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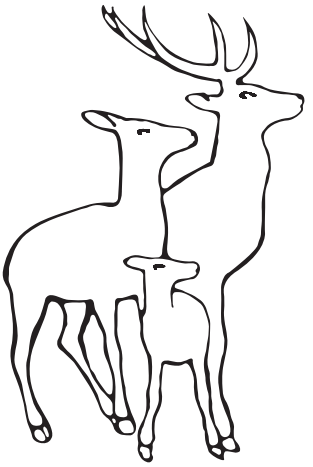

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Original Article

Assesment of obesogenic factors in school-age children

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ABSTRACT

Background. The prevalence of obesity in childhood is increasing all over the world and the World Health Organization (WHO) regards obesity as one of the most important public health problems. The aim of our study was to investigate the changes in body mass index (BMI) in children between 6 and 11 years of age and to evaluate the factors affecting this change in two different schools.

Methods. We conducted a cross-sectional epidemiological study between January and March 2016 in two different schools. School age children from two different ages (6 and 11 years) participated in the study. Children's sociodemographic characteristics and daily habits were evaluated by a questionnaire. Weight, height, body fat ratio were measured.

Results. Of all 495 students, 270 were in the 6-year old group. According to BMI classification 21.2% of students were overweight and 14.5% obese. From 6 to 11 years of age percentages of overweight and obese students increased slightly (1%). The mean daily screen time was high among overweight and obese students ($p<0.05$). The obesity rate (15.9%) was higher in public school, than in private school (6%). There was an obesogenic environment in the public school; sport facilities were limited, there was a canteen selling junk food and fizzy drink, but there was no free drinking water. Screen times of 11 year-old students were longer, and regular breakfast rates were lower than those of 6 year-old group ($p<0.05$).

Conclusions. In our study prevalence of obesity was 14.5%, and overweight was 21.2%. According to our findings obesogenic environment seemed to be a contributing factor of obesity. Screen time should also be considered in attempts to prevent obesity.

Key words: child, school age, obesity, obesogenic environment.

Obesity is an important public health problem that causes an increase in morbidity and mortality in childhood and adult age group.[1] Worldwide obesity has nearly tripled since 1975 and 41 million children under the age of 5 years and over 340 million children and adolescents aged 5-19 years were reported to be overweight or obese in 2016.[2]

According to Turkish Statistical Institute reports, obesity rates above 15 years old individuals were 19.9% in 2014.[3] According to childhood obesity surveillance initiative (COSI-TUR) 2016, 14.6% of 2nd grade elementary school children were overweight, 9.9% of them were obese in Turkey.[4]

Childhood obesity substantially is exogenous as a consequence of urbanization, increased calorie intake and diminished physical activity. According to Centers for Disease Control and Prevention (CDC) reports obesity increases from preschool period to school ages.[5,6] This slight increase by age is partly explained by some ultra-processed food consumption and dietary behaviors.[7,8] Studies are needed about the epidemiology of obesity and the impact of factors especially those can be modified, such as obesogenic environment.

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This study was presented as an oral presentation in the 1 st International Eurasian Congress of Social Pediatrics. 28th November-1st December 2018, İstanbul, Turkey.

The World Health Assembly welcomed the report of the Commission on Ending Childhood Obesity (ECHO) 2016 and its recommendations addresses the obesogenic environment and critical periods in the life course to tackle childhood obesity. According to ECHO, globalization and urbanization encourage obesity in all socioeconomic groups and children due to exposure to obesogenic environments.[9]

The aim of our study was to investigate the changes in body mass index (BMI) in children between 6 and 11 years of age, and to evaluate the factors affecting obesity, as well as to reveal the differences of environmental factors in two different schools.

Material and Methods

This cross-sectional epidemiological study was carried out in two schools, one was a private school representing high socioeconomic level, and the other was a public school representing middle and low socioeconomic level. Two different age groups (6 and 11 years) were included in the study.

Survey of Family Structure in Turkey 2011 content was used to question the socioeconomic level. Questions were developed with the help of a pediatric dietitian in creating the necessary items for evaluating the nutritional habits. By a questionnaire, information about the number of daily meals, breakfast habits, frequency and amount of consumption of snack foods and fizzy drinks, physical activity habits and daily and weekly screen time were asked. At the beginning of the study, a meeting was held in each school with school administration and council. The importance of the study was explained. At least 85% of the families filled the questionnaire forms in 4 days during the pilot study. Informed consent forms and questionnaires were delivered to the parents by class teachers 1 week before the measurements. The children whose parents agreed to participate in the study and filled the forms completely were included in the study. Height, weight and body fat ratios of each student were measured individually in a separate room.

Two schools were evaluated from the point of obesogenic environments during the study. This evaluation covered the type of food consumed during the school day, presence of gymnastic hall and free drinking water.

This is a social pediatrics doctoral thesis study.

This study was approved by the Istanbul University, Istanbul Medical School, Ethics Committee (approved number 25.01.2016-100).

Relevant permission was obtained at 04.12.2015, from Istanbul Governorship and Istanbul Provincial National Education Directorate.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its letter amendments or comparable ethical standards.

Informed consents were obtained from the patients and their parents according to institutional guidelines.

Anthropometric Measurements

All anthropometric measurements were carried out by one of the researchers with the help of a nurse. Height was measured using a portable stadiometer (SECA 213 Hamburg, Germany) recorded to an accuracy of 1 mm. Weight was measured using the Tanita BC-601 (Tanita Corporation, Tokyo, Japan). Weight was recorded to within 0.1 kg and students were asked to take off their shoes and socks or tights. Percentage of body fat (BFP) was estimated from leg-to-leg with bioelectric impedance analysis (using the Tanita Body Composition Analyzer BC-601).

Definitions

Socioeconomic levels of participants were classified according to the Survey of Family Structure in Turkey 2011, developed by the

Turkish Ministry of Family and Social Services.[10] Parental education level, parental occupation and characteristics of the accommodation were questioned for this purpose. The socioeconomic levels were classified as low, middle and high according to the scores.

Consumption of junk food and fizzy drink was asked based on the frequency and amount of crisps, gumdrop, candy, wafer, cracker, biscuit, chocolate and fizzy drink intake in a week. Exercise habits were asked as; 'how many times did your child have at least 60 minutes of physical activity in the last 7 days?' and 'does he attend a sports activity regularly?'. Mode of travel to school (school service, walking, private car) was also asked.

Body mass index values were evaluated according to the percentiles of Turkish children.[11] Values between 85 and 94 percentiles were classified as overweight and above 95 as obese. According to BFP curves of Turkish children, 85-94 percentiles were evaluated as overweight and over 95 was obese.[12]

Statistical Analysis

IBM SPSS Statistics 22 for statistical analysis (SPSS IBM, Turkey) programs were used for the analysis. Variables with normal distribution were evaluated by the Shapiro Wilks test. Kruskal Wallis and Mann Whitney U tests were used for quantitative variables. Qualitative variables were evaluated by Chi Square test.

Results

Of all students in public school 83.3% participated in the study, it was 67.7% in private school. In total, 80.8% of all students from two schools participated in the study. Recruitment of students are given in Figure 1.

Of 495 students in the study, 270 were in the 6-year old group, 225 were in the 11-year old group and 50.7% of them were female. Of all students, 50.9% were from low, 29% were from moderate socioeconomic level. The percentage of owning a mobile device was 43.0%, having television in bedroom was 15.8%. One third of the students ate junk food 1-2 times a week, 42.6% consumed fizzy drinks 1-3 times a week.

According to BMI classification 21.2% of the students were overweight and 14.5% obese. From 6 to 11 years of age percentages of overweight and obese students increased slightly (1%) (Table I). The participants' BFP was compatible with BMI percentiles. Therefore, only BMI values were used in the continuation of the study.

Among 11-year old students, the proportion of owning mobile electronic devices or computers was higher than that of 6-year old students, the difference was statistically significant ($p<0.05$). In the 6-year old group, the rate of having daily breakfast was significantly higher than that of the 11-year old group ($p<0.05$) (Table II). The daily and weekly screen time averages of 11-

Table I. BMI distribution of all students.

		n	%
BMI	Normal or low weight (all)	318	64.2
	6 year-old	176	65.2
	11 year-old	142	63.1
	Overweight (all)	105	21.2
	6 year-old	56	20.7
	11 year-old	49	21.8
	Obese (all)	72	14.5
	6 year-old	38	14.1
	11 year-old	34	15.1

BMI: body mass index

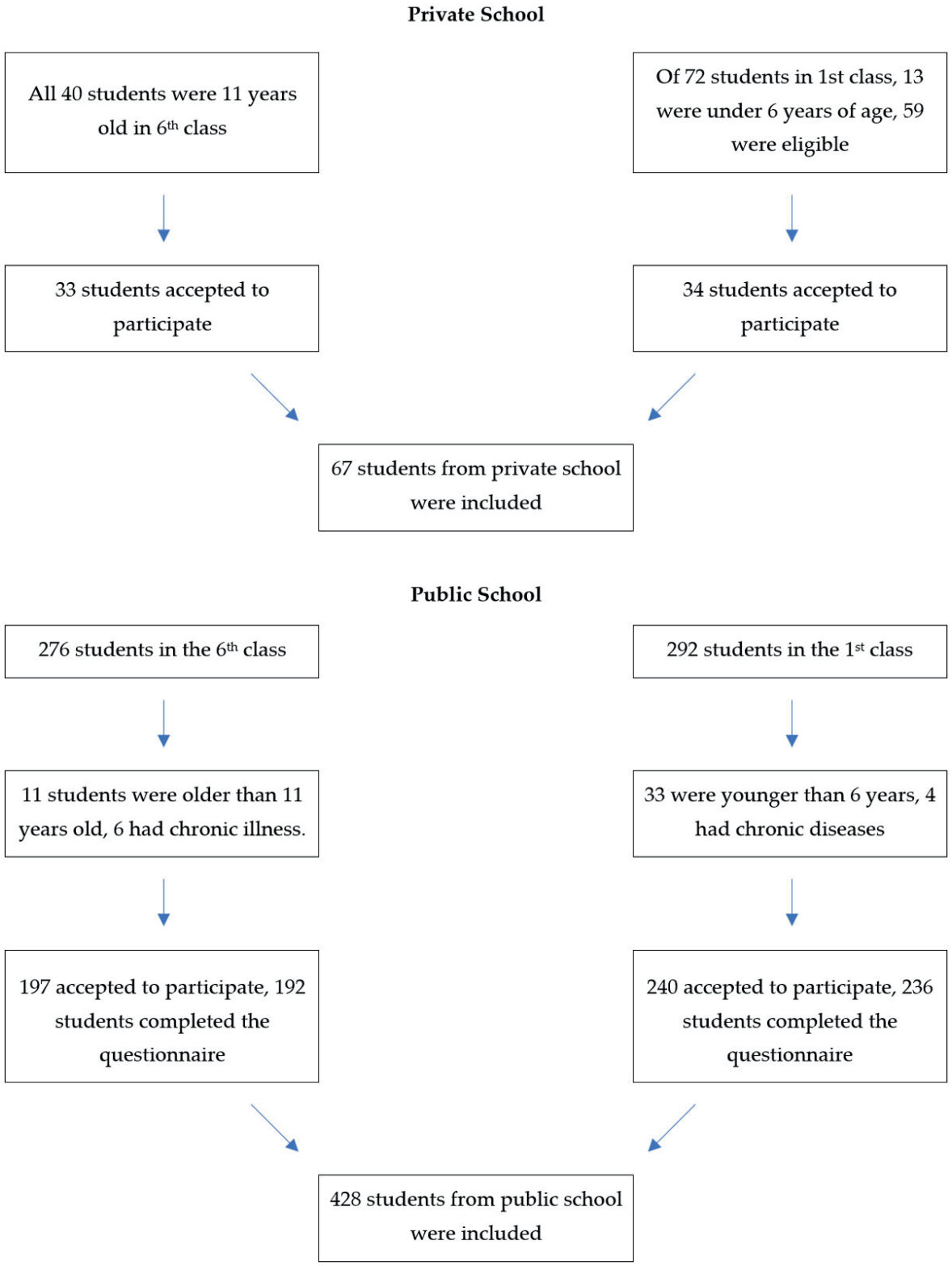

Fig. 1. Recruitment of children from two schools.

Table II. Descriptive data of two age groups.

		6 year-olds (n=270) n (%)	11 year-olds (n=225) n (%)	p
Owning mobile device or computer	Yes	60 (22.2%)	153 (68%)	0.001
	No	210 (77.8%)	72(12%)	
Breakfast frequency	No breakfast	4 (1.5%)	5 (2.2%)	
	1-3 times a week	22 (8.1%)	49 (21.8%)	0.001
	Most days of the week	40 (14.8%)	55 (24.4%)	
	Everyday	204 (75.6%)	116 (51.6%)	

Table III. Screen times of two age groups.

	6 year-olds (n=270) Mean ± SD	11 year-olds (n=225) Mean ± SD	p
Screen time during weekdays (hour)	1.81 ± 1.1	2.42 ± 1.2	0.001
Screen time during weekends (hour)	3.16 ± 2.04	3.38 ± 1.75	0.094
Average screen time (hour)	2.27 ± 1.32	2.61 ± 1.19	0.001

year old group were higher than those of the 6-year old group ($p<0.05$) (Table III).

Overweight and obese students, had longer screen time than those of normal and low weight students. This difference was statistically significant (Table IV).

Of all girls 24.7% were overweight and 13.1% obese. These figures were respectively 17.6% and 16% for boys. There was no statistically significant difference between genders.

All 67 students who were going to the private school, were at high socioeconomic level. Two hundred and fifty two (58.8%) students going to public school were at low, 99 (23.1%) were in the middle and 77 (17.9%) were in the high socioeconomic level. There was no statistically significant difference between socioeconomic level and being obese and overweight among students of the public school.

At the public school there was no indoor sports hall, there was no physical education teacher for the 1st class students. Physical education classes were carried out in the outdoor playground if the weather was good. The 1st class students were bringing their food from home, 6th class students were buying from the school canteen. In the canteen there was junk food, fruit juices, sandwich, water, yogurt drink and fizzy drinks. There was no free drinking water at the public school. At the private school there was an indoor sports hall, indoor swimming pool and outdoor playground. There were physical education teachers for both 1st and 6th class students and all physical education classes were routinely carried out. There was no canteen at the private school, breakfast, lunch and a snack were given in the cafeteria under the control of a dietitian. There was free drinking water at the private school .

Obesity rates (15.9%) were higher in students going to the public school than the private school (6%) (Table V).

Discussion

In our study we evaluated school children in two age groups from two different schools in their school environment. This is one of the few studies in Turkey on the change of obesity frequency among school children and the factors

Table IV. Screen time (hour) among obese, overweight and normal children.

	BMI			
	Normal or low weight (n=318) Mean ± SD	Overweight (n=105) Mean ± SD	Obese (n=72) Mean ± SD	*P*
Daily screen time (hour)	1.99 ± 1.12	2.27 ± 1.18	2.28 ± 1.41	0.030
Weekend screen time (hour)	3.17 ± 1.98	3.29 ±1.65	3.62 ± 1.95	0.116
Screen time a week (hour)	2.32 ± 1.25	2.53 ±.1.14	2.71 ± 1.51	0.029

BMI: body mass index

Table V. Comparison of two schools according to BMI levels.

BMI	Public school (n=428) n (%)	Private school (n=67) n (%)	p
Normal or low weight	268 (62.6%)	50 (74.6%)	
Overweight	92 (21.5%)	13 (19.4%)	0.069
Obese	68 (15.9%)	4 (6%)	

BMI: body mass index

influencing this change. We could not find any statistically significant change between 6 and11 years of age. We observed that the public school's environment was obesogenic and the number of obese and overweight students were higher in the public school.

WHO emphasizes that to successfully challenge childhood obesity; the obesogenic environment must be addressed.[13] In the UK Biobank study, it was found that obesogenic environment accentuates the risk of obesity in genetically susceptible adults.[14] Although the difference was not statistically significant, the obesity rate (15.9%) was higher in the public school than the private school (6%) (Table V). In the private school, there were both outdoor and indoor sports halls and an indoor swimming pool, but not in the public school. There was a physical education teacher for all the classes in private school, but there was only for 6th class students in the public school. Three meals were routinely given under the control of a dietitian and there was free drinking water at the private school, but these opportunities were not available at the public school. There was no canteen at the private school, but there was one at the public school. In previous studies it was found that higher consumption of water per weight was negatively associated with BMI and body fat, and obese children were less hydrated than normal peers, and they drink less water.[15,16] Compatible to other studies, we saw that these environmental factors create an obesogenic environment inducing obesity.[17-19]

There are conflicting results in the literature about socio-economic level and frequency of obesity. This may be due to the insufficient classification of socioeconomic level and/or due to the economic state of country.[20-23] Ozgüven et al.[21] evaluated 680 adolescents and found no socioeconomic level difference in prevalence of overweight and obesity. In a study from Turkey, being a member of high-income family was stated as a risk factor for childhood obesity.[22] Barriuso et al.[20] evaluated 158 articles on the relationship between socioeconomic level and childhood obesity, and found an inverse relationship between socioeconomic level and weight status. In our study, we classified the socioeconomic level according to the Survey of Family Structure in Turkey and had a comprehensive evaluation of the socioeconomic

level similar to the study of Barriuso et al.[10,20] In our study we could not find a relation between socioeconomic level and obesity.

Previous studies showed that the presence of television in the bedroom as a risk factor to become obese.[24,25] Nowadays children play games and spend time on social media on their mobile phones and tablets instead of watching television.[26,27] The proportion of owning a mobile phone/tablet or computer was generally 43.0% in our study. In the six-year old group it was 22%, and in the 11-year old group 68% (Table II). Thus, screen time of 11-year old students were longer than the 6 year-old group, and this difference was statistically significant (Table III). The overweight and obesity prevalence according to BMI increased as screen time increased, and this was statically significant like other studies.[28,29] (Table IV). Additionally, it was seen that one of the reasons of increased screen time was owning mobile devices as children get older. Intervention is needed at this point to decrease the screen time.

Unlike some studies, we could not find a correlation between skipping breakfast and obesity.[29,30] However, 75.6% of the students from 6-year old group were having breakfast every day, and it was 51.6% in the 11-year old group (Table II). The decline in the daily breakfast rates in the 11-year old group led us to think that as children grow up, their eating habits were changing in a wrong way.

There were some limitations of our study. The number of students from the private school was low. We could only measure the activity levels by self-report, and this may not reflect the true physical activity levels of the children.

In conclusion, our study was one of the few studies presenting important findings about the obesogenic school environment and obesity among students. Our findings led us think that the obesogenic school environment accentuates the risk of obesity; on the other hand, there was no single factor leading to obesity and many factors may interact with each other.

Acknowledgement

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Original Article

Aspirin-induced hepatotoxicity and anemia in children with acute rheumatic fever

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ABSTRACT

Background. The aim of this study was to investigate the frequency of anemia and hepatotoxicity associated with aspirin use in patients with acute rheumatic fever.

Methods. Patients with acute rheumatic fever followed at Erciyes University, Faculty of Medicine, Department of Pediatric Cardiology between 2015-2018 were reviewed retrospectively.

Results. A total of 286 patients with acute rheumatic fever were analysed. Aspirin treatment was started in 53 of the 286 patients (18.5%) due to arthritis. The mean age of the patients who used aspirin was 10.7 ± 2.5 years. Aspirin-induced hepatotoxicity developed in 9 (17%) of the 53 patients. Naproxen or ibuprofen was given to these patients as an alternative to aspirin. No side effects occurred in patients receiving naproxen or ibuprofen. In addition, 30% of 53 patients were initially anemic. The mean duration of aspirin use in the hepatotoxic patients who had anemia was longer than patients without anemia (p=0.02).

Conclusions. Patients with acute rheumatic fever should be closely monitored for aspirin hepatotoxicity. When aspirin hepatotoxicity develops, naproxen or ibuprofen treatment can be used safely.

Key words: acute rheumatic fever, arthritis, aspirin, aminotransferase elevation.

Acute rheumatic fever is a chronic, inflammatory and systemic disease caused by group A beta hemolytic streptococcal infections in genetically predisposed individuals. The disease manifests as a result of varying rates of inflammation of the heart, joints and basal ganglia. Rheumatic heart disease is mentioned in the presence of cardiac involvement and it is very important because it is responsible for mortality. Our country is among the middle-high risk populations in terms of acute rheumatic fever and rheumatic heart disease.[1] Therefore, early diagnosis and treatment of acute rheumatic fever are important for the prognosis of patients. Jones criteria were established in 1944 for acute rheumatic fever diagnosis and updated periodically. In the light of the latest updates in 2015, the importance of subclinical carditis in the diagnosis of acute rheumatic fever was emphasized and differences in terms of minor findings were highlighted in low and high risk populations.[2] Traditionally, the management of acute rheumatic fever is antibiotic therapy, anti-inflammatory therapy and resting. The aim of antibiotic use is the eradication of Streptococcal infection in tonsils. As an anti-inflammatory treatment, aspirin is used in arthritis, corticosteroid is used in carditis, and aspirin is added during the tapering of streoid therapy in carditis. Aspirin, a non-steroidal anti-inflammatory drug, reduces the synthesis of proinflammatory cytokines by inhibiting cyclooxygenase. Aspirin is often hepatotoxic when used in high doses. Aspirin, a direct, intrinsic hepatotoxin, causes hepatocellular hepatotoxicity. Besides the increase in alanine aminotransferase (ALT), bilirubin levels are generally in the normal range. The clinical picture of the patients is mostly mild and asymptomatic. Abnormalities

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in liver tests recover rapidly within days with discontinuation of aspirin. People with aspirin hepatotoxicity can safely use other non-steroidal anti-inflammatory drugs.[3] Naproxen treatment is an alternative treatment approach if intolerance develops during aspirin use.[4] When hepatotoxicity develops as a major side effect of aspirin, alternative non-steroidal anti-inflammatory drug or steroid therapy should be used.

The aim of this study was to evaluate the patients who developed aspirin-induced hepatotoxicity due to acute rheumatic fever and needed an alternative non-steroidal anti-inflammatory drug use and to investigate the rate of hepatotoxicity of aspirin, which is an important step in the treatment of acute rheumatic fever. In addition, the association of aspirin hepatotoxicity and anemia was also investigated in the present study.

Material and Methods

The files of the patients who were followed with the diagnosis of acute rheumatic fever between 2015 and 2018 at Erciyes University Faculty of Medicine, Department of Pediatric Cardiology were reviewed retrospectively. Patient records included age, sex, presence of arthritis, aspirin use, aspirin related side effects, serum aminotransferase levels, hemoglobin values, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), and non-steroid anti-inflammatory treatments other than aspirin. Patients with isolated carditis or chorea were excluded.

In the Department of Pediatric Cardiology, acute rheumatic fever was diagnosed according to the modified Jones criteria.[2] The first step in the anti-inflammatory treatment of arthritis in patients with acute rheumatic fever was aspirin treatment. If there was carditis together with arthritis, corticosteroid therapy (methyl prednisolone 1-2 mg / kg / day, maximum 48 mg / day) was given orally for two weeks. At the end of the treatment, the steroid dose was reduced and aspirin was added to the treatment. Steroid therapy was tapered in seven days. The biochemical evaluation of liver enzymes of the patients were performed before treatment. The dose of aspirin was started at a dose of 80-100 mg/kg/day divided four times daily, after a full dose was given for two weeks, it was reduced and discontinued in two weeks. After aspirin treatment was started, patients were asked to come for a check-up of liver enzymes in the first week of the treatment and then, control of liver enzymes was done weekly. Patients were monitored for side effects such as aspirin-induced hepatotoxicity and gastrointestinal bleeding. Elevated aminotransferase levels up to five times higher than normal was defined as mild, five to ten times higher than normal was defined as moderate and greater than ten times higher than normal limit after starting aspirin treatment was defined as severe aminotransferase elevations.[5] The upper limit of normal for aspartate aminotransferase (AST) and ALT is 40 U/L in the Biochemistry Laboratory in our hospital. In patients with carditis and arthritis, aspirin was started at a dose of 75-80 mg/kg/day one week before steroid discontinuation and the treatment duration was four weeks. In patients with arthritis, aspirin was discontinued and other non-steroidal anti-inflammatory drugs such as naproxen and ibuprofen were switched to aspirin. All patients with hepatotoxicity were evaluated for serum total and direct bilirubin, albumin, prothrombin time, INR, viral serology, ceruloplasmin and liver autoantibodies to rule out other causes of hepatitis. In the Biochemistry Laboratory in our hospital, the normal value for CRP was <5 mg/L and the normal value for ESR was <20 mm/h. Hemoglobin levels were <11.6 g/dL for children aged 8-12 years, <11.8 g/dL for girls aged 12-15 years and <12.3 g/dL for boys aged 12-15 years were defined as hemoglobin levels were below -2SD and the patients were accepted as anemic. Anemia etiologies of the patients were investigated from the file records.

Ethics committee approval was received from Erciyes University Faculty of Medicine for this

study (Date: 07.11.2018, Approval Number: 2018/573). Written and verbal consents were obtained from the parents of the patients.

Statistical analysis

In this study, the data were evaluated with descriptive statistics method (SPSS 22.0). Pearson $\chi 2$ analysis was used for the analysis of categorical data. The analysis of the data was carried out in TURCOSA (Turcosa Analytics Ltd. Şti, www.turcosa.com.tr) statistics software. The significance level was accepted as $p < 0.05$.

Results

The records of 286 patients with acute rheumatic fever were analysed. Aspirin treatment was started in 53 of 286 patients (18.5%) due to arthritis. The mean age of the patients who had used aspirin was 10.7 ± 2.5 years. The male to female ratio was 0.8. Echocardiographic evaluation was normal in 7 of the 53 patients (13.2%), while the remaining patients had first degree mitral regurgitation and/or aortic regurgitation. Nine of the 53 patients (17%) who received aspirin treatment were required to change medication due to aspirin-induced hepatotoxicity (naproxen in six patients and ibuprofen in three patients). The mean age of nine patients with aspirin hepatotoxicity was 10.5 ± 1.9 years. The remaining 44 patients received a full dose of aspirin. Naproxen or ibuprofen was preferred instead of aspirin in patients who developed aspirin induced hepatotoxicity. No side effects were reported in patients receiving naproxen or ibuprofen. Echocardiographic evaluation of aspirin-induced hepatotoxicity revealed seven patients with first-degree mitral regurgitation and two patients with first-degree mitral regurgitation and first-degree aortic regurgitation. Aspirin hepatotoxicity was asymptomatic and there were no accompanying symptoms such as abdominal pain or vomiting. Initially, CRP and ESR values were high in all patients receiving aspirin treatment. However, in patients who developed aspirin hepatotoxicity, serum total/direct bilirubin, albumin, prothrombin time, INR, ceruloplasmin levels were in the normal range. Viral serology and liver autoantibodies of these patients were also negative.

Elevated AST and ALT values were observed on average 10.9 ± 6.1 days when taking aspirin. Of nine patients with elevated aminotransferase levels, three had mild elevation, three had moderate elevation, and the remaining three had severe elevation. In patients who were started on a different non-steroidal anti-inflammatory drug after discontinuation of aspirin, aminotransferase elevation decreased to normal limits on average 12.4 ± 3.9 days. There was no significant difference in the time of normalization of liver enzymes between patients receiving naproxen or ibuprofen treatment ($p>0.05$). In patient 6, aspirin was discontinued and ibuprofen treatment was started because he had a chickenpox infection while taking aspirin. None of the patients with elevated aminotransferase developed drug-induced liver failure and no patients required a liver biopsy. Liver enzymes of patients returned to normal after discontinuation of aspirin. Table I shows the laboratory values of patients with aspirin hepatotoxicity.

Hemoglobin levels were below -2SD in four of nine patients with hepatotoxicity (44%). The mean duration of aspirin use in the hepatotoxic patients who had anemia was 15.7 ± 6.2 days. This period was 7.0 ± 1.4 days in hepatotoxic patients without anemia ($p= 0.02$). Hemoglobin levels were below -2SD in 12 (27%) of 44 patients who continued to receive aspirin but did not develop hepatotoxicity. Anemia was detected in 16 (30%) of 53 patients who were started on aspirin treatment. Two of the patients with anemia had folic acid deficiency and one patient had iron deficiency anemia. The etiology of anemia was not investigated in others. However, no statistically significant difference was found between the frequency of anemia in patients with and without hepatotoxicity ($p >0.05$). Folic acid or iron replacement was applied to those who had folic acid or iron deficiency, and it was determined that the anemia of others improved

Table I. Laboratory values of patients with aspirin hepatotoxicity.

Patients	Age (years)	CRP (mg/L)	ESR (mm/h)	Hb (g/dL)	Duration to onset of aspirin induced hepatotoxicity (days)	Elevated AST/ALT (IU/L) levels	Duration to improvement of aspirin induced hepatotoxicity (days)	NSAID
1*	15	10	26	11.4	17	77/357	12	Naproxen
2*	10	89	79	11.2	12	457/461	14	Naproxen
3	10	70.8	74	12	9	1076/1573	13	Ibuprofen
4*	9	52	104	10.8	10	250/260	9	Naproxen
5	11	34	56	13.4	7	107/112	14	Naproxen
6	8	105	62	13.6	5	24/106	4	Ibuprofen
7	10	30	78	14.5	7	76/113	14	Naproxen
8	11	45	51	12.8	7	489/402	18	Ibuprofen
9*	11	21	45	10.6	24	265/356	14	Naproxen

*: Patients with hemoglobin values below -2SD.
Hb: hemoglobin, ESR: erythrocyte sedimentation rate, NSAID: non-steroidal anti-inflammatory drug, CRP: C-reactive protein

Table II. Comparison of the clinical and laboratory data of the patients with and without hepatotoxicity.

	Patients with hepatotoxicity (n=9)	Patients without hepatotoxicity (n=44)	p
Age (years + SD)	10.5 ± 1.9	10.8 ± 2.7	>0.05
Gender (male/female)	0.8	0.9	>0.05
Mitral regurgitation, n	7	37	>0.05
Mitral regurgitation and aortic regurgitation, n	2	0	>0.05
ESR (mm/h, mean ± SD)	63.8 ± 22.8	65.7 ± 19.2	>0.05
CRP (mg/L, mean ± SD)	50.7 ± 31.8	56.3 ± 28.7	>0.05
Frequency of anemia, n (%)	4 (44%)	12 (27%)	>0.05

ESR: erythrocyte sedimentation rate, CRP: C-reactive protein

during the treatment process. Table II shows the comparison of the clinical and laboratory data of the patients with and without hepatotoxicity.

Discussion

For many years, aspirin has been used as the first-choice non-steroidal anti-inflammatory drug in the treatment of arthritis and mild carditis due to acute rheumatic fever.[6] Aspirin inhibits prostaglandin synthesis by irreversibly inhibiting cyclooxygenase 1 and thus has an anti-inflammatory effect. Aspirin is a drug with antipyretic, analgesic and anti-inflammatory properties as well as side effects such as dyspepsia, nausea, vomiting, abdominal pain, gastrointestinal ulceration, bleeding and hepatotoxicity.[7] Aspirin-induced hepatotoxicity has been described for more than 40 years.[8] Aspirin hepatotoxicity is dose-dependent, the higher the dose, the higher the risk of hepatotoxicity, although the mechanism of action of aspirin on the liver is unclear. Hepatotoxicity, which manifests itself as elevated aminotransferase levels, is rapidly recovered by discontinuation of aspirin.[9] Structural changes in hepatocytes similar to hepatotoxic drugs have been reported in the electron microscopic examination of a liver biopsy of a pediatric patient who developed hepatotoxicity while taking aspirin for acute rheumatic fever.[10]

In aspirin hepatotoxicity, aspirin is discontinued and switched to other non-steroidal anti-inflammatory drugs such as naproxen, ibuprofen, tolmetin. There is no consensus on which non-steroidal anti-inflammatory drugs will be given first. In the studies, aspirin treatment was primarily adopted as an anti-inflammatory treatment in patients with arthritis and mild carditis, however, the use of other non-steroidal anti-inflammatory drugs in case of aspirin intolerance is mentioned.[11-13] Cetin et al.[14] reported that they discontinued aspirin and administered naproxen because of aspirin-induced hepatotoxicity in 10% of children with acute rheumatic fever. In the study of Karademir et al.[15] they reported that tolmetin can be used effectively and safely in patients who cannot tolerate aspirin treatment. In this study, patients with aspirin hepatotoxicity were treated with naproxen or ibuprofen and no side effects were observed in the patients who were administered either as an alternative to aspirin.

Another serious side effect of aspirin is Reye's syndrome. Aspirin used during viral infection inhibits the fatty acid metabolism by causing mitochondrial damage and ultimately leads to liver failure accompanied by acute encephalopathy.[16] In our study, aspirin was discontinued and ibuprofen was started in one patient due to chickenpox infection while taking aspirin.

Rasa et al.[17] found that liver enzyme elevation was 12% in their study, and they recommended that the liver enzymes be checked every two days after starting aspirin, and weekly follow-up if there was no elevation. Yılmaz et al.[18] reported that aspirin hepatotoxicity developed after an average of 11 days from the beginning of aspirin treatment in pediatric patients with acute rheumatic fever, and liver enzymes of patients returned to normal values in an average of 17 days. Olgun et al.[19] reported that hepatotoxicity was observed approximately 14 days after the start of aspirin treatment, and liver enzymes returned to normal after an average of 16 days after aspirin was discontinued. Karademir et al.[15] reported that hepatotoxicity developed in the first week in patients using aspirin. In the present study hepatotoxicity secondary to aspirin in the patients was seen on average ten days after the initiation of aspirin treatment. Liver enzymes returned to normal within 12 days after discontinuation of aspirin.

There are limited studies on anemia associated with acute rheumatic fever and 42% and 62% of the anemia is mentioned in the studies.[17,20] Increased plasma volume, hemolytic anemia and decreased erythropoiesis are possible responsible causes of anemia in acute rheumatic fever.[17] It has been reported that inflammatory proteins such as TNF-α can cause anemia by decreasing erythropoiesis.[21] In the present study, the frequency of anemia was higher in patients who developed hepatotoxicity than those without hepatotoxicity (44% vs 27%), but it was not statistically significant. Hemoglobin values of the patients were not measured before the diagnosis of acute rheumatic fever. The etiology of anemia was investigated in only a few of the patients with anemia at the time of diagnosis, and the necessary vitamin and mineral supplements were given to the patients with folic acid or iron deficiency. However, it was found that the anemia of the patients with anemia improved during the anti-inflammatory treatment process. Most of the patients with arthritis also had mild carditis, and these patients were primarily given steroid therapy. Even though anti-inflammatory therapy may have contributed to the improvement of anemia, but also the improvement of anemia during the treatment period was associated with the improvement in nutrition as a result of increased appetite due to steroid therapy. Hemolytic anemia was not considered in the etiology of anemia because serum bilirubin levels of the patients were within the normal range. Similarly, Rasa et al.[17] reported that anemia in patients with acute rheumatic fever improved without treatment. Another finding in the present study was that aspirin hepatotoxicity occurred later in patients with anemia than in patients without anemia. In an animal study, it was noted that iron deficiency

can cause changes in drug metabolism.[22] In this study, the reason for the late occurrence of aspirin hepatotoxicity in the patients with anemia could not be explained.

The limitations of the study were a small sample size and absence of serum aspirin levels of the patients. However, the fact that no reason was found in the differential diagnosis of acute hepatitis in patients' laboratory tests and the aminotransferase levels returned to normal after aspirin was discontinued supported aspirin hepatotoxicity.

Patients with acute rheumatic fever were retrospectively evaluated in the present study and the rate of development of aspirin-induced hepatotoxicity was 17%. However, 30% of the cases had anemia. Although aspirin treatment is the first-line anti-inflammatory drug in patients with arthritis and mild carditis in acute rheumatic fever, hepatotoxicity and other systemic side effects should be considered. Naproxen and ibuprofen are safe non-steroidal anti-inflammatory drugs when aspirin-related side effects are observed in the treatment of acute rheumatic fever. In this study, it was determined that the rate of anemia was higher in patients who developed hepatotoxicity. Further studies are needed to determine the relationship between anemia and hepatotoxicity in acute rheumatic fever.

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Original Article

Upward trend in the frequency of community-acquired methicillin-resistant *Staphylococcus aureus* as a cause of pediatric skin and soft tissue infections over five years: a cross-sectional study

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ABSTRACT

Background. The increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and its resistance to multiple antibiotics has become a serious challenge since the early 2000s. Especially, community-acquired MRSA (CA-MRSA) infections that appear mainly as skin and soft tissue infections (SSTIs) tend to increase worldwide. The objective of this cross-sectional study was to evaluate the trends in the frequency of SSTIs due to community-acquired *S. aureus* among children.

Methods. All children with SSTIs caused by culture positive community-acquired *S. aureus* during the period from 2013 to 2018 were included in this study. Data of the outpatients were collected from medical records. Annual alteration in frequencies of CA-MRSA and community-acquired methicillin-sensitive *S. aureus* (CA-MSSA) were evaluated.

Results. A total of 112 cases was evaluated. Of these, 35 (31.25%) were CA-MRSA. The rates of CA-MRSA had emerged from an increasing annual frequency of 9.5 cases per 10,000 SSTIs as of 2014 to 96.8 cases per 10,000 SSTIs in 2018. The ratio of cases with CA-MRSA to cases with CA-MSSA was 0 – 0.09 in two years of the study period and increased to a maximum ratio of 0.6 – 0.72 in the last two years. Consequently, the frequency of *S. aureus* in cases with SSTIs was significantly higher in 2016 – 2018 compared to the initial study period within the years of 2013-2015 [p<0.001, relative risk increase: 7 (2.6-28.7) for CA-MRSA and p=0.002, relative risk increase: 2.1 (1.2-3.5)]. Cases with CA-MRSA increased approximately eight-fold during the six-year-study period.

Conclusions. The rates of CA-MRSA in SSTIs among children increased significantly compared to CA-MSSA. The clinical impact of this increase should be evaluated, especially in patients with SSTI who are unresponsive to empirical treatment.

Key words: Methicillin-resistant *Staphylococcus aureus*, child, skin diseases, infectious, soft tissue infections.

Staphylococcus aureus is a common pathogen that causes most skin and soft tissue infections (SSTIs). Despite the initial description of methicillin-resistant *Staphylococcus aureus* (MRSA) as a nosocomial pathogen in the 1960s,[1] it has become an outspread cause of community-associated infections. Since the early 2000s, the increasing prevalence of MRSA and its resistance to multiple antibiotics has become a serious challenge.[2] Besides invasive infections such as bacteremia, pneumonia, urinary tract infections; up to 96% of community-acquired MRSA (CA-MRSA) infections detected in children are SSTIs such as abscess, furuncle,

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and cellulitis.[3-6] The prevalence of CA-MRSA infections has been reported with different characteristics in different populations worldwide.[7,8] Recent studies showed the controversial rates of CA-MRSA infections, ranging from 70% in some regions of the United States to no cases in Finland.[7,8] While the rates of healthcare-associated MRSA infections have recently declined due to infection control programs, CA-MRSA infections tend to increase worldwide.[9]

In Turkey, the prevalence of SSTIs caused by CA-MRSA in children has been investigated in a limited number of studies and to date, there is no study focusing on a large-scale, long term alteration of the number of CA-MRSA infections in children. This cross-sectional study aimed to evaluate the changing frequencies of SSTIs caused by CA-MRSA among children in the study center, between the period of 2013-2018.

Material and Methods

Study subjects

This study includes outpatients with SSTI between January 1, 2013 and December 31, 2018 at Dr. Behçet Uz Children's Hospital a pediatric referral and tertiary care hospital in Izmir, Turkey, with an annual 600,000 outpatient visits and 24,000 hospitalizations in 2018. The medical records of the patients were obtained from the hospital electronic information system. All children diagnosed with L02 and its subsections (L02.0, L02.1, L02.2, L02.3, L02.4, L02.8, and L02.9) according to the International Statistical Classification of Diseases 10th Revision (ICD-10) were screened retrospectively from the medical records. Patients with SSTI in whom methicillin-sensitive *S. aureus* (MSSA) or MRSA were identified from wound swab or abscess cultures during outpatient clinic visits or hospital admissions. All culture positive patients compatible with the case definition mentioned below were enrolled in the final analysis of the study.

Definitions

Patients with community-acquired MRSA or MSSA infections were defined according to the criteria of the Centers for Disease Control and Prevention (CDC).[10] Those were the cases, culture-confirmed within 72 hours of admission; having no history of previous hospitalization, surgery, dialysis, or residence in a long-term care facility, and having no permanent indwelling catheter present at the time of culture.

Microbiological analysis

Identification of the isolates was performed by standard microbiological procedures. Organisms were identified to the species level by catalase and coagulase test. Phoenix automated system (BD, Sparks, MD, USA) and VITEK-2 compact automated system (bioMérieux, Marcy l'Étoile, France) were used for MRSA identification and susceptibility testing. Detection of MRSA in both systems is based on both oxacillin and cefoxitin minimum inhibitory concentrations, interpreted according to The Clinical and Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing.[11,12]

Statistical analysis:

The data were analyzed using SPSS Statistics 17.0 (International Business Machines Corp, Armonk, NY) and MedCalc Version 11.6 (MedCalc Software BVBA, Ostend, Belgium) software. The Kolmogorov-Smirnov test was used to determine whether the distribution of continuous variables was approximately normal, and the Levene test was used to determine whether the assumption of homogeneity of variance was met. Baseline characteristics were given as medians and interquartile ranges (IQRs) for continuous variables, and numbers of cases and percentages were calculated for categorical variables. The Mann- Whitney U test was used to examine differences between groups in non-normally distributed continuous variables. Categorical variables were evaluated

by the Fisher exact test for expected frequencies. Results were considered statistically significant when $p < 0.05$.

The number of CA-MRSA SSTI cases was calculated for each year. The rate of MRSA infections (with a 95% Poisson confidence interval) for each year and the relative risk reduction (determined by comparing groups) were calculated and are given as percentages. The relative risk ratio was also calculated, and a 95% confidence interval was used for the incidence rate.

Ethical approval was obtained from the Local Ethical Committee of Behçet Uz Children's Hospital on July 18, 2019. (Report number: 2019/320) All experiments were carried out in compliance with relevant laws and guidelines by following the ethical standards of the Declaration of Helsinki.

Results

During the study period, a total of 4,234,165 patients were recorded in the outpatient clinics and the emergency department. Skin and soft tissue infections were diagnosed in 6,718 cases. The annual numbers of outpatients and SSTI cases are reviewed in Table I. Of these, *S. aureus* grew in 155 cultures. Thirty-three patients who did not meet the case definition criteria and 10 patients due to the inadequate data were excluded. As a result, 112 cases accepted as SSTI caused by community-acquired *S. aureus* were enrolled in the final evaluation.

Among the 112 isolates, 35 (31.25%) were CA-MRSA, and 77 (68.75%) were CA-MSSA. Clindamycin resistance was found in two of 35 (5.7%) patients with SSTI due to CA-MRSA.

The trend in frequencies of CA-MRSA and CA-MSSA

In 2013, CA-MRSA was not detected in any of the SSTIs. However, CA-MRSA had emerged from an annual frequency of 9.5 cases per 10,000 SSTIs as of 2014 to 96.8 cases per 10,000 SSTIs in 2018. The frequencies of CA-MRSA and CA-MSSA are shown in Table I. The frequencies of CA-MRSA and CA-MSSA infections were calculated with the Poisson 95% confidence interval in each year and compared with the consecutive annual interval. No significant relative risk was present in these years ($p > 0.05$) meaning no increase compared with the previous year (data shown in Table II). However, the ratio of cases with CA-MRSA to cases with CA-MSSA was 0 – 0.09 in two years of the study period and increased to a maximum ratio of 0.6 – 0.72 in the last two years. Consequently, the frequency of CA-MRSA and CA-MSSA in cases with SSTIs was found to be significantly higher within the years of 2016-2018 compared to the initial study period 2013 - 2015 ($p < 0.001$

Table I. The annual rates of community-acquired *Staphylococcus aureus* in patients with skin and soft tissue infections.

Year	Total number of patients admitted to the hospital (n)	Number of cases with SSTIs (n)	MRSA positive cases (n)	MSSA positive cases (n)	Prevalence of MRSA per 10,000 cases with SSTI (n)	Prevalence of MSSA per 10,000 cases with SSTI (n)
2013	582,067	539	0	5	0	92.7
2014	636,396	1048	1	11	9.5	104.9
2015	657,147	1153	3	8	26	69.3
2016	752,123	1112	6	15	53.9	134.8
2017	788,702	1239	12	20	96.8	161.4
2018	817,730	1627	13	18	79.9	110.0
Total	4,234,165	6718	35	77	52.1	114.6

SSTI: skin and soft tissue infections, MRSA: methicillin-resistant *Staphylococcus aureus*, MSSA: methicillin-sensitive *Staphylococcus aureus*.

Table II. Comparison of poisson 95% confidence interval of cases with community-acquired Methicillin-resistant *Staphylococcus aureus* and Methicillin-sensitive *Staphylococcus aureus* by consecutive years.

Years	Comparison throughout consecutive years	
	CA-MRSA	CA-MSSA
2013-2014	p=0.4744 (95% CI: 0-75.82)	p=0.8 (95% CI: 0.2-2.7)
2014-2015	p=0.3651 (95% CI: 0.006-4.5)	p=0.36 (95% CI: 0.5-4.3)
2015-2016	p=0.2917 (95% CI: 0.44-12.8)	p=0.12 (95% CI: 0.7-5.2)
2016-2017	p=0.2351 (95% CI: 1.7-15.9)	p=0.5 (95% CI: 0.3-1.7)
2017-2018	p=0.63 (95% CI: 0.34-1.9)	p=0.2421 (95% CI: 0.34-1.36)

CA-MRSA: community-acquired methicillin-resistant *Staphylococcus aureus*, CA-MSSA: community-acquired methicillin-sensitive *Staphylococcus aureus*.

and p=0.002 consecutively.) Between the two periods, a 5-fold increase in the frequency of CA-MRSA and a 1.5-fold increase in the frequency of CA-MSSA were observed. During the six-year period, while the frequencies of the cases with SSTIs associated with CA-MSSA fluctuated, cases with CA-MRSA increased approximately eight-fold.

Discussion

Although infections caused by CA-MRSA is an emerging public health problem worldwide, a limited number of studies have been published on CA-MRSA prevalence in SSTIs in children.[13,14] This study is one of the largest series of children with SSTIs. It is also the first to investigate the changing frequency of CA-MRSA in the etiologic spectrum of pediatric SSTIs in Turkey. It clearly points out that the rate of CA-MRSA in SSTIs has steadily increased in less than a decade.

In the last few decades, CA-MRSA infections have increased worldwide. In a study from the United States, the incidence of CA-MRSA associated SSTIs in adult patients was reported to increase from 24.0 cases per 100,000 people to 164.2 cases per 100,000 people over a five-year study period.[13] In another study, similarly, the annual prevalence of adult patients with CA-MRSA was reported to have significantly increased from 8.9% in 1996 to 39.6% of MRSA cultures in 2005.[14] A study from Saudi Arabia which also included pediatric cases indicated that the prevalence of CA-MRSA infections increased from 9.9 to 67 per 10,000 admissions from 2001 to 2008, and the percentage of CA-MRSA in MRSA isolates increased from 20% to 59%.[15] As in adult studies, a study concerning children indicated that the rates of CA-MRSA infections increased from 12.2/10,000 to 145/10,000 during a 10-year period.[16] Compatible with the literature, the data of the current study also revealed that CA-MRSA rates in SSTIs had increased from 9.5 to 79.9 per 10,000 cases between 2014 and 2018, suggesting a trend similar to those worldwide.

In a 2017 report by World Health Organization (WHO), the consumption of antibiotics in Turkey was among the highest across the WHO European region.[17] The increase in the rates of CA-MRSA in SSTIs might be a reflection of the increased use of antibacterials. This resistance may pose a risk of treatment failure since most of the community-acquired SSTIs in children outpatients are treated with antimicrobial drugs such as sulbactam-ampicillin or amoxicillin-clavulanate which the community-acquired *S. aureus* strains tend to be resistant to.[18,19] Considering the high frequency of MRSA in the current study, it is thought that commonly used antimicrobials in our daily practice may not be effective in almost one-third of CA-MRSA-related SSTIs, suggesting that the selection of alternative antimicrobial drugs, such as clindamycin or rifampin is necessary for the initial treatment in anticipation of CA-MRSA. Only two of the 35 CA-MRSA cases in the study were resistant to clindamycin, suggesting

that clindamycin can be a favorable choice for SSTIs despite its side–effects and reported clinical failure due to inducible clindamycin resistance.[20]

Several considerations should be noted when interpreting the results. First, this was a retrospective study, which has inherent limitations when compared to randomized clinical trials. Second, the patients with SSTI were selected based on the current ICD codes, so the total SSTI numbers may be slightly different due to incorrectly entered diagnostic codes. Third, the study results reflect a single-center experience. Multicenter studies may yield more reliable data to support the increasing trend of resistance in community-acquired *S. aureus* infections.

In conclusion, this study showed that CA-MRSA frequency in the etiology of SSTI in children has increased compared to that of CA-MSSA. The clinical impact of this increase should be taken into account especially in the patients with SSTI, who are unresponsive to empirical treatment.

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Original Article

Altered gut microbiota is associated with feeding intolerance in preterm infants

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ABSTRACT

Background. Feeding intolerance (FI) is a common complication that may cause great harm to preterm infants. The mechanism of FI remains unclear, but probiotics may help prevent and alleviate its symptoms. We hypothesized that the alteration in gut microbiota may be associated with the development of FI. Our study aimed to investigate the association between gut microbiota and FI in preterm infants.

Methods. Ninety-seven preterm infants were divided into the FI group (N=42) and the feeding tolerance (FT) group (N=55) depending on whether the infants were diagnosed with FI. The fecal samples of each infant were collected on the 7th day after birth. Fecal microbiota was analyzed by 16S rRNA sequencing. Plasma motilin were detected on day-1, 7, 14, and 21.

Results. The microbial diversity of the FI group was significantly lower than that of the FT group. The abundance levels of phylum Proteobacteria, class Gammaproteobacteria, genera such as *Escherichia/Shigella* were higher in the FI group than in the FT group. The abundance levels of phylum Firmicutes, class Negativicutes, and genus *Veillonella* were higher in the FT group than in the FI group. The motilin levels on days 7 and 14 were negatively correlated with the FI-enriched genera *Planomicrobium* and *Vibrio*, respectively. Our study also found gut microbiota was correlated with FI clinical characteristics, including gestational age, birth weight, age of FI diagnosis, age of FI disappearance, and FI duration.

Conclusions. Altered gut microbiota is associated with FI in preterm infants. FI cases typically have lower microbial diversity, a decreased abundance of beneficial bacteria, and an increased abundance of pathogenic bacteria. Gut microbiota is correlated with the clinical characteristics of FI. The decrease in motilin secretion caused by some bacteria may lead to the occurrence of FI.

Key words: feeding intolerance, gut microbiota, 16S rRNA gene, preterm infant, motilin.

Feeding intolerance (FI) is a common gastrointestinal complication among preterm infants. The incidence of FI is about 60–70% in preterm infants with a birth weight (BW) of less than 2,000 g.[1] FI in preterm infants is mainly manifested by gastric retention, vomiting, abdominal distension, and feeding plan disruption. It often leads to inadequate nutrient intake and postnatal growth retardation in preterm infants.[2] Long-term parenteral nutrition accompanied with FI also increases the incidence of complications such as nosocomial infection, metabolic disorders, and liver damage.[3] FI is an important cause leading to prolonged hospitalization and increased complications of preterm infants.

The human gut harbors a large number of microbes, which are named ''gut microbiota.'' Normal gut microbiota plays an important role in human health. The gut microbiota of preterm infants differs dramatically from that of term infants, children, and adults.[4] In preterm infants, gut microbiota disorders are associated with many diseases, such as necrotizing enterocolitis (NEC) and sepsis.5 FI may be an

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early manifestation of NEC. Supplementation with probiotics may be helpful in preventing FI in preterm infants,[6] suggesting that gut microbiota plays a role in FI among preterm infants. However, this aspect has yet to be fully explored, and the relationship between gut microbiota and FI remains unclear. Our study aimed to investigate the relationship between gut microbiota and FI in preterm infants. It would help reveal new information related to the prevention and treatment of FI in preterm infants.

Material and Methods

Diagnostic criteria of feeding intolerance

Referring to the definition of FI by Moore et al.,[7] we defined infants who met two of the following conditions to have FI: (1) gastric residual volume (GRV) of more than 50% of the previous feeding volume (≥ twice within 24 h); (2) emesis, abdominal distention, or both; and (3) disruption of the patient's feeding plan presented as reduced or suspended feeding. We considered that FI disappeared when all of these conditions were no longer observed in 24 h. According to the 2015 Canadian Feeding Guideline,[8] GRV was checked only after a minimum feed volume (per feed) was attained (minimum volume: 500–749 g: 3 ml, 750–1000 g: 4 ml, >1000 g: 5 ml).

Study design and participants

This study is a single-center cross-sectional study. The inclusion criteria were as follows: (1) infants with gestational age (GA) of ≤34 weeks; (2) infants who were born between June and December 2018; and (3) infants who were admitted to the Neonatal Intensive Care Unit (NICU) of Peking University Third Hospital within 2 h after birth. The exclusion criteria included the following: (1) infants who were not fed within 24 h after birth; (2) infants who died or gave up treatment within 7 days of birth; (3) infants who suffered from congenital gastrointestinal malformations or hereditary metabolic diseases; and (4) infants whose guardians did not sign an informed consent.

All preterm infants in our study were fed in accordance with the 2015 Canadian Feeding Guideline.[8] Each infant was fed within 24 h after birth. Breast milk was the first choice for the infants; however, if their mothers could not produce breast milk, preterm formula was considered. In our study, no probiotics or erythromycin was given to the infants within 1 week. All the subjects who met the inclusion and exclusion criteria in our study were divided into the FI group and the feeding tolerance (FT) group in accordance with the diagnostic criteria of FI.

Blood sample collection and motilin detection

The residual blood samples of the infants were collected on day-1, 7, 14, and 21. A total of 178 blood samples were collected in our study. All blood samples were centrifuged and stored at –80°C until they were assayed. Enzyme-linked immunosorbent assay (Multiskan FC, Thermo scientific, USA) was used for the quantitative measurement of motilin.

Fecal sample collection and DNA extraction

Fresh fecal samples were collected from diapers by using a sterile spoon and placed in sterile tubes on day 7 after birth. Then, the fecal samples were transported immediately to the laboratory on ice and stored at −80°C prior to analysis.

DNA was extracted from each fecal sample by using a QIAamp fast DNA stool mini kit (Qiagen, Germany) in accordance with its improved protocol. In detail, 1 ml of InhibitEX buffer and a proper amount of glass beads (0.5 mm diameter, Qiagen) were added to 200 mg of each fecal sample. The mixture was homogenized and beaten at 60 Hz for 1 min. twice with a homogeneous instrument (FASTPREP-24, Aosheng Biotech, China). Afterward, DNA was purified in accordance with the manufacturer's instructions.

16S rRNA gene amplicon and sequencing

The V3 to V4 region of bacterial 16S ribosomal RNA genes were amplified through a polymerase chain reaction by using the barcoded primers 341F 5′-CCTACGGGRSGCAGCAG-3′ and 806R 5′-GGACTACVVGGGTATCTAATC-3′. Amplicons were extracted from 2% agarose gels and purified using an AxyPrep DNA gel extraction kit (Axygen Biosciences, Union City, CA, U.S.). All the quantified amplicons were pooled to equalize the concentrations for sequencing with Illumina MiSeq/HiSeq (Illumina, Inc., CA, USA). The 250 bp paired end reads were overlapped on their 3′ ends for concatenation into original longer tags by using PANDAseq (https://github.com/neufeld/pandaseq, version 2.9).

Ethical statement

This study was approved by Peking University Third Hospital's Medical Science Research Ethics Committee (Ethical Approval No.: 054-02), Beijing, China. The guardians of all the participants were informed of the purpose of the study, and they provided written informed consent in accordance with the Declaration of Helsinki.

Statistical analysis

SPSS (ver. 22.0, SPSS, Inc., Chicago, USA) and R (ver. 3.5.1, R Development Core Team, Vienna, Austria) were used for statistical analysis. Normally distributed quantitative data were represented as mean and standard deviation (SD), and Student's t-test for independent samples was used for comparison between groups. Qualitative data were represented as relative frequency and percent distribution, and chi-square test was used for comparison between groups. Microbiota data were summarized using α-diversity and β-diversity measures to compare the microbial communities between groups. Shannon and Simpson index measurements were conducted to analyze α-diversity and place weight on species richness and evenness. A Wilcoxon test ("wilcoxon.test" function in the R "stats" package) was used to determine significance. β-diversity indicated the shared diversity between bacterial populations in terms of ecological distance. Unweighted and weighted UniFrac distances were calculated for β-diversity analysis by using a table of operational taxonomic units (OTUs) and a phylogenetic tree. The unweighted UniFrac reflected the differences in community membership, whereas the weighted UniFrac mainly presented the differences in abundance. Ordination plots were generated through principal component analysis (PCOA). Differential abundance analysis was conducted at a genus level via a nonparametric Wilcoxon rank sum test, and adjusted for multiple testing by using the Benjamini–Hochberg procedure.[9] Linear discriminant analysis (LDA) effect size (LEfSe) analysis (LEfSe v1.0) was carried out to determine the organisms most capable of explaining differences between the two groups.[10] Different organisms with an LDA score cutoff of 2.0 were identified. Spearman correlation coefficients were calculated using R (package "stats"), and a correlation heatmap was drawn with R (package "corrplot"). For result interpretation, $p < 0.05$ was considered statistically significant.

Results

A total of 97 subjects met the inclusion and exclusion criteria in our study. In accordance with the diagnostic criteria of FI, 55 cases were included in the FT group and 42 cases were placed in the FI group. The age of FI diagnosis was 2.9 ± 0.9 days (2–5 days). The age of FI disappearance was 13.6 ± 5.3 days (8–26 days). The FI duration was 10.7 ± 5.2 days (4–24 days). There were no significant differences in gestational age, birth weight, gender, delivery mode, feeding type, and antibiotic exposure between the two groups ($p > 0.05$, Table I). Three infants in our study developed NEC (one in the FI group, two in the FT group) and one of them died of NEC. The details were shown in Table II.

Table I. Characteristics of the study subjects.

Characteristics	Groups		p-value
	Feeding intolerance (N = 42)	Feeding tolerance (N = 55)	
Gestational age (week)	30.0 ± 2.1 (25.3–34)	30.6 ± 1.9 (25.2–34)	0.132
Birth weight (g)	1270 ± 343 (700–2,070)	1375 ± 348 (600–1,970)	0.141
Male, n (%)	22 (52.4)	37 (63.7)	0.137
Caesarean section delivery, n (%)	26 (61.9)	41 (74.5)	0.181
Human milk feeding mainly, n (%)	11 (26.2)	13 (23.6)	0.773
Antibiotic exposure, n (%)	40 (95.2)	47 (85.5)	0.218
Duration of antibiotic exposure (day)	5.2 ± 2.3 (0–7)	4.4 ± 2.3 (0–7)	0.133

Continuous variables are shown as means ± standard deviation (minimum-maximum).
Human milk feeding mainly: human milk accounts for more than half of total milk intake.

Table II. Characteristics of the cases with necrotizing enterocolitis.

Case	Group	GA (week)	BW (g)	Age at diagnosis of FI (day)	Age at disappearance of FI (day)	Age of diagnosis of NEC (day)	NEC stage	Outcome of NEC
1	FI	28.6	1,100	2	15	26	II	Cured
2	FT	29.6	1,400	-	-	11	II	Cured
3	FT	28.1	910	-	-	12	III	Died

BW: birth weight, FI: feeding intolerance, FT: feeding tolerance, GA: gestational age; NEC: necrotizing enterocolitis -: non-existent.

Microbial composition and abundance analysis

The microbial composition of the two groups are shown in Fig. 1. At the phylum level, the top four phyla of the two groups were the same, and they were Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria. In the FI group, the abundance percentages were as follows: 80.9% Proteobacteria, 11.5% Firmicutes, 5.1% Bacteroidetes, and 1.9% Actinobacteria. In the FT group, the abundance percentages were as follows: 69.5% Proteobacteria, 19.7% Firmicutes, 5.8% Bacteroidetes, and 4.0% Actinobacteria. At the genus level, the top four genera and their abundance percentages in the FI group were 59.7% *Klebsiella*, 8.2% *Serratia*, 7.9% *Escherichia/Shigella*, and 3.9% *Bacteroides*. The top four genera and their abundance percentages in the FT group were 55.2% *Klebsiella*, 9.5% *Serratia*, 4.8% *Veillonella*, and 4.1% *Enterococcus*. The relative abundance levels of *Bifidobacterium* in the FI and FT groups were 1.3% and 3.2%, respectively. The abundance of *Lactobacillus* was very low and not among the top 20 genera found in both groups.

Diversity comparison between the two groups

Both α- and β-diversity indices were adopted to represent the comparison of microbiota diversity between the FI and FT groups. Simpson and Shannon indices were used to describe the α-diversity of the gut microbiota between the two groups. The Simpson index of the FI group was significantly lower than that of the FT group ($p = 0.016$; Fig. 2A). This result indicated that the diversity of the gut microbiota in the FI group was significantly lower than that of the FT group. The Shannon index of the FI group was lower than that of the FT group, but their difference was not statistically significant ($p = 0.053$; Fig. 2B). Significant difference in β-diversity between the two groups was detected by the weighted ($p < 0.05$, Fig. 2C) but not the unweighted ($p > 0.05$, Fig. 2D) PCOA (principal component analysis) based on the distance matrix of UniFrac. These results meant that the fecal microbial structure in the FI group was significantly different from that of the FT group because of the presence of OTUs.

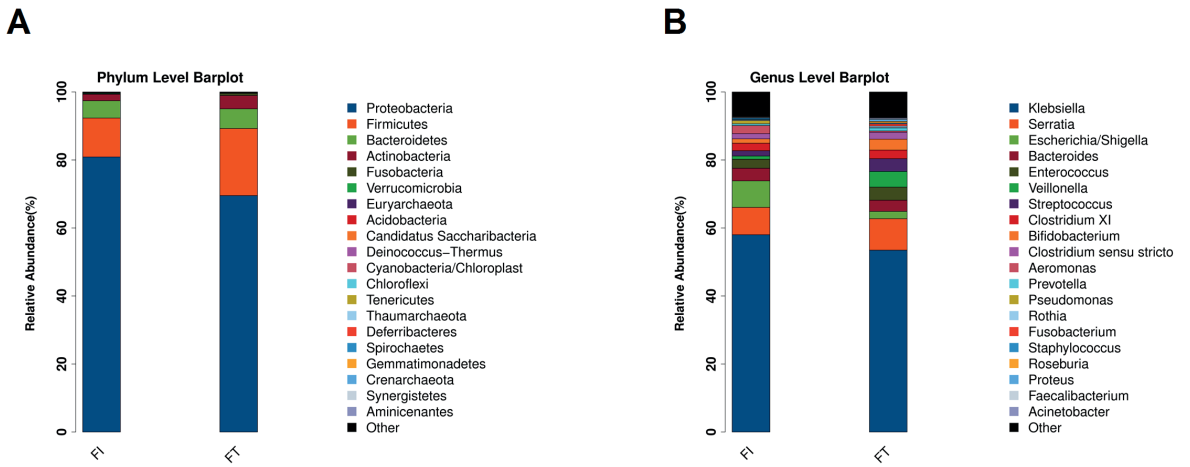

Fig. 1. Relative abundance of microbiota between the two groups at phylum and genus levels. The top 20 phyla **(A)** or genera **(B)** are displayed in different colors. The percentages of each taxon represent the relative abundance of the corresponding microbiota.

FI: feeding intolerance, FT: feeding tolerance.

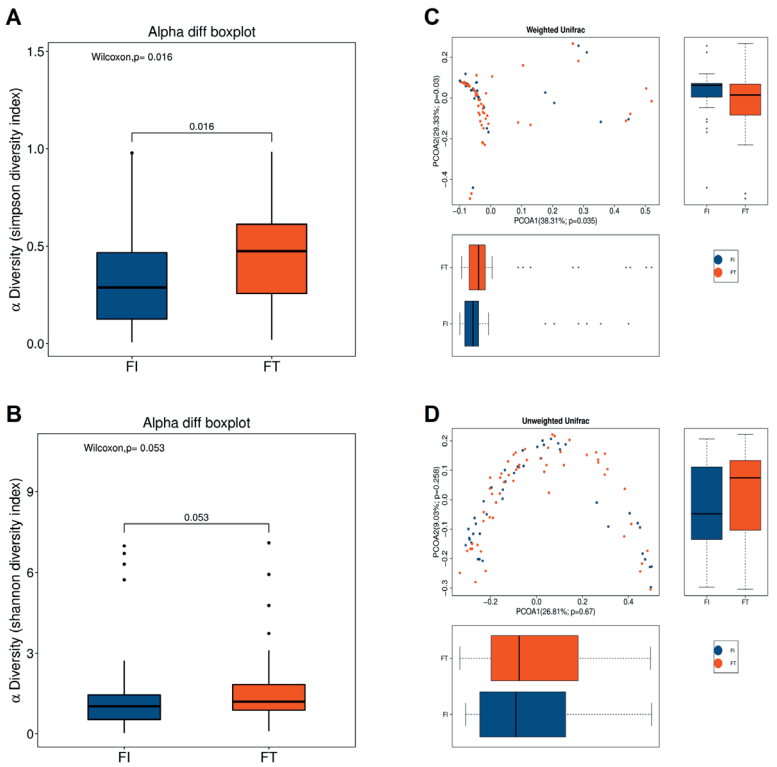

Fig. 2. Diversity of microbiota between the two groups. Box plots depict the differences in the fecal microbiome diversity indices between the FI (feeding intolerance) and FT (feeding tolerance) groups as indicated by the Simpson **(A)** and Shannon **(B)** indices based on OTU (operational taxonomic units) counts. Weighted **(C)** and unweighted **(D)** PCOA (principal component analysis) based on the distance matrix of the UniFrac dissimilarity of the fecal microbial communities in the FI and FT groups. Axes represent the two dimensions explaining the greatest proportion of variance in the communities. Each symbol represents a sample. The percentages in brackets respectively represent the contribution rate of each coordinate to the sample difference. Significant p values are indicated.

Differential microbiota between the two groups

The microbiota with differential abundances between the FI and FT groups were analyzed in our study. LEfSe analysis was conducted to identify the differential microbiota by determining the presence and effect size of region-specific OTUs among different groups. A logarithmic LDA score cutoff of 2.0 was utilized to identify the important taxonomic differences between the FI and FT groups. Several taxa of the microbiota with differential abundances were detected between the FI and FT groups at different levels (Fig. 3). At the phylum level, the abundance of Firmicutes was higher in the FT group than in the FI group, and the abundance of Proteobacteria was higher in the FI group than in the FT group. At the class level, the abundance of Gammaproteobacteria in the FI group was higher than that in the FT group, whereas the abundance of Negativicutes in the FT group was higher than that in the FI group. At the genus level, the abundance of *Veillonella* was higher in the FT group than in the FI group, whereas the abundances of some genera, including *Oceanisphaera, Janthinobacterium, Vibrio, Brachyspira, Oceanimonas, Petrobacter, Anaerovibrio, Planomicrobium, Cloacibacterium, Vampirovibrio, Leptotrichia, Actinomycetospora*, and *Escherichia_Shigella*, were higher in the FI group than in the FT group.

Correlation between motilin and the microbiota

Plasma motilin levels were detected on day-1, 7, 14, and 21. The correlation between motilin and the abovementioned differential bacteria was analyzed (Fig. 4). The results showed that the motilin level on day-7 was negatively correlated with *Planomicrobium* ($R = -0.285$, $p = 0.038$, Fig. 4B), and the motilin level on day-14 was negatively correlated with *Vibrio* ($R = -0.315$, $p = 0.028$, Fig. 4C). *Planomicrobium* and *Vibrio* were the FI group-enriched genera. The motilin levels on days-1 and 21 were not correlated with any of the differential bacteria.

Correlation between the clinical characteristics of feeding intolerance and the microbiota

A heatmap was used to demonstrate the correlation between the clinical characteristics of FI and the microbiota (all identified genera in the FI group). The heatmap showed that GA and BW were positively correlated with *Escherichia/Shigella* and *Staphylococcus* and

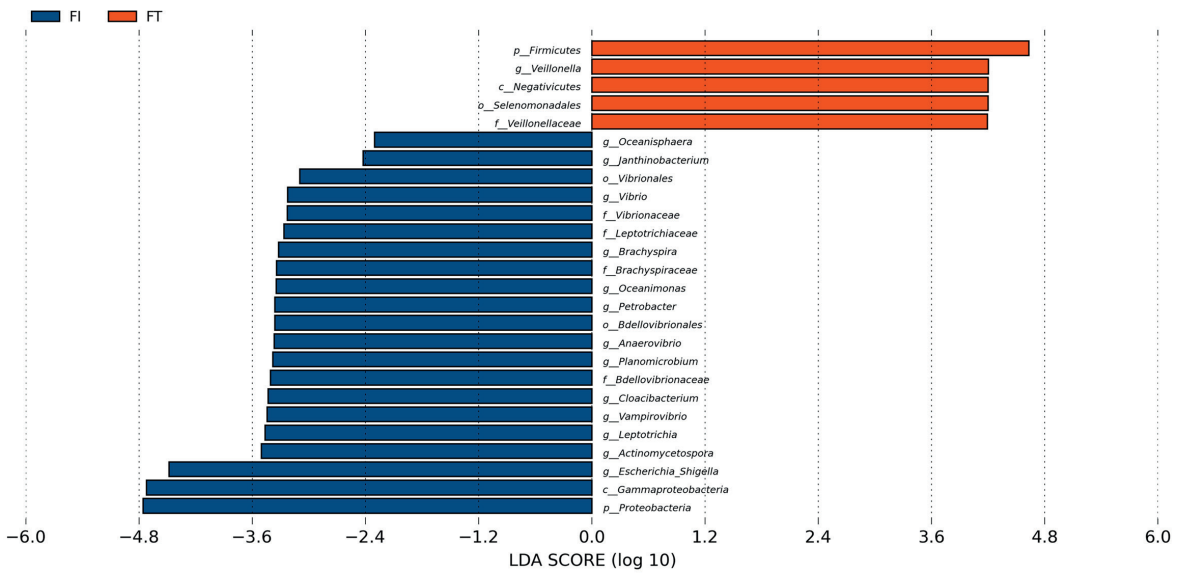

Fig. 3. Differential microbiota between the two groups. LEfSe analysis revealed significant bacterial differences in fecal microbiota between the FI (feeding intolerance) and FT (feeding tolerance) groups. LDA (linear discriminant analysis) scores (log10) > 2 and $p < 0.05$ were listed.

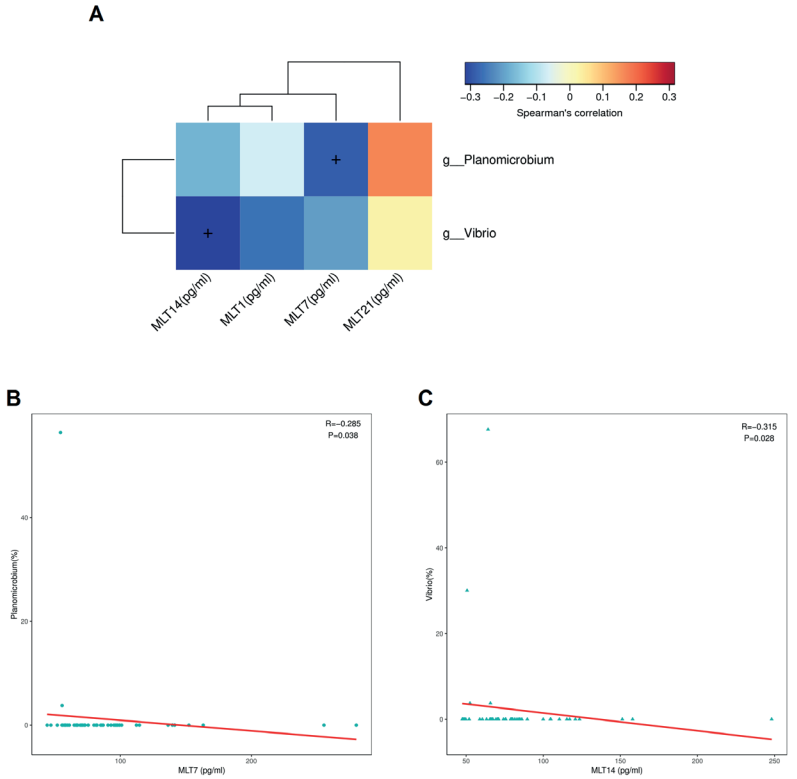

Fig. 4. Correlation between motilin and the microbiota. The correlation between motilin levels and the differential microbiota between the FI (feeding intolerance) and FT (feeding tolerance) groups was analyzed (A). The Spearman rank correlation (R) and probability (P) were used to evaluate statistical significance (B, C). The intensity of the color represents r (correlation; negative score, blue; positive score, red).

Spearman test, $^{+}$ p <0.05. MLT: motilin

negatively correlated with *Klebsiella* (Fig. 5). The age of FI diagnosis was positively correlated with *Fructobacillus* and *Gluconacetobacter* and negatively correlated with many genera such as *Staphylococcus*, *Acinetobacter*, and *Proteus*. The age of FI disappearance was negatively correlated with *Enterococcus* and positively correlated with several genera such as *Leptotrichia* and *Gordonia*. The FI duration was positively correlated with many genera such as *Leptotrichia* and *Gordonia*.

Discussion

Feeding intolerance has been a major problem for clinicians and infants' parents in recent years. The etiology and pathogenesis of FI in preterm infants are complicated and still unclear. The early colonization of the gut microbiota at birth can influence the correct ontogenesis of the gut barrier and motor and immune functions through a complex neuroendocrine cross-talk.[11] Therefore, gut microbiota is associated with FI in preterm infants. However, this aspect has yet to be fully explored. Our study aimed to investigate the relationship between gut microbiota and FI in preterm infants. Because the clinical manifestation of FI is similar to early NEC, we need to distinguish FI from NEC. Three infants in our study did have NEC, and two were in the FT group. One infant in the FI group had NEC, but NEC occurred 11 days after FI disappeared. A large number of studies have shown that the dysbiosis of gut microbiota is closely related to the occurrence of NEC. However, there are few studies on the relationship between gut microbiota and feeding intolerance, which is exactly what our study focused on.

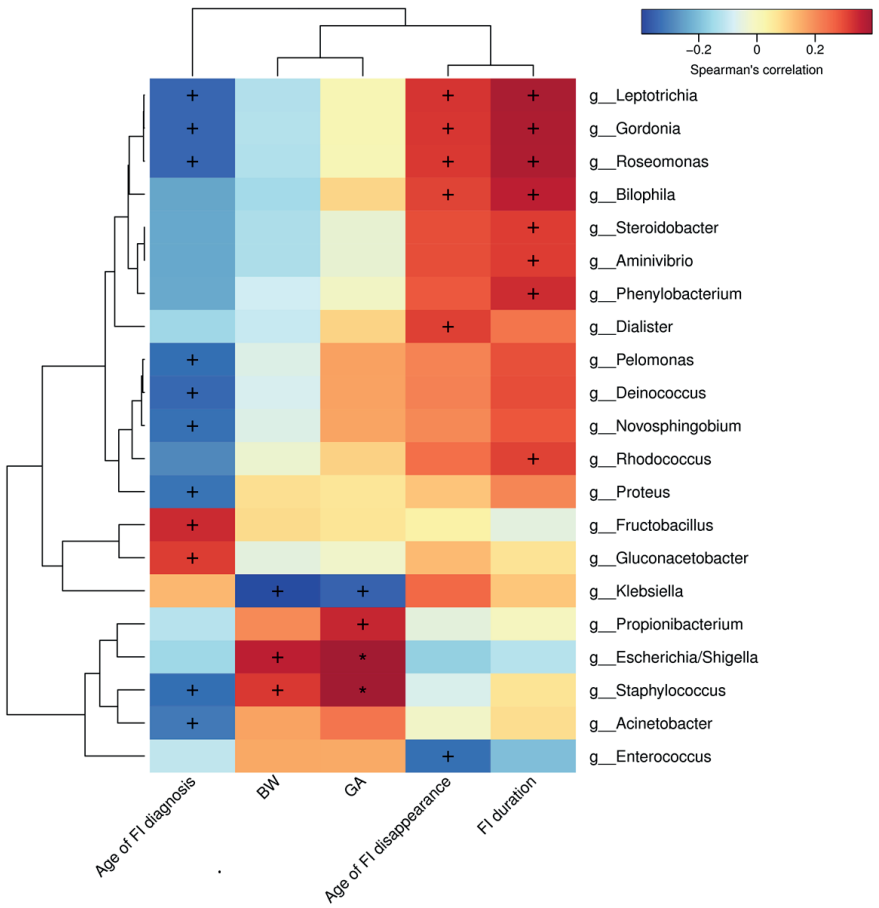

Fig. 5. Heatmap showing the correlation between the clinical characteristics of FI (feeding intolerance) and the microbiota. The heatmap shows the correlation between the clinical characteristics of FI and all the identified genera in the FI group. The intensity of the color represents r (correlation; negative score, blue; positive score, red).

Spearman test, $^{+}p < 0.05$, * $p < 0.01$.
BW: birth weight, GA: gestational age.

The early colonization of the gut microbiota in preterm infants is influenced by many factors, and the most important ones are delivery mode, feeding type, and antibiotic exposure.[12] Our study showed that there were no significant differences in GA, BW, delivery mode, feeding type and antibiotic exposure between the FI and FT groups. All of the subjects were hospitalized in one NICU and given similar treatment; hence, the gut microbiota of the two groups was comparable. The onset age of FI was 2–5 days, and the curing age was 8–26 days; as such, collecting the stool samples on the 7th day was appropriate.

Our study showed that the top four phyla of both groups were Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria (Fig. 1). These four phyla are the predominant gut microbiota in preterm infants.[4,13] However, the relative abundances of the four phyla in the FI and FT groups were different: the relative abundance of Proteobacteria was higher in the FI group than in the FT group (80.9% vs. 69.5%), and the relative abundance of the Firmicutes was higher in the FT group than in the FI group (19.7% vs. 11.5%). At the genus level, the top four genera in the FI group were *Klebsiella, Serratia, Escherichia/Shigella*, and *Bacteroides*, and the top four

genera in the FT group were *Klebsiella*, *Serratia*, *Veillonella*, and *Enterococcus*. These results showed that the composition and relative abundance of the gut microbiota were different between the FI and FT groups at different levels. *Bifidobacterium* and *Lactobacillus* are thought to be dominant in the gut of healthy infants after birth.[14,15] However, in our study, the abundance of these bacteria was very low mainly because of factors such as preterm birth, cesarean delivery, antibiotic exposure, lack of breast milk feeding, and living in NICU with enriched pathogenic bacteria.[16-19] Our results showed that the relative abundance of *Bifidobacterium* in the FI group was lower than that in the FT group (1.3% vs. 3.2%, respectively). This result might be related to the failure of gastrointestinal feeding in the FI group.

Microbial diversity plays an extremely important role in human health, that is, a low microbial diversity is associated with many diseases. In preterm infants, a low gut microbiota diversity is associated with late-onset sepsis.[20] Our study compared the diversity of the FI and FT groups. The results showed that the Simpson index of the FI group was significantly lower than that of the FT group ($p = 0.016$). β-diversity analysis revealed that the weighted UniFracs in the FI and FT groups were significantly different ($p < 0.05$). This result indicated that the gut microbiota diversity in the FI group was significantly lower than that in the FT group. Our study also showed that the Shannon index of the FI group was lower than that of the FT group, but the difference was not statistically significant ($p = 0.053$). This result might be related to our small sample size, which reduced the test efficiency. Microbial diversity significantly decreases when FI is present.[21] Up to now, studies on microbial diversity and FI are limited, but many studies have focused on the gut microbiota and NEC. FI may be an early manifestation of NEC.[22] The characteristics of the gut microbiota of patients with FI may be similar to those of patients with NEC. Microbial diversity decreases significantly in NEC and further decreases as the severity of NEC increases.[23-25] The results of our study also showed that gut microbiota diversity is closely related to FI in preterm infants.

The dysbiosis of the gut microbiota, often presented as an increase in pathogenic bacteria and a decrease in beneficial bacteria, is responsible for many diseases. Our study showed that the composition of the gut microbiota differed between the FI and FT groups at different levels. At the phylum level, the abundance of Proteobacteria in the FI group was higher than that in the FT group, and the abundance of Firmicutes in the FT group was higher than that in the FI group. A study of Yuan et al.[21] showed that a low relative abundance of Firmicutes and a significantly increased relative abundance of *Klebsiella* (belonging to Proteobacteria) are detected in preterm infants when FI is diagnosed. This result was consistent with our study. Mai et al.[26] reported that a 34% increase in the proportion of Proteobacteria and a 32% decrease in Firmicutes are observed in infants 1 week before the diagnosis of NEC. The expansion of Proteobacteria is a microbial signature of gut dysbiosis and epithelial dysfunction.[27] Besides the microbial difference at the phylum level, gut microbial differences at other levels were observed between the FI and FT groups. At the class level, the abundance of Gammaproteobacteria (belongs to phylum Proteobacteria) was higher in the FI group than in the FT group. By contrast, the abundance of Negativicutes (belonging to Firmicutes) was higher in the FT group than in the FI group. At the genus level, the abundance of *Veillonella* (belonging to Firmicutes) in the FT group was higher than that in the FI group. The abundance of several genera mainly belonging to Proteobacteria, including *Escherichia_Shigella*, in the FI group was higher than that in the FT group. *Veillonella* is considered a beneficial saccharolytic bacterial genus. And *Escherichia_Shigella* is a common pathogenic bacterial genus related to many diseases, including gastroenteritis[28] and NEC.[29] In general, the decreased abundance of beneficial bacteria and the increased abundance of pathogenic bacteria might be important factors leading to FI.

Motilin is a very important hormone in gastrointestinal tract. A study has shown that preterm infants with FI have significantly lower motilin levels than those without FI.[30] Gut microbiota may affect the secretion of gastrointestinal hormones. A randomized controlled study has demonstrated that preterm infants fed with prebiotic-enriched formula have significantly higher motilin levels, lesser gastric residues, and greater stool frequencies than those of infants fed with a common preterm formula.[31] In our study, the motilin levels on day-7 and 14 were negatively correlated with FI-enriched *Planomicrobium* and *Vibrio*, respectively. However, the motilin levels on days 1 and 21 were not correlated with any of the differential bacteria between the FI and FT groups. The average age of FI diagnosis was 2.9 days, and the average age of FI disappearance was 13.6 days. This result meant that microbiota was correlated with motilin levels in the course of FI but not when FI disappeared or did not occur. Our results suggested that some microbiota, such as *Planomicrobium* and *Vibrio*, might reduce motilin secretion, leading to FI. This finding provided new information that could contribute to the treatment of FI in preterm infants and recommended a new direction for future research.

Some bacteria were correlated with FI clinical characteristics, including GA, BW, age of FI diagnosis, age of FI disappearance, and FI duration. GA and BW were positively correlated with *Escherichia/Shigella* and *Staphylococcus* and negatively correlated with *Klebsiella*. This result indicated that the composition of the gut microbiota changed with different GA and BW in preterm infants with FI. Some bacteria were also correlated with the age of FI diagnosis, age of FI disappearance, and FI duration. For instance, *Leptotrichia*, *Gordonia*, and *Roseomonas* were negatively correlated with the age of FI diagnosis, but they were positively correlated with the age of FI disappearance and FI duration. This finding indicated that these bacteria might lead to early onset, slow recovery, and long FI course. Inhibiting these bacteria might prevent and treat FI. Our study first revealed the correlation between microbiota and the clinical characteristics of FI in preterm infants. Further studies on these correlations will provide additional information about the prevention and treatment of FI in preterm infants.

Our study has some limitations. We only compared the gut microbiota of the FI and FT groups on the 7th day, and we did not analyze the microbiota before and after FI. Therefore, we could not understand the dynamic changes in the gut microbiota during the occurrence of FI. Our study could show that the gut microbiota was associated with FI in preterm infants, but we could not determine the causal relationship between them. Another limitation was that we conducted a single-centered research with a small sample size; therefore, the evidence level of the research results was limited. These limitations might be considered in future research.

In summary, our study revealed that the gut microbiota is associated with FI in preterm infants, and microbial diversity is low in FI cases. Our study also detected a decreased abundance of beneficial bacteria and an increased abundance of pathogenic bacteria in the FI group. However, the causal relationship between beneficial and pathogenic bacteria and the mechanism between gut microbiota changes and FI occurrence remain unknown. Future research should focus on these directions.

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Original Article

Burkholderia cepacia complex bacteremia outbreaks among non-cystic fibrosis patients in the pediatric unit of a university hospital

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ABSTRACT

Background. *Burkholderia cepacia* complex (Bcc) comprises multi-drug resistant, Gram-negative, motile, and aerobic bacteria. Bcc causes severe nosocomial infections particularly in patients with intravascular catheters and in those with cystic fibrosis. We studied a Bcc outbreak in non-cystic fibrosis patients.

Methods. We analyzed data from six patients hospitalized at our center. Blood cultures identified as infectious were incubated onto 5% blood sheep agar, chocolate agar, and eosin methylene blue (EMB) agar. We examined possible sites that could be sources of infection at the clinic. We confirmed isolations with pulsed-field gel electrophoresis (PFGE) tests.

Results. The first patient was hospitalized due to left renal agenesis, urinary tract infection, and renal failure. Bcc was isolated in blood cultures obtained due to high fever on the third day of hospitalization. We stopped new patient hospitalizations after detecting Bcc in blood cultures of other five patients. We did not detect further positive specimens obtained from other clinic and the patient rooms. PFGE patterns were similar in all clinical isolates of Bcc indicating that the outbreak had originated from the source.

Conclusions. Bcc infection should be considered in cases of nosocomial outbreaks of multi-drug resistant organisms that require hospitalization at intensive care units. Control measures should be taken for prevention of nosocomial infections and required investigations should be done to detect the source of infection.

Key words: Burkholderia cepacia complex, child, outbreak, infection control, non-cystic fibrosis.

Burkholderia cepacia complex (Bcc) are aerobic, oxidase positive, motile, non-fermentative, spore-free Gram-negative bacilli that can cause opportunistic infections. They are commonly found in soil and humid environments. Bcc group includes at least 21 species that are phenotypically similar, but genotypically different. Identifying them is difficult with routine biochemical tests, and it may yield incorrect results. Therefore, confirmation and molecular tests should be performed in reference laboratories.[1] Bcc have emerged as pathogens leading to necrotizing pneumonia and bacteremia that is intrinsically resistant to most antibiotics, particularly in patients with cystic fibrosis and chronic granulomatous disease.[2,3] However, their pathogenicity is not limited to these patients. The bacteria may colonize and infect the respiratory tract, blood stream, and urinary tract in immunocompromised patients. Bcc infections have been reported in intensive care units, in patients on dialysis, transplant patients, newborns, and those with intravenous catheters. Majority of Bcc isolates are intrinsically resistant to aminoglycosides, polymyxins, and other beta-lactam antibiotics. These bacteria are highly contagious in hospital environments and early diagnosis and treatment of patients is essential.

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We investigated a bacteremia outbreak in six patients, who were detected to have Bcc bacilli growing in blood cultures and hospitalized in our pediatric clinic.

Material and Methods

We analyzed data from six patients hospitalized at our pediatric clinic between 18 and 25 February 2019, who had Bcc growing in blood cultures. The ethics committee of Tekirdağ Namık Kemal University Faculty of Medicine approved the study (date and report number: 2019-83.06.04).Written informed consent was received from all.

Epidemiologic research: We initiated our study rapidly after discussing the detection of Bcc-positive blood stream infections in six patients of our clinic with the infection control committee. We obtained clinical records of the patients. The staff of the clinic was informed about blood culture obtaining techniques. Compliance to infection control measures was checked. The training of health care workers, hand hygiene, use of gloves and skin antisepsis during blood culture, how environmental cleaning is done, disinfection and sterilization applications, surveillance studies were checked.

We obtained cultures from potential infection sources including intravenous fluid administration, vial stopper, disinfectants, antiseptics, nebulizer solutions, drugs, syringes, environmental surface, other patients, personnel, cotton balls, gauze, blood culture bottles, and distilled water. Humidified sterile swabs were used. Blood was directly cultivated in 5% sheep blood, eosin methylene blue (EMB), and chocolate agars and incubated at 36 ± 1°C for 48 hours. Fluid samples were additionally inoculated into automated blood culture vials and left for five days of incubation.

Microbiological analysis: The BACTEC 9120 (Becton Dickinson, USA) device was used for bacterial detections. Blood culture samples which yielded positive signals were cultivated onto 5% sheep blood agar and EMB agar and incubated at 36 ± 1°C for 24-48 hours. We used a Vitek 2 system (Biomerieux, Marcy L'etoile, France) and conventional methods for biochemical identification and antibiotic susceptibility testing.

Confirmation of isolates and PFGE tests were performed at the Ministry of Health, General Directorate of Public Health Presidency of Microbiology Reference Laboratories and Biological Products Department. Deoxyribonucleic acid (DNA) extractions were made from the colonies in the growth media. Afterwards we digested DNA samples with the restriction enzyme Fast Digest Spel (Thermo Scientific, USA). We performed a fingerprint assay using PFGE to investigate the clonal identity of the clinical isolates. Molecular weight standards were used as a control for PFGE experiment.

Results

Of the six patients detected to have Bcc growing in their blood cultures, four were female and two were male. Their ages ranged from 8 months to 14.5 years. *B. cepacia* was isolated from blood cultures of 6 patients between 18-25 February, 2019 (Table I).

The patients did not have a history of congenital anomaly, growth or developmental retardation, chronic diarrhea, immune deficiency, or cystic fibrosis. Their weights and heights were normal for their age. The first patient was hospitalized due to left renal agenesis, urinary tract infection, and renal failure. Bcc growth was detected in blood cultures obtained due to high fever on the third day after hospital admission. We stopped admitting new patients to the clinic after detecting Bcc growth in the blood cultures of the other five patients in the clinic. The third and fourth patients required mechanical ventilation at the intensive care unit after they developed respiratory failure.

The bacteria in the blood cultures of all patients were identified as Bcc. All strains were susceptible to trimethoprim/sulfamethoxazole

Table I. Clinical features of the cases.

Cases	Gender	Age	First diagnosis	Date for positive blood culture for *B. cepacia*	Ventilator care	Duration of hospital stay (days)
1	Female	14.5 years	Left renal agenesis, urinary infection	18.02.2019 (+)	No	12
				20.02.2019 (+)		
				22.02.2019 (+)		
				24.02.2019 (+)		
				27.02.2019 (-)		
2	Male	14.3 years	Vasculitis	20.02.2019 (+)	No	7
				27.02.2019 (-)		
3	Male	20 months	Pneumonia	22.02.2019 (+)	Yes	21
				02.03.2019 (+)		
				08.03.2019 (-)		
4	Female	8 months	Pneumonia and urosepsis	23.02.2019 (+)	Yes	10
				28.02.2019 (-)		
5	Female	4.7 years	Pneumonia	24.02.2019 (+)	No	7
				28.02.2019 (-)		
6	Female	3.5 years	Acute bronchiolitis	25.02.2019 (+)	No	14
				28.02.2019 (+)		
				03.03.2019 (-)		

and resistant to ceftazidime (minimum inhibitory concentration, MIC: 16 mg/L), and intermediately susceptible to meropenem (MIC: 4 mg/L).

Molecular typing of PFGE confirmed the clonal identical between the cepacia isolates from the blood of the 6 patients. PFGE patterns were similar in all clinical isolates indicating that the outbreak originated from a single source (Fig. 1). We did not detect growth from samples obtained from the clinic and patient rooms. We were not able to identify the primary source. The

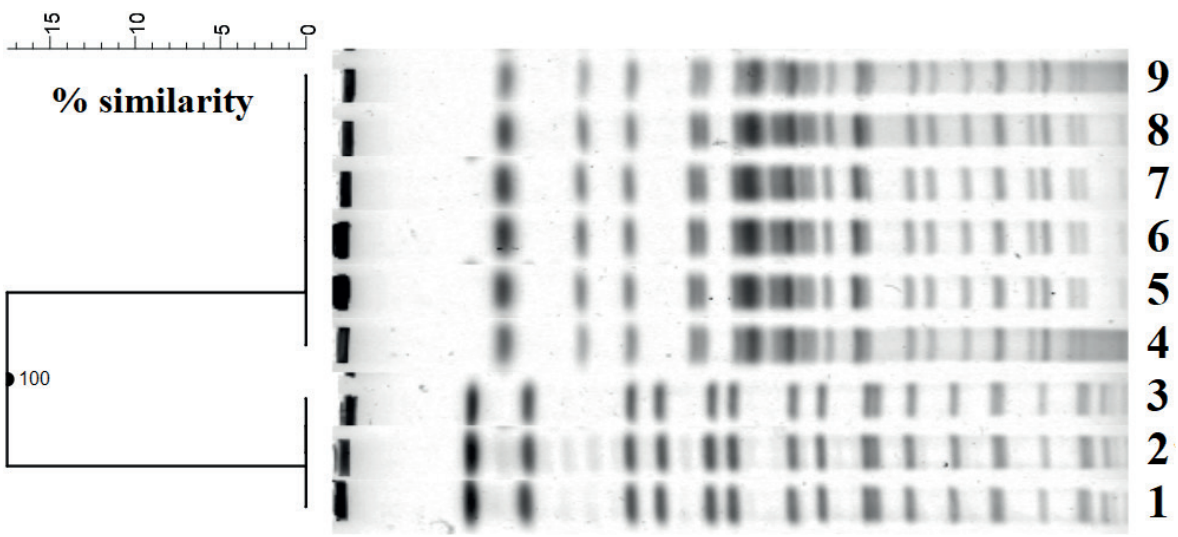

Fig. 1. Pulsed-field gel electrophoresis patterns for 6 clinical isolates. Lane 1-3, molecular weight standards; Lane 4-9, clinical isolates.

infected patients recovered after trimethoprim/sulfamethoxazole treatment within 7 to 21 days and were discharged (Table I). No growths were detected in control blood cultures after treatment.

Discussion

B. cepacia complex has recently been added to the group of non-fermentative Gram-negative bacteria including *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.[4] Bcc is found in water sources, soil, plants, and nature. It can lead to outbreaks through different sources such as contaminated taps, sinks, various intravenous and irrigation solutions, saline solutions, nebulizer drugs, respiratory devices using tap or distilled water, catheters, dialysis fluids and machines, blood gas measurement devices, thermometers, ventilator heat sensors, containers for enteral feeding, disinfectants (chlorhexidine), and antiseptics including povidone iodine, intravenous caffeine citrate, ultrasound gel, moisturizer, and benzalkonium chloride.[5-10] The source was detected in 22 of 30 Bcc outbreaks reported in non-cystic fibrosis patients in intensive care units.[1,11,12] However, the source in the remaining eight outbreaks could not be detected. We also failed to detect a source in the outbreak at our pediatric clinic despite detailed investigations done following reference literature recommendations.

The presence of a central venous catheter, hemodialysis-requiring renal failure, the requirements for multiple bronchoscopies, and recent surgeries have been reported as risk factors for Bcc bacteremia in case-control studies. The need for and duration of mechanical ventilator and the need for tracheostomy have been reported to increase the risk of acquiring Bcc infections compared to control groups.[13] Our patients did not have a history of central venous catheterization, invasive interventions, hemodialysis, surgery or bronchoscopy. The hospital stay was three weeks in the third patient as mechanical ventilation was indicated during treatment.

Bcc may spread from one person to another directly through infected excretions and droplets, or indirectly through contaminated devices and equipment. Isolation of the infected patient is of great importance. As a result of the outbreak at our hospital, we checked sterilization conditions, measures for isolation, hand hygiene, use of disposable gloves and masks. We shortened duration of visits, and improved health professional's education and environmental factors.

Bcc rarely leads to infections in healthy individuals and has a low mortality and morbidity despite having a high intrinsic resistance to many antimicrobial and antiseptic agents.[14,15] Bcc may lead to life-threatening opportunistic infections like urinary tract infection, septic arthritis, peritonitis, bacteremia, sepsis, osteomyelitis, meningitis, pulmonary abscess, and pneumonia in susceptible patients, particularly in patients in intensive care units and those with underlying diseases such chronic granulomatous disease, oncologic conditions, cystic fibrosis, or other immunocompromised patients, who are continually applied catheters/medical devices. Secondary urogenital infections may also be due to urogenital interventions.[6,16] The rates of intensive care unit hospitalizations were reported to be 61.9% and 52.9% by Dizbay and by Srinivasan, respectively . This rate was 33% in our patients. The mortality rate has been reported to be 41-83% in Bcc-related infections.[3,4]

While the vast majority of Bcc outbreaks originate in intensive care units, our patients were hospitalized at the pediatric clinic. We suspected that a 10-month old infant discharged after completion of her 10-day treatment for cystic fibrosis and recurrent pneumonia was the original outbreak source, but we did not detect bacterial growth in her samples. Our first Bcc (+) patient stayed in that room from which that cystic fibrosis case was discharged one week prior. There might be a connection between these two cases.

Bcc are among the nosocomial opportunistic microorganisms causing outbreaks in intensive care units due to their natural resistance to many antibiotics. They are associated with high mortality and morbidity in newborns, and in pediatric and adult intensive care units. Removing the main source, isolating patients, keeping hand hygiene, using disposable gloves and masks, keeping visits short, educating the staff, and cleaning and disinfecting the environment are important factors to prevent the spread of outbreaks. In our study, outbreaks were terminated in a short time through infection control measures taken rapidly after detecting Bcc bacteremia in pediatric clinics, even though their source could not be detected. Bcc infection should be considered in children hospitalized in general and intensive care units and in whom non-specific antibiotic treatment response is unsatisfactory.

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Original Article

The functional health status of children with cerebral palsy during the COVID-19 pandemic stay-at-home period: a parental perspective

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ABSTRACT

Background. Coronavirus disease 2019 (COVID-19) pandemic was effective all over the world. The stay-at-home period was proposed to protect against the pandemic. The aim of this study was to investigate the effects of the COVID-19 pandemic stay-at-home period on body structures and functions, activity and participation levels, and environmental factors of children with cerebral palsy (CP) from the parental perspective in Turkey.

Methods. A twenty-question survey, using the International Classification of Functioning, Disability and Health for Children and Youth set to understand the functional changes of children with CP during the stay-at-home period, was sent to parents in this prospective study. Motor function levels of children were determined by the Gross Motor Function Classification System parent report. The structural equation model was used for statistical analysis.

Results. One hundred and three parents of children with CP participated. At least one of four children with CP had increased levels of anxiety (41.8%), and increased level of a sensation of pain (34%) and sleep problems (25.2%). More than half of the children had increased tonus (67%), decreased range of motion (60.2%), decreased physical activity level (55.3%), and decreased support level of rehabilitation services (82.6%). During the stay-at-home period activity and participation levels and environmental factors of children explained the changes of body functions as 70% and 33% (RMSEA=0.077, $p<0.05$).

Conclusions. This study is the first study to examine the functional health of children with CP biopsychosocially during the COVID-19 stay-at-home period. According to the parents, the functional health of children with CP was affected at different levels during the COVID-19 pandemic. Body functions may also be affected positively if physical activity level, home program and environmental supports increase.

Key words: cerebral palsy, COVID-19, function, pandemic, stay-at-home, ICF-CY.

The Coronavirus disease 2019 (COVID-19) first appeared in Wuhan, China in late 2019 and created a pandemic that was effective all over the world, especially in Europe.[1] The COVID-19 outbreak was declared a "Public Health Emergency of International Concern" by the World Health Organization (WHO) on 30th January 2020[2] and a pandemic on 11th March 2020.[3] In this process, it was emphasized that people with disabilities who had a comorbidity were at a higher risk.[4]

Cerebral palsy (CP) is a chronic non-progressive neurodevelopmental problem that is caused by an injury in the immature brain; additionally, visual, hearing, sensory, perception, communication, nutrition, epilepsy may be accompanied by motor problems.[5,6] The overall prevalence of CP is 2.1 per 1000 live births in the world but 4.4 per 1000 in Turkey.[7,8] Along with all these problems, the activity and participation levels of children are also restricted

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over time. They need regular physiotherapy and rehabilitation, occupational therapy and speech-language therapy in order to increase functional health status biopsychosocially.[9]

During the COVID-19 process, in many countries in order to reduce the risk of contamination, environments where social participation is ensured, were temporarily restricted. In Turkey, special education and rehabilitation services were interrupted between 16th March and 15th June 2020. Children with CP were not able to go outside and receive their rehabilitation service.[10] WHO emphasized the importance of staying at home in order to be protected from the COVID-19 pandemic but also recommended being active physically.[11] Current studies suggest that activity-participation based approaches increase functional health of children with CP.[12] Just the opposite, it is believed that during pandemic stay-at-home period, children with CP were found to be restricted to the home environment which resulted in impairment of their body structures and functions, decreased level of daily life activities and social participation.

A parent is the key member of the home program and they spend the most time with their child. In the family-centered home-based approach, the therapist acts not only as a "practitioner" but also as an " advisor " and a "follower" in order to support the family and the child in their environmental setting.[13] During the COVID-19 process, the biopsychosocial health status of children and their parents, the applicability of a home program and restriction to health services were highlighted but to the best of our knowledge, no studies have been conducted that investigate which treatment models were used for children with CP to support their physical state during the COVID-19 process.

The International Classification of Functioning, Disability, and Health - Children and Youth version (ICF-CY) developed Cerebral Palsy core sets in order to define the health status of children with CP.[14] Longo et al.[15] emphasized that it was crucial to adapt a biopsychosocial model in rehabilitation, strengthening the role of the immediate environment (family members and home setting) during the COVID-19 pandemic period for children's rehabilitation. However, during the COVID-19 shielding process, no studies were conducted concerning the biopsychosocial effects of environmental restrictions on the functional health status of children with CP.

This study was carried out to investigate the effects of the COVID-19 stay-at-home period on the body functions, activity and participation status and environmental factors of children with CP from the parental perspectives using ICF-CY cerebral palsy core set. This study has three hypotheses: 1) during COVID-19 stay-at-home process, the functional health status of children with CP would be affected bio-psychosocially. 2) the number of changes in activity-participation levels and environmental factors would be related to the number of changes in body structures and functions of children during the stay-at-home period. 3) the changes in body structures and functions, activity and participation levels would differ according to the ability to walk.

Material and Methods

This prospective study was conducted during COVID-19 stay-at-home period between 16th March - 16th July 2020 by obtaining ethical approval from the Non-Interventional Clinical Research Ethics Committee (Project no: GO 20/420, 05.05.2020, decision no:2020/09-40) of Hacettepe University.

Participants

The study population consisted of children with CP and their parents who were followed at the Cerebral Palsy and Pediatric Rehabilitation Unit, Faculty of Physical Therapy and Rehabilitation in Hacettepe University, before the pandemic. Children who were diagnosed with CP, aged between 2-18 years and those who were at home because of COVID-19 since 16th March and who took part in therapy sessions twice

a week before the pandemic were included in the study. Children with another diagnosis in addition to CP and those with missing parent contact information were excluded from the study.

Parents of children with CP were contacted by e-mail or phone and invited to participate to the study. The study was explained to the parents and informed consent forms were taken from the participants who accepted to be part of the study. The first questionnaire was sent on the 16th May and the last acceptance date of the questionnaire was 30th June 2020. During the survey, parents who gave missing answers were excluded from the study.

Assessments

In this study, parents of children with CP were asked to complete a survey about the functional health status of their children during the COVID-19 stay-at-home process. Surveys were completed using web-based video conferencing.

COVID-19 Stay-at-Home Cerebral Palsy Parental Questionnaire

This survey was created to assess the functional health status of children with CP from a parental perspective. Under the leadership of a thirty-year experienced specialist pediatric physiotherapist, five pediatric physiotherapists (with at least ten-year experience) developed the questionnaire by making video conferences with a focus group at three different times.

At the first meeting, four physiotherapists created a questionnaire of 31 items based on the Comprehensive ICF Core Set for Children & Youth with Cerebral Palsy from birth to 18 age, 135 ICF categories) for the stay-at-home pandemic process and sent it to the focus group (ten pediatric physiotherapists with at least five-year experiences) for their expert opinion. At the second meeting, expert opinions were discussed. Content validity was examined by using Lawshe's Content Validity Ratio (0.80) and Content Validity Index (0.85).[16] Then eleven questions that were not necessary or similar were excluded. They were sent to twelve parents to ask about their intelligibility. At the third meeting, expert opinions and parental feedbacks were combined to create a survey of 20 questions including body functions (5 questions), activity and participation (12 questions), and environmental factors (3 questions). A Likert type survey pointed as *significantly decreased, decreased, not changed, increased, significantly increased* was selected in order to have two-way answers and not to put psychological pressure on parents when answering. Additionally, four descriptor items were added to the survey. The final version of the standardized form of the questionnaire was prepared by two physiotherapists by using Google Form (Table I).

Gross Motor Function Classification System Family Report Questionnaire (GMFCS-FR)

GMFCS is a classification system that determines mobility and locomotion of children with CP at five levels according to their age (0-4 years, 4-6 years, 6-12 years, 12 years and more).[17] "The GMFCS family report questionnaire was modified by Morris et al.[18] to define motor function level from a parental perspective. The questionnaire is available for four age bands of children and young people, specifically 2 to 4 years, 4 to 6 years, 6 to 12 years, and 12 to 18 years. The questionnaire was translated to Turkish by Kerem-Günel et al.[19] and can be found at https://www.canchild.ca/.

Statistical Analysis

Statistical analyses were conducted by using IBM SPSS 26.0 software (IBM Corp, Armonk, NY, USA) and LISREL 8.71 (Scientific Software International Inc., Lincolnwood, IL, ABD). At least 100 children were planned to be included in the study since the sample size should be at least 5 times the number of questions.[20,21] The variables were tested with visual (histograms/ probability plots) and analytical methods (Shapiro-Wilk's test) to check the normal distribution of variables. Demographic and clinical characteristics of the children were

described by using mean (standard deviation) or median (minimum-maximum) for the numerical variables and by using frequency (%) for categorical variables. The relationships between activity and participation, environmental factors and body functions were examined with a structural equation model (SEM).[22] Four goodness of fit statistics were used in the analysis: Root Mean Square Error of Approximation (RMSEA<0.08), goodness of fit index (GFI<0.90), Chi-square and degree of freedom. Primarily, it was relied on the RMSEA of <0.08 to assess model fit. In addition, since chi-square is sensitive to sample size, we used the relative chi-square test (chi-square divided by degrees of freedom) with < 3 indicating an acceptable goodness of fit. A probability level of p<0.05 was considered statistically significant.[23,24]

Results

One hundred eighty-five parents, who had a child with CP aged between 2-18 years, were contacted by phone. Of these, 148 parents received the questionnaire via e-mail (*n*=20) or smartphone (*n*= 128). Forty-five parents were excluded from the study because of no feedback or missing data. Finally, 103 children with CP (mean age 8y 3mo [4y 7mo], range 2-18 years) and their parents (mean age 37 y 6 months [5 y 9 months], range 25-60 years) were included in the study. The flowchart of the study is shown in Figure 1.

More than half of the children were male (n= 58, 56.3%), and most of the respondents (n =89, 86.4%) were mothers. The majority of children (*n* =78, 75.7%) were spastic type CP, others were dyskinetic (*n* = 23, 22.3%) or ataxic type (*n* = 2, 1.9%). Almost half of the children were bilateral spastic (quadriplegia) (*n* = 47, 45.6%). The majority of the children were classified in level V (*n* = 32, 31.1%) according to the GMFCS-family report questionnaire. The clinical and demographic characteristics of the children are shown in Table I.

The duration of staying at home for the children changed between a minimum of 8 weeks and a maximum of 14 weeks. Only one child had a parent diagnosed with COVID-19. None of the children were diagnosed with COVID-19. During the stay-at-home period, most of the parents (*n* = 62, 60.2%) continued their routine physiotherapy program that was prescribed to them by their physiotherapist before the pandemic: 24 (23.3%) of the parents, received physiotherapy services through tele-rehabilitation, while 8 (7.8%) received physiotherapist counseling services in their own home and 9 (8.7%) could not reach any physiotherapy services (Table I).

For the first hypothesis: The distribution of the responses given by families to "ICF-CY Codes for Children with Cerebral Palsy during COVID-19 Pandemic Stay-at-Home Process Parental Questionnaire" are shown in Table II.

Body structures [s]: All of the children had an impairment in their brain structure (s110) and lower extremity structure (s750); and almost 75% had upper extremity impairment (s730) (Table II).

Body functions [b]: According to the parental questionnaire, the sleep duration (b134) decreased in 18 children (17.5%) and increased in 26 children (25.2%); the stress and anxiety levels (b152) increased in 43 children (39.8%); the sensation level of pain (b280) increased in 35 children (33.9%); the range of movements (b710) decreased in 63 children (61.2%) and increased in 22 children (21.4%). Approximately 70% of parents thought that their child's overall muscle tone (b735) value increased, while about 10% thought their muscle tone decreased (Table II).

Activity and Participation [d]: According to the parents, 10.7% of children had a decrease in focusing attention skills and 43.7% ability to control their behavior. It is reported that six children had a decrease in hand arm use while four had an increase. During the pandemic period, approximately 20% of

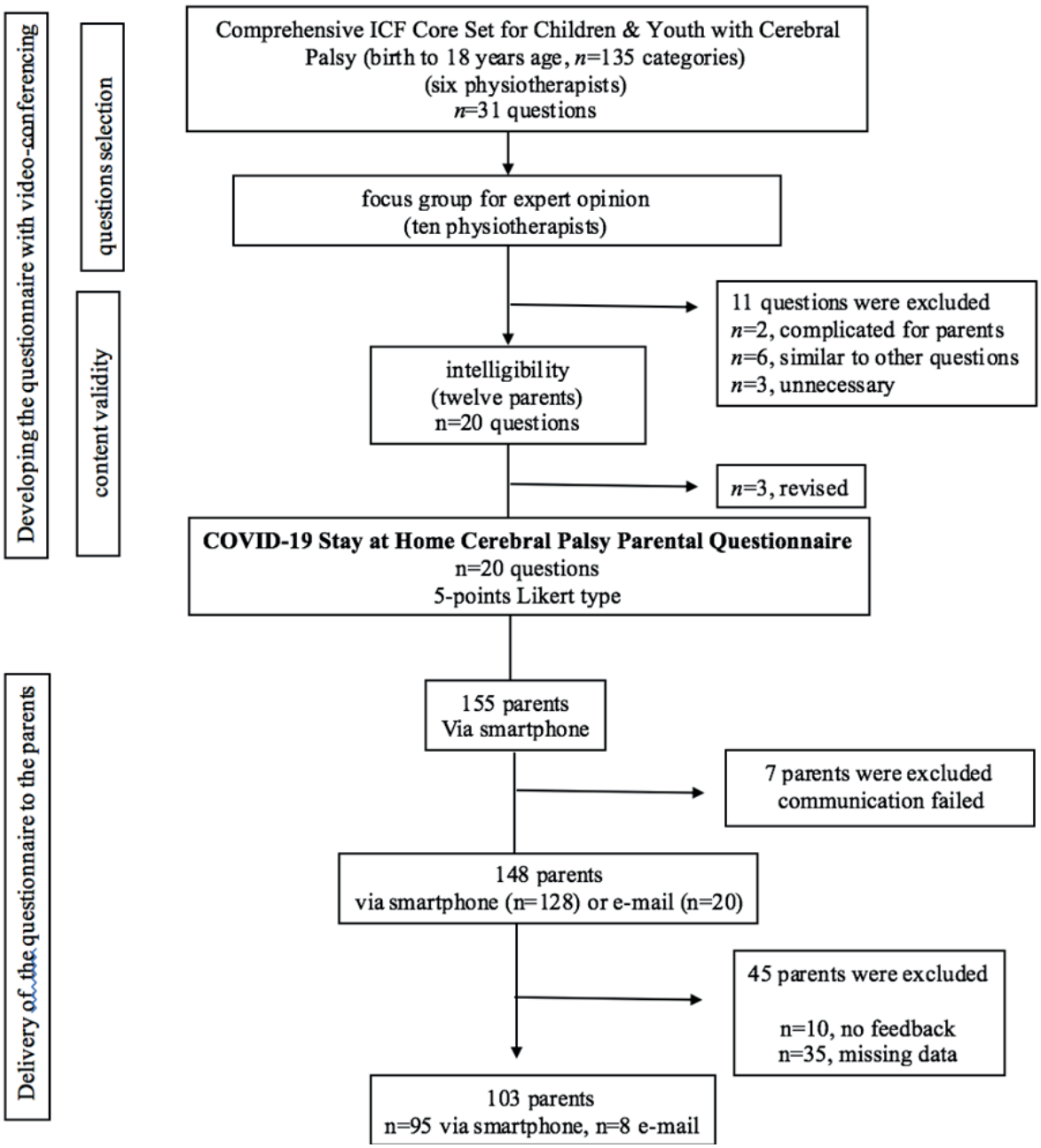

Fig. 1. Flow chart of the study.

children experienced limited walking skills. Furthermore, more than half of the children had a decrease in the level of physical activity. On the other hand, only three of the children had a decrease in their independence of self-care activities, while two of them had increased; ten children had a decrease in their independence of toileting, seven of them had a decrease in their dressing activity, and eleven children had a decrease in their eating skills. Communication skills improved in 30% of the children, but decreased in 20%. The engagement level in game-based activity at home increased in more than half of the children. Although a decrease was observed in the social participation level of outdoor activity in all children.

Table I. Demographic data of children with CP and their parents during COVID-19.

Children with CP	X (SD)	min-max
Mean age	8.26 (4.69)	(2-18)
Age distribution (GMFCS)	n	%
≤2 years	4	3.9
2-4 years	11	10.7
4-6 years	28	27.2
6-12 years	41	39.8
12-18 years	19	18.4
Sex	n	%
female	45	43.6
male	58	56.3
Parent	X(SD)	min-max
mean age	37.66(5.99)	(1-18)
	n	%
mother	89	86.4
father	14	13.6
Clinical type*	n	%
spastic	78	75.7
dyskinetic	23	22.3
ataxic	2	1.9
Limb distribution*	n	%
diplegia	27	26.2
hemiplegia	29	28.2
quadriplegia	47	45.6
GMFCS parent report	n	%
Level I	14	13.6
Level II	24	23.3
Level III	13	12.6
Level IV	20	19.4
Level V	32	31.1
duration of stay-at-home	n	%
8-12 weeks	94	91.2
>12 weeks	9	8.8
having a COVID-19 diagnosis in family members	n	%
yes	1	0.9
no	102	99
PT and rehabilitation during stay-at-home	n	%
none	9	8.7
home program	62	60.2
tele-rehabilitation	24	23.3
home visiting by a physical therapist	8	7.8

* from unit file

CP: cerebral palsy, COVID-19: coronavirus disease 2019, GMFCS: gross motor function classification system, PT: physiotherapy, X: mean, SD: standart deviation

Environmental Factors [e]: Orthosis and assistive device usage duration decreased in 36% of the children during the stay-at-home period, while it increased in 17%. Half of the parents reported that their support levels to the home program decreased, while it increased in 20%. Over 80% of parents reported that the support levels of rehabilitation/services decreased (Table II).

For the second hypothesis, the structural equation model was created to evaluate the relationships among the changes in body functions, the activity and participation levels, and environmental factors in children with CP during the COVID-19 stay-at-home period (Fig. 2). Walking (d450), moving around in different locations (d460) and engagement in play (d480) were selected to show the changes in activity and participation status, support levels of parents for the home program (e410) and support levels of the social services (e575) to show the changes in the environmental factors according to the model. Sensation of pain (b280), mobility of joint function (b710), muscle tone function (b735), sleep function (b134) and emotional function (b152) were chosen to demonstrate the changes in body functions. The changes in activity and participation status explained 73% of the changes in body functions. The proposed path model showed good fit indices (the root mean square error of approximation = 0.077, Goodness of Fit Index = 0.91, Chi-Square = 51.15, degree of freedom = 32, p<0.01). The changes in environmental factors explained 30% of the changes in body functions (Fig. 2).

For the third hypothesis, the comparison of the questionnaire results in terms of body functions, activity and participation levels and environmental factors in children with CP according to their mobility status is demonstrated in Table III. It was stated that the pain and muscle tonus increased and the range of joint motion decreased in both walking and non-

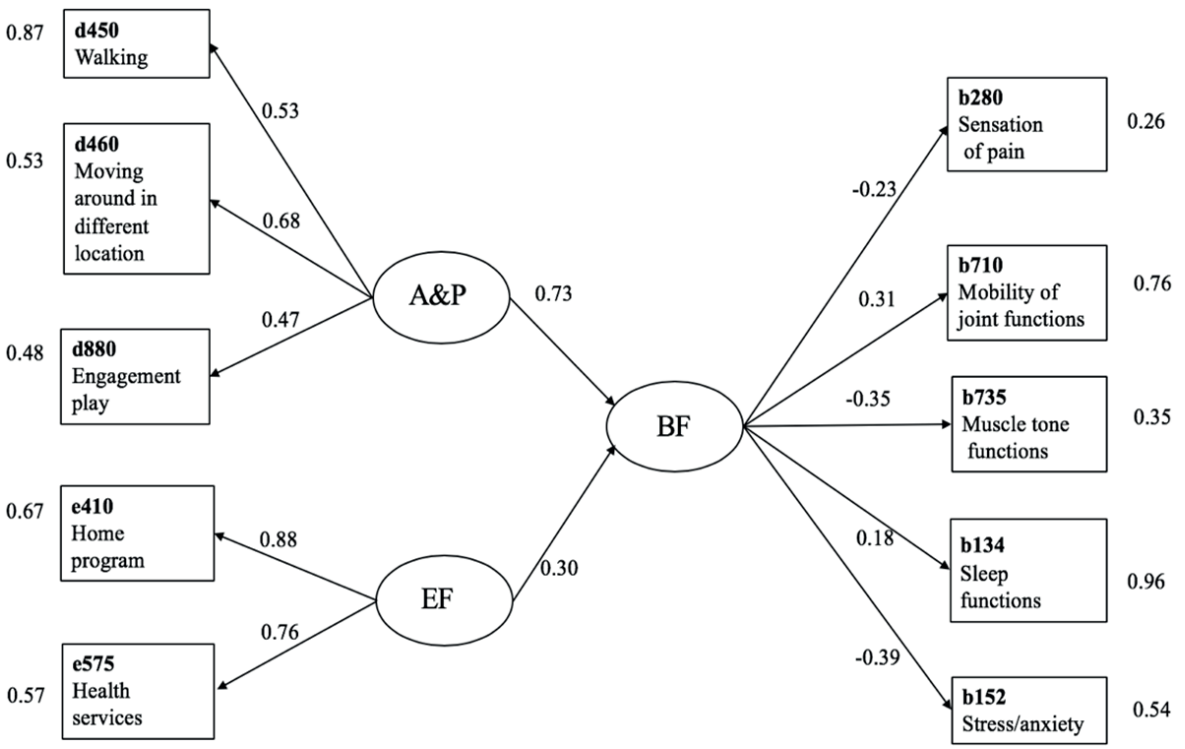

Fig. 2. According to the structural equation model, the relationship between the activity limitation and participation restriction between body functions and structures during the COVID 19 stay-at-home process (A&P; activity and participation, EF; environmental factors, BF; body functions and structures according to ICF-CY codes, Chi-Square=51.15, degree of freedom=32, Chi-Square/degree of freedom<3, RMSEA=0.077, p=0 .01).

Table II. The percentage distribution of responses to the COVID-19 Pandemic Stay-at-Home Process Parental Questionnaire for children with CP.

	Selected ICF-CY Codes	During COVID-19 Pandemic Stay-at-Home,	Qualifier (%), n=103				
			very decreased	decreased	not changed	increased	very increased
Body functions	b134 Sleep functions	how did your child's sleep duration change?	4.9	12.6	57.3	9.7	15.5
	b152 Emotional functions	how did your child's stress/anxiety level change?	1.9	8.7	47.6	34	7.8
	b280 Sensation of pain	how did your child's sensation level of pain change?	-	-	66	31.1	2.9
	b710 Mobility of joint functions	how did the range of motion of your child's joints change?	6.8	53.4	17.5	20.4	1
	b735 Muscle tone functions	how did your child's muscle tone change?	-	9.7	23.3	67	-
Activity and participation	d160 Focusing attention	how did your child's skills of focusing attention change?	-	10.7	89.3	-	-
	d250 Managing one's own behavior	how was your child's skill of managing of his/her behavior change?	6.8	36.9	50.5	2.9	2.9
	d445 Hand and arm use	how did your child's hand-arm use change?		5.8	90.3	3.9	-
	d450 Walking	how did your child's independence in walking skill change?	-	17.5	82.5	-	-
	d460 Moving around in different locations	how did your child's physical activity level change?	9.7	45.6	26.2	12.6	4.9
	d520 Caring for body parts	how did your child's independence in self-care activity change?	-	2.9	95.1	1.9	-
	d530 Toileting	how did your child's independence in toileting activity change?	-	9.7	89.3	0.9	-
	d540 Dressing	how did your child's independence in dressing activity change?	-	6.8	90.3	2.9	-
	d550 Eating	how did your child's independence in eating activity change?	1.9	8.7	86.4	2.91	-
	d710 Basic interpersonal interactions	how did your child's communication level change?	4.9	13.5	54.4	17.5	9.7
	d880 Engagement play	how did your child's engagement level in game-based activity at home change?	-	6.8	41.7	17.5	34
	d920 Recreation and leisure	how did your child's social participation level of outdoor activity change?	55.8	48.7	-	-	-
Environmental factors	e120 Products and technology for personal indoor and outdoor mobility and transportation	how did your child's duration of orthosis or assistive devices usage change?	9.7	26.2	47.6	11.6	4.9
	e410 Individual attitudes of immediate family members	how did your support level of home exercise program for your child change?	18.4	31.1	29.1	18.4	2.9
	e575 General social support services, systems and policies	how did support levels of social services (education/rehabilitation) for your child change?	21.4	61.2	7.8	9.7	-

walking children, while the decrease in range of motion (ROM) was statistically significantly higher in children who can walk according to the reports of the parents (p= 0.043). The highest tonus increase was in the calf muscles, inner and backside thigh muscles (adductor muscles, hamstrings). The joint ROM limitation mostly occurred in the hip, ankle and knee, respectively. Furthermore, pain mostly occurred in the ankle, hip and knee, respectively (Fig. 3). The number of body parts with increased muscle tone in the non-walking children (GMFCS level IV and V) was statistically significantly higher than the walking children (GMFCS level I, II and III) in 69 children with CP (p = 0.043). The number of restricted joints in non-walking children was statistically significantly higher than in walking children (p = 0.030). The changes in the range of joint motion values (p = 0.043), hand-arm usage (p = 0.049), and engagement in game activities (p = 0.018) were statistically significant, when comparing the changes in the activity and participation levels of walking and non-walking children (Table III).

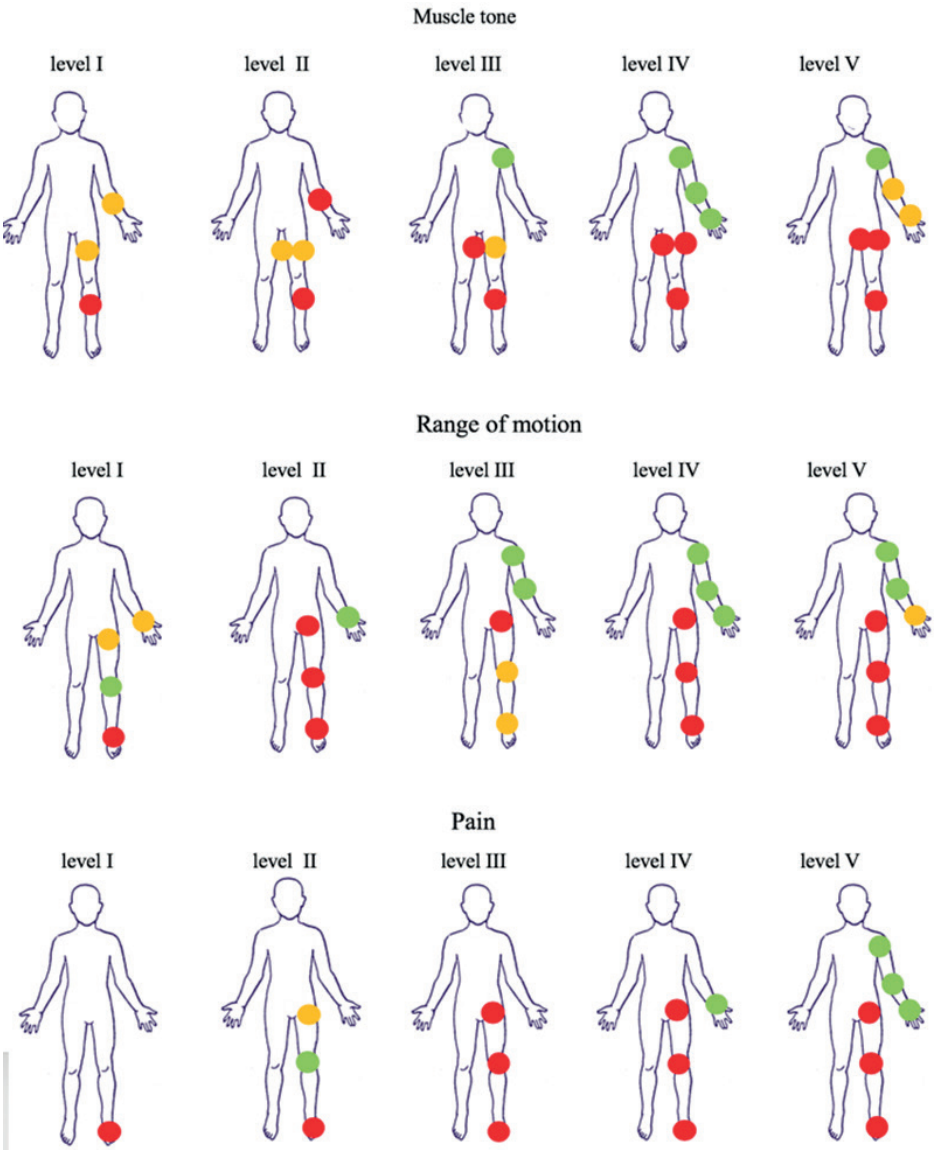

Fig. 3. Muscle tonus, range of joints motion and sensation level of pain: green (25% and below), yellow (25% - 50%) and red (50% and above) according to GMFCS levels during COVID-19 stay-at-home period.

Table III. Comparison of questionnaire scores in terms of body functions and structures, activity and participation and environmental factors of walking and non-walking children with cerebral palsy.

	walking	non-walking		
n=103	mean (SD)	mean (SD)	Z	p
Body functions				
Sleep duration	3.24(1.04)	3.13(0.9)	0-.606	0.544
Stress/anxiety level	3.30(0.70)	3.46(0.91)	-1.448	0.172
Sensation level of pain	3.32(0.47)	3.40(0.60)	0-.381	0.703
- number of body parts with increased pain (n=35)	3.27(0.89)	3.64(0.49)	0-.621	0.535
Mobility of joints	2.36(0.85)	2.91(0.97)	-1.962	0.043
- number of body parts with decreased ROM (n=63)	1.75(0.64)	2.55(1.62)	-2.147	0.030
Muscle tone	1.40(0.67)	1.44(0.66)	0-.303	0.762
- number of body parts with increased muscle tone (n=69)	1.29(0.58)	2.06(1.22)	-2.918	0.004
Activity and participation				
Focusing attention	2.96(0.19)	2.82(0.38)	0-.496	0.570
Hand arm use	3.04(0.34)	2.62(0.26)	-1.875	0.049
Walking	2.75(0.27)	2.90(0.97)	-1.448	0.148
Physical activity	2.46(0.90)	2.67(1.07)	0-.874	0.382
Self-care	3.02(0.31)	3(0)	0-.461	0.645
Toileting	2.90(0.34)	2.92(0.29)	0-.259	0.796
Dressing	2.60(0.36)	3.01(0.24)	0.796	0.042
Eating	2.96(0.19)	2.88(0.47)	0-.487	0.626
Communication	3.22(1.03)	3.05(0.84)	0-.496	0.620
Playing	4(0.96)	3.55(0.97)	-2.359	0.018
Social participation	1.56(0.59)	1.44(0.509)	-1.276	0.202
Environmental factors				
Orthosis and assistive devices	2.76(1.02)	2.76(0.89)	0-.456	0.649
Exercise program	2.18(1.02)	2.75(1.29)	0-.813	0.416
Supports of health/education services	226(0.95)	2.26(0.96)	-1.120	0.263

Mann Whitney U test, $p<0.05$
GMFCS: gross motor function classification system, ROM: range of motion, SD: standard deviation

Discussion

To the best of our knowledge, this study is the first study in the literature to investigate the functional health status of children with CP during the COVID-19 stay-at-home period. The questionnaire developed to assess the changes in body functions, activity and participation levels and environmental factors of children with CP was asked online to parents and it was found that the functional health status of children with CP was affected at various degrees. According to the parental reports, at least one of every four children had increased stress/anxiety levels, sensation level of pain, and sleep problems. More than half of the children had increased muscle tonus; whereas range of joint motion, duration of orthosis and assistive device usage, indoor and outdoor activity-participation level were found to be decreased. During the stay-at-home period, support levels of parents to the home program and support levels of rehabilitation services were found to be declined. Furthermore, limitations in activity and participation levels and changes in parental or rehabilitation service supports affected the changes in body functions of children. Accordingly, the walking ability of the severely affected children were found to be mostly impaired during the stay-at-home period.

The life habits of the children with CP had changed due to environmental constraints imposed under health policies. In this study, we found that the outdoor activities of all children were restricted. While most of the children continued previous home exercise programs, a quarter of them benefited from tele-rehabilitation programs by using smartphone applications, videoconferences and/or video-based telephone consultation. Goldschmidt et al.[25] stated that technology-based approaches should be used to provide social, emotional and functional wellbeing for children in the COVID-19 period. The use of tele-rehabilitation provides benefits such as better clinical outcomes, greater involvement and completion of interventions and greater service satisfaction compared to other interventions.[26,27] In the current study, there were positive changes or no change in the daily living activities of the children participating in tele-rehabilitation. On the other hand, nine children were not treated in any way, and their parents responded negatively.

As a result of prolonged physical inactivity in children with CP, morphological changes in muscles such as non-use atrophy may lead to impairment of muscles. Additionally, intramuscular protein synthesis, muscle fiber cross-sectional area and muscle mass decrease over time as a result of immobilization.[28] Lack of activity and participation during the COVID-19 period may cause problems in body structures and functions. Deterioration of bone structures, muscle weakness, muscle atrophy, muscle shortness and then contractures, also by developing joint limitation could lead to restriction or limitation of home activities if sufficient functional activity is not supported.[29] In this study, changes in activity and participation were seen to be related to the changes in body functions by 73%. Parallel to these findings, WHO emphasized the importance of physical activity during the pandemic period. Children who had an increase in indoor activities showed a decrease in muscle tonus, pain and stress levels, whereas the ROM and duration of sleep increased. In addition, it was observed that the communication skills of one of every four children increased, even though indoor activities of children decreased and body functions were affected negatively. Especially, during the stay-at-home period, it might be helpful to advise parents about age-dependent home-based activities for their children for being physically active.

Regarding the ICF-CY, the current conceptual rehabilitation models which covered participation in different life settings such as the home, school, and community participation became more obvious in accordance with the biopsychosocial approach. Recently, Novak et al.[30] in their systematic review stated that home program, play and activity-based interventions were found to be beneficial for functional health status. The emerging focus on the environment in therapy intervention is consistent with current evidence. Furthermore, a restricted environment may also negatively affect motor learning in children with CP.[31] Home arrangements, family support on exercise programs, social manners, access to health care affect activity and participation levels.[32] Espi Tella at al.[33] showed that social support of parents or friends and geographical conditions were the most common facilitators while insufficient physical environment, problems about transportation, politics, negative attitudes and inadequate support of service providers were the most common barriers of participation of children with CP. Longo et al.[15] reviewed the importance of home programs and the support of the immediate environment (parent and home environment) in children's rehabilitation in low- and middle-income countries during the COVID stay-at-home period. In this study, changes in supports level of parents to home programs and support level of rehabilitation services explained the changes in muscle tonus, sensation level of pain, joint movements, stress/anxiety level and duration of sleep by thirty percent. Additionally, environmental factors were found to be a predictor on body function impairment of children with CP during the

stay-at-home period. It is thought that home-based arrangements to reduce environmental barriers and increase of home program support to parents may have had positive improvements on body functions of children. Furthermore, rehabilitation professionals can use online transdisciplinary consultancy services to educate parents about the home organizations, coping with stress and home-based managements.

Palisano et al.[34] emphasized that in ambulatory or non-ambulatory children with CP, indoor and outdoor activity preferences and performance of chosen activities differ from each other. According to the current study, children with different mobility levels were affected differently from the pandemic period. According to parents, during the stay-at-home period, although indoor play activities of walking children increased, pain of the ankle joint increased and movements were seen to be decreased. In non-walking children, especially around the hip and ankle, all upper and lower extremities were affected in terms of muscle tonus, joint movement and pain.

Considering the pandemic stay-at-home period, which is likely to reoccur in the future, there may be a need for video-based technologic assessments and therapy programs that may offer indoor activities alternatively according to gross motor function levels of children with CP.

None of the children in the current study were infected with COVID-19. Only one of the mothers had contracted the virus. She reported that it had affected her child both physically and psychologically. Biopsychosocial changes in parents may also be related to the decrease in children's functional health and home program support.

This study has several limitations. The study was evaluated only in terms of physiotherapy and rehabilitation, and the content validity of the questionnaire consisted of physiotherapists and parents. Although we contacted at least five children with CP for each item, the number of samples to compare multiple groups according to GMFCS levels could not be reached. We were unable to find a valid and reliable home-based assessment scale to evaluate children's muscle tonus, joint movements, stress and sleep patterns in the home environment. The heterogeneous wide age distribution of children with CP and no detailed structural equation model about accompanying problems affecting functionality such as seizures, hearing, vision, emotional and behavioral problems were the other limitations of this study.

Future studies should investigate the effect of changes of the environment, activity and participation levels on functional health in all the children with neurodevelopmental problems who had to experience the stay-at-home period for different reasons according to different age range such as preschooler, schooler or adolescent. In addition, follow-ups should be evaluated after the restriction period. Considering that this lock-down process can be repeated, health politics should give priority to family-centered home-based tele-rehabilitation applications on health services. An increase of family stress/anxiety level and decrease in physical activity may affect the functional health status of children. In the future studies, there is need for biopsychosocial practices which evaluate the family under the transdisciplinary perspective including different disciplines like psychologist, occupational therapist and speech-language therapist mostly in the sample group. It should also be examined how functional health status has changed in families diagnosed with COVID-19 or in children with CP. All parents stated that they were affected emotionally and that their stress levels increased during the stay-at-home period when accepting to participate in the study. The increased level of depression or anxiety in parents should be evaluated with a short Likert type scale in future studies. In addition, the communication function levels of children may be classified according to Communication Function Classification System. The state of pain and anxiety should be assessed by asking the

children with appropriate age, communication level and cognitive status in future studies.

This study is the first study to examine the functional health of children with CP bio-psychosocially during the COVID-19 stay-at-home period. According to parent reports, during the lockdown period, body structures and function, activity and participation levels and environmental factors of children with CP were affected. Changes in life routines, physical activity and participation level affect changes of sensation level of pain, muscle tonus, joint movements, stress and sleep duration of children with CP. During the pandemic period, changes in support levels of parent and rehabilitation are related to changes in body functions. There is always a possibility that the pandemic will repeated itself, so in order to have no effect on the functional health of children with CP, there is an emergent need for home-based assessments concerning body functions and home-based therapy studies.

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Original Article

An examination of the characteristics of mothers neglecting their children

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ABSTRACT

Background. This study is aimed to recognize families who have neglected their children in the early period, to raise awareness among experts especially pediatricians-working in this field about the reasons contributing to the neglect.

Methods. For this purpose, data were collected by quantitative and qualitative methods. The mothers of 32 neglected children and 30 children who were not neglected were included in to the study. Sociodemographic Information Form, Neglect Assessment Tool, Close

Relationships Inventory, Perceived Social Support Scale, Marital Adjustment Scale, Childhood Trauma Survey Scale, Semi Structured Interview Form, SCID I and SCID II were used as data collection tools. T-test, ANOVA, Chi-square test, Kruskal Wallis, Pearson Correlation test were used for quantitative analysis and descriptive analysis method was used in the analysis of qualitative data.

Results. In the quantitative findings obtained; mothers of neglected children and control group mothers differed in terms of their age, income perceptions, multidimensional perceived social support, marital adjustment, physical and emotional abuse subscales on the scale of childhood abuse, adult attachment patterns, however; it was found that there was no difference in terms of mental illness and personality disorder, and scores on sexual abuse subscale of child abuse. Qualitative results demonstrated that most of the mothers did not consider themselves adequate for parenting, were not satisfied with their parenting role, had insufficient emotional investment in their children and could not balance their roles.

Conclusions. It was concluded that the mothers of neglected children had problems in the areas of social support, marital adjustment, adult attachment patterns and they were also exposed to physical and emotional abuse during their own childhood and these problems negatively affected the quality of their childcare. For this reason, it is important to provide support to neglectful families and family interventions should be established.

Key words: child neglect, maternal characteristics, risk factors for child neglect.

Child neglect occurs when a child's basic needs are not met.[1] Researches report that neglect can sometimes lead to more serious consequences than abuse. Neglected children are reported to have more physical, emotional or cognitive health problems, accidents, injuries or even death when there is a delay in treatment or safety precautions are not taken.[2-10] Since neglect can have tragic consequences, timely and effective interventions are crucial and as the primary caregiver, more attention was given to mothers in neglect.[5,11]

Studies have shown that there are significant differences between parents who neglect and

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do not neglect their children.[5,11] In many studies, low income, low education level, being a mother at a young age and single parent families are described as risk factors for neglect.[5,12-20] However, there are studies indicating that having adequate social support reduces the level of stress of mothers, which positively affects parenting attitudes and skills of mothers and as a result it facilitates their coping with problems and reduces their neglectful behavior.[21-25] In addition to demographic characteristics and social support, some psychological factors are also known to determine the quality of the mother's relationship with their children. It is also stated that most mothers who neglect their children have a history of one or more psychiatric disorders.[26-28] Similarly, it has been stated that parents who have conflict in the marriage cannot pay attention to their children's needs.[29] In addition, these parents have some features such as inadequate organization skills, less verbal communication, less expression of positive feelings and more expression of negative feelings.[30] The relationship between negligent mothers and their parents has also been mentioned, and it has been shown in many studies that the mother, who has a close, reliable relationship with her own parent, has secure attachment styles, meets the needs of her own child quickly and sensitively.[31-33] On the other hand, it has been shown in many studies that mothers' own childhood abuse experiences negatively affect the quality of caring for their own children.[34-38] MacPhee et al.[39] stated that the perception of parenting affects parental activities and plays a role in the development of children. Parental self-perception has been determined as to the extent to which an individual sees herself as a caregiver, has satisfaction with this role, invests in caregiving, and whether the individual can balance the mother role with other roles. Although there are studies conducted in the literature with the perception of parenting,[40-45] this issue has not been investigated in a sample of mothers of neglected children. It is thought that understanding perceptions of parenting is important to understand the cause of child neglect and prepare effective intervention programs.

Child neglect is an important issue to be considered and it is necessary to know which features of parents play a role in neglect.[12,46]The main goal of this research is therefore to raise awareness among professionals about the causes of child neglect. For this purpose, perceptions and experiences of motherhood, variables that may affect the caregiving competencies of the mothers whose children applied to the hospital due to another health problem and considered to be neglected in the evaluation, were examined. This research focuses on the various characteristics of mothers who are evaluated within the context of risk factors in child neglect.

Material and Methods

Participants and Procedure

The sample of the study consists of the mothers of children between the ages of 0-6 who were brought to different outpatient clinics of Hacettepe University Children's Hospital by their families.

Mothers of pre-school children were included in the study because of the fact that pre-school children are at higher risk of neglect and in these population, prevention and intervention strategies become important. The mothers in the study were divided into two groups as the mothers of neglected children and mothers of the control group. The mothers of 34 children who were considered to be neglected as a result of their history and examination (retardation in their cognitive, emotional and social development that could not be explained by medical findings, inadequate personal hygiene, application to the hospital with various accidents and injuries, disrupting their medical controls) were directed to the researcher. A home visit was made to these families by the researcher, and in cases of suspected negligence the observation and interview was carried out for 2 hours at home, using the "Graded

Care Profile" which targets understanding the severity of the situation and determine the areas of intervention. According to The Graded Care Profile, 2 mothers who did not meet the neglect criteria were excluded from the study and 32 mothers were included in the mothers of neglected children. The control group consisted of the mothers of 30 children who were brought to the hospital for reasons such as vaccination, medical consultation, and simple medical interventions. The criterion for inclusion in the control group was the absence of children's developmental problems and the lack of a history suggesting neglect, and also the mothers being parents who took care of their children and who have a healthy motherchild relationship. 8 of the mothers who neglected their children were selected for the qualitative analysis, giving importance to diversity according to some properties such as age, education, socioeconomic level, number of children, and history of psychiatric disorder. As a result, the qualitative sample of the study was created. In order to ensure that the group is homogeneous, the mothers being the primary caregiver, having a cognitive level that can understand and fill the data collection tools, the absence of a chronic illness or disability of their children, and living with their spouses were determined as the inclusion criteria of this research.

The study was conducted in accordance with the Helsinki declaration, and the Ethics Committee of the Non-Interventional Clinical Researches of Hacettepe University approved the study (decision number: 17/142-05, date: 2017/07) and consent form was taken from the participants.

Measurements

The Graded Care Profile: It was developed by Srivastava and Polnay[47] as a tool to identify and evaluate neglect in children. It was created as a descriptive, categorical tool in order to make reliable decisions about the quality of care taken by children and to make decisions not subjective and biased. It provides the opportunity to evaluate parents by observing four areas of care, including physical care, safety, love and esteem at home. The consistency coefficients of the tool between the different practitioners were found to be 0.89 for physical care subscale, 0.89 for safety subscale, 0.79 for love subscale, 0.87 for esteem subscale, and it was found to be a reliable tool for detecting neglect.[48] The scale in the original language was translated into Turkish by the two experts and the experts reviewed each other's scale. In the second step, the experts turned the questionnaire back to the original language and then the original language and the translated formats were compared with each other, and the translation was finalized by making the necessary corrections.

Sociodemographic Data Form: This form was prepared by the researcher to understand the sociodemographic features of mothers and some features related to their children.

Multidimensional Scale of Perceived Social Support, (MSPSS): The MSPSS is a scale developed in 1988 by Zimet et al.,[49] who subjectively assess the adequacy of social support from three different sources: "family", "friend" and "a special person". The factor structure, reliability and construct validity of the Turkish form of the scale were performed by Eker and Arkar.[50] Cronbach's alpha internal consistency coefficients of the scale ranged from 0.80 to 0.95.

Experiences in Close Relationship Scale: Brennan et al.[51] developed the Experiences in Close Relationship Scale which measured anxiety and avoidance dimensions in adult attachment.

Fraley et al.,[52] on the other hand, developed 18 anxiety and 18 avoidance items with the highest discrimination levels and developed the Experiences in Close Relationship Scale-II (EICRS-II). Both avoidance and anxiety dimensions have a high level of internal consistency, and the Cronbach alpha coefficients are 0.90 and 0.86 for these dimensions, respectively.

Marital Adjustment Test: It is a scale developed by Locke and Wallace[53] to measure the quality of marriage. It was adapted to the Turkish

culture in 1999 by Tutarel Kışlak.[54] The internal consistency coefficient calculated within the scope of the reliability study was 0.80. The scale not only measures general marriage satisfaction and quality, but also measures the type of agreement or disagreement with various issues.

Childhood Trauma Questionnaire: The scale was developed by Bernstein et al.[55] and adapted to Turkish by Aslan and Alparslan[56] in 1999. It has been developed to measure abuse experiences before the age of 18 years. It was determined that the scale has a factor structure that reflects physical, emotional and sexual abuse, and within the scope of the reliability study, Cronbach alpha internal consistency coefficients are 0.94 for physical abuse, 0.95 for emotional abuse and neglect, and 0.94 for sexual abuse, and 0.96 throughout the scale.

Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I): It is a clinical interview form structured by First et al.[57] for DSM-IV Axis-I disorders. Turkish adaption and reliability study were performed by Özkürkçügil et al.[58]

Structured Clinical Interview for DSM-III R Axis II Disorders (SCID-II): The scale developed by al. Spitzer and Williams was adapted to Turkish by Coşkunol et al.[59] in 1994, and its validity and reliability study was performed. This scale is a semi-structured interview tool applied by the interviewer to investigate personality disorders according to DSM-III diagnostic criteria.

Semi Structured Interview Form: It was prepared by the researcher in order to collect qualitative data. The questions were prepared by considering the 4 functions that were defined by MacPhee et al.[39] for self-perception of motherhood. The form was created with four titles: to what extent she sees herself as a mother, satisfaction with the role of motherhood, investments related to motherhood, whether she is in balance with motherhood and other roles. In order to check the suitability, understandability and feasibility of the prepared interview form, it was presented to experts (1 psychiatrist and 1 clinical psychologist) for their opinions.

Statistical Analysis

Quantitative data of the research were transferred to the SPSS 22 program, T-Test for the parametric statistics, Anova test, Chi-Square Test for nonparametric statistics and Kruskal Wallis Test, and descriptive analysis method was used in the analysis of qualitative data. The mothers' views on parenting perceptions were given directly under the main themes of parenting competence, satisfaction, investment and role balancing. The opinions of the participants were conveyed on the basis of confidentiality by using nicknames.

Results

The Sociodemographic Characteristics of Mothers

The findings regarding the sociodemographic characteristics of the mothers in the study are given in Table I. There is a statistically significant difference in the age and perceiving the income levels between the mothers who neglected their children and the mothers of the control group. There was no significant difference between the education levels of the mothers, family structures, the age, number and gender of their children. According to independent t test results ($t(60) = 2.49$ $p < .05$), the average age of mothers who neglected their children ($\overline{X} = 30.40$) was lower than the control group mothers ($\overline{X} = 33.96$) and they perceived their income levels as worse ($\chi^2(1) = 5.27$ $p < .05$).

The Variables of Mothers

When we examined the variables of mothers (Table II.), the difference between the mean scores of the mothers' marital adjustment was significant ($F1; 60) = 26{,}663$; $p < .05$), the marital adjustment of mothers of neglected children ($\overline{X} = 26.06$) was found to be more negative than that of the control group mothers ($\overline{X} = 43.23$). There was a difference between mean scores of family support ($F1; 60) = 40.780$; $p < .05$), a special person's support ($F1; 60) = 84.067$; $p < .05$) and friend's support ($F1; 60) = 17.761$;

Table I. Sociodemographic characteristics of mothers and their children.

Variables	Mothers of neglected children (n=32)		Mothers of control group (n=30)		χ^2
	N	%	N	%	
Education level of mothers					
Primary school	15	46.9	11	36.7	
Secondary school	10	31.3	7	23.3	χ^2 (6)= 2.39
High school	7	21.8	12	40.0	
Family structure					
Extended	27	84.4	23	76.7	χ^2 (1)= 0.589
Nuclear	5	15.6	7	23.3	
Perceived income level					
Middle	12	37.5	20	66.6	χ^2 (1)= 5.27**
High	20	62.5	10	33.4	
Number of chidren					
1	10	31.25	4	13.3	
2	12	37.5	14	46.7	χ^2 (1)= 2.84
3 and more	10	31.25	12	40.0	
Gender of children					
Female	13	40.6	15	50.0	χ^2 (1)= 0.550
Male	19	59.4	15	50.0	
Age of mothers	Mean	SD	Mean	SD	t (60) = 2.49**
	30.40	6.5	33.96	4.3	
Children's age	Mean	SD	Mean	SD	t (60) = 227
	3.63	1.4	3.70	1.4	

p <0.01 **p <0.05 SD: standard deviation χ^2:Chi-square

p <.05) perceived by mothers. Social support perceived by the mothers of neglected children was found to be more negative. In the mothers of neglected children anxious attachment (F1; 60) = 12.513 p <0.05) and avoidant attachment (F1; 60) = 10,268; p <0.05) was observed more. When the childhood traumatic experiences of mothers were evaluated, there was a significant difference between the mean scores of physical abuse (F1; 60) = 17.880 p <0.05) and emotional abuse (F1; 60) = 17,397 p <0.05).

Looking at Table III, it was found that the difference between the mean scores of sexual abuse of the mothers in both groups was not significant (χ^2 (1) = .113, p> 0.05). According to these results, it is understood that the mothers of neglected children experience more physical and emotional abuse during childhood.

In the evaluation made with the semi-structured interview form in mothers, the results regarding whether there was a mental illness and personality disorder are given in Table IV.

The Parenting Perceptions of Mothers

Interviews were made with the mothers of the neglected children to understand their parenting perceptions by using semi-structured questions based on four themes (how adequate they consider themselves as a mother, their satisfaction with maternity role, their investment in maternity, whether they balance maternity and other roles) created by McPhee et al.[39]

Some sub-themes were formed in line with the mothers' narratives (Table V).

Table II. Comparison of maternal psychosocial characteristics.

	Source of variance	Total sum of squares	SD	Mean square	F
Marital Adjustment	Between Groups	4565.226	1	4565.226	26.663*
	In-Groups	10273.242	60	171.221	
	Total	14838.468	61		
Family Support	Between Groups	1205.324	1	1205.324	40.780*
	In-Groups	1773.385	60	29.556	
	Total	2978.710	61		
Special Person Support	Between Groups	1672.053	1	1672.053	84.067*
	In-Groups	1193.367	60	19.889	
	Total	2865.419	61		
Friend Support	Between Groups	216.293	1	216.293	17.761*
	In-Groups	730.675	60	12.78	
	Total	946.968	61		
Avoidant Attachment	Between Groups	9489.630	1	9489.630	10.268*
	In-Groups	55449.919	60	924.165	
	Total	64939.548	61		
Anxious Attachment	Between Groups	5324.449	1	5324.449	12.513**
	In-Groups	25530.019	60	425.500	
	Total	30854.468	61		
Physical Abuse	Between Groups	2339.382	1	2339.382	17.880*
	In-Groups	7850.167	60	130.836	
	Total	10189.548	61		
Emotional Abuse	Between Groups	4754.363	1	4754.363	17.397*
	In-Groups	16397.185	60	273.286	
	Total	21151.548	61		

*p <0.01 **p<0.05 SD: standard deviation
OCD: Obsessive compulsive disorder

Table III. Mothers' childhood trauma questionnaire sexual abuse subscale scores.

Group	N	Mean	SD	χ^2
Mothers of neglected children	32	32.03	1	0.113*
Mothers of control group	30	30.93		

*p> 0.05 SD: standard deviation

Competence

Some mothers stated that their spouses are not interested in home and the child, their responsibilities are higher and marital problems are the reasons for not showing enough parenting skills:

… We never get along with my husband and I have no interest in taking care of the children because of the marital problems. I do not talk to my children, I do not want to play games. They spend most of their time on television. I feel sorry for not being able to but I'm not guilty of this. … (Mrs. Dilek)

…I am trying to do my best. For example, a child who does not eat, I force-feed, I try to keep him clean. Cooking and cleaning takes most of my time. My husband is not interested at all. Everything is dependent on me. Sometimes I get stressed, I get confused. I cannot deal with the child when there is stress at home. I put him to sleep until noon. ... (Mrs. Elif)

Table IV. Psychiatric disorders and personality disorders diagnosed in mothers.

Diagnoses	Mothers of neglected children (n=32)		Mothers of control group (n=30)		χ^2
	N	%	N	%	
Disorder					
Depression	6	75.0	2	50.0	
Anxiety	1	12.5	2	50.0	1.350 SD=1
OCD	1	12.5	-	-	
Total	8	100.0	4	100.0	
Personality disorder					
Dependent	3	42.9	-	-	
OCD	-		2	50.0	
Avoidant	3	42.9	2	50.0	0.774 SD=1
Histrionic	1	14.3	-	-	
Total	7	100.0	4	100.0	

p> 0.05 SD: standard deviation χ^2:Chi-square
OCD: Obsessive compulsive disorder

Table V. Distribution of how mothers of neglected children evaluate their parenting perceptions.

Main theme	Sub-theme	N
Competence as a mother	Sense of incompetence	4
	Competent	2
	Indecision	2
	Some conditions must be met	3
Satisfaction as a mother	Complicated feelings	1
	Abundance of difficulties	2
	Regret giving birth to children	2
	Good sense	2
Investments related to motherhood	Not need	5
	Need	3
Balancing the roles of mothers	Roles imbalanced	7
	Roles balanced	1

A mother stated that it was difficult for her to find time and give care to her child. She stated that she did not want to do so even if she had enough time: …*I can play games with my child, I can take more care of him. Actually, I have time for this. But I feel lazy to do these things. I don't want to do it either. I just want to lie down. I cook, I do housework and cleaning. I think these are enough for motherhood, but children want more. She wants compassion, she wants to play a game. My daughter is angry with me and she says: "You don't love me." We have problems with my husband, I am very unhappy. I also shout very much to my child. I am not a competent mother when I think about these.* … (Mrs. Zeynep) Two mothers stated that they found themselves competent. However, these mothers described competent motherhood as only meeting their children's physical needs such as food and hygiene, and to do whatever they want to prevent their children from getting upset:

…I'm a good enough mother. I cook my child's food, I keep their clothes clean, I do whatever they want so that they do not get upset, so it does not offend to me. ... (Mrs. Ayşe)

…I have four children, the eldest is 8, and the youngest is 3 years old. All of them can take care of themselves. They don't want anything from me anyway. I help them to take a bath, I cook. They eat their own food without help. They also help me with housework. There is nothing I have difficulty as a mother, I am enough for my children. … (Mrs. Gulsen)

Satisfaction

Some of the mothers stated that some conditions must be fulfilled in order to take pleasure from motherhood. They stated that motherhood is not enjoyable for them due to absence of these conditions at the moment and they face with more difficult aspects of motherhood: *…If I had been in a beautiful environment, I had no problems with my husband, motherhood would have been a nice feeling, but now I do not feel that way at all. I wish I hadn't given birth. …* (Mrs. Dilek)

…I didn't want to be a mother. I got married out of obligation. My pregnancy was unplanned. When there was a mother-in-law and a father-in-law at home, he became a friend to me. It made me move away from home a bit, but the tough parts of motherhood is more. … (Mrs. Elif)

Some mothers expressed the characteristics their children should have in order to get satisfaction from motherhood: *…Motherhood is a good feeling if she doesn't want to go wrong and have everything she wants. But I have difficulties because of her constant claims. ...* (Mrs. Zeynep)

…It is good to be a mother if he does not irritate me, does not have a temper, stubbornness. I want him to show himself in the community and not to be shy. But in these aspects, mother-hood was not what I expected. … (Mrs. Belma)

Mrs. Arzu complained about the unhappiness she experienced by motherhood limiting her and preventing her from fulfilling her wishes with the following expressions: *…I wish I had lived my life first, had waited 4-5 years, and had given birth after that. Sometimes I wish I gave birth to a stone instead of a child.…* (Mrs. Arzu)

Investment

It was found that mothers do not have behaviors such as reading, researching about motherhood and child development. Mostly, they behave in line with what they see from their families, and some mothers believe that the child will grow spontaneously:

… I have no time to read or learn anything, I don't need to. I prefer to do what I see from my environment. … (Mrs. Belma)

…We were nine siblings. I am rising my children just as my mother raised us. … (Mrs. Gulsen)

…I have never done research or reading about childcare. I did not think of it, I said the child grows by itself. I didn't ask anyone either… (Mrs. Arzu)

Balancing Roles

It is remarkable that mothers stated that they take all the time and energy to do housework and that they do not have time to do anything else while doing housework. It is understood that these housewives spent more time with housework such as cleaning and cooking than time to take care of their children. Expressions of mothers are given below:

…I spent most of my time doing housework. All my energy goes to household chores. I do not take time for myself, nor do anything with my children. … (Mrs. Zeynep)

…Most of my time passes by doing cooking, clearing the table, washing the dishes. It is important for me to cook and feed my child. I'm always in the kitchen. After dinner, cleaning comes…. (Mrs.Ayşe)

… More time goes to household chores. The whole day goes by cleaning. You have no time for other things.… (Mrs. Sevda)

…It is hard to do household chores and take care of children. He makes the house very dirty, I can never let him. He always comes near me while doing business. It makes me very angry. I want him to play with his toys on his own. I have to take care of the housework. If I take care of the child, housework

remains. It takes me a lot of time to eat. He wants his own food, but I can't allow it because he pollutes the house. I force him under my arm and put food in his mouth. There is no time for anything other than dealing with these.… (Mrs. Elif)

The most striking results among the qualitative findings were that mothers stated that they could not take care of their children due to the problems they had with their spouses, that their spouses did not help them. They had too many duties and responsibilities, they could not pay their attention to their children due to marital problems and confusion. As a result they could not be adequate mothers. It has been determined that household chores such as cooking and cleaning for mothers are perceived as a priority and more important than taking care of their children and spending time with them. In addition, understanding that mothers do not have sufficient information about child development and proper parenting are also important results of the qualitative findings.

Discussion

In this study, the characteristics of the mothers of the children who are neglected are tried to be understood by quantitative and qualitative methods from a holistic perspective. In our study, it was determined that there were differences between the age and perceived income levels of the mothers of neglected children and the control group mothers. On the other hand, there were no differences in the education levels of mothers, the age, number and gender of their children, and the types of families the mothers lived in. In the literature, it is stated that the low level of income, the education level of the mother, being a mother at a young age, and being a single parent are associated with neglect and abuse.[5,12-15,20] In his study with adolescent mothers, Barlet stated that whether these mothers had abusive experiences and whether they received good care during childhood is more of a determinant in their neglect behaviors compared to being young mothers. Similarly, in our study, it was found that there were more traumatic experiences during their childhood among the mothers who neglected their children. Although physical neglect is generally associated with poverty, it is observed that parents who receive financial support continue to neglect their children emotionally.[61] Despite the strong relationships between poverty and child neglect, low income alone does not fully explain this result. It is stated that although the adverse effects of financial difficulties on parents is known, income should not be handled alone, and variables such as parental self-efficacy and social support should be investigated together with variables that are thought to influence the effect of parental stress.[62,63] In our study, it was found that social support was perceived more negatively among the mothers who neglected their children in accordance with the literature.

According to the findings of our study, it was observed that mothers who neglected their children had poor marital adjustment, perceived inadequate social support, experienced more physical and emotional abuse during childhood, and had more anxious and avoidant attachment patterns. In addition, it was found that there was no difference between mothers in terms of psychiatric disorder, personality disorder and sexual abuse in childhood. Paavilainen and Astedt-Kurki[64] stated that families with neglect and abuse have a complex family structure. Unresolved problems, differences of opinion and violence are common in these families. Davies et al.[29] stated that parents who have marital problems cannot pay attention to the needs of their children. Parents who have marital problems and neglect their children are not sufficiently organized.[30] Coohey[65] stated that mothers who neglect their children define their relationships with their spouses more negatively and spend less time with their spouses than other mothers. The fact that negligent mothers in our study stated that they had more marital problems, that their communication with their children was inadequate, and that they did not spend qualified time with their children, is consistent with this information in the literature. There are

studies indicating that having adequate social support will reduce the stressful lives of mothers and this will positively affect the mothers' parenting attitudes and skills, facilitate their coping and reduce their neglect behavior.[21-25] The fact that social support perceived by the mothers who neglected their children were more negative in our study supports this information. It was found that mothers who neglected their children were diagnosed with psychiatric and personality disorders more frequently than the control group mothers, but the difference between the two groups was not statistically significant. It is stated in the literature that maternal psychopathology causes a decrease in the quality of childcare. Most of the mothers with psychiatric disorders abuse or neglect their children, and most of the mothers who show neglect behavior have a life-long history of psychiatric disorder.[26-28] As the most powerful determinants of child neglect, antisocial personality traits and using alcohol or drugs are described. However, these diagnoses were not detected in the mothers in our study.

Mothers who stated that they were neglected and abused by their own parents in their childhood neglect their children more and show more negative behaviors towards their children.[34-38] In our study, the fact that mothers who neglected their children stated that they experienced more physical and emotional abuse in their childhood is in line with literature. In the intergenerational transfer of maltreatment experienced in childhood, it is mentioned about learning by imitation, justifying the parenting style of parents and using it in their own parenting practices.[12,66,67] The effect of neglect and abuse experienced in childhood can continue throughout life. Without treatment, it can cause serious damage to the health of the mother and child after delivery, which is a particularly sensitive period.[68] Oshri stated that childhood maltreatment is a risk factor for insecure attachment.[69] In addition, Widom et al.[70] stated that physical abuse and neglect in childhood are associated with anxious attachment, depression, anxiety and low self-esteem in adulthood. In this study, the presence of more anxious and avoidant attachment patterns among mothers who neglected their children compared to other mothers supports this relationship between childhood traumatic experiences and adulthood attachment disorders.

According to qualitative findings, most of the mothers who neglected their children had negative transmissions related to parental perceptions. In the literature, mothers with a sense of parental competence will be able to demonstrate appropriate parental behaviors that will support the development of their children, they will establish a more sensitive, warm and mutual relationship with their children.[40,43,44,71] Mothers who have low parental competence are not interested in their children, and mistreat their children more.[42,72] The sense of competence affects the choice of behavior and helps to determine how much time, energy and effort a person will spend in parenting.[44] The mothers in our study did not mention the issues that are extremely important in the healthy development of children, such as establishing a positive relationship with their children, giving appropriate stimuli, and having a pleasant time with their children. This situation also suggests that mothers who neglect their children may have insufficient information about children's needs and development. The existence of life stresses (such as marital conflict, low family income) and whether they have effective coping skills and social supports affect parenting behaviors.[39,73,74] Consistent with this information in the literature, most of the participants, who did not find themselves competent as a mother, stated that they could not take care of their children due to the problems they had with their spouses. Their spouses did not help them, they were too responsible, could not pay any attention to their children, they were confused and therefore could not be adequate mothers. In the quantitative part of the study, it is understood that these data are in parallel with the mothers who neglect their children find their marital adjustment more negative than the control group mothers.

It is understood from the mothers' statements that most of the mothers who neglected their children were not satisfied with their motherhood. According to Lerner and Galambos,[75] feeling satisfied from the role of motherhood is an important indicator in mother-child relationship and being a positive parent. According to Corwyn and Bradley,[76] obtaining information about child development, making choices suitable for the benefit of the child, spending pleasant time with the child are examples of parent investments. In our study, the majority of mothers stated that they did not read any information about child care, child development or how to behave as a mother. They did not research and did not need these issues. Maintaining motherhood role and other roles in a balanced way is very important for child development.[75] It is remarkable that the mothers stated that they spent all their time and energy to housework and that they could not spare time for other things due to housework. It is understood that these mothers, who are housewives, give priority in housewife roles such as cleaning and cooking, and that they see domestic affairs as their main and most important duties. Barnett[77] states that women with different roles have better mental and physical health, and it is not satisfying for some women to perform roles such as housewife and spouse. He also states that working outside the home can be satisfying for women, which will enable women to fulfill their roles more effectively and to have a better qualified relationship with their children. According to Bornstein,[44] there is a mutual relationship between the feelings of their mothers, their satisfaction with parenting and the balancing of roles. Mothers who balance their roles increase their satisfaction from parenting, which increases their sense of competence. It is compatible with these findings that most of the participants have problems in balancing their parenting competencies, parenting satisfaction and roles.

It was determined that most of the participants had negative experiences in the fields of competence, satisfaction, investment and balancing roles, which constitute the four dimensions of the perception of motherhood. It is thought that this situation will directly or indirectly affect parenting skills and the healthy development of their children and will have an effect on neglect behavior. These variables, which are important in child neglect, should be evaluated from a holistic point of view, considering their relationships with each other. The traumatic experiences of mothers in their childhood may cause anxious and avoidant attachments in adulthood, difficulties in social and marital relations, and neglect of children. Moreover, the inability of mothers to get adequate support from their spouses and their surroundings, cultural household chores as a priority for mothers, and the fact that mothers do not have enough information about child development can also affect mothers' perception of parenting and affect child neglect.

In this research, the fact that only the neglect cases identified at the university hospital were taken and the relatively few participants were included in the study, limit the generalization of the results. Evaluating only mothers is another limitation in this research. It is among the limitations of the study that "Graded Care Profile", which is a descriptive, categorical tool, has not been examined for the 'inter-observer/rater reliability for our country. Focusing on mothers can lead to improperly accusing and labeling mothers. Neglect studies with father samples should be done, then fathers can be made a part of the solution. Further research is needed to understand the relationship between children's characteristics and neglect, as some of the difficulties and disabilities of children will also affect maternal behavior. Children of the mothers participating in the study were all in pre-school period. Research findings cannot be generalized for all age periods, as the needs of age-specific children and mothers' behavior may vary. It is also important to conduct neglect studies in different age groups, as neglect types may vary depending on the child's developmental stages and the family's ability to meet developmental needs specific to different age group.

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Original Article

Thrombin generation in children with febrile neutropenia

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ABSTRACT

Background. Febrile neutropenia (FN) is a common and serious complication in patients with leukemia. Hemostasis and inflammation are two interrelated systems in response to infection. We aimed to investigate the course of thrombin formation in febrile neutropenia attack of children with acute lymphoblastic leukemia (ALL).

Methods. Thrombin generation was monitored in children treated for ALL at diagnosis of febrile neutropenia (FN) (t_0), at 48th hour of FN (t_1) and after recovery from neutropenia (t_2).

Results. Twenty-nine patients and 50 healthy children as control were enrolled into the study. Mean endogenous thrombin potential (ETP) and mean peak value of thrombin results at t_1 were significantly higher than at t_0, t_2 and control groups, respectively. A positive but statistically nonsignificant correlation between ETP values at t_1 and duration of neutropenia was observed.

Conclusion. Although thrombin generation is enhanced both due to chemotherapy or malignancy itself, our results revealed that thrombin formation also increased in neutropenic infection of children with leukemia.

Key words: acute lymphoblastic leukemia, febrile neutropenia, thrombin generation.

Thrombin, which has a direct role in promoting and regulating clot formation, is also the key component of innate immunity. It stimulates a variety of responses by endothelial cells, including cell surface expression and secretion of cellular adhesion molecules.[1-4] Binding thrombin to protease-activated receptors (PAR) causes an increase in the production of cytokines and growth factors, ultimately macrophage activation, neutrophil infiltration and expression of proinflammatory cytokines.[5,6] It is known that bacterial infection and lipopolysaccharides could stimulate monocytes or vascular endothelial cells to express tissue factor (TF) a transmembrane glycoprotein that activates the extrinsic coagulation cascade.[7]

The aim of this cross-sectional study was to evaluate the alterations of thrombin formation during febrile neutropenia (FN) in patients with acute lymphoblastic leukemia (ALL).

Material and Methods

The study group included children diagnosed with ALL who were treated between January to August 2016 in our hospital. Healthy children who were admitted to the hospital for routine control consisted the control group. Febrile neutropenia was diagnosed and treated according to the guidelines by Turkish Pediatric Hematology Association and was classified

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as clinically or microbiologically documented infection or fever of unknown origin.[8] After the onset of FN attack, cultures were immediately obtained from peripheral blood and catheter lumens, and broad-spectrum antibiotics were initiated. Chemotherapy blocks were interrupted to all patients during the FN. Blood cultures were repeated once a day for the first 5 days of FN period whereas those placed in Bactec (Blood culture system, Becton Dickinson Diagnostic Instrument Systems) for at least 10 days. Newly diagnosed patients received ALL-BFM 2009 protocol and patients with relapsed ALL were treated with REZ BFM 2002 protocol.[9,10] Demographics, diagnosis, clinical and laboratory data of patients' were obtained from their medical records.

Venous blood sample was drawn into the standard tubes containing sodium citrate (3,2% trisodium citrate) for protrombin time (PT), activated thromboplastin time (aPTT) and for thrombin generation tests (TGT). Blood samples of patient group were collected at diagnosis of FN (t_0), at the 48th hour of FN (t_1) and immediately after recovery from neutropenia (t_2).

Samples for TGT analysis were immediately centrifuged at 3309g for 25 minutes in order to obtain platelet poor plasma (PPP) and PPP stored at −80°C until analysis. Thrombinoscope (BV, Maastricht, the Netherlands), which was calibrated and automated thrombogram device was applied with fluorogenic method using commercial kits for TGT. The maximum concentration of thrombin formed was defined as the 'thrombin peak' and the area under the curve represented the "endogenous thrombin potential (ETP)". "Lag time" referred the time from the start of analysis to time that thrombin started to generate. 'Time to thrombin peak' was the time from the start of thrombin generation until the maximum thrombin value was obtained. 'Thrombin tail' described the time, when the curve reaches the end.

The study was performed in accordance with the Declaration of Helsinki and approved by the local Ethical Committee. Informed written consent was obtained from all parents or guardians before the study. (Ethics committee approval: Pediatrics Hematology Oncology Training and Research Hospital of Ankara Health Sciences University, 01.18.2016, 2016/010)

Statistical analysis was performed with SPSS 15.0 statistical package program. Kolmogorov-Smirnov test was used to determine the normality of values. The variables of groups were compared with Mann-Whitney U test. Pearson's correlation analysis was performed to compare the relation between two continuous variables. The p- value <0.05 was considered significant.

Results

Twenty-nine children with ALL and 50 healthy children enrolled in the study. The sex ratio and mean age of patients and control group were 1.6, 1.7 and 8.3 ± 4.8 years, 8.8 ± 4.6 years, respectively. The groups were comparable with respect to age and sex (p>0.05). Treatment phase of ten patients were induction/reinduction. Ten patients were at consolidation phase and two patients were at maintenance phase of ALL-IC BFM 2009 protocol. Seven patients were followed as relapsed ALL. Twenty patients were in hospital at the onset of FN and the remaining patients admitted to hospital less than 24 hours after FN developed. Fifteen of 29 (51.7%) patients' neutrophil count was ≤ 100/mm^3 and 14 patients' (48.3%) neutrophil count was 100-500/mm^3. All patients had subcutaneous port catheter. The period between the last day of chemotherapy and development of FN was 4.2 ± 2 days (min-max 1-9 days). The period between the onset of FN and the recovery of neutropenia was 20.2 ± 11 days (min-max: 7-47 days). At the time of recovery of neutropenia all patients were in good condition and afebrile. Any complication was not noted and patients were continued on their chemotherapy schedule after FN attack.

Microbiologically documented infections were noted in eight of 29 (27.5%) febrile neutropenia attacks. Peripheral blood or catheter cultures revealed coagulase negative *Staphylococcus*, *Enterococcus* and *Stenotrophomonas maltophilia* in six, one and one patients, respectively. Severe sepsis and septic shock were not detected in any patient.

Protrombin time and aPTT tests were within normal ranges in both FN and CGs. Endogenous thrombin potential values at t_0 were higher than the CG, however it is not statistically significant (p>0.05). Mean ETP values of patients at t_1 were significantly higher than t_0, t_2 and CG (p<0.001). Mean ETP value at t_2 was higher than CG, but this difference was not statistically significant (p >0.05) (Table I). Mean thrombin peak at t_1 was also significantly higher than t_0 (p=0.002) and t_2 (p=0.001), respectively. Endogenous thrombin potential and mean thrombin peak values of patients and CG are shown in Table I. There was no significant difference between all groups in lag time, time to thrombin peak and thrombin tail times (p>0.05).

Absolute neutrophil count at t_0, t_1 and t_2 were 127 ± 133/mm^3 (min-max 0-400/mm^3), 134 ± 149 /mm^3 (min—max 0-600/mm^3) and 1748 ± 1075 /mm^3 (min-max 800-4100/ mm^3), respectively. Although not significant, a negative correlation between absolute neutrophil count and ETP at t_1 and t_2 was noted (p>0.05). A positive but statistically nonsignificant correlation between ETP at t_1 and neutrophil recovery duration was noted (p>0.05).

The t_0, t_1 and t_2 ETP values of relapsed patients were comparable with the newly diagnosed leukemia patients (p>0.05). However, significantly higher ETP level at t_1 (3042 ± 1629 nmol x minute) was noted in patients receiving induction/reinduction therapy than that of the other patients at t_1 (1784 ± 496nmol x minute) (p: 0.001). Endogenous thrombin potential and mean thrombin peak values were not different in patients that had microbiologically documented infections or not (p>0.05).

Discussion

Thrombin generation assays (TGA) have been used since the early 1950s.[11] The latest generation of the TGA calculate the parameters stemming from the thrombogram.[12] The area under the curve, defined as endogenous thrombin potential (ETP) and the peak value representing the highest thrombin concentration that can be generated, are two important parameters of the thrombogram.[13] Presence of hypocoagulability leads to low peak and low ETP whereas hypercoagulability leads to high peak and high ETP.[14]

Hemostasis and inflammation are tightly linked which have great influence on each other.[15] During infection, the damage to the endothelium and activation of leukocytes result in an increase in tissue factor expression and activation of the coagulation cascade. Thrombin is a multifunctional protein involved in coagulation, anticoagulation, platelet activation,

Table I. Mean neutrophil, ETP and thrombin peak values of patients with febrile neutropenia and control group.

Time	Mean neutrophil values (mm^3)	ETP (nmolxminute)	Mean thrombin peak (nmol/L)
T_0	127 ± 133 (0-400)	1460 ± 619	274 ± 118
T_1	134 ± 149 (0-600)	2131 ± 1080 *	408 ± 252**
T_2	1748 ± 1075 (800-4100)	1475 ± 552	292 ± 126
Control Group		1260 ± 267	212 ± 101

* Mean ETP values of patients at t_1 were significantly higher than t_0, t_2 and CG (p <0.001)
** Mean thrombin peak at t_1 was also significantly higher than t_0 (p= 0.002) and t_2 (p= 0.001)

endothelial activation, production of growth factors and proliferation of both smooth muscle cells and fibroblasts.[16] It is also a mediator of inflammation in diseases such as cancer.[17] On the other hand, thrombin leads to activation of the protein C system that has anti-inflammatory effects.

Infection is a major cause of morbidity and mortality during the neutropenic phase after intensive cytotoxic therapies for malignancies. Complex procoagulant and anticoagulant alterations in the bacterial infection modifies the inflammatory response.[18] A few studies, with a limited number of patients, have previously described TGA changes in sepsis with inconsistent results.[19-22] Mesters et al.[23] have evaluated prospectively coagulation measurements in patients with severe chemotherapy-induced neutropenia and reported that in neutropenic patients with septic shock has been associated with increased thrombin generation. Prothrombin fragment 1+2 was used as a marker of thrombin generation in that study.

In another study, though the patients were not neutropenic, initial thrombin generation in patients with sepsis had been noted to predict the development of multiorgan dysfunction and poor outcome.[19] Another study revealed no difference in ETP values in patients with sepsis, and the authors noted limited use of ETP in clinical practice.[20] A recent study that compared results in patients with severe sepsis to healthy controls have disclosed a significant difference regarding peak thrombin, lag time and time to thrombin peak. Thrombin peak has been higher in survivors compared to non-survivors at all time points. The lag time and time to thrombin peak has been shorter in non-survivors. No significant difference in ETP between survivors and non-survivors of sepsis has been noted. They reported that thrombin peak shows a positive correlation with survival and the ETP does not have any prognostic importance.[22] Picoli-Quaino et al.[23] investigated hypercoagulability status with TGA in adult patients with ALL during febrile neutropenia episode. In this study, TGA was performed at baseline, at the time of fever onset, and 48 hours thereafter. An increase in the time to peak thrombin, reflecting an impairment in TG generation, was observed during the first 48 hours of sepsis compared with baseline samples.[24]

Our results showed that thrombin generation was increased during the course of febrile neutropenia. Mean ETP and mean peak thrombin levels at t_1 are significantly higher than at t_0, and t_2. Also, there was a positive but statistically nonsignificant correlation between high ETP values at t_1 and duration of recovery from neutropenia. Duration and severity of neutropenia are important parameters of morbidity and mortality in patients with FN. Although all patients completed their treatment without any complication, neutrophil recovery time was longer in patients whose ETP values were high at t_1. Markers of inflammation are increased during chemotherapy. Platelet-derived, endotelial-derived, and tissue factor-positive microparticle levels are increased in children with ALL at diagnosis and after prednisone and L-asparaginase administration although tissue factor pathway inhibitor increases during the induction chemotherapy in leukemia patients at the same time.[25] Corticosteroids and L-asparaginase are widely used in induction and reinduction phase of ALL patients. As expected, the ETP value at t_1 of our patients in induction/reinduction was significantly higher than other patients. Although mean ETP and mean peak thrombin levels were particularly higher at the 48th hour than at diagnosis of FN and after recovery of neutropenia, it is not possible to define the main cause. Infection or previous chemotherapeutics might be related to thrombin generation.

To our knowledge, our preliminary study, which investigates the alterations of thrombin generation with TGA in children with ALL during FN attack, is unique in the English literature. Thrombin generation could not be evaluated in FN attacks in different chemotherapy phases of the same patient; this is a limitation of the cohort. Moreover, the

patient group was heterogeneous that consisted of children with leukemia who were at different stages of chemotherapy protocol. Another limitation was not assessing the TGT before FN attack of the patients and comparing the thrombin generation to the healthy children. Comparing thrombin generation during febrile neutropenia attack (both t_0 and t_1) to afebrile and normal absolute neutrophil count period (t_2) of the same patient may have improved this limitation.

In conclusion, thrombin generation increase during febrile neutropenia. Further studies in larger groups for the clinical significance of thrombin formation in pediatric leukemia patients with FN are warranted.

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Original Article

Transient positivity of anti-tissue transglutaminase IgA autoantibody in febrile children: a case-control study

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ABSTRACT

Background. Fever is a physiological response activated by integrative interactions between the neuronal and immune systems. The association of fever with the development of autoantibodies against various self-antigens is controversial. We here evaluated if fever was associated with increased levels of anti-tissue transglutaminase (tTG) IgA autoreactive antibodies in children.

Methods. This was a case-control study performed the Amir-Al-Momenin Hospital of Zabol City from January to December 2018. Febrile children (N=135) and apparently healthy counterparts (N=135) were included. Total IgA and anti-tTG IgA were measured by ELISA.

Results. From 270 children evaluated, 144 (53.6%) and 126 (46.4%) were males and females, respectively. The mean age was 4.7 ± 2.6 years. The mean total IgA titer was 208 ± 100 mg/dl, and the mean anti-tTG IgA titer was 15.9 ± 68 mg/dl. There was a significant difference in the mean titer of anti-tTG IgA between apparently healthy controls (1.97 ± 1.12 mg/dl) and febrile children (30.2 ± 94.9 mg/dl, p=0.002). Positivity for anti-tTG IgA was observed in 16 (11.8%) out of 135 febrile children while no subject in the control group had positive results. One out of the 16 positive cases showed persistent elevated levels after fever disappearance. On biopsy examination, this child was confirmed to have celiac disease.

Conclusions. We showed that fever can trigger the production of anti-tTG IgA autoantibody in children. It is recommended for pediatricians to be vigilant in interpreting anti-tTG IgA results during fever episodes and repeat positive cases after the cease of fever. It is also recommended to reassess anti-tTG IgA seropositivity in other clinical settings in future studies.

Key words: fever, tissue transglutaminase, autoantibody, immune system, celiac disease.

Fever is a physiological response activated by integrative interactions between the neuronal and immune systems.[1] Fever is in fact a regulated boost in the core body temperature to combat microorganisms and inhibit their growth within human tissues and cells. In this manner, fever is considered to be a major factor affecting physio-pathological features and the clinical course of human infectious diseases.[1]

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Overall, fever is a multifactorial adaptive response intercalating with various body systems. The role of immune system in promoting and regulating fever upon infections has been highlighted.[2] Immune components including immune cells and various cytokines are bridges linking immunological reactions to the nervous system to induce fever. These two systems act in a highly intercalated manner to promote a vigilant response against stresses and potential environmental threats.[3]

The cytokines involved in regulating the interaction between immune and nervous systems are known as "endogenous pyrogens". A wide variety of inflammatory cytokines

including tumor necrosis factor (TNF), interleukin (IL)-1, IL-6, and interferons can play a role as pyrogens. The main function of these mediators in neuronal system is to induce the production of prostaglandins which subsequently lead to hyperthermia and fever. However, induction of fever by pyrogens has been suggested to involve other parallel mechanisms which are poorly understood. The role of immune system receptors such as toll-like receptors has been suggested in these processes.[4,5] Nevertheless, these inflammatory cytokines also exert vast influences on immune system function.[6,7] Accordingly, fever induced inflammatory cytokines may promote exaggerated immune response leading to detrimental effects against self-tissues and autoimmunity.[8,9] Nonetheless, the association of fever with the development of autoantibodies against many self-antigens is unknown. In the present study, we evaluated if fever is associated with increased levels of anti-tissue transglutaminase (tTG) IgA autoreactive antibodies in febrile children.

Material and Methods

This case control study was performed in the Amir-Al-Momenin Hospital of Zabol City from January to December 2018. All children fulfilling our inclusion criteria were considered as the study population. Control subjects were recruited from age- and sex-matched apparently healthy children visiting the clinic for periodical checkups. The study was approved by the local Ethics Committee in Research of Zabol University of Medical Sciences (20th November 2018, Code: Ir.Zbmu.Rec.1397.115). Informed consent was acquired from the children's parents.

Inclusion and exclusion criteria

Children hospitalized in the pediatric ward of the hospital due to fever were included in the case group. Those with a previous diagnosis of celiac disease, a family history of celiac disease or other autoimmune diseases, and patients under treatment with anti-inflammatory drugs were excluded from both the case and control groups.

Sample size

The sample size was calculated based on the below formula.

$$n = (Z_{\alpha/2} + Z_{\beta})^2 * (p_1(1-p_1)+p_2(1-p_2)) / (p_1-p_2)^2$$

In this equation, $Z_{\alpha/2}$ (1.96) represented the coefficient of significance threshold ($p<0.05$). The Z_{β} was the coefficient related to the power of study (80%) and considered as 0.84. P1 and P2 represented the expected frequencies of positivity for anti-tTG IgA in the case and control groups (2% and 4%, respectively). Accordingly, the sample size for each group was obtained (N = 135).

Data acquisition

The demographic data and the past clinical history were obtained by interviewing parents. After that, blood samples (5 ml) were drawn from case and control children. The serum samples were separated in the hospital laboratory, and anti-tTG IgA and total IgA levels were determined using specific ELISA kits (Pars Azmoun Co, Iran).

Statistical analysis

SPSS 16 (Chicago Inc, USA) was used for statistical procedures. Shapiro-Wilk test was applied to screen the normal distribution. Independent sample Student t-test and Chi-square test were used for inferential statistics. P value <0.05 was considered as the statistical significance threshold.

Results

From 270 children evaluated, 144 (53.6%) and 126 (46.4%) were males and females, respectively. The mean age was 4.7 ± 2.6 years, and the mean weight was 19.7 ± 10.9 kg. Fever was the constant clinical finding in all the children in the case group. In 106 (78.5%) of the patients, fever was

accompanied with cough, and in 7 (5.1%) with diarrhea. Gastroenteritis and malnutrition each were observed in one patient. Recurrent fever was observed in 9 (6.6%). Mean hemoglobin level was 13.9 ± 1.9 g/dl, and the mean values of AST and ALT were 14.4 ± 2.1 IU/L and 11.8 ± 2.1 IU/L, respectively (Table I).

The mean total IgA value ranged from 7 to 677 mg/dl with the mean value of 208 ± 100 mg/dl. The mean anti-tTG IgA was obtained 15.9 ± 68 mg/dl. There was a significant difference in the mean titer of anti-tTG IgA between apparently healthy controls and febrile children (Table II). Based on the reference threshold, positivity for anti-tTG was noted in 16 out of 135 (11.8%) febrile while no subject in controls had positive results. From these 16 children, one case showed persistent elevated levels after fever disappearance (i.e. after discharge). On follow up biopsy examination, this child was confirmed to have celiac disease.

There were no significant differences comparing demographic or clinical variables between patients with positive or negative anti-tTG results except for the levels of alkaline phosphatase enzyme (p= 0.01, Table III).

Discussion

The main goal of the present study was to evaluate the levels of total IgA and autoreactive anti-tTG IgA antibodies in febrile children and compare them with healthy counterparts. Overall, 16 (11.8%) of 135 febrile children revealed positivity for anti-tTG IgA. One patient preserved positive results on follow-up which later was diagnosed with celiac disease based on intestinal biopsy examination. Therefore, the elevation of autoreactive anti-tTG IgA was temporary in most of the patients.

Although we found no similar studies assessing anti-tTG IgA autoantibodies in febrile children,

Table I. Demographic, clinical and laboratory features in febrile and non-febrile healthy children.

Parameters	Febrile (N=135)	Non-febrile (N=135)	P
Male/female, n/n (%/%)	66/69 (49.6/50.4)	77/58 (57.5/42.5)	0.13
Age (years)	5.8 ± 2.2	3.6 ± 2.4	<0.001
Weight (kg)	21.8 ± 11.6	17.5 ± 9.7	0.002
White blood cell (10^3/μl)	12.6 ± 1.7	13.6 ± 1.5	<0.001
Red blood cell (10^6/μl)	4.8 ± 0.3	4.8 ± 0.2	0.51
Hemoglobin (g/dl)	13.7 ± 2.6	14.1 ± 0.9	0.13
Hematocrit (%)	38.9 ± 1.9	40.1 ± 1.8	<0.001
Mean cell volume (fl)	79.5 ± 7.2	80.5 ± 2.1	0.11
Mean cell hemoglobin (pg)	28.8 ± 3.2	26.9 ± 0.6	<0.001
Platelet (10^3/μl)	356.8 ± 122.2	218.3 ± 61.7	0.005
Blood urea nitrogen (mg/dl)	14.1 ± 2.1	14.7 ± 1.3	0.21
Creatinine (mg/dl)	0.62 ± 0.79	0.54 ± 0.17	0.01
Aspartate aminotransferase (IU/L)	14.1 ± 2.7	14.8 ± 1.3	0.26
Alanine aminotransferase (IU/L)	11.6 ± 2.7	11.9 ± 1.4	<0.3
Alkaline phosphatase (IU/L)	139.7 ± 79.8	91.6 ± 45.9	0.73
Erythrocyte sedimentation rate (mm/h)	13.7 ± 5.2	13.4 ± 4.2	0.52

Table II. The mean titers of total IgA and anti-tTG IgA antibodies in febrile and non-febrile children.

Parameters	Febrile (N=135)	Non-febrile (N=135)	P
Total IgA (mg/dl)	211.5 ± 120.7 (range: 7-677)	204.5 ± 74.9 (range: 71-492)	0.59
Anti-tTG IgA (mg/dl)	30.2 ± 94.9 (range: 0.11-542)	1.97 ± 1.12 (range: 0.21-5.8)	0.002

tTG: tissue transglutaminase

Table III. Comparisons of demographic, clinical and laboratory features between anti-tTG IgA positive and negative patients among febrile children.

Parameters	Anti-tTG IgA		p
	Positive (N=16)	Negative (N=119)	
Male/female, n/n (%/%)	10/6 (62.5/37.5)	56/63 (47.1/52.9)	0.2*
Gastric symptoms (Yes), n (%)	1 (7.1)	18 (15.1)	0.6*
Recurrence of fever (Yes), n (%)	0	9 (7.6)	0.47*
Age (years)	6.5 ± 1.7	5.8 ± 2.3	0.24
Weight (kg)	24.1 ± 12.2	21.5 ± 11.5	0.41
White blood cell (10^3/μl)	12.8 ± 1	12.5 ± 1.8	0.69
Red blood cell (10^6/μl)	4.8 ± 0.3	4.8 ± 0.3	0.92
Hemoglobin (g/dl)	15.6 ± 6.7	13.4 ± 0.8	0.23
Hematocrit (%)	40 ± 2	38.8 ± 1.8	0.42
Mean cell volume (fl)	80.7 ± 2.5	79.3 ± 7.7	0.47
Mean cell hemoglobin (pg)	29.5 ± 1	28.7 ± 3.4	0.35
Platelet (10^3/μl)	362 ± 90.2	356 ± 127.6	0.85
Blood urea nitrogen (mg/dl)	13.6 ± 2.7	14.1 ± 2	0.34
Creatinine (mg/dl)	0.58 ± 0.17	0.63 ± 85	0.68
Aspartate aminotransferase (IU/L)	14.5 ± 2.7	14 ± 2.7	0.52
Alanine aminotransferase (IU/L)	12.6 ± 2.3	11.4 ± 2.8	0.09
Alkaline phosphatase (IU/L)	95.4 ± 61	146.7 ± 80.4	0.01
Erythrocyte sedimentation rate (mm/h)	13.8 ± 3.3	13.6 ± 5.5	0.92

*; Fisher's exact test, tTG: tissue transglutaminase

evidence from other diseases support a link between fever and autoimmune reactions. In fact, inflammatory cytokines can trigger leukocytes and other immune cells to promote the synthesis of pyrogens.[10] In one study on children with a family history of diabetes mellitus, fever episodes (either associated or independent of infections) within the first year of life predicted autoimmunity against pancreatic cells.[9] In another study with patients with scrub typhus infection, ANA autoantibodies developed within one week after initiation of fever.[11] These reports support our findings regarding that fever can be a trigger for development of auto-antibodies; however, the clinical significance and persistency of various autoantibodies should be further scrutinized in various clinical conditions.

Among immune cells, neutrophils have been known as major contributors to inflammatory fever associated with various infections. Neutrophils exposed to 45°C express a higher activity for 5-lipoxygenase, an enzymes involved in leukotriene synthesis.[12] Although fever-like temperatures have been shown to prevent lipopolysaccharide-induced activation of NF-κB transcription factor[13,14], neutrophils can promote the production of autoantibodies (e.g. anti-neutrophil cytoplasmic autoantibodies) through NF-κB-independent signaling pathways as well.[15] On the other hand, fever-like temperatures were noted to activate NF-κB pathway in macrophages.[16] Fever range temperatures also induce T lymphocytes to produce stress proteins (such as heat-shock proteins-Hsp).[17,18] In particular, Hsp90 protein can regulate T lymphocytes trafficking in feverish conditions.[17] Following infection with type A streptococcus bacteria, an interaction between interleukin-1β-granulocyte-macrophage colony-stimulating factor resulted in the proliferation of CD4+ T helper 1 lymphocytes. These events have been

suggested to participate in autoimmunity observed in acute rheumatic fever following group A streptococcal infections.[19,20] The roles of new identified mediators such as complement factor 5a and platelet-activating factor in fever induced immunity is yet to be divulged.[21] In a reciprocal manner, autoimmunity may be itself a reason for fever.[22] This is further supported by some reports noting that autoimmunity[23,24] and lymphoproliferative disorders[25] can be associated with fever of unknown origin. Fever can also regulate toll-like receptor (TLR) signaling pathways,[16] cytoplasmic phospholipase 2,[26] intercellular adhesion molecule 1 (ICAM-1), and CCL21 chemokine.[27]

We could not explain an isolated significant decrease in ALP in patients with anti-tTG positivity accompanying normal levels of AST and ALT with no significant deviations in other tests. This observation may be a basis for checking the fluctuations of this enzyme in these patients in future studies.

We showed that fever can transiently trigger anti-tTG IgA autoantibody production in children which probably is promoted by modulating cellular and humoral immunities. Based on these findings, it is recommended to pediatricians to be vigilant in interpreting anti-tTG IgA results during fever episodes and repeat positive cases after the cease of fever. It is recommended to reassess the association of anti-tTG IgA seropositivity with fever in other clinical settings in future studies as well.

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Original Article

The relationship between particulate matter and childhood respiratory complaints and peak expiratory flows in Harran agricultural area

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ABSTRACT

Background. In recent years, many studies have evaluated the increasing incidence of asthma and chronic respiratory diseases among children living close to rural areas with pesticide application. Pesticide exposure in 266 children (126 girls and 140 boys) in Şanlıurfa, a cotton-producing province in Turkey, was explored in this work. Four different villages spread over 40 km^2 were included.

Methods. Measurements of peak expiratory flow (PEF) in 266 children were conducted in late June, before intensive pesticide applications in the cotton-producing fields. The measurements were repeated for 72 of 266 children after pesticide application in late August. PEF, particulate matter with diameter less than 2.5 μm ($PM_{2.5}$), particulate matter with diameter less than 10 μm (PM_{10}), temperature, humidity, and wind speed were measured.

Results. After pesticide application, mean $PM_{2.5}$ and PM_{10} values were significantly increased compared to before pesticide application ($p < 0.001$ for both parameters). After pesticide exposure, nasal discharge, sneezing, burning and itching in the eyes, cough, sputum production, wheezing, shortness of breath and chest tightness were significantly increased ($p < 0.001$). The mean PEF value was demonstrated to decrease significantly after pesticide application ($p < 0.001$). Moreover, significant negative correlations were noted between PEF and PM_{10} and between PEF and $PM_{2.5}$ ($p < 0.001$).

Conclusions. Intensive pesticide application causes respiratory dysfunction and increased respiratory complaints in children living near the affected agricultural areas, and impacts quality of life adversely. The results of this work can be used to develop an early warning system and methods to prevent respiratory disorders in children residing in the study area.

Key words: pesticide exposure in children, Peak expiratory flow, PM_{10}, $PM_{2.5}$.

Pesticides have been used in agricultural applications to destroy pests and weeds. They include insecticides, herbicides, fungicides and rodenticides. Children are exposed to pesticides via air, food, dust, soil and touching surfaces contaminated with pesticides. In areas with widespread agricultural production, pesticides in powder form are known to affect children via inhalation, and the dimensions of pesticide particulate matter (PM) play a role in its movement and deposition. Regional transport over several kilometres may be responsible for unwanted effects on non-target species.[1]

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The amount of pesticide in dust collected from households near agricultural areas was found to be high, and increased concentrations of pesticide metabolites have been reported in the urine of children residing near such areas.[2,3] The health effects of pesticide exposure on children include nausea, vomiting, seizures, bloody urination and skin lesions, and different systems of the body, including the respiratory system, are affected. Clinical manifestations such as aspiration pneumonia and asthma have also been observed.[1] Organophosphate pesticides result in decreased forced vital capacity (FVC) and forced expiratory volume in one second (FEV_1) in children.[4]

PM is a contaminant group consisting of a mixture of organic and inorganic substances in solid or liquid form in air. It can reach harmful concentrations in indoor and outdoor environments during pesticide application. The aerodynamic diameters of PM_{10} and $PM_{2.5}$ are smaller than 10 μm and 2.5 μm, respectively.[5] While emissions of motor vehicles are the main source of $PM_{2.5}$, PM_{10} consists of a mixture of carbon and organic compounds, acids and fine metal particles. Pesticides can be found in both PM_{10} and $PM_{2.5}$, or cling to the surfaces of these particles.[6,7]

Saharan dust outbreaks in the Eastern Mediterranean region occur predominantly during winter owing to the cold-core mid-latitude Mediterranean cyclones, and during spring due to the *Sharan* cyclones.[8,9] The frequency of synoptic-scale meteorological events and, accordingly, dust transport cases increase significantly in spring and autumn. Both the number of patients with respiratory complaints and PM_{10} levels in air reach their peaks in late January and early February, respectively. Contrary to expectations, the number of patients decrease significantly during June, when only dry dust events prevail. Processes that help to wet the organic fraction of the desert dust curtain play an important role in health.[9]

As children breathe at a faster rate than adults, they inhale more air in relation to their body weight.[10] The respiratory tract in a child is shorter, smaller and more permeable to air pollutants, and therefore, such pollutants, including PM, easily enter children's lungs and cause irritation, inflammation and acute or chronic respiratory problems.[11] Children living in urban areas are exposed to air pollution because of heavy exhaust fumes. Children residing in rural neighbourhoods are exposed to PM generated by biomass burning in their homes and the application of pesticides in agricultural areas. Children of immigrant and asylum-seeking families are exposed to smoke from stoves used in tents.[12] A follow-up study of a Swedish cohort of children from 1992–2001 showed a decrease in the frequency of chronic cough, bronchitis, colds, dry cough and conjunctivitis with a reduction in PM_{10} levels.[13]

After increased admissions of children living in agricultural areas with respiratory complaints to our hospital during the pesticide application season, we decided to investigate whether the complaints were related to the pesticide application in these regions. Thus, the purpose of this study was to measure the peak expiratory flow (PEF) rates before and after pesticide application, record upper and lower respiratory tract complaints among children residing in those areas, and explore the relationship of these data with PM_{10} and $PM_{2.5}$ levels.

Materials and Methods

Study population

Following the approval of the Ethics Committee of Harran University, Turkey (May 6, 2016/18), we designed a population-based prospective cohort study to investigate the role of environmental risk factors in increased respiratory symptoms in children living in agricultural areas during pesticide application. A total of 266 volunteers (126 girls and 140 boys) from Şanlıurfa, a cotton-producing

province in Turkey, were recruited from 4 different villages spread over 40 km^2. The mean age of the 266 children who participated in the study before pesticide application was 10.7 ± 2.6 years (minimum: 6 years, maximum: 16 years), while the median age was 11 years. The mean age of the 266 children who participated in the study after pesticide application was 10.5 ± 2.4 years (minimum: 6 years, maximum: 16 years), while the median age was 10.5 years. Moreover, 133 of the children (ages 6–10 years) were primary school students, while the remaining 133 children (ages 10–15 years) attended middle school. Eight (3%) of the 266 children who participated in the study before pesticide application had a diagnosis of asthma. Measurements for the respiratory function of the 266 children were performed in late June, before intensive pesticide application to the cotton-producing fields. The parents of the children who participated in the study provided their informed consent. This study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Application area

The study was conducted in Harran city of Şanlıurfa province, southeast Turkey, between June and August 2016. Cotton farming is one of the main sources of livelihood in Şanlıurfa province. As mentioned previously, the research was conducted with children living in the following 4 villages scattered over an area of 40 km^2: Giyimli (N36.56 E39.00), Küçük Minareli (N36.55 E39.00), Büyük Minareli (N36.56, E38.09) and Bozyazı (N36.51, E39.05). The children were evaluated before the application of the pesticides (Fig.1). Pesticide application was conducted over a time interval of 10–12 d. The application started in the third week of June and continued until the end of August. Tractors with agricultural sprayer machines were used for pesticide spraying.

Fig. 1. Study location (the red maker indicates intensive pesticide application area).

Applied pesticides

The pesticide was a mixture of lambda-cyhalothrin (7 g/daa; grams per decare), emamectin benzoate (4 g/daa), nitrogen (40 g/daa), phosphorus pentoxide (200 g/daa) and zinc (120 g/daa). It was applied to kill green worms (Helicoverpa armigera), which are harmful to cotton plants.

Measurement of particulate matter ($PM_{2.5}$ and PM_{10}) Two pDR-1500 cyclones (Personal DataRAM Pdr, Thermo Scientific, USA) were used to measure PM_{10} and $PM_{2.5}$ concentrations (in $\mu g/m^3$). The necessary calibration apparatus was used for instrument calibration before each sampling. Post-pesticide PEF measurements were conducted on the second day of pesticide application. Before starting the sampling, the coordinates and altitude of the working area were determined with a GPS device (Magellan, USA). Fifteen-minute measurements of PM_{10} and $PM_{2.5}$ were made with the cyclones in the four villages before pesticide application (in June) and after it (in August). Weather parameters (temperature, humidity and wind speed) were also recorded on the sampling dates.

Children and/or their parents were asked to report any issues pertaining to the nose, throat and eyes, as well as cough, sputum production, wheezing, dyspnea and chest tightness before and after pesticide application. PEF is the maximum expiration rate recorded during a forced expiration. PEF measurements are frequently used in the follow-up of asthma and epidemiological studies. They also serve as a suitable method of respiratory function assessment in children. However, PEF measurements are affected by anthropometric parameters such as age, height, weight, gender, air quality, climate (temperature and humidity), atmospheric air pressure (wind and altitude) and nutritional status.[14-16] PEF rates were measured in the children before and after pesticide application with a Mini-Wright Peak Flow Meter (Clement Clarke International, UK) in a closed environment (village rooms, schools and reeve rooms). Post-pesticide PEF measurements were conducted on the second day of pesticide application. All the measurements were taken by the same four people (ZS, SH, PA and BO). The measurements were conducted with the subject in standing position. At least three technically acceptable PEF measurements were collected, and the highest absolute PEF value was recorded.

Statistical analysis

The data collected were analysed with the Statistical Package for Social Sciences (SPSS) software (version 21.0, SPSS Inc., US). An independent samples *t*-test was used to compare independent groups, while paired samples *t*-test was applied to compare the dependent groups. The distribution of categorical variables in both groups was compared with Pearson's chi-square test. The data were expressed as mean ± standard deviation (SD). Categorical variables were expressed as frequencies and percentages. Pearson's correlation analysis was used to detect the relationships between age, PM_{10} and $PM_{2.5}$, wind speed, humidity, heat and PEF rates. A linear regression analysis was used to detect the independent effects of PM_{10} and $PM_{2.5}$, wind speed, humidity and heat on the PEF rates. Statistical significance was assumed for $p < 0.05$.

Results

The children were evaluated for respiratory functions and symptoms related to agricultural pesticide exposure before and after pesticide application in the study area. Of the study subjects in the four villages, 140 (52.6%) were male and 126 (47.4%) were female. Moreover, 104 (39.1%), 102 (38.3%), 37 (13.9%) and 23 (8.6%) came from Giyimli, Küçük Minareli, Büyük Minareli and Bozyazı, respectively. After pesticide application, 72 children from the initial group were re-evaluated in late August. Of these 72 children, 33 (45.8%), 15 (20.8%), 17 (23.6%) and 7 (9.7%) were from Giyimli, Küçük Minareli, Büyük Minareli and Bozyazı, respectively.

The measurements of $PM_{2.5}$, PM_{10}, wind speed, temperature and humidity before and after pesticide application are shown in Table I. Before pesticide application, the mean $PM_{2.5}$ and PM_{10} values were 4.7 and 11.7 for Giyimli, 5.19 and 27.8 for Küçük Minareli, 14.5 and 27.8 for Büyük Minareli, and 12.2 and 23.8 μg/m^3 for Bozyazı. After pesticide application, the corresponding values changed to 13.2 and 334 for Giyimli, 11.2 and 328 for Küçük Minareli, 15 and 313.5 for Büyük Minareli and 13.21 and 319 μg/m^3 for Bozyazı. The regions with pesticide-sprayed fields showed significantly increased values of $PM_{2.5}$ and PM_{10} compared to those before the pesticide application. The air temperature during the study period (June to August) showed an increase, in line with normal seasonal variation. Humidity levels doubled as a result of the irrigation of fields surrounding the study area. Wind speed was found to have decreased during pesticide spraying in the study period.

Examination of the respiratory functions of the participating children, as well as their eye, nose, throat and respiratory complaints before and after the pesticide application are shown in Table II. All of these complaints increased significantly after pesticide application due to the unpleasant smell resulting from the applied pesticide. Table III shows the incidences of eye, nose, throat and respiratory complaints of 72 children who were tested before and after the pesticide application. It was observed that all respiratory complaints of the 72 children increased significantly after the pesticide application. Unfortunately, the children could not be evaluated in groups due to decreased participation after pesticide application. The mean PEF of the 256 children before the application of pesticide was 261.0 ± 75.7 mL/s (minimum: 120 mL/s, maximum: 530 mL/s), while the corresponding value after the application was 231.1 ± 65.7 mL/s (minimum: 60 mL/s, maximum: 450 mL/s) ($p < 0.001$). The PEF values were measured in 72 children after pesticide application. The mean PEFs of the same 72 children before and after pesticide application were 263.1 ± 67.0 mL/s (minimum: 120 mL/s, maximum: 480 mL/s) and 231.1 ±

Table I. Environmental measurements before and after pesticide application.

	Pesticide application		p
	Before	After	
PM2.5 (μgr/m^3)	6.9 ± 3.6	12.7 ± 1.2	<0.0001
PM10 (μgr/m^3)	18.2 ± 5.8	327.9 ± 7.3	<0.0001
Temperature (°C)	32.5 ± 1.2	37.9 ± 0.5	<0.0001
Humidity (%)	26.6 ± 2.6	53.1 ± 0.9	<0.0001
Wind speed (m/s)	27.3 ± 4.8	21.0 ± 0.0	<0.0001

*the values show mean±SD

Table II. Frequency of respiratory symptoms before and after pesticide application.

Symptoms	Pesticide application				p
	Before (n=266)		After (n=72)		
	n	%	n	%	
Mouth-nose-throat burning	29	10.9	45	62.5	<0.0001
Eye burning	31	11.7	47	65.3	<0.0001
Cough	34	12.8	46	63.9	<0.0001
Sputum	14	5.3	38	52.8	<0.0001
Wheezing	10	3.8	43	59.7	<0.0001
Dyspnea	15	5.6	47	65.3	<0.0001
Chest tightness	19	7.1	43	59.7	<0.0001

Table III. Evaluation of respiratory symptoms within same individuals before and after pesticide application.

Symptoms	Pesticide application				p
	Before (n=72)		After (n=72)		
	n	%	n	%	
Mouth-nose-throat burning	9	12.5	45	62.5	<0.0001
Eye burning	9	12.5	47	62.5	<0.0001
Cough	10	13.9	46	65.3	<0.0001
Sputum	3	4.2	38	52.8	<0.0001
Wheezing	3	4.2	43	59.7	<0.0001
Dyspnea	4	5.6	47	65.3	<0.0001
Chest tightness	10	13.9	43	59.7	<0.0001

Table IV. Relationship between PEF and environmental factors before pesticide application.

	PM2.5	PM10	Heat	Wind speed	Humidity
PEF					
r	0.094	0.071	0.019	0.137	-0.026
p	0.137	0.266	0.766	0.030	0.681

Table V. Correlations between PEF values and environmental factors after pesticide application.

	PM2.5	PM10	Heat	Humidity
PEF				
r	0.217	-0.295	0.135	-0.135
p	0.067	0.012	0.259	0.259

65.7 mL/s (minimum: 60 mL/s, maximum: 450 mL/s), respectively ($p < 0.0001$). No statistically significant differences were observed between the 194 children who participated in the first PEF measurement and the 72 children who participated in the second half of the study in terms of average age (10.8 ± 2.7 to 10.5 ± 2.4 years; $p = 0.457$) and gender distribution (while the rate of girls participating in the study before pesticide application was 50.5%, after application it dropped to 39%, and the corresponding rates for the boys were 49.5 to 61.5%; $p = 0.060$). These results were excluded from the discussion that follows, as these exerted no effects on the results of this study.

A significant positive correlation was observed between the PEF values measured before ($r = 0.680$, $p < 0.001$) and after ($r = 0.438$, $p < 0.001$) pesticide application. There was no significant difference between the mean PEF values of girls (255.8 ± 71.4 mL/s) and boys (265.4 ± 79.3 mL/s) before pesticide application ($p = 0.318$). The mean PEF value of girls after pesticide application (202.8 ± 63.3 mL/s) was significantly lower than that of the boys (249.1 ± 61.3 mL/s) ($p = 0.03$). PEF values can be affected by $PM_{2.5}$ and PM_{10} concentrations, wind speed, temperature and humidity in the pesticide application area. For this reason, the PEF values were assessed with environmental factors to determine the correlation among them. Before pesticide application, PEF and wind speed alone were positively related (Table IV). After pesticide application PEF was found to be significantly negatively correlated with PM_{10} (Table V and Fig. 2). The linear regression analysis showed that PM_{10} measured after pesticide application was independent of PEF ($B = -2.706$, $t = -2.425$, and $p = 0.018$). While no significant differences were observed for PEF ($p = 0.059$) and wind speed ($p = 1.000$) among four villages after pesticide application, significant differences

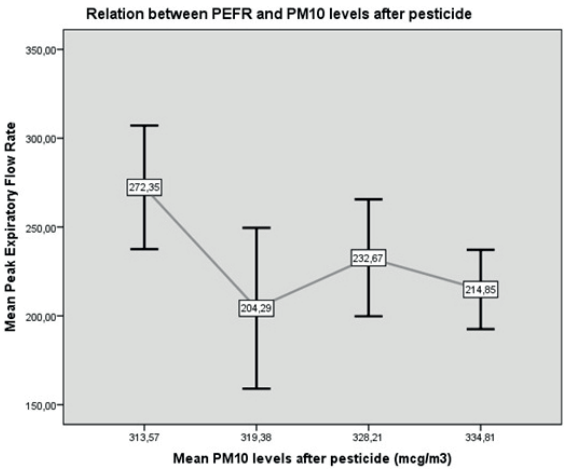

Fig. 2. Negative relationship between PEF and PM10 levels after pesticide application.

existed for temperature ($p < 0.001$), humidity ($p < 0.001$), PM_{10} ($p < 0.001$) and PM ($p < 0.001$) according to the results of the Kruskal–Wallis test.

Discussion

It is natural to believe that rural farmers are blessed with clean air and a naturally healthy environment. However, farming is rife with the hazards posed by pesticide application and many other respiratory hazards. Approximately five billion pounds of pesticides are consumed worldwide annually.[17] Among these pesticides, organophosphate and carbamate insecticides are the most commonly used.[18] Toxic pesticides are widely applied to control pests and pest-related diseases in agriculture, fishery, forestry and the food industry, leading to their dissemination to the environment.[16] Although non-toxic or less toxic and harmless strategic alternatives exist, they are often overlooked. The toxic effects of pesticides are particularly intense among children, elderly and women living in agricultural areas due to their heightened exposures.[18-23] Awareness about the risks of pesticide exposure to human life, and especially later ages in the case of children, is lacking. Pesticide exposure can cause asthma, allergic rhino-sinusitis and chronic bronchitis in the respiratory tract.[24-30] Our study investigated the acute effects of exposure to pesticide, because the long-term effects of pesticides on humans are yet to attract sufficient attention. In addition to pesticide-related complaints in children, PEF measurements were performed in order to make it easier for the children to adapt to the pulmonary function tests. The PEF values before and after pesticide application varied due to the irritation caused by the pesticide exposure, particularly in the respiratory tract. The most well-known outdoor allergens in Şanlıurfa are meadow and grain pollen. Meadow pollen levels are at their highest level in May.[31] Although the time period for the pollination of grain pollen varies regionally, it typically begins in March and continues until the end of May.[32]

Our study revealed that children living near areas with agricultural pesticide application suffered from a significant increase in upper and lower respiratory tract complaints and a significant decrease in PEF values after pesticide application. The decrease in PEF was significantly negatively correlated with the increase in PM_{10} values. In children exposed to PM originating from a mixture of lambda-cyhalothrin (7 g/daa) and emamectin benzoate (4 g/daa), the upper and lower respiratory tract complaints increased in the short term, while the respiratory function decreased. Our study observed that the average PEF values of girls decreased to a greater extent than those of boys after pesticide application. A review investigating whether boys or girls are more affected by air pollution, be it outdoors or indoors, showed that children's hormonal levels, growth rates and respiratory abilities were adversely affected by air pollution, and that girls tend to be affected to a greater extent than boys.[33]

Lambda -cyhalothrin is an insecticide belonging to the pyrethroid family.[34,35] Some studies report a burning sensation in the face and irritation in the upper respiratory tract after exposure to cyhalothrin.[36,37] A study conducted in Tanzania showed that pesticide spray application can lead to irritation in the nose and throat as well

as a burning sensation in the face and periorbital area within a few hours after application, and the duration of these complaints could last for a day.[38] Our study revealed that cough, sputum production, wheezing, dyspnea, chest tightness as well as a burning sensation in the eyes, mouth, head, neck and throat could be observed in 59.7–88.8% of the children on the second day after cyhalothrin application outdoors (7 g/daa). While such complaints did not last for more than a day in the previously cited study[38], the symptoms continued to exist 2 d after the pesticide application in our case. Most importantly, these symptoms were also accompanied by a decrease in mean PEF values of the children. Moreover, the affected person in the previously cited study[38] was exposed to cyhalothrin (25 mg/m^2) an indoor environment, and was less affected than the children in our study although the latter took place outdoors and the dose of cyhalothrin dosage was lower (7 mg/m^2). This difference can be explained by the fact that children are more severely affected by pesticide application than adults. The possible explanations for this result include higher respiratory rates and shorter airways in children compared to adults.

A previous study in Ethiopia also showed significantly lower FEV_1 and FVC values due to pesticide application in a group of young subjects aged 15–24 years.[39] In our study, the PEF values of the children showed significant negative correlations with PM_{10} levels.

A previous work exploring the effects of organophosphate application in 279 children over 7 years in California, US, reported a significant decrease in the FEV_1 and FVC values as a result of exposure at an early age to pesticides (dialkyl phosphate levels were measured in the urine).[4] The American study also showed a long-term decline in respiratory function. Similarly, the significant accelerated decrease in the PEF values shortly after pesticide application in our study revealed that pesticides cause acute and adverse respiratory health outcomes in children.

Although our study was initiated with 266 children as participants, it eventually continued with only 72 children. Given the discomfort caused by the intensive pesticide application in this region, the farmers chose to shift temporarily to other regions during the pesticide application season. The data obtained from the 266 children before pesticide exposure and the pre-exposure data of the remaining 72 children were compared. The statistical comparisons provided similar results. Thus, one of the most important limitations of our study was that only one-third of the population before pesticide application continued participation after the application. The absence of a control group was another important limitation. Moreover, the pesticide ratios in the PM could not be measured.

In this study, the colour of the bare soil of the fields before pesticide application in June was red. After the cotton plants grew by July or August, the fields turned green and lush with the crop. The concentrations of airborne PM were expected to decrease with the increased amounts of moisture provided by irrigation, the decreased wind speed and the greening of the environment. However, the pesticide application increased PM levels.

The potential routes of pesticide exposure in children residing near these areas included contact with pesticide-contaminated clothing of the adults who applied the pesticides, air inhalation, contact with soil, crops and pesticide application devices as well as water consumption.[2,40] The acutely impaired respiratory functions of the children in our study may be prevented by various approaches. The first approach involves temporarily shifting people from the agricultural area during periods of pesticide application to prevent exposure via air inhalation. Nonetheless, exposure to pesticide residues in the soil and environment is unavoidable. The second and most radical approach involves replacing pesticides with alternative substances. The use of pesticides should be controlled, and non-toxic pesticides should be encouraged. New harmless methods for protection against

pests should be investigated. Another option involves cultivating pest-resistant plants. Further, to minimise human and environmental exposure arising from airborne pesticide residues, the potential for losses to the air should be considered when selecting pesticide formulations and application methods.

In addition to these measures, an early warning system can be established to detect respiratory disorders by tracking annual PM_{10} data in the region.[41] Such a system can ensure the use of the best scientific tools to inform all those concerned about possible health exposures in a timely manner. Such a warning system can help in the planning of temporary relocation of the affected inhabitants of the region.

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Original Article

The assesment of prothrombotic potential using thrombin generation assay in pediatric patients with nephrotic syndrome: preliminary study

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ABSTRACT

Background. Nephrotic syndrome (NS) is a common kidney disease associated with an increased risk of thrombotic events. The aim of this study was to assess the prothrombotic potential of patients with NS using the thrombin generation assay (TGA).

Methods. A total of 35 patients with NS, who were followed in the Division of Pediatric Nephrology in Behcet Uz Children's Hospital, were included in the study. After the patients with Steroid Resistant NS (n:3) were excluded, 32 patients in total were evaluated for TGA. Patients were primarily classified according to their response to corticosteroid therapy. The control group consisted of 34 healthy volunteers with similar gender and age distribution to the patients. Blood urine nitrogen, creatinine, albumin, triglyceride, cholesterol, 24-hour proteinuria, platelets, erythrocyte sedimentation rate, C-reactive protein and thrombin generation values in activation and remission period of NS were compared. Moreover, TGA values of the patients in their remission period were compared with the values of those in the control group.

Results. Endogenous thrombin potential (ETP) and peak thrombin levels were significantly higher in the activation period than remission period of NS. Additionally, after the patients achieved remission, their ETP was still higher than the control group. There was a negative correlation between both ETP and peak thrombin levels of patients with serum albumin, whereas a significant positive correlation was detected with platelet levels. Thromboembolic events were not observed in any of the patients during follow-up.

Conclusions. Nephrotic syndrome is strongly associated with hypercoagulopathy as assessed by TGA during active NS. The present study reinforces the usefulness of TGA as a marker of hypercoagulability in pediatric patients with NS. Further studies are needed in this regard.

Key words: nephrotic syndrome, thrombin generation assay, children, hypercoagulability.

Nephrotic syndrome (NS) is a disorder of the kidneys which originates from increased permeability of the glomerular filtration barrier. It is characterized by proteinuria, hypoalbuminemia, edema and hyperlipidemia.[1] NS is associated with hypercoagulability and an elevated risk of thromboembolic events leading to increased morbidity and mortality.[2] The thromboembolic event incidence in NS has been reported as 3-44% depending on the extent of the diagnostic screening.[3] Pulmonary embolism and renal vein thrombosis have been reported as common complications (20-35%) in patients with NS.[4-6] The incidence of subclinical pulmonary embolism by scintigraphic pulmonary ventilation and perfusion studies was found to be 28% in children with NS.[7] Additionally, most patients (adults and children) with pulmonary embolism (84%) were asymptomatic.[8-11]

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The risk of thrombosis in patients diagnosed with NS can be affected by multiple factors and can be divided into two categories; urinary loss of proteins and increased hepatic synthesis of procoagulant proteins.[12,13] The urinary loss of proteins, such as antithrombin, plasminogen, protein C and S and increased synthesis of factors I, II, V, VII, VIII, X, and XIII promote thrombosis.[13] Thrombocytosis and increased platelet aggregation in NS can also play a role in thrombosis.[14] Furthermore, hyperviscosity and hyperlipidemia can also be responsible for the prothrombotic state.[15]

Treatment of NS such as corticosteroids, diuretics and usage of a central venous catheter, can also result in an increased risk of venous thromboembolism (VTE). Many studies have demonstrated the relationship between the hemostatic status of patients in activation and remission period of NS with healthy individuals.[13-17] However, no study has yet been reported on the thrombin generation assay (TGA) which indicates global hemostasis and the result of these changes during NS in children.

Thrombin is a multifunctional protein and a key enzyme in the coagulation system. It is involved in coagulation, anticoagulation, endothelial activation and proliferation of fibroblasts. With the use of TGA, the endogenous capacity of the overall hemostatic potential can be evaluated. Both thrombotic conditions due to high thrombin production and the hemorrhagic conditions due to low thrombin production can be assessed. It is possible to evaluate the relationship between both proteases and inhibitors by measuring the thrombin capacity with TGA.[18,19]

In this study, we aimed to evaluate the prothrombotic potential increase in the activation and remission periods of patients with NS with TGA, a test that measures hypercoagulation by measuring thrombin formation capacity.

Material and Methods

The study was carried out from March 2015 to August 2015 and included 35 patients in total, who were followed with NS in the Division of Pediatric Nephrology in Behcet Uz Children's Hospital. This retrospective analysis was approved by the Institutional Review Board and approval was received from the local Ethics Committee (2015/04-04). The study was approved on 12.03.2015. Following approval from the ethics committee; patients, volunteers and their families were informed, and written consent was received.

Patients who were admitted to hospital during the study period in activation of idiopathic NS were included and the following data was collected: age, gender, response to therapy, duration of follow-up, additional diseases, and history of personal and/or familial thromboembolic events. The activation period was described as nephrotic range of proteinuria, hypoalbuminemia and edema; remission of the disease was described as urinary protein analysis in 24 hours < 4 mg/m^2/hour or urine protein to creatinine ratio < 0.2 mg/mg creatinine for 3 consecutive days, and normal values of serum albumin. Patients were primarily classified by their response to corticosteroid therapy as; corticosteroid responsive (steroid sensitive): if complete remission with corticosteroid therapy was attained; or steroid resistant (SR): if there was inability to induce a remission within 4-6 weeks of daily corticosteroid therapy (2 mg/kg/day or 60 mg/m^2/day). Steroid responsive patients who relapse during the dose tapering or within 2 weeks of discontinuation of steroid therapy are termed steroid dependent NS. Patients diagnosed with SRNS (n:3) were excluded and 32 patients were evaluated for TGA in the study (Fig 1). None of the patients or their parents had a history of thrombosis. During the study period, the anticoagulant prophylaxis was not used in the patients.

Urea nitrogen, creatinine, albumin, triglyceride, cholesterol, 24-hour quantitative proteinuria, platelets, erythrocyte sedimentation rate,

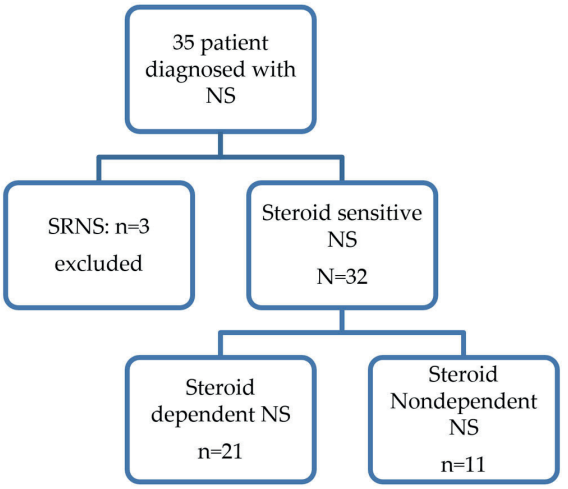

Fig. 1. Diagnoses of patients in study.
NS: nephrotic syndrome

C-reactive protein and TG were assessed during hospital admission with activation of NS.

After the patients achieved remission, TG was assessed again for comparison. The patients' and healthy volunteers' blood pressures were measured during their visits. The control group consisted of 34 healthy children with no glomerulopathy, acute infection or family history of thrombosis. They were admitted to the pediatric nephrology clinic for urological pathologies (monosymptomatic enuresis or isolated hydronephrosis) with normal renal function and biochemical parameters and did not use any medication.

Blood sampling procedure and organizing Platelet-Poor Plasma (PPP): Peripheral venous blood was collected into vacutainer tubes containing 0.129 mol/L tri-sodium citrate. With a double centrifugation at 2500g for 15 minutes, PPP was collected from the upper half of the plasma supernatant, frozen and stored at -80 ºC. For the TGA test, aliquoted PPP was stored for 3 months at -80 ºC and all samples were assessed simultaneously. The Calibrated Automated Thrombogram method was used for TGA.[14] Recombinant relipidated tissue factor Innovin was used to activate thrombin generation. The assays were performed according to the manufacturers' instructions for thrombin generation with a mixture of 5 pM TF and 4 μM phospholipids for PPP. Fluorescence was measured using an Ascent Reader, and thrombin generation curves were calculated with the Thrombinoscope™ software package. Thrombin generation parameters include the following: lag time (time to 16.7% of peak concentration; min), endogenous thrombin potential (ETP; area under the curve; nMminute), peak thrombin (nM), time to peak (min), and start to tail (min).[20] The 24-hour urine samples which were collected from the patients were used to determine protein excretion. Thrombin generation parameters of patients during the active NS period were compared with the parameters during the remission period. The interval between activation and remission was a minimum of four weeks. Thrombin generation parameters in remission period of the patients were compared with the thrombin generation parameters of the healthy volunteers, and it was evaluated whether the patients were susceptible to hypercoagulability even in the remission period. Patients with steroid-dependent NS and non-dependent NS were also compared among each other.

Statistical analyses were carried out using SPSS 18.0. Normality of the variable distribution was evaluated using parametric tests. Variables were analyzed by means of nonparametric tests if not normally distributed. Measurements normally distributed were reported as the mean ± SD; non-normally distributed data were expressed as median and minimal–maximal value. The Student's t test or Mann–Whitney U test were used in statistical analysis to compare differences between groups. Pearson correlation analysis was used for correlation of the parameters in active disease and TGA values. P values < 0.05 were considered statistically significant.

Results

The study group consisted of 32 patients with NS (18 male, 14 female). The mean age of the participants was 128 ± 53 months. There was no history of thromboembolic events in any

participants or their parents. The control group was comprised of 16 males and 18 females. Similarly, the mean age was 125 ± 53 months (Table I). The laboratory results of patients with NS in activation and remission period are presented in Table II.

From the evaluation of TGA parameters in activation and remission periods of patients showed that; ETP and peak thrombin levels were significantly higher during the activation period compared to remission period (P < 0.001, and P < 0.001, respectively) (Table III). Although patients achieved remission, their ETP levels were still higher than the control group (P = 0.001). Mean lag time of the patients in activation period was significantly shorter than in remission (p < 0.001). Additionally, mean time to peak and mean start tail levels of the patients were shorter in remission compared to activation (P < 0.001 and P < 0.001, respectively) (Table III). Conversely, lag time and time to peak of the patients with remission were longer than the control group (P = 0.001 and P= 0.017, respectively) (Table III). In a comparison of the TGA parameters of patients diagnosed with the subgroups of steroid sensitive NS, there was no significant difference between steroid dependent and non-steroid dependent disease groups (Table IV).

A significant converse correlation was found between serum albumin and both ETP and peak thrombin of the patients (P < 0.001 and P = 0.005, respectively), while there was a positive correlation between serum albumin and start tail (P= 0.010). In addition, there was a negative relationship between urine protein of the patients and lag time, time to peak and start tail, while a significant positive correlation was

Table I. The demographic and clinical data of patients with NS and healthy volunteers.

	NS patients (n: 32)	Healthy volunteers (n: 34)	p
Age (month)	128 ± 53	125 ± 54	0.78
Gender			
Female	14	18	0.40
Male	18	16	
Other chronic disease	-	-	
Tromboembolic event	-	-	
Tromboembolic event in family members	-	-	

NS: nephrotic syndrome

Table II. The laboratory data of patients with NS and healthy volunteers.

Laboratory parameters	NS patients (n: 32)	
	Active disease period	Remission period
BUN (mg/dl)	13.0 ± 7.4	9.6 ± 2.6
Creatinine (mg/dl)	0.51 ± 0.12	0.50 ± 0.10
Albumine (g/dl)	2.5 ± 0.7	4.0 ± 0.39
Total cholesterole (mg/dl)	331.3 ± 107.3	207.5 ± 70.4
LDL (mg/dl)	206 ± 8.0	128 ± 56
HDL (mg/dl)	63 ± 16	69 ± 17.8
Triglycerides (mg/dl)	243 ± 145	207 ± 70
Platelets (/mm^3)	390.228 ± 100	337.571 ± 76.050
Sedimentation (mm/hour)	47.2 ± 1.9	20.7 ± 13.6
24 hour urine protein (mg/m^2/hour)	140 ± 77	2.2 ± 1.4

NS: nephrotic syndrome, mg: milligram, dl: deciliter, m: meter

Table III. Comparison of TGA parameters in patients with NS during activation, remission and healthy control group.

	Activation of NS Mean±SD	Remission of NS Mean±SD	Control Mean±SD	p1 A/R	p2 R/C
Lag time (min)	4.4 ± 1.2	5.9 ± 2	4.5 ± 1.1	<0.001	0.001
ETP (nM/dk)	2550 ± 494	1715 ± 467	1355 ± 386	<0.001	0.001
Peak thrombin (nM)	356 ± 96	209 ± 91	197 ± 80	<0.001	0.575
Time to peak (min)	8.9 ± 1.7	11.2 ± 3.0	9.3 ± 3.2	<0.001	0.017
Start to tail (min)	20.2 ± 3.0	28.2 ± 8.9	24.3 ± 8.5	<0.001	0.073

TGA: thrombin generation assay, ETP: endogenous thrombin potential, NS: nephrotic syndrome, A: activation, R: remission, C: control, SD: standard deviation, min: minute
(p1 value for: statistical significance between TGA parameters of patients during activation and remission periods, p2 value for: statistical significance between patients' TGA values during remission and healthy children's TGA values.)

Table IV. Comparison of TGA parameters in patients with steroid dependent NS and steroid responsive (not dependent) NS patients during active disease period.

	Steroid dependent NS patients Mean ± SD (n: 21)	Steroid not dependent NS patients Mean ± SD (n: 11)	p
Age	10.36 ± 3.5	11.52 ± 5.0	0.502
Gender			
Female/Male	13/8	1/10	
Follow up with NS period (year)	7.02 ± 5.4	5.13 ± 2.9	0.297
Other chronic disease	-	-	
Tromboembolic event	-	-	
Tromboembolic event in family members	-	-	
Lag time (min)	4.3 ± 1.1	4.1 ± 0.98	0.598
ETP (nM/dk)	2599 ± 579	2510 ± 348	0.646
Peak thrombin (nM)	348 ± 116	380 ± 55.5	0.302
Time to peak (min)	8.9 ± 1.9	8.3 ± 0.85	0.963
Start to tail (min)	20.5 ± 3.5	20.5 ± 3.5	0.350

TGA: thrombin generation assay, ETP: endogenous thrombin potential, NS: nephrotic syndrome, SD: standard deviation, min: minute

detected between proteinuria and both ETP and peak thrombin (Table V). A significant positive correlation was found between triglyceride of the patients and both ETP and peak thrombin (P = 0.003, and P= 0.006, respectively) (Table V).

A significant relationship between peak thrombin and cholesterol was also observed (P= 0.007), whereas no correlation between cholesterol and ETP was found (P= 0.428).

Thrombocytosis is also defined as a platelet count greater than two standard deviations above the mean or greater than 450,000/mm^3.[21] According to this definition, there were no patients with thrombocytosis in our study; but platelet counts of the patients with NS in activation (390.228 ± 100/mm^3) were significantly higher than the control group (337.571 ± 76/mm^3) (p<0,001). There was a significant positive correlation between platelet counts of the patients and both ETP and peak thrombin (P= 0.001 and P= 0.006, respectively) (Table V).

Thromboembolic event was not observed in any of the patients during follow-up.

Table V. The correlation between serum total cholesterol, triglyceride, platelets of the patients and TGA parameters.

	T. cholesterol		Triglyceride		S. albumine		U. protein		Platelets	
	R	P	R	P	R	P	R	P	R	P
Lag time(min)	-0.143	0.238	-0.134	0.270	0.158	0.191	-0.239	0.470	-0.340	0.778
ETP (nM/dk)	0.000	0.428	0.346	0.003	-0.477	<0.001	0.561	<0.001	0.404	0.001
PT (nM)	0.322	0.007	0.325	0.006	-0.331	0.005	0.561	<0.001	0.327	0.006
Time to peak (min)	-0.099	0.415	-0.175	0.147	0.132	0.277	0.298	0.120	-0.138	0.254
Start to tail (min)	-0.288	0.016	-0.230	0.056	0.307	0.010	-0.404	0.001	-0.162	0.180

TGA: thrombin generation assay, ETP: endogenous thrombin potential, PT: peak thrombin, T.cholesterol: total cholesterol, S. albumine: serum albumin, U. protein: urine protein
(R <0.2: too weak correlation, 0.2-0.4: Poor correlation, 0.4-0.6: Moderate correlation, 0.6-0.8: High correlation, :0.8> Very high correlation)

Discussion

NS is a common kidney disease associated with a significantly increased risk of thrombotic events. The important changes in plasma hemostatic proteins (such as increased factor V, factor VIII, fibrinogen, factor XI, α2 macroglobulin, decreased protein C, protein S and antithrombin) in patients with NS have been shown in several studies.[17-19,22] Although hemostatic potential is increased in NS, there are very few studies about direct examination of global hemostasis.

Kerlin et al.[12] reported that thrombotic capacity is correlated with disease severity, but evident thrombosis may necessitate vascular injury (two-hit hypothesis). In the same study, they have shown that TGA has a significant correlation with the severity of hypoalbuminemia and proteinuria.[13] Their results suggest that the increased thrombin–generating capacity is responsible for susceptibility to thrombotic events and therefore, the risk of thrombosis increases gradually depending on the severity of the disease. Similar to the report of Kerlin et al.[12]; urinary protein excretion, serum albumin and triglyceride levels correlated significantly with ETP and peak thrombin levels of the patients in our study. ETP and peak thrombin levels of the patients in activation were significantly higher than those in remission. Mean lag time, time to peak and start tail of the patients in activation was significantly shorter than in remission. Additionally, although the patients achieved remission, mean ETP levels were still higher than the control group. In recent studies, it has been reported that the increase in anticoagulant factors and decrease in procoagulant factors return to normal levels after remission.[22-24] However, the persistence of hypercoagulability after remission and the changes in factor levels have not been reported.

Despite a significant high thrombotic capacity in patients with NS in activation, thromboembolic event was not observed during follow-up. These results may be correlative with a two-hit hypothesis of thrombus expansion, which has been offered for other prothrombotic disorders (e.g., antiphospholipid syndrome). An acquired prothrombotic coagulopathy (first hit) is not adequate to induce thrombosis in the absence of a second event, such as vascular injury (second hit).[25] Consequently, more studies should examine the multifactorial effects of both disease severity and other prothrombotic conditions such as cardiovascular disease, catheter–related venous endothelial injury, trauma or venous stasis related to edema.

Increased thrombin has been shown in a single human study (adults) of NS.[26] Mahmoodi et al.[26] reported that peak thrombin, in the presence of thrombomodulin, was significantly lower during the antiproteinuric treatment period compared to the placebo. Additionally, ETP, peak thrombin and time to peak demonstrated

a more procoagulant state in patients with NS than controls. However, mean levels of lag time and time to peak were similar during the antiproteinuric treatment and the placebo period.[26] Similarly, in this study, TGA parameters in NS with activation were significantly higher than both in remission and control groups. Although the patients achieved remission, their ETP was still higher than the healthy control group. These findings may indicate that even if remission is obtained in NS, the prothrombotic state goes on. Conversely, lag time and time to peak of the patients in remission were longer than the healthy control group. Because of this discrepancy in the findings about lag time and peak thrombin, these TGA parameters may not be useful to demonstrate nephrotic hypercoagulopathy.

When the TGA parameters in activation of corticosteroid dependent, corticosteroid resistant and relapse groups were compared with each other, there was no significant difference. These results may show that activation of the disease is the basis of hypercoagulability condition in NS rather than response to corticosteroid. Both severity of hypoalbuminemia and proteinuria have been accompanying factors to thrombotic risk.[27] In another pediatric study, worsening proteinuria is directly correlated with increasing venous thrombosis probability.[3] However, hypoalbuminemia, which is closely correlated to proteinuria severity, was reported not to be a significant marker in a recent large pediatric cohort.[3] We revealed that ETP and peak thrombin significantly correlated with serum albumin and proteinuria of the patients. Although TGA may be a marker for thrombotic risk, its clinical practice has been limited due to lack of standardization among test techniques.[26,28] Thus, proteinuria and/or serum albumin may become suitable and useful biomarkers for identifying those patients with an increased risk of thrombosis.[27,29]

Hyperlipidemia is a known factor underlying atherosclerosis. Nevertheless, it is unclear whether it is a cause of thrombosis or not. Paraskevas et al.[30] revealed that statins are able to lower the risk of venous thrombosis in NS patients when compared with a placebo.[30] These actions of statins have been explained with their anti-inflammatory effects, prevention of atherosclerotic vascular diseases and modulation of the coagulation cascade.[31] In this study, we showed that there was a significant positive correlation between triglyceride of the patients and both ETP and peak thrombin. Total cholesterol also had a significant relationship with peak thrombin, but there was no correlation with ETP. These results indicate that hyperlipidemia in activation period of NS may increase thrombin generation, which is the reason for the hypercoagulable condition. Increased platelet counts and hyperactivity have been detected in children with NS.[30,32] Although these findings may show increased risk for thrombotic event especially in adults with atherosclerotic disease, reactive thrombocytosis is rarely a cause for thrombotic event in children.[33-35]

The underlying linkage between platelet alterations and the occurrence of thromboembolic event is not completely understood. However, Eneman et al.[14] have shown that the mechanisms underlying platelet abnormalities are probably due to changes in plasma levels of platelet-interfering proteins and lipids, as a result of NS.[11] The pathogenesis of platelet hyperactivity is also associated with hypoalbuminemia, hyperfibrinogenemia, high levels of cholesterol and low-density lipoprotein.[36-38] In this study, even if platelet function of the patients was not assessed, their platelet counts in activation were significantly higher than the control group. There was also a significant positive correlation between platelets of the patients in activation and both ETP and peak thrombin. Since platelet-rich plasma was not used for TGA, this correlation was thought to be related to the increase in platelet count as well as the increase in ETP and peak thrombin levels at the time of activation.

This study has several limitations. First, the number of patients was low. Second, the other inherited thrombosis risk factors that may be

responsible for the prothrombotic state in NS (such as fibrinogen, FV, FVIII, FXI, AT, PS, PC, α2 macroglobulin, and plasminogen levels) were not assessed. Third, if TGA was also performed with platelet rich plasma, we could attain more information regarding nephrotic hypercoagulopathy.

In conclusion, this is the first pediatric study that shows prothrombotic state by TGA in NS. This study will help to identify children with NS who are at high risk of thrombosis. We think that the patients who have other prothrombotic circumstances (such as trauma, obesity, cardiovascular disease, venous catheter–related endothelial injury, or bed rest) should be closely monitored for thrombotic events. TGA seems a promising laboratory tool for investigating patients with risk of thrombosis or VTE recurrence but further studies with standardized TGA are needed to make it applicable in clinical practice.

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Original Article

How effective is family counselling on screen exposure of pre-school children?

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ABSTRACT

Background. Excessive screen viewing and background TV exposure are common problems all over the world. Therefore, intervention studies have gained importance. This study aims to investigate the effectiveness of family-based, developmental pediatrics clinic setting counseling in reducing screen time in typically developing children and to compare them with neurodevelopmental disorders.

Methods. Children (aged 24-62 months) who were exposed to screen viewing for at least 2 hours/day were included. Parents were given three counseling sessions to reduce excessive screen time. Parents reported daily screen time, co-viewing, background TV exposure, the duration of reading books and playing with their child.

Results. The study included 105 children (median age: 34 months IQR:28-41). Before counseling, the screen viewing time and the percentage of co-viewing among typically developing children (n=22) and children with a neurodevelopmental disorder (n=83) were similar. There was a statistically significant decrease in screen time in both groups after the intervention. A higher impact was shown in the neurodevelopmental disorder group. The increase in percentages of co-viewing, as well as the increase in the time spent playing with their children, were statistically significant in the neurodevelopmental disorder group.

Conclusions. The study demonstrated that three pediatric office-setting counseling sessions including media use recommendations of the American Academy of Pediatrics are effective to decrease screen time for children who are either typically developing or with a neurodevelopmental disorder.

Key words: background TV exposure; excessive screen time; family counseling; neurodevelopmental disorder, pediatric office setting.

In recent years, children are being brought up in a digital world. Excessive screen time was found to be associated with various negative child health outcomes including language delay[1], attention problems[2], cognitive developmental delay.[3] Since 2016 the American Academy of Pediatrics (AAP) has recommended no more than 1 hour per day of screen exposure for children aged 2-5 years.[4] However, studies from various countries have shown that children's exposure to a screen is more than 2 hours per day.[5,6] In the literature, there is conflicting evidence about screen-based activities of children with neurodevelopmental disorders (NDD).[7-10]

Not only screen viewing but also background TV exposure is associated with negative outcomes[11,12] Although not emphasized in studies related to screen intervention, background TV exposure is an important problem all over the world. A study in which low-income Mexican American parents were asked "How often their child was playing in the same room or near a TV that is on?" 43% of the parents reported that their child was often,

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very often and always doing so, in other words, exposed to background TV.[13]

Therefore, screen time intervention studies are important. The intervention studies showed that longer duration (>6 months) and having high parent involvement, in studies in which the types of interventions were health promotion counseling, behavioral interventions and used electronic TV monitoring devices were effective strategies for reducing screen time.[14,15] Preschool care setting, healthcare center/pediatric office setting interventions are less successful at reducing children's screen time than the other interventions.[15] In the literature, only a limited number of intervention studies target reduction of screen time in pre-school children and the effects of interventions in reducing screen time are contradictory.[15-17] Moreover, we could not reach any article in the published literature about the effectiveness of screen intervention in an office setting tackling background exposure beside screen viewing time and targeting both typically developing pre-school children and those with NDD.

The aim of this study was to evaluate the effectiveness of three consecutive family-based (high intensity)[18], developmental pediatrics clinic setting counselling sessions, based on the media use recommendations of the American Academy of Pediatrics (AAP), in decreasing screen time, background TV exposure and in increasing development promoting activities of pre-school children with typically developing (TD) and NDD.

Material and Methods

This was a retrospective case control study conducted at Hacettepe University Ihsan Doğramacı Children's Hospital, Developmental Pediatrics Clinic. The study was approved by the Ethics Committee of Hacettepe University Faculty of Medicine on September 2018 (GO 18/317-27). The study also conforms to the provisions of the Declaration of Helsinki in 1995.

Population

Patients aged between 24-62 months admitted to the clinic between March 2014 - December 2017, who were exposed to screen viewing (using a computer, watching television or videos, mobile phone and playing video or computer game) for 2 hours or more whose parents were provided with counselling on three consecutive visits (high intensity)[18] were included in the study.

Ages and Stages Questionnaire for Turkish children (ASQ-TR)[19] and Bayley Scales of Infant and Toddler Development III[20] with clinical observation were both used to evaluate the development level of children in the present study. Global developmental delay was defined as a delay in at least two developmental domains (communication, gross and fine motor skills, problem solving, and personal-social skills) in the standard development tests mentioned above. Autism Spectrum Disorder (ASD) was diagnosed via a clinical evaluation by a child psychiatrist and developmental pediatrician according to DSM-V criteria. Language development delay (LDD) was diagnosed based on a clinical evaluation by a developmental pediatrician and language speech therapist using one of the following scales; Bayley Scales of Infant and Toddler Development III, Preschool Language Scale Edition-5.[21] Children admitted to the clinic due to parental concern regarding their development but who were considered as typically developing constituted the TD control group.

Feeding difficulty was defined as caregiver concern about child's feeding according to the study by Kerzner.[22]

Study design

During the first admission interview, information on the demographic data (age, gender, parental age, parental disease, parental education, mother's employment status, number of siblings), attending pre-school, feeding difficulties were all recorded.

Screen time, background TV exposure, co-viewing, promotion development activities, feeding difficulties were collected by a questionnaire specifically designed and asked for the study by the researchers. Screen time was measured using the question "how many hours is your child usually exposed to a screen (computer, television, mobile phones, etc) daily?". The background exposure was evaluated by asking parents "how many hours a day does your child usually spend next to a turned-on TV although he/she is not watching?". The percentage of co-viewing was measured by asking the parents "what is your daily percentage of watching the screen together?". The development promotion activities were asked by "how much did reading books and playing together changed after intervention?". Response options were no change, decrease, increase. Feeding difficulties were reported by the parents' answers to the question "do you have any concerns about your child's feeding?" If the caregiver answered yes, it was accepted that the child had a feeding difficulty.

Intervention

Screen time interventions are classified according to their intensity as low and high intensity. High intensity interventions are those having at least three interactions with family/child or the presence of an electronic monitoring device to limit screen time.[18] The intervention in the study is high intensity as it included three consecutive counselling sessions in a developmental behavioral pediatric clinic. In each session, at least one parent received about 10-15 minutes behavioral counselling intervention face to face by a developmental pediatrician after the medical visit. The counselling included the AAP recommendations about screen viewing, information on the health and developmental effects of screen time on children, strategies to reduce screen time (reading books or playing together) and an explanation about the detrimental effects of background TV exposure.

Statistical Analyses

The statistical analysis was performed with IBM SPSS 22.0. Numerical variables were given as mean ± standard deviation or median (interquartile ranges). Categorical variables were displayed as frequencies and percentages. Categorical variables were compared by chi square test. Normality of the continuous variables was evaluated by the Kolmogorov Smirnov test. Differences between the subgroups of NDD as they were not normally distributed according to continuous variables were determined by the Kruskal Wallis test. Differences between the TD and NDD groups according to daily median screen time and duration of playing together and reading books were determined by the Mann Whitney U test. Differences between the daily median screen time and median percentage of co-viewing before and after the intervention within each TD and NDD groups were determined by the Wilcoxon test. A p value of less than .05 was considered significant.

The power of the study, which included 105 children, was found to be 99% for screen duration and 81% for co-viewing with a 5% error rate.

Results

One hundred five children were included in the study, 83 with NDD and 22 TD pre-school aged children. The NDD group mainly comprised of children with three developmental disorders: Autism Spectrum Disorders (ASD) (n= 24), Language Development Delay (LDD) (n= 34), Global Developmental Delay (GDD) (n=25).

The demographic characteristics of the participants of the NDD and TD groups are shown in Table I. In the TD group, the mean age of children was 37.82 ± 10.2 months, 59% (n= 13) were boys, 55% (n= 11) of the mother's education level was less than 8 years and more than half of the mothers' (n= 13) were unemployed. In the

NDD group, the mean age of children was 34.8 ± 8.8 months, 68.6% (n= 57) were boys, 51.2% (n= 41) of the mother's education level was less than 8 years and more than half of mothers' (n= 59) were unemployed. There was no statistically significant difference between the groups except for the NDD mothers being younger than the TD mothers (p = 0.005) (Table I).

The mean duration between each counselling session was 4.1 ± 1.2months.

The baseline median screen time before the intervention was 5.0 hours a day for all participants (Interquartile range (IQR): 4-9), after the intervention the median screen time for all participants decreased to 2.0 hours a day (IQR:1-3) (p<0.001). Before the intervention, the median percentage spent co-viewing was described as 12.5% of screen time, compared to 40% of screen time after the intervention (p=0.007) for all participants.

While background screen time was described as "whole day" in 89.8% (n=89) of all participants (n=99) before the intervention, this decreased to 16.1% after the intervention. Before the intervention, parents of children in the TD and NDD groups reported that whole day

Table I. Comparison of the sociodemographic characteristics of children in the neurodevelopment disorder (NDD) and typically developing (TD) groups on admission.

	TD (n=22)	NDD(n=83)	P-value
Age, month, mean ± SD	37.82 ± 10.2	34.8 ± 8.8	0.184
Gender (female/male)	9/13	26/57	0.449
Maternal age, mean ± SD	35.19 ± 4.99	31.47 ± 5.51	0.005
Paternal age, mean ± SD	38.90 ± 5.55	36.58 ± 6.42	0.107
Maternal education, N (%)			0.807
≤8 year	11 (55.0)	41 (51.2)	
9-12 year	3 (15.0)	22 (27.5)	
>12 year	6 (30.0)	17 (21.3)	
Paternal education, N (%)			0.186
≤8 year	9 (50)	27 (33.8)	
9-12 year	6 (33.3)	31 (38.8)	
>12 year	3 (16.7)	22 (27.5)	
Maternal employment, N (%)			0.463
Employed	4 (23.5)	10 (14.5)	
Unemployed	13 (76.5)	59 (85.5)	
No. of sibling, N (%)			0.717
None	6 (27.3)	25 (30.5)	
One sibling	9 (40.9)	38 (46.3)	
Two or more sibling	7 (31.8)	19 (23.2)	
Attending to preschool education N (%)			0.603
Yes	2 (9.1)	4 (4.8)	
No	20 (90.9)	79 (95.2)	
Feeding difficulties, N (%)			0.809
Yes	12 (54.5)	49 (59)	
No	10 (45.5)	34 (41)	
Parental disease, N (%)			0.591
Yes	7 (31.8)	21 (25.3)	
No	15 (68.2)	62 (74.7)	

background TV exposure was 72.7% (n=16) and 92.8% (n=77) respectively. After the intervention, these percentages decreased to 16.7% (n=3/18) and 16.3% (n=13/80) of children respectively.

Before the intervention only, the median screen time was statistically significantly different in families indicating a feeding difficulty (Table II).

At the beginning of the study only 6 children were attending preschool. After the intervention, 49 children started attending preschool. However, no difference was found for these children either at the baseline or at the end of 3 visits compared to those not attending preschool for the median duration of screen viewing (5.0 [IQR= 4.0-8.0], 5.0 [IQR= 4.0 - 10.0]; 2.0 [IQR= 1.0 - 3.6], 2.0 [IQR= 1.0 - 3.0]), respectively. After the intervention the only statistically significant demographic characteristic for longer screen time was found as NDD (Table II).

The screen viewing characteristics of the TD and NDD children before and after the intervention are displayed in Table III. The median baseline viewing durations of TD and NDD groups were 5.5 hrs. (IQR: 3.75-10.0), 5.0 hrs. (IQR=4.0-8.0), respectively (p= 0.820). After the intervention the median viewing durations of TD and NDD groups were 3.0 hrs. (IQR:2.0-3.62), 2.0 hrs. (IQR=1.0-3.0), respectively, (p= 0.039). The intervention decreased screen viewing time in both groups with statistical significance, however, the decrease was much greater in the NDD group children. The percentages of families in the NDD and TD groups reporting an increase in reading books after the intervention were 45.3% and 47.1% respectively. An increase of 78.5% in time spent playing with their child for the NDD group whereas this increase was 44.4% for the TD group. (p=0.008) (Table III).

Discussion

The study focused on decreasing background TV exposure besides screen viewing time and increasing the duration of household development promotion activities (reading books, playing with their child) after three consecutive counselling sessions to parents of pre-school children with NDD and TD in a developing country. The counselling sessions, which took place in a developmental behavioral pediatric clinic, were based on AAP media use recommendations. This intervention was effective in reducing the total screen time and background television exposure in both NDD and TD children.

Before the intervention, only children having a feeding difficulty were found to have higher screen time. This finding is consistent with the literature. The previous studies showed that TV exposure was associated with disordered eating and insufficient consumption of vegetables and fruits.[23,24] Longer background TV exposure was associated with opening the TV during meal times.[13] According to previous studies low household income, lower parental education and female gender were associated with high-screen time.[25,26] Parental education, gender, age, occupations of parents were not associated with high screen time in this study.

The screen viewing time and the percentage of co-viewing before counselling were similar among typically developing children and children with neurodevelopmental disorder. The study by Montes[12] recorded no difference for screen viewing time between children with ASD and children without ASD. In this study, it was demonstrated that not only children with ASD but also a group of children with NDD had similar screen viewing duration to TD children.

The majority of the parents in both NDD and TD groups reported "whole day" background TV exposure before the intervention. Our observation likewise that in Thompson's study[13], is that most parents did not know the detrimental effects of background exposure.

After the intervention, there was also a statistically significant decrease in screen time and background TV exposure in both groups. In this study, a higher impact was shown in the neurodevelopmental disorder group. Montes[10]

Table II. Screen time covariates before and after intervention for all participants.

	Before intervention (n=105)	After intervention (n=105)
	Daily Median Screen Time-hour (IQR)	Daily Median Screen Time-hour (IQR)
Gender		
Male (n=70)	5.0 (4.0 - 7.25)	2.0 (1.0 - 3.0)
Female (n=35)	6.0 (4.0 - 10.0)	2.0 (1.0 - 3.0)
Maternal age		
≤35 y (n=64)	5.0 (4.0 - 10.0)	2.0 (1.0 – 3.0)
>35 y (n=38)	5.5 (4.0 – 7.25)	2.0 (1.0 - 3.0)
Paternal age		
≤35 y (n=36)	5.5 (4.0 - 10.0)	2.0 (1.12 - 3.37)
>35 y (n=65)	5.0 (4.0 - 7.5)	2.0 (1.0 - 3.0)
Maternal education		
<8 years (n=52)	6.0 (4.0 - 9.5)	2.0 (1.0 - 3.0)
9-12 year (n=25)	5.0 (4.0 - 10.0)	2.0 (1.0 - 3.5)
>12 year (n=23)	5.0 (4.0 -12.0)	2.0 (2.0 - 3.0)
Paternal education		
<8 year (n=36)	6.0 (4.0 – 10)	2.5 (0.62 - 3.75)
9-12 year (n=37)	5.0 (4.0 - 9.0)	2.0 (1.0 - 3.0)
>12 year (n=25)	5.0 (4.0 - 7.0)	2.0 (1.0 - 3.0)
Maternal employment		
Employed (n=14)	4.5 (3.75 - 12.0)	2.0 (1.0 - 3.0)
Unemployed (n=72)	5.0 (4.0 - 10.0)	2.0 (1.0 - 3.0)
No.of sibling		
None (n=20)	5.5 (4.0 - 10.0)	2.0 (1.0 - 3.0)
one sibling (n=37)	5.0 (4.0 - 10.0)	2.0 (1.0 - 3.0)
two or more sibling (n=17)	4.0 (3.5 – 6.5)	2.0 (1.75- 3.0)
Feeding difficulties*		
No (n=61)	5.0 (4.0 - 6.0) *	2.0 (1.0 - 3.0)
Yes (n=44)	6.0 (4.0 - 11.5) *	2.0 (1.1 - 3.0)
Parental disease		
No (n=77)	5.0 (4.0 - 10.0)	2.0 (1.0 - 3.0)
Yes (n=28)	5.0 (4.0 - 7.5)	2.0 (1.0 - 3.0)
Developmental status**		
Typically developing (n=22)	5.5 (3.75 - 10.0)	3.0 (2.0 – 3.62) **
Neurodevelopmental disorder (n=83)	5.0 (4.0 – 8.0)	2.0 (1.0 – 3.0) **

* p<0.05 before intervention
** p<0.05 after intervention
no differences to determine for other comparison
IQR: Interquartile range

recommends that pediatricians should take into account the fact that children with ASD and their families are quite similar to families of children without ASD with regard to the use of screens so the same recommendations should be given to the parents of both groups. This study demonstrated that the same intervention could be effective in reducing total screen time of all of the NDD groups and TD children statistically significantly as suggested by Montes.

Table III. Screen viewing characteristics of typically developing children (TD) and children with neurodevelopmental disorder [NDD- Autism Spectrum Disorder (ASD), Language Development Delay (LDD), Global Development Delay (GDD)] before and after intervention.

	TD	NDD TOTAL	ASD	LDD	GDD
Daily Median Screen Time hour (IQR)	n= 22	n= 83	n= 24	n= 34	n= 25
Before intervention	5.5[a](3.75-10.0)	5.0[a] (4.0-8.0)	6.0 (4.0-11.5)	4.5(4.0-6.0)	6.0 (4.0-10.0)
After intervention	3.0[bc](2.0-3.62)	2.0[b] (1.0-3.0)	1.0[cd](0.5-2.0)	2.0(1.0-3.0)	2.0[bd](2.0-4.0)
p* value	<0.01	<0.01	<0.01	<0.01	<0.01
Median Percentage of Co-viewing (%) (IQR)					
Before intervention	0.0 (0-32.50)	20.0 (0.0-50.0)	22.5 (0 -50)	12.5 (0 - 50)	20.0 (0-50)
After intervention	25.0 (0–80.0)	40.0(0.0 -70.0)	50.0 (5-100)	22.5(0- 65)	50.0 (10-70)
p* value	0.117	0.022	0.097	0.393	0.125
Families reporting increase Reading Book After Intervention n (%)					
no change	9 (52.9)	35 (54.7)	12 (54.5)	13 (54.2)	10 (55.6)
increase	8 (47.1)	45 (45.3)	10 (45.5)	11 (45.8)	8 (44.4)
Families reporting increase Playing Together After Intervention n (%)					
no change	10 (55.6)	14 (21.5)	6 (27.3)	6 (22.2)	2 (12.5)
increase	8 (44.4)[ef]	51 (78.5)[f]	16 (72.7)[e]	21 (77.8)[e]	14 (87.5)[e]

p* dependent sample Wilcoxon tests were used for before and after intervention in each group,
a: p=.820 independent samples Mann-Whitney test were used
b: p=.039 independent samples Mann-Whitney test were used
c: p=.002 independent samples Kruskal- Wallis test were used
d: p=.004 independent samples Kruskal- Wallis test were used
e: p=.034 independent samples Kruskal wallis test were used
f : p=.008 independent samples Mann-Whitney test were used
no differences to determine for other comparison

While in the literature office setting interventions were found to be less successful than others[15], office setting interventions are preferred by health professionals because these interventions require less time and have more applicability than home, community and primary care settings. The study by Birken et al.[16] which made only one brief behavioral counseling intervention to pre-school children in a pediatric office setting showed no effect of the intervention on overall screen time. Our study differed from this by giving three consecutive interventions, which have shown to be effective, and by lasting for more than one year. In the study by Downing et al.[15] it was also recommended that an effective intervention study should last for more than a 6-month period. The other two office setting intervention studies were associated with diet, physical activities and reducing screen time during well child visits. Overweight and obese children were included to these studies. The study by Taveras et al.[27] showed that intervention participants who completed at least 2 intervention activities had a greater decrease in the duration of TV and video viewing. A study by van Grieken et al.[28] was a low intensity obesity intervention, which focused only on TV viewing, showed no difference between the intervention and control group TV viewing. This study differed from the other two by focusing mainly on screen viewing time, while promoting book reading, play in NDD children, and succeeded in reducing total screen time in this population significantly.

The effectiveness of the intervention which was conducted in a developmental behavioral pediatric clinic, may also be related to the increased awareness of the families about the detrimental effects of screen viewing on developmental delay, on language and communication fields as well as behavioral problems. Therefore, as recommended in the review by Downing et al.[15] it is important to design the intervention to be family-based in order to be successful. The family plays the primary role in the social environment and the behavioral development of the child.[29] Limiting screen time and active role model behavior are key factors that prevent excessive time spent on screen-based behavior.[29] Therefore, families who took part in the study were asked to limit the screen time and become role models for screen-based behavior.

As mentioned above, repeating counselling and extending it for more than one year were another reason for effectiveness.

It is worth pointing out that the reliance on parental reports for children's screen-based habits may be a limitation of the current study. However, parent- reported screen time was used in many previous studies[17,30] and a high correlation was found between parent-reported screen time and screen time of children.[31] Screen time was not evaluated after each counselling but only after the three sessions as previous studies recommend and consider as an effective high intensity intervention. The small sample size is another limitation of the study.

Limiting screen time has been recommended by the AAP for more than a decade, but there has been limited success in reducing children's screen time. This study reveals the use and effectiveness of a developmental pediatrics clinic setting intervention via parents, across three sessions including the AAP media recommendations in NDD children, which are applicable, accessible and cost-effective. There is an apparent need for larger sample group studies among children with NDD. Further research is necessary to define the long-lasting effects of such an intervention.

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Original Article

Demographic, ocular and associated neurological findings in corpus callosum malformations

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ABSTRACT

Background. The corpus callosum is a primary commissural part of the brain which connects the two hemispheres. Processing sensory, motor, visuo-motor and cognitive functions are related to a healthy connection. In this study, we aimed to evaluate the ocular, neurologic and other systemic findings of corpus callosum malformations and to focus on the association between the ocular and neurological findings and the type of callosal malformation according to cranial magnetic resonance imaging (MRI).

Methods. A retrospective chart review of 57 patients with corpus callosum malformation was performed. Demographic features, neurologic, ocular and other systemic findings were noted. Patients were divided into 3 groups according to the severity of corpus callosum malformation on MRI (total agenesis, partial agenesis and hypoplasia) and also evaluated as a part of a genetic disorder/syndrome or not. The differences between demographic features, ocular and neurological findings between these 3 groups and also between syndromic and non-syndromic groups were evaluated statistically.

Results. Only 35.1% of patients had fixation and following pattern of visual acuity. Anterior segment pathologies were observed in 6.9% of patients. However, 57.9% of patients had posterior segment malformations. Only 19.3% of patients had a normal ocular alignment. There was no statistically significant difference of demographic features, ocular and neurologic findings between the 3 groups or between the syndromic/non-syndromic groups.

Conclusions. Ocular findings can be reliable depending on the severity of the corpus callosum malformations. However, delay in fixation reflex development or loss of fixation should remind us of central nervous system pathologies especially corpus callosum malformations.

Key words: agenesis, corpus callosum, hypoplasia, low vision, magnetic resonance imaging.

The corpus callosum is the largest white matter structure in the human brain and it is responsible for normal communication and cooperation between the two hemispheres.[1] Interhemispheric connections have an importance for the functional integration of sensory, motor, visuo-motor and cognitive processes.[2,3] Corpus callosum malformations may occur as a result of any step in the development of the corpus callosum. Disruption of neurogenesis, telencephalic midline patterning, neuronal migration and specification, axon guidance and post-guidance development are the steps that can be affected by the development.[2] Acquired conditions, including inflammatory, vascular, demyelinating and ischemic causes can also affect the corpus callosum.[4]

Corpus callosum malformations can be isolated or accompany other cranial malformations. Additionally, these malformations can be associated with different genetic disorders or syndromes.

In this study, we aimed to evaluate the ocular, neurologic and other systemic findings of patients with corpus callosum malformations, to investigate concomitant syndromes and

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genetic disorders in these patients and to focus on the association between the ocular and neurological findings and type of callosal malformation according to cranial magnetic resonance imaging (MRI).

Material and Methods

This retrospective study included patients with corpus callosum malformations who were examined at Ankara University Hospital. Patients whose corpus callosum malformation were confirmed by cranial MRI were included in the study. A retrospective chart review of 57 patients was performed. Demographic features of patients, including; age, gender, follow-up period, presence of consanguinity, birth week, accompanying systemic and neurological diseases were reviewed. The presence of motor delay, intellectual disability and epilepsy were also noted. Patients were evaluated as syndromic and non-syndromic according to whether the corpus callosum malformation was part of any genetic disorder or syndrome and systemic and radiologic findings of these two groups were evaluated separately.

Ophthalmic examination findings including; visual acuities (VA), anterior and posterior segment evaluation and measurement of ocular alignment were noted. Due to the preverbal age of the study group and the presence of accompanying intellectual disability, VAs were assessed by fixation and following pattern of light. Cranial MRI results were evaluated and patients were divided into 3 groups according to the severity of the corpus callosum malformation on MRI. Patients with total corpus callosum agenesis consisted of group 1, group 2 with partial corpus callosum agenesis, group 3 with corpus callosum hypoplasia. Patients with no development of any part of the corpus callosum, were considered as total agenesis, while those with shorter anterior-posterior length as a result of missing segment(s) such as the splenium and/or the rostrum were considered as partial agenesis. In spite of normal anteroposterior length, patients with thinner than normal corpus callosum constituted the hypoplasic group. The differences between demographic features, ocular and neurological findings between these 3 groups were evaluated statistically.

Statistical analyses

Categorical parameters were presented as number and percentage. Continuous variables were presented as median and interquartile range 25-75. Categorical parameters were compared using the Chi square test and Kruskal Wallis H tests were used to compare continuous variables between the groups. SPSS 22 software for Windows (SPSS Inc., Chicago, IL) was used for all statistical analysis. A *P* value less than 0.05 was considered statistically significant.

The study was conducted in line with the dictates of the Declaration of Helsinki and approved by the local ethical committee of Ankara University Faculty of Medicine (Report number: İ2-46-19, Date: 18 July 2019).

Results

Of 57 patients included, 36 (63.2%) were male and 21 (36.8%) were female. The median age was 13 (8-24) months and the median follow-up period was 11 (5-24) months. Ten patients (17.5%) had a history of consanguineous marriage. The median birth week of patients was 39 (34-40) weeks. Fifty-three (93%) patients had another concomitant neurological disorder. There exists motor delay in 51(89.5%) patients and intellectual disability in 41 (71.9%) patients. The number of patients with epilepsy was 37 (64.9%).

Twenty patients (35.1%) had no fixation and response to light. There was no fixation in 10 patients (17.5%), but they had a response to light. There were 7 patients (12.3%) with only fixation, 20 patients (35.1%) with fixation and following pattern. When the ocular alignments of patients were evaluated, only 11 patients (19.3%) were orthophoric. Seventeen patients (29.8%) had wandering eye movements. Thirteen (22.8%)

were esotropic and 16 (28.1%) was exotropic. In the anterior segment examinations of the patients; 2 patients (3.5%) had ptosis, 1 (1.7%) had megalo-cornea and blue sclera, and 1 (1.7%) had shallow orbit. Fundus examination of 24 patients (42.1%) was normal. The most common pathological findings were optic disc pallor and optic atrophy with a percentage of 42.1%. Other findings were; optic disc hypoplasia (8.8%), chorioretinal scars and pigmentary changes (3.5%) and optic disc coloboma (1.7%).

Group 1 consisted of 15 patients (26.3%), group 2 consisted of 12 (21.1%) and group 3 consisted of 30 (52.6%) patients. When the differences of demographic features, ocular and neurologic findings were assessed between the 3 groups, there was no statistically significant difference in any parameters (Table I).

Corpus callosum malformation in 14 patients (24.6%) was part of a genetic disorder or syndrome. The findings of these patients are seen in Table II. Demographic features, ocular and neurological findings were similar in patients with or without a genetic disorder / syndrome. Other accompanying systemic disorders and MRI findings of patients without a genetic disorder/syndrome are shown in Table III and Table IV.

There were 4 patients (7%) without any neurological abnormalities. Of these, 3 (5.2%) had isolated corpus callosum agenesis and 1 (1.8%) had papillo-renal syndrome. When findings of these 3 patients with isolated corpus callosum agenesis were evaluated, all of them had normal ocular, neurologic and MRI findings except callosal agenesis. Two (3.5%) had partial agenesis and 1 (1.8%) had total agenesis. One of the patients with partial agenesis had short-duration fixation and following pattern and the other had only fixation. The patient with total agenesis had short-duration fixation and following pattern.

Table I. Demographic features, ocular and neurological findings of patients with corpus callosum total agenesis, partial agenesis and hypoplasia.

Variable	Group 1 Total Agenesis (n: 15)	Group 2 Partial Agenesis (n: 12)	Group 3 Hypoplasia (n: 30)	p
Admittance age (month)	19 (9-43)	9 (5-22)	14 (9-22)	0.243
Birth week	39 (39-40)	39 (37-40)	38 (33-40)	0.192
Gender				
Female (n)	5 (33.3)	2 (16.7)	14 (46.7)	0.181
Male (n)	10 (66.7)	10 (83.3)	16 (53.3)	
Consanguineous marriage (n)	1 (6.7)	4 (33.3)	5 (16.7)	0.191
Motor retardation (n)	14 (93.3)	10 (83.3)	27 (90.0)	0.725
Intellectual disability (n)	11 (73.3)	6 (50.0)	24 (80.0)	0.143
Epilepsy (n)	8 (53.3)	6 (50.0)	23 (76.7)	0.156
Neurological findings (n)	14 (93.3)	10 (83.3)	29 (96.7)	0.314
Syndromic (n)	6 (40)	3 (25)	5 (16.7)	0.257
Non-syndromic (n)	9 (60)	9 (75)	25 (83.3)	
Strabismus type				
None	4 (26.7)	2 (16.7)	5 (16.7)	0.773
Esotropia	5 (33.3)	3 (25)	5 (16.7)	
Exotropia	3 (20)	4 (33.3)	9 (30)	
Wandering eye movements	3 (20)	3 (25)	11 (36.7)	
Fundus pathology				
Normal	5 (33.4)	8 (66.7)	11 (36.7)	0.128
Pathologic	10 (66.7)	4 (33.3)	19 (63.3)	

Table II. Findings of patients with corpus callosum malformation as part of a genetic disorder or syndrome.

Patients age (month) /gender/type of corpus callosum malformation	Genetic disorder or syndrome	Ocular findings	Systemic findings	Radiological findings
9m / male / Hypoplasia	18q deletion	Megalocornea Blue sclera	Microcephaly Motor retardation Intellectual disability	Delay in myelination Dilatation of lateral ventricles
19m / male / Total agenesis	Dandy-Walker syndrome	Optic atrophy	Hydrocephalus Motor retardation Intellectual disability	Hypoplasia of inferior cerebellar hemispheres Enlargement of ventricles Colpocephaly Cerebral atrophy
49m / male / Total agenesis	Dudley syndrome	Optic atrophy	Macrocephaly Deafness Motor retardation Intellectual disability Hypothyroidism	Dysmyelination of white matter Cerebral and cerebellar atrophy
13m / female / Hypoplasia	Aicardi syndrome	Chorioretinal scars Retinal pigmentary changes	Infantile spasm Motor retardation Intellectual disability	Chronic subdural hematoma at left frontal cortex Dilatation of lateral ventricles Loss of volume in periventricular white matter
10m / male / Partial agenesis	de Morsier syndrome	Optic atrophy Optic nerve hypoplasia	Epilepsy Motor retardation Intellectual disability Contracture of feet	Loss of volume in periventricular white matter Bilaterally thinning of optic nerve and chiasm
15m / female / Hypoplasia	Pearson syndrome	Chorioretinal scar	Sideroblastic anemia Mitochondrial myopathy Motor retardation Intellectual disability	Delay in myelination Large cerebral sulci
5m / male / Total agenesis	Under investigation	Optic atrophy Strabismus	Colpocephaly Microcephaly Deafness Motor retardation Intellectual disability Hypertrichosis Cerebral palsy Cryptorchidism	Shallow silvian fissure Decrease in normal cerebral sulcation Colpocephaly

Table II. Continued.

Patients age (month) /gender/type of corpus callosum malformation	Genetic disorder or syndrome	Ocular findings	Systemic findings	Radiological findings
4m / female / Total agenesis	Aicardi syndrome	Retinal pigmentary changes	Hydrocephalus Motor retardation Intellectual disability Infantile spasm	Periventricular encephalomalacia
19m / female / Hypoplasia	18th exon c40936> T nonsense heterozygot mutation	Ptosis Optic disc hypoplasia	Syndromic face Esophageal atresia	Delay in myelination
1m / female / Partial agenesis	Under investigation	Shallow orbit	Cleft palate Agenesis of left kidney Ventricular septal defect Malnutrition Motor retardation Intellectual disability	-
36m / female / Hypoplasia	6q deletion (Interstitial deletion at the region of q21-q22.33)	Optic atrophy	Scoliosis Hallux valgus Tetralogy of Fallot	-
26m / male / Partial agenesis	Pierre Robin syndrome	Ptosis Optic atrophy Strabismus Retinal pigmentary changes	Micrognatia Laryngomalacia High arched palate Motor retardation Intellectual disability	Loss of volume in white matter Deformation of vermis and pons
43m / female / Total agenesis	Under investigation	-	Cleft lip/ palate Deafness Pilor obstruction Motor retardation Intellectual disability	-
15m / female / Hypoplasia	Papillorenal syndrome Chromosome 10 deletion (q25.1-q26.13)	Optic disc coloboma	Deafness Hypothyroidism Motor retardation Intellectual disability	Agenesis of septum pellucidum

Table III. Accompanying systemic disorders of patients without a genetic disorder/syndrome.

Disorder	Number of patients n / %
Hydrocephalus	8 / 18.6
Cerebral palsy	4 / 9.3
Microcephaly	3 / 7
Hydrocele	3 / 7
Cardiac pathology	2 / 4.7
Meningomyelocele	2 / 4.7
Otism	1 / 2.3
Macrocephaly	1 / 2.3
Hypothyroidism	1 / 2.3
Scoliosis	1 / 2.3
Hearing loss	1 / 2.3

Table IV. Radiological findings of patients without a genetic disorder/syndrome.

Radiological findings	Number of patients n / %
Colpocephaly	10 /23.3
Loss of volume in periventricular white matter	9 / 20.9
Diffuse loss of volume in white matter	7 / 16.3
Oligogyria	4 / 9.3
Chiari malformation	3 / 6.9
Defect of septum pellucidum	3 / 6.9
Hypoplasia of inferior vermis	2 / 4.6
Cerebellar hypoplasia	2 / 4.6
Loss of volume in brainstem	1 / 2.3

Discussion

In this study, we evaluated the systemic and ocular findings in patients with corpus callosum malformations including agenesis and hypoplasia and also assessed the relationship between genetic and radiological findings. Anterior segment pathologies were observed in only 6.9% of patients. However, 57.9% of patients had posterior segment malformations. Only 19.3% of patients had a normal ocular alignment. When we evaluated the differences of all demographic, neurologic and ocular parameters between the 3 groups, there was no statistically significant difference. In addition, whether the corpus callosum malformation was part of a genetic disorder or syndrome, did not make any difference on these findings.

The corpus callosum develops between the 8th and the 20th week of gestation.[5] Although the number of callosal fibers and final shape of the corpus callosum are completed by the 20th week of gestation, axonal growth continues until 2 months after birth[6] and structural changes due to fiber myelination, redirection and pruning, continue in childhood and adolescence.[7] Since the myelin tissue, which is the protective tissue of the corpus callosum, is less than in other brain tissues, the corpus callosum is affected more easily by external factors. Different studies found that the corpus callosum is one of the most common parts of the brain affected by demyelination and axonal loss.[8,9] Therefore, the corpus callosum may also be affected by postpartum pathologies such as hydrocephalus, ischemia and demyelination. With an embryologic developmental aspect, the eye is required for the development of callosal terminals.[10,11] Additionally, the corpus callosum is necessary for the maturation of the visual cortex.[12]

This interhemispheric connection has a role in visual perception. There exists a connection between callosal fibers and the visual cortex. Neuronal changes caused by callosal inputs in the visual cortex have been demonstrated by electrophysiological tests.[13] If these inputs are eliminated by cooling or unilateral GABA injection into the hemisphere, a decrease in neuronal response occurs in the visual cortex. Therefore, callosal fibers play a role in the visual process. Additionally, it is thought to have a complex relationship between the parts of the corpus callosum and visual function. Kwinta et al.[14] found that there is a correlation between the function of rostrum, genu and body of the corpus callosum and stereoscopic vision. Dougherty et al.[15] showed projections between the occipital lobe and splenium of the corpus callosum. These projections may differ, as the shape and location of the splenium vary between individuals. If these projections are damaged, visual deficits such as visual recognition and identification can be seen. In our study, because of the age of patients and accompanying intellectual disability, we could only evaluate the visual functions of patients by measuring fixation and following patterns. Thirty-five % of patients had no fixation and response to the light. Only 33.3% of patients had normal fixation and following pattern.

Although corpus callosum malformations can be isolated, they are more commonly associated with other cerebral or extra-cerebral abnormalities.[16] Therefore, it is difficult to say that the cause of neurological and ocular disorders in these patients is mainly due to corpus callosum malformations. Existing neurological and ocular disorders may also be due to accompanying cerebral pathologies. Therefore, in order to evaluate the effects of corpus callosum pathologies, it would be more appropriate to investigate patients with isolated corpus callosum malformation without any accompanying cerebral or extra-cerebral abnormalities. In our study, 94.7% of patients had other cerebral abnormalities. The most common pathology associated with the corpus callosum malformation was hydrocephalus (18.6%) and cerebral palsy (9.3%) in patients without a genetic disorder or syndrome. Additionally, colpocephaly (23.3%) and loss of volume in periventricular white matter (20.9%) were the most common radiological findings in this group. Only 3 of 57 patients (5.3%) had an isolated corpus callosum malformation with a normal neurologic examination and MRI findings except callosal agenesis. These 3 patients had normal anterior and posterior segment findings with a good ocular alignment. While one patient with total agenesis had a weak fixation and follow-up pattern, one patient with partial agenesis had only fixation. Although these findings may suggest that the connections of the corpus callosum may differ between individuals, they cannot be conclusive because of the small number of patients.

When we evaluated the association between the ocular and neurological findings and type of callosal malformation, there was no difference between the 3 groups. Because of the complex structure of the corpus callosum and accompanying other cerebral pathologies, these groups may have similar findings. It would be more appropriate to evaluate these 3 groups in patients with isolated corpus callosum malformations. Moutard et al.[17] evaluated the neurological findings of isolated corpus callosum agenesis and they showed that isolated corpus callosum agenesis could have a favorable outcome that was independent of corpus callosum agenesis type. In our study, one of the patients with isolated corpus callosum agenesis had total agenesis with a visual function of weak fixation and following pattern and the other had partial agenesis with only fixation. These findings suggest that isolated corpus callosum malformations should be evaluated in terms of ocular findings.

Corpus callosum malformations can also be seen as a part of any genetic disorder or syndrome. More than 200 genetic syndromes with corpus callosum malformations have been described.[2] Rubinstein-Taybi, Marden-Walker syndrome, Aicardi syndrome, Bardet-Biedl syndrome,

Joubert syndrome and septo-optic dysplasia are some of these syndromes. Thirty- forty-five % of patients with corpus callosum agenesis had a genetic disorder. Of these, the cause of 20-35% is single gene mutations.[16,18] Some of these syndromes have ocular findings, including aniridia, iris and optic nerve coloboma, optic nerve hypoplasia, chorioretinal lacunae and congenital fibrosis of the extraocular muscles.[19-22] In our study, 14 patients (24.6%) had a genetic disorder or syndrome. Ocular findings observed in these patients were as follows: megalocornea, blue sclera, ptosis, shallow orbit, optic disc atrophy and hypoplasia, chorioretinal scar and pigmentary changes.

One of the main limitations of this study was the small number of patients with isolated corpus callosum malformations. Most patients had other accompanying cerebral pathologies. Therefore, the effect of the corpus callosum on visual functions could not be evaluated as isolated. In addition, visual functions of the corpus callosum such as fusion, stereopsis and binocular visual field could not be evaluated because of the age and mental status of patients.

This study has importance in determining the frequency of concomitant ocular, neurological and syndromic disorders in patients with corpus callosum malformations, due to the large study population. Children with loss of fixation or delay in fixation reflex development should be evaluated for central nervous system pathologies including corpus callosum malformations. Additionally, in the presence of accompanying neurological pathologies in children with visual dysfunction, further neuro-radiological examinations should be performed. This study will be a guide for a future study, including the evaluation of visual functions of patients with isolated corpus callosum malformation according to the type and severity of the corpus callosum malformation.

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Original Article

Evaluation of ultrasonographic optic nerve sheath diameter and central retinal artery Doppler indices by point-of-care ultrasound in pediatric patients with increased intracranial pressure

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ABSTRACT

Background. Measurement of the optic nerve sheath diameter (ONSD) with point-of-care ultrasound (POCUS) is a non-invasive and radiation-free technique that can be used to assess increased intracranial pressure (ICP). Ophthalmic artery and central retinal artery Doppler indices can be used like transcranial Doppler to evaluate increased ICP. This study aims to examine the diagnostic value of ONSD measurements and central retinal artery Doppler indices in the evaluation of pediatric patients with increased ICP.

Methods. This was a prospective, case-controlled single center study. The study group was comprised of a total of 38 pediatric patients with increased ICP and the control group included 19 healthy children. Ophthalmic ultrasound was performed and ONSD and central retinal artery Doppler indices were measured.

Results. The mean age of the study group was 80.84 ± 65.12 months. The mean ONSD was 5.9 ± 0.8 (3.6–8.1) mm in the study group and the mean resistive index (RI) was 0.71 ± 0.08 (min:0,55–max:1) and was significantly greater than the control group ($p<0.001$ and $p<0.001$, respectively). In terms of predicting increased ICP, the ONSD measurement was the strongest parameter, with its area under the curve: 0.767 (95 percent confidence interval: 0.68–0.85). In the study group, the cut-off value for ONSD was 5.8 mm (66 percent sensitivity, 100 percent specificity) and the cut-off value for RI was 0.68 (63 percent sensitivity, 83 percent specificity).

Conclusions. Point-of-care ultrasound is a noninvasive and important tool in pediatric intensive care units. Our study is significant as one of the few pediatric studies where central retinal artery Doppler indices are evaluated in addition to OSND, in patients with increased ICP.

Key words: central retinal artery, Doppler indices, optic nerve sheath diameter, point-of-care ultrasound, pediatric, resistive index.

Increased intracranial pressure (ICP) can emerge as a result of many different neurological conditions, such as trauma, infection and intracranial mass.[1] Early diagnosis and treatment of increased ICP in critically ill pediatric patients receiving treatment in pediatric intensive care units (PICU) is crucial for the prevention of neurological damage and mortality.[2] In the evaluation of increased ICP, the gold standard is the measurement of pressure using a catheter placed in the brain parenchyma or ventricle.[3] However, this method is invasive, difficult to implement and carries a risk of various complications, such as bleeding and infection; therefore, performing this method is not always possible and new, non-invasive diagnosis methods are emerging.[4,5]

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The methods of cranial computed tomography (CT) and magnetic resonance imaging (MRI) are frequently used; however, the requirement of sedation, difficulties in the transportation of hemodynamically unstable intensive care patients and radiation exposure of CT are limitations of these methods.[6]

In recent years, the use of point-of-care ultrasound (POCUS) by non-radiologist clinicians has become widespread.[7] For specialists working in pediatric emergency departments and PICUs, POCUS has become widely used for physical examinations due to the rapid results it offers for critically ill patients.[8] Ultrasonographic measurement of ONSD is especially important for the clinical follow-up of patients with increased ICP.[9] The fact that an increase is detected in ONSD in the early phase in case of increased ICP due to its direct relation with subarachnoid distance has been revealed in many studies.[10,11]

The effect of increased ICP on ophthalmic veins is not completely understood. Ophthalmic artery and central retinal artery Doppler indices can now be used in addition to the transcranial Doppler to evaluate increased ICP.[12] With ultrasonography, the central retinal and ophthalmic arteries can be readily visualized deep in the orbital cavity, in the area where they cross the optic nerve.[13] Peak systolic velocity (PSV) and end diastolic velocity (EDV) measurements are performed in colored Doppler images. Resistive index (RI) is used to measure resistance against the arterial bloodstream and is calculated using an ultrasound device through a formula with colored Doppler measurements [PSV-EDV)/ PSV].[14]

This study aims to examine the diagnostic value of ONSD measurements and central retinal artery Doppler indices in the evaluation of PICU patients with increased ICP and to draw attention to the increased usage rate, popularity and importance of the use of POCUS by pediatric intensivists.

Material and Methods

Patients

A total of 38 pediatric patients, who were treated in our tertiary PICU and for whom increased ICP was suspected clinically (change in consciousness level, dilated or nonreactive pupil, loss of brain stem reflexes, injury of cranial nerve, Cushing triad) or radiologically (shift, deletion in the sulcus, ventricular collapse, cistern pressure) were prospectively included in this single-center study, over eight months. In addition to the study group, 19 children, who applied to the hospital's general pediatric outpatient clinic for different reasons and who did not show symptoms of increased ICP, were included in the study as the control group. Because the Doppler ultrasound is an extremely difficult method, to eliminate measurement error, low dose sedative drugs were administered to the PICU patients in the study group. For the control group, non-pharmacological sedation methods were used, such as non-nutritive suckling or the use of breast milk or glucose as sedatives for infant subjects. For subjects able to communicate, the method required for obtaining accurate ultrasonographic measurements was explained. Three measurements were taken from each patient in the study group to control for measurement error and the mean value of these measurements were recorded for the last analysis. Similarly, two measurements were taken from each child in the control group and the mean value of these measurements were recorded for the last analysis. The mean value of each child's ONSD and central retinal artery Doppler indices were recorded. Following a radiology expert's evaluation of image quality, 88 measurements from the study group and 23 measurements from the control group were selected for analysis.

The study was conducted following the ethical criteria of the 1964 Helsinki Declaration and was approved by a Çukurova University Faculty of Medicine clinical research ethics committee (Date: January 4, 2019; Meeting number: 84).

Written informed consent was obtained from the families of the patients.

Ophthalmic ultrasound method

All ophthalmic ultrasonographic measurements were performed by an experienced pediatric intensive care fellow (N.A.) who completed a POCUS course provided by the Turkish Pediatric Emergency and Intensive Care Society and performed at least 300 ophthalmic ultrasound measurements before the study. The Mindray ultrasound device (Resona7, Mindray Bio-Medical Electronics Co., Ltd., China) 5.1–12.5 MHz linear probe was used for the measurements. The patients were placed in the supine position by raising their heads by 20–30 degrees. The ultrasound system controls acoustic output so as not to exceed a mechanical index (MI) level of 1.9, a spatial peak, temporal average intensities (ISPTA.3) of 50 mW/cm^2 or a thermal index (TI) value of 1.0.[15,16] After applying sterile gel at room temperature, the ultrasound probe was placed on the right superior eyelid of the patient's closed eye and the ONSD measurement was performed in B mode, 3 mm behind the optic disc (Fig. 1). First, the central retinal artery was identified in the optic nerve using color Doppler sonography. Afterward PSV and EDV measurements were performed on the saved colored Doppler images (Fig. 2). By using these measurements, RI was automatically calculated by the ultrasound device.

Statistical analysis

The IBM SPSS Statistics Version 20.0 packaged program was used for the statistical analysis of the data. The categorical measurements were summarized in numbers and percentages and numerical measurements were summarized as mean and standard deviation (median and minimum–maximum when necessary). The Chi-square test statistic was used to compare the categorical measurements between the groups. The Mann-Whitney U test was used to compare the numerical measurements between two groups that did not show normal distribution. ROC analysis was performed to detect the most effective parameter in terms of foreseeing increased ICP and to determine the appropriate breakpoints of ONSD. A statistical significance level of 0.05 was implemented for all tests.

Results

In this study, 88 ophthalmic ultrasound measurements were analyzed on 38 (14 females, 24 males) patients with increased ICP and 23 measurements were analyzed on 19 (nine females, 10 males) patients from the healthy control group. When the underlying causes of increased ICP in the study group were

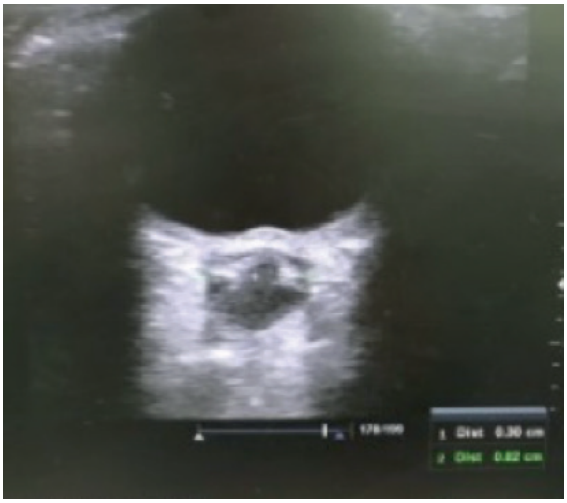

Fig. 1. Ultrasonographic OSND measurement.

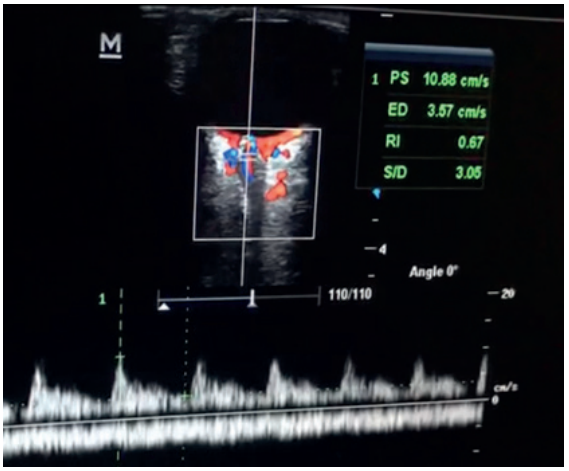

Fig. 2. Measurement of ophthalmic artery Doppler indices.

examined, it was apparent that nine (23.7%) patients had traumatic causes and 29 (76.3 %) patients had non-traumatic causes. The underlying diagnoses of the patients are given in Table I. The mean age was 88.47 ± 64.98 (min: 3–max: 204) months in the whole group. The mean age was 80.84 ± 65.12 months in the study group and 97.74 ± 64.95 months in the control group. No significant difference was found between the patient and control groups in terms of age and gender (p=0.397 and p=0.313, respectively). While the mean ONSD was 5.9 ± 0.8 (3.6–8.1) mm in the study group, the mean RI was 0.71 ± 0.08 (min:0,55–max:1) and was significantly greater than the control group (p<0.001 and p<0.001, respectively) (Table II). In terms of predicting increased ICP, the ONSD measurement was determined as the strongest parameter, with its area under the curve: 0.767 (95 percent confidence interval: 0.68–0.85) (Fig. 3). In the study group, the cut-off value

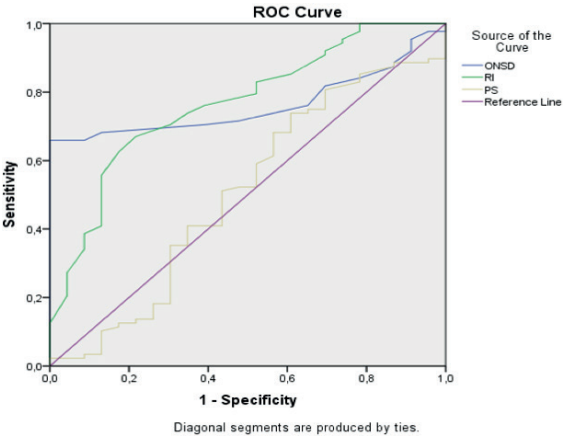

Fig. 3. The ROC curve for factors which predict increased ICP.

Table I. Underlying causes of increased ICP in the patient group.

Underlying causes of patients	Number (%)
Traumatic causes	9 (23.7)
Nontraumatic causes	29 (76.3)
Meningoencephalitis	5 (17.3)
Intracranial mass	9 (31)
Postarrest care	3 (10.3)
Hydrocephalus- ventriculoperitoneal shunt dysfunction	6 (20.7)
Other	6 (20.7)

Table II. Comparison of ultrasonographic measurements of the patient and control groups.

Variables	Patient group N=38, number of measurements=88) Mean ±SD Median (min-max)	Control group N=19, number of measurements:23) Mean ±SD Median (min-max)	*p*
ONSD (mm)	5.9 ± 0.8 6.1 (3.6-8.1)	5.2 ± 0.3 5.3 (4.4-5.7)	<0.001
Resistive index	0.71 ± 0.08 0.71 (0.55-1)	0.62 ± 0.07 0.64 (0.51-0.79)	<0.001
Peak systolic velocity	16.02 ± 8.05 13.98 (6.91-55.92)	16.59 ± 9.03 13.39 (7.94-36.97)	0.925
End diastolic velocity	4.3 ± 1.86 3.77 (0-13.89)	6.01 ± 3.06 5.66 (2.83-12.76)	0.017

ONSD: optic nerve sheath diameter

for ONSD was determined as 5.8 mm with 66 percent sensitivity and 100 percent specificity and the cut-off value for RI was determined as 0.68 with 63 percent sensitivity and 83 percent specificity.

Discussion

POCUS is a very important method for intensivists and its usage has become widespread since it offers the opportunity to make cost-effective, non-invasive, radiation-free and repeated measurements of ONSD.[12,17] Evaluation of myocardial contraction and cardiac index by critical care echocardiography, rapid diagnosis and treatment of pneumothorax by lung ultrasound, detection of pleural fluid and drainage by ultrasound, decreasing radiation exposure in the radiological follow-up of pneumonia by substituting for chest radiography, clinical follow-up of the patient with increased ICP by measuring the ONSD by ophthalmic ultrasound, determination of the fluid around the liver, spleen and kidney with rapid abdominal evaluation (and, if necessary, drainage of the fluid by ultrasound-guided paracentesis), placement of central venous catheter by ultrasound, evaluation of the volume status of the patient by measuring vena cava inferior diameters and guiding the fluid treatment to be applied are among the usage areas of POCUS by pediatric intensivists.[9,13,18-21]

ONSD has an ongoing character with dura mater, arachnoid mater and pia mater.[18] When ICP increases, it is directly reflected on ONSD and an increase may be detected even before the development of papillary edema.[14,22] As a rapid and noninvasive method, the ultrasonographic ONSD measurement has become a new tool in monitoring elevated ICP for pediatricians in emergency and intensive care departments.[11] The usage of ocular ultrasound for the indirect assessment of ICP by measuring ONSD is already well known.[9] POCUS, which is distinguished from other diagnosis methods for being fast, easy, cheap, repeatable and especially for being non-invasive, is preferred as a new method for the detection of increased ICP and clinical follow-up.[22,23]

Rehman Siddiqui et al.[24] conducted a pediatric study and reported that the limit for ONSD was >4 mm in newborns, >4.71 mm in those between 1–10 years of age and >5.43 in those above 10 years (sensitivity 100 percent, specificity 60–66.7 percent). In another study, Irazuzta et al.[25] evaluated 13 patients diagnosed with pseudotumor cerebri syndrome and stated the mean ONSD as 5.5 ± 1.2 mm for the right eye and 5.4 ± 1 mm for the left eye. In a study evaluating 72 pediatric patients who received invasive ICP catheters, ONSD cut-off value was stated as 5.28 mm for intracranial pressure >15 mmHg and 5.57 mm for intracranial pressure >20 mmHg.[26] In the current study, the mean ONSD was 5.9 ± 0.8 mm in the group with suspected increased ICP and 5.2 ± 0.3 mm in the control group. Our results showed the cut-off value as 5.8 mm (sensitivity: 66 percent, specificity: 100 percent) for ONSD in the patient group.

In the literature, the exact effect of increased ICP on ophthalmic veins is not well known.[23] In a study with adult subjects, Ebraheim et al.[27] looked at colored Doppler ophthalmic ultrasound on 24 adult patients diagnosed with pseudotumor cerebri syndrome and did not detect a significant difference to the control group measurements. On the contrary, Karami et al.[28] performed colored Doppler ophthalmic ultrasonographic measurements on patients diagnosed with increased ICP and noted a significant elevation in the Doppler indices of the patient group. Gura et al.[29] determined a significant correlation between retinal RI and intracranial pressure measured with a catheter inside the ventricle. In another study by Riggs et al.[30] that looked at the ophthalmic artery Doppler ultrasonographic measurements conducted on 13 pediatric patients with brain death, a significant decrease was observed in PSV and the RI was determined as one in all patients. In another adult study conducted by Tarzamni et al.[23] the Doppler measurements of 30 patients with increased ICP and 30 healthy individuals

in the control group were compared and ONSD, PSV and RI were found to be significantly elevated. In the current study, ONSD and RI were also found to be significantly elevated in the study group and the cut-off value for RI was determined as 0.68 with 63 percent sensitivity and 83 percent specificity.

For ophthalmic diagnostic applications, the US Food and Drug Administration (FDA) has set maximum recommended exposure levels of MI, TI and ISPTA.3.[15] These are very significant limits to ensure safe evaluation and the protection of the optic globe. As determined by the FDA, there are different maximum limits for different types of ultrasonographic methods. In an article about the safety considerations of ophthalmic ultrasound, Harris G.R.[16] recommends the safety limits for the use of ophthalmic ultrasound. The FDA's maximum recommended acoustic output level guidelines have been adhered to and the technical characteristics of the device used have been carefully monitored throughout this study.

The major limitation of this study lies in describing increased ICP without using an invasive ICP catheter. The gold standard for measurement of ICP is by a catheter placed in the brain parenchyma or ventricle. However, since this method is invasive and has various complications, it cannot be applied easily. This study opts for the use of clinical and radiological methods to measure an increase in ICP. Unfortunately, our study group is a suspected increased ICP patient group. Another limitation is the number of patients. To compensate for the low number of participants, additional measurements were evaluated. In addition to ONSD, the central retinal artery Doppler indices (RI, PSV, EDV) were measured and compared to the healthy control group's ultrasonographic measurements.

In conclusion; POCUS may become the new stethoscope for pediatric intensive care specialists because of its wide usage area. One of the most important usage areas of POCUS is ophthalmic ultrasound. In follow up for critically ill pediatric patients with increased ICP, ultrasonographic OSND and ophthalmic and central retinal artery RI measurements, which are non-invasive and can be repeated pending clinical necessity, appear promising for the future in terms of the early diagnosis of increased ICP and essential emergency intervention. This research is important because it is one of the few pediatric studies where central retinal artery Doppler indices are evaluated in addition to OSND in patients with increased ICP. There is a need for further studies with larger series to evaluate the acceptance of POCUS as a noninvasive and radiation-free technique for measure increased ICP by the medical community.

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Original Article

Is there a relationship between joint hypermobility and gastrointestinal disorders in children?

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ABSTRACT

Background. The main aim of the study was to assess the association between joint hypermobility (JH) and gastrointestinal (GI) disorders in children.

Methods. All children aged 4-17 years attending the clinics of the participating Pediatric Gastroenterology Centres for functional GI disorders (FGIDs) and inflammatory bowel disease (IBD) were screened for joint laxity. JH diagnosis was inferred using the Beighton Score. JHS diagnosis was inferred based on the Brighton Criteria. Rome III Diagnostic Criteria were used to diagnose possible FGIDs. Ulcerative colitis and Crohn's disease diagnoses were made according to the Porto Criteria. Age and sex- matched healthy children were enrolled as controls.

Results. One-hundred-seventy children with GI disorders (70 with FGIDs, 50 with Crohn's disease, and 50 with ulcerative colitis) and 100 healthy controls were enrolled in the study. JH was reported in 7/70 (10%) children with FGIDs (p=0.26 compared to controls), 4/50 (8%) children with Crohn's disease (p=0.21 compared to controls) and 15/50 (30%) children with ulcerative colitis (p=0.09 compared to controls; p=0.01 compared to FGIDs; p=0.01 compared to Crohn's).

Conclusions. JH is more prevalent in patients suffering from ulcerative colitis compared to the healthy general population, yet the difference did not reach statistical significance. Likely, a proportion of children with ulcerative colitis and JH may show connective tissue abnormalities. However, whether JH can be considered a possible feature of pediatric GI disorders deserves further investigation.

Key words: joint hypermobility, joint hypermobility syndrome, inflammatory bowel disease, ulcerative colitis, children.

Joint hypermobility syndrome (JHS) is the most prevalent of the hereditary disorders of connective tissue characterized by joint hypermobility (JH), the others being Marfan syndrome, Ehlers–Danlos syndrome, and *osteogenesis imperfecta*. JHS is defined as "musculoskeletal symptoms in a hypermobile individual in the absence of systemic rheumatological disease" and its prevalence is around 0.5% of the population.[1] JH, besides being a common clinical feature of the hereditary disorders of connective tissue, is also a common phenomenon in the healthy general population and represents one extreme of the physiological range of joints laxity.[2]

It has been recently pointed out that most of the features of JHS, such as symptom-based diagnosis in the absence of validated biomarkers, youth

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and female gender prevalence, and association with other diseases like fibromyalgia, migraine and sleep disturbance, are common features in most functional gastro-intestinal disorders (FGIDs).[3] FGIDs include a combination of chronic or recurrent gastrointestinal age-dependent symptoms not explained by known biochemical or structural abnormalities.[4] They represent a challenging group of conditions that are frequently misdiagnosed in children and are associated with significant morbidity and high health care costs, accounting for more than 50% of the consultations in pediatric gastroenterology practice and from 2 to 4% of all general pediatric office visits.[5] Over the last years, interest in the study and recognition of FGIDs in children has escalated. Despite some promising developments, more research is needed to further advance the science underlying the pathophysiology and treatment of these conditions, which remain one of the unmet needs in the modern pediatric gastroenterological practice.

Aside from FGIDs, over the last years a few reports have shown an association between JH/JHS and organic diseases as well, such as Crohn's disease and celiac disease, thus raising the hypothesis that collagen varieties may play a role in their pathogenesis.[6,7] Evidence in pediatric age is still very limited.

The primary aim of our study was to assess the relationship between JH and both functional and organic GI disorders in a pediatric population. The secondary objective was to identify a possible subgroup of children with GI disorders who are more likely to suffer from JH.

Material and Methods

All consecutive children and adolescents aged between 4 and 17 years attending the clinics of the participating Pediatric Gastroenterology Centres for FGIDs and inflammatory bowel disease (IBD) from November 2014 to May 2015 were eligible for the study. All patients were screened for JH by one of the study investigators, using the 9-point Beighton score as described in Table I. JH diagnosis was assumed as having a Beighton score higher than 4.[8] A proportion of patients who were found to have JH was referred to a tertiary referral clinic run by a rheumatologist for further evaluation. JHS diagnoses were inferred only by the rheumatologists based on the Brighton criteria.[9] Quality of life of all the enrolled children was scored by the use of a validated questionnaire.[10,11]

The GI diagnostic evaluation included pH-impedance, gastric emptying, bowel transit time, manometric or endoscopic evaluation, and blood and fecal laboratory tests (complete blood count, inflammatory markers, anti-transglutaminase and anti-endomysial antibodies, fecal calprotectin), according to children's clinical picture and to the pediatric gastroenterolgist's free judgement. Rome III diagnostic criteria were used to diagnose possible FGIDs. Ulcerative colitis and Crohn's disease diagnoses were made according to the Porto Criteria.[12]

After a functional or organic diagnosis was confirmed, patients with JH were compared in terms of clinical characteristics to those without JH. Patients who had a diagnostic reassessment

Table I. Nine-point Beighton hypermobility score.

Maneuvers	Score
Passive dorsiflexion of fifth metacarpophalangeal joint to ≥90°	1 point each for left and right
Opposition of thumb to volar aspect of ipsilateral forearm	1 point each for left and right
Hyperextension of elbow to ≥10°	1 point each for left and right
Hyperextension of knee to ≥10°	1 point each for left and right
Place hands flat on floor without bending knees	1 point

A score >4 out of 9 is consistent with joint hypermobility.

or whose diagnosis had been questioned during the study period were excluded from the study.

Age and sex-matched healthy children were enrolled as negative control group among brothers or sisters of children attending the clinics or from children attending the clinics for well-child routine visits, and were screened for both JH and JHS, once the presence of any GI disorder was excluded.

Statistical analysis

Study data were entered into Excel (Microsoft, Redmond, WA) and analyzed with GraphPad PRISM software, version 5.01. Results are expressed as mean ± standard error and percentages. Statistical analyses include Student's t-test, chi-square test, and Fisher's exact test, with significance accepted at the 5% level. The sample size was computed considering an expected difference in the studied variable between the 2 groups of approximately 15-20% (power 80%; confidence interval 95%; first type error 0.05).

The study was approved by the Independent Ethics Committee of the University of Naples Medical School (rep. num. 0187/2015) and was conducted in accordance with the Declaration of Helsinki and Guidelines for Good Clinical Practice. An informed consent was obtained at enrolment from parents of all children younger than 10 years, and from both parents and children, if older than 10 years.

Results

One-hundred and eighty-six children and their parents agreed to participate and were enrolled in the study. Of these, only 170 (89 boys; mean age 120.6 ± 51 months; age range: 49-204 months) were included in the analysis since 16 had a diagnostic reassessment during the study period or, alternatively, were lost to follow-up. One-hundred age- and sex-matched healthy children were recruited as the control group (55 boys; mean age 123.5 ± 44 months; age range: 58-199 months).

Twenty-six out of 170 (15.3%) patients of the study group and 17 out of 100 (17.0%) children of the control group had clinical evidence of JH according to the Beighton score (p=0.73). Among the study group, only 1/170 (0.6%) also fulfilled the Brighton criteria and was therefore diagnosed as having JHS while no healthy children met the criteria for JHS (data too small to be analyzed). Compared to those without evidence of JH, children with JH had a similar age (133.3 months vs. 133.5 months, respectively) and were more frequently female (54.3% vs. 45.7%, respectively; p = 0.72 in the study group whereas were older (135.9 months vs. 105.5 months, respectively; p = 0.44) and more frequently female as well (66.7% vs. 33.3%, respectively; p = 0.18) in the control group.

Among the study group, 70 children fulfilled the criteria for FGIDs, mainly functional constipation (FC; n=32) and irritable bowel syndrome (IBS; n=19) and 100 suffered from IBD, 50 of which from ulcerative colitis and 50 from Crohn's disease. JH was reported in 7/70 (10%) children with FGIDs (p=0.26, compared to controls), 4/50 (8%) in children with Crohn's disease (p=0.21, compared to controls) and 15/50 (30%) in children with ulcerative colitis (p=0.09, compared to controls; p=0.01, compared to FGIDs; p=0.01 compared to Crohn's). Figure 1 summarizes the prevalence of JH among children with different GI diagnoses compared to controls.

The mean score of the quality of life was 97.0/100 among the control group, 88.4/100 among patients with FGIDs, 91.4/100 among patients with Crohn's disease, and 92.1/100 among patients with ulcerative colitis. Children with JH had a mean quality of life score of 96.4/100, whereas children without JH had a mean score of 94.3/100 (no statistically significant difference detected).

Discussion

This study evaluated the association between JH and both functional and organic GI

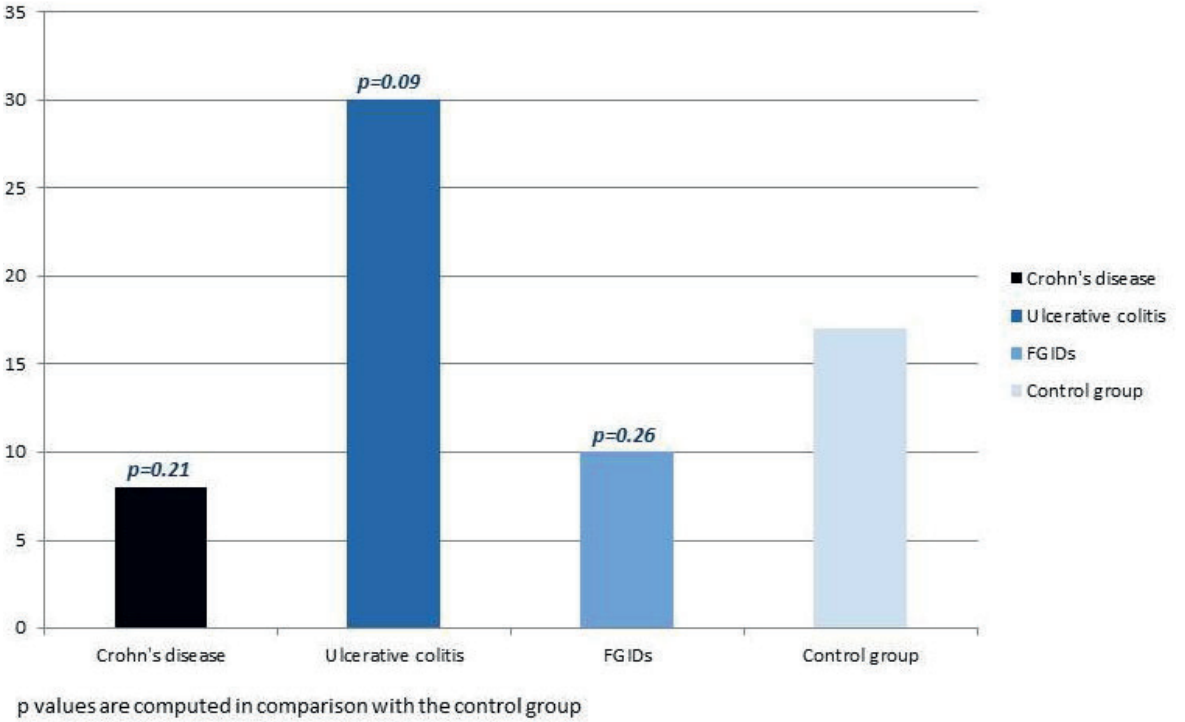

Fig. 1. Prevalence (%) of joint hypermobility in children with Crohn's disease, ulcerative colitis, functional gastro-intestinal disorders (FGIDs) vs. control group.

disorders in a cohort of pediatric patients attending a tertiary care gastroenterology unit. JH was more prevalent in patients suffering from ulcerative colitis compared to the healthy general population, although the difference did not reach statistical significance. Conversely, children suffering from other GI disorders, such as Crohn's disease and FGIDs, showed a lower rate of JH. When compared to these two groups of patients, the rate of JH in children with ulcerative colitis reached statistical significance.

The association between JH and GI symptoms in adults was first described 10 years ago by Hakim and Grahame.[13] Since that landmark study, other studies in specialist hospital settings worldwide have confirmed that GI symptoms are common in patients with an existing diagnosis of JH.[14-16] Direct evidence for an association between FGIDs and hypermobility comes from a single retrospective observational study in an adult tertiary gastroenterology setting.[3] The Authors of this study used the validated 5-point hypermobility questionnaire to screen 129 consecutive patients attending a neurogastroenterology clinic. The prevalence of JH in these patients was 49%, about 3 times higher than the prevalence in healthy controls (17%). Subjects with JH were more likely to have GI symptoms without a known underlying structural, biochemical, metabolic, or autoimmune cause compared to those without JH. A subgroup of these patients was further assessed by a rheumatologist and was found to have JHS. These patients suffering from JHS tended to have motility problems in their gut on physiologic testing, such as small bowel dysmotility, delayed gastric emptying, and delayed colonic transit. This study confirmed that, in a tertiary neurogastroenterology setting, JH was strongly associated with unexplained GI symptoms. Furthermore, it showed that GI dysmotility is common in patients with GI symptoms and JHS, suggesting that these patients may have a neuromuscular basis for their symptoms. A few years later Zweig et al.[17]

provided further evidence about the possible link between JH and FGIDs, collecting data from a population of IBS patients. Their main finding was the significantly higher prevalence of JH in patients with constipation predominant IBS compared to patients with diarrhea predominant IBS.

In recent years, two studies published by an Italian and a Greek study group highlighted a possible association between hypermobility and GI organic disorders, such as Crohn's disease and Celiac disease, as well.[6,7] Finally, Fikree et al.[18] reported that, compared to patients without hypermobility, those with JH, experience more reflux and dyspepsia, and also more commonly complain from chronic pain, fibromyalgia, and autonomic symptoms.

In 2015, Castori et al.[19] reviewed all the available evidence and confirmed a strong relationship between a variety of GI disorders and JHS.[19] According to the authors' opinion, given the relatively high frequency of this condition compared to other heritable connective tissue disorders, JHS emerges as a model for studying the pathophysiologic basis of such an association and, reasonably, identifying more tailored management and treatment approaches. Moreover, these studies emphasize the relevance of raising the scientific interest in this field. Indeed, accumulated evidence on the non-casual association between JH and many potentially disabling GI disorders opens a novel approach for interpreting highly prevalent complaints in humans.

Evidence in pediatric age is still very limited. A 2008 study by an Australian group showed a higher prevalence of JH in young boys with slow transit constipation compared with those without constipation, suggesting a possible relationship between the two conditions.[20] More recently, Kovacic et al.[21] analyzed a cohort of children with FGIDs, showing a high prevalence of both JH and comorbid symptoms, including sleep disturbances, chronic fatigue, migraine headache, dizziness, chronic nausea, and fibromyalgia, that markedly affected the subjects' social life.

The main findings of the present study are partially in disagreement with the results of the aforementioned studies, since we found an increased rate of JH only in children with ulcerative colitis, whereas we reported a lower prevalence in children with Crohn's disease and FGIDs. However, the rate of JH that we found among healthy children is quite comparable to that reported in previous studies.[20-22] Likewise, the higher prevalence of JH in females has already been reported.[21] The frequent objective evidence of JH in many children with ulcerative colitis leads to the intriguing hypothesis of a new possible contributing etiologic factor. How could connective tissue defects be related to a chronic inflammatory condition is still to be determined. Perhaps alterations in the integrity and mechanical properties of the intestinal wall and distensibility of the gut may lead to altered motility and pain perception. Nevertheless, the biochemical mechanisms underlying these GI symptoms remain to be studied.

The prevalence of JH in our FGIDs and Crohn's disease study groups is comparable to that reported in healthy children. This finding contradicts the results of the aforementioned studies that reported a higher prevalence in adults and children with these disorders. Since the diagnostic tools and the sample sizes are similar among the different studies, we are not able to provide any possible explanation about the controversial results.

Finally, the evaluation of the quality of life in all the enrolled patients showed no difference between patients with and without JH. Furthermore, it allows an intriguing analysis of the impact of the different GI disorders. As already reported by recent papers, children's social and mental health was not affected by the functional or organic nature of the diagnosis, being slightly lower in children with FGIDs than IBD.[23,24]

Our study has possible shortcomings. Although it is acknowledged that anxiety and social stressors play key roles in both functional and organic disorders, the study lacks an accurate evaluation of these factors, limiting study interpretation and particularly the role of behavioral symptom amplification. Future prospective studies should include validated, psychosocial measures, such as an anxiety/depression evaluation. Moreover, our tertiary care cohort likely suffers from a referral bias with higher symptom severity and complexity than a community population with GI disorders, thus limiting our findings. Finally, even if the overall sample size of the present study is adequately large, it would lack power if we wanted to analyze patients with different FGIDs, such as IBS and FC.

On the other hand, our study shows several strengths. The objective nature of the hypermobility assessment, performed by the same physician per Centre for all the patients, rules out any possible bias related to different unreliable interpretations. Moreover, the physician who performed the joint laxity assessment was usually unaware of the diagnosis thus avoiding another major bias. Since JH has been reported to vary widely according to both age and gender, the present study included an age- and sex- matched control group, which allowed a relevant comparison, an essential feature conferring a clinically relevant meaning to connective tissue abnormalities in GI diseases.

In conclusion, our study shows that the prevalence of JH among children with FGIDs and Crohn's disease is comparable to that reported in healthy children, whereas children with ulcerative colitis were shown to have a higher prevalence of JH, although not reaching statistical significance vs. healthy children. Although preliminary, our data concur in making a step forward in a new and promising area of research. The existence of a possible overlap between JH and ulcerative colitis suggests that the two conditions may share a common pathophysiology with collagen tissue changes exerting a contributory role. New, larger studies could help explain this relationship, providing a better understanding of the possible role of abnormal connective tissue in the GI tract.

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Case Report

Brown Vialetto Van Laere syndrome: presenting with left ventricular non-compaction and mimicking mitochondrial disorders

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ABSTRACT

Background. Brown-Vialetto-Van Laere syndrome (BVVLS) is a rare, treatable neurodegenerative disorder with a variable clinical presentation, caused by mutations in three different riboflavin transporter genes.

Case. An 11-year-old-boy presented with respiratory insufficiency and a rapidly progressive muscle weakness. He was the fifth child of a consanguineous marriage with a medical history of hearing loss. He was peripherally week with a reduced muscle tone. Upper extremity muscles were effected more than lower limbs. He deteriorated rapidly and became quadriplegic. Brain magnetic resonance imaging and magnetic resonance spectroscopy were normal. Echocardiography revealed left ventricular non-compaction. A homozygous c.1088C>T (p.363L) missense mutation was identified in *SLC52A2* gene. Significant clinical improvement was seen with high dose riboflavin.

Conclusion. This is the first reported BVVLS case presented with left ventricle-non compaction which may be caused by a secondary respiratory chain deficiency. Riboflavin transporter deficiencies should be considered in the differential diagnosis of mitochondrial disorders and secondary respiratory chain deficiencies should be thought during the follow-up of BVVLS.

Key words: Brown-Vialetto-Van Laere syndrome, riboflavin, left ventricle-non compaction, mitochondrial disorders.

Brown-Vialetto-Van Laere syndrome (BVVLS), a rare motor neuron disorder, is caused by three different riboflavin transporter (RFVT) genes; *SLC52A3*, *SLC52A2*, and *SLC52A1* which codes RFVT3, RFVT2 and RFVT1, respectively.[1-3] While *SLC52A3* and *SLC52A2* are the main responsible genes, there is only one neonatal case with a mutation in *SLC52A1* gene, probably due to the defective placental expression.[2] Defects of RFVTs, decreases cellular uptake of riboflavin and may cause defects in energy production and cellular function.[2] BVVLS is in the same spectrum with Fazio-Londe syndrome, which may present with cranial nerve palsies, bulbar palsy, respiratory compromise, sensorineural deafness, limb weakness and upper motor signs.[1,4] There are some phenotypical differences between RFVT3 and RFVT2 deficiencies. While hearing loss and muscle weakness are the most common presenting symptoms, abnormal gait and/or ataxia is often a presenting feature of RFVT2 deficiency.[5] Although the vast majority of patients are presented in early childhood, late onset presentation is more characteristic for a *SLC52A3* mutation.[5] Optic atrophy, retinitis pigmentosa, intellectual delay and nystagmus have been reported as other rare neurological features.[6] Moreover, there are several other accompanying non-neurological findings such as diabetes insipidus, hypogonadism and hypertension.[6] Due to this clinical variability,

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patients may initially be misdiagnosed. They are suspected to have other neurological diseases such as mitochondrial myopathies and they experience a longer clinical journey.[7] Patients may also have abnormal acyl carnitine profiles, mimicking multiple acyl-CoA dehydrogenase deficiency.[8] Some of the current publications report varying degrees of improvement in clinical outcome or stabilisation in the symptoms with high doses of riboflavin replacement especially when rapid treatment started in the earlier stages.[1,9-11]

Here we describe the clinical course and responsiveness to riboflavin with left-ventricular non-compaction which is a rare manifestation of mitochondrial diseases and has never been reported in BVVLS.

Case Report

An 11-year-old-boy was admitted to our paediatric intensive care unit because of respiratory insufficiency and a rapidly progressive muscle weakness. He was the fifth child of a consanguineous marriage and there was a history of early infantile death in the elder two male siblings after a similar respiratory deficiency. His past medical history was unremarkable except some minor hearing difficulties in the previous 2 years. He had been seen by a general practitioner and hearing loss was thought to be associated with infections. Audiological examination was suggested but the family did not consider it and it has been dismissed. During the admission, he was in the status of respiratory failure requiring intubation and mechanical ventilation. He was peripherally weak with a reduced muscle tone. Upper extremity muscles were more involved than the lower extremities and he suffered from shoulder girdle and distal hand muscle weakness. Deep tendon reflexes were bilaterally absent at the knees and ankles. Babinski sign was bilateral negative. Audiologic evaluation demonstrated neurosensorial loss. Within the next few days, he rapidly deteriorated, dysphagia progressed and a feeding tube was also placed for nutrition. He became quadriplegic and could not walk. Ammonia, serum lactic acid, hepatorenal function, thyroid function tests and microelements were normal. His acyl carnitine profile and amino acid screens were also normal. His urine organic acid analysis showed mild etylmalonic and suberic aciduria. Cerebrospinal fluid protein levels were mildly elevated but it was acellular. The findings from electroencephalogram and ophthalmology assessments were normal. Brain magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS) were also non-significant. Echocardiography revealed left ventricular non-compaction without any cardiac anomalies. There were no changes in the electrocardiogram. He was initially diagnosed with a post infectious neuropathy and due to the possible autoimmune aetiology intravenous immunoglobulin and corticosteroid treatments were given. There was no significant improvement and based on the concern that his phenotype was compatible with a mitochondrial disorder, coenzyme Q10, riboflavin and carnitine were commenced. Despite this mitochondrial cocktail, there was no clinical improvement. Considering BVVLS in the differential diagnosis, oral riboflavin therapy was gradually increased in two weeks to 75 mg/kg/day. One week after increased riboflavin therapy the child was extubated to room air, two weeks later was feeding orally and in a month he was walking without any support, he has independent mobility. There are no reported side effects.

According to the clinical features and this dramatic response to riboflavin treatment, BVVLS was suspected. Peripheral venous blood was collected from the proband and his parents. A homozygous c.1088C>T (p.P363L) missense mutation was identified in *SLC52A2* gene (Fig. 1). Both parents were heterozygous for this mutation. This sequence change replaces proline with leucine at codon 363 of the protein (p.Pro363Leu). It has been classified as likely pathogenic by the ACMG guideline.

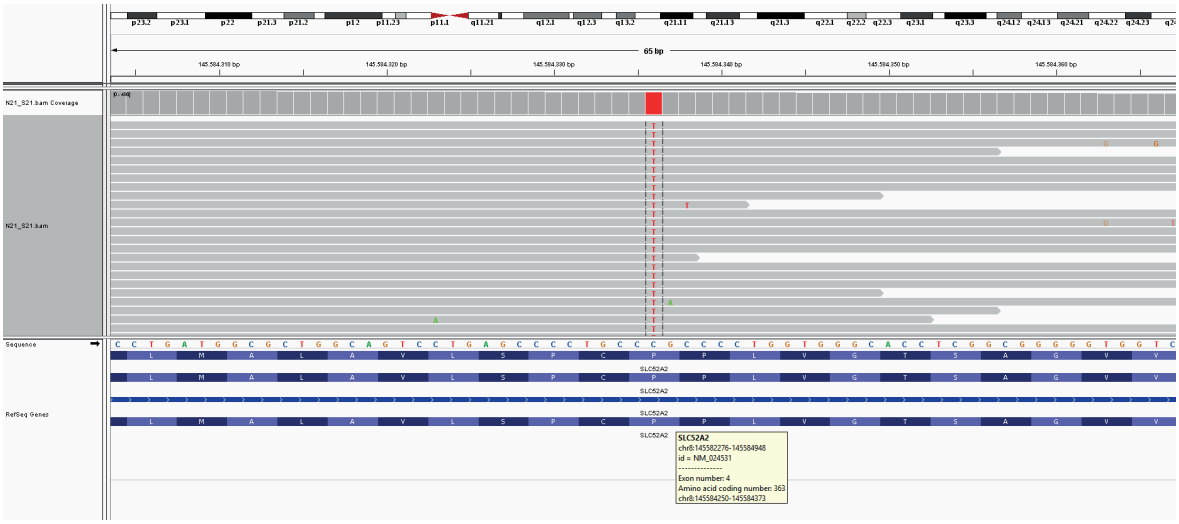

Fig. 1. c.1088C>T (p.363L) mutation in the *SLC52A2* gene.

Genetic analyses in this study were approved by the ethics committees of Cukurova University. Written informed consent was obtained from both of the patient's parents.

Discussion

BVVLS is a rare, neurodegenerative disorder characterised by progressive pontobulbar palsy, sensorineural deafness and respiratory insufficiency.[11] There is a clinical heterogeneity; while milder forms present in the second or third decade, severe forms may die in the early infantile period.[3,6] Mean age of presentation is (4.1–8.2 years).[1,2] Our patient presented slightly older than this when we consider the minor hearing difficulties as the main presenting symptom. Besides the variability in age of onset, clinical findings may differ from patient to patient including the characteristic features of lower cranial nerve involvement (nerves VIII—XII), lower and upper motor neurone limb signs, feeding difficulties and respiratory failure, facial weakness, sensory ataxia and optic atrophy.[1,6] Here, in our case sensorineural hearing loss was an insidious feature, thus the clinical picture has been dismissed till the development of respiratory insufficiency. Therefore, the diagnosis and the treatment were delayed.

There are clinical differences in the phenotype of patients depending on the gene which has the causative mutation. Early onset and prominent weakness in the upper extremities is almost always seen in patients with mutations in *SLC52A2*, in contrast to those patients with *SLC52A3* mutations, in whom the onset of weakness is often more generalized.[3,8,9] Another typical presentation with *SLC52A2* mutations is the absence of upper motor neuron signs in the lower limbs which is very common for *SLC52A3* mutations.[4] Correlated with that, our patient had a more significant weakness in upper limbs.

Manole et al.[12] reported a Brazilian girl who presented with sensorineural hearing loss, optic atrophy, respiratory insufficiency and sensorimotor neuropathy. She was compound heterozygous, besides c.1088C>T she had c.383C>T mutation in the *SLC52A2* gene.[12] Her phenotype was similar to our patient except the optical atrophy.

The flavoproteins which contain a nucleic acid derivative of riboflavin acts essentially in normal respiratory chain functions, and especially prominent for complex II- succinate dehydrogenase.[13] Foley et al.[9] reported two patients who had proven respiratory chain deficiency. One of them had slightly decreased complex IV activity (0.012; reference range:

0.014–0.034) and the other one had decreased complex I activity (0.089; reference range: 0.104–0.268).[9] They both had mutations in the *SLC52A2* gene. Nimmo et al.[13] reported two patients who have complex II deficiency and red ragged fibres in histochemistry of muscle biopsy consistent with mitochondrial myopathy with both *SLC52A2* and *SLC52A3* mutations. Our patient had a phenotype which is suggestive for a mitochondrial myopathy. Although we could perform neither a muscle biopsy nor a respiratory chain enzyme activity measurement, this could be because of a possible respiratory chain enzyme deficiency. Thus, in the differential diagnosis of neuromyopathy, as well as the mitochondrial disorders riboflavin transporter defects should be considered in the early stages and high doses of riboflavin treatments should be given.

Cardiac presentation is not common for BVVLS. Several cardiac complications have been reported due to the hypoxia caused by respiratory insufficiency, diaphragmatic weakness or prolonged seizures.[14] The cardiac finding of our patient, non-compaction of the left ventricle, has not been previously reported among patients with BVVLS. Left ventricular non-compaction is a rare manifestation of mitochondrial disorders mostly related with mitochondrial complex II deficiencies and Barth syndrome.[15] This is the most interesting finding of our patient which may be due to a secondary mitochondrial defect.

In conclusion, this case highlights the importance of suspicion on the way to the early diagnosis. This led to early initiation of high dose riboflavin which probably provided a significant improvement in our case. Life-saving treatment should be started immediately without awaiting the genetic confirmation. Further studies will clarify the underlying mitochondrial defects in the metabolic pathway. In addition, genetic counselling and genetic screening should be recommended to affective families. Prophylactic riboflavin treatment may be beneficial for preventing symptoms.

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Case Report

Rare presentation of levamisole-induced leukoencephalopathy in a pediatric patient: seizure

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ABSTRACT

Background. Levamisole is an imidazole derivative used in the treatment of various cancers, dermatological diseases, and parasitosis. Illegal use of levamisole by mixing it with cocaine in order to increase the psychotropic effects has also increased in recent years. Leukoencephalopathy is one of levamisole's most prominent neurological side effects.

Case. Here we present the clinical, laboratory, imaging findings, treatment, and follow-up information of a 12-year-old girl who presented with seizures due to levamisole, which was prescribed to treat vitiligo.

Conclusion. Levamisole-induced leukoencephalopathy should be considered in the differential diagnosis of demyelinating diseases, the neurotoxic effects of the drug should be well understood, and treatment should be initiated as soon as possible.

Key words: levamisole, demyelinating diseases, leukoencephalopathies, seizure, vitiligo, cocaine.

Levamisole is the L-isomer of tetramisole. It was first used as an anthelmintic agent in 1966 and was later used in inflammatory and oncological diseases due to its wide immunomodulatory effects.[1] Levamisole has also been used to treat many dermatological diseases.[2,3] It was approved by the United States Food and Drug Administration in 1991 as an adjuvant therapy in the treatment of colorectal cancers. However, over time, various side effects such as agranulocytosis, thrombocytopenia, and leukocytoclastic vasculitis have been observed and it was withdrawn from use. The most notable neurological side effect was multifocal inflammatory leukoencephalopathy (MIL), which was first seen in patients receiving a combination of 5-fluorouracil and levamisole therapy for colon cancer treatment. Since treatment of non-cancer diseases with levamisole alone also caused leukoencephalopathy, it was thought to be responsible for causing this side effect.[4] Due to all these side effects, the pharmacological use of levamisole in humans has become very limited. However, in recent years, levamisole has been illegally used to increase the psychotropic effect of cocaine. Sixty-five percent of cocaine worldwide is contaminated with levamisole and it has been shown that leukoencephalopathy developing in individuals using cocaine is due to levamisole.[5] Neurological symptoms such as altered consciousness, ataxia, aphasia, hemiparesis, quadriplegia, seizure, and facial palsy that manifest as a result of leukoencephalopathy can be treated with steroids, immunoglobulin and plasmapheresis treatments.[4] Although there are reports regarding levamisole induced leukoencephalopathy (LIL) in the literature, most recent studies have focused on leukoencephalopathy that develops in cocaine users. As far as we know, there is only one study that reported two pediatric patients with LIL.[6] Here, we present a case of a 12-year-old patient that developed LIL.

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Case Report

A 12-year-old girl, who did not report any previously known diseases, presented with generalized tonic-clonic seizures. It was learned that the patient had previously fainted a week ago and since brain tomography and electroencephalography (EEG) examination performed at that time were normal, the patient was diagnosed with syncope. The patient, who was born at 38 weeks by Caesarean section and weighed 2840g, did not have any problems during the postnatal period. She reached all her developmental milestones at appropriate ages and her family history was unremarkable. The patient had a 6.5-year-old healthy sister. The patient had recently experienced emotional lability, light-headedness and mild headache. The physical examination was normal, except for hypopigmentation in the right lower quadrant of the abdomen. When questioned, it was learned that the patient was started on oral levamisole treatment 6 weeks ago due to vitiligo, and that she had taken a total of 720 mg levamisole.

Although repeated one-hour sleep and wakefulness EEG examination was found to be normal, levetiracetam treatment was started, since the patient had experienced two episodes within one week. Brain MRI revealed T2 and FLAIR hyperintensities in the bilateral periventricular regions and right anterior section of centrum semiovale. Some of these lesions showed gadolinium enhancement, one lesion in the right frontal region was shaped like a ring (Fig. 1A-C).

No cells were seen in the patient's cerebrospinal fluid (CSF) and her biochemistry results were normal. CSF cultures were sterile. The CSF viral serology panel that included HSV, adenovirus, enterovirus and CMV was negative. The patient received 1x1gr pulse methylprednisolone treatment for three days and her CSF oligoclonal band was negative and IgG index was 0.49 (n: <0.7). The serology results of TORCH, hepatitis,

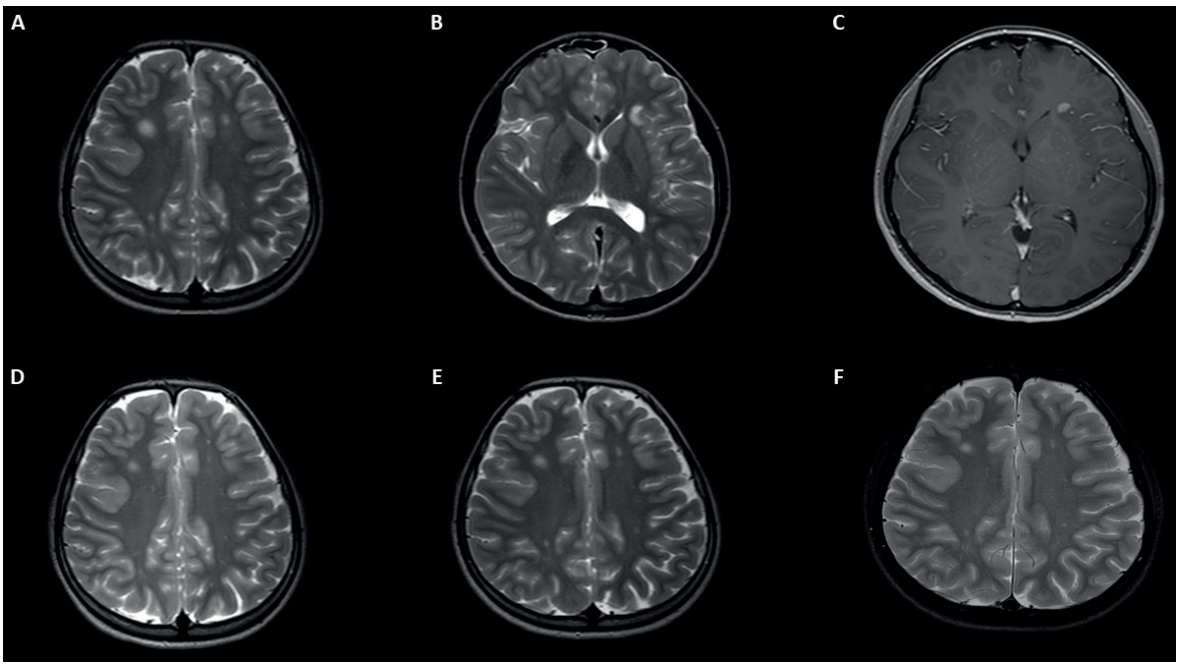

Fig. 1. A-C. MR images at admission. Axial T2-weighted images showed white matter lesions in both cerebral hemispheres (A, B); Axial T1-weighted images after gadolinium administration demonstrated enhancements and also ring enhancement of right frontal subcortical lesion (C). **D-F.** Axial T2-weighted images at one month, one year and 2,5 years later (respectively), lesions were significantly decreased and contrast enhancement after gadolinium administration had disappeared. There was no new lesion.

lyme and mycoplasma were negative. Moreover, serum neuromyelitis optica and myelin oligodendrocyte glycoprotein antibodies were negative. The brain MR angiography performed for the differential diagnosis of vasculitis was found to be normal and the patient's erythrocyte sedimentation rate was 4 mm / h (n: 0-20). Antinuclear antibody, anti-ds DNA, anti-cardiolipin, and lupus anticoagulant were negative. Since the patient did not have any abnormal test results, the white matter lesions were thought to be associated with levamisole treatment. Oral methylprednisolone treatment was reduced and discontinued in one month.

No clinical complaints were observed during the 2.5-year follow-up. No new lesions were detected in brain MRI examinations and regression was observed in existing lesions over time (Fig. 1D-F). The patient, whose levetiracetam treatment was discontinued, continues to be followed without treatment. Informed consent was received from the family.

Discussion

Levamisole, which is a synthetic antihelmintic drug, triggers cellular immunity by changing the balance of T helper-1 cell / T helper-2. While it increases the levels of some interleukins (IL-2 and IL-12) it causes others to decrease (IL-4, IL5, and IL-10). Its toxic effects are generally mild. Common side effects are gastrointestinal disorders, skin manifestations, and hematological disorders. There is a wide potential for neurological side effects such as headache, vertigo, vomiting, aphasia, blurred vision, diplopia, weakness that can be seen in 1.3-5% of patients.[3]

LIL is a delayed demyelinating leukoencephalopathy.[7] Although the cause has not been fully revealed, it has been reported that in dogs treated with levamisole perivascular areas in the brain and meninges are surrounded by mononuclear cells. Levamisole has not been shown to cause inflammation or demyelination in mice, therefore it is thought that it does not have a toxic effect on myelin. However, administration of levamisole increased inflammation and demyelination in mice infected with a virus that can cause demyelination. Therefore, levamisole is thought to cause leukoencephalopathy by increasing inflammation that occurs against the presence of a symptom-free antigen.[3] LIL has been reported in patients receiving cancer treatments or individuals using levamisole for anthelmintic purposes, however recently there has been a surge of LIL cases in cocaine users. There are only two pediatric LIL cases reported in the literature.[6]

LIL usually occurs subacutely within the first two months of levamisole use and cognitive, motor and less frequently sensory clinical findings (ataxia, consciousness change, hemiparesis / quadriplegia, facial palsy, diplopia, dysarthria) can be seen, while seizures are rare.[7] In our patient, the symptoms were seen in the subacute period, 6 weeks after the use of levamisole. Interestingly, our patient's most prominent neurological finding was a seizure. Apart from our patient, seizures have been reported in only four patients in the literature: in two adults that used levamisole as an anthelmintic due to ascaris, in another adult that used it due to colon cancer, and in one adolescent patient.[6-8] The adolescent patient experienced epilepsia partialis continua due to the use of levamisole and was treated with levetiracetam.[8]

LIL has many similarities with acute disseminated encephalomyelitis (ADEM) and multiple sclerosis (MS). The diagnosis of LIL is made clinically, while CSF examinations, EEG and MRI are ancillary studies. Levamisole has been reported to cause ADEM, where it develops acutely or subacutely following a febrile illness. Inflammation findings are expected in CSF analysis and serological examinations. Although EEG abnormalities are common, they are non-specific. Diffuse slowing, which mostly refers to cortical dysfunction, are observed.[7] Our patient did not have a febrile infection history, there were no signs of inflammation and serological

examinations were normal. EEG examination was normal at sleep and wakefulness. Since rheumatological tests and MR angiography were normal, vasculitis was ruled out.

The MR images of LIL patients often indicate multifocal demyelinating changes in subcortical white matter and periventricular areas. Frontal and parietal lobes are affected more often. Ring-like enhancement of the lesions is typical.[6] It may not be easy to differentiate these LIL MRI findings from MS and ADEM. However, unlike MS and ADEM, gray matter involvements such as basal ganglia, thalamus and brainstem are not expected, and the corpus callosum is generally preserved.[7] In the MR examination of our patient, demyelinating lesions, some with circular enhancement, were observed in the subcortical white matter and periventricular areas in frontal and parietal lobes of both hemispheres, while the gray matter involvement was not observed.

Levamisole treatment is used in many different dermatological diseases such as verruca, aphthous ulcers, pemphigus, erythema multiforme, and vitiligo.[2] In LIL, the symptoms and lesions appear independent of the levamisole dose, which indicates that its toxic effect is idiosyncratic.[3] Although symptoms are most commonly seen as subacute within 8 weeks of use, there have been cases where symptoms began 1 day or months later. The symptoms can be seen after months of chronic use, or after a single dose of 50 mg.[6,7] As with most cases presented in the literature, in our case the symptoms appeared in the sixth week with a total of 720 mg levamisole use. The treatment of LIL is aimed at suppressing the immune response and good long-term results have been reported with steroids, IVIg, and plasmapheresis treatments. Permanent problems such as ataxia and quadriplegia may be encountered if the treatment is delayed or neglected.[7,9] No recurrence of seizures was observed in our patient after steroid treatment, and the imaging findings improved after one month. In the subsequent 2.5-year follow-up, no clinical findings have developed, patient's lesions have significantly regressed and no new lesions were observed.

In conclusion, the neurotoxic effects of levamisole can occur regardless of the dose. Patients that have taken levamisole may present with seizures and their encephalopathy symptoms may be mild. If possible, we suggest avoiding the use of levamisole for the treatment of dermatological diseases.

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Case Report

Unexpected condition in a rare disease: encephalopathy in early-onset sarcoidosis

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ABSTRACT

Background. Granulomatous autoinflammatory diseases are monogenic syndromes caused by mutations in the region encoding the nucleotide-binding domain of the nucleotide-binding oligomerization domain-containing 2 gene. Blau syndrome and early-onset sarcoidosis are familial and sporadic forms of the same disease and are very rare. Many organ systems may be involved; however, neurologic involvement is infrequent. We reported a case of encephalitis in a 12-year-old girl followed with a diagnosis of early-onset sarcoidosis.

Case. The patient was diagnosed with juvenile idiopathic arthritis at 3 years of age. We considered drug-induced sarcoidosis at 6 years of age with granulomatous inflammation of liver and kidney. Small joint involvement and camptodactyly developed during follow-up. M315T mutation was detected in the NOD2 gene supporting the diagnosis of early-onset sarcoidosis. The patient suffered from encephalopathy when she was under methotrexate, infliximab, and systemic steroid treatment at 12 years of age. Cerebrospinal fluid limbic encephalitis antibody panel was negative.

Conclusion. Encephalopathy is not common in Blau syndrome and early-onset sarcoidosis. The cause of encephalopathy in our patient was interpreted as autoimmune encephalitis.

Key words: Blau syndrome, early-onset sarcoidosis, encephalopathy, sarcoidosis.

Blau syndrome (BS) and early-onset sarcoidosis (EOS) are granulomatous autoinflammatory diseases defined as familial and sporadic forms of the same clinical condition, respectively. These syndromes are caused by a mutation in the nucleotide oligomerization domain 2 (NOD2) gene localized in the 16q12 chromosome. Clinical symptoms are characterized by the clinical triad of granulomatous dermatitis, symmetric arthritis, and recurrent uveitis with onset before 4 years of age.[1-3] Non-caseating granulomatous inflammation in affected organs is a hallmark of the disease.[4] Other expanded manifestations of BS/EOS are fever, pneumonitis, bronchial granulomas, hepatosplenomegaly, hepatic granulomas, sialadenitis, erythema nodosum, leukocytoclastic vasculitis, transient neuropathies, arterial hypertension, pericarditis, pulmonary embolism, granulomatous glomerular and interstitial nephritis, and chronic renal failure.[5] In this report, we present a patient who developed encephalitis while being followed with EOS diagnosis.

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Case Report

A 12-year-old girl was diagnosed with juvenile idiopathic arthritis at 3 years of age. During that time, she had arthritis on both knees and received methotrexate (MTX) therapy. Because MTX response was inadequate, etanercept (ETN) was added. While she was on MTX and ETN treatment, at 6 years of age, she was hospitalized because of fever, neutropenia, and liver and renal failure. Multiple organ failure

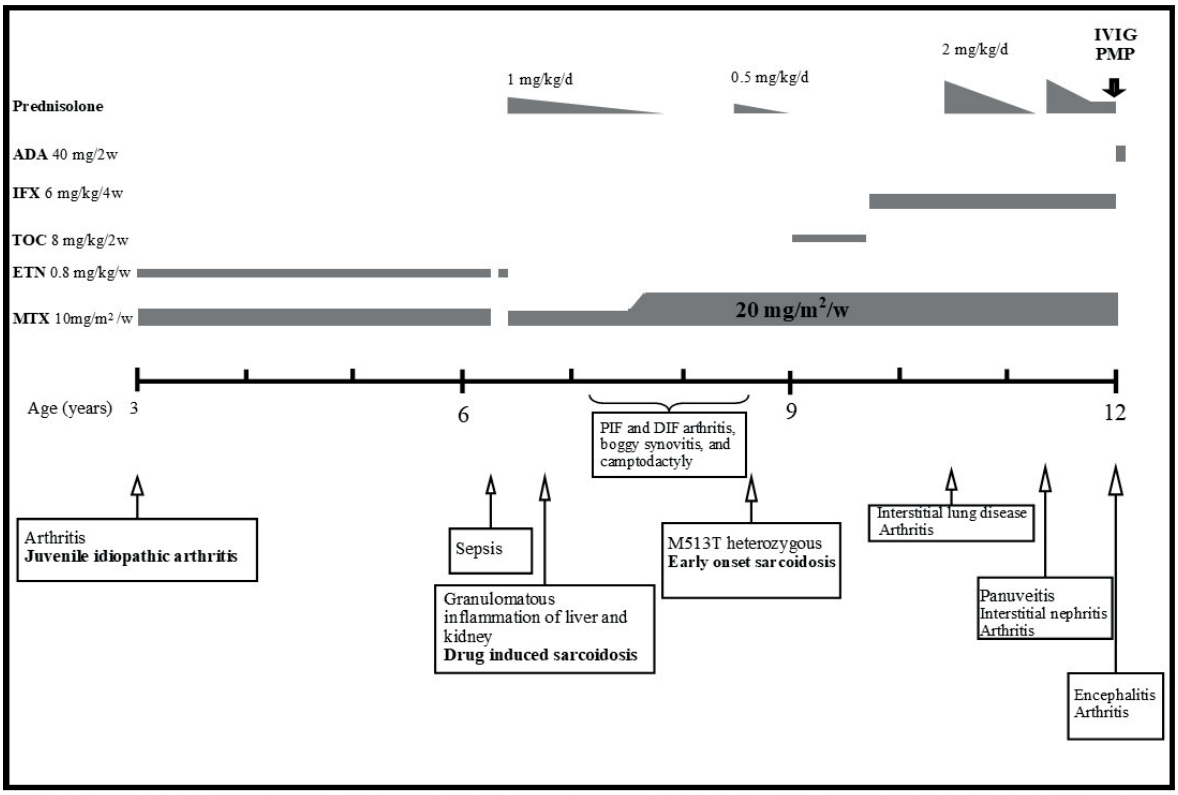
secondary to sepsis was considered. MTX and ETN were discontinued. After an antibiotic and supportive therapy, she improved in three weeks. She was hospitalized again two months later, because of fatigue and weakness. Physical examination revealed hepatomegaly and splenomegaly. Her hemoglobin level was 6.2 g/dl. The blood smear was consistent with normochromic and normocytic features. She had no evidence of nutritional and hemolytic anemia. Bone marrow aspiration showed normocellular marrow findings. She had renal failure (blood urea nitrogen 40 mg/dl, creatinine 2.0 mg/dl) without oliguria and hypertension. Proteinuria was 23 mg/m^2/hour. We performed liver and kidney biopsies; non-caseating granulomatous interstitial nephritis and non-caseating granulomatous inflammation of liver were detected. Urinary calcium/creatinine ratio and serum angiotensin-converting enzyme levels were normal. Tuberculin skin test was anergic, PCR for *Mycobacteria* of the liver tissue sample, and QuantiFERON test were negative. Since the patient was treated with ETN, we considered drug-induced sarcoidosis, and we discontinued ETN. Oral prednisolone was given and tapered. MTX was continued. Involvement of bilateral proximal interphalangeal joints and distal interphalangeal joints, boggy synovitis, and camptodactyly developed in the following year.

We reevaluated all clinical signs, and *NOD2* gene analysis was performed. M513T heterozygous mutation was detected. There was consanguinity, but no family history. Thus, we diagnosed EOS and added infliximab (IFX) treatment. In the last three years, the disease activations occurred as arthritis, interstitial lung disease, interstitial nephritis, and bilateral panuveitis. Systemic steroids were added on attack periods, MTX, and IFX were continued. She was admitted to our clinic with a headache and vomiting in June 2018. These complaints were present for two weeks and were increasing, especially in the morning. She was still on MTX (15 mg/week), prednisolone (10 mg/day), and IFX (200 mg per month) treatment. High fever, high blood pressure, lethargy, diplopia, and bilateral papilledema were detected on physical examination. Laboratory examination revealed leukocytosis, increased erythrocyte sedimentation rate and C-reactive protein, and pyuria. *Escherichia coli* was isolated in the urine culture. Antibiotic treatment was started for urinary tract infection, and then fever resolved. Brain magnetic resonance imaging (MRI) and MRI angiography were normal. The patient underwent repeated lumbar punctures; opening pressures were 23 cm H_2O and 38 cm H_2O, respectively. Study of cerebrospinal fluid (CSF) revealed pleocytosis (120 leukocyte / mm^3 with lymphocyte predominance), slightly elevated protein level (35 mg/dl), normal chloride, and glucose level. CSF cultures were negative. The serology for viruses and *Mycobacterium tuberculosis* were negative. Electroencephalography (EEG) showed delta brush waves compatible in the temporal-occipital and the parietal-occipital regions. The patient was diagnosed as autoimmune encephalitis. Cerebrospinal fluid limbic encephalitis antibody panel that included NMDA, AMPA1, AMPA2, CASPR2, LGI1, GABA B antibodies were negative. Anti-nuclear antibody (ANA), ANA subgroups, anti-phospholipid antibodies, anti-neutrophil cytoplasmic antibodies, and direct Coombs test were negative. Although intravenous immunoglobulin was given at a dose of 400 mg/kg/day for 5 days, lethargy, diplopia, and papilledema were not resolved. Following pulse methylprednisolone (1 g/day for 5 days) her neurological and ophthalmologic findings improved rapidly (Fig. 1). MTX, prednisolone (40 mg/day) were continued, but IFX was switched to adalimumab.

The written consent form for publishing was obtained from the patient's parents.

Discussion

We reported an EOS patient with joint involvement, granulomatous inflammation, uveitis, who developed encephalopathy in the



ADA: Adalimumab, DIF: Distal interphalangeal, ETN: Etanercept, IVIG: Intravenous immunoglobulin, IFX: Infliximab, MTX: methotrexate, PIF: Proximal interphalangeal, PMP: Pulse methylprednisolone, TOC: Tocilizumab

Fig. 1. Diagram of the patient's course of disease and treatments.

follow-up. Our patient was diagnosed as JIA and received JIA treatment at first because her initial finding was only arthritis. While the patient was receiving ETN and MTX, liver and kidney involvement with granulomatous inflammation in the biopsy was identified. After tuberculosis was excluded, we diagnosed drug-induced sarcoidosis as the development of sarcoidosis in patients treated with ETN has been demonstrated in previous studies.[6] However, later, the diagnosis was reconsidered, because the patient's joint involvements in follow-up were boggy synovitis and camptodactyly. It is known that common joint involvements of BS/EOS are camptodactyly and tenosynovitis.[7,8] Thus, we requested a genetic analysis. M513T heterozygous mutation was detected in the NOD2 gene. NOD2 gene localized in 16q12 chromosome encodes NOD2 protein. NOD2 protein, one of the pattern recognition receptors, is activated by muramyl dipeptide.[9,10] It is involved in response to bacterial infections. NOD2 protein comprises of caspase activation and recruitment domain, NOD, leucine-rich repeat domains (LRRs). While mutations in LRRs lead to Crohn's disease, BS/EOS has associated with mutations in NOD.[11-14] More than 80% of patients with BS/EOS had R334Q/W mutation. M513T is a rare NOD mutation and seen only in EOS.[4,15] Mutations in the domain of M513T inhibit folding, so the protein constantly remains in an active state. Active NOD2 protein has initiated pathways of NF-κB and stress kinase and has played a role in inflammation.[16] In the functional analysis in EOS patients with M513T mutation, NF-κB activation increased compared to wild type.[17,18] NF-κB activation is hypothesized to be responsible for granulomatous inflammation[19], also, which was detected in our patient's biopsy. But we did not perform functional analysis. The clinical findings of our patient were consistent with BS/EOS, and sporadic NOD2 mutation strongly supported the diagnosis of EOS.

The classical triad of BS/EOS is rash, uveitis, and arthritis.[1,2,20] When our patient was diagnosed with BS/EOS, there was only arthritis from the classic triad and granulomatous inflammation in the biopsy. Uveitis, another component of the triad, developed in our patient under treatment. Similar EOS cases with uveitis developing under treatment were reported.[21]

Encephalopathy was a remarkable event because neurologic involvement was infrequent in BS/EOS. Neurologic involvement of EOS, central nervous system infections, and posterior reversible encephalopathy syndrome (PRES) were considered in the differential diagnosis. Hypertension is a common cause of PRES.[22] But, in our patient, high blood pressure was seen in first examination and did not persist. Also, brain MRI was not compatible with PRES. Fever and elevated acute phase reactants were suggestive for infections and EOS activation; we considered urinary tract infection as the cause of fever as it resolved following treatment for urinary tract infection. In addition, CSF examination had no significant findings for central nervous system infection. EOS reports were not informative about CSF findings in neurologic involvement. But, in neurosarcoidosis, raised protein and lymphocytosis were detected.[23] Therefore, we thought that CSF findings in our patient could be due to EOS. Reported neurological involvements of EOS were cranial neuropathy, papilledema, and steroid-sensitive sensorineural hearing loss.[15,24-26] Emaminia et al.[27] presented a BS patient who had seizures, with normal neurologic examination, cranial MRI, and EEG. They could not verify that seizures were associated with BS. In another case, seizure was detected with NOD2 mutation. This case had left middle cerebral artery and bilateral renal artery stenosis was regarded as Takayasu arteritis.[28] EOS patients with M513T mutation reported in the literature had a fever, rash, arthritis, and uveitis. Also, renal calcification and erythema nodosum were seen except for the classical triad. However, there were no neurologic abnormalities in these patients with M513T mutation.[17,18]

We detected delta brush waves in EEG. There are case reports and case series with a delta brush pattern in patients with autoimmune encephalitis.[24,25] However, Baykan et al.[29] showed that this pattern was seen for various reasons in patients followed in intensive care unit. The detection of specific autoantibodies helps in the definitive diagnosis of autoimmune encephalitis and the differential diagnosis of atypical cases. The absence of autoantibodies does not exclude autoimmune encephalitis, and the positive titer does not always imply an accurate diagnosis.[30]

In a study, NOD2-deficient mice and wild type mice were immunized with muramyl-dipeptide and human serum albumin. Then the researchers assessed the antigen-specific serum immunoglobulins. Human serum albumin specific antigens were detected in wild-type mice but not in NOD2 -/- type mice. These results suggested that NOD2 activates the adaptive immune system by acting as an adjuvant receptor for antibody production directly or by increasing the production of immune-stimulatory molecules.[31] Shaw et al.[32] showed that resistant autoimmune encephalitis dependent on peptidoglycan in NOD1 -/-, NOD2 -/-, RIPK2 -/- mice. The authors also reported that the RIP2 signaling pathway taken part in the self-activation of the central nervous system infiltrated dendritic cells, not in the migration of T cells and dendritic cells. Our patient did not reveal any autoantibodies. Although our patient's clinical and EEG findings were compatible with autoimmune encephalitis, we could not be sure whether the autoimmune mechanism works in our patient or disease affect itself.

In conclusion, central nervous system involvement can be seen as encephalitis apart from classical findings of BS/EOS. We think that with the clarification of immune mechanisms in the pathogenesis of the disease, we can understand the cause of autoimmune encephalitis in our patient.

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Case Report

The role of plasma exchange in acute liver failure of autoimmune etiology

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ABSTRACT

Background. Autoimmune hepatitis (AIH) is characterized by increased immunoglobulin G (IgG) levels, the presence of autoantibodies, and various degrees of lymphocyte predominant inflammation and fibrosis histologically. Immunosuppressive therapy induces remission in approximately 80% of those affected. However, liver transplantation is indicated in patients with acute liver failure with encephalopathy at presentation. Liver supporting systems, including plasma exchange (PE) allow bridging patients to transplantation or spontaneous recovery in the setting of liver failure. The role of these systems has not been assessed in children with liver failure of autoimmune etiology.

Case. Herein, we report three cases of AIH with fulminant presentation, with marked symptom resolution with PE as an adjunct therapeutic option to immunosuppressive treatment.

Conclusion. In the setting of AIH, PE may have a special therapeutic role by removing autoantibodies and cytokines, therefore preventing further liver damage and decompensation, and allowing time for recovery

Key words: children, plasma exchange, treatment, autoimmune hepatitis.

Autoimmune hepatitis (AIH) is an immunologically mediated inflammatory liver disorder of unknown etiology. It is characterized by increased immunoglobulin G (IgG) levels, the presence of autoantibodies, and interface hepatitis described as dense inflammatory infiltrate composed of lymphocytes and plasma cells, which crosses the limiting plate and invades the surrounding parenchyma and fibrosis histologically. AIH is particularly aggressive in children and progresses rapidly unless immunosuppressive treatment is started promptly. The mode of AIH presentation includes acute hepatitis, chronic liver disease and its complications, incidental finding of raised transaminases, insidious onset characterized by nonspecific symptoms and fulminant AIH.[1] Pediatric acute liver failure is described as biochemical evidence of acute liver injury, and hepatic-based coagulopathy defined as a prothrombin time (PT) ≥ 15 seconds or international normalized ratio (INR) ≥ 1.5 not corrected by Vitamin K in the presence of clinical encephalopathy or a PT ≥20 seconds or INR ≥2.0 regardless of the presence or absence of encephalopathy.[2] It is seen in approximately 3% of patients with type-1 AIH and 25% of patients with type-2 AIH. With appropriate treatment, 80% of patients achieve remission and long-term survival. However, liver transplantation is indicated in patients who present with fulminant hepatic failure (with encephalopathy) unresponsive to steroid treatment and those who develop end-stage liver disease.[1,3] Liver supporting systems, including plasma exchange (PE) allow bridging patients to transplantation

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or spontaneous recovery in the setting of liver failure.[4] The role of these systems has not been assessed in children with liver failure of autoimmune etiology. Herein, we report three cases of AIH with fulminant presentation, with marked symptom resolution with PE adjunct to immunosuppressive treatment.

Case 1

A 6-year-old girl was admitted to the hospital with jaundice for the previous 6 days. There was no history of a particular illness or drug use. Family history was unremarkable. She was icteric, with slight hepatomegaly. Laboratory findings were aspartate aminotransferase (AST) 1314 IU/L, alanine aminotransferase (ALT) 837 IU/L, total bilirubin (TB) 28.17 mg/dl, direct bilirubin (DB) 24.31 mg/dl, INR 3.65 (unresponsive to vitamin K) and ammonia 57 mg/dl. Further investigations revealed IgG 4245.7 mg/dl (633-1280), ceruloplasmin 49.4 mg/dl, and a positive antinuclear antibody (ANA), titer ≥1/100. Viral serologies of Hepatitis A, B, C, E, Epstein-Barr Virus (EBV), and cytomegalovirus (CMV) were all negative. She rapidly progressed to grade 3 encephalopathy under prednisolone (2 mg/kg/day) and PE was started. After three daily sessions of PE, the patient was non-encephalopathic. After the second week of prednisolone treatment, azathioprine was added. Liver biopsy performed one month after hospitalization demonstrated minimal portal inflammation, giant cell formation, cholestasis, and grade 2 to 3 portal fibrosis. At the end of one month, the patient was tolerating the azathiopurine and the prednisolone dosage was tapered. She is in complete remission longer than 2 years with no recurrence (Table I).

Case 2

A 10-year-old boy presented with abdominal pain and jaundice. Laboratory tests revealed elevated transaminase levels. INR was 1.96 (unresponsive to vitamin K). Viral serologies were negative for Hepatitis A, B, C, EBV, and CMV. The anti-smooth muscle antibody (ASMA) test was positive (titer >1/160), and the serum IgG value was 4745 mg/dl (608-1572). A liver biopsy was not performed owing to coagulopathy. Prednisolone was started at a dosage of 2 mg/kg/day. On the 4th day of prednisolone administration, the patient developed grade 2 encephalopathy and the INR was 2.1. After three PE sessions, the patient showed improvement. Liver biopsy performed on the 20th day of prednisolone treatment demonstrated resolution of acute hepatitis, pericentral and periportal bridging fibrosis and necrosis along with portal mononuclear dominant mixed inflammatory cell infiltration. These biopsy findings under immunosuppressive treatment together with clinical and laboratory findings were supporting the diagnosis of AIH. He

Table I. The course of ALT, bilirubin and INR values.

	Days	Initial*	3	7	15	30	60	90
	ALT (IU/L)	711	316	225	513	197	99	87
Case 1	T. bilirubin (mg/dl)	28.11	9.85	11.30	12.30	3.90	1.30	0.92
	INR	3.67	2.23	2.34	1.71	1.32	1,27	1.21
	ALT (IU/L)	501	60	96	63	92	34	31
Case 2	T. bilirubin (mg/dl)	4.00	1.42	1.34	1.00	0.98	0.910	1.15
	INR	2.1	1.48	1.64	1.4	1.18	1.07	1.33
	ALT (IU/L)	405	120	318	351	253	47	56
Case 3	T. bilirubin (mg/dl)	17.60	13.40	10.9	7.9	4.3	0.8	0.3
	INR	3.51	2.02	2.49	1.91	1.24	1.26	1.13

*Day 0; first day of plasmapheresis
ALT: alanine aminotransferase, INR: international normalized ratio.

was discharged on prednisolone treatment and all laboratory findings were normalized by the second month of treatment. He is now in complete remission without any recurrence longer than 3 years under low dose methyl-prednisolon and azathiopurine (Table I).

Case 3

A 2-year-old girl was referred to the hospital with grade 2 encephalopathy. She had a three-month history of jaundice. Physical examination showed slight hepatomegaly and splenomegaly. Laboratory findings at presentation were as follows: AST 427 IU/L, ALT 412 IU/L, TB 15.43 mg/dl, DB 12.23 mg/dl, INR 3.51, and ammonia 187 mg/dl. Viral, toxic, and metabolic causes were excluded. IG value was 1888 mg/dl (453-916). She tested positive for ANA (titer 1/160) and ASMA (titer 1/80). Prednisolone and PE treatments were commenced immediately. After three daily sessions of PE, she was non-encephalopathic. Despite the correction of the INR values, transaminases did not decrease and cyclosporine therapy (PO) was started. Liver biopsy demonstrated resolution of acute hepatitis along with slight portal mononuclear cell infiltration and pericentral and periportal fibrosis. Transaminase levels were declining and the INR value was completely normalized at the end of the first month following admission, while she was on prednisolone and cyclosporine. By the 3rd month of presentation, she was under the cyclosporine maintenance treatment. Unfortunately, she died of severe pneumonia and sepsis 6 months later while she was in complete remission (Table I).

Discussion

The mainstay of treatment in AIH is immunosuppressive therapy and should be instituted promptly to avoid progression to cirrhosis. The conventional treatment consists of prednisolone 2 mg/kg/day, which is gradually tapered in due course within 4-8 weeks, in parallel to decline in transaminase levels, to a maintenance dose of 2.5 to 5 mg/day.[5,6] In the pediatric age, remission is defined as complete clinical recovery with transaminase levels within the normal range and is achieved in 60% to 90% of patients, except fulminant presentation with encephalopaty.[3] The management of AIH with fulminant presentation is controversial. Steroid treatment in fulminant patients is considered to be of little benefit and increase septic complication in adults.[7] On the other hand, recovery was reported in 4 out of 9 children referred to the transplant center while the remaining 5 required liver transplant despite steroids.[8] Smolka at al. reported complete response in all 6 children presenting with AIH with encephalopathy.[9] In a report from India, 2 out of 4 children with fulminant AIH responded to steroid treatment, the other 2 died due to progressive encephalopathy and infectious complications.[10] Karakoyun et al.[11] reported AIH with encephalopathy in 2 (4.3%) out of 44 patients who did not respond to steroid treatment and underwent living-related liver transplantation.

In the setting of acute liver failure, decompensation of liver function results in decreased biotransformation and excretion of toxic substances as well as synthetic functions. Most liver support systems are based on detoxification of water-soluble (ammonia, lactate, urea, GABA, amino acids, and cytokines) or albumin-bound (bile acids, bilirubin, free fatty acids, aromatic amino acids, indoles, phenols, mercaptans, and endogenous benzodiazepines) toxins. PE removes toxic substances and replaces essential substances by separating the patient's plasma from formed elements and replacing it with the same amount of fresh frozen plasma.[12] High volume PE is considered as first line therapy in the setting of liver failure in adults, either as primary stand-alone treatment or in conjunction with other modes of treatment.[13] Although PE is not recommended as standard of care for children with liver failure, it can be valuable as a temporizing measure because it rapidly removes large amounts of copper in patients with liver failure due to Wilson disease. Few studies of liver support systems

have focused on pediatric patients and are mainly case series and retrospective studies. Most case series in children or adults with acute liver failure suggests that PE might improve coagulation profiles, vasopressor requirements, and encephalopathy grade scores.[14-16] It can significantly decrease mortality in acute or acute-on-chronic liver failure by temporarily supporting liver functions until functional recovery or liver transplantation.[17,18] On the other hand, Chien et al.[19] reported in a case series of 23 pediatric patients that plasma exchange for more than six times probably offers little benefit with regard to patient survival in the absence of a timely liver transplant. In the present study, the first 2 cases developed encephalopathy under steroid treatment. The third case was already encephalopathic at admission. After three daily sessions of PE, all cases were clinically non-encephalopathic, coagulation profiles and liver biochemistry improved and did not progress to encephalopathy in due course. Sogo et al.[20] recently reported 4 children that presented with fulminant hepatic failure all survived by PE therapy initiated together with steroid and cyclosporine treatment. The data is limited regarding the role of PE in children with fulminant AIH. Moreover, no such data are available in adults with fulminant AIH refractory to medical treatment but improved by plasmapheresis. Dumortier et al.[21] are reported a case of a liver transplanted adult female patient who presented a severe de novo AIH, refractory to tacrolimus, mycophenolate mofetil and steroids, and who was treated with plasmapheresis. Only one case presenting with severe AIH-systemic lupus erythematosus overlap despite massive corticosteroid administration, and who improved with plasmapheresis therapy, has been reported.[22]

Similar to the other liver supporting systems, PE allows bridging patients with acute liver failure to transplantation or spontaneous recovery. In the setting of AIH, however, it may have a special therapeutic role as an adjunct to immunosuppressive treatment by removing autoantibodies and cytokines, therefore preventing further liver damage and decompensation, and allowing time for recovery. However, the data is limited and future well-designed studies are needed exploring the role of PE in fulminant AIH.

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Case report: early onset narcolepsy initially misdiagnosed as obstructive sleep apnea syndrome

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ABSTRACT

Background. Narcolepsy is a chronic neurological syndrome, which is characterized by excessive sleepiness, cataplexy, hypnagogic hallucinations, and sleep paralysis during the rapid eye movement period of sleep. This disease is commonly diagnosed within adulthood. However, the first symptoms often appear in childhood and/or adolescence. Pediatric cases of narcolepsy generally remain unrecognized and undiagnosed. Clinical heterogeneity, prolonged onset and diverse symptoms contribute to the delay in diagnosis and treatment in childhood.

Case. This report describes a case of narcolepsy in an 8,5-year-old male patient who was misdiagnosed as obstructive sleep apnea syndrome and many other diagnoses at different hospitals over a period of 3 years before the correct diagnosis was made.

Conclusions. Narcolepsy in children is a rare neurological syndrome, which can occur with uncommon and atypical clinical presentations. In our case report we aimed to highlight pediatric narcolepsy, which could help to make more appropriate approaches and prevent misdiagnoses or diagnosis delay in these cases.

Key words: narcolepsy, polysomnography, multiple sleep latency test, misdiagnosis.

Narcolepsy is a rare chronic neurodegenerative disease caused by autoimmune destruction of hypocretin (orexin) producing neurons in the lateral hypothalamus. It's characterized by chronic excessive daytime sleepiness and cataplexy; which is loss of muscle tone that is typically triggered by strong emotional stimuli. Hypnagogic or hypnopompic hallucinations, behavioral changes, sleep paralysis, vivid dreams and frequent nocturnal awakening, weight gain, and cognitive impairment are less commonly associated symptoms.[1] Usually, patients experience some, but not all of these symptoms; only 10% of individuals with narcolepsy have the four cardinal symptoms of narcolepsy all together (paroxysmal sleep, cataplexy, hypnagogic hallucination, and sleep paralysis).[1]

Even the exact etiology of narcolepsy is still unclear, multiple genetic and environmental factors may underlie the mechanism of hypocretin neuron loss, through autoimmune processes. After the adjuvanted pandemic influenza A (H1N1) in 2009, there was a significant increase in narcolepsy prevalence within both infected and vaccinated populations.[2] Many studies showed evidence of autoimmune and molecular mechanisms associated with flu antigens, modulated by genetic factors, which can trigger narcolepsy.[3]

The clinical heterogeneity and relative rarity of narcolepsy in childhood often leads to a misdiagnosis of narcolepsy as depression, epilepsy, and other disorders. Misdiagnosis of children with narcolepsy can interfere with their normal growth and puts them at increased risk of life-threatening accidents.

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We will present a case report of early onset pediatric narcolepsy, with a history of multiple previous misdiagnoses over a period of 3 years till the proper diagnosis and treatment were made in Ağrı State Hospital's child psychiatry and pediatric neurology clinics.

Case Report

Our patient was an 8,5-year old male, who was brought to our outpatient clinic by his parents due to excessive daytime sleepiness with no gross nocturnal symptoms.

According to his family; the patient's complaints had started at the age of 5 and they first sought medical advice after 6 months of the symptom's onset. Initially, they reported low energy levels, desire to sleep, and loss of interest in daily activities. Sudden and recurrent episodes of sleep started over time, gradually increasing to 2-3 times per day, with about 30 minutes as duration for every episode. Once his family tried to wake him up during these sleep attacks, the patient became irritable and agitated.

According to his medical history, a wide range of tests and examinations were conducted at three different hospitals over a period of 3 years, and many diagnoses were considered, like iron deficiency anemia and depression.

Finally, through only Polysomnography (apnea-hypopnea index was found as 13.9), he was diagnosed with severe obstructive sleep apnea syndrome due to adenoid hypertrophy and referred to an ear, nose, throat (ENT) clinic, where tonsillectomy and adenoidectomy were performed in 2017. According to his parents, after the operation his symptoms gradually worsened and sleep attacks increased. Over the course of time, he became more irritable, forgetful, less active, and started to gain weight despite having a normal diet.

His father reported similar symptoms during his childhood, which spontaneously stopped at the age of 14 without receiving any medical advice. Laboratory method insufficiency prevented the autoimmune workup evaluation for the father. The patient was 142cm tall (>90th percentile) and weighed 38 kg (>90th percentile). On mental status examination, he was irritable and had difficulty concentrating but was fully conscious and orientated. His behavior was appropriate and well-coordinated with normal intelligence and insight appropriate for his age. During the examination, he had slurred speech and on occasion his eyes closed with no response to loud noises.

Blood tests (including CBC, blood chemistry, iron, ferritin, vitamin D and B12), chest radiograph, and electrocardiogram were normal. Cranial CT/MRI performed to rule out intracranial pathologies (like encephalitis and multiple sclerosis) were also normal. No epileptiform discharges were found in the electroencephalogram (EEG).

The overnight polysomnography (PSG) showed an increased number of awakenings, with a total of 7 minutes of wakefulness after sleep onset. The latency to sleep onset was 5.1 minutes, and rapid eye movement (REM) sleep latency was 4.5 minutes, without sleep apnea or periodic limb movements (Table I). After getting enough sleep (≥6h), the daytime Multiple Sleep Latency Test (MSLT) revealed severe daytime sleepiness with sleep onset REM periods (SOREM) in all 5 naps on the MSLT (Table II).

Autoimmune work up including human leukocyte antigen (HLA) typing was not done to confirm the diagnosis (the proper method was

Table I. Sleep study results.

Total sleep time	451 min
Wake after sleep onset	7 min
Sleep onset	5.1 min
Sleep efficiency	97.4%
Number of awakenings	10 min
NREM 1	5.1 min
NREM 2	7.1 min
NREM 3	25.6 min
REM Latency	4.5 min
Apnea-hypopnea index	0.4

Table II. Mean sleep latency test (MSLT) results.
MSLT/Nap Sleep Data

Start time	Stop time	Sleep latency (min)	REM latency (min)	Total sleep time (min)
08:01	08:17	0.5	1.5	16
10:00	10:17	1.0	0.5	15.5
12:08	12:26	0.0	2.0	17.5
14:01	14:18	0.0	5.5	17.5
15:48	16:09	2.5	5.5	19
	Average:	0.8	3	17.1

not available in our facility's laboratory), but the diagnosis of narcolepsy without cataplexy (type 2) was obvious based on medical history, PSG, and MSLT results.

With written consent of his parents the patient was started on modafinil 50 mg/day (stimulant agent), his symptoms resolved, and his diurnal sleep was significantly reduced. No significant difficulties in his daily life or school attendance remained.

Discussion

Narcolepsy is a lifelong disorder that most commonly begins in the first or second decade of life. About one-third of patients become symptomatic at the age of 15 years, and up to 5% of cases begin before the age of five years.[2] Our patient's symptoms started at the age of 5 years.

In a retrospective study, the time between symptoms onset and diagnosis in patients with narcolepsy was found to be about 10 years on average.[4] In our case this duration was about 3 years.

The prevalence of narcolepsy in the general population varies between 20 to 50 cases per 100,000 across different studies with a bimodal peak for age at onset (15 years and 35 years) with no detectable male-female differences.[2] A study estimated the incidence rate in children aged 5-19 years as 0.83/100,000 person-years in Europe.[5] In an American study the overall incidence of narcolepsy was found as 0.74 in 100,000. The incidence rates were found variable among different age groups, with highest incidence rate in the second decade (3.84 per 100,000), followed by the 3rd and 4th decades, and the lowest incidence rate was found in the 1st decade of age.[6]

Narcolepsy is classified as type 1 (narcolepsy with cataplexy) or type 2 (narcolepsy without cataplexy). Reduction in the numbers of hypocretin-producing neurons, and low levels of hypocretin-A in the cerebrospinal fluid (CSF) was founded in about 90% of cases of type 1.[7] The cause of narcolepsy type 2 is unknown, although only 20-30% of these patients have low CSF orexin-A levels.[7] This disorder may result from less extensive loss of the orexin neurons, about half of these individuals may later develop cataplexy, suggesting progression of the disease.[8]

Hypocretin is an excitatory neuropeptide transmitter, which plays a role in promoting wakefulness, energy consumption and food intake, by modulating serotonin, histamine, and other neurotransmitters.[2] The loss of hypocretin producing neurons occurs in narcolepsy is secondary and specific to patients with HLA-DQB1*0602 allele, which may act as an antigen presenter to the T cell receptors. Most narcolepsy type-1 patients (76-98%) and 40-60% of patients with narcolepsy type-2 are positive for HLA DQB1*0602 allele.[2]

Compared to normal the population, the risk of narcolepsy in patient's first-degree relatives is increased by 10-40 folds (1-2%) which suggests a genetic predisposition to narcolepsy.[9] Even though our patient's father reported similar

symptoms during his childhood, his diagnoses was not clear.

The exact underlying mechanism of hypocretin neurons loss is unknown, but recent studies suggest that autoimmune-mediated mechanisms may cause this loss, with major roles of both environmental and genetic factors.[3] Multiple environmental factors are considered as possible triggers for narcolepsy, including upper respiratory tract infections, influenza, neurotoxic metals, major psychological stress, hormonal changes during puberty and smoking.[10-12]

Auto-antibodies against Tribbles Homolog 2 (TRIB2) were appointed in narcolepsy patients, supporting the autoimmune hypothesis.[13] Also, elevated anti-streptolysin O (ASO) antibodies and anti-DNAase B (ADB) antibodies in patients with narcolepsy were found, suggesting an autoimmune process triggered by streptococcal infection.[14]

This underlying mechanism, can be triggered by other infections too, such as in H1N1. Many recent studies have reported increased cases of narcolepsy in Europe and Asia after the H1N1 pandemic, especially in children and adolescents. This increment seems to be related to influenza infection itself in China, and Pandemrix vaccination in Europe.[5,10,15] A cross immune reaction occurs after flu infection, involving antigens of multiple hypocretin-producing neuron epitopes, and possibly CD8+ T cell cytotoxic killing of hypocretin-producing neurons.[3]

According to the American Academy of Sleep Medicine (2005)[16], symptoms of narcolepsy with cataplexy include: (a) recurrent daytime naps that occur almost daily for at least three months, and (b) sudden loss of postural muscle tone in association with intense emotion (cataplexy). Administering the Multiple Sleep Latency Test (MSLT) after overnight polysomnography (PSG) can assist in the diagnosis of narcolepsy. The presence of one or both of the following confirms the diagnosis: (a) a mean sleep latency of <8 minutes and two or more sleep onset REM periods (SOREMPs) based on MSLT performed after at least six hours of sleep during the previous night. (b) CSF Hypocretin-1 concentration <110 pg/ml.

In our case, MSLT revealed severe daytime sleepiness with SOREM in all 5 naps and mean sleep latency of 0.8 minutes, which confirm the diagnoses of narcolepsy without cataplexy.

The cardinal symptoms of narcolepsy include paroxysmal sleep (100%), cataplexy (70%), hypnagogic hallucination (25%), and sleep paralysis (5%), as in our case usually, patients experience some, but not all, of these four symptoms.[17] About two-thirds of patients experience transient paroxysmal sleep only, and one-third of patients have one of the other three symptoms in addition to paroxysmal sleep.[17]

Obesity and precocious puberty are common in children with narcolepsy, in addition within a few years of diagnosis, approximately two-thirds of these children become overweight or obese, which can cause severe and irreversible secondary health problems in these cases.[18]

At the initial examination our patient's height and weight were found to be over the 90th percentile, which became within normal ranges (50-75 percentile) after 7 months of modafinil treatment. Also, narcolepsy could be secondary to systemic disease or neurological insults targeted at the hypothalamus, such as multiple sclerosis, brain injury, neurocysticercosis, encephalomyelitis, tumors, and cerebrovascular accidents. Carbon monoxide poisoning and congenital disorders like Prader-Willi, myotonic dystrophy, and Neimann-Picks have also been associated with narcolepsy.[19] Secondary narcolepsy was ruled out in our case by running blood tests, EEG and imaging studies.

Due to the prolonged onset and atypical symptoms in children, the diagnosis of narcolepsy could be more difficult and could result in delayed diagnosis and treatment. Associated with immunological mechanisms, there is a potential increased risk of narcolepsy

during or after the Covid-19 pandemic, such as that which occurred in the H1N1 pandemic. This case highlights the wide presentational variety of uncommon psychiatric and neurological conditions of pediatric narcolepsy. After excluding secondary narcolepsy; to rule out the possibility of narcolepsy and to decrease complications and similar misdiagnosis; we recommend conducting sleep monitoring, polysomnography, and multiple sleep latency tests in all cases with excessive sleep of unknown etiology in children. Accurate diagnosis and treatment depend on taking detailed medical histories and being aware of the atypical presentations of uncommon psychiatric/ neurological conditions, particularly in children.

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Case Report

Infantile systemic lupus erythematous presenting as nephrotic syndrome in a 12-month-old boy: a case report

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ABSTRACT

Background. Systemic lupus erythematous (SLE) is extremely rare in infants and has been reported to be a much more severe disease with higher prevalence of critical organ involvement. Herein we present the clinical and laboratory features of infantile SLE (iSLE) with an onset of nephrotic syndrome (NS) during the first year of life.

Case. A 12-month-old boy was suffering from generalized edema for two months. He had thrombocytopenia, hemolytic anemia with positive direct and indirect Coombs tests and proteinuria of nephrotic-range. Other laboratory studies revealed slightly decreased C3, low C1q and normal ANA and C4 levels; anti-phospholipid and anti-cardiolipin antibodies were also found to be negative. Renal biopsy revealed Class IV lupus nephritis. The patient also suffered from massive pulmonary thromboembolism. Complete remission was achieved with steroid, cyclophosphamide, mycophenolate mofetil and anticoagulant therapy.

Conclusion. iSLE should be kept in mind especially in infantile NS with multisystem involvement. Renal biopsy is mandatory for early diagnosis. Although the disease was reported to have poor prognosis, complete remission could be achieved with intensive immunosuppressive therapy.

Key words: infantile lupus, systemic lupus erythematous, lupus nephritis, infantile nephrotic syndrome.

Systemic lupus erythematous (SLE) is a multisystemic auto-immune disease characterized by immune dysregulation and formation of auto-antibodies. The disease has childhood onset in 15-20% of the cases and is uncommon before the age of 5 years.[1] In infants, SLE is extremely rare and has been reported to be a much more severe disease with higher prevalence of critical organ involvement. Herein we present the clinical and laboratory features of infantile SLE (iSLE) with an onset of nephrotic syndrome (NS) during the first year of life.

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Case Report

A 12-month-old male patient was admitted to our hospital with a 2-month history of generalized edema. His mother noticed dark urine for one month. Previously he was a healthy child, born at term with birth a weight of 3,450 gr to non-consanguineous parents and family history did not reveal a significant disease.

On admission, the height and weight of the patient were within normal range. He was pale and blood pressure was measured as 110/70 mmHg. Physical examination revealed anasarca edema including eyelids, face and pretibial regions and ascites (Fig. 1). Laboratory investigations were as follows: BUN 9 mg/dl, creatinine 0.09 mg/dl, creatinine clearance 389 ml/min/1.73m^2, total protein 2.82 g/dl, albumin 1.5 g/dl, LDH 534 U/L, total cholesterol 350 mg/dl, hemoglobin 6.7 g/dl, WBC 18,800/mm^3, platelet 52,000/mm^3 and reticulocyte 6.8%.

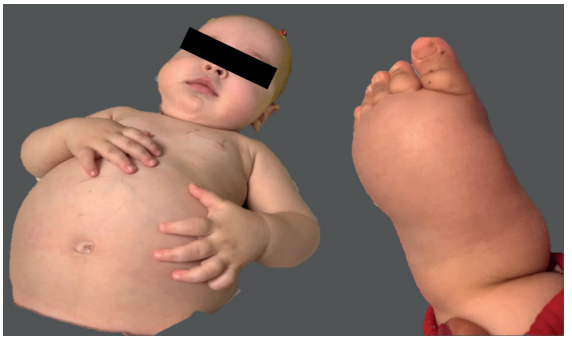

Fig. 1. Pictures of the patient showing anasarca edema and ascites.

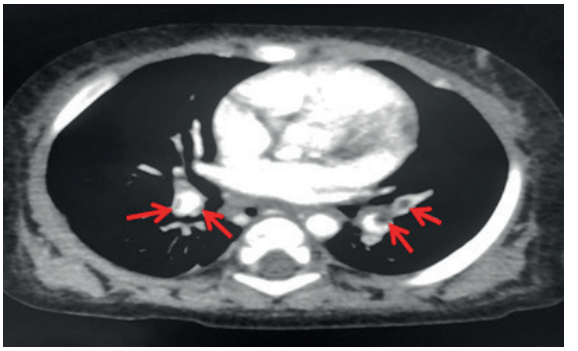

Fig. 2. Pulmonary thromboembolism on computed tomography.

Blood smear was consistent with hemolysis. Direct and indirect Coombs tests were found to be positive. Bone marrow aspiration revealed no evidence of malignancy. On the urinalysis, urine density and protein were 1,028 and 1,000 mg/dl, respectively. Direct microscopy of the urine revealed 545 RBC/hpf and 83 WBC/hpf. Spot urine protein/creatinine ratio and 24-hour proteinuria were calculated as 21.4 mg/mg and 496 mg/m^2/hour, respectively. Serum C3 level was 0.73 g/L (N:0.86-1.79) whereas C4 level 0.30 g/L (N:0.14-0.48).

He was diagnosed as NS with generalized edema, nephrotic range proteinuria, hypoalbuminemia and hypercholesterolemia. Renal ultrasonography (USG) showed an increase in both kidney sizes and parenchymal echogenicities.

ANA, anti-dsDNA, lupus anticoagulants, nuclear auto-antibodies and serologic tests for infectious etiologies including EBV, CMV and syphilis were found to be negative. He had a slightly decreased C3 and normal C4 levels, while C1q levels was found to be low. Anti-phospholipid and anti-cardiolipin antibodies were also found to be negative.

Steroid treatment (8 mg/kg/d) was commenced for autoimmune hemolytic anemia and thrombocytopenia, and tapered to 2 mg/kg/d in 10 days. Echocardiography was normal whereas, thoracoabdominal computed tomography (CT) revealed massive pulmonary thromboembolism (Fig. 2).

Urgent intravenous (IV) heparin infusion was started. Doppler USG showed additional thrombosis on left deep crural veins. Thrombophilia tests revealed heterozygote mutations for factor V Leiden and methylenetetrahydrofolate reductase. Anti-thrombotic therapy was continued with enoxaparin. However, respiratory distress started and tachypnea increased soon after. Oliguria developed and hypertension (HT) persisted. His clinic condition was stabilized with repeated blood transfusions, IV albumin infusions and anti-hypertensive medications. Renal biopsy revealed diffuse podocyte hypertrophy, glomerular basal membrane thickening, endocapillary and mesangial proliferation and "full-house" positivity on immunofluorescence staining (including IgG, IgA, C3, C1q, fibrinogen, lambda and kappa) which is consistent with Class IV lupus nephritis (Fig. 3). Pathological vascular findings were not detected on renal biopsy.

In addition to steroid therapy which was slowly tapered after a month of 2 mg/kg/d dose, cyclophosphamide (CYP) (500 mg/m^2/dose per month for 6 months) was given. At the third month of therapy, he achieved complete remission with spot urine protein/creatinine ratio 0.3 mg/mg, serum albumin 3.5 mg/dl, and normal renal function tests, hemoglobin, platelet and C3 levels. Mycophenolate mofetil (MMF) (1000 mg/m^2/day) was commenced as maintenance therapy, after 6 monthly doses of CYP were completed. Enoxaparin was given

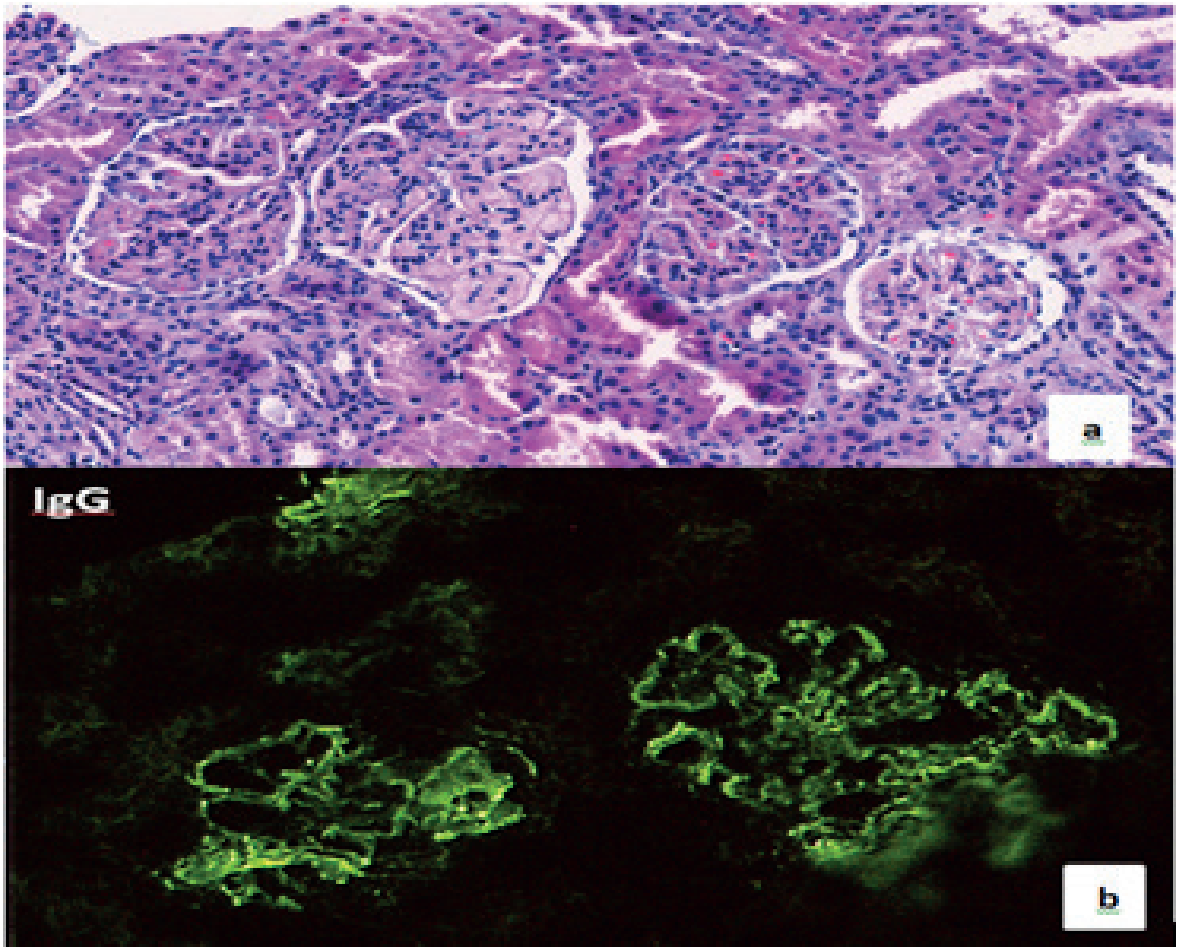

Fig. 3. Renal biopsy specimens: a) endocapillary and mesangial proliferation on light microscopy; b) Ig G depositions on immunofluorescence.

for 6 months and discontinued when control radiologic images (CT and USG) revealed thrombosis-free lungs and extremities.

The last visit of the patient was at the 20th month of the therapy and he is still in complete remission without edema and HT, and with totally normal renal function. Additional genetic work-up for infantile NS (a comprehensive glomerular disease NGS panel) did not reveal any mutations.

Informed consent was obtained from the patient for whom identifying information is included in this article.

Discussion

Our patient presented with infantile NS developing below 1 year of age which is a rare but severe clinical condition that may occur due to primary or secondary causes.[2] In regions where consanguineous marriages are prevalent, like our country, primary infantile NS with genetic or syndromic origin is more common. However, he had Coombs positive autoimmune hemolytic anemia and thrombocytopenia that lead us to further investigate for secondary causes. We performed a renal biopsy as soon as possible which revealed lupus nephritis. In spite of his seronegativity for SLE, according

to SLICC criteria, he was diagnosed as iSLE with his biopsy proven lupus nephritis (LN), autoimmune hemolytic anemia, autoimmune thrombocytopenia and hypocomplementemia.[3] Though full-house immune staining can also occur in other conditions, he did not have any symptoms suggesting other autoimmune diseases like mixed connective tissue disease (no rash, arthritis or myopathy), Sjögren syndrome (no keratoconjuctivitis sicca), hypocomplementemic urticarial vasculitis syndrome (no typical urticarial rash), IgG4-related nephritis, and rheumatoid arthritis (no arthritis).

Infantile lupus with onset before the age of two years is an uncommon disease. It has a more severe disease course as compared with older onset patients. Female predominance that is typical for adult SLE patients is not evident in iSLE with nearly equal incidences in both genders. The interval between the onset of symptoms and diagnosis was found to be shorter and multisystem disease at the time of diagnosis was reported more frequently than older age groups. Noticeably, renal, cardiopulmonary and neurologic involvements have the highest incidence. As a result, iSLE is believed to cause higher morbidity and mortality.[1] In line with the literature, our patient was male and had two important system involvements at the time of the diagnosis. Additionally, pulmonary thromboembolism which is an important complication of NS aggravated his clinical condition and complicated our treatment.

There are several single case reports about iSLE.[4-9] Infantile SLE patients presenting with congenital NS as early as 6 weeks of age was reported and all of them had severe progressive multisystemic involvement.[4,5] Kreindler et al.[6] presented a 1 month of age male infant with pulmonary hemorrhage and glomerulonephritis. As our patient, he needed an aggressive immunotherapy with a combination of prednisone, intermittent CYP and MMF for a complete serological and clinical remission at 30-month follow-up. The possibility of association between iSLE and Epstein-Barr virus (EBV) infection was suggested in a 14-month-old Japanese boy who had biopsy proven lupus nephritis and hepatitis after an EBV infection.[7]

To our knowledge, there is only one case series about iSLE reported by Zulian et al.[10] which included 13 patients with SLE diagnosed in the first year of life. Most common laboratory findings of these patients were anemia, thrombocytopenia and hypocomplementemia which are similar in our patient. Renal disease was the most common organ involvement seen in 92% of their patients and the most frequent renal pathology was World Health Organization class IV LN. Mortality rate was reported to be high (38%) and 62% of the survivors had residual damage.

Lupus nephritis is believed to be one of the most important predictors of morbidity and mortality in iSLE. Class IV LN, younger age, HT, impaired renal function and low C3 levels at the time of diagnosis are reported to be indicators of poor prognosis and low treatment response in LN.[11] While corticosteroids and CYP are the mainstay of induction therapy; azathioprine, MMF, rituximab and tacrolimus are other options which are shown to be efficient.[12] Following the induction phase, all patients with LN should be administered maintenance therapy to sustain remission. Azathioprine and MMF were shown to be superior to CYP as a maintenance drug, with less serious side effects.[11-13] Regarding general approach to treatment of infantile LN, our patient was treated with CYP and high dose steroids in induction phase followed by MMF and low dose steroids as maintenance therapy. Despite having severe clinical and laboratory features at presentation, he achieved remission in the third month of therapy and still in remission with good growth.

Although infantile NS usually develops secondary to genetic and syndromic disorders, rare conditions such as iSLE might be the underlying etiology. The possibility of iSLE should be kept in mind especially in infantile NS patients who have multisystemic involvement.

Renal biopsy is mandatory for early diagnosis. Although the disease was reported to have poor prognosis, complete remission was achieved with intensive therapy. Careful long-term follow up is necessary for early diagnosis of recurrences.

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