of results: YK, BK, AED; draft manuscript preparation: YK, BK, AED, MÖ, KE. 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

The challenge to prove a rare cause of secondary arterial hypertension. A case report of a pediatric renal solitary fibrous tumor

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ABSTRACT

Background. Childhood hypertension is getting more attention in recent years. We present a case report of a rare cause of secondary arterial hypertension in a teenage girl - a solitary fibrous tumor of the kidney. The case demonstrates that standard imaging techniques, computed tomography and magnetic resonance imaging, are not fully reliable in the diagnosis of renovascular hypertension.

Case. A 15-year old girl was admitted to the Pediatric Department because of episodes of stiffness in the limbs, accompanied by pale skin and lips, dated 4 months back. During these episodes, high blood pressure up to 160/100 mmHg was measured. A 24-hour blood pressure monitoring demonstrated arterial hypertension stage II. Renovascular hypertension was suspected, but the computed tomography examination of the abdomen showed normal-sized renal arteries. In the left kidney hilum, an intraparenchymal formation was discovered. The data presented a non-specific lesion with a wide differential diagnosis. Given the fact that the patient had been treated with an ACE-inhibitor, serum renin level could not be correctly interpreted. The lesion was removed through a laparoscopic intervention. Intraoperatively, the tumor was compressing a small intra-renal vessel - a finding that hadn't been discovered by the previous imaging studies. The final pathologist diagnosis was: solitary fibrous tumor. During the next six months of follow-up, the maximal blood pressure values of the patient were up to 120/80 mmHg.

Conclusions. Solitary fibrous tumors of the kidneys are infrequent in children. The presented case displays a rare form of initial clinical manifestation of this tumor. It is also a demonstration that standard imaging techniques are not able to get a precise visualization of the small intra-renal vessels. At the same time, the decision of whether or not to perform a more invasive procedure should be based on the clinical conditions and risks of the individual patient.

Key words: hypertension, renal solitary fibrous tumor.

The current definition of hypertension (HTN) in children includes systolic and/or diastolic blood pressure above the 95th percentile for age, sex, and height. Children with values between the 90th and 95th percentile are classified as having high - normal blood pressure (BP).[1] Arterial hypertension is divided into primary, or essential, and secondary, in which an underlying disease is present. It is well-known, that primary hypertension is more common in adolescents, with the majority of those affected being overweight and/or with a family history of being overweight.[2] Secondary hypertension is considered more common among younger children, especially in those under 6 years of age.[3,4] The smaller the child, and the more severe and rapid the rise in the BP, the more likely they have secondary hypertension. However, recent reports demonstrate that the incidence of

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secondary hypertension in adolescents is not to be neglected.[3,4]

We present a case report of a rare cause of secondary hypertension in a teenage girl, which demonstrates that standard imaging techniques, computed tomography (CT) and magnetic resonance imaging (MRI), are not fully reliable in the diagnosis of renovascular hypertension due to the inability of incomplete visualization of small vessels.

Case Report

A 15-year-old girl was admitted to the Pediatric Department because of episodes of stiffness in the limbs, accompanied by pale skin and lips. During these episodes, high BP up to 160/100 mmHg was measured. The family history revealed that the grandfather (paternal) had arterial hypertension and the mother suffered from nephrolithiasis. Her complaints dated 4 months back and were accompanied by severe occipital and frontal headaches. Repeatedly, higher values of BP had been measured, up to 160/120 mmHg. Blood pressure remained high (140/80 mmHg) in between these periods. Angiotensin receptor blocker therapy was started but due to a lack of efficacy, the therapy was cancelled after a month. Subsequently, a combined medication was prescribed [angiotensin-converting enzyme inhibitor (ACEi) and calcium channel blocker (CCB)].

Upon admission, the patient was in satisfactory condition. Her stature and body mass index (BMI) for age and sex were normal (BMI 18.8 kg/m^2). The cardiac evaluation showed a rhythmic heart rate (95 bpm) and clear heart sounds. BP was 140/95 mmHg. Her pulse was well palpable, symmetrical on the upper and lower extremities. As pointed above, the current treatment was with a combined antihypertensive drug (ACEi + CCB).

The following examinations were performed:

- Complete blood count (CBC), renal function tests (BUN, creatinine, uric acid), liver enzymes (AST, ALT), and electrolytes - all within the reference values.
- Thyroid function (FT4 15.4 pmol/L, TSH 1.73 uIU/ml) - all within the reference values
- Serum cortisol levels and cortisol circadian rhythm were also in the normal range. 24-hour urine cortisol test showed elevated values: 306.80 μg/24h and 344.30 μg/24h (normal range up to 286 μg/24h). In this regard, the ACTH was repeatedly examined and showed normal values.
- Urinalysis - clear urine sample; protein/creatinine ratio was 8.98 mg/mmol (normal range up to 7.9 mg/mmol).
- Electrocardiogram and echocardiography - no abnormalities.

Given the treatment with an ACEi, serum renin and aldosterone levels could not be correctly evaluated.

A 24-hour blood pressure monitoring demonstrated arterial hypertension stage II. The mean daily BP values were 141.7/99.1 mmHg, night BP values were 117/80.6 mmHg, as 39.7% of the measured systolic values were above 140 mmHg. Maximal BP was 165/16 mmHg.

Renovascular hypertension was suspected, and a computed tomography (CT) examination of the abdomen was performed. The results showed normal-sized renal arteries, normal adrenal glands, and right kidney. In the left kidney hilum, an intraparenchymal mass was discovered. The tumor was oval-shaped, with smooth borders, sized 11/11 mm, located in the middle to the lower third of the parenchyma, projecting slightly to the hilum (Fig. 1). Such a lesion implied a broad differential diagnosis, so a magnetic resonance imaging (MRI) was performed. The tumor was iso- to slightly hypointense in comparison to the renal parenchyma, and after contrast injection, remained hypocontrasted to the rest of the parenchyma. The data presented a non-specific lesion with a wide differential diagnosis and included a renal cell carcinoma or another solid tumor (Fig. 2).

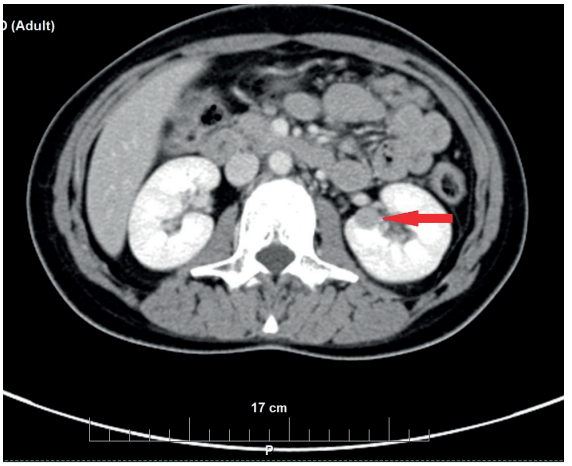

Fig. 1. Venous phase-contrast enhancement abdominal computed tomography scan showing a small homogenous well-defined hypovascular cortical neoplasm (marked by arrow).

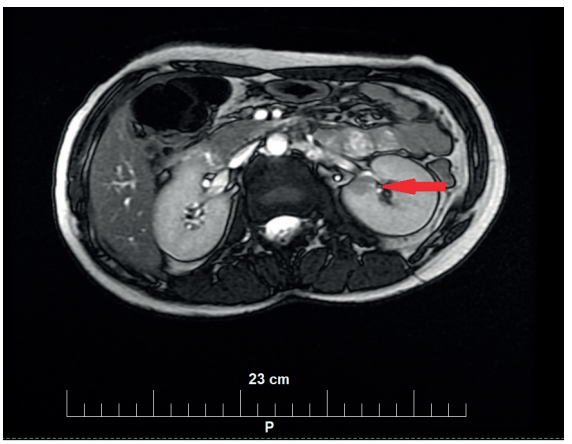

Fig. 2. Magnetic resonance imaging revealed a hypointense (on T2 images) well-defined lesion in the left kidney hilum (marked by arrow). No signal intensity changes found on the gadolinium-enhanced images, slightly increased signal intensity on DWI, slightly decreased signal intensity on ADC map (not presented).

A decision to act immediately was taken, and a laparoscopic intervention was performed to remove the lesion. Intraoperatively, the tumor was well-circumscribed with a grey color, compressing a small intra-renal vessel - a finding that hadn't been discovered by the previous imaging studies. A second smaller satellite nodule with the same morphology was also found nearby and removed together with the larger one.

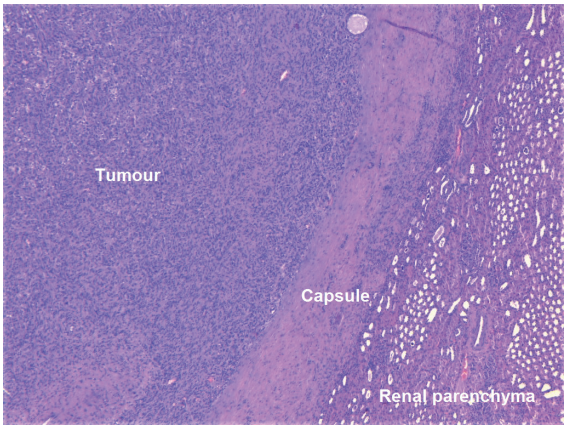

Fig. 3. Tumor biopsy (H&E, x40) showing a well-demarcated tumor with a thick fibrous capsule, partially surrounded by renal tissue.

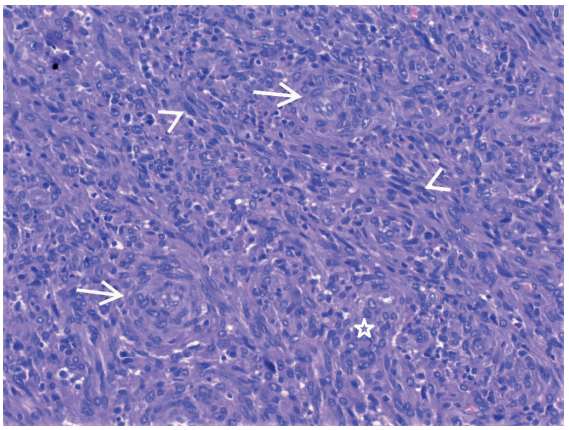

Fig. 4. Tumor biopsy (H&E, x200) showing. a tumor composed of intersecting bundles (arrowheads) and whorls (arrows) of spindle and round cells (star) without nuclear atypia and mitotic activity.

Histology revealed a well-demarcated, round tumor with a thick fibrous capsule, partially surrounded by renal tissue (Fig. 3), composed of intersecting bundles and whorls of spindle and round cells without nuclear atypia and mitotic activity (Fig. 4). Immunohistochemistry showed diffuse positivity for CD34 (Fig. 5), focal for CD99 and bcl2, sparse single S-100 positive cells, negative CK AE1/3, Ki-67 in less

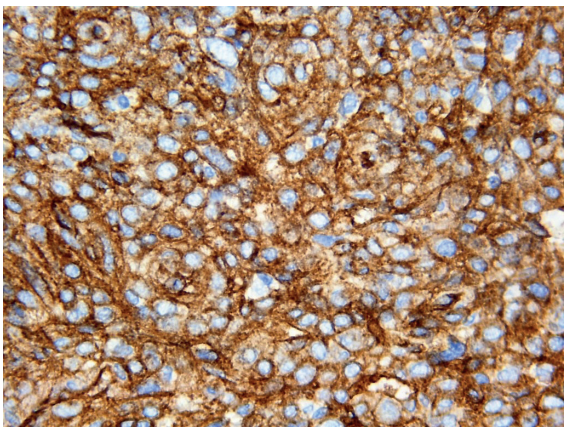

Fig. 5. Tumor biopsy with diffuse positivity for CD34 stating (x400).

than 10% of the nuclei. The smaller tumor had the same characteristics. The surrounding renal parenchyma appeared normal.

The differential diagnosis was required to rule out a renin-producing juxtaglomerular tumor that had a similar histomorphology. Since the juxtaglomerular cells have a smooth muscle cell origin, they show SMA expression. In our case, SMA marked only stromal vessel walls, and the tumor cells were SMA negative. The final pathologist's diagnosis was: solitary fibrous tumor.

The postoperative period went smoothly with rapid normalization of blood pressure values on the 1st postoperative day, with the possibility of a quick withdrawal of antihypertensive treatment. During the next six months of follow-up, the patient was feeling fine, with maximal BP values up to 120/80 mmHg.

Written informed consent for publication of the patient's clinical details and/or clinical images was obtained from the parent of the patient.

Discussion

Due to the patient's specific clinical presentation, a secondary renovascular HTN was suspected. The challenge was in the results of the imaging studies - CT and MRI - renal arteries without stenosis, and the presence of a tumor in one of the kidneys.

Renal pathology (renoparenchymal and renovascular) is the leading cause of secondary HTN in all age groups of the pediatric population and accounts for up to 80% of cases.[3,5,6] Glomerulonephritis causes secondary HTN in 42% of patients.[3] Reflux nephropathy ranks second. Frequent urinary tract infections and subsequent chronic kidney injury are considered major risk factors for the development of HTN.[7] In our patient, in particular, there were neither clinical nor laboratory markers for renal parenchymal disease.

Renovascular disease causes 6 to 10% of all childhood hypertension.[5,6,8] Renal artery stenosis accounts for up to 5% of all cases of secondary HTN, and fibromuscular dysplasia is considered the most common cause of renal artery stenosis in children.[3,6] Renal artery stenosis can be isolated (unilateral, bilateral, stenosis of a small intra-renal vessel), or can affect the abdominal aorta, as well.[9] Renovascular HTN should be considered in every single child with high-grade HTN, symptomatic HTN (presented with hypertensive encephalopathy, cardiac failure), and difficult to control HTN (with more than two antihypertensive drugs). This was the case in the presented patient, in whom a renovascular HTN or hormone-secreting tumor was discussed. However, the conducted imaging studies, CT and MRI, showed normal diameters of renal arteries and didn't confirm stenosis.

A CT scan with contrast demonstrates a high percentage of sensitivity and specificity for renal artery stenosis in children.[10] It is not yet fully understood how reliable the information is about small renal vessels in cases of suspected renovascular HTN.[8,10] MRI examinations also have some limitations concerning the diagnosis of renovascular HTN, mainly due to an uncooperative patient who won't hold their breath.[8,11] Conventional angiography remains the gold standard for a detailed

evaluation of small renal vessels. It provides precise information not only about the lumen of the renal arteries but also about their small branches. On the other hand, despite the obvious advantages of this imaging technique, it is an invasive procedure that carries risks. The main disadvantage is considered to be the higher levels of radiation in comparison to a properly conducted CT scan, and the risk of vascular damage.[8] In our patient, the information from this procedure would not have influenced the therapeutic decision since the tumor formation was subject to removal.

Given the presence of a tumor in the kidney that could potentially press a renal vessel or produce renin, the next step in the diagnostic process would be serum renin evaluation. Neuroblastoma, Wilms tumor, hemangiopericytoma, and other different types of tumors could press a renal vessel with a subsequence of kidney hypoperfusion. This could be verified through the evaluation of serum levels of renin and aldosterone. Although extremely rare (100 cases were reported in the literature), the juxtaglomerular cell tumor is capable of producing renin to a high concentration and presents clinically with moderate to severe HTN and symptoms such as headache, dizziness, and nausea.[12] Furthermore, there was an obvious macroscopic resemblance between the juxtaglomerular tumor and the tumor in our patient - well-circumscribed formation with a fibrous capsule and a grey-whitish color. Given the fact that the patient had been treated with an ACEi, serum renin level could not be correctly interpreted. Following the oral administration of an ACEi, plasma renin levels increase several times due to the feedback mechanism that exists between renin and angiotensin II.[13]

The most common endocrine disorders that cause secondary HTN are catecholamine secreting tumors (pheochromocytoma and paraganglioma), Cushing syndrome, hyperthyroidism, primary hyperaldosteronism, and congenital adrenal hyperplasia. Arterial HTN, palpitation, and headache are the most common initial clinical manifestations of catecholamine-secreting tumors, due to their ability to secrete adrenalin, noradrenalin, and dopamine in high concentrations in the serum.[14] In our patient, a 24-hour urine test for catecholamine wasn't performed because of the persistent manner of the HTN and the lack of a tumor in the adrenal glands or other parts of the sympathetic/parasympathetic nervous system. Besides, there was clear evidence of a tumor located in the left kidney, which was considered to be the potential cause of renovascular hypertension.

HTN is one of the main clinical manifestations of Cushing syndrome in up to 47% of cases.[15,16] Elevated 24-h urine cortisol in our patient didn't match with any clinical criteria such as stunted growth, pubertal arrest, visceral obesity, etc. Laboratory investigation also didn't reveal any other signs of increased glucocorticoid secretion. That is why increased urinary cortisol was considered more as a secondary event.

The histology result showed one very rare tumor in childhood: a solitary fibrous tumor of the kidney. This is a spindle cell neoplasm with a mesenchymal origin, firstly described in the pleura.[17] An extrapulmonary location has also been described, yet kidney origin represents one of the rarest locations. Typically manifested in adulthood, solitary fibrous tumors of the kidneys are extremely rare in children.[17,18] As far as we know, there are only a few pediatric cases described in the literature.[18-20] The current case is the only one clinically manifested with secondary arterial hypertension. The clinical presentation varies from an accidental finding to a palpable abdominal mass, abdominal pain, gross hematuria, and intermittent hypoglycemia.[17,21] The macroscopic appearance of the tumor shows a well-circumscribed mass, with a grey or tan-white surface. Histopathology resembles hemangiopericytoma. Differentiation between the solitary fibrous tumors and other spindle cell neoplasm is based on immunohistochemistry: they show high positivity for CD34, CD99, and bcl-2. In most of the described cases, the course is benign with no recurrences after removal.[18]

Malignancy occurs only rarely, but should not be neglected. A continuous follow-up is recommended.[21]

We think that the most probable cause for the high-grade HTN in the presented patient was the compression of a small renal vessel by the tumor, thus causing secondary renovascular HTN. As a shortcoming in the differential diagnosis, we take into account the fact that a conventional angiography wasn't performed. It would have given us more information regarding small vessels in advance. On the other hand, the procedure itself is not completely safe and carried some additional risks. To speed up the cure, our patient went directly to surgery. The assessment of whether to perform angiography or not should be based on the clinical condition of the individual patients and the impact of the resultant therapeutic behavior.

Childhood HTN is getting more and more attention in recent years. With the increase of obesity in the pediatric population, cases of essential hypertension increase proportionally, especially among adolescents. Nevertheless, secondary hypertension remains a diagnostic and therapeutic challenge.

In summary, renovascular HTN should be considered in every single child with high-grade HTN, symptomatic HTN (presented with hypertensive encephalopathy, cardiac failure), and difficult to control HTN (with more than two antihypertensive drugs). A clinician should keep in mind that standard imaging techniques, CT and MRI, are not fully reliable in the diagnosis of renovascular HTN, due to the inability of complete visualization of small vessels.

Ethical approval

Written informed consent was obtained from the parent of the patient for publication of the patient's clinical details and clinical images.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: KG, PS, VI; data collection: KG; analysis and interpretation of results: KG, PS, VI, BB; draft manuscript preparation: KG, VI. 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

Sirolimus treatment of a PTEN hamartoma tumor syndrome presenting with melena

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ABSTRACT

Background. PTEN hamartoma tumor syndrome (PHTS) is an umbrella term including Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS), PTEN-related Proteus syndrome (PS), and PTEN-related Proteus-like syndrome. One of the disorders in PHTS spectrum, CS is characterized by macrocephaly, mucocutaneous findings, gastrointestinal system (GIS) polyposis and an increased lifetime risk of GIS, breast, thyroid and other cancers.

Case. In this study, we report an adolescent patient presenting with recurrent life-threatening upper GIS bleeding as a result of hamartomatous polyposis. Genetic studies revealed a known pathogenic nonsense mutation confirming the initial diagnosis of CS.

Conclusions. Additionally, we describe our therapeutic intervention to improve the patient's clinical symptoms with sirolimus, which its use is infrequently addressed in the literature for pediatric age group harboring PTEN mutations.

Key words: PTEN hamartoma tumor syndrome, gastrointestinal system bleeding, PTEN mutation, sirolimus.

PTEN hamartoma tumor syndrome (PHTS) is an umbrella term including cancer predisposition and overgrowth syndromes such as Cowden syndrome (CS), Bannayan-Riley-Ruvalcaba syndrome (BRRS), *PTEN*-related Proteus syndrome (PS) and *PTEN*-related Proteus-like syndrome.[1] Cowden Syndrome (OMIM 158350) is the first of the PHTS spectrum disorders to be associated with autosomal dominantly inherited germline "Phosphatase and Tensin Homologous) *PTEN* mutations.[2-4] Syndrome is characterized by macrocephaly, mucocutaneous findings such as trichilemmomas, papillomatous papules, acral keratosis and penile freckling, multiple hamartomas in various tissues including gastrointestinal system (GIS) polyposis and an increased lifetime risk for benign and malignant tumors of breast, thyroid, genitourinary and GIS.[2,5-7] Polyps are predominantly hamartomatous, but mixed histologic types including hyperplastic, inflammatory and adenomatous types could also be seen.[1] Polyps may be asymptomatic or complicated by bleeding, obstruction, invagination, and infarction.[8] The prevalence of CS in the general population is thought to be 1 in 200,000, and although the cases are usually diagnosed in the third decade, there are rare cases in early childhood.[8,9]

PTEN is a lipid phosphatase, a negative regulator of the phosphatidylinositol-3-kinase (PI3K) / protein kinase B (AKT)/ the mammalian target of rapamycin (mTOR) signaling pathway and plays an active role in cell cycle and apoptosis

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by controlling phosphoinositol triphosphate levels.[3] Loss of function of this tumor suppressor gene leads to inappropriate activation of the PI3K/AKT /mTOR pathway, uncontrolled cell growth and proliferation causing benign and malignant tumor formations.[3,10] In recent years, successful outcomes have been reported in the treatment of benign and malignant tumors by targeting the inhibition of PTEN-related PI3K/AKT/mTOR pathway with an mTOR inhibitor, sirolimus.[11,12] Here, we report an adolescent CS patient presenting with life-threatening recurrent upper GIS bleeding. Additionally, we describe our therapeutic intervention to improve the patient's clinical symptoms with sirolimus, which its use is infrequently addressed in the literature for pediatric age group with PTEN mutations.

Case Report

A fourteen-year-old male patient with melena was referred to our hospital for the evaluation of GIS bleeding. From his medical history, it was learned that he had a stomach ache for the last two months, and malaise and darkening of the stool color for the last week.

His parents were first-degree cousins and he had no further significant finding in his family history. On his physical examination, body weight, height and head circumference were compatible with 25p, 10p, and >97 p, respectively. His skin and mucous membranes were pale. He had multiple skin-colored soft papules on his face, axillary region, upper extremity and back (Fig. 1a). Additionally, a hyper-pigmented macule with irregular edges in the glans penis was observed (Fig. 1b). Other system findings were normal. His laboratory examinations revealed iron deficiency anemia; Hgb 7.6 g/dL (9.5-13.3 g/dL), serum iron 24 μg/dL (45-182 μg/dL), unsaturated iron binding capacity 358 μg/dL (155-300 μg/dL), ferritin 9 ng/mL (23-70 ng/ mL), white blood cell count 11.7 $x10^3$/μL, platelet count 339 $x10^3$/μL. In his upper GIS endoscopic examination, locally eroded, ulcerated and pedunculated or non-pedunculated polyps with diameters ranging from 0.3-2 cm, localized mostly in the antrum-corpus junction and antrum were observed (Fig. 1c). Colonoscopy showed a decreasing number of polyps from rectum to sigmoid and no polyps were observed in proximal segments of colon. Histopathologic examination demonstrated that the polyps located in the stomach were compatible with mix histology consisting of both hyperplastic and hamartomatous types, and those in the colon were hyperplastic polyps (Fig. 1d).

Genetic studies were performed after obtaining written informed consent from the patient's parent. Genomic DNA of peripheral blood leukocytes was extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Libraries were prepared using a capture-based target enrichment kit (Hereditary Cancer Solution™, Sophia genetics, Switzerland) containing 27 genes (Supp. File: *ATM, APC, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, FAM175A, MLH 1, MRE11A, MSH2, MSH6, MUTYH, NBN, PALB2, PIK3CA, PMS2, PMS2CL, PTEN, RA D50, RAD51C, RAD51D, STK11, TP53* and *XRCC2*). Next-generation sequencing (NGS) was performed on an Illumina MiSeq System (Illumina, USA). After sequencing, data were analyzed using Sophia DDM software v.5.7.7. (Sophia Genetics, Switzerland). Analysis revealed a known heterozygous nonsense variant c.388C>T; p.Arg130Ter in the 5th exon of *PTEN* (NM_000314). No pathogenic variants were found in APC and MUTYH responsible for different polyposis syndromes. The identified variant in the patient was not observed in his mother. Segregation of the variant could not be done for the father, as he could not be reached.

Ultrasonography of the thyroid, abdomen and scrotum were normal. Cranial magnetic resonance imaging for the presence of AV-malformations was also normal. Upper GIS bleeding was treated with proton pump inhibitors (PPIs) and octreotide infusion therapy. Occult blood positivity in the stool continued, although his major symptoms

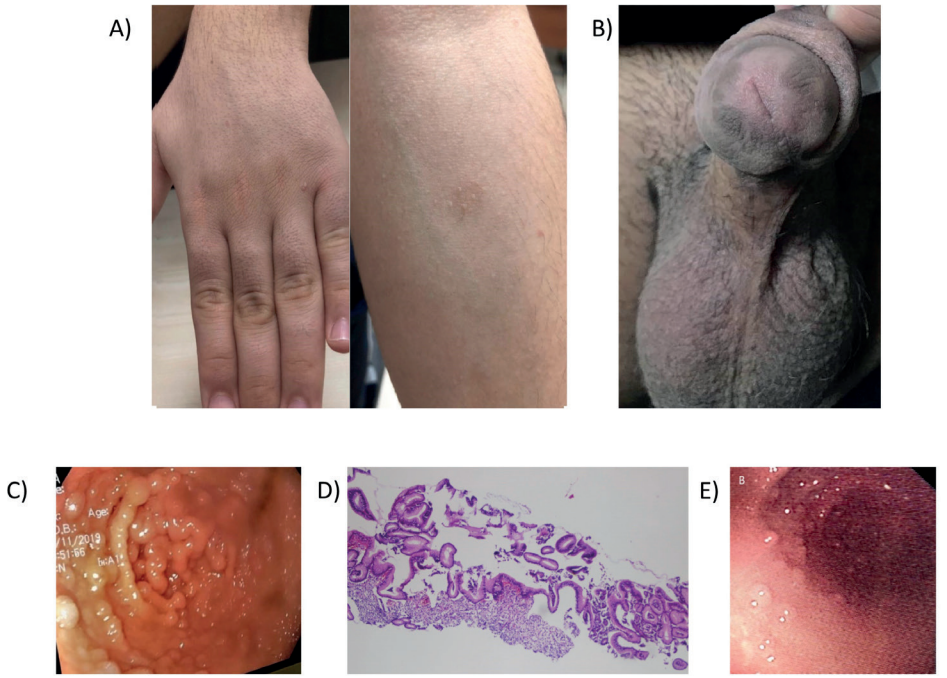

Fig. 1. A) Multiple skin-colored soft papules on the upper extremity. **B)** Hyper pigmented macule with irregular edges in glans penis. **C)** Upper gastrointestinal system endoscopic examination demonstrating locally eroded, ulcerated and pedunculated or non-pedunculated polyps with diameters ranging from 0.3-2 cm, localized in the entire gastric mucosa. **D)** Gastric hyperplastic polyp with visible elongated foveolar epithelium (Hematoxylin and eosin stain, ×20 magnification.) **E)** Significant decrease in the number of polyps and size of the polyps in the third month of treatment.

regressed. Sirolimus treatment was planned in the patient whose macroscopic bleeding recurred twice during the follow-up, as conventional treatments were insufficient. Sirolimus treatment was initiated at a 0.8 mg/m² dosage and was administered per oral twice daily. During the treatment, complete blood cell count, hepatic and renal function tests, lipid profile and serum levels of sirolimus were monitored regularly. Serum sirolimus levels were between 6-9 ng/ml during the follow-up. The upper and lower endoscopic examinations were repeated in the third month of the treatment. There was a significant decrease in the number and size of the polyps (Fig. 1e). He continued on a therapeutic dose of sirolimus for a total of 6 months without any major adverse effects. He had only transient mild hypercholesterolemia. Sirolimus was tapered down in two months. The patient was followed for two years and GIS bleeding did not recur.

Discussion

We report an adolescent patient with PHTS whose clinical, laboratory and endoscopic findings were successfully treated with sirolimus. His clinical features (macrocephaly, wart-like papules on his skin and hyperpigmented penile macules) and hamartomatous GIS polyposis were compatible with the clinical diagnostic criteria for PTEN Hamartoma Tumor Syndrome based on the National Comprehensive Cancer Network (NCCN) Guidelines.[13] Clinical diagnosis is also confirmed with the identification of c.388>T p. (Arg130Ter) nonsense variant by molecular studies. PTEN protein consists of two important domains that are required for the tumor suppressor function. First domain is the phosphatase (catalytic) domain that is located between the amino acids 14 to 185, and the second is the C2 lipid membrane-binding

domain, which participates in membrane binding, placed in between the amino acids 190 and 350.[14] Exon 5 is a hotspot for germline mutations as it harbors the tyrosine phosphatase signature motif H123CKAGKGR130 and within this loop, the C124 and R130 residues are essential for the physiological function of PTEN in tumor suppression.[15]

Identified variant in the present patient is located in signature motif of the catalytic domain at the 130th position. This variant has been observed in several individuals affected with PHTS.[16] The truncating variant is expected to undergo nonsense-mediated decay, supporting haploinsufficiency as the cause of the clinical features of the present patient.

Main GIS findings of CS are non-neoplastic polyps. Although GIS polyps are predominantly of hamartomatous histopathology, hyperplastic or adenomatous polyps and mixed polyposis with malignancy risk are also reported.[17] Multiple gastric, ileal and colorectal polyps were signs of major GIS involvement in our patient. Although the polyps detected in the patient were of hyperplastic and hamartomatous histopathology, long-term follow-up was continued due to increased risk for the development of malignancies, especially in the colon and thyroid. Thus, detection of *PTEN* mutation changed the management of the patient not only in terms of follow-up but also in terms of treatment options.

Although polyposis is rare in the pediatric age group, it is diagnosed more frequently with the more widespread use of endoscopic diagnostic examinations.[18] Invasive procedures are often required for the treatment of polyps complicated by severe life-threatening bleeding and anemia. Although our patient's polyps were benign in nature, they caused severe and repetitive GIS bleeding, and profound anemia requiring aggressive transfusion. Polypectomy was not considered as there were too many polyps to be resected.

PTEN alterations result in an enhanced PI3K/AKT/mTOR signaling, causing an uncontrolled cell proliferation and suppression of this pathway represents a rational therapeutic target in PHTS.[10] In recent years significant success has been achieved in the treatment of PHTS patients with sirolimus, an mTOR inhibitor. Beneficial results using sirolimus were first demonstrated in 2008 in both experimental animal models and a 26-month-old male patient with *PTEN*-related Proteus syndrome (PS).[12,19] In this patient with PS, oral sirolimus treatment at a dose of 0,1 mg/kg/d for 2 months was effective in reducing the size of hamartomatous masses with prominent clinical improvement.[12] Three years later, another report involving a treatment attempt with sirolimus, demonstrated that a 6-year-old male patient with BRRS regained pain free full mobility as a result of a reduction in the size of vascular masses, with only minor side effects.[20] Clinical improvements reported in abovementioned studies provide a rationale for sirolimus therapy in patients with disorders in the PHTS spectrum. As a result of this rationale, trials have begun to demonstrate the efficacy of sirolimus for patients harboring *PTEN* mutations with complex vascular anomalies. The majority of studies reported full or partial response to sirolimus such as an amelioration in pain scale scores, an improvement in the appearance and the cutaneous discoloration of the vascular lesions and an increase in patients' performance and quality of life.[21-25] In addition to vascular anomalies, there is a growing number of cases in the literature reporting successful outcomes with sirolimus in various clinical presentations associated with *PTEN* mutations including oral hamartomatous lesions, thymus hyperplasia, abdominal lipomatosis, isolated infiltrative soft tissue

lesions, ganglioneuromatosis, Lhermitte Duclos disease and hypoinsulinemic hypoglycemia.[26-31] Moreover, mTOR inhibitors were shown to inhibit polyp formation and prolong the process of the development of dysplasia in mouse models.[32,33] Regarding the efficacy of sirolimus on GIS findings in patients, successful results in decreasing the number and size of polyps have been demonstrated not only for *PTEN* point mutations but also for intragenic *PTEN* deletions and larger deletions encompassing both *PTEN* and *BMRP1A*.[26,34-37] In another recent interventional study by Komiya et al.[11], a 56-day course of sirolimus treatment was well tolerated in patients with PHTS and was associated with some evidence of improvement in symptoms, skin and GI lesions, cerebellar function, and decreased mTOR signaling. Thus, mTOR inhibitor treatment was initiated in the present study, as a potent role of sirolimus was shown in several studies in patients with PHTS. Table I summarizes the comprehensive details of the main studies available in the literature to date.

During the treatment, hemoglobin and hematocrit values increased to reference values and significant decrease of the size and number of polyps was observed after a period of 6 months. Red blood cell transfusion was not needed during the 2-year follow-up.

Table I. Studies in which sirolimus was used for the treatment of PHTS associated clinical findings.

Study[Reference]	Disorder / Genetic result (NM_000314.8)	Sirolimus dose	Outcomes / Side effects
Komiya et al., 2019[11]	PTHS (18 patients) / *PTEN* mutations were located in exons 1 through 8	A loading dose of 6 mg, followed by a 2 mg dose	Regression of skin and GI lesions, improvement in cerebellar function score at 1 month / Abnormalities in liver enzymes (39%), electrolytes (33%), and anemia (33%). Two individuals developed grade 3 toxicities (hypophosphatemia and lymphopenia)
Marsh et al., 2008[12]	Proteus syndrome (PTHS) / c.507delC (p.Ser170ValfsTer13)	0.1mg/kg/d, serum levels were maintained between 5–10 ng/ml	Increase in the patient's serum albumin levels and reduction in soft tissue masses. At the age of 5 years and 6 months the patient began walking independently / No side effects
Iacobas et al., 2011[20]	BRRS (PTHS) / c.913_914insT (p.Ser305MetfsTer7)	0.8 mg/m^2/d aiming a serum level of 10–15 ng/ml	The pain was reduced and patient regained pain-free full mobility. Decrease in size of the vascular masses / Minor side effects: few oral ulcers and mild hypercholesterolemia
Adams et al., 2016[21]	2 patients with AVM, 4 patients with overgrowth + VA (PHTS) / NA	0.8 mg/m^2/d Target serum levels of 10 to 15 ng/mL	Partial response in 5 patients, stable disease course in 1 patient with overgrowth / NA
Triana et al., 2017[22]	VA (PHTS) / NA	0.8 mg/m^2/12hour	Partial response / Insignificant side effects
Pimpalwar et al., 2018[23]	Vascular anomalies (PHTS) (4 patients)	0.8 mg/m^2/d Target serum levels of 7 to 10 ng/mL	Pain improvement. Sirolimus did not prevent increase in the size of the hamartoma or development of VA / oral mucositis and elevated triglycerides
Sandbank et al., 2019[24]	AVM (PHTS) / NA	NA	Improvement in appearance and cutaneous discoloration of lesion, betterment in pain scale score with increased performance capacity / Grade 1 mouth sores

AVM: Arteriovenous malformation, BRRS: Bannayan-Riley-Ruvalcaba syndrome, d: day, kg: kilogram, m2: square meter, mg: milligram, ml: milliliter, NA: Not assessed, ng: nanogram, PHTS: PTEN hamartoma tumour syndrome, VA: Vascular anomaly *Asterix represents the cases who did not receive oral sirolimus treatment.

Table I. Continued

Study[Reference]	Disorder / Genetic result (NM_000314.8)	Sirolimus dose	Outcomes / Side effects
Schmid et al., 2014[26]	PHTS Heterozygous intragenic PTEN deletion of the exons 2–9	0.1mg/kg/d, serum levels were maintained between 5–10 ng/ml	Improved somatic growth and reduced thymus volume. After 1 year of treatment, the patient was able to walk. These effects diminished over the treatment period of 19 months / No side effects
Zak et al., 2017[27]	Lhermitte Duclos Disease (PHTS) / NA	Via gastrostomy tube at 0.6 mg/kg/d*	Regression of the mass, improvement of clinical status and quality of life / NA
Rosenfeld et al., 2019[28]	Ganglioneuromatosis (PHTS) / c.741dupA (p.Pro248ThrfsTer5)	NA	Improvement of rheumatologic manifestations. / NA
Botsali et al., 2019[29]	Oral hamartamatous lesions (PHTS) / NA	Topical sirolimus 0.5% once daily*	Sustained benefit, lesions did not recur / NA
Siklar et al., 2020[30]	Recurrent severe hypoglycemia (PHTS) / c.395G>T (p Gly132Val)	Beginning dose was 0.5 mg/m^2/d and increased to 4 mg/m^2/d to reach serum level between 4 to 12 mg/dl	Hypoglycemia was controlled / No side effects
Hill et al., 2021[31]	PHTS c.1028T>A (p.Val343Glu)	0.8 mg/m^2/d Target serum levels of 10 to 15 ng/mL	Marked improvement in pain, softening of the lesion, and decrease in neuropathy, no decrease in size of the lesion / NA
Busoni et al., 2019[34]	Juvenile polyposis / Deletion of *PTEN* and *BMPR1A*	0.8 mg/m^2/d Target serum levels of 6 to 8 ng/mL	Scarce distribution and smaller size of polyps, improved severe protein-losing enteropathy / NA
Quaranta et al., 2019[35]	Juvenile polyposis / Deletion of *PTEN* and *BMPR1A*	Blood concentration of 5 ng/mL	Decrease in number and size of polyps, weight improvement / No side effects
Taylor et al., 2021[36]	Juvenile polyposis / Deletion of *PTEN* and *BMPR1A*	Target serum level of 5 ng/mL	Improved protein-losing enteropathy and chronic gastrointestinal bleeding / Well tolerated no side effects
Bell et al., 2022[37]	Juvenile polyposis / Deletion of *PTEN* and *BMPR1A*	1.5 mg/m^2, targeting a serum trough level of 6–8 ng/ml	Improvement in protein-losing enteropathy, decreased intestinal blood loss, and improved weight gain. Decrease in gastrointestinal polyp burden / NA

AVM: Arteriovenous malformation, BRRS: Bannayan-Riley-Ruvalcaba syndrome, d: day, kg: kilogram, m2: square meter, mg: milligram, ml: milliliter, NA: Not assessed, ng: nanogram, PHTS: PTEN hamartoma tumour syndrome, VA: Vascular anomaly *Asterix represents the cases who did not receive oral sirolimus treatment.

Frequently reported side effects of sirolimus are abdominal pain, dyspepsia, mucositis, diarrhea, constipation, fatigue, headache, delayed wound healing, skin rash, lymphopenia, electrolyte imbalances, hyperglycemia and dyslipidemia.[38] No undesirable effects were observed in our patient, except for mildly elevated cholesterol levels, at the 24th week of the treatment.

There is no specific recommendation for the duration of sirolimus treatment in the literature as far as we know. Treatment period differs among previous studies ranging from 56 days to 40 months.[11,39]

Due to the rarity of similar cases, information regarding all aspects of sirolimus treatment is limited. Improved post-treatment clinical,

laboratory and endoscopic findings contribute to the favorable outcome of sirolimus treatment for the GIS findings of PHTS patients. Nevertheless, monitoring the treatment outcomes of future patients will further delineate the long-term effects of sirolimus in PHTS patients and contribute to the establishment of standard therapy protocols.

Ethical approval

Genetic studies were performed after obtaining written informed consent from the patient's parent.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: GES, FOH; data collection: GES, NGL, AEG; analysis and interpretation of results: GES, SY, NGL, AEG, GS; draft manuscript preparation: GES, SY. 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

Recovery of cyanosis after esophageal intubation in a neonate with tracheal agenesis: a case report

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ABSTRACT

Background. Tracheal agenesis (TA) is a rare congenital defect that consists of a complete or partial absence of the trachea below the larynx, with or without tracheoesophageal fistula (TEF). It is a severe congenital defect with a very high mortality rate. The recommended surgical approach is esophageal ligation and gastrostomy. Despite the progress in reconstructive surgical techniques, the outcome of the anomaly is still very poor. We described a case of TA with a TEF in a female newborn with a hemivertebra, single ventricle, single atrioventricular valve, single atrium, and cardiac left isomerization.

Case. The patient, who was born at 37 weeks of age, was diagnosed with imaging methods, as the cyanosis did not improve despite being intubated many times in the delivery room; the cyanosis improved after esophageal intubation. Despite all life support treatment, the patient died on the fourth day of life. At autopsy, tracheal agenesis was diagnosed.

Conclusions. In newborns who cannot be intubated in the delivery room or whose lungs cannot be ventilated despite being intubated and whose cyanosis cannot be corrected, tracheal agenesis should be considered and ventilation with esophageal intubation should also be tried.

Key words: tracheal agenesis, newborn, heterotaxy.

Tracheal agenesis (TA) is a rare congenital defect that consists of complete or partial absence of the trachea below the larynx, with or without a concomitant tracheoesophageal fistula (TEF). This usually lethal defect is also the rarest anomaly among tracheal anomalies with an incidence of 1 per 50,000 newborns. Male patients are more likely as twice of female patients to be effected.[1]

The recommended surgical approach is esophageal ligation and gastrostomy. Despite the progress in reconstructive surgical techniques, the outcome of the anomaly is still very poor. In neonates with tracheal agenesis, severe respiratory distress and cyanosis occur just after birth and no audible cry, typically cyanosis decreases with bag-mask ventilation, but not improves or worsens by intubation. We described a case of TA with a TEF in a female newborn with vertebral anomalies (hemivertebra), congenital heart anomalies (single ventricle, single atrioventricular valve, single atrium), and cardiac left isomerization (radial ray defects).

Case Report

A female newborn was delivered from 32-year-old healthy woman by caesarean section because of fetal distress at 37 weeks and 5 days of gestation at a local hospital. The baby was the third child of healthy nonconsanguineous parents. Her brother and sister were healthy.

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At the delivery room, although spontaneous breathing movements were present, crying sounds were not heard. The newborns weight was 2400 g, her head circumference was 33 cm and Apgar scores at 1, 5, and 10 min. were 4, 5, and 5, respectively. Endotracheal intubation was performed because of severe cyanosis and bradycardia, however her oxygen saturation decreased after intubation. Therefore, bag and mask ventilation was started and her oxygen saturation increased. Tracheal agenesis and TEF were suspected and esophageal intubation was performed, oxygen saturation improved to 80% and her lungs were ventilated bilaterally. On thorax computerized tomography, a short air column at the upper part of distal trachea was visualized and disappeared at the lower part of distal trachea. As the air column was re-visualized just before the carina and main bronchia, presence of the connection between carina, main bronchi and distally esophagus were revealed.

The patient was referred to our hospital on the third day of life. Chest X-ray showed the air-filled esophagus, hemivertebra of the twelfth thoracic vertebra and bilaterally normal ventilated lungs (Fig. 1) Although the mechanical ventilation support was gradually increased, respiratory acidosis and partial carbondioxide pressure increased persistently. Echocardiography showed a functional single ventricle (single atrium, single atrioventricular valve), atrioventricular septal defect, left atrial isomerism, and patent ductus arteriosus.

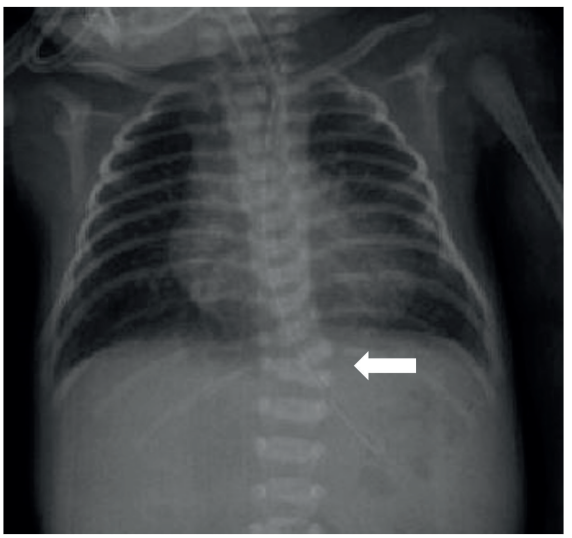

Fig. 1. Chest X-ray showed hemivertebra (white arrow) of the twelfth thoracic vertebra and bilaterally normal ventilated lungs.

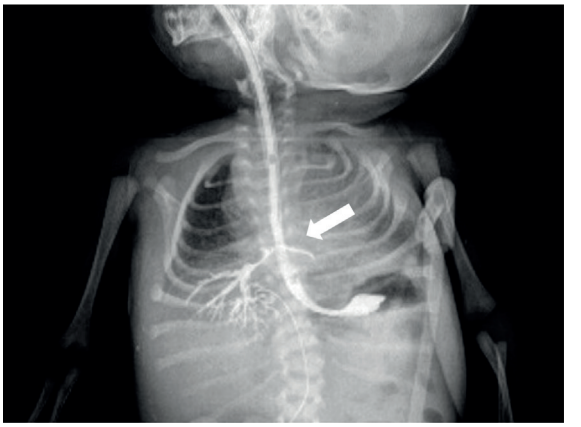

Fig. 2. The trachea was blind-ended and both main bronchi (white arrow) originated from esophagus.

Flexible laryngoscopy examination showed that the trachea was blind-ended beyond the vocal cords. Expansion of the distal esophagus was observed in esophagoscopy. In order to reduce the amount of air entering the stomach and to provide better ventilation, a Foley balloon catheter with guide wire was inserted through the esophagus into the stomach. After the balloon was inflated and pulled upward, oxygen saturation decreased more, so it was deflated and removed. It was concluded that it compressed the junction of the esophagus and main bronchia and therefore decreased the air flow to the main bronchi.

The initial surgical plan was to perform a distal esophageal ligation and gastrostomy after hemodynamic stabilization. However, despite all supportive treatment, the patient died on the fourth day of life. After the written autopsy permission was taken from the patient's family, barium sulfate was given, the trachea was blind-ended and both main bronchi originated from esophagus were demonstrated (Fig. 2).

At autopsy, TA was diagnosed. The larynx was normal but blind-ended; the pharynx continued with the esophagus which showed a connection through a TEF with two main bronchia at the bifurcation level originated from esophagus (Fig. 3). TA type was found to be type 2 according to Floyd classification and type D according to Faro classification.

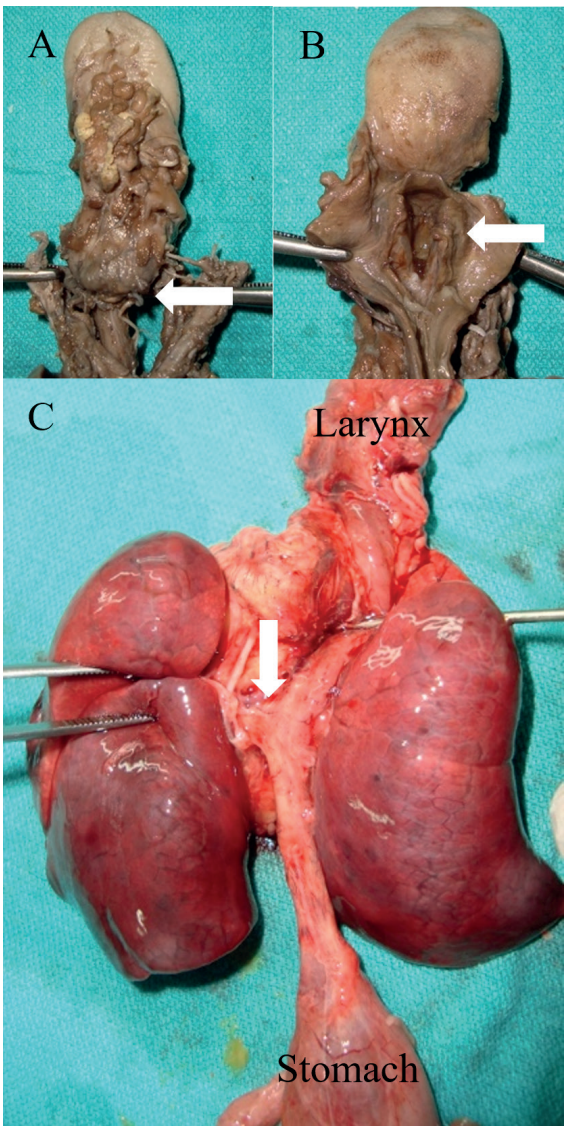

Fig. 3. A, B: At autopsy, the larynx was normal but blind-ended (white arrow). C: The pharynx continued with esophagus which showed a connection with two main bronchi at the bifurcation level through a tracheoesophageal fistula (white arrow).

The heart had single ventricle with one atrium and one atrioventricular valve. In addition, an accessory spleen and slightly dilated ureters were seen while gross examination of other organs were normal. Light microscopy revealed intra-alveolar hemorrhage, atelectasis and emphysema in lungs, and severe congestion in other organs because of hypoxia. Karyotype analysis from fibroblast cultures was 46, XX. With these results, left heterotaxy syndrome was considered in our patient.

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

Discussion

De Groot-van der Mooren et al. reviewed in 2012, forty-nine cases of TA who have been reported since 1900.[1] Among these cases, diagnosis of TA was presumed prenatally only in five cases based on congenital high airway obstruction syndrome (CHAOS). Prenatal diagnosis in these five cases of TA was confirmed by fetal magnetic resonance imaging or detailed ultrasound examination.[1] Prenatal diagnosis is only possible in the absence of TEF with the findings of CHAOS. In other cases without prenatal diagnosis, ultrasonography showed altered amniotic fluid status, mainly polyhydramnios.[1] Our case did not have abnormal ultrasonography findings, therefore the patient could not be diagnosed prenatally.

Common clinical symptoms are respiratory distress with breathing movements without an audible cry. An interesting finding in these cases is the decreasing oxygen saturation level despite endotracheal intubation, whereas temporarily increasing oxygen saturation level when bag and mask ventilation is started. After esophageal intubation, marked abdominal distension develops, and air passes through the fistula into the lungs.[1]

Two different classifications of TA cases were made by Floyd and Faro.[2,3] While there are

types 1, 2, 3 in the Floyd classification, there are 7 types in the Faro classification as A, B, C, D, E, F, G. Floyd type 2 and Faro type D are seen most frequently. In our case, Floyd type 2 was classified as Faro type D.

Associated malformations were reported in 94% of cases with TA. Most accompanying anomalies are related to cardiovascular (64%), distal respiratory system (45-64%), gastrointestinal tract (47-50%), genitourinary tract (35-49%), musculoskeletal (19-38%), and nervous system (7%).[1,4,5] Associated malformations can be a part of the VACTERL association (Vertebral defects, Anal atresia, Cardiovascular defects, Tracheoesophageal fistula and/or Esophageal atresia, Renal defects, and Limb defects) or TARCD association (Tracheal Agenesis/Atresia, Radial ray defects, Complex congenital cardiac abnormalities, and Duodenal atresia).[4-6] In a case series including 6 patients with TA, one patient had gastrointestinal, genitourinary, cardiac anomalies and hemivertebra.[7] In our case, hemivertebra and congenital heart anomaly were accompanied by TA.

It was reported that chromosomal analysis was done in 18 cases of TA.[1] Abnormal karyotype was detected in only two cases; mos, 47, XY+mar(43,3)/46XY(56,5) and 5q11.2 deletion.[8,9] In our case, there were no apparent dysmorphic features and karyotype analysis from fibroblast cultures was 46,XX.

Radiographic findings may show posterior location of the endoctracheal tube and absence of the tracheal shadow.[1] Computerized tomography is the preferred imaging method due to giving the best and the fastest results for delineating the anatomy.[10] Radiologic imaging studies with contrast media may demonstrate a blind laryngeal/tracheal end and a TEF.[1]

The primary management includes esophageal ventilation and hemodynamic stabilization, definition of the TA with radiographic and endoscopic evaluation, followed by the initial surgical approach of esophageal ligation at the site distal to the TEF and gastrostomy. Esophagus could be used as a pseudo-trachea through this surgical procedure. Double barrel esophagostomy is to be recommended as the distal end for intubation and the proximal end for salivary drainage. An external esophageal stent can be used to avoid the esophageal collapse. Gastrostomy should be performed for feeding as a next therapeutic step. Reconstruction with small intestine, colon interposition, or gastric pull-up has been demonstrated to be feasible and compatible with survival.[11-13] Because infants with TA died shortly after birth due to severe asphyxia, surgery could not be performed in these cases. The largest review in the literature, comprising 49 cases of TA, reported that tracheostomy had been attempted in 38% of the cases to explore the options of surgical reconstruction.[1]

Unfortunately, TA has a very high mortality rate. In the largest case series of TA, mortality was high, as 34 of 40 (85%) children died within 2 days.[13,14] Despite improvements in surgical management, it was reported that only two children were still alive at 10 months and 4 years, respectively.[13,14] Soh et al.[15] reported in 1999 that among the reported cases the longest living case of TA lived up to the age of 6 years and 10 months through tracheal reconstructive surgery.[15] In 1994, a case reaching the age of 4 years with TA, proximal TEF and bronchioesophageal fistula was reported.[16] After tracheostomy was performed and long T-tube was placed, the patient was discharged from the hospital.[16] But, most of the cases of TA do not have a favorable anatomy for tracheostomy, therefore tracheostomy and T-tube do not work in these patients.

If TA is prenatally suspected, ex-utero intra-partum therapy (EXIT) should be planned.[17] EXIT procedure means providing and assuring the continuity of upper airway by tracheostomy just before clamping the umbilical cord in the delivery room.[17]

For effective tracheal repairment, special materials compatible with children's growth which are homolog tissues such as pericardium, esophagus, bladder or synthetic materials such as silicon, dacron, elastane need to be produced.[18,19] Unfortunately, biomedical technology has not yet been developed sufficiently all over the world today. Despite the progress in reconstructive surgical techniques, outcome of the TA is still very poor.

In conclusion, in newborns who have respiratory distress with breathing movements without appropriate air entry, no audible cry, and failed endotracheal intubation or whose cyanosis cannot be corrected in the delivery room, tracheal agenesis should be considered; and ventilation with esophageal intubation should also be tried.

Ethical approval

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

Author contribution

The authors confirm contribution to the paper as follows: case report conception and design: HTÇ, MŞA, DB, MY; data collection: HTÇ, MŞA, DB; analysis and interpretation of results: HTÇ, EŞY, MH, AES, KŞG, MY; draft manuscript preparation: HTÇ, MŞA, EŞY, MY. All authors reviewed the results and approved the final version of the manuscript.

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The authors declare the study received no funding.

Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

AA amyloidosis presenting with acute kidney injury, curable or not?

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ABSTRACT

Background. Amyloidosis is a group of disorders with extracellular accumulation of autologous fibrillary insoluble proteins in various tissues and organs such as the kidneys, liver, spleen, heart and gastrointestinal tract leading to impairment of normal organ function. Childhood amyloidosis is an exceedingly rare entity mainly caused by familial Mediterranean fever (FMF) and the other autoinflammatory diseases such as mevalonate kinase deficiency (MKD).

Case. A 16-year-old male was referred to pediatric nephrology for coincidentally discovered proteinuria. He had no significant findings on physical examination except for urochromic color. He had nephrotic range proteinuria with 109 mg/m2/h and serum creatinine was 1.35 mg/dl. Kidney biopsy was performed because of nephrotic range proteinuria with acute kidney injury. In hematoxylin-eosin-stained tissue sections, amyloid was suggested as extracellular amorphous material that is lightly eosinophilic in the glomeruli. Diagnosis was confirmed by Congo red positivity, with apple-green birefringence under polarized light. MEFV gene mutation was negative and a compound heterozygote mutation found in mevalonate kinase gene. A 6-month-trial of colchicine, enalapril, and losartan combination was not successful; Canakinumab was started thereafter. Proteinuria and creatinine decreased to 7 mg/m2/h and 0.6 mg/dl respectively 4 years after treatment.

Conclusions. Amyloidosis should be considered especially in children presenting with proteinuria and with a history of recurrent fever. This report also emphasizes the efficacy of canakinumab to prevent or decelerate chronic renal failure in these patients although it does not reduce tissue deposition in long-term use.

Key words: amyloidosis, kidney injury, mevolonate kinase deficiency, canakinumab.

Nephrotic range proteinuria in the setting of acute kidney injury is a serious and alarming clinical problem. It can be secondary to acute glomerulonephritis, focal segmental glomerulosclerosis, infections, acute tubular necrosis, interstitial nephritis, nephrotoxic medications and renal vein thrombosis. One of these, renal amyloidosis is an exceedingly rare progressive disease in children caused by the deposition of insoluble amyloid fibrils. The diagnosis is confirmed by microscopic examination of faintly red amyloid fibrils on Congo red staining also showing typical apple-green birefringence under polarized light. Childhood amyloidosis is mostly in AA form, mainly caused by familial Mediterranean fever (FMF) and the other autoinflammatory diseases such as mevalonate kinase deficiency (MKD).[1]

MKD is an autoinflammatory disease characterized by recurrent episodes with fever, abdominal pain, mucoid and cutaneous lesions, conjunctivitis, and arthralgia. Episodes occur more frequently in children than adults and people with MKD may develop long-term complications including AA amyloidosis although it is rare in childhood period.[2]

In this report, a case with incidental nephrotic proteinuria diagnosed as amyloidosis after renal biopsy and diagnosed as MKD with a favorable response to canakinumab was presented.

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Case Report

A 16-year-old male presented with dry skin was referred to pediatric nephrology for coincidentally proteinuria. He denied any complaints including fever, fatigue, diarrhea, vomiting, cough and abdominal or joint pain with no notable past medical history. There was no consanguinity between parents and no known family history of kidney or inflammatory disease. On examination, he had urochrome color. The temperature was 36.9°C, the pulse was 85 beats/min, the blood pressure measured as 120/70 mmHg and the respiratory rate was 20 breaths/min. His body weight was measured as 57.1 kg (10-25 percentile), height as 164 cm (3-10 percentile). Other system examination was normal.

Laboratory tests revealed: serum urea 40 mg/dl (normal: 5-20 mg/dl), serum creatinine 1.35 mg/dl, uric acid 8.9 mg/dl (normal: 0-7.0 mg/dl), albumin 2.8 g/dl (normal: 3.8-5.4 g/dl), cholesterol 315 mg/dl (normal: 0-170 mg/dl). Other biochemical findings were normal as well as C-reactive protein (CRP), sedimentation rate (ESR), C3, C4, anti-nuclear antibody (ANA) and anti-double-stranded-DNA (anti-dsDNA) titers. Urinalysis showed 3+ proteinuria with no leukocytes, erythrocytes, and glycosuria. Nephrotic range proteinuria was confirmed by a 24-hour urine test (109 mg/m^2/h). Abdominal ultrasonography revealed increased renal echogenicity as grade 1.

Nephrotic range proteinuria with acute kidney injury indicated a kidney biopsy. In hematoxylin-eosin-stained tissue sections, amyloid was suggested as extracellular amorphous material that is lightly eosinophilic in the glomeruli, resulting in expansion of mesangial areas and thickening of capillary basement membranes. Tubular epithelium showed bubbly appearance due to protein resorption droplets (Fig. 1). No interstitial fibrosis or tubular atrophy was seen in biopsy specimens. The Congo red stain, special for the diagnosis of amyloidosis, showed reddish or intense orange deposits (Fig. 2). Diagnosis was confirmed by Congo red positivity, with apple-green birefringence under polarized light. Histologic identification of AA Amyloidosis was also performed using immunohistochemistry (Fig. 3).

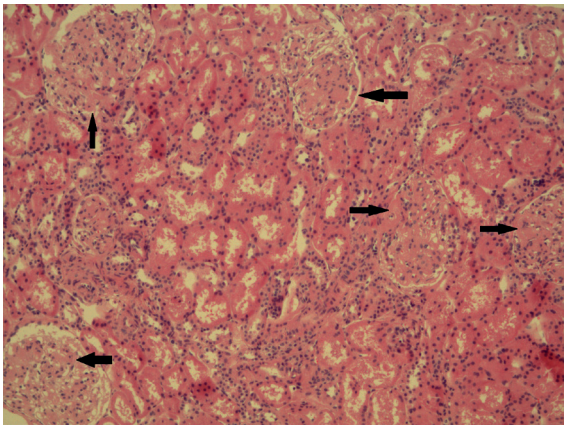

Fig. 1. H&E-stained tissue sections from first biopsy with amyloid, amorphous, acellular eosinophilic material in the glomeruli (hematoxylin and eosin, x100).

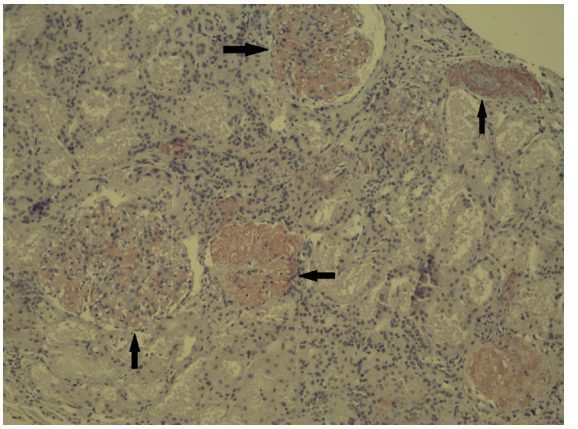

Fig. 2. Positivity with Congo red staining (Congo red, x100).

After the diagnosis of AA amyloidosis, M-mode echocardiography and tissue Doppler echocardiography was performed and showed no signs of systolic and diastolic dysfunction as well as hypertrophy. The additional laboratory workup revealed unexpectedly normal level of fibrinogen measured as 274 mg/dl and above normal level of serum amyloid-A (SAA) as 10.7 mg/L (normal <6.4 mg/L). Chronic infections including tuberculosis, brucella, salmonella, hepatitis A, hepatitis B, hepatitis C and human

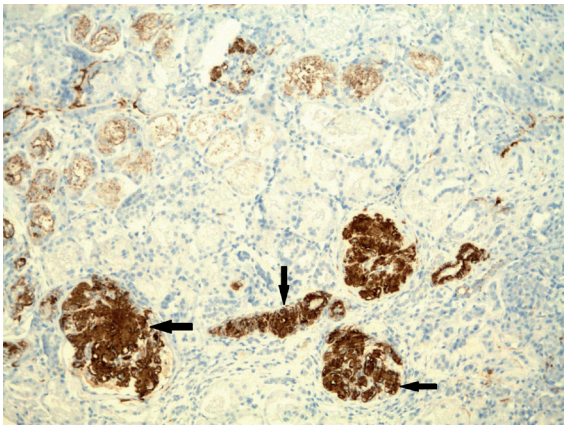

Fig. 3. Positivity with anti-AA immunohistochemistry (x100).

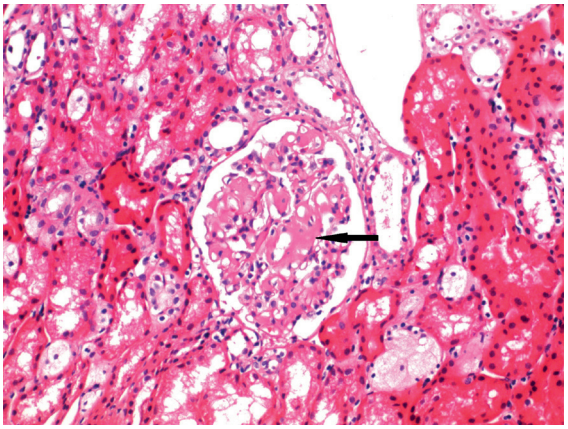

Fig. 4. Second biopsy after 4 years of canakinumab treatment with persisting amyloid accumulation (hematoxylin and eosin).

immunodeficiency virus (HIV) were ruled out by negative serology tests, negative PPD and normal chest X-ray. He had no relevant history of arthritis or arthralgia associated with rheumatoid arthritis or spondyloarthropathy with negative test results of rheumatoid factor (RF). Symptoms of FMF, the most frequent of all hereditary autoinflammatory syndromes, were reevaluated. Although there were no mentioned symptoms of an autoinflammatory disease or family history, insistent questioning revealed recurrent fever attacks every two weeks through infancy and chronic kidney disease in three relatives of the father. Genetic analysis of FMF revealed no *MEFV* gene mutation. A compound heterozygote mutation with p. Val377Ile (c.1129G>A) and p. Arg388*(c.1162C>T) variants in *mevalonate kinase gene* (MVK -NM_000431) was found.

Colchicine (2 mg/day), enalapril (10 mg/day) and losartan (25 mg/day) were given. There was no decrease in nephrotic range proteinuria after six months; and canakinumab treatment was started at monthly intervals as 150 mg subcutaneously. Nephrotic range proteinuria decreased to 48, 23.4, 15 and 7 mg/m^2/h through 7th, 9th, 36th and 48th months of the treatment, respectively. A control renal biopsy showed persistent tubular protein depositions, thick capillary basement membranes and expansion of mesangial areas (Fig. 4).

A written informed consent was received from the parents for the publication.

Discussion

Acute kidney injury (AKI), presenting with nephrotic range proteinuria , is a major and severe clinical problem especially in children older than 10 years old and kidney biopsy should be considered in these conditions. Since our patient was 16-year-old at presentation and he had massive proteinuria and acute kidney injury, kidney biopsy was performed immediately. Amyloid fibril deposits were identified with Congo red positivity and apple-green birefringence under polarized light in biopsy material and AA type was confirmed with immunohistochemistry.

Amyloidosis is a group of disorders with extracellular accumulation of autologous fibrillary insoluble proteins in various tissues and organs leading to impairment of normal organ function. AA type amyloidosis, probably the most common type in pediatrics, is associated with chronic inflammatory diseases such as FMF, juvenile rheumatoid arthritis, ankylosing spondylitis, inflammatory bowel disease, chronic infections such as tuberculosis, and chronic granulomatous disease.[3,4] Proteinuria may be the earliest clinical manifestation of AA amyloidosis in chronic inflammatory conditions

and may precede to nephrotic syndrome and renal insufficiency.[5] Although FMF was the most common cause of secondary amyloidosis in childhood, its incidence had decreased dramatically with colchicine therapy. However other autoinflammatory diseases like MKD and cryopyrin-associated periodic syndrome (CAPS) are becoming more frequent in the etiology of seconder amyloidosis. Common causes of AA amyloidosis such as tuberculosis, inflammatory bowel disease, juvenile rheumatoid arthritis, brucella, salmonella, hepatitis A, hepatitis B, hepatitis C, HIV and malignancies were excluded with appropriate diagnostic tests in our patient.

MKD is defined as a periodic fever syndrome (PFS) presented by periodic, recurrent fever caused by recurrent inflammatory episodes with autosomal recessive inheritance. Hyperimmunoglobulinemia D and periodic fever syndrome (HIDS) and mevalonic aciduria (MA) both result from mutations in the gene encoding mevalonate kinase (MK) and both are part of the MKD spectrum. They result from mutations in the gene encoding mevalonate kinase (MK).[2,6-9] Rare but the most devastating complication of the MKD is type AA amyloidosis.[6,10] Secondary amyloidosis due to MKD had been reported in 3% of patients, which is more rare than reported for the other monogenic autoinflammatory syndromes.[11,12] A review declared amyloidosis prevalence at about 6%[13], which is compatible with the 5% of MKD patients with amyloidosis in a recently published article in which the genotypes of 15 of the 17 patients included at least one p.(Val377Ile) variant similar with our patient.[14] p.(Val377Ile), which is the most frequent variant likely and mainly reported in MKD-HIDS, is also one of the heterozygote mutations described in our patient. Although the genotypes including p.(Val377Ile) (homozygous or compound heterozygous) are more frequent in mild systemic forms they are also sometimes related with severe disease leading to amyloidosis. Our patient had no systemic inflammation due to MKD at the diagnosis but infantile periodic fever history supports the systemic inflammation occurring through infancy which resulted as amyloidosis.

Colchicine treatment is the backbone therapy for the protection against amyloidosis in FMF whereas in other autoinflammatory syndromes such as MKD, this protection is often achieved with biologic treatment like anti-TNF, anti-IL-1 therapy.[1] In our case; although creatinine decreased immediately after treatment, proteinuria persisted within the nephrotic range for 6 months. Therefore, canakinumab was started at monthly intervals as 150 mg subcutaneously. After four years of canakinumab treatment, a major regression in proteinuria occurred whereas amyloid depositions persisted in control renal biopsy. Although there are previous reports claiming regression of proteinuria by biologic treatments, there is no evidence of ceased progression of amyloidosis at the tissue level.[1,5,15-17] The decrease in proteinuria may evoke regression of amyloid deposition. However, both Topaloglu et al.[16] and Yıldırım et al.[18] could not demonstrate a significant change in extent of amyloid accumulation in control renal biopsy even though there was complete remission of proteinuria, just like in our patient. They concluded that the incompatibility between clinical improvement and lack of change in amyloid deposition may be explained with the suppressed inflammation resulting in a decrease in glomerular permeability despite persistence of amyloid deposition.[16,18]

In this report it was emphasized that there was ongoing amyloid burden at tissue level in control biopsy performed four years after anti-IL1 therapy. Therefore, although canakinumab treatment has been shown to reduce proteinuria in these patients, it is unknown whether this treatment initiated after amyloid accumulation prevents chronic renal failure.

Ethical approval

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

Author contribution

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

Optic neuritis in CD59 deficiency: an extremely rare presentation

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ABSTRACT

Background. CD59 is the principal cell inhibitor of complement membrane attack on cells. Stroke, peripheral neuropathy, and recurrent central nervous system attacks have been reported in patients with inherited CD59 deficiency. In this paper, we report a patient with CD59 deficiency associated with two attacks of demyelinating peripheral neuropathy and the third attack as an isolated optic neuritis.

Case. An 8-month-old girl whose sibling died at 12th month of age with recurrent weakness episodes responsive to intravenous immune globulin treatment, presented with weakness in legs and poor sucking. Weakness episodes with neurogenic electromyography suggested CD59 deficiency. Immunophenotypic analysis with flow cytometry showed CD59 deficiency. Sanger sequencing of CD59 gene revealed a homozygous *c146delA* (p.Asp49Valfs*32) mutation. First two attacks were treated with intravenous immunoglobulin therapy without any sequalae. Third attack was an isolated optic neuritis which could not be explained by any other entity. The patient had no response to intravenous immunoglobulin but benefited from pulse steroid therapy. Eculizumab was started every two weeks in order to prevent possible advanced attacks and to reduce their severity.

Conclusion. Although it is a rarely reported disease, better recognition of CD59 deficiency by pediatric neurologists is necessary because it is curable. In addition to different presentations reported, optic neuritis may also be a manifestation of CD59 deficiency.

Key words: complement inhibition, immune dysregulation, demyelinating, eculizumab, optic neuritis.

The complement system (CS) is responsible for recognition and neutralization of bacteria and starting phagocytosis through opsonization. Once complement system is activated, the formation of C3 convertase complexes leads to cleave C3 into two subunits, C3a and C3b. The latter is essential for C5 convertase activity to cleave C5 to C5a and C5b. Freshly activated C5b starts the formation of membrane attack complex (MAC), which is tightly regulated by CD59 through inhibiting recruitment of C9, thereby keeping the cell lysis under control.[1] The relationship between C5 and both the central and peripheral nervous system demyelinating diseases has been shown in many studies.[2-4]

Optic neuritis (ON), an inflammatory condition of the optic nerve causing visual impairment, which can occur as an isolated, monophasic condition, also recurs without signs of any other manifestations of chronic demyelinating diseases such as multiple sclerosis (MS), myelin oligodendrocyte glycoprotein antibody (MOG-Ab) associated disease or neuromyelitis optica spectrum disorders (NMOSD).[5]

Stroke, peripheral neuropathy and central nervous system (CNS) demyelinating disorders have been reported in CD59 deficiency.[6-9] CD59 deficiency has been shown to play an important role in the pathogenesis of NMOSD in mice and rats.[10,11] However, to our knowledge, only

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two siblings have been reported to present optic neuritis, which was thought to be due to CD59 deficiency demonstrated in postmortem studies.[12] We report a patient with CD59 deficiency presenting with first two attacks of early-onset demyelinating peripheral neuropathy and third attack as ON.

Case Report

First attack

An 8-month-old girl presented with a three-day history of generalized weakness and poor sucking. The patient was prescribed amoxicillin-clavunic acid treatment for acute upper respiratory tract infection one week prior. The patient was born at term by cesarean section without any complications and had age-appropriate neurodevelopment. Parents were relatives with four children. The first child had recurrent episodes of weakness after febrile episodes which were responsive to intravenous immunoglobulin (IVIg) treatment and died at 12 months of age without a specific diagnosis. The mother's cousin was also under follow-up at another center with a similar disease. The pedigree of the patient is presented in Figure 1. Neurological examination revealed absent deep tendon reflexes (DTR) and decreased muscle strength [Medical Research Council (MRC) grading system: 3/5] of lower extremities, normoactive DTR and decreased muscle strength (MRC: 4/5) of upper extremities and tongue fasciculation. The patient was unable to hold her head. Clinical features in each attack are shown in Table I. Complete blood count, biochemical tests, vitamin B12, creatine kinase, contrast-enhanced brain magnetic resonance imaging (MRI), contrast-enhanced spinal MRI, cerebrospinal fluid (CSF) examination, and extensive metabolic investigations were normal. Table II shows the laboratory and neuroimaging findings in each attack. Nerve conduction studies were normal but needle electromyography revealed diffuse neurogenic changes. With IVIg (1 g/kg/day for -two days) treatment, sucking improved, head control was gained, but weakness in the lower extremities continued. Weakness episodes with neurogenic electromyography findings suggested CD59 deficiency. Immunophenotypic analysis with flow cytometry showed CD59 deficiency. Sanger sequencing revealed a homozygous, pathogenic variant in the CD59 gene (MIM: 107271), NM_203331: *c146delA* (p.Asp49Valfs*32) (rs587777149). The same variant was found in the mother's cousin. No evidence for hemolysis was detected. Eculizumab treatment could not be given because parental consent could not be obtained. Seven days after IVIg treatment, sucking and other motor functions recovered without any sequelae. Treatment options, durations and benefits during attacks are shown in Table III.

Second attack

The patient who did not have any attacks in the following four months and continued to

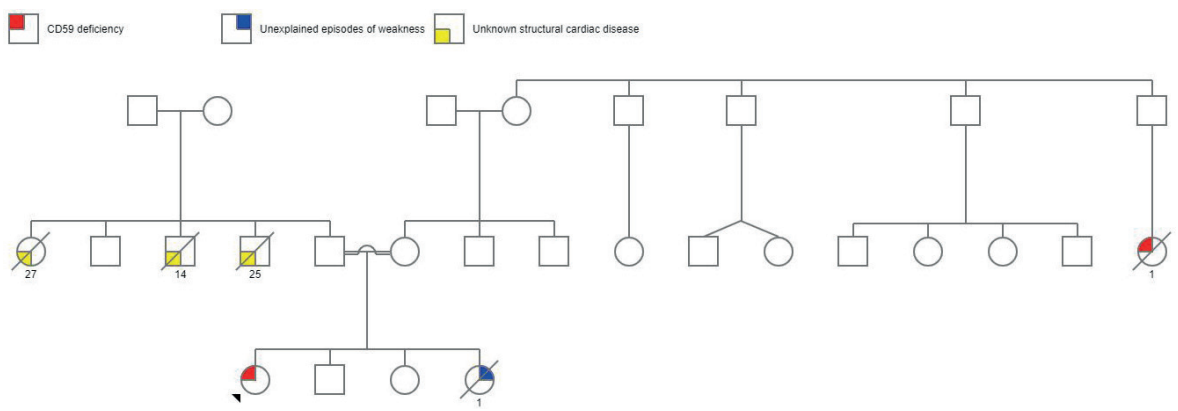

Fig. 1. The pedigree of the patient.

Table I. Clinical features in attacks.

	First attack	Second attack	Third attack
Age (month)	8	12	20
Preceeding febrile infection	(+)	(+)	(-)
Hypotonia	(+)	(+)	(-)
Tongue fasciculation	(+)	(-)	(-)
Optic neuritis	(-)	(-)	(+)
Attack duration before treatment (day)	5	1	1
Attack duration after treatment (day)	7	3	30
Sequelae in muscular system	(-)	(-)	(-)
Sequelae in other systems	(-)	(-)	Optic atrophy

Table II. Laboratory and radiological findings in attacks.

	First attack	Second attack	Third attack
Hemoglobin (g/dL)	12.7	13.4	10.9
Reticulocyte count (%)	NA	1.15	1.41
LDH (U/L)	216	NA	236
Haptoglobin (g/dL)	NA	NA	0.169 (Normal: 0.3-2)
Total bilirubin (mg/dL)	0.7	NA	0.9
Direct Coombs test	(-)	NA	(-)
Peripheral blood smear	No hemolysis	No hemolysis	No hemolysis
Creatine kinase (U/L)	108	81	76
CSF protein (mg/dL)	44.7	NA	29.1
Immunoglobulin index	NA	NA	(-)
MOG-Ab	NA	NA	(-)
AQP4-Ab	NA	NA	(-)
Brain MRI	Normal	NA	Suspicious enhancement in the bilateral olfactory nerves
Spinal MRI	Normal	NA	Normal
Orbital MRI	NA	NA	Increased signal intensity and enhancement in bilateral optic nerves, increased chiasm thickness, heterogeneity in the orbital fatty tissue

CSF: cerebrospinal fluid, MOG-Ab: myelin oligodendrocyte glycoprotein antibody, AQP4-Ab: aquaporin 4 antibody, MRI: magnetic resonance imaging, NA: not available

Table III. Treatment options, dosages, durations and benefits.

	First attack			Second attack			Third attack		
	Duration (day)	Dosage	Benefit	Duration (day)	Dosage	Benefit	Duration (day)	Dosage	Benefit
IVIg	2	1 g/kg/day	(+)	2	1 g/kg/day	(+)	2	1 g/kg/day	(-)
Pulse steroid		NA			NA		10	30 mg/kg/day	(+)
Maintenance steroid		NA			NA			NA	
Eculizumab		NA			NA			Started after the attack	

IVIg: intravenous immunoglobulin, NA: not administered

gain neurodevelopmental milestonesin time, presented with weakness in the legs and poor sucking. The patient had an upper respiratory tract infection 15 days prior and received oseltamivir and clarithromycin treatments. Neurological examination revealed absent DTR in upper and lower extremities, decreased muscle strength of lower and upper extremities (MRC: 3/5). Intravenous immune globulin was given at the same dosage. On the 3rd day, the complaints regressed and the neurological examination findings improved.

Third attack

Despite the advice for close follow-up, the patient was admitted eight months after the second attack, presenting with sudden vision loss and dilated pupils noticed by the mother. Detailed ophthalmological examination revealed loss of light fixation with fixed dilated pupils and no light reflex; however, dilated fundoscopy was normal (Fig. 2). The patient was diagnosed with bilateral ON, since the remainder of the neurological examination and CSF assessment were normal. Orbital MRI showed increased signal intensity on T2 weighted sequences and increased enhancement on contrast enhanced T1 images at the bulbar and retrobulbar level of the bilateral optic nerves **(**Fig. 3A), more prominent on the right, increased thickness of chiasm, heterogeneity in the orbital fatty tissue on the right and suspicious enhancement in the bilateral olfactory nerves (Fig. 3B). Visual evoked potential could not be performed because of patient's poor compliance. Nasopharyngeal swab polymerase chain reaction and serum antibody tests for Coronavirus disease 2019 were negative. Cerebrospinal fluid MOG-Ab and aquaporin 4 antibody (AQP4-Ab) were negative. Rheumatological tests, viral and bacterial serology resulted negative. Pulse steroid (30 mg/kg/day for ten days) and intravenous immunoglobulin (1 g/kg/day for two days) were given without any benefit. After the parental consent, eculizumab treatment was planned biweekly. Routine vaccination scheme and meningococcemia prophylaxis were collocated. Five days after the steroid therapy, the patient was uncomfortable with light and avoided objects while walking. Ophthalmological examination revealed bilateral optic disc pallor with the lack of optokinetic nistagmus (OKN). Subjective complaints about vision disappeared. The neuro-ophthalmological examination revealed bilateral normoisochoric pupils with central, steady, and maintained fixation on the 30th day of the beginning of symptoms (15th day of the end of steroid treatment). Radiologic examination after one month revealed regression of optic and olfactory nerve findings but three new cerebellar demyelinating small lesions on T2 images without clinical correlation (Fig. 3C, Fig. 3D). Considering that meningococcal vaccination would delay optimal treatment, although the second dose of

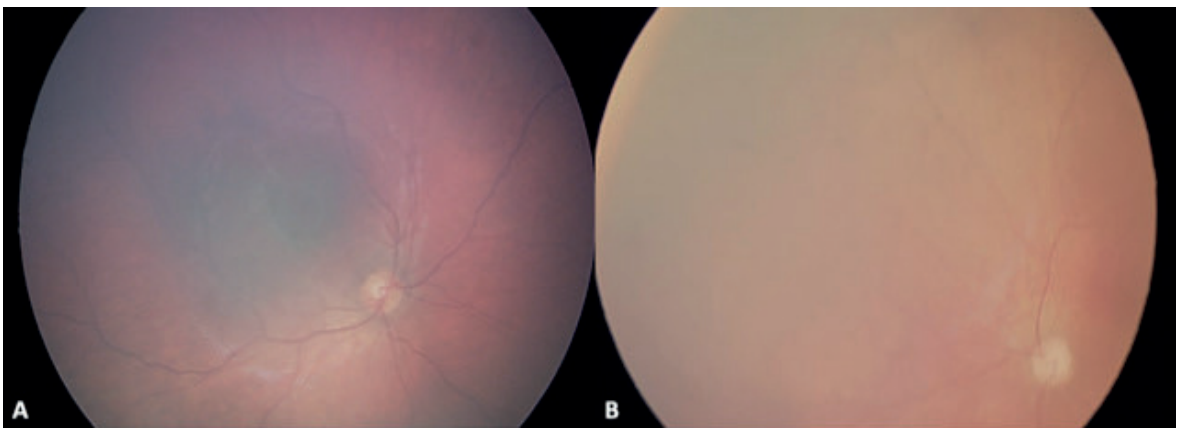

Fig. 2. Dilated fundoscopy revealed normal findings.

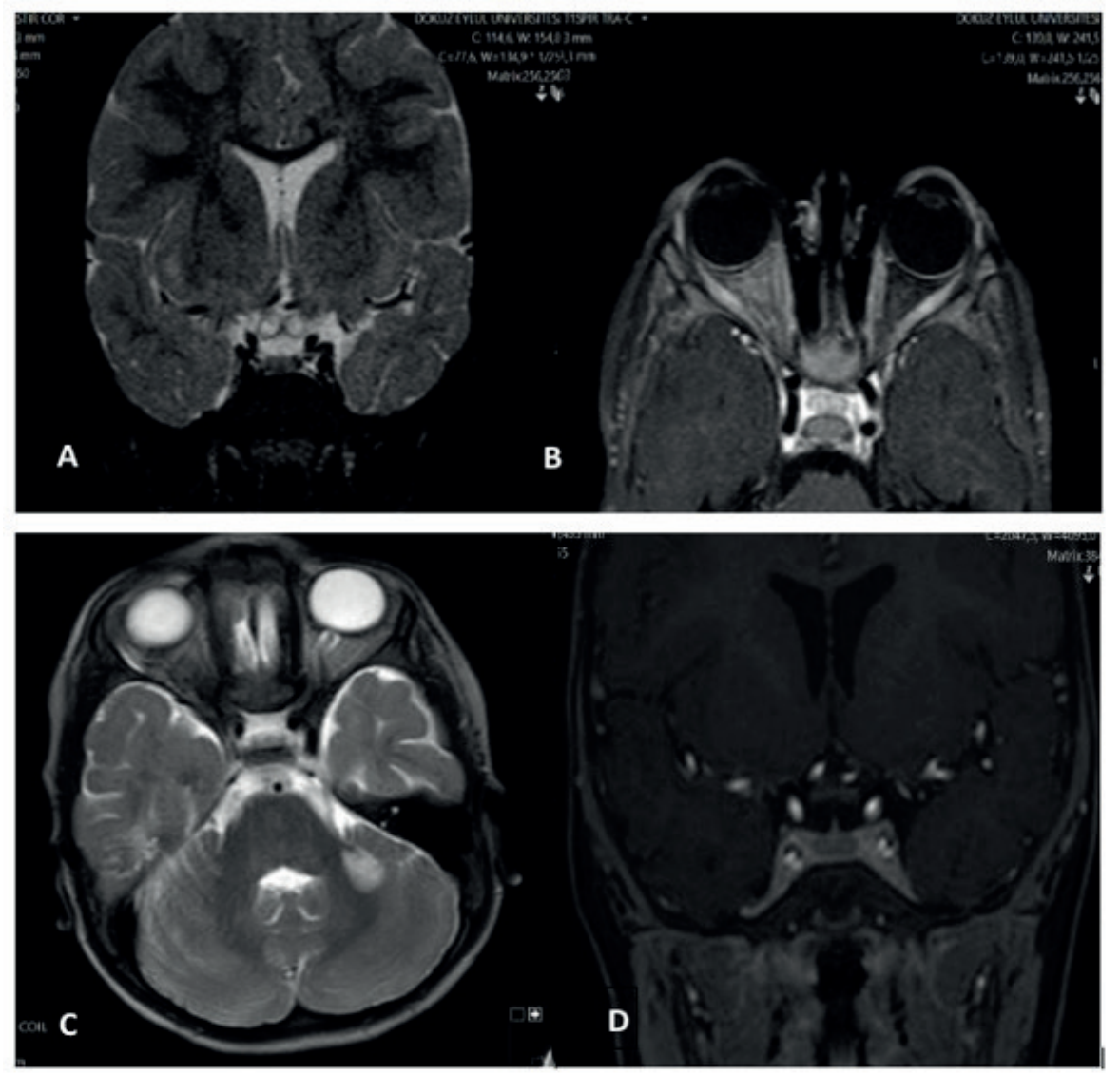

Fig. 3. (A) Retrobulbar optic nerves appearing hyperintense and swollen on coronal T2 weighted sequence. **(B)** Heterogeneity in the orbital fatty tissue on the right and increased enhancement bilateral optic nerves on axial contrast enhanced T1 sequence. **(C)** Cerebellar demyelinating lesion on axial T2 weighted sequence. **(D)** Regression of optic and olfactory nerve enhancement on T1 weighted sequence.

vaccine had not been administered yet, the first dose of eculizumab (300 mg, intravenous) was administered on the 35th day of the symptom onset with penicillin V prophylaxis. Detailed ophthalmological examination performed after the sixth dose of eculizumab treatment revealed positive OKN, bilateral central, steady, and maintained fixation with normoisochoric pupils and positive light reflexes on both eyes; however, dilated fundoscopy depicted bilateral optic atrophy (Fig. 4) Cycloplegic refraction was found as -1.75 diopters (D) of myopia with -3 D of astigmatism in the right eye and -3.5 D of astigmatism in the fellow eye. The patient is still followed-up with no further attacks after eculizumab which is administered biweekly. The parents gave their informed consent for this publication.

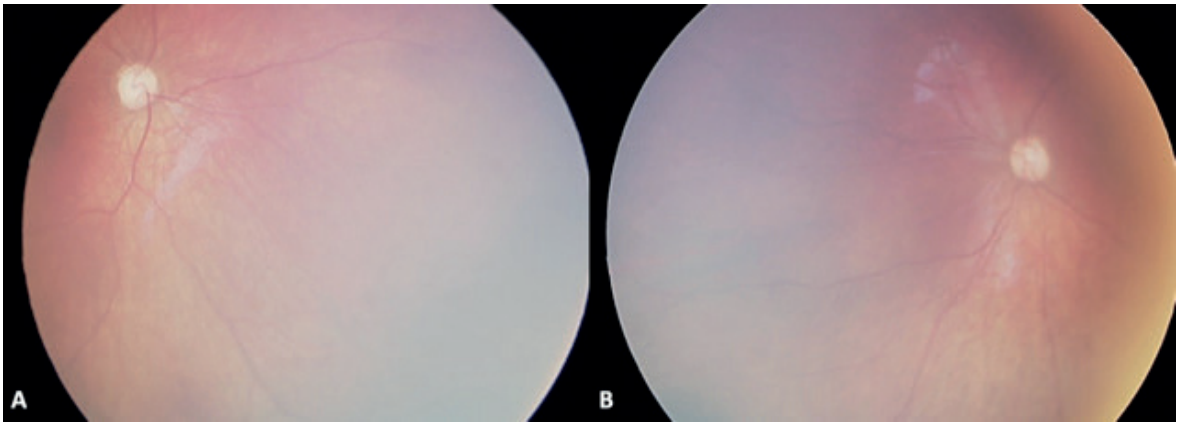

Fig. 4. Bilateral optic atrophy was diagnosed by dilated fundoscopy.

Discussion

As interest in MOG-Ab has increased, there is an increase in pediatric ON studies. Myelin oligodendrocyte glycoprotein antibody was thought to be most commonly associated with acute disseminated encephalomyelitis (ADEM) in children; however, recent studies have shown ON to be the predominant phenotype. Although MOG-Ab was previously thought to identify a benign MS subtype, it appears to be predictive for a non-MS disease and is detected in patients with AQP4-Ab negative NMOSD. Now MOG-Ab associated disease has been reassessed as a distinct entity.[5] Despite all these new antibodies and classifications, CD59 deficiency associated with nervous system involvement has been described in a small number of patients in the literature. CD59 deficiency has been reported to cause Coombs-negative hemolysis, early-onset recurrent peripheral neuropathy, chronic inflammatory demyelinating polyneuropathy, and stroke.[6-8] A homozygous missense mutation, p.Cys89Tyr in CD59 gene was identified in five infants from four unrelated families of North-African Jewish origin with Coombs-negative hemolysis accompanied by sensorimotor, demyelinating, or axonal peripheral polyneuropathy.[13] Based on these results, Ben-Zeev et al.[12] reported two historical unsolved siblings who carried the same mutation and died 17 years before the date of report. The patients were reported to have a similar course but also developed recurrent strokes, bilateral optic atrophy, and retinal involvement as hypopigmented retina and depigmented scar-like retinal lesion in one eye each. While the authors speculated that the pathogenesis of optic nerve damage may be similar to the course of the peripheral neuropathy in CD59 deficiency, macular damage secondary to MAC activation has been proposed as the mechanism of retinal involvement in the fashion of age-related macular degeneration.[14] Apart from this report, CNS involvement is relatively rare in the literature. In two siblings reported from Turkey, one had peripheral neuropathy while the other had ADEM-like presentation.[15] Similarly, a case with isolated recurrent CNS inflammatory disease has been reported.[9]

Besides ON caused by CD59 deficiency were reported in two siblings, an association between CD59 deficiency and NMOSD was also demonstrated in animal models.[10,11] Aquaporin 4 antibody binds to aquaporin 4 channel on astrocytes and activates the classical CS resulting demyelination in NMOSD.[16] Zhang and Verkman[10] showed NMOSD pathology produced by AQP4-Ab and complement following CD59 inactivation in mice. However, a major limitation of mice as models of NMOSD is the almost-zero activity of their classical CS because of complement inhibitory factors in

mice serum. Yao and Verkman[11] showed the role of CD59 deficiency in the NMOSD pathogenesis by using rats and transferring passive AQP4-Ab to models with CD59 deficiency, as they have human-like CS.

Eculizumab is a humanized monoclonal antibody that inhibits the terminal complement protein C5 to prevent its cleavage into C5a, which is proinflammatory, and C5b, which is responsible for the MAC formation.[17] Thereby, eculizumab prevents overactivation of MAC and is used in the treatment of inherited CD59 deficiency and other disorders caused by defective complement regulation, such as paroxysmal nocturnal hemoglobinuria, hemolytic uremic syndrome, and CD55 deficiency.[18] Eculizumab seems to stabilize or improve neurological symptoms and be beneficial for discontinuing other immunomodulating treatments in CD59 deficiency.[19] Eculizumab is the first drug to be approved in the European Union, United States of America, Canada and Japan specifically for use in adults with AQP4-Ab seropositive NMOSD.[17] Even though demyelinating polyneuropathy attacks were partially responsive to high dose steroids and IVIg in the patients reported by Ben-Zeev et al.[12], the effects of these treatments on optic atrophy or retinal involvement were not mentioned. Although eculizumab was not used in these patients, the authors suggested that eculizumab is necessary to prevent multisystem involvement in patients with CD59 deficiency.[12] We suggest that eculizumab can also prevent possible future attacks or reduce their severity in our patient.

CD59 deficiency may be a relatively common autosomal recessive disease in Turkey. Child neurologists should know classical findings of the disease because the attacks are preventable with eculizumab. Optic neuropathy may be a manifestation of CD59 deficiency.

Ethical approval

The parents gave their informed consent for this publication.

Author contribution

The authors confirm contribution to the paper as follows: study conception and design: CG, EY, EY, ASHK, GSU, TO, AY, UY; data collection: CG, EY, EY, ASHK, GSU, TO, AY, UY; analysis and interpretation of results: CG, UY, draft manuscript preparation: CG. 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

Fucosidosis: clinical and molecular findings of Turkish patients

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ABSTRACT

Background. Fucosidosis is a rare, autosomal recessive lysosomal storage disease caused by alpha L- fucosidase enzyme deficiency in all tissues. Here, we identify a patient with a novel homozygous pathogenic variant and atypical clinical findings and summarized the clinical and molecular features of Turkish patients reported in the literature and present.

Case. The patient was born to consangineous parents at the 28th week of gestation. He had developmental delay that was attributed to prematurity. At he age of 2.5 years, brain magnetic resonans imaging revealed hyperintensities of symmetrical periventricular, subcortical, centrum semiovale and corona radiata regions on T2 and FLAIR weighted images. He developed seizures and showed developmental regression at he age of 3,5 years. Beside, coarse facial features and hepatomegaly were detected on phsyical examination. Lysosomal enzyme analysis revelaed alfa fucosidase deficiency and molecular genetic analysis identified a novel homozygous pathogenic p. Lys431 fs variant in FUCA1 gene.

Conclusions. In Turkish patients no distinguishable clinical and radiologic finding could be established. Molecular analysis was performed in few patients. Increasing of molecular and biochemical facilities might enable to make diagnosis and increase the prevalence of the disease in countries with high rate of consanguineous marriages. Moreover, it will provide genetic counseling, and enlighten the therapeutic effects of hematopoietic stem cell transplantation.

Key words: fucosidosis, *FUCA1*, developmental regression, coarse face.

Fucosidosis is a very rare autosomal-recessive lysosomal storage disease. Deficiency of α-L-fucosidase due to biallelic pathogenic variants in FUCA1 gene leads to the accumulation of glycoproteins, glycolipids, oligosaccharides, and mucopolysaccharides in various tissues including both the central and peripheral nervous system.[1]

Clinical manifestation are typically characterized by delayed motor and cognitive functions followed by progressive neurological deteriorations, associated with systemic features of a coarse face, dysostosis multiplex, recurrent respiratory infections, angiokeratoma corporis diffusum, organomegaly, ocular abnormalities, hearing loss, growth retardation, contractures, spasticity, seizures and consequently early death.[2-4]

The diagnosis is confirmed by demonstration of reduced enzyme activity and, preferably, mutation analysis.[5,6] Hematopoietic stem cell transplantation (HSCT) may reduce the severity and slow the progression of the neurological features.[7]

Here we present a patient with a novel pathogenic mutation and atypical clinical findings and identify an overview of Turkish patients in the literature.

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Case Report

Our patient was born as a first child to consanguineous parents at the 28th gestation week with the weight of 1150 gr by cesarean delivery. He was admitted to the neonatal intensive care unit and developed grade I-II intracranial bleeding. During his follow up, developmental milestones were delayed. Holding his head in a prone position was possible at the age of 8 months and he developed sitting without support and speech at 12 months. He had recurrent respiratory infections and his sweat chloride test was performed and found abnormal. However cystic fibrosis (CF) molecular analysis was normal. He underwent an inguinal hernia operation at 1,5-year-old and he was consulted to the Department of Orthopedics for kyphosis. At the age of 2,5 years, brain magnetic resonance imaging (MRI) showed hyperintensities of symmetrical periventricular, subcortical, centrum semiovale and corona radiata regions on T2 and FLAIR weighted images (Fig. 1). Then he developed seizures and after one week he showed rapid developmental regression as losing his sitting and speech abilities. Whole exome sequencing (WES) analysis was planned, and he was referred to our clinic at the age of 3,5 years. On physical examination, his anthropometrics were as follows: height: 92cm (3p), weight: 13.5 kg (12p) head circumference: 50 cm (35p). He had a coarse face with broad nasal bridge, hypertelorism, wide ala nasis, and minimal hepatomegaly. He was not able to cooperate and had a lack of eye contact and no response to social interaction. He had axial hypotonicity, he was not able to sit without support, he had poor head control on traction and spasticity in all four extremities. He also had dysostosis multiplex, kyphosis and pectus carinatum (Fig. 2). Physical examination and clinical findings indicate an oligosaccharidosis and his WES analysis revealed a novel pathogenic homozygous c. 1290_1299delGAAGTGGTCC (p. Lys431 fs) variant in FUCA1 gene that may result in a frameshift mutation. Leukocyte enzyme analysis showed almost a non-detectable enzyme levels which confirmed the diagnosis. Transthoracic echocardiography and fundoscopic evaluation were normal, however audiological assessment identified bilateral hearing loss.

Written informed consent was obtained from the parents of the child.

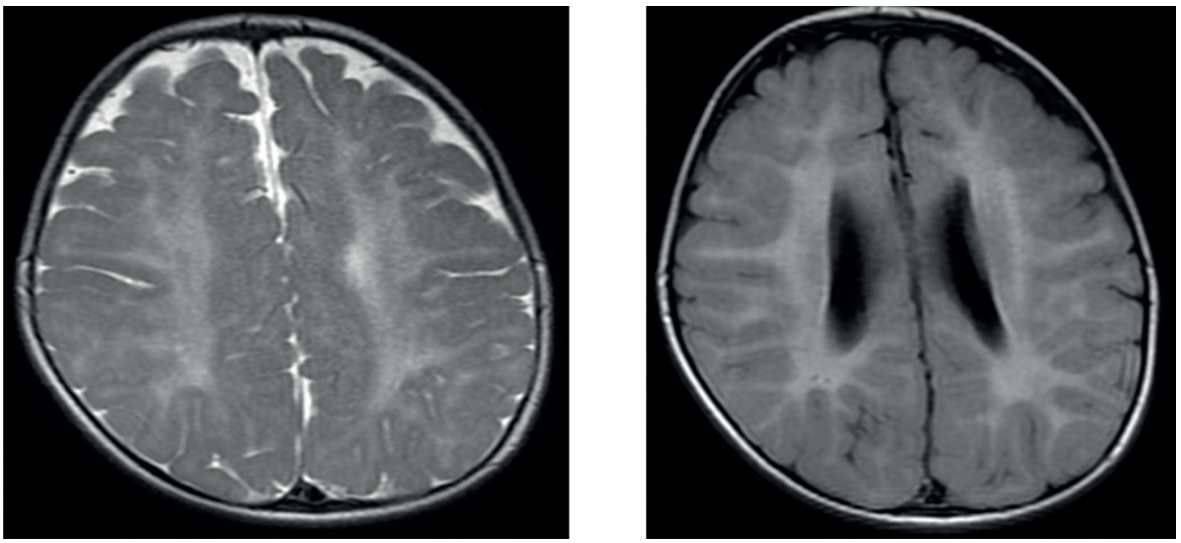

Fig. 1. Magnetic resonance imaging (MRI) T2-weighted axial images showing almost symmetrical hperintensities involving the peritrigonal, periventricular, subcortical, centrum semiovale, corana radiata regions.

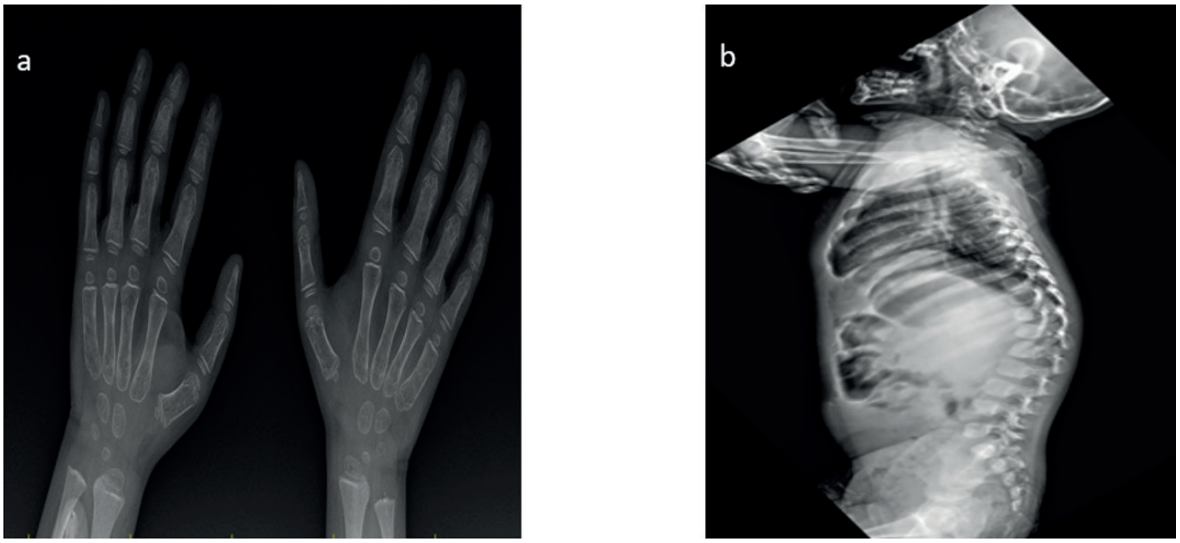

Fig. 2. Dysostosis multiplex of hands and vertebra. **a.** indicates irregularities of proximal metacarpals and bullet shaped distal phalanges; **b.** indicates anterior beaking of the vertebral body and gibbus deformity.

Discussion

Alpha L-fucosidase enzyme is a homotetramer composed of subunits of different masses (50 to 60 kDa), which causes variations in N-glycosylation and proteolytic processing. 19,20 As a result of the hydrolytic enzyme deficiency, incomplete catabolism of N- and O-glycosylproteins leads to the accumulation of fucose-containing glycolipids and glycoproteins in various tissues and urine. Fucose (C6H12O6), is found in most of the plasma glycoproteins in the mucopolysaccharides and mucolipids of various human and animal tissues.[8]

Fucosidosis is a very rare disease with a very low incidence of <1/200000. 12 To date, around 120 patients were identified.[9-11] The highest incidence has been described in Italy, Mexico, Colorado and Cuba.[9,12] We could obtain clinical and molecular manifestations of 14 Turkish patients after a detailed literature investigation. The mean age of the patients was 6 years and male/female ratio was 9/5. Consanguinity was present in 11/12 families. 9 (81%) patients had developmental delay. All patients had coarse facial features, intellectual delay, developmental delay and developmental regression. Apart from one patient, all patients (90%) had growth retardation. Recurrent respiratory infections were present in 63% of the patients. Angiokeratoma corporis diffisum and dysostosis multiplex were seen in 54% of the patients. Seizure and organomegaly were accompanied in 27% of the patients. Our patient was the only patient with hearing loss involvement. Brain MRI findings of 12 patients demonstrated periventricular and, subcortical white matter abnormalities in 7 and 4 patients, cortical and midbrain involvement in 1 and 2 patients respectively. Hypointensities on T2 and hyperintensities on T1- weighted images of globus pallidus was detected in 4 patients and hypointense areas were shown in 3 patients. Cerebellar volume increasing was demonstrated in 3 patients in early stage of the disease. (Alpha L- fucosidase enzyme analysis was performed in 9 patients and low levels were detected in a total of the patients. Only 3 patients underwent molecular analysis of the FUCA1 gene and pathogenic mutations were determined in all of the patients.[13-20]

Willems et al.[2] and Wali et al.[7] reported clinical and molecular findings of 77 and 89 patients respectively. Comparison of clinical features of those patients with Turkish patients is shown in Table I. Our findings were more consistent with Willems et al.[2] report. Almost all of the percentages of clinical manifestations in the

Table I. Comparision of prevelance of clinical findings in the literature.

	Wiliam et al.[2]	Wali et al.[7]	Turkish Cases
Mental retardation	73 (95%)	50-60%	14 (100%)
Neurologic regression	68 (88%)	50-60%	14 (100%)
Coarse face	61 (79%)	70-80%	11 (100%)
Growth retardation	60(78%)	30-40%	10 (90%)
Recurrent infections	60 (78%)	30-40%	7 (63%)
Dysostosis multiplex	45 (58%)	50-60%	6 (54%)
Angiokeratoma	40 (52%)	40-50%	6 (54%)
Seizure	29 (38%)	10-20%	3 (27%)
Organomegaly	23 (30%)	30-40%	3 (27%)
Hearing loss	9 (12%)	10-20%	3 (27%)
Hernia	7 (9%)	-	1 (9%)
Ophthalmologic findings	5 (6%)	<10%	-

study by Wali et al.[7] were lower than the Willems et al.[2] 's and ours. Particularly growth retardation was present in 30-40% of the patients in the report by Wali et al.[7], however, in Willems et al.[2] and in our report the percentages were 78% and 90% respectively. Growth retardation becomes more prominent with age and severity of the disease. The difference in the growth retardation rates in different studies might be associated with the difference in age and disease severity. The most common finding was intellectual disability in Willems et al.[2] and our report, however it was coarse face in Wali et al.[7] Hearing loss, hernia and ophthalmologic findings are the least frequent findings in all reports. Amongst the Turkish patients our patient was the only patient with hearing loss. In addition, hypothyroidism in Case 2, and angiokeratoma only on the tongue and gingiva in case 12 were the remarkable distinct findings.

In our patient, a striking finding was the positive sweat chloride test. Several authors have noted possible links between fucosidosis and CF. Both disorders are associated with high sweat electrolytes and recurrent infections of the respiratory tract without a recognized defect in systemic immunity.[21] Fucosidosis patients often have recurrent infections confined to areas of mucus-secreting ciliated epithelia. It was proposed that the terminal sugars fucose and sialic acid play a major role in defining the viscoelasticity of mucus. Therefore, alterations in the enzymatic cleavage of these sugars affect mucus cross-linking and its viscoelasticity. Without cross-linking, cilia would flail about ineffectively in watery secretions.[8]

Classical MRI findings include bilateral globi pallidi hyperintensities on T1- and marked hypointensity on T2-weighted images. In addition, there may be diffuse symmetric white matter hyperintensities on T2-weighted images with normal appearance on T1-weighted images, indicative of hypomyelination.[7] In Turkish patients all of the MRIs were abnormal, and the manifestations were heterogenous (Table II). In case 12 there were hyperintense signal alterations on T1-weighted imaging and hypointense signal alterations on T2-weighted imaging in bilateral globus pallidus, substantia nigra, and nucleus ruber. Corpus callosum thinning and superior vermian atrophy was observed. This finding caused misdiagnosis of the elder brother as neurodegeneration with brain iron accumulation disorders (NBIA) which is one of the diseases that can present itself with the same bilateral pallidal hypointensity image on T2; whereas, it does not cause hypomyelinization and atrophy like fucosidosis.[19] In case 2 in addition to cortical atrophy, focal nodular signal abnormalities in the brainstem on T2- weighted images were shown. Interestingly, in 3 siblings reported by

Table II. Clinical, molecular and radiologic features of Turkish patients.

References	Case No	Sex	Age of Onset (y)	Consang.	Developmental history	Enzyme analysis/Mutations	Cranial Imaging
Seo et al.[13]	1	M	1	+	Diagnosed with fucosidosis at the age of 1 due to progressive neurological regression, spasticity, contractures, coarse facies, hepatosplenomegaly, growth retardation, and angiokeratoma corporis diffusum. He did not develop any speech skills and was bedridden until his death at the age of 22	Negligible enzyme activity in cultured skin fibroblasts/ c.773delA, p.(Glu258Glyfs*3)	NA
Mungan et al.[14]	2	M	1,5	+	He smiled to his mother at three months, sat with support at 12 months, and walked at 18 months. Afterwards his developmental delay became more obvious. He developmentally regressed, losing his ability to walk. He never uttered any meaningful words. His past medical history was also remarkable for recurrent pulmonary infections and myoclonic seizures	No enzyme activity in leukocytes/NA	Cortical atrophy, and focal nodular signal abnormalities in the brainstem on T2- weighted images
Kanitaksis et al.[15]	3	F	9	+	She presented severe growth and mental retardation and angiokeratomas predominating over the lower trunk, the abdominal wall, the buttocks, external genitalia, and upper thighs. Smaller, lesions were also found on the gingiva and the lips	Almost complete absence of leokocyte enzyme lactivity/ NA	NA
Öner et al.[16]	4	F	6	+	She demonstrated moderate growth and mental retardation until 3 years of age, never developing expressive language or walking without support. She then showed rapid developmental regression and psychomotor deterioration, subsequently becoming severely spastic with dystonic movements of her right arm and jaw and losing all psychosocial responses	Almost complete absence of leukocyte enzyme activity/ NA	Symmetrical hyperintensity in the periventricular and subcortical white matter on T2-weighted images, hyperintensity on T1- and hypointensity on T2-weighted imaging in bilateral globus pallidus,

Consang: consanguinity, y: year, m:month, a and b indicate siblings, NA: Non available

Table II. Continued.

References	Case No	Sex	Age of Onset (y)	Consang.	Developmental history	Enzyme analysis/Mutations	Cranial Imaging
Kılıç et al.[17]	5[a]	F	8	+	Mental Motor retardation, subsequently psychomotor deterioration	Low enzyme activity in cultured fibroblast cells/NA	Atrophy of pervebtricular white matter and hypointensities in thalamus
	6[a]	F	16	+	Mental Motor retardation, subsequently psychomotor deterioration	Low enzyme activity in cultured fibroblast cells/NA	Atrophy of pervebtricular white matter and hypointensities in thalamus
	7[a]	M	12	+	Mental Motor retardation, subsequently psychomotor deterioration	Low enzyme activity in cultured fibroblast cells/NA	Atrophy of pervebtricular white matter and hypointensities in thalamus
Kau et al.[1]	8[b]	M	25 m	+	Developmental delay and regression	Low enzyme activity an mutation analysis	İncreased cerebellar volume
	9[b]	M	20 m	+	Developmental delay and regression milder than the twin brother case 8 and underwent bone marrow transplantation		İncreased cerebellar volume
	10[b]	M	2m	+	Daignosed at 2 months of age and underwent bone marrow transplantation at 4 months of age		
Kılıç E et al.[18]	11	M	12	+	His early developmental milestones were normal until two years of age . He then began to deteriorate in all developmental fields. At age 12, he had deteriorated significantly. There was intellectual delay with no speech, an inability to walk without support and an evident pattern of behavioral irritability.	Low leukocyte enzyme activity/NA	Symmetric periventricular white-matter hyperintensities contrasting with low intensities on the basal ganglia on T2 weighted images

Consang: consanguinity, y: year, m:month, a and b indicate siblings, NA: Non available

Table II. Continued.

References	Case No	Sex	Age of Onset (y)	Consang.	Developmental history	Enzyme analysis/Mutations	Cranial Imaging
Zübarioğlu et al.[19]	12	F	7	-	She developed normal until 12 months of age. She began unsupported walk at 15 months and became gradually unsteady and was lost soon after 5 years of age. She had a complete loss of voluntary movements including head control. Choreathetoid movements, especially marked on arms. There were red streaks on gingivae and blue-brown spots on tongue	Low enzyme activity in plasma, leukocytes, and cultured fibroblasts	Mild hyperintensity in cerebral deep white matter and subcortical areas bilaterally, hyperintensity on T1- and hypointensity on T2-weighted imaging in bilateral globus pallidus, substantianigra, and nucleus ruber. Corpus callosum thinning and superior vermian atrophy was also observed
Ediz et al.[20]	13	M	4	+	He started to seat with unassisted after one year and to walk at age of 27 months. He could not speak any meaningful words. He was referred with recurrent upper respiratory tract infections in four months and treated like an asthma patient. There was hypertonicity in lower extremities	NA/ c.244C > T, p.Gln82* Nonsense mutation, truncated protein	Combination of hypointensity in the medial and lateral pallidal segments of the globus pallidus and hyperintensity in its laminae on T2-W.
Our patient	14	M	3,5	+	Developmental delay, seizure and neurologic regression at 2,5 years of age	Almost complete absence of leukocyte enzyme activity/ c. 1290_1299delGAAGTGGTCC (p. Lys431 fs)	Symmetric white-matter hyperintensities on T2 weighted images on periventricular and subcortical, centrum semiovale, corona radiata regions

Consang: consanguinity, y: year, m:month, a and b indicate siblings, NA: Non available

Kau et al.[1], increased cerebellar volume was detected in early childhood which was a novel finding.

The diagnosis of fucosidosis is made by measuring the enzyme activity or by molecular analysis of FUCA1 gene. To date, 41 pathological variants of FUCA1 gene encoding alpha L-fucosidase has been identified (www.hgmd.cf.ac.uk; updated 03 October 2021).[22]

All mutations result in an almost total absence of enzyme activity suggesting that clinical heterogeneity is associated with not only residual enzyme activity but also with unknown factors.[22]

In Turkish patients, only 3 molecular analysis was reported and our patient is one of them. We report a novel homozygous deletion leads to a frameshift mutation. The other mutations were nonsense and frameshift mutations.

The management of fucosidosis is challenging. There is still no approved therapy for neurologic findings of the disease and the main management strategy is supportive with physiotherapy and other allied health input. The multidisciplinary team usually involves metabolic physicians, physiotherapists, ophthalmolog, orthopedic, cardiology and neurology specialists. Enzyme replacement therapy via cerebrospinal fluid and substrate inhibition therapies are under preclinical stages. HSCT via umbilical cord blood and bone marrow transplantation are the treatment options. HSCT was applied in a small number of patients with fucosidosis with symptoms stabilization in some early diagnosed cases however the long-term results are controversial.[8] In addition, individuals are often considered unsuitable for transplantation because they are diagnosed with advanced disease. HSCT was applied to 2 of the Turkish patients. They were siblings and case 9 had mild disease, and case 10 underwent HSCT at the age of four months before the development of clinical manifestations. However, there was no data regarding their neurologic progress. HSCT in our patient is controversial as he has neurologic manifestations.

We report a fucosidosis patient with seizure onset developmental regression. In lysosomal storage diseases seizure is generally a feature of late stages of the disease. The patient is also distinguished from the other patients by having hearing loss and a hernia. In addition, in our patient despite symmetrical periventricular white matter abnormalities, there was no globus pallidus involvement.

In Turkish patients, there were no clinical and radiologic findings to distinguish them from those in the literature. Patients had common clinical features such as coarse face, developmental delay, neurologic regression, and non-common features such as angiokeratoma, seizure and hearing loss. On the other hand, radiologic features also showed heterogeneity among the patients; some patients only had hypomyelination while some also had basal ganglia and brain stem involvement..

Ethical approval

Written informed consent was obtained from the parents of the child.

Author contribution

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