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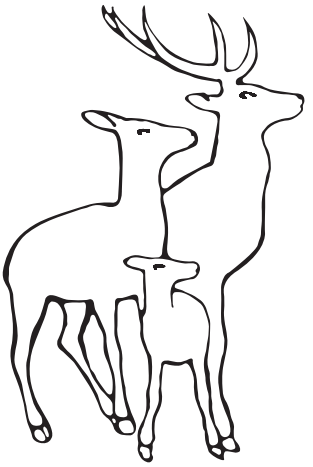

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YAYIN SAHİBİ
Yayınlayan Kurumlar Adına
Murat YURDAKÖK

SORUMLU YAZI İŞLERİ MÜDÜRÜ
Enver HASANOĞLU

DÖKÜMANTASYON
Ali DÜZOVA

YAYINLAYAN
Türkiye Milli Pediatri Derneği,
Hacettepe Üniversitesi Çocuk Sağlığı Enstitüsü
Uluslararası Çocuk Merkezi

EDİTÖR ADRESİ
Çocuk Sağlığı ve Hastalıkları Dergisi
P.K. 36, Samanpazarı 06240 Ankara, Türkiye
Faks: (312) 305 22 64

YAYIN İDARE MERKEZİ
The Turkish Journal of Pediatrics Editör Ofisi
Hacettepe Üniversitesi
İhsan Doğramacı Çocuk Hastanesi
06100 Ankara
Tel : (312) 305 26 76
Faks: (312) 305 22 64

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BASIM YERİ
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Tel: (312) 266 44 10 (Pbx)

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Ali DÜZOVA

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Original Article

Risk factors of intravenous immunoglobulin resistance and coronary arterial lesions in Turkish children with Kawasaki disease

Serkan Türkuçar[1], Kaan Yıldız[2], Ceyhun Acarı[1], Hatice Adıgüzel Dundar[1], Mustafa Kır[2], Erbil Ünsal[1]

Divisions of [1]Rheumatology and [2]Cardiology, Department of Pediatrics, Dokuz Eylül University Faculty of Medicine, İzmir, Turkey.

ABSTRACT

Kawasaki disease (KD) is the most common cause of childhood coronary artery disease. The incidence of coronary artery lesions (CALs) has declined with the routine use of intravenous immunoglobulin (IVIG) treatment, but there is still considerable risk for resistance to IVIG treatment and development of CALs. The present study was aimed to determine the risk factors in Turkish children with IVIG resistant KD and coronary artery involvement. Clinical, laboratory and echocardiographic data were retrospectively analyzed in 94 Kawasaki patients. IVIG resistant and responsive groups were compared. The IVIG resistant group had a higher rate of CALs compared to the IVIG responsive group ($p<0.05$). Duration of fever ≥ 9.5 days, C-reactive protein (CRP) ≥ 88 mg/L and Neutrophil/lymphocyte ratio (NLR) ≥1.69 were the best cutoff values for predicting IVIG resistance before treatment. The criteria for at least two of these three predictors were considered to be statistically significant risk factors for detecting IVIG resistance in KD before treatment (76.47% sensitivity, 71.05% specificity and 95% CI were 50.1-93.19% and 59.51-80.89%, respectively). Based on the clinical and laboratory features, we established a new risk-scoring system for predicting IVIG resistance in a cohort of Turkish children with KD. This may be useful for choosing optimal treatment for KD to prevent coronary artery involvement.

Key words: Kawasaki disease, IVIG resistance, risk score.

Kawasaki disease (KD) is an acute, systemic, febrile vasculitis that occurs during infancy and is the most common cause of childhood coronary artery disease.[1] Although, its etiology has not been definitively determined, recent studies have focused on the increased inflammatory cytokines in KD pathology.[2-4] The clinical features include an existence of fever persisting more than five days with mucocutaneous and lymphatic manifestations that are commonly self-limiting. However, the most serious complication is the coronary artery involvement that can be mortal. With the routine use of intravenous immunoglobulin (IVIG) treatment, the incidence of coronary artery lesions (CALs) has declined from 23% to 8%.[5] On the other hand, some patients are at risk for resistance to IVIG treatment and development of CALs.[6] In Turkey, KD was reported as the second most common type of vasculitis in a nationwide study by Ozen et al.[7], despite the real prevalence being unknown. Previous studies about KD from Turkey reported higher prevalence of coronary arterial involvement than Japanese children based studies.[8-12]

Many studies have previously been conducted about the risk factors of KD, and patients with atypical age presentation, elevated acute phase reactants and liver function tests were reported as high risk.[10-12] The Japanese-based risk scoring systems such as that by Kobayashi, Egami, and Sano was reported as inadequate for fully determining the risks for IVIG resistance and CALs in Western populations living in North

✉ Serkan Türkuçar
serkan_turkucar@hotmail.com

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America, Israel and the UK.[13-15] We had a similar concern, when we tried to estimate the risk of IVIG resistance using the Japanese risk scoring systems for our patients in Turkey. The sensitivity and specificity were 17.65% and 92.11% for Egami[10], 40.00% and 89.66% for Kobayashi[11], and 40.00% and 94.44% for Sano[12], regarding our patients. Also, a recent paper by Yang et al.[16] had a different scoring system for Chinese children with KD. We also evaluated our patients using the related score, and found the sensitivity and specificity as 43.75% and 80.43%, respectively. These scoring systems have limited predictive capacity for IVIG resistance of KD in Turkish children.

The present study was aimed to determine the risk factors in Turkish children with IVIG resistant KD and coronary artery involvement for early effective treatment.

Material and Methods

This study was approved by Dokuz Eylul University Non-Interventional Research Ethics Committee (2018/21-07, 02.08.2018).

Subjects and Definitions of KD: This retrospective study was performed by reviewing the medical records of 94 Kawasaki patients who had received IVIG treatment in a tertiary center between 1996 and 2018. Patients were diagnosed with following six major clinical signs: i) fever persisting for five or more days (≥38.0 °C); ii) bilateral conjunctival congestion; iii) changes of the lips and oral cavity; iv) polymorphous exanthema; v) changes of peripheral extremities; and vi) acute non-purulent cervical lymphadenopathy.[17] Complete KD (cKD) was diagnosed when subjects had at least five of the six clinical signs, and incomplete KD (iKD) was defined as having four or fewer major signs, with or without cardiac lesions.[18] Laboratory findings including leukocytosis, anemia, elevation of erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP), elevated alanine aminotransferase (ALT) levels, and sterile pyuria were considered while diagnosing iKD. According to these definitions, patients were classified as 61 cKD and 33 iKD. Demographic data including age and sex, and clinical information such as the duration of fever before IVIG treatment were noted.

Treatment and Definition of IVIG Resistance

All patients were treated with IVIG (2 gr/kg) and high dose acetyl salicylic acid (80-100 mg/kg/day). The IVIG resistance was defined as persistent fever (≥ 38.0 °C) 48 hours after administration of initial dose of IVIG.[19] Second dose of IVIG was given to IVIG resistant patients, and high dose steroids (IV methyl prednisolone 30 mg/kg dose) were administered in patients who were resistant to recurrent IVIG treatments.

Laboratory Assessment

Hemogram parameters such as white blood cell count (WBC), absolute neutrophil count (Neu), absolute lymphocyte count (Lym), hemoglobin count (Hb), absolute platelet count (Plt), mean platelet volumes (MPV), and acute phase reactants such as CRP and ESR were recorded prior to the IVIG treatment and 48-hours following the treatment. In addition, biochemical parameters such as serum albumin (Alb) and total bilirubin (T-bil), liver function tests (LFT), and electrolyte levels of sodium (Na^+), potassium (K^+) and calcium (Ca^{++}) were noted. Neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratios (PLR) were calculated from the available data. The difference between the assessments related to ESR, CRP and WBC values were recorded as delta values (ΔWBC, ΔESR and ΔCRP).

Echocardiographic Assessment and Definition of CALs

Echocardiographic assessment was performed on all patients at the time of diagnosis and in the subacute phase (two weeks after IVIG). Coronary artery involvement was determined in 31 patients (33%) before initial IVIG treatment. Coronary artery lesions were

listed as perivascular echogenicity, ectasia/dilatation and aneurysm, according to severity. Perivascular echogenicity was defined as echogenicity of pericoronary tissue minus blood pool. Coronary artery ectasia and dilatation were defined if the internal diameter was up to 1.5 times that of the adjacent coronary artery, and as presenting a luminal dilatation up to 3 mm in children under the age of 5 years or 4.0 mm in children 5 years of age or older. Coronary artery aneurysms were defined and classified according to the criteria established by the Japanese Ministry of Health and Welfare guidelines as follows: Small aneurysm: localized dilatation showing an inner diameter ≤ 4 mm (in children ≥5 years: the internal diameter of a segment < 1.5 times compared to an adjacent segment). Medium aneurysm: aneurysm showing an inner diameter > 4 mm and < 8 mm (in children ≥5 years: the internal diameter of a segment 1.5~ 4 times compared to an adjacent segment). Giant aneurysm: aneurysm showing an internal diameter ≥ 8 mm (in children ≥5 years: the internal diameter of a segment > 4 times compared to an adjacent segment).[20] The CALs were sorted according to this severity classification such that perivascular echogenicity 19.35%, ectasia and dilatation in coronary arteries 61.30% and coronary artery aneurysms 19.35%.

Statistical Analysis

Statistical analysis was performed using SPSS 20 software. Chi-square or Fisher's exact test (when expected count was below 5 in any of the cells) was used to compare categorical variables. Shapiro-Wilk test was performed to evaluate homogeneity of the values. Homogeneously distributed values were presented as mean±SD and heterogeneously distributed values were presented as median and interquartile ranges (25%-75%). Homogenous values were estimated by one-way ANOVA and independent T-test, while heterogeneous values were tested by nonparametric tests. Variables having statistically significant differences among groups were evaluated by receiver operating characteristic (ROC) curves to determine the optimal cut-off values, and relevant odds ratios (OR) were calculated. $p<0.05$ was considered as statistically significant.

Results

Of the 94 patients included in the study, 55 (58.5%) were male and 39 (41.5%) were female and the ratio was 1.41. The median age at the time of diagnosis was 35 (19-52)* months. CALs were observed at echocardiographic evaluation in 31 patients (33%). Seventeen patients (18.1%) were IVIG resistant. 61 patients were evaluated as cKD (64.9%) and 33 patients as iKD (35.1%). No statistically significant difference was found between complete and iKD groups in terms of age, gender, laboratory and echocardiographic data (Table I).

Risk Factors and Predictive Tools for IVIG Resistance

The IVIG resistant group had a higher duration of fever before treatment, and a higher rate of CALs compared to the IVIG responsive group ($p<0.05$). There was no significant difference in the distribution of severity of coronary artery lesions between groups (Table I).

When two groups were compared prior to IVIG, neutrophil/lymphocyte ratio (NLR) and CRP parameters were statistically higher in the IVIG resistant group. Also in the same group, other inflammatory markers such as ESR, and Neu count were found to be higher, and Hb and Lym count values were lower than the IVIG responsive group ($p>0.05$). In further evaluations two days following initial IVIG administration, NLR values were still found to be statistically higher in the IVIG-resistant group; while the Lym count, Na, K, and Ca values were statistically lower (Table II). There was no significant difference between the two groups in terms of delta values including ΔWBC, ΔESR and ΔCRP.

Table I. Comparison of demographic, clinic and echocardiographic data between IVIG responsive and IVIG resistant groups.

	Parameters	IVIG responsive n= 77 (81.9%)	IVIG resistant n=17 (18.1%)	p value
Age	months	34 (21-54) *	36 (12-49) *	0.426
Gender	male/female	1.40	1.42	0.572
Clinic Type	(complete/incomplete)	1.75	2.4	0.466
Duration of fever before IVIG	days	7 (6-10) *	10 (7-20) *	0.039
CALs		21 (45.7%)	10 (54.4%)	0.009
Perivascular echogenicity		4 (5.2%)	2 (11.8%)	0.208
Coronary ectasia /dilatation		13 (35.3%)	6 (16.9%)	0.191
Aneurism		4 (5.2%)	2 (11.8%)	0.208

p<0.05, *median (25%-75%)
IVIG: intravenous immunglobulin, CALs: coronary artery lesions

The predictive value of the variables before initial IVIG treatment revealed duration of fever (p=0.039), CRP (p=0.017) and NLR (p=0.029) as independent predictors of resistance to treatment for KD. ROC curves applied to each variable revealed cut-off values as; duration of fever≥9.5 days, CRP≥88 mg/L and NLR ≥1.69. The criteria for at least two of the three predictors were considered to be statistically significant risk factors for detecting IVIG resistance in KD before treatment (76.47% sensitivity, 71.05% specificity and 95% confidence intervals were 50.1-93.19% and 59.51-80.89%, respectively). The predictive values and OR of each parameters are shown in Table III, and related ROC-curve is presented in Figure 1.

Risk Factors and Predictive Values of Coronary Arterial Lesions

Regarding demographic-clinical data and echocardiographic findings, increased duration of fever prior to IVIG treatment, and IVIG resistance were found as significant determiners in patients with CALs (p <0.05) (Table IV).

Regarding the laboratory parameters, no statistically significant risk factor for coronary artery involvement was detected prior to IVIG treatment. On the other hand, after first IVIG treatment, lower hemoglobin and higher Plt were determined to increase the risk of CALs (p<0.05) (mean Hb count was 10.2±1.5 gr/dL and median Plt count was 583 (409-706) x10^3/uL in

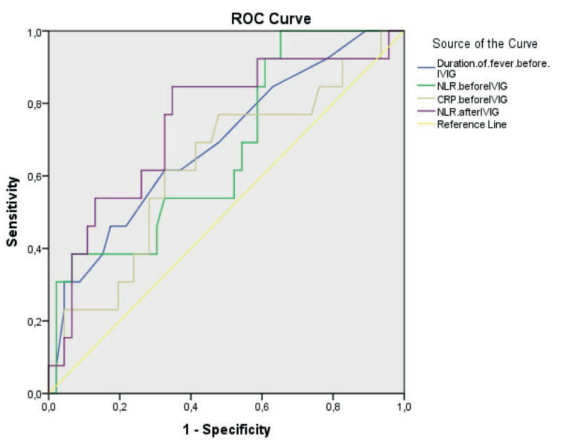

Fig. 1. ROC-curve of the factors for predicting IVIG resistance.

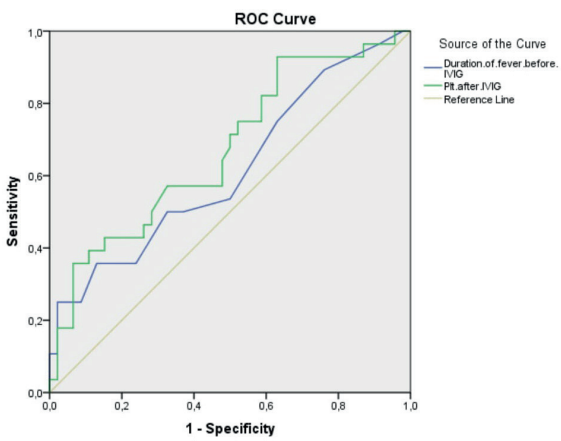

Fig. 2. ROC-curve of the factors for predicting CALs.

Table II. Comparison of laboratory values between IVIG responsive and IVIG resistant groups. Evaluation of before and after IVIG values.

Parameters		Before IVIG			2 Days After IVIG		
		IVIG response n= 77 (81.9%)	IVIG resistant n=17 (18.1%)	P value	IVIG response n= 77 (81.9%)	IVIG resistant n=17 (18.1%)	P value
Hb	g/dL	11.2 ±1.5**	10.6 ±1.3**	0.109	10.98 ±1.29**	10.18 ±1.59**	0.047
WBC	10^3/uL	14.75 ±6.15**	15.08 ±5.95**	0.838	10.4 (8.0-12.5) *	11.6 (7.0-15.1) *	0.671
Plt	10^3/uL	395 (325-514) *	352 (284-649) *	0.551	458 (387-641) *	397 (298-502) *	0.064
Neu	10^3/uL	8.2 (4.3-13) *	9.87 (7-15) *	0.065	4.4 (3.3-7.2) *	5.65 (3.80-10.20) *	0.191
Lym	10^3/uL	3.6 (2.6-5.5) *	3.0 (1.6-3.82) *	0.137	4.40 (3.40-6.20) *	3.60 (2.50-4.50) *	0.045
NLR	Neu/Lym	1.69 (0.74-3.32) *	3.0 (2.0-7.13) *	0.029	1.07 (0.53-1.78) *	1.79 (1.27-3.38) *	0.023
PLR	Plt/Lym	101.76 (80.36-157.04) *	122.15 (98.89-361.11) *	0.097	104.56 (78.65-153.18) *	101.32 (80.67-161.36) *	0.774
MPV	fL	7.3 (6.7-8.0) *	7.9 (7.9-8.1) *	0.068	7.3 (6.9-7.7) *	7.7 (7.4-8.3) *	0.197
CRP	mg/L	54.50 (23.30-116.55) *	108.20 (86.0-158.0) *	0.017	13.3 (5.0-25.0) *	25.5 (4.0-84.3) *	0.191
ESH	mm/h	58 (38-82) *	73 (49-102) *	0.155	65.89±39.76**	72 ± 48.16**	0.239
ALT	U/L	25 (15-44) *	31 (24-59) *	0.173	26 (17-37) *	20 (12-121) *	0.954
AST	U/L	30 (21-43) *	25 (17-45) *	0.468	33 (19-42) *	29 (20-59) *	0.440
T. bil	mg/dL	0.32 (0.20-0.61) *	021 (0.20-1.00) *	0.946	0.30 (0.19-0.41) *	0.29 (0.20-0.80) *	0.571
Alb	g/dL	3.8 (3.4-4.1) *	3.7 (3.1-4.2) *	0.525	3.6 (3.4-3.9) *	3.5 (3.2-3.6) *	0.153
Na	mmol/L	137 (135-139) *	135 (132-137) *	0.066	138.04 ± 3.23**	135.64 ± 2.09**	0.012
K	mmol/L	4.47 ± 0.59**	4.48 ± 0.17**	0.428	4.68 ±0.59**	4.00 ± 0.64**	0.001
Ca	mg/dL	9.38 ± 0.07**	9.16 ± 0.17**	0.426	9.44 ± 0.56**	8.99 ± 0.58**	0.023

p <0.05, *Median (25%-75%), **Mean ± Standard deviation
Hb: hemoglobin, WBC: White blood cell, Plt: platelet count, Neu: absolute neutrophil count, Lym absolute lymphocyte count, NLR: neutrophil/lymphocyte ratio, PLR: platelet/lymphocyte ratio, MPV: mean platelet volume, CRP: C reactive protein, ESH: erythrocyte sedimentation rate, AST: aspartate aminotransferase, ALT: alanine aminotransferase, T. bil: total bilirubin, Alb: albumin, Na: sodium, K: potassium, Ca: calcium.

Table III. ROC analyses and odds ratios for best cut-off values of variables for predicting IVIG resistance before treatment.

Variable	Value	Sensitivity	Specificity	Discriminative ability	Odds ratio
Duration of fever before IVIG	9.5 days	58.8%	72.7%	0.667±0.080 (95% CI: 0.510-0.824, p=0.032)	3.809 (95% CI: 1.28-11.31, p=0.016)
CRP before IVIG	88.0 mg/L	70.0%	64.7%	0.669±0.076 (95% CI: 0.520–0.817, p=0.032)	4.40 (95% CI: 1.38-13.97 p=0.012)
NLR before IVIG	1.69	93.3%	43.4%	0.679±0.070 (95% CI: 0.541–0.817, p=0.031)	4.40 (95% CI: 1.38-13.97 p=0.012)

p<0.05
IVIG: intravenous immunglobulin, CRP: C-reactive protein, NLR: neutrophil/lymphocyte ratio

CALs (+) group, and 11.2±1.2 gr/dl and 453 (342-511) x10^3/uL in CALs (-) group, respectively).

The predictive value of the variables revealed duration of fever before initial IVIG treatment (p=0.050) and Plt counts two days after IVIG treatment (p=0.014) as independent predictors of coronary artery involvement. ROC curves applied to each variable revealed cut-off values

Table IV. Comparison of demographic-clinical data and echocardiographic findings.

	Parameters	CALs (-) 67.01% (n=63)	CALs (+) 32.9% (n=31)			p value
			Perivascular echogenicity 19.35% (n=6)	Ectasia/ Dilatation 61.30% (n=19)	Aneurism 19.35% (n=6)	
Age	months	36 (27-57)*	29 (14-40)*	33 (16-54)*	23 (6-42)*	0.367
Gender	m/f	1.07	5	2.16	1	0.337
Clinic	(cKD/iKD)	1.54	2	3.75	5	0.413
Duration of fever before IVIG	days	7 (5-10)*[a]	13 (5-25)*[b]	8 (7-14)*[b]	21 (10-25)*[b]	0.050
IVIG resistance	%	12.5[a]	33.3[b]	31.6[b]	33.3[b]	0.021

p <0.05, *Median (25%-75%)
IVIG: intravenous immunglobulin, CALs: coronary artery lesions, iKD: incomplete Kawasaki disease, cKD: complete Kawasaki disease.

as; duration of fever≥9.5 days before IVIG, which had an OR 3.41 (51.6% sensitivity and 71.4% specificity), and Plt count after IVIG ≥ 670x10^3/uL which had an OR 5.5 (35.7% sensitivity and 95.3% specificity) for CALs. ROC-curve of the factors for predicting CALs is presented in Figure 2 for predicting CALs.

Discussion

Recent literature about KD in children reports better morbidity and mortality rates related with decreased incidence of coronary arterial disease. On the other hand, there is still a significant number of patients with IVIG resistance, and 15-25 % of them have CALs.[1,21] In the literature, there are many risk-scoring systems from different countries to predict IVIG resistance and CALs. However, using the same parameters for all countries is inadequate due to differences in genetic and environmental factors. There is no risk-scoring system of KD in Turkish children to the best of our knowledge. In the current study, we designed a risk-scoring system to predict IVIG resistant cases with 76.47% sensitivity, and 71.05% specificity.

Resistance to the initial IVIG treatment is a high-risk factor for CALs.[22] Kobayashi et al.[11] showed that each of the variables indicating IVIG resistance was also a risk factor for CALs. The current study determined the rate for developing CALs as 33%, and there was a positive correlation with IVIG resistance. The rate was similar to other Turkish studies, but more frequent than Japanese studies.[8-11,23]

Duration of fever before IVIG was found to be significantly higher in IVIG resistant and CALs (+) groups, in this study. This period was ≥ 9.5 days, which had an OR of 3.8 for IVIG resistance (58.8% sensitivity and 72.7% specificity) and 3.4 for CALs (51.6% sensitivity and 71.4% specificity). On the other hand, Egami[10] and Kobayashi's[11] risk scoring systems reported early administration of IVIG to be a strong predictor for IVIG resistance and coronary artery involvement. They defined fever ≤ 4 days before IVIG as a risk factor. They speculated that, patients who were earlier diagnosed and treated were probably sicker and had greater inflammation. However, Chantashiriwan et al.[24] reported duration of fever ≥ 8 days before IVIG as a predictor of coronary artery aneurism, similar with our results. Also, Gulhan et al.[22] suggested administrating initial IVIG treatment within 7 days of illness to prevent cardiac complications. We considered that delayed diagnosis and treatment may cause prolonged inflammation of vessel walls before IVIG treatment, which may create a high risk for IVIG resistance and CALs.

In the literature, CRP ≥ 100 mg/L (Kobayashi et al.[11]) and ≥ 70 mg/L (Sano et al[12]) was defined as a risk factor for IVIG resistance. Regarding IVIG resistant cases which had higher CRP values, we compared the related values with IVIG resistant and IVIG responsive groups in the current study. CRP values ≥ 88 mg/L had an OR 4.4 (70% sensitivity and 64.7% specificity) for IVIG resistance. In a recent study, Yang et al.[16] reported this cut-off value as 90 mg/L, which was similar to our results.

Studies about prognostic factors of many infectious and inflammatory disorders suggested that instead of WBC count, using absolute neutrophil and lymphocyte counts (Neu and Lym) and neutrophil/lymphocyte ratio (NLR) might be more predictive for IVIG resistance in KD.[25-27] Zahorec et al.[27] reported that higher Neu and NLR with lower Lym were associated with severe inflammatory response. Kawamura et al.[28] reported that NLR value before IVIG ≥3.83 and one day after IVIG ≥1.27 were the best cut-off values for predicting IVIG resistance. Similarly, a study by H-J Cho et al.[29] reported that higher NLR values before initial IVIG treatment were associated with increased risk for IVIG resistance and lower NLR changes before and 2 days after IVIG were a predictive tool for coronary artery abnormalities. In current study, we reported best cut-off values of NLR value for IVIG resistance as NLR ≥1.69 before IVIG which had an OR of 4.40 (93.3% sensitivity and 43.4% specificity) and ≥1.25 two days after IVIG which had an OR of 11.29 (85.7% sensitivity and 65% specificity) for IVIG resistance.

Increased pro-inflammatory cytokines stimulate megakaryocyte proliferation leading to an increase in platelet counts. Plt during the acute phase tend to decrease in patients with severe KD.[30] Egami[10] and Kobayashi[11] speculated that it might be related with intravascular consumption, reflecting greater inflammation. They also stated that Plt ≤300x10^3/mm^3 was a predictor for IVIG resistance. We found no statistically significant difference in the IVIG resistant group. Patients had lower Plt both before and after IVIG. However, Plt two days after first IVIG was ≥ 670x10^3 /uL (OR: 15.5) in CALs (+) group, which was significantly higher than CALs (-) group. This cut-off value had a 35.7% sensitivity and 93.5% specificity.

Hyponatremia has been associated with increased vascular permeability and inappropriate antidiuretic hormone secretion due to increased inflammatory cytokine levels, in the course of KD.[31] Sodium values under 133 mmol/L and 135 mmol/L was defined as a risk factor for Kobayashi[11] and Yang[16], respectively. In our study, lower sodium levels were detected in the IVIG resistant group, both before and after IVIG treatment, without significance.

This study defined three criteria for IVIG resistance in KD prior to treatment: Duration of fever before IVIG ≥ 9.5 days, CRP ≥ 88 mg/L and NLR value ≥ 1.69. Following initial therapy with IVIG, if NLR value was ≥ 1.25, it also predicted ongoing IVIG resistance, possibly implying a need for steroid therapy instead of IVIG.

In summary, based on the clinical and laboratory features, we established a new risk-scoring system for predicting IVIG resistance in KD. This may be useful for choosing optimal treatment for KD before coronary artery involvement. Our findings should be supported by multicenter studies in Turkey.

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Original Article

Gross motor development of preschool children: effects of socioeconomic status and maternal education

Cemil Özal[1], Birgül Bayoğlu[2], Sevilay Karahan[3], Mintaze Kerem Günel[1], Banu Anlar[4]

[1]Department of Physiotherapy and Rehabilitation, Hacettepe University Faculty of Health Sciences, Ankara; [2]Department of Child Development, Bayındır Hospital, Ankara; Departments of [3]Biostatistics and [4]Pediatric Neurology, Hacettepe University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Motor development reflects the general health status of the child and affects other areas of development. It is influenced by biological and family characteristics especially in infancy and early childhood, and by environmental conditions in preschool age. We assessed the effect of several family and environment characteristics on gross motor developmental items included in the Denver-II test on 2,042 healthy children. Increasing maternal age and education were associated with later achievement in several items after age 12 months while socioeconomic status, sex and birth rank did not show a clear effect. Our observations suggest in a relatively homogenous urban population, few external factors affect gross motor development in preschool children.

Key words: gross motor development, children, maternal, socioeconomic factors.

Surveillance of psychomotor development is an important part of pediatric care especially in the first years of childhood. Early detection of possible delays allows early intervention and, in certain etiological groups, medical treatment.[1] Gross motor development is frequently affected by the child's general health status such as vitamin and mineral deficiency or chronic systemic disorders. Therefore, gross motor delays may constitute a warning sign for medical conditions. On the other hand, primary developmental problems such as those in motor control and perception affect up to 6% of school-age children and their early detection can lead to appropriate educational interventions.[2] Physicians should therefore possess knowledge about factors affecting motor development, the degree and nature of their effects, and the range and limits of normal variation.

✉ Cemil Özal
cemilozal@hotmail.com

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Development is ideally evaluated using standardized tests which in clinical practice consist of parental questionnaires or screening tests.[1]

Developmental screening tests are standardized tools used for identifying children who need more detailed evaluation and if used appropriately are useful.[3] Since screening is used for identifying children who will receive the benefits of more professional evaluation or treatment, it is recommended that all children be screened for developmental delays.[3] There are many developmental screening tools which are based on achieving developmental milestones at specific chronological ages. Denver Developmental Screening Test II (DDST-II) is one of the examples for such formal tools.[4] In order to differentiate between abnormal children and normal children who have slower rates of achieving developmental skills, these developmental screening tools must be reliable and valid, as well as have acceptable sensitivity and specificity.[5]

DDST-II is a formal developmental screening tool that assesses children from birth to 6 years of age. DDST-II is a brief and validated screening tool; although there is doubt about its limited specificity (43%) and risks of over referral, it has high rate of sensitivity (83%) and identifies children with developmental delays.[5-6]

Developmental assessment should take into account the family and environmental factors that strongly affect the results.[7-9] In this study we examined developmental screening test results in pediatric outpatient settings and the role of possible variables such as maternal age, education and socioeconomic status as factors affecting gross motor development of urban children.

Material and Methods

Participants

Healthy children were sought in community health centers, well-baby clinics, private practices, kindergartens and day care centers in 7 metropolitan districts of Ankara during the collection of normative data for the standardization of the Denver II Developmental Test for Turkey (Denver II –Turkey) between 2011 and 2012.

Exclusion criteria were, 1) prematurity <37 weeks gestational age, 2) birth weight under 2,500 g, 3) past history of hospitalization, 4) congenital malformation, 5) any illness during testing time. Only one child per family was tested in order to avoid over-representation of any particular factor. Total 2,042 children, 1,041 girls (51%) and 1001 boys (49%), aged 0 to 72 (minimum 3, maximum 72, mean 24.8±20.4) months were included. Each participants' parents signed informed consent. Ethical approval was obtained from Faculty of Medicine ethics committee (46954233-604.02).

Four levels of maternal education were defined, illiterate (n: 41, 2%), schooling of ≤8 years (n: 734, 35.9%), 8-12 years (n: 778, 38.1%), or ≥12 years (n: 489, 23.9%). Birth rank of the child was compared in three groups: first child (n: 1035, 48.8%), second child (n: 737, 34.8%), and third child or above (n: 270, 16.4%) in the family.

The socioeconomic status of the family was recorded on a questionnaire designed by a sociologist based on parents' occupation, years of schooling, household income, residential area, participation to cultural, leisure and sportive activities. Principal component analysis was applied and three different socioeconomic groups were formed according to the standards of the Turkish Institute of Statistics: low (n: 760, 37.2%; at least 0.5 standard deviation [SD] below the mean), medium (n: 796, 39.0%; mean ± 0.5 SD), and high (n: 486, 23.8%; at least 0.5 SD higher than the mean) socioeconomic levels.[10,11]

Assessment tool

The Denver II test standardized for Turkey comprises 134 items from age 0 to 78 months, of which 37 are in the gross motor domain. Children's "pass" or "fail" status were recorded for each item, and the mean age each item was passed was calculated. Testing was done in a quiet room in the presence of the tester, the child, and the primary caregiver, usually the mother, using standard test material.

Inter and intra-rater reliability

The test was administered by 4 students of the departments of psychology or child development in Hacettepe University. They were all trained in the use of Denver II by attending a one-week course and had been using the test for at least 3 months prior to the initiation of the study. Examiners reached at least 90% inter- and intra-rater concordance at the beginning of the project and reliability was re-checked two months later. Data collection was completed in 3 months.

Statistical analysis

The mean age children passed each gross motor item, standard deviation of the mean, minimum and maximum values were calculated. The results of tests were expressed as the number

of observations (n), mean ± standard deviation (SD). Homogeneity (Levene's) and normality (Shapiro Wilk) tests were used to choose statistical methods. Groups with normal distribution and homogeneous variances were assessed by using Pearson's correlation coefficient. As parametric test assumptions were not available for some variables, these were assessed by using Spearman rho correlation coefficient. All statistical analyses were performed with the SPSS software (SPSS ver. 17.0; SPSS Inc., Chicago IL, USA), and $p<0.05$ was considered statistically significant.

Results

Mean ages when gross motor milestones were accomplished are shown in relation with socioeconomic level, maternal education and maternal age in Tables I, II and III.

There was no significant difference between boys and girls in any items. Birth order did not affect any gross motor item except 'kick ball forward' (p: 0.049), which was later in the first child. Children of high socioeconomic level (HSL) accomplished items 'lift head', 'bear weight on legs', 'stand 10 seconds', 'walk independently', 'stoop and recover' and 'heel to toe walk' at significantly younger ages than the other two groups (Table I). On the other hand, 'stand holding on', 'walk holding onto furniture', 'stand 2 seconds', 'ride tricycle' and 'broad jump' were accomplished significantly later by children of HSL ($p<0.001$).

Between maternal education groups, 'stand 10 seconds' (p: 0.018) was significantly earlier in children of less educated mothers compared to university graduate mothers. Items 'broad jump', 'catch bounced ball' and 'run' were performed later by children of mothers with ≥12 years education compared to other groups (p: 0.004; p: 0.03; p <0.001, respectively). 'Balance each foot 1 second' (p: 0.037) and 'balance each foot 7 seconds' (p: 0.011) items were earlier in high educated group than other groups (Table II).

According to maternal age, 'run' (p <0.01), 'jump up' (p: 0.003), 'ride tricycle' (p: 0.001), 'balance each foot 2 seconds' (p: 0.002), 'broad jump' (p <0.01), 'catch bounced ball' (p: 0.011) and 'balance each foot 9 seconds' (p: 0.021) items were related with maternal age and as mother's age increased, children tended to be later in these items (Table III).

Discussion

Motor development influences the child's social adjustment, learning, and school performance, and motor delays may be a sign of a global developmental problem where early intervention improves outcome.[12] Knowledge about factors affecting development allows the clinician to identify adverse practices and environments.[13]

In the young child, the environment mainly consists of the family. Parental, especially maternal education and mother-child interaction affect the amount of cognitive and emotional stimulation given to the child, which is strongly related to motor development. In a study on 6 months-old infants from Sweden, older maternal age and having older siblings, together with maternal depression and feelings of loneliness, affected several developmental areas.[14] Our results showed older maternal age associated with later motor development in the toddler age group.

Income is an important indicator of the family's assets and opportunities offered by the home environment. Freitas et al.[14] observed positive correlation between the dimensions of the home (daily activities and play materials) and global motor performance. Adverse environmental factors are more likely to be found in socioeconomically disadvantaged communities. Although our study was done in central-metropolitan areas of Ankara city where families living below poverty line are rare, the socioeconomic structure of the city varies among different districts, mostly due to the effect of rapid urbanization. Cultural

Table I. Mean age (months) of gross motor skills according to socioeconomic level.

	Socioeconomic levels						
	Low		Medium		High		
Test items	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	p value
Equal movements	1	0.07	1	0.03	4	0.12±0.09	>0.05
Lift head	39	1.82±0.46	18	1.81±0.39	23	1.33±0.64	**0.015**
Head up 45 degrees	73	3.43±0.90	58	3.56±0.87	44	3.74±0.77	>0.05
Sit head steady	71	3.56±1.01	75	3.46±0.96	55	3.81±0.87	>0.05
Head up 90 degrees	49	4.31±0.64	37	4.34±0.85	35	4.61±0.65	>0.05
Chest up with arm support	42	5.29±0.97	47	5.03±1.05	39	5.04±0.87	>0.05
Sit no support	-	-	6	7.57	1	7.5	>0.05
Stand holding on	38	9.19±0.38	41	9.09±0.76	9	9.31±0.74	**<0.001**[a]
Get to sitting	33	9.22±0.37	37	9.26±0.59	8	9.65±0.39	>0.05
Weight bearing on legs	94	7.90±1.44	110	7.71±1.40	68	7.01±1.23	**<0.001**[a]
Pull to stand	37	9.39±0.58	30	9.58±0.77	20	10.39±0.50	>0.05
Stand 2 seconds	36	9.47±0.71	36	9.81±0.91	28	10.71±0.53	**<0.001**[a]
Walk holding onto furniture	32	9.76±0.90	28	10.28±0.87	33	10.97±0.61	**<0.001**[a]
Stand 10 seconds	31	12.58±1.15	53	12.61±0.85	24	11.90±0.74	**<0.001**[a]
Walk well	33	13.69±1.03	49	13.16±1.10	21	12.71±1.35	**0.006**[a]
Stop and recover	37	13.94±1.11	48	13.34±1.10	20	12.99±1.43	**0.007**[a]
Walk backwards	73	15.98±2.18	110	16.11±2.35	50	15.76±2.76	>0.05
Kick ball forward	45	14.96±1.45	60	14.68±1.68	26	14.62±1.82	>0.05
Walk up steps	57	15.73±1.56	56	16.00±1.61	18	15.78±1.28	>0.05
Throw ball overhead	39	17.17±1.41	67	17.14±1.43	22	17.51±1.38	>0.05
Runs	106	28.74±5.44	126	29.85±6.64	67	30.56±6.93	>0.05
Jump up	80	28.74±2.54	80	28.57±2.64	33	29.53±2.94	>0.05
Ride tricycle	61	28.27±2.77	41	28.78±2.97	25	30.33±1.94	**0.009**[a]
Balance each foot 1 second	28	31.78±3.84	29	31.73±3.29	11	32.86±2.61	>0.05
Balance each foot 2 seconds	5	32.05±3.91	7	34.59±3.55	5	33.92±2.07	>0.05
Balance each foot 3 seconds	14	36.61±2.84	12	36.18±3.80	9	36.96±4.54	>0.05
Broad jump	96	34.85±5.05	104	35.93±5.20	71	37.04±5.26	**0.041**
Balance each foot 4 seconds	7	39.37±5.00	18	40.30±5.20	8	42.24±5.12	>0.05
Catch bounced ball	88	38.91±5.65	88	38.96±5.41	62	40.00±5.25	>0.05
Balance each foot 5 seconds	10	42.80±5.44	9	42.32±4.63	12	47.21±4.88	>0.05
Hops one foot	50	46.11±5.90	63	46.27±5.33	49	47.27±5.54	>0.05
Balance each foot 6 seconds	6	44.92±7.62	11	47.12±5.37	6	45.36±5.63	>0.05
Balance each foot 7 seconds	15	53.42±6.40	7	48.28±6.79	6	49.36±3.65	>0.05
Heel to toe walk	55	59.33±5.45	79	59.92±5.54	51	56.75±7.04	**0.031**
Balance each foot 8 seconds	6	55.27±4.91	11	54.91±7.28	5	51.23±7.83	>0.05
Balance each foot 9 seconds	74	60.04±6.80	81	61.77±6.19	34	60.14±7.29	>0.05
Backward heel to toe walk	30	67.15±5.40	47	67.19±5.16	28	69.31±5.99	>0.05

n: count, SD: standard deviation, bold characters indicate $p < 0.05$, [a]: $p < 0.01$

Table II. Mean age (months) gross motor skills according to maternal education groups.

	Maternal Education (school years)								
	Illiterate		Eight years or less		8-12 years		More than 12 years		
Test items	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	p value
Equal movements	-	-	-	-	6	0.094	-	-	-
Lift head	6	1.82±0.30	35	1.85±0.36	31	1.48±0.69	8	1.57±0.53	>0.05
Head up 45 degrees	5	3.91±0.50	66	3.53±0.96	82	3.60±0.77	22	3.36±0.93	>0.05
Sit head steady	6	3.36±1.14	77	3.71±1.01	95	3.47±0.91	24	3.69±0.98	>0.05
Head up 90 degrees	3	3.88±0.50	47	4.47±0.73	55	4.34±0.74	16	4.54±0.64	>0.05
Chest up with arm support	2	6.62±0.02	46	4.97±0.81	56	5.15±0.97	24	5.22±1.13	>0.05
Sit without support	-	-	3	7.5±0.26	3	7.63±0.42	1	7.5	>0.05
Stand holding on	1	8.5	33	9.15±0.67	35	9.17±0.63	19	9.18±0.56	>0.05
Pull to sitting	-	-	30	9.23±0.54	33	9.34±0.51	15	9.25±4.10	>0.05
Bear weight on legs	5	7.26±0.78	98	7.73±1.44	122	7.48±1.14	47	7.67±1.53	>0.05
Pulls to stand	-	-	37	9.65±0.82	37	9.82±7.02	13	9.41±0.58	>0.05
Stand 2 seconds	-	-	38	9.83±0.86	48	10.15±0.94	14	9.52±0.71	>0.05
Cruising	-	-	33	10.44±0.90	50	10.38±0.98	10	9.86±0.81	>0.05
Stand 10 seconds	3	13.33±0.15	34	12.07±1.17	49	12.47±0.81	20	12.84±0.75	**0.018**
Walk well	3	13.33±0.15	28	13.00±1.16	50	13.25±1.12	20	13.59±1.13	>0.05
Stoop and recover	3	13.33±0.15	28	13.50±1.33	50	13.31±1.23	22	13.96±1.05	>0.05
Walk backwards	7	15.96±2.58	74	16.20±2.47	108	15.73±2.45	43	16.39±1.99	>0.05
Kick ball forward	4	14.1±1.54	35	15.07±1.63	65	14.70±1.73	26	14.67±1.40	>0.05
Walk up steps	4	15.23±2.24	44	15.95±1.51	60	15.90±1.51	23	15.64±1.63	>0.05
Throw ball overhead	2	18.25±0.64	46	17.56±1.40	60	16.88±1.43	20	17.32±1.23	>0.05
Run	5	26.42±2.13	95	28.20±5.11	115	28.54±5.97	83	32.90±7.02	**<0.001**[a]
Jump up	2	31.30±1.98	67	28.91±2.38	87	28.35±2.64	40	29.41±3.04	>0.05
Ride tricycle	1	32.7	45	28.62±2.51	49	28.17±2.96	31	30.06±2.57	0.08
Balance each foot 1 second	-	-	20	32.77±3.73	32	31.91±3.80	17	31.81±2.54	**0.037**
Balance each foot 2 seconds	-	-	5	36.68±2.31	8	32.10±3.33	3	32.20±0.35	>0.05
Balance each foot 3 seconds	-	-	14	36.67±2.80	12	35.60±3.56	9	37.63±4.60	>0.05
Broad jump	2	33.977±1.79	85	35.40±5.29	74	34.02±4.90	107	37.61±4.94	**0.004**[a]
Balance each foot 4 seconds	-	-	9	42.37±5.72	7	41.00±4.58	19	39.86±5.13	>0.05
Catch bounced ball	4	40.50±7.91	80	39.80±5.57	53	36.85±5.60	102	39.76±4.81	**0.03**
Balance each foot 5 seconds	-	-	8	44.75±5.58	2	41.98±1.86	22	44.10±5.70	>0.05
Hops	3	52.07±2.17	56	46.12±5.29	32	46.26±5.90	69	46.67±5.73	>0.05
Balance each foot 6 seconds	-	-	7	47.52±6.19	7	47.85±7.24	10	45.56±6.80	>0.05
Balance each foot 7 seconds	1	49.63	14	54.43±6.26	3	58.46±6.57	12	47.41±3.90	**0.011**
Heel to toe walk	3	62.70±3.21	66	59.26±5.96	44	59.85±5.54	74	58.00±6.30	>0.05
Balance each foot 8 seconds	-	-	10	53.82±7.90	6	52.64±6.27	6	56.29±5.50	>0.05
Balance each foot 9 seconds	4	61.97±7.23	77	60.24±6.92	47	61.12±6.11	61	61.58±6.79	>0.05
Backward heel to toe walk	1	64.67	36	67.48±5.72	26	66.56±5.93	44	68.28±5.36	>0.05

n: count, SD: standard deviation, bold characters indicate $p < 0.05$, [a]: $p < 0.01$

Table III. Correlation analysis between developmental milestones and maternal age.

Milestones	N	P*	T
Equal movements	13	0.64	0.243
Lift head	86	0.34	-0.107
Head up 45 degrees	180	0.96	0.004
Sit head steady	205	0.97	-0.03
Head up 90 degrees	124	0.23	-0.110
Chest up with arm support	130	0.98	-0.002
Sit no support	25	0.66	-0.205
Stand holding on	107	0.83	-0.023
Pull to sit	95	0.57	0.066
Bear weight on legs	307	0.63	-0.030
Pull to stand	105	0.44	0.084
Stand 2 seconds	117	0.80	-0.026
Cruise	109	0.49	0.073
Stand 10 seconds	123	0.49	0.067
Walk well	117	0.69	0.040
Stoop and recover	120	0.77	-0.029
Walk backwards	247	0.17	0.090
Kick ball forward	143	0.57	0.050
Walk up steps	140	0.13	0.134
Throw ball overhead	134	0.11	0.143
Run	304	**<0.01**[a]	0.250
Jump up	202	**0.003**[a]	0.208
Ride tricycle	139	**0.001**[a]	0.295
Balance each foot 1 second	20	0.32	0.259
Balance each foot 2 seconds	69	**0.002**[a]	0.371
Balance each foot 3 seconds	35	0.93	0.015
Broad jump	278	**<0.01**[a]	0.347
Balance each foot 4 seconds	35	0.75	-0.055
Catch bounced ball	245	**0.011**	0.164
Balance each foot 5 seconds	32	0.35	0.172
Hop	159	0.95	0.005
Balance each foot 6 seconds	25	0.81	-0.052
Balance each foot 7 seconds	30	0.64	0.090
Heel to toe walk	187	0.68	0.031
Balance each foot 8 seconds	22	0.61	-0.011
Balance each foot 9 seconds	189	**0.021**	0.168
Backward heel to toe	109	0.28	0.105

Bold characters indicate $p < 0.05$; [a]: $p < 0.01$.
* Pearson correlation analysis

factors restricting spontaneous play activities may influence gross motor abilities. In non-urban areas low socioeconomic level may be associated with more opportunity for the child's exploration of his/her environment, facilitating motor development.[15-16]

Higher maternal education was associated with earlier development in several studies.[13] Although in our previous work we found children of more educated mothers developed earlier in gross motor skills[17], in the current study this difference was observed less prominently and was even observed later in high educated mothers in some items. This difference by years in the same community may reflect differentiations of life styles that more high educated mothers have and welfare conditions in different educational groups. In a study from Greece, maternal educational level and the caregiver being a grandparent or babysitter were found to affect infants' gross motor development assessed by the Alberta Infant Motor Scale, whereas gender, birth order, maternal age, paternal educational level and income were not significant factors.[18] Koutra et al.[18] did not find any significant association between gross motor development and maternal education. These findings are not comparable with our study because their age group was up to 18 months, and we observed the main differences associated with maternal education in older children.

Sex was not found to affect motor items in our study, as in others.[19] Birth rank also lacked any effect in our study. Certain reports emphasized the negative effect of the presence of older siblings.[20] On the other hand, Berger and Nuzzo[19] observed having an older sibling provides developmentally more advanced motor development models.

Collaborative systems in motor development include musculoskeletal components, central sensorimotor integrative mechanisms, environment, and motivation. As expected from such a multifactorial function and from heterogeneous populations, our results do not show uniform trends. For instance, increasing educational level of women is expected to increase their employment rates and consequently, socioeconomic status. However, the effect of these two factors is not in the same direction in Turkey.[21,22] This can be explained by working mother's leaving the child with a non-professional caregiver from low educational background, and the child spending more time in closed, indoor spaces with less quality time. This effect is more important in urban areas of developing countries where standard preschool education and day care centers are not widely available.[23] Providing an enriched and safe environment and experience of motor activities via recreational activities are important for bringing out and enhancing the developmental potentials of children.[24]

Educational or socioeconomic factors appeared to affect certain motor items, although the effect was variable and no specific trend towards one direction was observed. Notably, socioeconomic status appeared effective on functions acquired after age 12 months. Developmental inabilities result from the combination of biological, social and environmental factors. Knowledge of the relationship between motor development and environmental stimulation, and the role of the family to bring out the motor abilities are important for the planning of developmental interventions. Development of motor skills in early childhood can influence future life. Gross motor development affects other developmental domains probably through acquisition of experience and opportunity for exploration. Gross motor items were found to be related to cognitive performance; in particular, subtests of working memory and processing speed in a study using the Ages and Stages Questionnaire, an infant and preschool screening tool based on parental report.[25]

The main limitation of this study is the absence of validation of our screening test results with a diagnostic test. On the other hand, our aim was to examine results applicable in well-baby or primary health care clinics, which provide the earliest opportunity for developmental

screening. Identifying factors affecting gross motor results in Denver II, the most commonly used screening test in Turkey, helps primary care physicians' approach and correct interpretation of the test, allowing the undertaking of appropriate measures. Our results pertain to Ankara, a city of 5 million inhabitants, and their applicability to smaller towns with different social structure remains to be investigated.

In conclusion, familial and environmental factors are effective on gross motor functions of preschool period. Specific socioeconomic factors seem to influence the infants' motor development. Gender and birth order did not affect gross motor development while maternal education was more effective at 8-16 months and socioeconomic level, in the 10-60 month old period.

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Original Article

Detection of allele frequencies of common c. 511C>T and c.625G>A variants in the *ACADS* gene in the Turkish population

Mustafa Kılıç[1], Bekir Ergüner[2], Can Koşukçu[3], Rıza Köksal Özgül[3]

[1]Division of Metabolism, Dr. Sami Ulus Children Hospital, Ankara; [2]TÜBİTAK, BİLGEM, Kocaeli; [3]Division of Metabolism, Hacettepe University Children Hospital Ankara, Turkey.

ABSTRACT

Short-chain acyl-CoA dehydrogenase deficiency (SCADD) is a rare inborn error of mitochondrial fatty acid oxidation and protein misfolding disorder. Our aim was to detect the number of Turkish patients diagnosed with SCADD in the literature and to determine the allele frequencies of two common variants (c.511C>T and c.625G>A) in the Turkish population. Five Turkish patients with SCADD were reported in the literature from four unrelated families. We also investigated allele frequencies of common variants of c.511C>T and c.625G>A, which confer susceptibility to SCADD, which were found to be 1.7% and 20.2%, respectively. Both of these susceptibility variants were found to be high in the Turkish population as they are worldwide.

Key words: short-chain acyl-CoA dehydrogenase deficiency, ACADS, SCAD, SCADD, SCAD deficiency, ethylmalonic aciduria.

Short-chain acyl-CoA dehydrogenase (SCAD, OMIM 606885) deficiency (SCADD, OMIM 201470) is a rare autosomal recessive inherited disorder of mitochondrial fatty acid oxidation encoded by *ACADS* gene, located on chromosome 12q24.[1-12] It has variable clinical phenotypes ranging from asymptomatic individuals to severe neurological presentation.[1-12] The brain is the the most commonly affected organ, but muscles, liver, heart involvement are also reported.[1-12] Birth prevelance of SCADD from newborn screening was reported to be approximately 1 in 35.000-50.000 live births.[5-6] SCADD was first described in 1987 and thereafter the responsible gene was identified in 1990.[13,14] To date, nearly 70 pathogenic mutations and two common variants, c.511C>T and c.625G>A have been reported in the *ACADS* gene.[1-14] Genotype-phenotype correlation is weak and poorly understood.[1-14] Our aim was to detect the number of Turkish patients diagnosed with SCADD in the literature and to determine the allele frequencies of two common variants (c.511C>T and c.625G>A) in the Turkish population.

Material and Methods

Turkish patients diagnosed with SCADD through metabolic screening and mutation analyses were searched in the literature. Literature search was made via PubMed with the following key words: 'short-chain acyl-CoA deficiency', 'SCAD deficiency', 'SCAD', 'SCADD', '*ACADS*', 'ethylmalonic aciduria'. We used TUBITAK, IGBAM in-house exome database to find out the allelic distributions of c.511C>T and c.625G>A variants in the *ACADS* gene in the Turkish population. These two variants were questioned anonymously in a total of 1182 individuals. This exome database cohort includes a mixed group of unrelated individuals and some randomly selected family members. It is composed of patients with

✉ Mustafa Kılıç
kilickorkmaz@yahoo.com.tr

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undiagnosed diseases, their healthy parents and healthy and/or affected siblings. In addition to these data, we analysed these allele frequencies in a control group of 89 healthy individuals to fulfill the Hardy-Weinberg equation.

Results

In the literature, we found five Turkish patients from four families. All five patients were diagnosed with metabolic screening followed by Sanger sequencing of *ACADS* gene (Table I). Age at diagnosis was 2 months-14 years. Male to female ratio was 2:3. Two of three families were consanguineous and consanguinity was not reported in one of the families. All patients except one had increased butyryl carnitine levels and all had increased ethylmalonic acid in urine. Two siblings had the homozygous c.1138C>T pathogenic variant and the homozygous c.625G>A susceptibility variant. One patient had a different homozygous c.1147C>T pathogenic mutation. Two patients had homozygous c.625G>A susceptibility variant, but no other causative mutations. All of the patients except one were symptomatic with mostly neurological problems. Three of them had neurological sequela.

In the in-house exome data of 1182 individuals, the heterozygote ratio of c.625G>A allele (pGly209Ser) was found to be 44% (hetRatio= 0.4391) and the homozygote ratio was calculated as 13% (homRatio= 0.1320). The heterozygote ratio of c.511C>T (p.Arg171Trp) variant was 3% (hetRatio= 0.0305) and the homozygote ratio was 0%. In 89 unrelated healthy Turkish individuals (control group), the allele frequency of c.625G>A (pGly209Ser) susceptibility variant was 20.2% and the c.511C>T (p.Arg171Trp) susceptibility variant was 1.7%.

Discussion

The clinical spectrum of SCADD is extremely heterogeneous. It can vary from being asymptomatic to having feeding problems, ketotic hypoglycaemia, metabolic acidosis, lethargy, weakness, hypotonia, microcephaly, developmental delay, speech delay, behavioural disturbances, epilepsy, myopathy, neuropathy, dysmorphic features and rarely optic atrophy, hepatic dysfunction, Reye syndrome, cardiomyopathy, arrhythmia.[1-18] Acute fatty liver of pregnancy, pre-eclampsia, maternal HELLP syndrome and premature birth have also been reported. SCADD is generally diagnosed when investigating neurological disorders and/or hypoglycaemia, by selective screening. Therefore, these patients were diagnosed later by selective screening and showed severe clinical symptoms including microcephaly, developmental delay, epilepsy and dysmorphic features. However, in recent years newborn screening by tandem mass spectrometry has led to identification of mostly asymptomatic newborns and the prevalence appeared higher than it was estimated.[3,6]

In spite of many patients already known in the literature, only five Turkish patients from four unrelated families were reported.[15-18] Bok et al.[15] reported a homozygous pathogenic c.1138C>T mutation with a homozygous c.625G>A susceptibility variant in two Turkish siblings, one of whom had transient cholestasis, maternal HELLP syndrome, premature delivery but normal mental development, slight hypotonia, active behaviour at three years of age while the other sibling was asymptomatic. Kiykim et al.[16] reported a homozygous c.625G>A susceptibility variant in a Turkish patient with speech delay, epilepsy and behavioural disturbances, without any other causative mutations. Okuyaz et al.[17] reported a homozygous c.625G>A susceptibility variant in a Turkish patient with infantile hypotonia, also without other causative mutations. We also recently reported a homozygous c.1147C>T pathogenic mutation in a Turkish patient with microcephaly, developmental delay, epilepsy and dysmorphic features.[18] All five patients were diagnosed with metabolic screening followed by direct sequencing of *ACADS* gene (Table I). Neurological problems were found in the majority of the patients and are accepted as the most important clinical findings.

Table I. Turkish patients diagnosed with SCADD in literature.

Patient No	References	Age at diagnosis (month)	Sex (Male/ Female)	Consanguinity	Tandem mass analyses (C4: N<1.1µmol/L)	Urine organic acid analyses (ethylmalonic acids: mmol/ molcreatinine; N<18)	Brain MRI	Mutation (*ACADS* gene)	Protein effect	Prognosis
I[a]	Bok et al	2	M	+	2.4/3.91/4.7	124-380	NA	c.1138C>T; c.1138C>T and c.625G>A;c.625G>A	p.R380W;p. R380W and p.G209S;p. G209S	Prematurity, cholestasis, hepatomegaly, maternal HELLP syndrome. Normal mental development, slight hypotonia, active behaviour at three years of age
II[a]	Bok et al	2	F	+	2/6.25	25-58	NA	c.1138C>T; c.1138C>T and c.625G>A;c.625G>A	p.R380W;p. R380W and p.G209S;p. G209S	Asymptomatic
III	Kiykim et al	168	M	NA	2.14	Increased ethylmalonic acid and methylsuccinic acid.	NA	c.625G>A;c.625G>A	p.G209S;p. G209S	Delayed speech, epilepsy and behavioral disturbances
IV	Okuyaz et al	8	F	-	N	Significant increase	N	c.625G>A;c.625G>A	p.G209S;p. G209S	Hypotonia, developmental delay
V	Kilic et al	48	F	+	2.32	720	Microcephaly and arachnoid cyst	c.1147C>T;c.1147C>T	p.R383C; p.R383C	Microcephaly, severe developmental delay, epilepsy, dysmorphic features

N: normal, NA: not available M: male, F: female, a: siblings

There is a remarkably high prevalence of homozygosity for *ACADS* variants in the general population, with frequencies of approximately 0.3% for the c.511C>T (p.R171W) and 5.5% for the c.625G>A (p.G209S) variant.[19,20] Additionally, 7% of the population was found to be homozygous for one of these variants or compound heterozygous for each. In Europe, two common variants c.511C>T;p.Arg171Trp and c.625G>A;p.Gly209Ser were reported as polymorphisms.[1,21] Each variant accounts for 3-8% and 22-43% of normal population, respectively. In a population study conducted in the United States of 694 samples, the allele frequency of the c.625G>A variant was found to be 22% and that of the c.511C>T variant was 3%.[19] Sequence analyses of the *ACADS* gene in 100 alleles from Danish controls revealed allele frequencies of 8% for c.511C>T and 21% for c.625G>A.[22] When the ExAc browser (The Exome Aggregation Consortium) was searched, the c.625G>A allele frequency was 25.9 % and the c.511C>T allele frequency was 3.1%. To date, there is no data for the allele frequencies of these two common variants in the Turkish population. In our study, the allele frequency of c.625G>A (pGly209Ser) variant was 20.2% and the c.511C>T (p.Arg171Trp) variant was 1.7% in our Turkish population based on the healthy control group composed of 89 unrelated individuals.

These two variants have not been directly associated with SCADD although they were reported to confer disease susceptibility when co-occurring with environmental or genetic factors.[1,3,21] Homozygosity for these variants solely or together with homozygous or heterozygous known pathogenic mutations, increase susceptibility to symptoms in certain environmental situations.[1,21] Although individuals with these two common genetic sensitivity variants in the general population are mostly asymptomatic, they can also present with severe neurological abnormalities. Environmental (e.g. fever, infection, metabolic stress, starvation, hypoglycaemia) and genetic (modifier genes) factors may be responsible for the clinical and genetic variability of this disease.

Our observation from our patient and the reported clinical cases suggest that patients with higher ethylmalonic acid (EMA) levels usually had more of a severe clinical condition. We agree that elevated EMA excretion is related to mitochondrial dysfunction and oxidative stress.[23-25] Homozygosity for one of these polymorphisms is associated with an increased incidence of elevated EMA excretion.[10]

In conclusion, SCADD is a rare fatty acid oxidation disorder. The allele frequencies of c.625G>A (pGly209Ser) and c.511C>T (p.Arg171Trp) susceptibility variants in the Turkish population are found to be very similar to that in the literature. In the future, population-specific mutations and genotype-phenotype correlations will be clearer when the number of reported patients increase.

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Original Article

Clinical and laboratory predictors of survival for pediatric patients on non-postcardiotomy extracorporeal membrane oxygenation (ECMO)

Güntülü Şık[1], Asuman Demirbuğa[1], Agageldi Annayev[1], Bahar Temur[2], Selim Aydın[2], Halil İbrahim Demir[3], Ersin Erek[2], Agop Çıtak[1]

Departments of [1]Pediatric Intensive Care, [2]Cardiovascular Surgery and [3]Pediatric Cardiology, Acıbadem Mehmet Ali Aydınlar University, İstanbul, Turkey.

ABSTRACT

Extracorporeal membrane oxygenation (ECMO) is used in pediatric patients with severe cardiopulmonary failure who do not respond to conventional therapy; only a few studies have been conducted in Turkey. We present the experience of pediatric ECMO with the aim of showing factors affecting mortality. We retrospectively reviewed our ECMO database to identify patients who received ECMO from October 2015 to March 2018. Our population comprised 30 pediatric patients. The mean patient age was 41.31±53.35 months and 17 (56.7%) patients were male. The median duration of ECMO support was 8.9 (6.6-10.8) days. The rates of successful ECMO weaning and survival to discharge were 70.0% (n=21) and 66.7% (n=20), respectively. Indications for ECMO were respiratory failure (40.0%), cardiac failure (33.3%), and sepsis (26.7%). We found that pre-cannulation values of pH (p=0.034), leukocytes (p=0.029), C-reactive protein (p=0.045), creatinine (p=0.047), chloride (p=0.001) and post-cannulation pH (p=0.0001), bicarbonate (p=0.014), lactate (p=0.002), chloride (p=0.0001) were associated with mortality. The results showed that preexisting sepsis and renal conditions contributed to poor outcomes. Indications, ECMO onset time, and pre- and post-cannulation laboratory values such as leukocytes, CRP, creatinine, bicarbonate, lactate, and chloride are factors that affect outcomes.

Key words: extracorporeal membrane oxygenation, pediatric intensive care, chloride, lactate.

Extracorporeal membrane oxygenation (ECMO) is an effective treatment method performed in patients with severe cardiopulmonary failure who are resistant to medical treatment, and provides temporary cardiopulmonary support. This technique involves oxygenation of the blood outside the body, thereby obviating the need for gas exchange in the lungs, and it can also aid cardiovascular circulation if necessary.

The first successful ECMO treatment was performed in an infant with congenital heart defects undergoing cardiac surgery in 1970 by Baffes et al.[1]

In 1975, Bartlett et al.[2] reported the first successful use of ECMO in neonates with severe respiratory distress. Since then, ECMO has increased gradually in pediatric cardiac–respiratory support and showed certain benefits. Currently, ECMO is one of the most important treatment modalities worldwide for cardiac and pulmonary failure in children.

In the present study, we studied children who received venoarterial (VA) and venovenous (VV) ECMO support in a pediatric intensive care unit. We tried to describe the current status of ECMO in children for both cardiac and pulmonary support and the risk factors associated with their outcomes.

✉ Güntülü Şık
drguntulu@hotmail.com

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Material and Methods

Study population

All patients (n=30) aged younger than 18 years who received VV or VA-ECMO support in the pediatric intensive care unit (PICU) of Acibadem Mehmet Ali Aydınlar University School of Medicine between October 2015 and March 2018 were retrospectively evaluated. Ethics board approval of Acibadem Mehmet Ali Aydınlar University was granted (31.05.2018, 2018-8/8).

ECMO setting

Maquet or Medos ECMO systems were used. Cannulation was performed through cardiovascular surgery. The cannula size was selected in accordance with the age, height, and weight of the patient. The right internal jugular vein-femoral vein was used in all patients who had VV-ECMO support, and the right common carotid artery-right internal jugular vein was used in 18 patients who received VA-ECMO. The right common carotid artery-left femoral vein was used in 1 patient, and the femoral artery-femoral vein was used in 5 patients.

A perfusionist is present 24 hours per day in our hospital. Before cannulation, patients received heparin at a loading dose of 50 U/kg. During ECMO, heparin infusion continued by following the activated clotting time (ACT). The target was to keep ACT between 170-220 sec, hemoglobin levels > 10 g/dl, and platelet count >100.000/mm^3

Adjunct flow volume was adjusted according to the hemodynamic insides and was maintained at 100-150 ml/kg/min to keep venous oxygen saturation at >70% for patients with VA-ECMO. Patients were evaluated through daily echocardiographic and chest X-rays. All patients received pressure-controlled synchronized intermittent mechanical ventilation. Throughout ECMO, the following parameters were kept between these values: fractional inspired oxygen (FiO_2) 30-45%, frequency 10-18/min, positive end-expiratory pressure (PEEP) 8-12 cm H_2O.

The indications were divided into three groups as cardiac failure, respiratory failure, and sepsis. Ten patients were diagnosed as having cardiac failure [congenital heart disease (CHD), myocarditis, cardiomyopathy (CMP), or intractable arrhythmia], 12 patients had hypoxemic respiratory failure (bacterial pneumonia, pneumonia and acute respiratory distress syndrome) and 8 patients had sepsis.

VA-ECMO indications for patients were evidence of inadequate end organ perfusion and oxygen delivery resulting from inadequate systemic cardiac output;

- Hypotension despite maximum doses of two inotropic or vasopressor medications,
- Low cardiac output with evidence of end organ malperfusion despite medical support as described above: persistent oliguria, diminished peripheral pulses.
- Low cardiac output with mixed venous, or superior caval central venous (for single ventricle patients) oxygen saturation <50% despite maximal medical support.
- Low cardiac output with persistent lactate >4.0 mmol/L and persistent upward trend despite optimization of volume status and maximal medical management.

Patients who underwent ECMO for hypoxemic respiratory failure had an oxygenation index (OI) >16 or oxygenation saturation index (OSI) >12.3, despite the use of lung protective mechanical ventilation strategy, low tidal volume ventilation (5-8 ml/kg), alveolar recruitment maneuvers with high PEEP, prone positioning, high-frequency oscillatory ventilation (HFOV) and neuromuscular blockage agents.

Oxygenation index (OI) was defined as ([FiO_2 × mean airway pressure (Paw) × 100]/PaO_2) and oxygenation saturation index (OSI) ([FiO_2 × Paw × 100]/SpO_2).

Definitions for variables

Pre-cannulation, we recorded the following information: age, sex, weight, pediatric risk of mortality score (PRISM), pediatric logistic organ dysfunction score (PELOD), time from PICU admission to cannulation, the pre-ECMO vasoactive-inotropic score (VIS). Inotropic score (IS) = dopamine dose (mcg/kg/min) + dobutamine dose (mcg/kg/min) + epinephrine dose (mcg/kg/min) × 100. Vasoactive-inotropic score (VIS) = IS + 10 × milrinone dose (mcg/kg/min) + 10,000 × vasopressin dose (units/kg/min) + 100 x norepinephrine dose (mcg/kg/min). The pre- and post-cannulation heart rate, systolic and diastolic pressure levels, and laboratory variables were recorded. The routinely measured laboratory parameters were white blood cell count, hemoglobin, platelets, prothrombin time, activated partial thromboplastin time, urea, creatinine, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), albumin, chloride, lactate dehydrogenase (LDH), C-reactive protein (CRP), pH, partial pressure of O_2 in the arterial blood (paO_2), partial pressure of CO_2 in the arterial blood (pCO_2), sodium bicarbonate (HCO_3), and lactate.

Overall outcomes including the successful weaning rate and the survival to discharge rate were calculated.

Complications due to ECMO were grouped according to the The Extracorporeal Life Support Organization (ELSO) registry in the following categories: 1) metabolic, 2) cardiovascular, 3) infectious, 4) renal, 5) hemorrhagic, 6) neurologic, 7) pulmonary, and 8) mechanical.

Weaning

Cannula position, cardiac functions, effusions and air leakage were evaluated through daily bedside echocardiography and chest radiography. The patients were prepared for weaning when cardiac and pulmonary function improved. During the weaning period, patients required low-dose catecholamine and the mechanical ventilation parameters were increased.

Statistical analysis

The statistical analysis was performed using the Number Cruncher Statistical System (NCSS) 2007 Statistical Software package program (Utah, USA). Independent t-test was used in the comparison of paired variables with normal distribution, and Mann-Whitney U test was used in the comparison of paired groups with variables with non-normal distribution. Chi-square test was used in the comparison of qualitative data in addition to descriptive statistical methods (mean, standard deviation) in the evaluation of the data. Logistic regression analysis was used to identify factors that affected patient loss. The results were evaluated with a significance value of $p<0.05$.

Results

A total of 30 patients comprising 13 girls (43.3%) and 17 boys (56.7%) were included in the study between October 2015 and March 2018. The demographic data of the patients are shown in Table I and Table II.

The mean age was 41.31±53.35 months. The mean weight of all patients was 11.96±8.97 kg; 12.65±9.92 kg in the survivors, and 10.55±7.01 kg in the non-survivors. In addition, 80% (24 of 30) of the patients were on VA-ECMO, whereas 20% (6 of 30) were on VV-ECMO. Altogether, 70% (21 of 30) of the patients were successfully weaned from ECMO and the survival to discharge rate for all patients was 66.7% (20 of 30). The survival rate in both groups receiving VA (16/24) and VV (4/6) ECMO was 66.7%. Survival frequency was not significantly affected by ECMO support type, sex, and weight.

ECMO was performed due to cardiac causes in 10 patients, respiratory causes in 12 patients, and catecholamine-resistant sepsis in 8 patients. The most common ECMO indication was hypoxemic respiratory failure. The most frequent cardiac cause was acute myocarditis.

Table I. Demographic data of patients who received ECMO support.

Features	Survivors (n=20)	Non-survivors (n=10)	p
Age, months	38.17±46.64	47.61±67.53	0.757*
Weight, kg	12.65±9.92	10.55±7.01	0.816*
Timing of ECMO initiation, hours	3.85 (2.88-18.75)	31.3 (5.05-53.35)	0.012
Vasoactive inotropic score	61 (41.25-178.75)	157 (112.38-165.75)	0.115
CRP, mg/dl	4.13 (1.025-7.85)	7.73 (5.68-19.71)	0.045*
Procalcitonin, ng/ml	8.59 (2.163-24.275)	9.09 (2.515-102.85)	0.697
Albumin, g/dl	2.51±0.79	2.1±0.8	0.325*
Troponin-I, ng/ml	2.8 (0.3855-13.966)	3.65 (0.938-27.65)	0.739
Creatine kinase-MB, IU/ml	84 (48.5-236)	183.5 (82.33-533)	0.279
LVEF	27 (18.75-50)	47.5 (18-54.25)	0.441
PRISM score	23 (15-30)	24 (19-31)	0.689
PELOD score	21 (12-32)	21 (20-28.75)	0.851

*Independent t-test (mean±SD), ‡Mann-Whitney U test (median, IQR), +Chi-square test
ECMO: extracorporeal membrane oxygenation, LVEF: left ventricular ejection fraction, PRISM: pediatric risk of mortality score, PELOD: pediatric logistic organ dysfunction.

Table II. Outcomes of patients who received ECMO support.

Parameters	All patients (n=30)	VA-ECMO (n=24)	VV-ECMO (n=6)
Median ECMO time, days	8.9 (6.6-10.8)	9.35 (6.8-12.2)	7.3 (6.1-9.4)
Weaning, n (%)	21 (70%)	17 (70.8%)	4 (66.7%)
Survivor, n (%)	20 (66.7%)	16 (66.7%)	4 (66.7%)
CPR before ECMO	8 (26.7%)	6 (25.0%)	2 (33.3%)

CPR: cardiopulmonary resuscitation, ECMO: extracorporeal membrane oxygenation, VA: venoarterial, VV: venovenous

Table III. Underlying diagnoses and outcomes of extracorporeal membrane oxygenation.

Diagnosis	Total, n	Survivors, n	%
Cardiac reasons			
Congenital heart disease	3	2	66.7
Acute myocarditis	5	4	80.0
Resistant Arrhythmia	1	1	100.0
Cardiomyopathy	1	1	100.0
Respiratory causes			
Hypoxemic respiratory failure	12	10	83.3
Other			
Sepsis	8	2	25.0

Three patients were diagnosed as having congenital heart disease. The patient with transposition of great arteries diagnosis underwent reconstruction of the arcus aorta with arterial switch operation, due to arcus aorta hypoplasia. 18 months after operation, venoarterial ECMO was performed with the diagnosis of heart failure due to aortic re-coarctation. The patient was operated under ECMO support, underwent coarctation repair. He weaned from ECMO successfully. The first patient with total anomalous pulmonary venous

drainage (TAPVD) diagnosis, pulmonary vein stenosis and heart failure developed 6 months after total correction. ECMO was the bridge for transplantation, but he died due to multi-organ failure during ECMO support. The second patient with TAPVD diagnosis was operated on 8 months ago. Venoarterial ECMO was performed with the diagnosis of sepsis, pulmonary hypertension and heart failure. She weaned from ECMO after 8 days support.

Sepsis, eventually superimposed with cardiac or respiratory conditions, was the indication for ECMO in approximately 26.7% (n=8) of cases. The highest mortality was detected in patients who were performed ECMO due to sepsis; 6 patients died, and 5 patients in this group were bone marrow transplant patients. The indications of ECMO are shown in Table III.

The median ECMO period was 8.9 (6.6-10.8) days; 9.35 (6.8-12.2) days for VA-ECMO and 7.3 (6.1-9.4) days for VV-ECMO. Cardiopulmonary resuscitation (CPR) was performed for 8 patients before starting ECMO. CPR was continued during cannulation in 4 patients (E-CPR); 2 of these 4 patients survived, and 2 died. Continuous renal replacement treatment was performed over the ECMO set in 25 patients.

Hemorrhagic complications were detected in 5 (16.7%) patients, neurologic complications were observed in 2 (6.7%) patients, and 3 (10.0%) patients had mechanical complications. Hemorrhagic complications were surgical site bleeding, nasopharyngeal bleeding, hematuria, and hemothorax. Neurologic complications (n=2) were intracranial bleeding and intracranial infarct; decompressive bone surgery was performed to the infarct due to secondary bleeding and due to intracranial pressure increase syndrome. The patient was discharged with mild neurologic sequela after bone correction surgery. Mechanical complications were cannula perforation and position disorder, which required decannulation and recannulation. Recannulation was performed with no need for the set to be changed in any patients.

Survivor and non-survivor patients are compared in Table I. The vasoactive inotropic score was 157 in non-survivor patients at admission to the PICU, and the vasoactive inotropic score was 61 in survivors; however, there was no statistical difference in the comparison of vasoactive VIS, left ventricular ejection fraction (LVEF), PRISM, PELOD, procalcitonin, troponin I, and creatine kinase-MB (CK-MB) values between the two groups. CRP levels were found to be higher in non-survivor patients (p=0.045). Early ECMO initiation was associated with improved survival when compared with late ECMO (p=0.012).

Table IV shows the vital signs and laboratory values for survivors and non-survivor patients on ECMO pre-cannulation and one day after cannulation. Our analysis shows no statistically significant difference between two groups for the following values at pre-cannulation: heart rate, systolic and diastolic blood pressure, arterial oxygen saturation, pCO_2, paO_2, sodium bicarbonate, lactate, hemoglobin, platelet count, urea, albumin, and LDH. On the other hand, white blood cells (WBC) (p=0.029) and C-reactive protein (CRP) (p=0.045) and laboratory values associated with kidney function [pH (p=0.034), creatinine (p=0.047), and chloride (p=0.001)] were statistically significantly different between both groups precannulation.

Furthermore, pH (p=0.001), pCO_2 (p=0.043), HCO_3 (p=0.014), lactate (p=0.002), LDH (p=0.039), and chloride (p=0.001) values were statistically different between the survivors and non-survivors on day-1 after cannulation.

In addition, the comparison of data of pre-cannulation and day-1 showed that the improvement in heart rate beat (p=0.0001), systolic blood pressure (p=0.013), diastolic blood pressure (p=0.001), arterial oxygen saturation (p=0.0001), pH (p=0.0001), pCO_2 (p=0.002), paO_2 (p=0.0001), HCO_3 (p=0.007), and lactate (p=0.002) levels were statistically significant in the survivors compared with the non-survivor patients.

Table IV. The vital signs and laboratory values for survivor and non-survivor patients on ECMO.

Parameters		Survivors (n=20)	Non-survivor (n=10)	p
Heart rate	Initial	166.71±37.45	163.25±33.91	0.827*
	Day-1	121.53±19.52	129±32.2	0.477*
	p†	0.0001	0.005	
Systolic BP	Initial	76.35±19.65	79.75±23.85	0.710*
	Day-1	89.94±13.8	81.63±29.74	0.343*
	p†	0.013	0.877	
Diastolic BP	Initial	43.18±10.82	50.63±18.17	0.210*
	Day-1	55.53±8.78	50.63±22.78	0.440*
	p†	0.001	0.999	
Arterial oxygen saturation	Initial	71.71±18.26	81.38±11.25	0.183*
	Day-1	92.94±2.93	95±3.85	0.157*
	p†	0.0001	0.017	
pH	Initial	7.16±0.14	7.03±0.14	0.034*
	Day-1	7.38±0.04	7.15±0.14	0.0001*
	p†	0.0001	0.086	
pCO_2	Initial	64.3±25.55	78.25±32.67	0.256*
	Day-1	41.72±6.03	50.41±14.07	0.043*
	p†	0.002	0.035	
paO_2	Initial	32.78±19.32	46.23±16.96	0.106*
	Day-1	99.31±32.07	85.6±32.47	0.336*
	p†	0.0001	0.013	
HCO_3	Initial	18.09±7.73	15.3±6.01	0.379*
	Day-1	21.82±5.12	15.55±6.01	0.014*
	p†	0.007	0.924	
Base excess	Initial	-5.8 (-12-0.85)	-8.65 (-12.33-3.85)	0.662‡
	Day-1	-2.4 (-6.25-2.175)	-7.6 (-12.38-1.425)	0.158‡
	p‡	0.009	0.889	
Lactate	Initial	5.1 (2.13-6.85)	6.1 (3.9-10.5)	0.610‡
	Day-1	1.8 (1.3-2.25)	4.5 (2.93-9.6)	0.002‡
	p‡	0.002	0.441	
WBC	Initial	13,170 (5,480-16,480)	23,805 (14,387-52,440)	0.029‡
	Day-1	10,190 (6,030-10,190)	15,615 (8,197-38,587)	0.159‡
	p‡	0.033	0.077	
Hemoglobin	Initial	11±2.32	9.78±2.34	0.223*
	Day-1	9.9±1.82	8.75±0.95	0.106*
	p†	0.077	0.146	
Platelet count	Initial	136,000 (57,000-294,000)	137,000 (74,500-386,000)	0.958‡
	Day-1	109,000 (90,000-176,000)	101,000 (61,500-195,575)	0.671‡
	p‡	0.176	0.263	
Urea	Initial	35 (26.5-57.5)	36.5 (19.75-70.25)	0.838‡
	Day-1	32 (11-62.5)	26 (14-93.25)	0.884‡
	p‡	0.420	0.999	

Table IV. Continue.

Parameters		Survivors (n=20)	Non-survivor (n=10)	p
Creatinine	Initial	0.7 (0.38-0.86)	0.98 (0.72-1.20)	0.047‡
	Day-1	0.54 (0.40-0.78)	0.64 (0.55-1.04)	0.157‡
	p‡	0.138	0.515	
Albumin	Initial	2.78±0.78	2.33±0.69	0.175*
	Day-1	3.12±0.49	2.48±0.64	0.064*
	p†	0.093	0.131	
Chloride	Initial	103.89±4.84	111.67±4.9	0.001*
	Day-1	102.94±3.76	112.67±6.42	0.0001*
	p†	0.148	0.412	
LDH	Initial	716.5 (462.75-2,293.75)	317 (201-3,979.5)	0.391‡
	Day-1	710 (413-1,797)	2,721 (1,448-7,593)	0.039‡
	p‡	0.110	0.999	

*Independent t-test (mean±SD), † Paired t-test, ‡Mann-Whitney U test (median IQR), ‡Wilcoxon test
ECMO: extracorporeal membrane oxygenation, BP: blood pressure (mm/Hg);
pCO_2: partial pressure of CO_2 in the arterial blood (mm Hg); paO_2: partial pressure of oxygen in the arterial blood, platelet count (K/μl), HCO_3: bicarbonate (mmol/L), lactate (mmol/L), WBC: white blood cell count (K/μl), hemoglobin (g/dl), platelet count (K/μl); urea (mg/dl), creatinine (mg/dl), albumin (g/dl), chloride (meq/L), LDH: lactate dehydrogenase (U/L)

Discussion

Mechanical circulatory support has been widely used in the treatment of cardiopulmonary failure in pediatric patients, and many recent studies have reported its outcomes.[3,4] However, many studies have been conducted to determine the indications and factors affecting mortality.[5] The aim of this study was to report our experiences with pediatric critical patients placed on non-postcardiotomy ECMO in the PICU and to determine the variables associated with hospital mortality. We achieved an overall survival rate of 66.7% in our two and a half years' experience, which is comparable with that of the Extracorporeal Life Support Organization (ELSO) registry.[6]

In hypoxemic respiratory failure, ventilation with low tidal volume (5-8 ml/kg), alveolar recruitment with high PEEP, prone positioning and early initiation of neuromuscular blocking agents are recommended. Also, HFOV should be considered as an alternative ventilatory mode in hypoxic respiratory failure in patients in whom plateau airway pressures exceed 28 cm H_2O in the absence of clinical evidence of reduced chest wall compliance. However, in patients who do not benefit from these treatment approaches, hypoxemia may not improve and morbidity and mortality increase. ECMO support therapy is life-saving in patients with severe ARDS, refractory hypoxemia or hypercapnia.

In our study, hypoxemic respiratory failure was detected in 12 patients. The oxygenation index (OI) was >16 or the oxygenation saturation index (OSI) was >12.3 in all patients. High PEEP, lung recruitment maneuvers, prone position and neuromuscular blocking agent were used in all of these patients. In patients with pulmonary hypertension, inhaled nitric oxide was given. HFOV was used in clinically appropriate patients but ECMO supportive treatment was performed because the expected response could not be achieved and hypoxia could not be corrected. VV-ECMO was performed in 6 patients, and VA-ECMO was performed in 6 patients due to accompanying pulmonary HT, catecholamine need, and the effect on cardiac function. The survival rate was 83.3% in 12 patients who received ECMO due to hypoxemic respiratory failure. The survival rate of VV-ECMO was 66%. Carpenter et al.[7] jointly evaluated the pediatric and neonatal patients in their study, and VV-ECMO was performed for 83 patients, and 49 patients (59%) survived.

The most frequent indication was detected as pneumonia in patients who received VV-ECMO in this study. Kim et al.[8] detected the most frequent indication as pneumonia in their study (57.1%), and VV-ECMO was performed in 25 pediatric patients; the survival rate was found as 52%.

Joffe et al.[9] performed a review of 1,755 children with VA-ECMO and found that the cumulative survival after cardiac ECMO in children was 788/1755 (45%). Thourani et al.[10] reported an in-hospital survival rate of 88% in a group of cardiac ECMO patients with cardiomyopathy-myocarditis-arrhythmia, not related to congenital heart diseases (CHD). Kim et al.[8] reported that the survival rate of patients without CHD was 56.5%. Twenty-four patients received VA-ECMO in our study. The survival rate was 66.7%, and the most frequent diagnosis was myocarditis in patients who had ECMO support due to cardiac indications. Patients with myocarditis had acute onset and short time before pediatric intensive care admission. Furthermore patients had no underlying additional disease. All these factors affect the prognosis positively. If we considered any sign of a lack of response to medical treatment, we decided to begin ECMO support. We think that high survival rates in patients with myocarditis are due to early ECMO indication and the short duration between indication and implementation of ECMO. CHD was detected in only 3 patients. (TAPVD in two patients, transposition of great arteries in one patient). The low number of patients with CHD who received ECMO was one of the factors that improved the results.

Laboratory values consistent with impaired kidney function are also common in patients on ECMO. For instance, markers associated with acute kidney injury such as pH, lactate, creatinine levels are among parameters which distinguish outcomes.[11-15] Our study has shown that laboratory values of kidney function pre-cannulation (pH, creatinine) and day-1 (pH, bicarbonate) are associated with poor outcomes similar to other studies.[16] Moreover, our multivariate analysis indicated that pH was an independent predictor of mortality. Chloride, regulate the acid-base balance in the body, and the balance is organized by the kidneys. Chloride is a strong anion that provides the negative load in extracellular fluid and it has a significant role in the acid-base balance. It decreases the glomerular filtration rate and renin activity by performing renal vasoconstriction in patients with hyperchloremia. Hypo- and hyper-chloremia were associated with poor results in critical patients.[16-20] These results showed that kidney dysfunction contributed to mortality in critical pediatric patients.[21]

Urine output declines due to the renal perfusion decrease in the presence of low cardiac flow. The amount of extravascular and interstitial fluid increases, tissue oxygenation, and oxygen presentation to the lungs deteriorates. Blood lactate levels increase as a consequence of poor tissue perfusion in patients.[12,22] Thus, the presence of low cardiac flow and kidney dysfunction result with increased morbidity and mortality.

In our study, metabolic acidosis before and during ECMO was a risk factor of mortality. Consistent with previous studies, pre-ECMO arterial pH was related to survival.[23,24] Severe metabolic acidosis and low arterial pH indicate inadequate circulation, which could cause critical complications and death.[24] Therefore, it is necessary to closely monitor the acid-base status of high-risk patients to decide upon early mechanical circulatory support insertion before irreversible organ damage occurs.

Sepsis remains a major cause of morbidity and mortality in children. The use of predictive and prognostic biomarkers has the potential to improve early recognition and timely intervention of patients with sepsis leading to improved outcomes. We showed that CRP and leucocyte were high in patients who were non-survivors. Leukocytosis and CRP are traditional inflammatory markers for the diagnosis of infection.[26,27] Leukocyte and CRP levels rise in response to infection and high levels have been

found to correlate with the severity of infection, development of multiple organ dysfunction syndrome (MODS), and worse outcome in various studies.[28,29]

We evaluated the trends and predictive value of leukocytosis and CRP levels during treatment of pediatric patients on ECMO. We showed that leukocytosis and high CRP levels were effective factors in mortality, similar to other pediatric patient groups.

In addition, the patients who received ECMO support due to sepsis had the lowest survival. VA-ECMO was performed in 8 patients due to sepsis, and 6 patients died. Three patients who died as having bone marrow transplant, and they were immunosuppressed patients. Zabrocki et al.[30] reported that children with sepsis who were supported with ECMO had a higher risk of mortality than those without sepsis, and Skinner et al.[31] reported that children with sepsis who were on ECMO had an overall survival rate of 68%. Also, immunosuppression is an accepted risk factor for ECMO-associated mortality. Grupta et al.[32] reported that survival of immunocompromised children supported by ECMO was 31%, which was significantly lower than that of non-immuncompromised children (57%).

The ideal timing of ECMO for pediatric patients remains unclear. Steimer et al.[33] reported that adult patients with acute respiratory distress syndrome (ARDS) who were cannulated within 48 hours of admission had an 80% survival rate at 90 days. Liao et al.[34] showed that early establishment of ECMO might improve the prognosis for patients with refractory cardiogenic shock. Our study has shown that early ECMO initiation was associated with improved survival when compared with late ECMO.

In conclusion, this study's collective review of ECMO cases in our pediatric intensive care unit, shows an increasing trend in pediatric ECMO utilization. Although the number of patients is low, we present an acceptable survival rate, low complication rates, and reasonable short-term neurologic outcomes in pediatric ECMO support. In addition, although early-initiated ECMO was demonstrated to improve the results, we argue that leukocytosis, low pH, high CRP, high creatinine, and hyperchloremia at admission are the factors that negatively affect prognosis. Future studies are needed to identify risk factors and strategies to improve outcomes.

There were several limitations to this study. First, patient selection was limited because our study was a retrospective, observational, single-center study. Second, it is limited by the detail and completeness of the collected data. Thirdly, postop cardiac patients were not included in the study because they were followed up in a different intensive care unit. Therefore, the number of patients were limited with 30 patients.

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Original Article

Cord blood delta neutrophil index values of term neonates

Melek Büyükeren[1], Hasan Tolga Çelik[1], Oytun Portakal[2], Erdem Fadıloğlu[3], Gökçen Örgül[3], Şule Yiğit[1], M. Sinan Beksaç[3], Murat Yurdakök[1]

[1]Division of Neonatology, Department of Pediatrics, [2]Department of Biochemistry, [3]Division of Perinatology, Department of Obstetrics and Gynecology, Hacettepe University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

In this study, we aimed to demonstrate cord blood immature granulocyte (IG) count and delta neutrophil index (DNI) values for term neonates. This retrospective study consisted of 126 term newborns born between July 2017 and December 2017. Cord blood samples were collected during delivery and IG count together with DNI values were obtained. "Beckman Coulter DXH800 System Hematology Analyzer" was used for analysis and calculations. The median DNI value was found to be 1.0 (interquantile range(IQR) 0.5-1.8%) and the median gestational age at delivery was 38.4 (IQR 37.6-39.0) weeks. The median birth weight and IG count were 3250 (IQR 2955–3593) g and 66 (IQR 26.5-112.3)/mm^3, respectively. In conclusion, we believe that determining the normal laboratory reference values of IG count or DNI, which are important potential diagnostic markers for neonatal sepsis, will contribute to future studies on the diagnosis of neonatal sepsis.

Key words: cord blood, neonate, immature granulocyte ratio, delta neutrophil index, neonatal sepsis.

Neonatal sepsis is one of the major causes of mortality and severe morbidity in neonatal intensive care units. The overall incidence of neonatal sepsis ranges from one to five cases per 1000 live births.[1] Due to its rapid and fatal course, early diagnosis and immediate initiation of appropriate antimicrobial therapy are critical. Globally, neonatal sepsis and other severe infections accounted for approximately 15 percent of all neonatal deaths.[1]

The clinical signs and symptoms of sepsis in newborns are nonspecific. The gold standard diagnostic method for sepsis is the isolation of the causative microorganism (bacterium or fungus) in one or more blood cultures.[2] However, because the culture results are not immediately availiable and the procedure has certain limitations in newborns, blood cultures are not that useful for early diagnosis of sepsis during the neonatal period.[3] This situation has led to the development of several laboratory methods to facilitate the rapid and accurate diagnosis of neonatal sepsis. The most commonly used biomarkers are the immature-to-total neutrophil ratio (I/T), procalcitonin, C-reactive protein and interleukin-6 values.[4-6] Moreover, immature granulocyte (IG) ratio and delta neutrophil index (DNI) values seem to be potential predictive markers for neonatal sepsis.[7,8] DNI is defined as immature granulocyte fraction provided by a blood cell analyzer which is determined by subtracting the fraction of mature polymorphonuclear leukocytes from the sum of myeloperoxidase-reactive cells.[9] IG count and DNI are reported to be used widely in diagnosis of sepsis and determination of disease severity in critically ill patients.[10]

Recent technological improvements have enabled automated hematology analyzers to determine the IG count and fraction. The term IG is used to describe the myelocytes, promyelocytes and metamyelocytes (i.e., neutrophil precursors) that are found in the bone marrow but not in the peripheral blood

✉ Erdem Fadıloğlu
erdemfadiloglu@hacettepe.edu.tr

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after the neonatal period.[11] On the other hand, it has been reported that immature neutrophil forms enter the circulation during infection.[10] In addition to providing results in a shorter period of time, automatic IG counts are more accurate and precise than manual counts.[11] Cord blood (CB) IG count and DNI are candidates to be used as sepsis markers. However, the normal range of these counts in neonates has not yet been established for CB.

We believe that determining the normal laboratory reference values of IG count and DNI values as important potential diagnostic markers for neonatal sepsis will contribute to future studies on the diagnosis of neonatal sepsis.

Material and Methods

This retrospective study consisted of 126 term normal pregnancies that were delivered between July 2017 and December 2017. The inclusion criteria for CB sampling in this study were as follows: 1) Presence of written maternal informed consent for CB collection; 2) Absence of familial inherited diseases; 3) Negative hepatitis B, hepatitis C, Human immunodeficiency virus (HIV) and human T-cell leukemia virus type-I/II (HTLV) serology; 4) Term pregnancy; 5) No history of early rupture of membrane; 6) Absence of clinical and/or laboratory findings suggesting infection; 7) Absence of prenatally detected chromosomal anomalies and congenital abnormalities in the fetus; 8) Absence of placental abnormalities; 9) Absence of multiple gestations; 10) Absence of maternal chronic inflammatory diseases; and 11) Absence of maternal metabolic and immunological disorders. Pregnant women who did not meet these criteria were excluded from CB sampling.

Cord blood samples were collected from the umblical vein into EDTA tubes after delivery of the fetus and IG count together with DNI values were obtained. The complete blood count data was obtained using a UniCel DxH 800 hematology analyzer (Beckman Coulter, Inc., Brea, CA, USA), and the DNI values were automatically calculated by this device. The UniCel DxH 800 uses electrical impedance, radiofrequency conductivity, and volume, conductivity and multiangle light scattering to count and distinguish between the leukocyte subpopulations.[12]

A total of 179 healthy full-term neonates (born between $37^{0/7}$ weeks and $42^{6/7}$ weeks of gestation) were initially recruited for this research. Of those, fifty-three neonates suspected of having sepsis and/or with missing laboratory data were excluded. Thus, the remaining 126 subjects were included in this study.

Necessary clinical and demographic data were drawn from the electronic registry of Hacettepe University Hospital. All statistical analyses were performed using SPSS version 23.0. A descriptive analysis was performed for the whole cohort and non-parametric tests were used according to performed visual and statistical normality tests. Median values were given for whole parameters and Interquartile Ranges were given for the distrubition of the cases. Prior to conducting this study, ethical approval was obtained from the Hacettepe University Ethics Committee (GO-17/824-29).

Results

The demographic and neonatal characteristics of the 126 patients included in this study are presented in Table I. The median gestational

Table I. Demographic and neonatal data.

Characteristics	n (%)
Gender (M/F)	57/69 (45.2% / 54.8%)
Gestational age (weeks)*	38.4±1.0 (38.4; 37.6–39.0)
Apgar score (5 min)*	9.7±0.8 (10; 10–10)
Resuscitation at birth	4 (3.17%)
Birthweight (g)*	3272±452 (3250; 2955–3593)
SGA/LGA	5/4 (3.96% / 3.17%)

*Mean ± standard deviation (median; 25^{th}–75^{th} percentile)
SGA: small for gestational age, LGA: large for gestational age

Table II. Complete blood count values.

Parameters	Mean ± SD (median; 25th–75th percentile)
Hemoglobin (g/dL)	14.8±1.6 (14.9; 13.5–15.8)
Hematocrit (%)	46.2±5.3 (46.0; 42.0–49.6)
Platelet count (/μL)	239730±70077 (244500; 203500–278500)
White blood cell count (/μL)	11393±4120 (10600; 8900–13250)
ANC (/μL)	6550±3240 (5959.5; 4483.8–7763.0)
DNI (%)	1.3±1.0 (1.0; 0.5–1.8)
IGC (/μL)	94±127 (66; 26.5–112.3)

ANC: absolute neutrophil count, DNI: delta neutrophil index, IGC: immature granulocyte count.

age was 38.4 weeks (interquartile range (IQR) 37.6–39.0 weeks), and the median birth weight was 3250 (IQR 2955–3593) g. Fifty-seven (45%) of the neonates were males (Table I). Complete blood count values of the neonates are shown in Table II. The median DNI value was found to be 1.0 (IQR 0.5-1.8%). Immature granulocyte count was 66 (IQR 26.5-112.3)/mm^3.

Discussion

Sepsis is common in neonatal intensive care units, and it is responsible for significant mortality and morbidity. When treating sepsis, it is critical that therapy targeting the causative agent is initiated as early as possible. However, isolating the causative agent requires time, and it is not always feasible in newborns. Therefore, sepsis markers are more important in this patient group.[2,3] One of the most significant markers is the left shift of neutrophils in the peripheral blood smear, which refers to an increase in the I/T ratio. A value greater than 0.2 reflects an increase in the number of immature neutrophils, which is suggestive of sepsis.[13] Although the I/T ratio is a valuable marker in the diagnosis of neonatal sepsis, it can be unreliable since it is determined based on counting 100 cells in a peripheral blood smear. Therefore, its accuracy is directly proportional to the observer's (counter's) experience.[12-14] We believe that novel neonatal sepsis markers are necessary for better clinical practice.

Nahm et al.[15] reported no statistical difference between the immature neutrophil counts obtained from an automated hematology analyzer and those obtained from a hematologist who manually counted 200 cells from a peripheral blood smear. Lee et al.[7] observed a statistically significant difference in the DNI values between 24 newborns with sepsis and a control group consisting of 48 newborns without sepsis (mean gestational age: 37.0±7.2 weeks, mean birth weight: 2910±650 g), with the sepsis group showing higher values at the time of diagnosis, 24 hours after the diagnosis, and 72 hours after the diagnosis. In the same study, the DNI values at the time of diagnosis in the septic newborns who died, those who survived and the control group were 6.5±2.4%, 3.7±1.8% and 1.1±0.7%, respectively. Cimenti et al.[12] determined a DNI cut-off value of 1.3% based on the DNI values of 21 infants diagnosed with sepsis and 112 controls. In a study by Wiland et al.[8] , the IG percentage of infants with a mean gestational age of 39±1.6 weeks and a mean birth weight of 3187±520 g ranged between 0% and 8.4% within the first 48 hours of life, and it was greater than 1% in 70% of the samples before 12 hours. Therefore, they concluded that the IG percentage threshold of 1% that is used to diagnose sepsis in adults and children is not suitable for newborns during the first 48 hours of life. In their study, Wang et al.[16] reported that the I/T value was the highest at birth (0 hours) in newborns with very low birth weights.

In our study, the median DNI of the CB samples of 126 healthy full-term neonates with a median gestational age of 38.4 weeks (IQR 37.6–39.0 weeks) and a median birth weight of 3250 (IQR

2955–3593)g was 1.0 (IQR 0.5-1.8%). The lowest DNI value was 0% and the highest was 3.9%. The median immature granulocyte count was 66(IQR 26.5-112.3)/mm^3 and it ranged from a minimum of 0/mm^3 to a maximum of 1153/mm^3. The results of this preliminary study may be used in clinical practice for the early prediction of neonatal sepsis. We believe that, further studies are necessary in this field.

The limitations of this study were the lack of a neonatal sepsis group and the small number of cases. On the other hand, this was the first study to demonstrate the normal IG counts and fractions exclusively in the CB of healthy full-term newborns. The fact that preterm infants were not taken into the study is one of the limitations.

We believe that determining the normal laboratory reference values of IG count and DNI values in CB as important potential diagnostic markers for neonatal sepsis will contribute to future studies on the diagnosis of neonatal sepsis.

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Original Article

Evaluation of pentraxin 3 level and cardiac functions in psoriatic children

Mecnun Çetin[1], İbrahim Halil Yavuz[2], Göknur Özaydın Yavuz[2], Kamuran Karaman[3]

Departments of [1]Pediatric Cardiology, [2]Dermatology and [3]Pediatric Hematology, Van Yüzüncü Yıl University Faculty of Medicine, Van, Turkey.

ABSTRACT

Psoriasis is a chronic inflammatory disorder affecting the skin, nails, and joints. Its lifetime prevelance has been estimated to be at 1% to 3%. This study was designed to examine the association between serum pentraxin 3 (PTX3) and cardiovascular function in psoriatic children. Thirty-three children who were diagnosed with psoriasis, and 29 healthy children, between 4 and 18 years of age, were included in the study. Both patient and control group was evaluated by the pulsed wave tissue doppler imaging (TDI) echocardiography as well as with conventional Doppler echocardiography (CDE). PTX3 values of the groups were evaluated. There was no difference between cases and controls for age (9.67±3.72, 9.60±2.84 years, p=0.916, respectively). In evaluation of the left ventricle (LV) CDE; A wave, isovolumic relaxation time (IVRT) and myocardial performance index (MPI) were significantly higher in the study group (p<0.05). Ejection time (ET) was significantly lower in the study group compared to the control group (p<0.05). In evaluation of LV TDI; Deceleration time (DT'), IVRT', E/E' and MPI' were found to be significantly higher in the study group (p<0.05). In addition to, E', E'/A' and ET' were siginificantly lower in study group. PTX3 level was significantly higher in the study group compared to the control group (p=0.009) (Table III). However, no correlation was found between PTX3 level and cardiovascular parameters. In conclusion; both doppler echocardiography and PTX3 may be useful tools for the screening of cardiovascular (CV) risk in these patients. Psoriasis itself may be an independent risk factor for cardiac dysfunction in the pediatric population.

Key words: tissue doppler echocardiography, pediatric, psoriasis, pentraxin 3.

Psoriasis is a chronic inflammatory disease affecting the skin, nails, and joints. Its lifetime prevelance has been estimated to be at 1% to 3%.[1] Currently, psoriasis is accepted as a systemic inflammatory disorder associated with various medical comorbidities.[2] Several epidemiological investigations have reported that adult patients with psoriasis have elevated prevalence of well-known cardio-metabolic risk factors such as diabetes, hypertension, dyslipidemia, obesity and metabolic syndrome compared with the general population, and importantly a clinically marked increased risk of cardiovascular (CV) disease and cardiovascular mortality.[3-5]

✉ Mecnun Çetin
drmecnun@hotmail.com

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Data related with cardiovascular risk in childhood psoriasis is limited, however there are many studies that shows the childhood psoriasis is also associated with an increased occurrence of cardiovascular comorbidities.[6]

PTX3 is an inflammatory molecule and a member of pentraxin superfamily which contains C-Reactive Protein (CRP) and serum amyloid P.[7] PTX3 is produced by different kinds of cells, mainly by dendritic cells, macrophages, fibroblasts, activated endothelia, and by other tissues. It is synthesized in response to proinflammatory stimulants, including bacteria, IL-1, and TNF-alpha produced by primarily endothelial cells, neutrophils, and macrophages.[8] PTX3 has similarity with regards to the structure and actions of CRP which is known as an acute phase reactant. Higher

PTX3 levels were detected during infections, autoimmune diseases, inflamatory conditions and various vasculitis.[9] Serum level of PTX3 especially in children with psoriasis has not yet been investigated.

The association between psoriasis and cardiovascular disease may be explained by sharing chronic systemic inflammatory processes. Investigations with psoriasiform experimental models have showed that sustained skin-specific inflammation is related with increased aortic root vascular inflammation and arterial thrombosis.[10] The present study was designed to examine the association between serum PTX3 and cardiovascular status in psoriatic children.

Material and Methods

Thirty tree children with psoriasis and 29 age and sex matched controls were recruited from Pediatric Cardiology and Dermatology Clinics. Patients with a murmur in the pediatric cardiology outpatient clinic that had no other illnesses were accepted in the control group.

Psoriasis was diagnosed by history, clinical examination and histopathological findings. Psoriasis Area Severity Index (PASI) scoring was performed for the severity of the disease. The patients accepted to the study were those who had not received any treatment for the last month. Height, weight, body mass index (BMI), heart rate and blood pressure were measured in both groups.

Both patient and control groups were evaluated using the pulsed wave tissue doppler echocardiography, as well as with conventional doppler echocardiography (CDE). The PTX3 values of both the patient and control group were evaluated.

Patients with any cardiac disease, systemic inflammatory disease, infectious disease, obesity, or immunological disease were excluded from the study.

All patients' parents gave informed consent to be involved in this study after the study protocol was explained to them before the initiation of the study.

Echocardiography

Echocardiography was performed in the left lateral decubitus and supine position with an ultrasound machine GE Vivid 6S system (GE-Vingmed Ultrasound AS, Horten, Norway) and 3S-RS (3.5 MHz) probe. Averages of three consecutive cycles for all echocardiographic data were measured. Images were obtained from parasternal and apical views using 2D, M-mode and doppler echocardiographic techniques. Examinations were performed by a single pediatric cardiologist. M-mode measurements were evaluated according to standards outlined by the American Society of Echocardiography.[11] Interventricular septum diameter (IVSD) and left ventricular posterior wall diameter (LVpWD) was measured at the end of diastole in parasternal long axis. Left ventricular end-diastolic and end-systolic diameter was measured at the end of systole and diastole in parasternal long axis. Left ventricular (LV) global systolic function was assessed as the mitral annular plane systolic excursion (MAPSE) by the 2-dimensional difference of the end- diastolic and end-systolic lines traced between the center of the ultrasound fan origin and the junction of the LV lateral mitral annulus in the apical 4C view

Conventional doppler echocardiography and Tissue Doppler imaging (TDI) values for all patients were recorded.

Conventional Doppler echocardiography examination: The cursor was placed in the parasternal long-axis view at the junction of the LV exit pathway with the anterior mitral valve to obtain mitral flow and LV outflow pathway simultaneously. E-velocity, A-velocity and deceleration time (DT), isovolumic relaxation time (IVRT) were measured from diastolic function parameters. E velocity; The peak velocity of the fast filling phase of LV, A

velocities; was taken as the peak velocity of late ventricular filling flow and the E/A ratio was calculated. DT was measured as the time between peak E velocities and the point at which the fast filling ends. S-wave, ejection time (ET) and isovolumic contraction time (IVCT) of systolic function parameters were measured.

TDI was recorded from the apical four-chamber view with the pulse wave Doppler sample volume placed on the mitral lateral annulus. Peak systolic (S') velocity, peak early (E'), the deceleration time of E' wave (DT') and peak late diastolic myocardial annular velocity (A'), isovolumic relaxation time (IVRT'), and isovolumic contraction time (IVCT') were measured. Myocardial performance index (MPI') of LV was calculated with the Tei index Formula.[12] (Fig. 1).

Pentraxin 3 (PTX3)

Samples for PTX3 were taken into anticoagulant-free biochemical tubes, the samples were centrifuged at 1500 rpm for 10 minutes after the coagulation process was completed, separated into their sera and stored at -40 C until the day of assesment. The levels of PTX3 was measured with the immunoabsorbent method bound to double antibody enzyme sandwich model in ELISA (Eastbiopharm®, Zhejiang, China). The results were measured as ng/ml for PTX3.

Ethics approval was obtained from the Regional Ethics Board with the decision number 98 of 04.08.2017.

Statistical analysis

The studied variables (characteristics) were presented as mean, minimum and maximum values. Student t test was used to compare Control and Patient group means for the studied variables. Pearson correlation analysis was carried out to examine linear relationships among the variables were considered as 5% and SPSS (ver: 21) statistical program was used for all statistical computations.

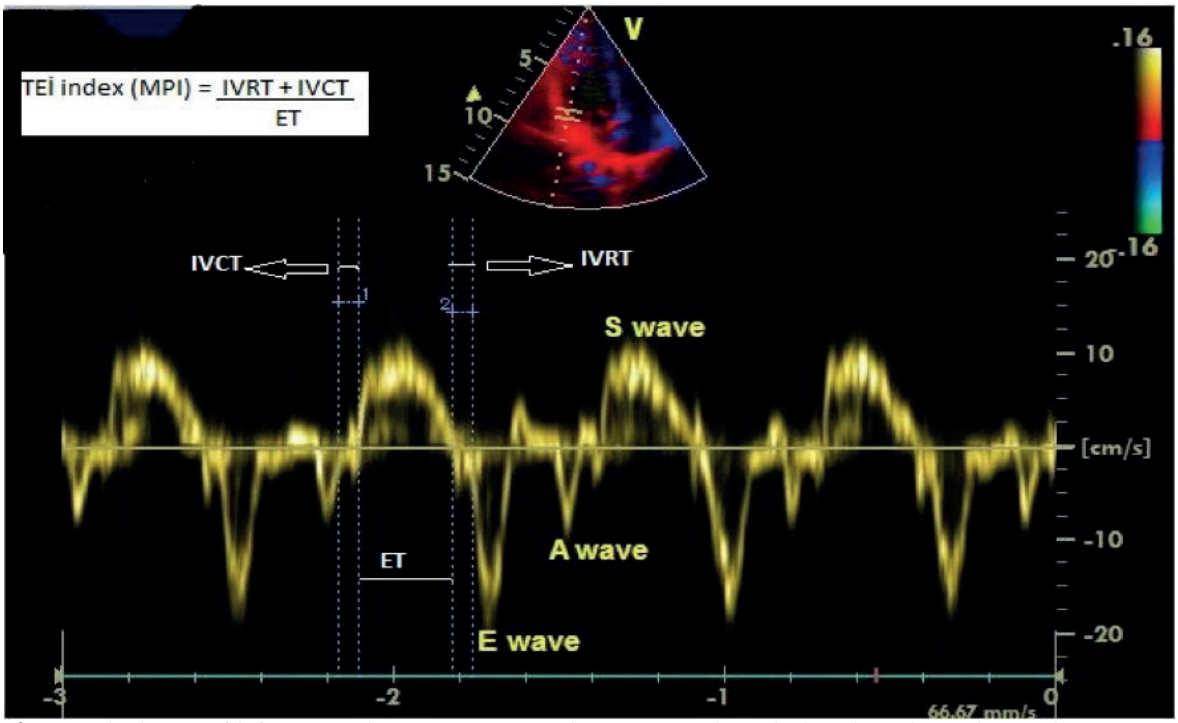

Fig. 1. Calculation of left ventricular MPI, waves and time intervals with doppler echocardiography. S wave, systolic myocardial velocity; E wave, early diastolic myocardial velocity; A wave, late diastolic myocardial velocity; IVRT, isovolumic relaxation time; IVCT, isovolumic contraction time; ET, ejection time; MPI, myocardial performance index.

Results

This study included 33 psoriatic children and 29 healthy controls. There was no difference between cases and controls for age (9.67 ± 3.72, 9.60 ± 2.84 years, p = 0.916, respectively). Mean psoriasis duration time was 2.6 ± 1.9 years. Systolic blood pressure (sBP) was significantly higher in study group. Comparison between weight, BMI, height, dBP and heart rate revealed no statistically significant difference betwen groups (Table I).

Echocardiographic data

Comparison between LVedD, LVesD, LVpWD, IVSD and MAPSE revealed no statistically significant difference between groups in M-mode echocardiographic examination (p>0.05) (Table I).

In evaluation of LV convantional doppler echocardiography; A wave, IVRT and MPI were significantly higher in study group (p <0.05). ET was significantly lower in study group compared to control group (p <0.05). No significant difference were detected between the study group and control group regarding all other variables (p>0.05) (Table II).

In evaluation of LV TDI; DT', IVRT', E/E' and MPI' were found to be significantly higher in the study group (p <0.05). In addition, E', E'/A' and ET' were siginificantly lower in the study group (Table III).

PTX3 level was significantly higher in the study group compared to the control group (p = 0.009) (Table III). Although the PTX3 level was higher in the study group, no significant correlation was found between PTX3 and MPI, MPI', PASI, disease duration, MAPSE and other echocardiographic parameters (Table IV).

Discussion

This study was designed to examine the association between serum PTX3 and cardiovascular condition in psoriatic children. Our study is the first study which measure PTX3 level and examine cardiac functions with TDI in psoriatic children. In our study, we documented the occurrence of subclinical systolic and diastolic deterioration in psoriatic children.

Table I. General characteristics and m-mode echocardiographic findings of groups.

	Study (n = 33)	Control (n = 29)	P
Age (years)	9.69 ± 3.72	9.60 ± 2.84	0.916
Sex (M/F)	17/16	14/15	
Weight (kg)	33.39 ± 16.33	32.38 ± 14.23	0.557
Height (cm)	132.5 ± 20.5	135.0 ± 17.3	0.615
BMI (kg/m^2)	17.8 ± 3.9	16.6 ± 2.6	0.151
Psoriasis duration (y)	2.6 ± 1.9		
PASI	5.9 ± 4.2		
sBP (mmHg)	109.0 ± 10.2	102.2 ± 7.9	0.006
dBP (mmHg)	67.6 ± 11.4	65.5 ± 8.3	0.401
HR (beat/min)	88.5 ± 19.7	84.8 ± 12.4	0.398
LVedD (mm)	38.3 ± 9.1	36.6 ± 3.8	0.352
LVesD (mm)	23.7 ± 4.5	22.8 ± 2.9	0.372
LVpW (mm)	4.4 ± 1.0	4.0 ± 0.7	0.069
IVSD (mm)	4.2 ± 1.0	3.9 ± 0.7	0.125
MAPSE (mm)	12.9 ± 1.8	13.0 ± 1.7	0.745

BMI: Body mass index; sBP: sistolic blood pressure; dBP: diastolic blood pressure; HR: heart rate; LVedD: left ventricle end-diastolik diameter; LVesD: left ventricle end-sistolik diameter; LVpWD: left ventricle posterior wall diameter; IVSD: interventricular septum diamater; MAPSE: mitral annular plane systolic excursion.

Table II. Conventional doppler echocardiographic parameters of groups.

Parameter	Study (n = 33)	Control (n = 29)	P
E (cm/s)	82.1 ± 11.6	80.4 ± 11.7	0.569
A (cm/s)	49.9 ± 13.1	43.2 ± 9.5	0.026
E/A	1.73 ± 0.46	1.93 ± 0.45	0.095
S (cm/s)	79.6 ± 16.26	85.3 ± 10.8	0.117
DT (ms)	89.7 ± 19.5	84.5 ± 12.9	0.234
ET (ms)	241.1 ± 19.4	257.5 ± 17.0	0.001
IVRT (ms)	71.2 ± 16.5	58.9 ± 12.4	0.002
IVCT (ms)	66.9 ± 16.4	68.0 ± 13.0	0.768
MPI	0.57 ± 0.10	0.49 ± 0.09	0.002

E: early diastolic myocardial velocity; A: late diastolic myocardial velocity; S: systolic myocardial velocity; DT: deceleration time; IVRT: isovolumic relaxation time; IVCT: isovolumic contraction time; ET: ejection time; MPI: myocardial performance index.

Table III. Tissue doppler imaging parameters and PTX3 values of groups.

Parameter	Study (n = 33)	Control (n = 29)	P
E′ (cm/s)	15.33 ± 3.41	17.55 ± 2.64	0.006
A′ (cm/s)	7.21 ± 1.89	6.72 ± 1.19	0.239
E′/A′	2.21 ± 0.62	2.67 ± 0.52	0.003
E/E′	5.71 ± 2.21	4.67 ± 0.94	0.023
S′ (cm/s)	9.81 ± 2.44	9.06 ± 1.22	0.141
DT′ (ms)	74.90 ± 12.59	64.03 ± 11.62	0.001
ET′ (ms)	256.5 ± 29.3	271.7 ± 20.8	0.024
IVRT′ (ms)	60.30 ± 10.36	52.65 ± 11.19	0.007
IVCT′ (ms)	66.51 ± 15.33	61.89 ± 9.23	0.163
MPI′	0.50 ± 0.11	0.42 ± 0.07	0.003
PTX3 (ng/ml)	5.89 ± 5.00	3.10 ± 2.61	0.009

E′: early diastolic myocardial velocity; A′: late diastolic myocardial velocity; S′: systolic myocardial velocity; DT′: deceleration time; IVRT′: isovolumic relaxation time; IVCT′: isovolumic contraction time; ET′: ejection time; MPI′: myocardial performance index; PTX3: pentraxin 3.

Table IV. Correlation between parameters.

		MPI	MPI′	PASI	Duration	MAPSE	IVRT′	IVRT	E/A	E′/A′
PTX3	r	+.130	+.008	+.056	-.073	+.071	-.014	+.169	+.167	+.226
	p	0.471	0.965	0.759	0.686	0.693	0.940	0.348	0.353	0.205

E: early diastolic myocardial velocity; A: late diastolic myocardial velocity; IVRT: isovolumic relaxation time; MPI: myocardial performance index; PTX3: pentraxin 3; PASI: Psoriasis area severity index; MAPSE: mitral annular plane systolic excursion.

In recent decades, the association of psoriasis with several comorbidities, such as obesity, metabolic syndrome, systemic hypertension, dyslipidemia, type 2 diabetes, malignancies and inflammatory bowel diseases have been documanted. This association between psoriasis and comorbidities, especially cardiovascular and metabolic diseases, may be related to their chronic and inflammatory nature, especially due to elevated pro-inflammatory cytokines that are mandatory for the occurrence the pathophysiology of such disorders.[10,13,14]

Psoriasis is accepted as a systemic inflammatory disease, several studies have evaluated PTX3 and CRP levels in psoriatic patients. Numerous studies have shown that plasma PTX3 levels are increased in patients with psoriasis.[7,15,16]

In our study, PTX3 was significantly increased in psoriatic patients (pointing to possible role in pathogenesis of psoriasis). However, there was no significant correlation between PTX3 and risk factors of psoriasis such as PASI and psoriasis duration. There was also no significant correlation between PTX3 and doppler parameters (Table IV). The finding of no correllation between PTX3 and PASI found in our study may be due to the occurence of systemic inflammation earlier than phenotypic presentations in psoriatic children.

The results of our study showed that echocardiographic parameters were negatively affected in the study group compared to those in the control group. Conventional doppler echocardiography and TDI are important techniques to evaluate cardiac function. Prolongation of IVRT and DT, decrease in maximum E wave velocity and E/A ratio, and elevation in maximum A wave velocity show diastolic dysfunction.[17] Prolongation of IVCT and shortening of ET showed systolic dysfunction. MPI, which is a indicator for both systolic and diastolic functions of the heart, is a parameter which is easy-to-apply, reproducible parameter independent of ventricular geometry, blood pressure, and heart rate changes.[18] An abnormal increase in MPI, measured by CDE and TDI, indicates ventricular global dysfunction (both systolic and diastolic).

To the best of our knowledge, there are no studies evaluating psoriatic childhood heart with tissue doppler echo. There is only one study done by Salam et al.[10] with conventional doppler echocardiography. Salam et al.[10] found that IVRT and DT values were significantly higher in children with psoriasis than the control group. They reported that diastolic dysfunction developed in psoriatic children. In a study by Ozden et al.[19] which evaluated the adult psoriatic patients with doppler echocardiography stated that there is significant changes in parameters that indicate subclinical cardiac dysfunctions (High MPI, low E/A, and E/E ratio). In our study, parameters indicating diastolic dysfunction; E' wave and E'/A' ratio were lower in the study group, DT', IVRT' and A wave were higher in the study group. ET, the indicator of systolic dysfunction, was significantly lower in the study group. In our study, MPI values measured with both TDI and CDE were significantly higher in study group than the control group (Table II, III). Thus, our result in psoriatic patients shows the systolic and especially diastolic dysfunction of the heart.

Moreover, we can say that MPI which is practically easy to perform can be used as an early marker of cardiac dysfunction in the follow-up of these patients.

In this study, a relatively new and more sensitive method, TDI, was used to detect subclinical cardiac involvement in psoriatic childhood patients. Both doppler echocardiography and PTX3 may be useful tools for the screening of CV risk in these patients. However, there was no significant relationship between TDI and PTX3 levels. This can be explained by the population size of the study. In these patients, psoriasis may have an independent CV risk factor, causing cardiac disfunction. In addition, larger and longer-term studies are necessary to evaluate clinical implications of our finding.

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Original Article

Infection risk after paediatric liver transplantation

Mukadder Ayşe Selimoğlu[1], Samime Kaya[1], Şükrü Güngör[1], Fatma İlknur Varol[1], Harika Gözde Gözükara Bağ[2], Sezai Yılmaz[3]

Departments of [1]Pediatric Gastroenterology, Hepatology, and Nutrition, [2]Biostatistics and [3]Liver Transplantation Institute, İnönü University Faculty of Medicine, Malatya, Turkey.

ABSTRACT

Infections after liver transplantation (LT), despite prophylactic therapy, are still important causes of morbidity and mortality in children. Although underlying disease and immunosuppression along with the complexity of LT procedure are the major predispositions to infections, there still might be under recognised factors predisposing infections in paediatric LT. In this study, we retrospectively analysed the risk factors of bacterial, viral, and fungal infections after LT in a series of 167 children (median =5 yr.). Of all children, 112 (67%) experienced infections: 93 (55.7%) bacterial, 56 (33.5%) viral and 15 (9%) fungal. Multilogistic regression analysis showed that the need of immunosuppressive switch increased total, bacterial, and viral infection risk 5.3, 2.5, and 2.5 times, respectively, (p=0.001, p=0.021, and p=0,019, respectively). Re-LT increased bacterial infection risk 4.2 times (p=0.040). Viral infection risk was 10 times higher in children who had more than two re-laparotomies (p=0,002). Children who had post-LT, cytomegalovirus (CMV) infection had 5.6 times increased risk for fungal infection (p=0.035). In conclusion, infection is still an important morbidity in paediatric LT and is in close relationship with other morbidities such as surgical complications. CMV infection, itself, is an independent risk factor for fungal infection.

Key words: children, infection, liver transplantation.

Infections after liver transplantation (LT), despite prophylactic therapy, are still important causes of morbidity and mortality in children. Of patients undergoing LT, 80% experience at least one infection episode within the first year of LT, of which some result in death.[1,2] Nevertheless infections are the most common cause of mortality after LT in some centres.[3] Underlying disease and immunosuppression along with the complexity of LT procedure are the major predispositions to infections.[1] However, there still might be under recognised factors predisposing to infections in paediatric LT.

To the best of our knowledge, there is not any paediatric study investigating the risk of all infections, bacterial, viral, and fungal, after LT. In this study, we retrospectively analysed the risk factors of bacterial, viral, and fungal infections after LT in a series of 167 children.

✉ Mukadder Ayşe Selimoğlu
ayseselimoglu@hotmail.com

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Material and Methods

One hundred and sixty seven children who underwent LT for acute or chronic liver failure (ALF and CLF, respectively) between January 2011 and December 2015 at the Liver Transplantation Institute of İnönü University Faculty of Medicine were included in that study.

Medical reports and culture results were analysed retrospectively. Any sign or symptom suggesting infection including fever, hypothermia, graft dysfunction, or clinical detoriation were the indications of sampling for culture. Only culture proven bacterial infections were included. Ampicillin sulbactam is routinely used for the prophylaxis after liver transplantation and tacrolimus or cyclosporine along with corticosteroids is used

for immunosuppression. Corticosteroids were used for only 3 months. Drug switch was made when acute rejection or adverse effects occurred. Prophylactic acyclovir was used for 3 months.

Viral serology for cytomegalovirus (CMV) and Epstein–Barr virus (EBV) was studied every three months and in case of graft dysfunction. Only PCR positive cases were analysed.

The study protocol was approval by the Institutional Ethics Committee of İnönü University (No: 2017/3-7). SPSS version 17.0 (SPSS Inc. Chicago, IL) was used for statistical analysis; student's t test and Mann-Whitney-U test for the comparison of parametric and nonparametric data of two groups, respectively, and ANOVA and Kruskal Wallis tests for the comparison of more than 2 groups. Multilogistic regression analysis was used to confirm the risk factors. $p< 0.05$ was considered as significant.

Results

Mean age of the children was 7.1±5.4 years (5 months-18 years; median=5.5 yr.). Of the children, 90 (54%) were boys and 77 (46%) were girls. While 79 (46.7%) had had end stage liver disease and underwent elective LT, the remaining 88 (53.3%) had had ALF which necessitated urgent LT. Living donor LT (LDLT) and deceased donor LT (DDLT) were performed in 118 (70.7%) and 49 (29.3%) patients, respectively. Mean intensive care stay was 15.1 ± 23.0 (median=8; range=2-173) days and ward stay was 21.0 ± 18.2 (median=16; range=0–81) days. Vascular (thrombosis/stenosis) and biliary complications (leak/stenosis) developed in 36 (21.5%) and 11 (6.6%) patients, respectively; 4 (2.4%) experienced both. Laparotomy and ostomy were performed in 78 (46.7%) and 13 (7.8%) patients, respectively, after LT. Fifteen patients (9%) needed re-LT.

Of all children, 112 (67%) experienced infections: 93 (55.7%) bacterial, 56 (33.5) viral and 15 (9%) fungal (*all Candida spp.*). The most common bacterial agents were *Escherichia coli* and *Klebsiella spp*, respectively. CMV and EBV infections were detected in 23.4% and 15.6% of children, respectively. Effects of demographic, pre- and post-LT features on the development of post-LT infections were evaluated.

Demographic and pre-LT features

Bacterial infection frequency was higher in girls compared to boys (65% vs. 48%; p=0.026). Mean age and primary diagnosis distribution were not different in those with or without bacterial infection (p>0.05). However, mean ages of those with or without viral infection was different (62.4±58.8 mo. vs. 97.2±64.8 mo.; p=0.001). As a supportive finding, viral infection was more common in children younger than 2 years old (p=0.01) (Fig. 1).

Mean height Z score was lower in those who had bacterial infection (p=0.039; -1.1 ±1.8 vs. -0.5 ±1.5). Children were classified as having weight Z score under or above -2 SD (underweight or not) and height Z score under or above -2 SD (stunted or not). Infection frequency was not different regarding underweight or stunting (p=0.905 and p=0.45). Viral and fungal infection prevalence was higher in children with cholestatic liver disease (50% vs. 30%; p=0.028, and 21.9% vs. 5.9%; p=0.01, respectively).

Type of LT, LDLT or DDLT, urgent or elective, did not affect the frequency of total, bacterial or viral infection (p>0.05). However, in children with DDLT, fungal infections were more common compared to LDLT (16.5% vs. 6%, p=0.041).

While presence of pre-LT ascites increased the risk of total and bacterial infections (p=0.01 and p=0.02, respectively), encephalopathy had no effect (p>0.05). Mean Pediatric End-Stage Liver Disease/ Model for End-Stage Liver Disease (PELD/ MELD) and Child-Pugh scores were higher in children with infection (all) (p=0.02 and p=0.06). Mean PELD/MELD score was also higher in those with viral infection (p=0.04).

While children with congenital heart defects had higher fungal infection prevalence (23% vs. 5.3%, p=0.017), presence of hepatopulmonary syndrome did not affect the frequency (p>0.05).

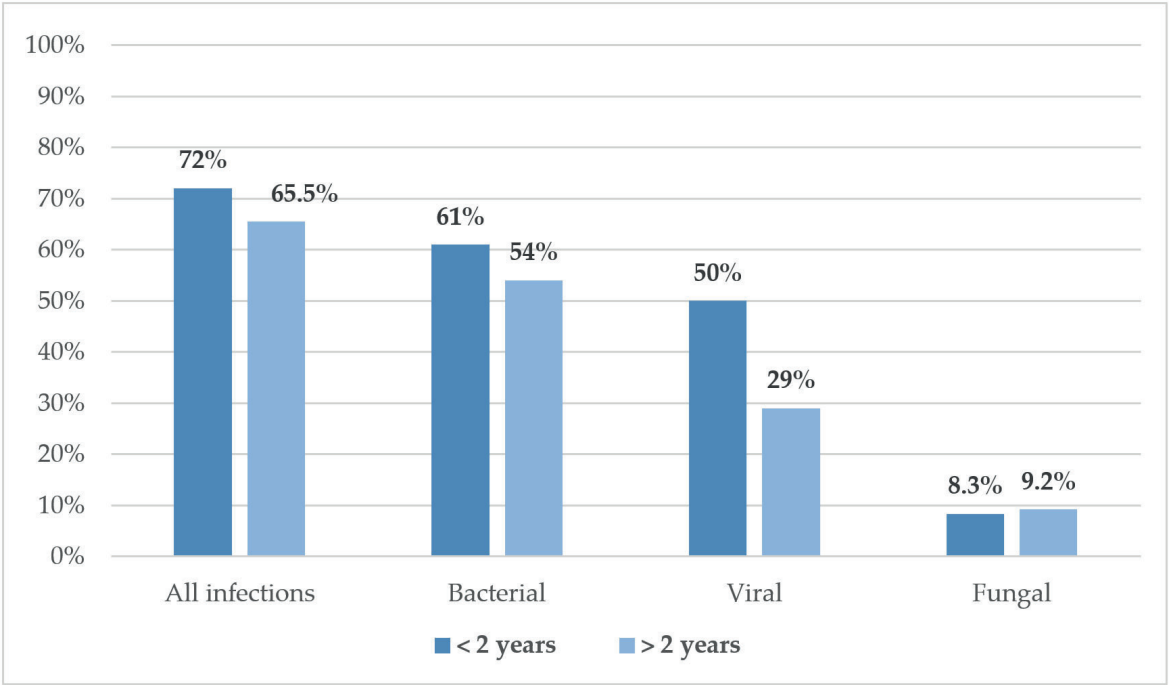

Fig. 1. Infection rates in respect with the age of the children.

Mean pre-LT hospital stay was not different in children with or without infection (p>0.05). Having a positive culture result for bacteria before LT was not an indicator of post-LT infection (p>0.05). Pre-LT seropositivity for EBV or CMV did not affect post-LT viral infection prevalence (p>0.05).

Post-LT features

The longer the intensive care unit (ICU) stay, the higher total infection, bacterial, viral, and fungal infection rates (Table I). Bogota bag usage was not associated with higher infection rates (p>0.05). Biliary complications increased total and bacterial infection rates (p=0.02 and p=0.01, respectively), and intestinal perforation increased viral infection rate (p=0.017). Re-laparotomy was associated with higher total and viral infection rates (p=0.003, and p=0.03, respectively). Re-LT increased only the bacterial infection rate (80% vs. 53.3%, respectively; p=0.047).

Table I. Relationship between hospital stay and infection.

		ICU Stay (D)		Ward Stay (D)	
		Mean ± SD	p	Mean ± SD	p
Infection	(+)	18.5 ± 27.0		24.3 ±20.0	
	(-)	8.0 ± 6.0	<0.001	14.2±11.0	0.02
Bacterial Infection	(+)	20.0 ± 29.0		25.0 ± 20.6	
	(-)	9.0± 7.0	<0.001	16.1±13.3	0.005
Viral Infection	(+)	20.8 ±30.2		25.8 ± 20.0	
	(-)	12.2 ±18.0	0.03	18.6± 16.8	0.02
Fungal Infection	(+)	26.5±30.0		28.0 ± 22.4	
	(-)	14.0 ± 22.0	0.001	28.0 ± 22.4	0.16

ICU: Intensive care unit

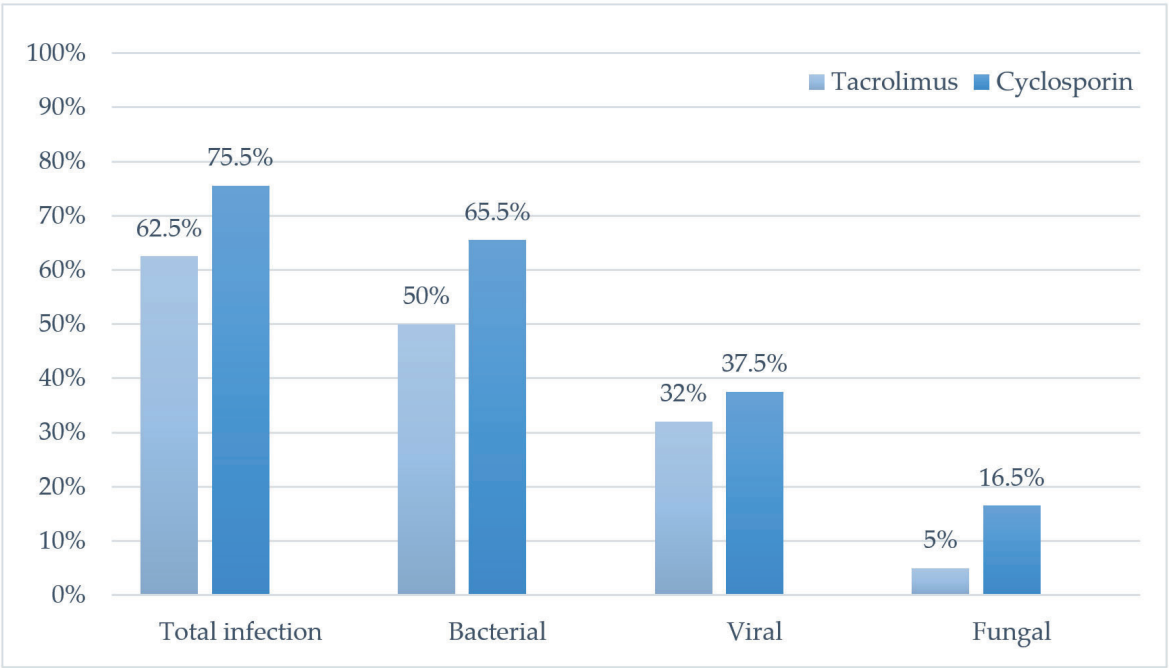

Fig. 2. Infection rates in respect with the type of immunosuppression.

Type of immunosuppressive agent did not increase the total infection, bacterial or viral infection risk (p>0.05) but fungal infection rate was higher in those on cyclosporine compared to tacrolimus (16.5% vs. 5%, p=0.01) (Fig. 2). Need for immunosuppression switch was associated with higher total, bacterial, viral, and fungal infection rates (p<0.001, p=0.003, p=0.002, and p=0.03, respectively).

It was observed that 20.5% and 5.5% of children with or without post-LT CMV, respectively, experienced fungal infection (p=0.004); there was no effect on bacterial infection rate (p=0.16).

Data were analysed with multilogistic regression analysis and it was found that need of immunosuppressive switch had 5.3, 2.5, and 2.5 times, respectively, increased total, bacterial, and viral infection risk (p=0.001, p=0.021, and p=0,019, respectively) (Table II). Re-LT increased bacterial infection risk 4.2 times (p=0.040) (Table II). Viral infection risk was 10 times higher in children who had re-laparotomy more than 2 (p=0,002) (Table II). Children who had post-LT CMV infection had 5.6 times increased risk for fungal infection (p=0.035) (Table II).

Discussion

This study showed us that 67% of children experienced infections, most of which were bacterial (55.7%) and viral (33.5). Pre-LT characteristics have important influences on post-LT morbidity and mortality. In this study we found mean PELD/MELD score to be higher in those who developed post-LT infection, which means that the disease severity is one of the factors predisposing to infections. In a study performed on adult patients MELD score was not found effective on infection development.[4]

In accordance with the findings of Avkan-Oğuz et al.[5], we did not find pre-LT encephalopathy to be a risk for post-LT infection. On the other hand, re-laparotomy and biliary complications were reported as risk factors for infection[6,7], like we found in our study.

When bacterial infections were evaluated, it was observed that none of the factors including primary diagnosis, disease severity, age at LT, or pre-LT hospitalization duration was responsible. While Kim et al.[8] reported similar findings in 144 LT patients, in another study it

Table II. Risk factors for post-LT infection according to logistic regression analysis.

	OR	95% Confidence Interval	p
Total Infection Risk			
Immunosuppressive Switch	5.351	1.942-14.748	0.001
Bacterial Infection Risk			
Immunosuppressive Switch	2.561	1.150-5.703	0.021
Re-LT	4.298	1.071-17.257	0.040
Viral Infection			
Immunosuppressive Switch	2.539	1.167-5.521	0.019
Re-Laparotomy >2	10.125	2.361-43.420	0.002
Fungal Infection			
Post-LT CMV Infection	5.618	1.133-27.867	0.035

LT: Liver transplantation
CMV: Cytomegalovirus

was shown that bacteraemia risk was higher in patients with higher Child Pugh score.[9] We found that total infection (bacterial, viral, and fungal) frequency was correlated with the score (p=0.038).

Nafady-Hega et al.[10] reported a correlation between bacterial infections and length of pre-LT hospital stay as opposed to the findings of our study. They observed no relationship between pre-LT ascites and bacterial infection[10] but we observed that pre-LT ascites increased both total and bacterial infection frequency (p=0.01 and p=0.02), though no spontaneous bacterial peritonitis was detected.

Most of the LT candidate children with chronic liver disease have malnutrition and it is accepted that malnutrition, especially chronic type, increases the complications and mortality.[11] Moukarzel et al.[12] showed that children having a height Z score under -1 were at risk of bacterial sepsis, invasive fungal infection, surgical complication, and re-LT. Likewise we found that mean height Z score, an indicator of chronic malnutrition, was lower in those who had bacterial infection (p=0.039) but that was not the case in viral or fungal infections.

Among post-LT factors, long ICU stay increased the bacterial infection risk as expected and shown before.[8] However it is not clear whether bacterial infections cause longer ICU stay or vice versa. Re-laparotomy may be one of the causes of longer ICU stay and hence higher infection rates, which was observed in our and others' studies.[5] Re-laparotomy may well represent the surgical complications such as biliary leak and intestinal perforations; which were also shown to increase bacterial infections after LT.[10,13] Not intestinal perforation but biliary leak was found to increase bacterial infection in our series (p=0.01). Length of post-LT ward stay was also longer in those with bacterial infection.

Viral infections are also common after LT due to immunosuppression mostly during post-LT 1-6th months and the most common viral pathogen is CMV as shown in many studies.[14,15] We found that age at LT, disease severity, cholestatic nature of the disease, intestinal perforation, and the length of ICU and ward stay were effective factors for the development of viral infection. Hadley et al.[16] reported the relationship between ICU stay and viral infections, as well.

Prevalence of fungal infection after LT was reported to be 5-40%, mostly of *Candida spp*.[17,18] We found the prevalence as 9%; all *Candida spp*. CMV is a well-defined risk factor for post-LT fungal infections.[19] In our study, fungal infection was detected in 20.5% and 5.5% of patients with or without CMV infection, respectively

(p=0.004). We found CMV infection to be an independent risk factor which increased fungal infection risk 5.6 times (OR=5.618, p=0.035). Fungal infection rate was higher in DDLT compared to LDLT (16.5% vs. 6%, p=0.03). This was not the case in an adult series.[20]

While Hadley et al.[16] revealed that type of immunosuppressive used had no statistical effect on the fungal, viral, or bacterial infection rate, we found that children on cyclosporine had higher fungal infection rate (p=0,01).

In the final evaluation, the need of immunosuppressive switch was the independent risk factor for total, bacterial and viral infection development. Re-laparotomy and re-LT were also independent risk factors for bacterial and viral infections, respectively.

In conclusion, infection is still an important morbidity in paediatric LT and is in close relationship with other pre- and post-LT morbidities such as malnutrition, disease severity, and surgical complications. Starting before LT, strategies aiming at reducing those morbidities could lead to lower infection rates.

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Original Article

Red blood cell variables and correlations with body mass components in boys aged 10-17 years

Jasmina Pluncevic Gligoroska[1], Serjoza Gontarev[2], Vesela Maleska[1], Ljudmila Efremova[1], Daniela Shukova Stojmanova[2], Sanja Manchevska[1]

[1]Institute of Physiology and Anthropology, Faculty of Medicine, and [2]Faculty of Physical Education, Health and Sport, University Ss Cyril and Methodius, Skopje, Republic of Macedonia.

ABSTRACT

The objective of this study was to analyze the hematologic parameters and their correlation with body composition components in healthy boys at pubertal age. One hundred and ninety physically active male subjects, aged 10 to 17 years, mean age 13.87 ±4.5 years, were included in the study. Capillary blood was drawn from all subjects and the following hematologic parameters were measured: RBC, Hct, Hb, MCV, MCH, MCHC. The following body components derived from Matiegka anthropometric method were assessed: muscle mass (MM), bone mass (BM), and body fat mass (BF). The mean values (±SD) of hematologic parameters were: RBC= 4.87±0.41 x 10^{12}/L, Hb=14.24±1.24 g/dL, Hct=43.83±3.8%. Anthropometric characteristics were as follows: body mass index (BMI) = 20.26± 3.27 kg/m^2, relative muscle mass (MM%) = 53.18± 3.19%, bone mass (BM%) = 18.83± 2.4% and body fat percentage (BF%) = 15.19± 2.64%. Correlation analysis between hematologic parameters and body composition showed a moderate to strong correlation between RBC, Hb and Hct and all body components. The strongest correlations were found between Hb and Hct, and muscle mass (r= 0.60; r= 0.61) and lean body mass (r= 0.59). The body fat mass showed also a positive association with RBC (r=0.47); Hb (r=0.47) and Hct (r=0.48). Our findings showed that the relationship between anthropometric measures and RBC variables in healthy physically active boys were positively correlated, but the level of association was higher with skeletal muscle mass.

Key words: red blood cells, hemoglobin, hematologic indices, body composition, boys.

Changes in the physical appearance that happen during the rapid growth periods of life are accompanied with developmental changes in all physiological systems. Normal physical development of young people is reflected in the changes of body components, increase in muscle and bone mass, and decrease in fat component.[1-3] Blood as the vitally important physiological system also undergoes big changes in the adolescent period, which are manifested with a significant increase in the hematologic markers and attaining levels established in the adult age.[4]

✉ Jasmina Pluncevic Gligoroska
jasnapg965@yahoo.com

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During puberty there is often an imbalance between the increased bodily needs for energetic and building food components and the acquisition of inappropriate food habits, which could lead to different deficiencies, such as iron deficiency anemia.[5-7] Therefore, the assessment of nutritional status in adolescents is very important for proper assessment of growth of adolescents. The complete nutritional status includes estimation of body composition and various biochemical analyses, especially evaluation of hematologic parameters.[8] Hematologic parameters such as hemoglobin, hematocrit and red blood cell count (RBC) are useful for the assessment of nutritional status in healthy individuals.[9] The association between hematologic markers, especially hemoglobin and serum iron level, with body composition

are investigated in different populations, regarding age, sex and health status.[10,11] The findings predominantly have shown a positive association between hematologic parameters and skeletal muscle mass and an inverse association with body fat and body mass index.[12-14]

The aim of this study is to investigate the correlation between skeletal muscle mass (SMM) and RBC variables. We hypothesized that the association between RBC variables and lean body mass (LBM) is stronger than the association with fat mass. Therefore, it was our aim to determine the strength of association between body mass components and hematologic parameters in healthy adolescent boys.

Material and Methods

The study population included 190 male participants, physically active boys, aged between 10 and 17 years, randomly selected from the subjects who participated in regular pre-participation examination (PPE) in the Center of Sports Medicine, Institute of Physiology, Medical Faculty, UKIM, Skopje, Republic of Macedonia between March and July 2016.

Hematologic analysis

Hematologic testing was part of the complete medical check–up for sport pre-participation screening, during morning hours (from 8:00 to 12:00 am) in a controlled laboratory with constant temperature (between 20°C and 24°C) and humidity. The blood samples were collected from capillary vessel using sterile plastic containers with anticoagulant (EDTA K3) incorporated in its walls. An experienced evaluator was in charge of the collection procedures. Analysis was done by automated hematology analyzer ABX Micros 60-OT (ABX hematology, Montpelier, France). The following hematologic parameters were analyzed: red blood cell (RBC) count, hematocrit (Ht), hemoglobin concentration (Hb), mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC).

Anthropometric measures

Body composition was assessed using the anthropometric measurement protocol by Matiegka. Besides the standing height and weight, the following anthropometric parameters were also measured: Five limb circumferences (upper arm relaxed and flexed, forearm, thigh and calf), four limb diameters (wrist, elbow, ankle and knee) and seven skinfolds (biceps, triceps, forearm, subscapular, thigh, calf, suprailiac). All measurements were performed on the right side of the body. Participants' height was measured to the nearest 0.1 cm with a fixed stadiometer (Holtain Ltd., Crymich, U.K.) and body weight to the nearest 0.1 kg using the SECA beam balance (Seca, Hamburg, Germany). Harpenden skinfold caliper (British indicators Ltd., Luton) was used to measure skinfold thickness with 0.1 mm accuracy and the ankle diameter was measured using Vernier caliper. Elastic tape was used to measure circumferences with 0.01 accuracy. An anthropometry expert performed the anthropometric measurements according to the guidelines of the International Society for the Advancement of Kinanthropometry. Personal information of the participants (full name, date of birth, activity record) as well as anthropometric data were filled in on special forms. The final parameters from Matiegka's body composition assessment were muscle mass (MM), bone mass (BM) and fat mass (FM) expressed as absolute values in kilograms and the same parameters expressed as relative values in percentage of the whole body mass: MM%, BM% and FM%. Lean body mass was also assessed (LBM).

Ethics

The study was undertaken in compliance with the Declaration of Helsinki and approved by the Human Research Ethics Committee of the Medical Faculty, UKIM, Skopje (IRB number: 03-5338/5). The children and their parents gave

written informed consent after having been introduced to the procedures, benefits and possible risks of participation in the study.

Statistical Analysis

Data were analyzed using the statistical program for Windows, STATISTICA, version 7.1. Data were inspected for normal distribution using the Kolmogorov-Smirnov test. Results are expressed as mean ± SD (standard deviation), and minimum and maximum values are also presented. Multivariate analysis was used to assess significant differences for anthropological and hematologic parameters between age different groups ($p<0.05$). The strength of the association between the variables was estimated by Pearson correlation coefficient. Statistical significance was set at $p \leq 0.05$. Multiple regression analysis between RBC variables as dependent variable and body composition parameters as predictors (constant) was made to assess the influence between analyzed variables.

Results

Table I outlines anthropometric characteristics and hematologic parameters of the participants. The mean age of the participants in this study was 13.37 ± 2.77 years (10-17). All analyzed parameters were in the normal value range for a specific parameter. Anthropometric characteristics were as follows: Body mass index (BMI) = 20.26 kg/m^2, relative muscle mass (MM%) = 53.18%, which suggested that the muscle mass was well developed. The mean value of bone mass (BM%) was 18.83, suggesting that the subjects were in an active growth phase. The body fat percentage (BF%) was 15.19%, which is considered as an optimal value for this parameter for physically active boys. 95.8% of all participants had normal red blood count (RBC) within the interval of normal range for this parameter (3.87 to 6.32 x 10^{12}/L), with RBC mean value of 5.02 x 10^{12}/L. Hemoglobin concentration varied from low levels (11g/dL) in only eight subjects (4.2%) to very high levels of 17.9 g/dL (in one subject),

Table I. Anthropometric characteristics, body composition and hematologic parameters of male adolescents (N=190).

	Mean	SD	min	max
Age (year)	13.87	4.5	10	17
Height (cm)	163.83	15.8	129.6	186
Weight (kg)	55.68	16.9	26	100
BMI (kg/m^2)	20.26	3.27	14.38	33.62
LBM (Lean Body Mass) kg	46.56	14.23	14.9	80.07
MM (Muscular mass) kg	30.02	9.73	12.93	60.1
MM%	53.18	3.19	40.85	61.81
BM (Bone mass) kg	10.45	2.8	5.56	23.45
BM%	18.83	2.14	12.43	26.13
BFM (Body fat mass) kg	8.64	3.64	3.55	31.22
BF%	15.19	2.64	11.06	28.33
RBC (10^{12}/dl)	4.87	0.41	3.87	6.32
Hb (gr/dl)	14.24	1.24	11	17.9
Hct (%)	43.83	3.8	34	53.8
MCV (μm^3)	86.47	3.74	72	94
MCH (pg)	28.15	1.7	19.9	31.8
MCHC (g/dl)	32.54	1.25	27.7	35.8

MM: muscular mass; BM: bone mass; FM: Fat mass; LBM: lean body mass; BMI: body mass index; RBC: red blood cells; Hb: hemoglobin, Hct: Hematocrit; MCV: Mean corpuscular volume; MCH: mean corpuscular hemoglobin, MCHC: mean concentration of hemoglobin

within narrow confidence interval of 14.08 g/dL to 14.63 g/dL, with mean level of 14.24 g/L. The mean values of hematologic indices, MCV (which measures mean cell volume), MCH (which shows the amount of hemoglobin in one average erythrocyte) and MCHC (which shows mean cell Hb concentration), were in the range of normal reference values.

Table II summarizes anthropometric and hematologic parameters for three age different subgroups: group U12: boys aged 10 to 12 years, group U14: 12 to 14 years, and U17: 14 to 17 years old. Since the age span included a broad period of growth and development (10-17 years), due to the lack of physical examination of puberty stage (Tanner's staging) we divided the participants into three groups which covered the different stages of growth. The majority of anthropometric and hematologic parameters were statistically different between the different age groups, with a tendency to rise with increasing age. Only bone mass percentage was higher in younger groups. The body fat percentage (BF %) was insignificantly different in all three groups.

The values of the Pearson correlation coefficient (r) between RBC variables and body mass components which are expressed in kilograms (MM – muscle mass; BM - bone mass; FM - fat mass) and relative body mass components, expressed as percentage of whole body weight (MM%; BM%; FM%) are displayed in Table III. Correlation analysis between hematologic parameters and body composition components showed moderate to good correlations between red blood cell counts, hemoglobin levels and hematocrit levels with all body components. The strongest correlations were found between hemoglobin and hematocrit levels and muscle mass (r = 0.60; r = 0.61) and lean body mass (r = 0.59). Negative correlations were found between all hematologic parameters and relative bone mass (BM%).

Table IV shows the results of the multivariate regression analysis between RBC variables as

Table II. Anthropometric characteristics, body composition and hematologic parameters of male adolescents divided into different age groups (N=190).

	U12 (N=70)	U14 (N=70)	U17 (N=50)
Weight (kg)	40.91 (9.89)^{bc}	57.35 (15.85)^{ac}	66.41 (12.21)^{ab}
Height (cm)	148.29 (8.59)^{bc}	165.08 (10.48)^{ac}	177.21 (7.83)^{ab}
MM (kg)	20.87 (5.13)^{bc}	28.72 (7.62)^{ac}	35.97 (8.22)^{ab}
MM (%)	51.21 (2.72)^{bc}	52.77 (2.77)^{ac}	53.76 (3.37)^{a}
BM (kg)	7.91 (1.5)^{bc}	9.51 (2.75)^{a}	9.45 (1.25)^{a}
BM (%)	19.82 (1.71^{c}	19.08 (2.28)^{c}	18.21 (4.11)^{ab}
BF (kg)	6.59 (2.73)^{bc}	9.10 (4.05)^{ac}	9.75 (2.87)^{a}
BF (%)	16.02 (3.02)$^{\&}$	16.10 (3.71)$^{\&}$	15.94 (6.21)$^{\&}$
LBM (kg)	33.24 (6.82)^{bc}	44.96 (9.80)^{ac}	54.89 (9.52)^{ab}
BMI (kg/m^2)	18.75 (3.01)^{bc}	20.39 (3.57)^{ac}	21.65 (3.42)^{ab}
RBC (10^{12}/dl)	4.83 (0.33)^{bc}	5.08 (0.40)^{ac}	5.21 (0.51)^{a}
Hb (gr/dl)	13.31 (0.96)^{bc}	14.32 (1.15)^{ac}	14.92 (1.19)^{ab}
Hct (%)	40.84 (4.89)^{bc}	44.20 (3.37)^{ac}	45.91 (3.52)^{ab}
MCV (μm^3)	84.24 (3.54)^{bc}	86.88 (4.49)^{a}	87.97 (4.80)^{ab}
MCH (pg)	27.09 (1.82)$^{\&}$	27.34 (2.06)$^{\&}$	28.38 (2.36)$^{\&}$
MCHC (g/dl)	32.51 (1.25)$^{\&}$	31.42 (2.90)$^{\&}$	31.98 (1.56)$^{\&}$

U12: 10 to 12 years; U14: 12-14 years; U17: 14- 17 years old

* significant differences between different age groups was significant at level $p < 0.005$

$^{\&}$ no significant difference with other groups; ^{a} significantly different from U12; ^{b} significantly different from U14; ^{c} significantly different from U17

Table III. Correlations between hematological parameters and body components.

	MMkg	MM%	BMkg	BM%	FMkg	FM %	LBM	BMI
RBC (10^{12}/dl)	0.474*	0.114	0.416*	-0.321*	0.424*	0.109	0.439*	0.374*
Hb (gr/dl)	0.603*	0.254*	0.577*	-0.301*	0.477*	0.019	0.586*	0.352*
Hct (%)	0.615*	0.177*	0.574*	-0.329*	0.487*	0.024	0.590*	0.397*
MCV (μm^3)	0.380*	0.163*	0.401*	-0.101	0.212*	-0.156*	0.399*	0.124
MCH (pg)	0.246*	0.212*	0.283*	-0.006	0.133	-0.110	0.268*	0.014
MCHC (g/dl)	-0.021	0.171*	0.006	0.066	-0.018	-0.003	-0.005	-0.109

* Pearson correlation coefficient was significant at the 0.05 level
MM: muscular mass; BM: bone mass; FM: Fat mass; LBM: lean body mass; BMI: body mass index; RBC: red blood cells; Hb: hemoglobin, Hct: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin, MCHC: Mean concentration of hemoglobin.

Table IV. Multiple regression analysis between RBC variables as dependent variable and body composition parameters as predictors (constant).

Dependent variable	Sum of Squares	Mean Square	F	p
RBC (10^{12}/dl)	7.246	1.208	8.895	0.0001
Hb (gr/dl)	99.950	16.658	15.896	0.0001
Hct (%)	1008.765	168.127	17.929	0.0001
MCV (μm^3)	505.571	84.262	7.456	0.0001
MCH (pg)	54.841	9.140	3.293	0.004
MCHC (g/dl)	8.602	1.434	0.882	0.509

RBC: red blood cells; Hb: hemoglobin; Hct: Hematocrit; MCV: Mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean concentration of hemoglobin.

a dependent variable and body composition parameters as predictors (constant). At multivariable level there was a statistically significant influence on analyzed variables. Only one hematologic variable, MCHC, showed a non-significant association with body composition parameters, as it was the case in the correlation analysis as well. Regression analysis at univariate level showed an inverse relationship between Hb, Hct, MCV and BMI.

Discussion

To the best of our knowledge this is the first study that investigated the relationship between body composition and hematologic parameters in healthy Macedonian boys. Our goal was to determine the strength of the association between body components and red blood cell variables in this population group. The main finding of our study is that higher lean body mass and skeletal muscle mass are associated with higher levels of hemoglobin, red blood cell counts, hematocrit and hematologic indices.

Participants in this study were healthy young children/adolescents who were actively involved in physical activities, that is, they participated in different sports on a regular basis. The body composition analysis showed average values of body components indicating a good level of growth and body development in our young participants. The eventual influence of physical activity on body components and hematologic status was not taken into consideration in this study.

Regarding the correlations between red blood cell variables and body components, we found positive associations between all items of both groups of parameters. Total erythrocytes count or RBC showed a moderate positive correlation with all three body mass components (expressed in kilograms): muscle mass (MM) r=0.47, bone mass (BM) r=0.41 and body fat mass (BF)

r=0.42. Hemoglobin concentration and Hct showed almost the same level of association with hematologic parameters: Strong positive correlation with MM (r=0.60), lean body mass (LBM, r=0.59), bone mass (r=0.57) and moderate positive correlation with BF (r=0.48) and BMI (r=0.35). Hematologic indices showed weaker associations with body components. MCV and MCH, showed stronger associations with active body components, LBM, MM and BM than with BF. However, we should bear in mind that the participants were healthy physically active adolescents, who predominantly had optimal body composition, which might explain why all body components, including the fat one, have shown a positive correlation with hematologic parameters.

In the literature, the largest number of studies related to the association between hematologic parameters and anthropometric indices have been conducted in adults and overweight or obese children. However, very few studies have examined individuals with normal body weight, and even more scant are data presenting results about children populations between the ages of 10 and 17 years regarding this issue. The majority of data about the association between body composition and hematologic parameters refer to the correlation between body fat mass and serum iron level.

Rates of iron deficiency are higher in overweight children and adults compared to their normal weight counterparts.[13-16] Although the reason for this is not completely clear, the theory that increased circulating hepcidin-induced iron intake in gastrointestinal system is a scientifically appropriate explanatory mechanism.[17] It has been noticed that obese children and adolescents may be prone to anemia despite their excessive dietary and caloric intake.[18,19] The prevalence of anemia in underweight and overweight children is higher than in subjects with desirable weight.[20] The rate of iron deficiency (ID) among obese and overweight children is twice the rate of children with normal weight. Almost one of every ten overweight adolescent has ID.[13] In his study (thesis) Ahlgrim[21] found that high fat mass was associated with small blood volume per kg. The amount of adipose tissue, which is almost avascular, strongly influences the standardization of values of blood volume per kilogram body mass weight.[22] Some authors suggest that FFM (fat free mass) could be used as an anthropometric reference for the blood parameters because of the high correlation coefficient between them.[23]

Studies have reported that obesity has an adverse effect on iron metabolism, which is registered with low serum iron and low hemoglobin levels.[25] A large study confirmed that overweight children were twice as likely to be iron deficient comparing to normal weight children.[13] In their study conducted among school children in Iran, Moayeri et al.[19] found that the prevalence of iron deficiency increased with subject's body mass index. A similar study was performed in the adult population in Iran, but the correlation between BMI and hemoglobin, MCV, serum iron and other hematologic indices was not found.[24] In the Third National Health and Nutrition Examination Survey (NHANES III) the association between serum ferritin and adiposity was analyzed. The study reported a moderately positive correlation between serum ferritin and obesity indices: WHR (r=0.36); BMI (r=0.34); waist circumferences (r=0.34) and sum of skinfolds (r=0.32).[25] There has been substantial evidence suggestive of an association between iron status and body fat mass. However, a few studies failed to determine the association between iron deficiency and BMI and body fat. The contradictory findings of this study may be due to the good health and nutritional status of the investigated group (young women, military recruits).[26] The results obtained in our study showed a positive relationship between majority of RBC variables and body fat mass, but lower intensity regarding the relationship with active body components, LBM, muscle and bone components.

In the published literature there are few data on the relationship between lean body mass i.e. muscle mass and hematologic markers. Our results are in agreement with the existing

ones in similar studies. The level of association between RBC variables and BMI, LBM and muscle mass were similar to the other reported findings. In a study of the relationship between body composition and complete blood count in Korean university students, a moderate positive correlation was found between RBC, Hb, Hct and hematologic indices and weight, height, BMI, skeletal muscle mass and basal metabolic rate. The strongest correlation coefficient was found between SMM and Hb (r=0.663), Hct and weight (r=0.536). Body fat percentage (BF%) showed weak to moderate negative correlation with RBC, Hb, Hct and indicies (-0.39; -0.434; -0.432; -0.169, respectively).[27] A correlation analysis between body composition and blood parameters carried on a group of one hundred students revealed a high correlation coefficient between hemoglobin and body weight (r=0.65) and lean body mass (r=0.69).[28] Higher levels of hemoglobin were associated with higher muscle density, higher muscle area and lower fat area.[22] In a study that analyzed the association between anthropometric parameters and biochemical profile, positive associations between RBC, Hb and Hct and BMI were observed (r=0.53, 0.65, 0.61; all $p<0.001$).[24] Hemoglobin levels are associated with muscle body component (obtained by peripheral quantitative computed tomography, pQCT), and occur in the presence of anemia in elderly people.[25]

The findings of other studies predominantly showed a positive association between hematologic parameters and skeletal muscle mass and an inverse association with body fat and body mass index. Our results demonstrated that young healthy physically active boys with proper body weight and composition showed positive correlations between hematologic parameters and all body components (muscle mass, bone mass and body fat), but the strongest correlation was found between hemoglobin level and hematocrit with skeletal muscle mass and lean body mass. The knowledge and understanding of the relationship between RBC variables and body composition parameters could be important in healthcare planning for children and youth.

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Original Article

Mother-child interaction and the development status of children who have been accidentally poisoned

Ganime Ayar[1], Tülin Köksal[1], Funda Kurt[1], Aysun Kara Uzun[1], Bahar Çuhacı Çakır[1], Birgül Bayoğlu[2], Sıddıka Songül Yalçın[3]

[1]Ankara Children's Hematology Oncology Training and Research Hospital, University of Health Sciences, Ankara; [2]Unit of Child Development, Bayındır Hospital, Ankara; [3]Division of Social Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Positive parent-child interaction, in particular bond between mother and child, is important for the mental and behavioral development of children. The aim of this study was to evaluate both mother-child interactions as well as the developmental status of children admitted to the pediatric emergency department with accidental poisoning using Parenting Interactions with Children: Checklist of Observations Linked to Outcomes tool (PICCOLO) and Denver Developmental Screening Test-II (DDST-II). Children between ages 1 to 5 years who were admitted to the emergency department with accidental poisoning were included in the study alongside a control group selected from healthy volunteers. A ten-minute video recording was obtained both for the case and control groups, while the mother and her child played together in a separate room. The interaction of mother-infant pair was assessed using the PICCOLO tool. The children's development was examined using the DDST-II. The video recordings of 115 children (n=65 in the case group and n=50 in the control group) were evaluated. A high score of PICCOLO-teaching domain (≥9 points) was associated with a 3.3-fold increase in terms of risk of poisoning [p<0.05, at 95% confidence interval (CI) of 1.34-8.37]. Multivariable analysis revealed that the PICCOLO-teaching domain was a significant factor. A high proportion of cases had either abnormal or questionable DDST-II scores (p<0.05). In order to improve the bond between mother and child, drug poisoning prevention training must be meticulously provided to both mothers and children alike. Developmental assessments of these children as a holistic approach also should not be forgotten.

Key words: child, intoxication, mother-child interaction.

Poisoning is one of the most common medical problems that children are exposed to.[1] It is more so encountered in children under 5 years of age. Accidental poisoning accounts for 67.4% of all poisoning cases, of which mortality accounts for 2% of cases. Among these, the mortality rate of drug-related poisoning cases is 1.3%.[2] These rates indicate the importance of preventing accidental poisoning. It is certain that classical measures are crucial, and that they should be implemented in order to prevent poisoning.[3] In addition, it is well documented that parental behavior influences a child's health and their social outcomes. Positive parenting practices are associated with the positive social skills of children; moreover, good parental practices can protect children from an unfavorable outcome.[4] The basis of a healthy mother-child relationship depends upon how the mother behaves, which in turn shapes personality traits of the child. The quality of this interaction with the child determines how children ultimately behave.[5-7] The literature fails to show whether there is any relationship between accidental poisoning and the bond between parent and child. Therefore, we set out to evaluate this, as well as assess the developmental status in children presented with accidental poisoning.

✉ Ganime Ayar
ganimeayar@gmail.com

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Only part of the results (preliminary study) were presented as a poster presentation at the 15th WAIMH Conference (World Association for Infant Mental Health), May 29-June 2, 2016, Prague, Czech Republic (P551 ID 706).

Material and Methods

After approval from the Institutional Ethics Committee (Ankara Child Health and Diseases Hematology Oncology Training and Research Hospital, protocol code: 2015-017), we prospectively included asymptomatic children under the age of 5 and who were admitted to the emergency department between July 2015 and June 2016 due to accidental poisoning or accidental drug intake. The mother-child bond was assessed using a tool known as the Parenting Interactions with Children: Checklist of Observations Linked to Outcomes (PICCOLO). The developmental steps of both the healthy as well as poisoned children were assessed using the Denver Developmental Screening Test II (DDST-II). The control group comprised of healthy children within the same age range who were admitted to the outpatient clinic. Those who were enrolled in both groups included children with a gestational age of >37 weeks, a birth weight of >2500 g, and no background of any genetic syndromes, neurological diseases, or other chronic diseases. Parents were informed about the study, and written informed consent was obtained. Those who did not give permission or consent to be videotaped were excluded from the study.

The characteristics of the children, their families, and the details relating to the poisoning events were learned through a questionnaire developed by the researchers.

A room filled with children's toys in which the mother and the child could play together was provided both to the control group as well as the poisoning cases. The same environment and same toys were used for all of the participating children. Ten-minute videotapes were made while the mothers played with their children. After filming, DDST-II was performed on the children. Then, the mothers were provided education on child development and care depending on their respective scores.

PICCOLO is a scoring system that evaluates a healthy child-parent interaction and identifies problems, that also gives anticipatory guidelines. The video footage was evaluated using the PICCOLO tool created by Roggman et al,[8] with a checklist of 29 observable behaviors in order to assess the parenting interaction in four domains: affection, responsiveness, encouragement, and teaching. Each of the four PICCOLO domains included seven to eight items of observable parenting behavior, each with a short label and a more detailed description of the behavior. Each item was scored as 0 (absent, no behavior was observed), 1 (barely, brief, minor, or emerging behavior was observed), or 2 (clearly, definite, strong, or frequent behavior was observed). A score of 42 and above was considered to be a high for PICCOLO-total, 11 and above was considered to be high for PICCOLO-affection, PICCOLO-responsiveness, and PICCOLO-encouragement, and 9 and above was considered to be high for PICCOLO-teaching. It has been translated into Turkish and validated.[9] We used the Turkish version of the tool.[9]

Statistical analysis

The sample size calculation for the present study was performed using G*Power V3.1.9. Given that no study of a similar nature presently exists in the literature, the effect size could not be calculated. In taking the mean effect size as suggested by Cohen as 0.5[10] with a 5% error and 80% power, the sample size required in the independent groups was determined to be 51 for each group and 102 in total. Statistical analysis was performed using IBM SPSS Statistics for Windows V 22.0 (IBM Corp., Armonk, NY, USA). The scores were compared to the predetermined values, and the mean scores were calculated. The chi-square and Mann-Whitney U tests were used for the comparison of data upon seeking normality. Multiple logistic regression analysis was performed after controlling gender, children's age, mother's age range, mother's employment status, mother's educational level being of high school or higher, being the first child, presence of a child aged ≤5 years at home, and number of households being ≥5. A p value of ≤0.05 was considered as being statistically significant.

Results

The video footage of all 115 children was examined, and the PICCOLO scores were calculated. Baseline variables are mentioned in Table I. There were no significant differences between the case and control groups in terms of gender and age of the children, the mother's age, level of educational, employment status\ being the first child, having a sibling or a child under the age of 5 years at home and whether the child is being cared for at home or attending kindergarten or not (Table I).

Analgesics, antipyretics, and decongestants ranked first at a rate of 29.9% among the drugs ingested accidentally. Two cases involved the ingestion of more than one drug. Of these cases, 75% had ingested the drugs within their own homes (Table II).

The relationship between accidental drug ingestion and the PICCOLO scores for each domain alongside the PICCOLO-total score is summarized in Table III. A high score of PICCOLO-teaching domain (≥9 points) was associated with a 3.3-fold increase in the risk of poisoning [$p<0.05$, at 95% confidence interval (CI) of 1.34-8.37]. However, high PICCOLO-affection scores, high responsiveness, and high encouragement domain, and PICCOLO total scores were not significant ($p>0.05$). Multivariable analysis revealed that PICCOLO-teaching domain was a significant factor after adjusting the children's gender and age groups, the mother's age and level of education, birth

Table I. Comparison of the case and control groups in terms of general characteristics.

Characteristics	Case Group (n=65) n (%)	Control Group (n=50) n (%)	p
Children's gender, male	33 (50.8)	26 (52.0)	0.896
Children's age ≤24 months	25 (38.5)	26 (52.0)	0.373
Birth order, being the first child	26 (40.0)	26 (52.0)	0.200
Presence of a sibling	38 (58.5)	31 (62.0)	0.701
Presence of a child aged <5 years at home	16 (24.6)	10 (20.0)	0.557
Number of households ≥5	24 (36.9)	19 (38.0)	0.906
Mother's age ≤24 years	17 (26.2)	11 (22.0)	0.774
Mother's educational level, high school or higher	24 (36.9)	26 (52.0)	0.106
Mother as the caregiver	60 (92.3)	41 (82.0)	0.137
Mother's employment status, employed	5 (7.70)	8 (16.0)	0.163

Table II. Data on poisoning.

Parameters	n (%)
Drug taken	
Analgesic, antipyretic, decongestant	20 (29.9)
Non-corrosive chemical substance or methyl alcohol	9 (13.4)
Antipsychotic, antiepileptic	6 (9.0)
Iron preparation	4 (6.0)
Insecticide	3 (4.5)
Other	25 (37.3)
Total (two cases had more than one poison ingested)	67 (100)
Place of poisoning	
Home	49 (75.4)
Outside home	16 (24.6)

Table III. Relationship between drug ingestion and PICCOLO scores.

	Univariable analysis OR (95% CI)	p	Multivariable analysis* OR (95% CI)	p
PICCOLO-Affection (≥11 points)	0.68 (0.18-2.61)	0.580	0.59 (0.14-2.34)	0.450
PICCOLO-Responsiveness (≥11points)	1.89 (0.57-6.26)	0.298	2.27 (0.61-8.46)	0.221
PICCOLO-Encouragement (≥11 points)	1.80 (0.49-6.58)	0.373	1.32 (0.33-5.24)	0.696
PICCOLO-Teaching (≥9 points)	3.35 (1.34-8.37)	0.010	3.22 (1.17-8.80)	0.023
PICCOLO-Total (≥42 points)	1.01 (0.22-4.54)	0.983	1.66 (0.33-8.17)	0.533

CI: confidence interval, DDST-II: Denver Developmental Screening Test II, OR: odds ratio.
*Adjusted for gender, children's age, mother's age range, mother's employment status, mother's educational level being of high school or higher, being the first child, presence of a child aged ≤5 years at home, and number of households being ≥5.

order, the presence of a child at home under 5 years of age and the number of households.

A comparison of the median scores of PICCOLO for each domain alongside PICCOLO-total score between the case and control groups were summarized in Table IV. The PICCOLO-responsiveness, encouragement, teaching and PICCOLO-total scores were significantly higher in the case group. When it came to whether the PICCOLO scores were high or not between the case and control groups, there was a significant difference with the exception of the PICCOLO-affection. The frequency of an abnormal or questionable result obtained from the DDST-II was 61% in the case group, and 40% in the control group. A higher proportion of cases had abnormal DDST-II scores ($p<0.05$) (Table V).

Discussion

Previous studies have indicated that poisoning more commonly occurs due to the ingestion of medications and caustic/corrosive substances.[11] Just as the literature indicates, we found that one-third of the cases were poisoned only due to medical drugs, whereas nearly half of the cases were poisoned due to a mixture of

Table IV. Comparisons of scores of PICCOLO for each domain and PICCOLO-total score between the case and control groups.

PICCOLO Scores	Case Group (n=65)	Control Group (n=50)	p
PICCOLO-Affection	12 (9-13); [6-14]	10 (7-13); [4-14]	0.094
PICCOLO-Responsiveness	13 (11-14); [5-14]	11 (9-13); [2-14]	0.011
PICCOLO-Encouragement	12 (7.8-12); [3-14]	10 (9-13.5); [1-14]	0.010
PICCOLO-Teaching	9 (6.5-12); [1-15]	6.5 (4-10); [0-14]	0.001
PICCOLO-Total	45 (36.5-51); [22-56]	39.5 (28.8-46); [11-55]	0.002

Data is presented as median (Q1-Q3); [minimum - maximum]

Table V. Comparison of high PICCOLO scores and DDST-II between two groups.

	Case Group, n (%)	Control Group, n (%)	X^2	p
PICCOLO-Affection (≥11 points)	41 (63.1)	23 (46.0)	3.34	0.068
PICCOLO-Responsiveness (≥11 points)	52 (80.0)	30 (60.0)	5.53	0.019
PICCOLO-Encouragement (≥11 points)	44 (67.7)	22 (44.0)	6.49	0.011
PICCOLO-Teaching (≥9 points)	38 (58.5)	14 (28.0)	10.59	0.001
PICCOLO-Total (≥42 points)	43 (66.2)	22 (44.0)	5.64	0.018
DDST-II (Abnormal or Questionable)	40 (61.5)	20 (40.0)	5.25	0.022

DDST-II: Denver Developmental Screening Test II, X2: Chi-square.

medical drugs and chemicals. Moreover, our results demonstrated that 75.4% of the cases were accidentally poisoned at home, which was also similar to the literature.[12]

According to the main outcomes of our study, there were no significant differences between the case and control groups in terms of mother's age, level of education, or employment status. Moreover, none of these parameters were found to be a risk factor for the occurrence of accidental poisoning among the children. Based on the outcomes of a recent study on the epidemiological characteristics of accidental poisoning during childhood revealed that children living in households where addictive substances were used and as children whose mothers' were employed (and who are not a housewife) were at higher risk for accidental poisoning.[13] Another study reported that parents who were insufficient when it came to supervising their children posed a greater risk when it came to poisoning children under 6 years of age.[14] The studies have mainly highlighted the critical role of parents' inattentiveness.[15]

The common conclusion in the studies on accidental childhood poisoning was that the necessary preventive measures should be taken, not only at a national level and but also at home, and that they should be implemented adequately in order to prevent children from accidentally poisoning themselves.[16] In our study, this issue has been investigated from the perspective of parental interactions with their children. This approach is based on the fact that the behavior, action, and skills of parents while interacting with their children have been shown to be quite effective in correcting children's behavioral problems.[17] Different methods such as the dependency theory, learning theory, the social learning theory all focus on the improvement of parental interactions and are targeted towards preventing child abuse and negligence.[18]

Evaluating how well parents interact and communicate with their children is another critical sub branch of this subject. In our study, the degree of communication between parents and children was evaluated using the PICCOLO tool, as it provides concrete data on developmental guidance between parent and infant, helps determine and organize the potential needs of both the mother and children, and encourages the strengthening communication while identifying problems.[19-22] In our study, the PICCOLO scores were significantly higher among the poisoned group presented than among the control group. In particular, a high score within the domain of mother-child-teaching (≥9 points) was associated with a 3.3-fold increase in the risk of ingested medical drugs. According to these results, the parent-child interaction was higher among the case group than among the control group. This finding was contradictory to the literature, whereby it suggests a lower level of poorer parent-child interaction in the case group. The literature suggests that family negligence, the supervision of the child by people other than parents, and parents not paying attention to dangerous activities of children at home were underlined in being some of the reasons behind the occurrence of accidental childhood poisoning.[23] According to the results of our study, a high proportion of children who experienced poisoning had either abnormal or questionable DDST-II results. What is more, the PICCOLO teaching scores of mothers were higher in the group with intoxication. There might be various underlying factors behind this: a) unfavorable health problems as experienced by the child might have led to an increased amount of care by the family towards the child, b) the high interaction with their children might have led children to act more bravely, to be more inquisitive and curious, and to be more prone to taking risks, c) in considering the absence of children who showed poisoning-associated symptoms or who were seriously intoxicated within the course of the present study, it could be thought that the mother group with a high PICCOLO score had exhibited overprotective behavior, and moreover had frequently visited the hospital on suspicion of their child being poisoned.

The fact that we performed PICCOLO after the occurrence of the poisoning event makes it difficult to completely understand the cause of this situation.

There are some strengths and limitations of the study. How mothers behave towards their children may change because they are more likely to feel guilty at the moment of poisoning. In being mindful of this, the videotapes were made prior to the patients being discharged in order to minimize bias. In addition, 10 min video recording is necessary to evaluate parent-infant interaction. During this long time of one on one interaction the capacity of parent about the domains of affection, responsiveness, encouragement, and teaching can become visible. There are studies in which this tool was used that have been done involving different groups, including children with developmental retardation, children from different ethnicities and low-income dyads.[7,8,24] However, this study is the first of its kind to use PICCOLO for accidental poisoning cases. PICCOLO is not a tool that is able to predict the future, it cannot define any pathology in parents, it is an evaluation tool that relies on observations, and it only focuses on positive parent behavior.[9]

In conclusion, encouragement without adequate measures being taken towards prevention of accidents may lead to an increase in the number of accidents. In order to improve the bond and therefore interaction between parent and child, education must be provided in a meticulous manner in order to prevent poisoning accidents. In addition, it appears that poisoning conditions tend to be observed more among children with developmental delays, and therefore it is felt that their developmental status ought to be evaluated more closely as well.

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Original Article

Factors affecting the success of pediatric extracorporeal shock wave lithotripsy therapy: 26-year experience at a single institution

Fuat Kızılay[1], Turan Özdemir[1], Burak Turna[1], Nursen Karaca[2], Adnan Şimşir[1], Işık Alper[2], Oktay Nazlı[1]

Departments of [1]Urology and [2]Anaesthesiology, Ege University Faculty of Medicine, İzmir, Turkey.

ABSTRACT

Despite its widespread use, few studies have evaluated the success of extracorporeal shock wave lithotripsy (ESWL) in pediatric patients with several parameters and a large group of patients. In the present study, we aimed to analyze the factors that affect the outcomes of pediatric ESWL treatment, which we have practiced for 26 years. This study included 1012 pediatric patients who underwent ESWL between March 1991 and November 2017. Pre-procedure radiological evaluations were performed using kidney-ureter-bladder and/or urinary system ultrasonography. Demographic data, stone characteristics, and ESWL treatment data and complications were recorded and univariate and multivariate analyses were performed for the stone-free rate (SFR). Receiver operating characteristic (ROC) analysis was performed to determine the cut-off values for stone size to predict ESWL success for both kidney and ureteral stones. Age, body mass index (BMI), congenital renal anomaly, stone location, stone size, number of stones, and stone composition significantly affected the SFRs in univariate analysis; however, only age, BMI, stone location, and stone size were significant in the multivariate analysis. If no residual fragments were detected after three sessions of ESWL application, the procedure was considered successful. The cut-off stone size values for the kidney and ureter that predicted treatment success were 96.28 mm^2 and 44.16 mm^2, respectively. ESWL is an effective and safe treatment in the pediatric age group that provides high SFRs. Age, BMI, and stone location, size, and composition are particularly critical factors that can predict the success of ESWL.

Key words: urolithiasis, pediatric urology, extracorporeal shock wave lithotripsy, stone disease, ureteral stone.

The aim of urinary stone treatment is to provide stone elimination with the least morbidity and greatest success rate.[1] Technological advances have replaced invasive procedures, such as open surgery, with more non-invasive methods, such as extracorporeal shock wave lithotripsy (ESWL), ureteroscopy, and percutaneous nephrolithotomy. ESWL has been used worldwide since Chaussy et al.[2] first used ESWL to treat kidney stones in 1980.

✉ Fuat Kızılay
fuatkizilay@gmail.com

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The first ESWL applications in pediatric patients was carried out in 1986 by Newman et al.[3] After this series, several short-term studies were published regarding the use of ESWL in pediatric patients.[4,5] In children, ESWL has significant advantages that make it the first treatment option in eligible patients: its non-invasive nature, outpatient applicability, lower complication rates compared to surgical approaches, replicability (because stone recurrence is more common in children than in adults), and the ease of passage of ESWL-broken fragments in children.[6,7]

Despite its widespread use, few studies have evaluated the use of ESWL in pediatric patients with several parameters in large patient groups. The number of samples was small in

some studies[8] and, in some of them, factors that could affect the results, such as stone side and size, were missing.[9] In the present study, we analyzed the data, over a 26-year period, of 1012 pediatric patients who underwent ESWL treatment and aimed to evaluate the factors that affected ESWL outcomes in our cohort.

Material and Methods

Patient selection and preparation

Between March 1991 and November 2017, the data of 1012 pediatric patients who underwent ESWL treatment were retrospectively analyzed. Patients with a kidney stone smaller than 2000 mm^2 and ureteral stone smaller than 144 mm^2, with complete imaging and laboratory data and patient records, without previously failed ESWL history (a total of 3 sessions), accepting the risks of ESWL and general anesthesia, and who were fit for anesthesia, were included in the study. ESWL was not performed in patients with active urinary infection, uncontrolled bleeding diathesis, or distal obstruction or who were unsuitable for general anesthesia. All patients were assessed via urinalysis, complete blood count, blood chemistry, and bleeding and clotting time prior to ESWL. Pre-procedure radiological evaluations were performed using X-ray plain abdominal film of kidney-ureter-bladder (KUB) and/or urinary system (US) ultrasonography. US ultrasonography, KUB and both of them were performed in 685, 150 and 177 patients, respectively. Informed consent was obtained from all parents before the procedure. The study was conducted in accordance with the Helsinki declaration, and the Institutional Review Board of Ege University approved the study (decision number: 19-3/2, date: 18.12.2017).

Anesthesia method

Anesthesia was induced via a facemask with 8% sevoflurane in 100% oxygen (O_2) and the rate of sevoflurane was gradually reduced without spontaneous respiratory depression and closed after an intravenous cannula was indwelled. Afterward, an intravenous infusion of 0.9% sodium chloride (NaCl) was started and 10 μg/kg atropine, 0.05 mg/kg midazolam, and 0.5-1 mg/kg ketamine as a slow bolus over 60s were administered and 5-6 L/min O_2 support was given via face mask during the procedure. Anesthesia was maintained with an additional dose of 0.5 mg/kg ketamine given according to clinical parameters, such as moving or moaning from pain induced by shock waves. Patients were discharged once they were fully recovered from anesthesia and their vitals were stabilized, nausea, vomiting, and pain were controlled, and when they reached their first time consciousness score.

ESWL procedure

ESWL was performed using a Dornier MPL 9000 from March 1991 to November 2010 in 607 patients and an ELMED Multimed Classic from November 2010 to November 2017 in 405 patients. Two urologists who were experienced in pediatric stone disease treatment supervised all ESWL procedures (O.N. and B.T.). In the ESWL procedure, shock waves were boosted up to a maximum of 20-22 kV energy starting from low values. The total number of shock waves applied per session generally exceeded 2000 pulses. The number of sessions was in the range of 1 to 4 and applied at 15-20 day intervals. During the ESWL procedure, we stopped the therapy when the maximal number of predetermined shocks was reached in the absence of a visualized stone. If any stones remained un-fragmented at the end of 3 sessions, the ESWL was considered a failure and other treatment options took place. However, after 3 sessions, after a while, additional ESWL sessions were applied to some children whose parents did not accept surgical treatment options and/or whose stones were partially fragmented. Stone analysis could be obtained in a small proportion of patients and further treatment was initiated. Patients were checked with KUB and/or US ultrasonography at intervals of 20 days and the ESWL procedure was repeated when indicated. Indications for repeat-ESWL were residual fragment detection

in control imaging. If the child was found to be out of stone, the next control was carried out at 6 months and one every 6 months thereafter. After a total of 3 ESWL sessions, children without residual fragments were considered stone-free; otherwise, the procedure was considered unsuccessful. Stone free was accepted as the absence of any fragments in control imaging methods. Fragments less than 4 mm were considered clinically insignificant residual fragments. However, the criteria for success and statistical analysis was stone free status. The patients were also divided into two groups (Group A and B) according to lithotriptor devices used and stone-free rates were compared between the two groups.

Data collection

The stone size was calculated in square millimeters by multiplying the longest diameter of the stone by the longest perpendicular diameter detected in the imaging method. In the case of multiple stones, total stone burden was calculated by adding up the volume of each stone. The following values were retrospectively analyzed: age, gender, body mass index (BMI), family history, previous surgery history, congenital kidney anomaly status of the patients, the location and size of the stone in the kidney or ureter, stone composition, double J stent (DJS) requirement, hydronephrosis status, shock wave number and energy applied per session, total number of sessions, outcome (stone-free, fragmented, or ineffective), control method for stone-free, anesthesia method, complications, and residual stone number and size. The family history of patients with stone history in the first degree relatives, was accepted as positive. The primary outcome measurement of the study was the stone-free rate; identifying which variables affected the stone-free status was the secondary outcome measurement. Since the control of stone-free status with only KUB might affect the stone-free rate, 150 patients undergoing only KUB after ESWL were then excluded from the study and a subgroup analysis of the remaining 862 patients was performed.

Statistical analysis

Categorical measurements were recorded as number and percentage, whereas continuous measurements were recorded as the mean and standard deviation (median and minimum-maximum when necessary). The Shapiro-Wilk test was used to test the normality of the variables. Student's t-test was used to compare continuous measures between stone-free and non-stone-free groups and the Chi-squared test was used to compare categorical variables. Logistic regression analysis was performed to identify the independent risk factors that affected the success rate. Multivariate analysis was performed for variables that were significant in univariate analysis to determine the predictive factors. A cut-off value was also determined for the statistically significant values among the groups and the area under the receiver operating characteristic (ROC) curve was evaluated by calculating the sensitivity and specificity values. SPSS 23.0 was used for statistical analysis. Statistical significance was accepted as $p < 0.05$.

Results

A total of 1012 patients were treated with ESWL. The mean age of children was 6.6±1.18 years (8 months - 18 years). The vast majority of patients were boys (644/368). ESWL treatment was most commonly applied for kidney stones (915/1012) with a mean stone size of 118.5 mm^2 and a mean number of shock wave count of 2949. Complications (*steinstrasse*, the German word for "stone street", describing a possible complication of ESWL for urinary tract calculi wherein a column of stone fragments forms that blocks the ureter) were seen in only 20 patients (1.97%). Conservative medical treatment was initiated for the patients with *steinstrasse*. However, 8 patients did not benefit and underwent ureteroscopy. Ureteral catheters were placed at the time of surgery to help to locate the stone in 96 patients (9.5%).

Stone-free-rate (SFR) was higher in younger children with lower BMI (p: 0.024, p: 0.018,

respectively) but significantly lower in children with congenital kidney anomalies (p: 0.032). Thirteen children had horseshoe kidneys, 13 children had duplex collecting systems, and 7 children had pelvic kidneys obstruction anomalies. After ESWL treatment, the total number of stone-free children for both kidneys and ureter stones was higher than children with residual stones (p: 0.015, p: 0.029, respectively). The stone's location in the urinary system affected the SFR: in the kidney, SFR was higher for stones in the renal pelvis, upper calyx, and middle calyx (p: 0.011, p: 0.048 and p: 0.014, respectively), while it was higher for the proximally located stones in the ureter (p: 0.035). In both the kidney and ureter, the mean stone volume was lower in the stone-free group (p: 0.019 and p: 0.022, respectively). When the number of stones was evaluated for ESWL success, there were significantly fewer stones in the kidney in the stone-free group than in the non-stone-free group (p: 0.017); nevertheless, there was no significant difference between the groups regarding ureteral stones (p: 0.355). Stone analysis was obtained from passing stones in sixty-six patients and the SFR was lower in calcium phosphate, calcium oxalate, and cystine stones (p: 0.012, p: 0.038 and p: 0.044, respectively). Children who underwent stone analysis were referred to the pediatric nephrology with the aim of prophylactic treatment. Patient and stone data and univariate analysis of the predictive variables for ESWL success and information on ESWL procedure and complications are given in Tables I and II.

Multivariate analysis of the variables for stone-free status are summarized in Table III and age, BMI, renal pelvis location, upper calyx location, proximal ureter location, and stone size were independent predictors of SFR. The cut-off kidney and ureteral stone size values for treatment success were 96.28 mm^2 and 44.16 mm^2, respectively, as shown in Table IV (for ROC curves, see Figs 1 and 2).

In the subgroup analysis of 862 patients [patients evaluated with only kidney-ureter-bladder (n: 150) were excluded) who underwent ultrasonography post-ESWL, we found that age, stone location and size were important factors affecting SFR and those data were summarized in Table V. We have not added ureteral stones to this analysis because the diagnostic efficiency of ultrasonography in ureteral stones is low.

Patients were divided into two groups in order to determine whether there was device dependent variability in SFRs, and SFR was higher in patients treated with the new generation device (92% vs. 73%, p: 0.042). The comparison of the two groups according to the lithotriptor device used is summarized in Table VI.

Discussion

Pediatric urolithiasis is a significant disease with frequent recurrences and significant morbidity in children. Nowadays, modern treatment methods of this disease are minimally invasive interventions in both children and adults. ESWL therapy has not been approved by the Food

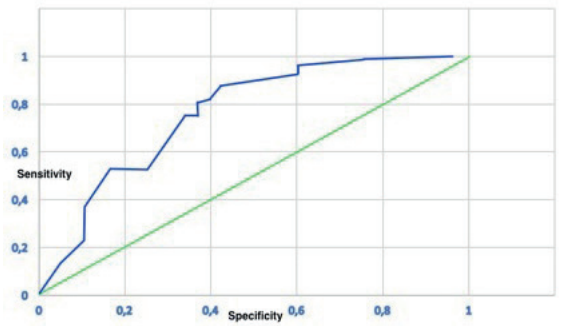

Fig. 1. ROC curve of cut-off value for kidney stone size.

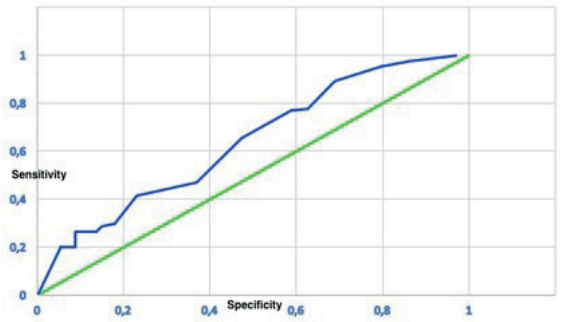

Fig. 2. ROC curve of cut-off value for ureteral stone size.

Table I. Patient and stone characteristics and univariate analysis of the variables.

Characteristics	Total	Stone-free	Non-stone-free	p value
Number of patients, n (%)	1,012 (100)	815 (80.5)	197 (19.5)	*0.038*
Age, years*	6.6 (0.58-18)	5.6 (0.58-12)	8.2 (1.2-18)	*0.024*
Gender, n (%)				0.166
Boys	644 (63.6)	302 (46.8)	342 (53.2)	
Girls	368 (36.4)	172 (46.7)	196 (53.3)	
Body mass index, kg/m^2 *	23.2 (17.3-28.6)	19.4 (17.8-22.5)	24.2 (23.6-28.6)	*0.018*
Family history, n (%)				0.362
Yes	237 (23.4)	125 (52.7)	112 (47.3)	
No	775 (76.6)	393 (50.7)	383 (49.3)	
Previous surgery, n (%)				
Yes	155 (15.3)	81 (52.2)	74 (47.8)	
No	857 (84.7)	442 (51.5)	415 (48.5)	
Congenital kidney anomaly, n (%)				
Yes	33 (3.2)	12 (36.3)	21 (63.7)	*0.032*
No	979 (96.8)	512 (52.3)	467 (47.7)	
Stone location, n (%)				
Kidney	915 (90.4)	777 (84.9)	138 (15.1)	*0.015*
Renal pelvis	584 (63.8)	523 (89.2)	61 (10.8)	*0.011*
Upper calyx	172 (18.8)	149 (86.6)	23 (13.4)	*0.048*
Middle calyx	122 (13.4)	89 (72.3)	33 (27.7)	*0.014*
Lower calyx	37 (4.0)	19 (52.2)	18 (47.8)	0.560
Ureter	97 (9.6)	84 (87.2)	13 (12.8)	*0.029*
Proximal ureter	76 (78.3)	62 (81.5)	14 (18.5)	*0.035*
Distal ureter	21 (21.7)	12 (57.2)	9 (42.8)	0.228
Stone side, n (%)				0.644
Kidney	915 (90.4)	450 (49.2)	465 (50.8)	
Right	487 (53.2)	252 (51.7)	235 (48.3)	
Left	428 (46.8)	198 (46.2)	230 (53.8)	
Ureter	97 (9.6)	43 (44.3)	54 (55.7)	
Right	52 (53.6)	22 (42.3)	30 (57.7)	
Left	45 (46.4)	21 (46.6)	24 (53.7)	
Stone size, mm^2 *	118.5 (12-1,680)			
Kidney		78.5 (12-98)	118.4 (28-1,680)	*0.019*
Renal pelvis	127 (25-1,680)			
Upper calyx	90.7 (25-300)			
Middle calyx	74.7 (12-274)			
Lower calyx	75.4 (25-625)			
Ureter		38.6 (20-68)	72.4 (34-121)	*0.022*
Proximal ureter	61.4 (20-121)			
Distal ureter	31.4 (25-50)			

*: data is presented as mean (minimum-maximum)
p values <0.05 are given in italics

Table II. Stone number, structure and information on extracorporeal shock wave lithotripsy (ESWL) procedure and complications.

Features	Total	Stone-free	Non-stone-free	p value
Number of stones*	1.29 (1-5)			
Kidney		1.02 (1-2)	1.89 (1-5)	*0.017*
Renal pelvis	1.04 (1-3)			
Upper calyx	1.16 (1-5)			
Middle calyx	1.13 (1-5)			
Lower calyx	1.34 (1-3)			
Ureter		1.08 (1-2)	1.26 (1-2)	0.355
Proximal ureter	1.08 (1-2)			
Distal ureter	1 (1-1)			
DJS before intervention, n (%)				
Yes	96 (9.5)	45 (47.8)	51 (52.2)	
No	916 (90.5)	462 (50.4)	454 (49.6)	0.286
Number of shock waves *	2,949 (200-18,131)	3,126 (448-18,131)	2,825 (200-16,625)	0.290
Stone composition, n (%)	66 (6.52)			
Ca-phosphate	26 (39.4)	10 (38.4)	16 (61.6)	*0.012*
Ca-phosphate/Ca-oxalate	16 (24.2)	7 (43.7)	9 (56.3)	*0.744*
Ca-oxalate	11 (16.6)	3 (27.2)	8 (72.8)	
Cystine	5 (7.5)	1 (25.0)	4 (75.0)	
Mg ammonium phosphate	4 (6.0)	2 (50.0)	2 (50.0)	*0.038*
Ca-carbonate	3 (4.5)	2 (66.6)	1 (33.4)	*0.044*
Xanthine	1 (1.5)	1 (100)	-	
Additional intervention, n (%)			62 (6.12)	
Percutaneous nephrolithotomy			17 (27.4)	
Cystolithotripsy			2 (3.2)	
Ureterorenoscopy			43 (69.3)	
Complication, n (%)			20 (1.97)	
Steinstrasse			20 (1.97)	

Ca: calcium, DJS: double J stent, Mg: magnesium
*: data is presented as mean (minimum-maximum)
p values <0.05 are given in italics

and Drug Administration in the United States because of insufficient data on the long-term side effects in the pediatric population; however, it has been widely accepted worldwide since its first reported application[3] and is currently being applied as a first-line treatment in urolithiasis treatment. In the present study, we aimed to analyze the factors that predict the efficacy of this method in a large group of patients.

In the literature, it is stated that the child's age is not a limiting factor for ESWL and that even infants can be treated easily.[10] In the present study, both univariate and multivariate analyses showed that the mean age of the children in the stone-free group was lower. Better success in these children may be due to decreased stone burden, softer stone composition, better ureteral compliance, and less distance between the shock wave generator and the stone.[11] In Alsagheer et al.'s[12] study, ESWL was more successful in younger children and age was the only independent predictor of success in the multivariate analysis. More recently, Dogan et al.[11] have developed a new nomogram for

Table III. Multivariate analysis of statistically significant variables for stone-free status for extracorporeal shock wave lithotripsy.

Variable	HR (95% CI)	p value
Age (years)	1.13 (0.56-2.65)	*0.028*
Body mass index (kg/m^2)	0.96 (0.68-1.88)	*0.015*
Congenital kidney anomaly	2.35 (0.84-11.21)	0.752
Stone location		
Kidney		
Renal pelvis	1.04 (0.91-1.21)	*0.027*
Upper calyx	0.88 (0.72-1.05)	*0.019*
Middle calyx	1.35 (0.42-9.43)	0.168
Ureter		
Proximal ureter	1.21 (0.83-1.38)	*0.021*
Stone size (mm^2)		
Kidney	1.19 (0.90-1.31)	*0.048*
Ureter	0.98 (0.82-1.18)	*0.011*
Number of stones		
Kidney	1.52 (0.52-11.49)	0.788
DJS before intervention	1.36 (0.32-9.96)	0.684

CI: confidence interval, DJS: double J stent, HR: hazard ratio,
p values <0.05 are given in italics.

Table IV. Cut-off values calculated for kidney and ureteral stone size predicting shock wave success.

Parameter	Cut-off	AUC	Sensitivity	Specificity	p value
Kidney stone size	96.28 mm^2	0.726	72.6%	68.8%	0.005
Ureteral stone size	44.16 mm^2	0.768	70.8%	67.2%	<0.001

AUC: area under the curve

prediction of outcome of pediatric ESWL and stated that age is a risk factor for stone-free status in multiple logistic regression analysis and they included the age factor in their nomogram.

ESWL success is low in adult obese patients but obesity has not been shown to significantly affect the success of fragmentation in ESWL in the pediatric patient group.[13,14] In our study, we found that, unlike current data, obesity is an important factor in ESWL success in multivariate analysis, likely because the age range in our patient group is very wide, the children are from different regions, and obesity in our society is seen in almost one in every three children.

Some studies show that congenital anomalies and even anomaly types in adult patients are important factors that affect ESWL success.[15,16] In fact, in some studies, the presence of renal abnormalities was an exclusion criterion for the study.[12] We found that renal anomalies were effective factors in univariate, but not multivariate analysis, likely because only a few patients had congenital anomalies and the majority of these anomalies were anomaly types that do not interfere with the passage of fragments.

Stone location is assumed to be an important factor affecting ESWL success; however, contradictory results exist in the literature about the effect of stone location on ESWL success, especially in ureter stones. The conclusions of the Bader et al.[17] review were consistent with our results in that the SFR of proximal ureteral

Table V. Patient and stone characteristics and univariate analysis of the variables in patients whose control was performed with urinary system ultrasonography [150 patients with only X-ray plain abdominal film were excluded].

Variable	Total	Stone-free	Non-stone-free	p value
Number of patients, n (%)	862 (100)	678 (78.6)	184 (21.4)	*0.022*
Age, years*	6.8 (0.61-17.5)	5.2 (0.61-14.4)	8.4 (1.0-17.5)	*0.013*
Stone location, n (%)				
Kidney	765 (88.7)	662 (86.6)	103 (13.4)	*0.029*
Renal pelvis	509 (66.5)	443 (86.8)	66 (13.2)	*0.007*
Upper calyx	155 (20.3)	137 (88.2)	18 (11.8)	*0.033*
Middle calyx	92 (12.0)	68 (73.4)	24 (26.6)	*0.041*
Lower calyx	9 (1.2)	5 (53.8)	4 (46.2)	0.618
Stone side, n (%)				0.824
Kidney	765 (88.7)	377 (49.2)	388 (50.8)	
Right	414 (54.1)	209 (50.4)	205 (49.6)	
Left	351 (45.9)	168 (47.8)	183 (52.2)	
Stone size, mm^2*	182.6 (38-1,590)	74.9 (10-104)	124.2 (39-1,590)	*0.038*
Renal pelvis	141 (49-1590)			
Upper calyx	88.6 (41-322)			
Middle calyx	70.2 (38-221)			
Lower calyx	66.7 (39-191)			

*: data is presented as mean (minimum-maximum)
p values <0.05 are given in italics

stones was higher than that of the distal stones. On the other hand, Lu et al.[18] showed that SFR rates after ESWL were similar for proximal, middle, and distal ureteral stones. Our overall success rate was 87.2% for the ureteral stones. Important factors explaining the success of ESWL in children are: although the child ureter has a narrower lumen than the adult ureter, it is shorter, more elastic and stretchable, making the passage of the fragments easier and ureteral stone impaction more difficult, and shock wave transmission in the child's body is better.[19] It is estimated that 10-20% of the shock wave energy disappears as it passes through every 6 cm of body tissue.[20] The important effect of stone location and calyx anatomy on stone clearance has been revealed previously[21,22] ; in particular, lower calyx location was noted as a negative factor for stone clearance and some authors mentioned the importance of the infundibulopelvic angle.[23] The European Association of Urology (EAU) 2017 Pediatric Urology guidelines state that renal pelvis and upper calyx stones respond better to ESWL than other stones.[24] The SFR was about 90% for the renal pelvis and upper ureteral stones but between 50% and 62% for the lower calyx stones.[25] Although we did not measure the infundibulopelvic angle in our patients, our SFRs were consistent with the guidelines and the rate was around 84-89% in the renal pelvis and upper ureter and 52% in the lower calyx.

In the EAU guidelines, SFRs for stones <1 cm, 1-2 cm, and >2 cm and overall are reported to be around 90%, 80%, 60%, and 80%, respectively. In addition, as the stone size increases, the necessity of additional interventions also increases.[25,26] We found that stone size is an important factor for stone-free status in multivariate analysis. Our study proposes a different measurement of cut-off values for both kidney and ureteral stones for pediatric ESWL success. In our study, we found that SFR was higher in patients treated with the new generation device. Although the effect of developing technology is undeniable, we believe that the lower BMI of the patients

Table VI. Comparison of stone-free rates and demographic characteristics of patients according to different lithotriptor devices.

Variable	Total	Group A	Group B	P value
Number of patients, n (%)	1012 (100)	607 (60.0)	405 (40.0)	*0.039*
Stone-free rate, n (%)	815 (80.5)	443 (73.0)	372 (92.0)	*0.042*
Age, years*	6.6 (0.58-18)	6.9 (0.58-16)	6.4 (1.9-18)	0.207
Gender, n (%)				0.311
Boys	644 (63.6)	311 (51.2)	333 (82.2)	
Girls	368 (36.4)	296 (48.8)	72 (17.8)	
Body mass index, kg/m^2 *	23.2 (17.3-28.6)	26.8 (19.1-29.0)	22.1 (17.3-27.1)	*0.008*
Family history, n (%)				0.544
Yes	237 (23.4)	118 (19.4)	119 (29.3)	
No	775 (76.6)	489 (80.6)	286 (70.7)	
Previous surgery, n (%)				*0.027*
Yes	155 (15.3)	129 (21.2)	26 (6.4)	
No	857 (84.7)	478 (40.8)	379 (55.2)	
Congenital kidney anomaly, n (%)				
Yes	33 (3.2)	20 (3.3)	13 (3.2)	0.051
No	979 (96.8)	587 (96.7)	467 (96.8)	
Stone location, n (%)				
Kidney	915 (90.4)	535 (88.1)	380 (94.0)	*0.021*
Renal pelvis	584 (63.8)	305 (57.0)	279 (73.4)	*0.034*
Upper calyx	172 (18.8)	44 (8.2)	128 (33.7)	*0.029*
Middle calyx	122 (13.4)	40 (7.4)	82 (21.6)	*0.047*
Lower calyx	37 (4.0)	20 (3.7)	17 (4.4)	0.628
Ureter	97 (9.6)	52 (8.5)	45 (11.8)	0.424
Proximal ureter	76 (78.3)	37 (71.1)	39 (86.7)	*0.011*
Distal ureter	21 (21.7)	15 (28.9)	6 (13.3)	*0.038*
Stone size, mm^2 *	118.5 (12-1680)			
Kidney		128.6 (34-1680)	89.1 (12-1240)	*0.023*
Renal pelvis	127 (25-1680)			
Upper calyx	90.7 (25-300)			
Middle calyx	74.7 (12-274)			
Lower calyx	75.4 (25-625)			
Ureter		45.8 (28-102)	56.6 (20-121)	0.319
Proximal ureter	61.4 (20-121)			
Distal ureter	31.4 (25-50)			

Group A: patients treated with the Dornier MPL 9000 between March 1991 and November 2010.
Group B: patients treated with the ELMED Multimed Classic between November 2010 and November 2017.
*: data is presented as mean (minimum-maximum)
p values <0.05 are given in italics

in this group, the smaller number of patients with previous surgery, the greater proportion of patients with renal pelvis and upper-middle calyx stones and smaller size of kidney stones might ultimately have significantly impacted this result. With the development of surgical technique, ESWL has been replaced with percutaneous surgery in the modern era.

In pediatric patients, both KUB and ultrasonography are commonly used methods for post-ESWL evaluation. Because of the lower diagnostic capability of ultrasonography in ureteral stones, more accurate results can be obtained by using these two methods in combination. In our study, we found that the factors affecting the SFR were similar in the subgroup analysis after excluding the patients evaluated with only KUB.

Two important studies have revealed contradictory results regarding the number of stones. Dogan et al.[11] showed significantly lower stone-free rates in multiple stones in a comparative analysis of the effective factors for stone clearance after a single session, whereas Alsagheer et al.[12] showed that stone number is not an important predictor for ESWL success in univariate analysis. Two nomogram studies have indicated that the presence of a single stone is a favorable factor for stone clearance in the pediatric ESWL.[11,27] The number of stones in our patients ranged from 1 to 5 and we found that the number of stones did not affect SFR in the ureteral stones, though it affected the SFR in the kidney stones significantly. In the multivariate analysis, the effect of the number of stones for SFR was statistically non-significant. We believe that the effect of the number of stones was not significant in the ureteral stones because the maximum number of ureteral stones was two and the overall SFR in the ureter was higher than in the kidney.

The pre-ESWL DJS placement rate is up to 15.4% in the literature. This intervention requires general anesthesia and has mild complications, meaning that one should perform it only in the case of absolute indications. The stent does not affect the SFR, but the overall complication rate is higher and the hospital stay is longer in patients who are not stented.[28,29] The prevalence of DJS application before ESWL was slightly lower (9.5%) in our patient group than in the literature. Steinstrasse was seen in only 20 children who underwent ESWL and only three of them had a DJS. The mean stone size was 239 mm^2 in patients who underwent pre-procedural DJS, well above the overall average.

The response of cystine, calcium oxalate monohydrate, and calcium phosphate stones to ESWL is quite poor.[29] In our study, the SFR was significantly lower in the calcium phosphate, calcium oxalate, and cystine stones, consistent with the literature. The reason why there was no significant difference in multivariate analysis was that stone analysis could be performed in only 66 (6.52%) patients. Patients known to have these stone compositions might be better directed to other treatment alternatives. The main reason for why stone analysis was conducted in such a small group of patients was the referral of the patients to an external center because the analysis could not be performed in our hospital. Another reason is the difficulty in obtaining stone fragments in this age group.

This study has several limitations. First, it's retrospective nature. Second, the ESWL procedure was performed by a different urologist each month; this factor can also affect the results. Another limitation is the lack of metabolic evaluation data of patients. Metabolic evaluation is absolutely mandatory in pediatric stone patients. However, after ESWL, we refer our patients to the pediatric nephrology clinic for metabolic evaluation and further treatment. Therefore, this data was not available. The strengths of our study are its long-term extent, excessive patient number, and inclusion of several parameters that have not been found together in many studies.

We concluded that ESWL is an effective and safe treatment modality in the pediatric age group that provides high SFRs. However, sufficient technical equipment and increased experience affect the outcomes positively, and age, BMI, and stone location, size, and composition are significant factors that predict the success of ESWL.

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Original Article

Cardiorespiratory parameters in newborns during sedation with chloral hydrate

Petja Fister[1], Jerneja Pecek[1], Barbara Gnidovec Strazisar[2], Darja Paro-Panjan[1]

[1]Division of Neonatology, Department of Pediatrics, University Medical Centre, Ljubljana; [2]Department of Pediatrics, General Hospital Celje, Slovenia.

ABSTRACT

We commonly use chloral hydrate sedation in newborns, though its cardiorespiratory side effects have not yet been fully investigated. Our study aimed to analyze the impact of chloral hydrate on cardiorespiratory parameters in term newborns. We performed a prospective, pre-post single-arm interventional study in 42 term, respiratorily and hemodynamically stable newborns. Oxygen saturation (SpO_2), end-tidal CO_2 ($ETCO_2$), the apnea-hypopnea index and the respiratory and heart rates were recorded by polygraphy, starting 0.5-1 hour before oral administration of chloral hydrate at a dose of 40 mg/kg and ceasing 4 hours post-administration. After administration of chloral hydrate, the mean basal SpO_2 dropped by 2.0% (from 97.1% to 95.1%; $p<0.001$) and the mean basal $ETCO_2$ increased by 3.9 mmHg (25.6 to 29.5 mmHg; $p<0.001$). We found a significant decrease in the minimal SpO_2 values ($p<0.001$) and an increase in the percentage of time spent with SpO_2 <95% and <90% ($p<0.001$). The mean increase in the estimated apnea-hypopnea index was 3.5 events per hour ($p<0.001$). The mean respiratory and heart rates were significantly lower 150 min after the administration of chloral hydrate when compared with pre-sedation values (51/min and 127/min versus 61/min and 138/min respectively; $p<0.001$). A considerable number of patients exhibited changes in cardiorespiratory parameters that differed considerably from the normal ranges. In conclusion, SpO_2, $ETCO_2$, the estimated apnea-hypopnea index and the respiratory and heart rates changed after the administration of chloral hydrate. They remained within normal limits in most newborns, but the inter-individual variability was high in the studied population.

Key words: chloral hydrate, newborn, oximetry, respiration, sedative.

Chloral hydrate is frequently used for light sedation of newborns to facilitate painless diagnostic procedures that require the patients to be motionless, such as various radiological and neurophysiological tests.[1-5] The recommended dose of chloral hydrate for newborns and infants is 25-50 mg/kg.[6] The sedative effects of chloral hydrate become clinically apparent approximately 20 minutes after oral or rectal administration[7-9] and last between 90[7,8] and 165[10] minutes, although they may be prolonged, especially in preterm infants.[2]

In some previous pediatric studies, it has been suggested that chloral hydrate sedation in infants and children is effective and safe, with a relatively low risk of severe respiratory and hemodynamic adverse effects.[1,3,4,8,9] On the other hand, some have reported severe complications of chloral hydrate sedation in children and even death.[11] Chloral hydrate may, like many other sedatives, affect respiratory and cardiovascular function and pose a risk of respiratory depression and hypoxia.[12-14] This is especially important in newborns, who have unstable breathing patterns due to the immature control center, and in whom the elimination of chloral hydrate may be prolonged.[6,15] It has been implied that the risk of hemoglobin desaturation during chloral hydrate sedation is relatively high in newborns[16] and studies have shown that younger age is a risk factor for sedation-related adverse effects.[3,17] Still, little data exist on the cardiorespiratory side effects of chloral hydrate in newborns. Previous studies have mainly

✉ Darja Paro-Panjan
darja.paro@kclj.si

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included older infants and children[3,5,8,9], relied on intermittent measuring of vital signs[3,4,8,9] or were mostly retrospective, focusing only on clinically apparent adverse events.[1,4,16]

To further clarify the impact of chloral hydrate on cardiorespiratory function in term newborns, we analyzed continuously recorded cardiorespiratory parameters, including arterial oxygen saturation (SpO_2), end-tidal CO_2 ($ETCO_2$), the apnea-hypopnea index and the respiratory and heart rates, before and after the administration of oral chloral hydrate at a dose of 40 mg/kg.

Material and Methods

Patients

A prospective, pre-post intervention study on a single group of patients, where each newborn served as his or her own control, was conducted between May 2015 and June 2016 at the Department of Neonatology, Division of Pediatrics, University Medical Center Ljubljana, Slovenia. Ethical approval was obtained from the National Medical Ethics Committee on May 26, 2015 (number 40/05/15).

The inclusion criteria were: postmenstrual age at least 37 weeks, chronological age less than 4 weeks and the need for sedation with chloral hydrate for auditory brainstem response (ABR) audiometry. We excluded newborns receiving drugs that have known interactions with chloral hydrate (furosemide, phenytoin, flumazenil and amiodarone) and newborns with congenital cyanotic heart disease, liver failure, renal insufficiency and signs of infection or respiratory compromise.

Parents of all newborns signed an informed consent form prior to their enrollment in the study.

Methods

Polygraphic recordings of cardiorespiratory parameters were started half an hour to one hour prior to the administration of a single dose of oral chloral hydrate of 40 mg/kg. A second dose was never given. Half an hour later, the ABR test was performed, during which the recording remained uninterrupted and was continued until 4 hours after the administration of chloral hydrate. During the monitoring, newborns were under constant supervision.

Cardiorespiratory parameters were recorded by an Embletta MPR PG, Natus Medical Incorporated polygraphy device. The respiratory effort was measured by respiratory inductance plethysmography (XactTrace Single Use Cut-to-Fit Embla RIP Belts), nasal airflow by nasal thermistor (Embla Breath Sensor Airflow Thermistor Preemie), SpO_2 and the heart rate by pulse oximetry (Nonin Xpod External OEM Pulse Oximeter) and ventilation by $ETCO_2$ (Microstream CapnoLine H Infant/ Neonate nasal cannulas and Comdek Portable Color Oxi-Capnography MD-850, calibrated to assure accuracy). The thermistor and nasal cannulas were positioned in a way that they did not obstruct the nares of the newborns, conforming to the manufacturer's instructions. The newborns were breathing room air and there was no supplemental oxygen given. The noninvasive side-stream capnometry through nasal cannulas has previously been proven to be an accurate method for estimating $ETCO_2$ in nonintubated newborns without pulmonary disease.[18-20]

Data processing

Polygraphic recordings were displayed and analyzed using Embla RemLogic-E Sleep Diagnostic Software version 3.4.1, calibrated for newborns, infants and children.

Cardiorespiratory parameters were determined for every newborn and for each part of the study (before and after the administration of chloral hydrate). The primary outcomes were: 1) basal SpO_2 as a marker of oxygenation; and 2) basal $ETCO_2$ as a marker of ventilation. The secondary outcomes included: 1) percentage of time with SpO_2 <95%; 2) percentage of time

with SpO_2<90%; 3) minimal SpO_2; 4) estimated apnea-hypopnea index; 5) respiratory rate; and 6) heart rate. Normal ranges for these cardiorespiratory parameters were defined according to the published centiles for healthy term newborns.[21-25]

The Embla RemLogic-E Software allowed automatic calculation of the basal and minimal SpO_2 and the percentage of time spent in a specified SpO_2 zone or below a chosen threshold in selected parts of the recordings.

The basal $ETCO_2$ was estimated manually from the capnometry curve by comparing $ETCO_2$ values before and after the administration of chloral hydrate and determining the most accurate basal value for these two periods.

Apneas and hypopneas were scored manually, according to the American Academy of Sleep Medicine criteria.[26] We also categorized apneas as central, obstructive or mixed. The apnea-hypopnea index was defined as the number of apneas and hypopneas per hour of estimated total sleep time. Total sleep time was estimated by manually excluding all parts of the recordings with motion artifacts on account of arousals, movement or handling of newborns.

The respiratory and heart rates were counted in the period before the administration of chloral hydrate and then at 30-minute intervals during sedation. The average respiratory rate over the 30-minute period was counted by analyzing the breathing waveform, derived from the signal from the respiratory inductance plethysmography: we counted the number of breaths in 60 seconds, when the breathing was calm and the respiratory pattern stable. The mean heart rate was calculated automatically by the Embla RemLogic-E Software.

Statistical analysis

All calculations were performed using Microsoft Excel 2013 and IBM SPSS Statistics version 21. Data are presented either as the mean and standard deviation or the median and interquartile range, depending on the normality of the distribution of observed variables.

For all of the observed variables, the difference between the value before and after the administration of chloral hydrate, as well as the relative change from the baseline pre-sedation value, were calculated for each individual patient. The differences were statistically analyzed using the two-tailed Student's t-test for paired data and the two-tailed Wilcoxon signed-rank test. The level of significance (alpha) of 0.05 was used. Because of multiple comparisons, this significance level was adjusted using the Bonferroni correction. Hence, each individual hypothesis was tested at $\alpha = 0.006$. Confidence intervals were adjusted accordingly.

Results

Out of 49 newborns enrolled in the study, seven newborns were excluded due to the poor quality of the recordings. Measurements of $ETCO_2$ were available for 27 newborns. The characteristics of the study population are presented in Table I. Adequate depth of sedation to perform the ABR testing was achieved in all newborns. After we stopped the recording of cardiorespiratory parameters, newborns were awake and back to their baseline alertness.

In most newborns, basal SpO_2 was lower and basal $ETCO_2$ was higher after the administration of chloral hydrate compared with pre-sedation values (Table II). The mean basal SpO_2 level dropped by 2.0% (from 97.1% to 95.1%) and the mean basal $ETCO_2$ increased by 3.9 mmHg (from 25.6 to 29.5 mmHg). The maximum decrease in basal oxygen saturation was 5%. The maximal $ETCO_2$, recorded during sedation, was 38 mmHg. We observed a >20 % increase in $ETCO_2$ in 9 of 27 newborns (33%).

The decrease in oxygenation was additionally manifested by a higher proportion of time spent at lower SpO_2 levels and a decrease in minimal SpO_2 during sedation with chloral hydrate.

We also observed an increase in the estimated apnea-hypopnea index. Of all the recorded

Table I. Baseline characteristics of participating newborns (n: 42).

Patient Characteristics	Results
Male sex, n (%)	28 (66.7%)
Age at the time of the study, days	16 ± 16
Gestational age, weeks	38 ± 3
Postmenstrual age at the time of the study, weeks	40 ± 2
Birth weight, gr	3,107 ± 803
Weight at the time of the study, gr	3,350 ± 605
Head circumference at birth, cm	34 ± 3
Head circumference at the time of the study, cm	35 ± 1
Apgar score at 5 min,	8 ± 3
Apgar score of > 8 at 5 min, n (%)	22 (52.4%)

Table II. Primary outcome measures.

Variable	Before Sedation[a]	During Sedation[a,b]	P[c]	Difference (99.4% CI)
Basal SpO_2, %	97.1 ± 1.9	95.1 ± 2.4	<0.001	-2.0 (-2.7 to -1.4)
Basal $ETCO_2$, mmHg	25.6 ± 3.3	29.5 ± 3.9	<0.001	3.9 (2.1 to 5.7)

[a]Mean ± SD. [b]In the 4-hour period after administration of chloral hydrate. [c]Two-tailed Student's t-test for paired data.
ETCO2: end-tidal CO2, SpO2: arterial oxygen saturation.

Table III. Secondary outcome measures.

Variable	Before Sedation[a]	During Sedation[a,b]	P[c]	Difference (99.4% CI)[d]
Time with SpO_2<95%, %	11.0 (0.8 to 26.9)	30.1 (12.4 to 55.0)	<0.001	19.1 (11.6 to 26.6)
Time with SpO_2<90%, %	0.0 (0.0 to 0.7)	1.9 (0.6 to 7.8)	<0.001	Not applicable
Minimal SpO_2, %	90.0 (87.5 to 93.0)	83.0 (81.0 to 85.0)	<0.001	Not applicable
Apnea-hypopnea index, n/h	0.0 (0.0 to 2.1)	3.4 (0.8 to 6.9)	<0.001	3.5 (1.7 to 5.2)
Respiratory rate, min^{-1}	60.9 ± 14.0	51.3 ± 11.1[e]	<0.001	-9.6 (-16.7 to -2.5)
Heart rate, min^{-1}	138.1 ± 17.0	126.8 ± 13.0[f]	<0.001	-11.3 (-18.0 to -4.6)

[a]Median (IQR) or mean ± SD. [b]In the 4-hour period after administration of chloral hydrate. [c]Two-tailed Student's t-test for paired data or two-tailed Wilcoxon signed-rank test. [d]Calculated when data were normally distributed. [e]At 150 minutes after administration of chloral hydrate, when the mean respiratory rate was the lowest. [f]At 150 minutes after administration of chloral hydrate, when the mean heart rate was the lowest.
SpO_2: arterial oxygen saturation.

apneas, 89% were central in origin, 3% were obstructive, 1% were mixed and in 7%, the type of apnea could not be reliably determined. Lastly, chloral hydrate sedation affected the respiratory and heart rates (Table III and Figs 1 and 2).

The mean respiratory rate and the mean heart rate gradually decreased during the course of sedation and were at their lowest 150 minutes after the administration of chloral hydrate (Fig. 2). The mean maximum decrease in the respiratory rate was 10±15 breaths per minute and the mean maximum decrease in the heart rate was 11±14 beats per minute (Table III). A >20% decrease in the respiratory rate was observed in 13 newborns (34%) and a >20% decrease in the heart rate was seen in five newborns (13%). Of these, one newborn (3%) exhibited an abnormally low respiratory rate, while an abnormally low heart rate was measured in three newborns (8%), according to the normative data (lower 10th percentiles for respiratory and heart rates in healthy term newborns).[21,22]

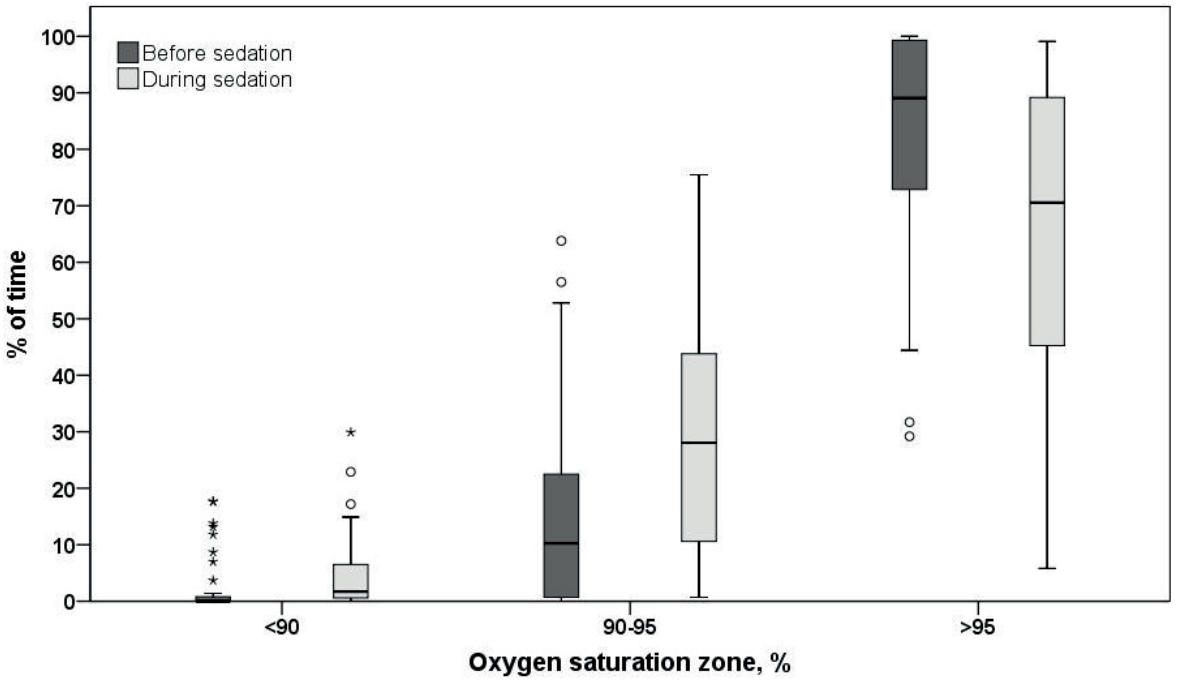

Fig. 1. Box plot of time spent with SpO2 within certain zones before sedation with chloral hydrate (dark gray) and during sedation with chloral hydrate (light gray) at a dose of 40 mg/kg. The line across each box represents the median value. The bottom of the box represents the 25th percentile and the top of the box represents the 75th percentile. The lower and upper bars that extend from each box (whiskers) extend to 1.5 times the height of the box or to the minimum or maximum value. The closed circles indicate outliers and the asterisks are extreme outliers (values more than three times the height of the boxes).

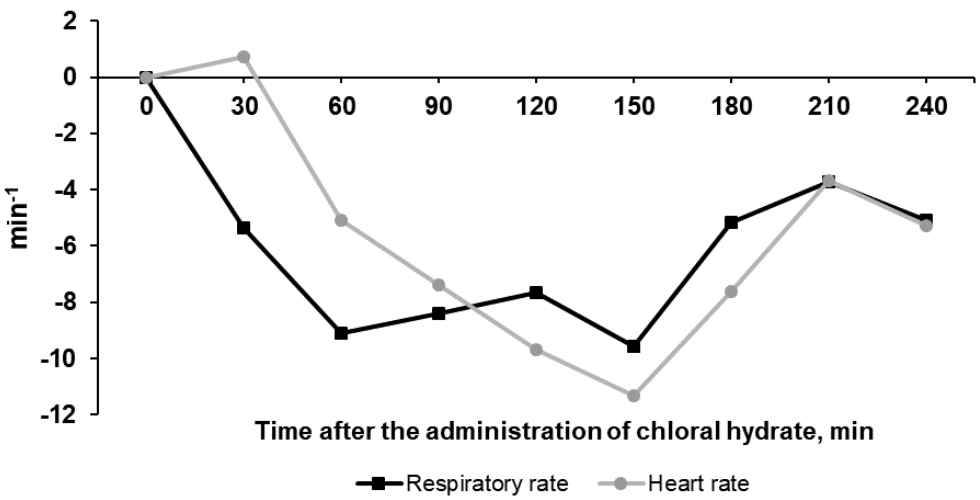

Fig. 2. Time course of the mean changes in the respiratory rate (black line) and heart rate (gray line) during sedation with chloral hydrate at a dose of 40 mg/kg relative to the mean baseline values before sedation (starting point is marked as a zero value).

Discussion

Our study confirmed the impact of chloral hydrate at a dose of 40 mg/kg on cardiorespiratory parameters in newborns. All primary and secondary outcomes changed significantly after the administration of chloral hydrate. Nevertheless, the cardiorespiratory parameters remained within the normal ranges in the majority of newborns. For instance, one of our primary outcomes – mean basal SpO_2– decreased by 2%, but the mean value after the administration of chloral hydrate remained above 95%. At the same time, there was a small but important number of patients in whom we measured changes in cardiorespiratory parameters that differed considerably from the accepted normal ranges.

We also investigated the impact of chloral hydrate on the noninvasively measured $ETCO_2$, which has been shown to be an accurate measure of ventilation in nonintubated newborns with normal respiratory function.[18-20] Changes of $ETCO_2$ during sedation with chloral hydrate have previously only been investigated in two retrospective studies, both of which included older children.[3,10] The increase in $ETCO_2$ to an average of 30 mmHg and to a maximum of 38 mmHg with a decrease of respiratory rate from 61 to 51 breaths per minute in our study may indicate bradypneic hypoventilation.[18,19,26] However, it is important to note that we measured only basal $ETCO_2$ and that our results do not provide information on the percentages of time during which $ETCO_2$ was above the expected levels. The equipment we used also does not allow quantitative measurement of tidal voulmes. We therefore cannot conclude whether our patients had hypopneic hypoventilation or not.[26,27] A >20% increase in basal $ETCO_2$ was found in 9 of 27 of our studied newborns (33%). In contrast, in their retrospective study, Heistein et al.[3] reported a >20% increase from the baseline $ETCO_2$ measurement after the administration of chloral hydrate in only 40 of 603 patients (6.6%). This discrepancy could be attributed to the differences in age – the age of the patients in the study by Heistein et al.[3] was between 1 month and 3 years, whereas our study included only newborns.

We also observed significantly higher percentages of time when SpO_2 was <95% after the administration of chloral hydrate in comparison with pre-sedation values. At the same time, the percentages of time when SpO_2 was <90% remained very low in most newborns both before and during sedation with chloral hydrate. There are currently no reference data on the normal percentages of time spent in each saturation zone for healthy full-term infants.[23] The cut-off level of 95% was chosen because this was reported as the 5th percentile of basal SpO_2 for healthy term newborns.[24]

Our study also showed a significant increase in the estimated apnea-hypopnea index during sedation with chloral hydrate. The mean apnea-hypopnea index in the period after the administration of chloral hydrate was 3.4 events per hour. However, apneas of short duration are common in healthy term newborns, and are mostly central.[23,28] The median central apnea index for 1-month-old newborns was reported to be 5-10 events per hour and the 95th percentile was 45.[23] Thus, given the fact that most of the recorded apneas were central in origin, the apnea-hypopnea index remained within normal limits even during sedation with chloral hydrate. It should be emphasized, however, that we used a nasal thermistor instead of a nasal pressure transducer to detect hypopneas, hence it is possible that we underestimated the number of hypopneas.[29] Furthermore, since we did not objectively determine the total sleep time by analyzing neurophysiological variables, our calculation of the apnea-hypopnea index was only an approximation based on the number of apneas and hypopneas per hour of estimated sleep time.

During the course of sedation, the respiratory and heart rates decreased in most patients. The mean maximum decrease in the respiratory rate from 61 to 51 breaths per minute and the mean decrease in the heart rate from 138 to 127 beats per minute do not represent a deviation from the

reported normal ranges.[21,22] It is less plausible that the decrease in respiratory and heart rates was secondary to hypoxia or hypercapnia since both changes were clinically non-significant and because the mean SpO_2 and $ETCO_2$ remained within normal limits. However, the effects of chloral hydrate varied considerably among the newborns. While the respiratory and heart rates actually increased in some of the studied newborns, one newborn experienced a clinically important decrease in the respiratory rate and three participants experienced a clinically important decrease in heart rate. These findings are consistent with the report by Treluyer et al.[8], who observed a decrease in the respiratory rate outside the normal limits for age in three of 19 children aged 2.13±1.43 years. Our results are also compatible with the study by Heistein et al.[3], where alterations in heart rate beyond the published normal ranges occurred in a minority (1.4%) of the children between one month and three years of age, sedated with chloral hydrate.

In our study, the impact of chloral hydrate on the respiratory and heart rates was greatest 150 minutes after administration of chloral hydrate. Our results are not in agreement with the previously reported time-course of chloral hydrate sedation in patients aged between six months and six years, where the maximum clinical sedative effect was observed after 30 minutes, following which it gradually decreased.[8] In another study in patients aged between three months and twelve years, the effects of sedation completely disappeared within the span of about 60 minutes.[5] This disagreement could be attributed to the prolonged terminal serum elimination half-life of chloral hydrate and its active metabolite in newborns, compared to the older population.[6,15] Our findings imply that newborns should be monitored for at least three hours after sedation with chloral hydrate. Further studies should explore the temporal association between the clinically apparent effects of sedation and the measured effects of chloral hydrate on cardiorespiratory parameters.

It should also be emphasized that there was high inter-individual variability in our study, as evident from the large statistical dispersion of the measured variables. Furthermore, the variability of some of the cardiorespiratory parameters (e.g. time with SpO_2 <95%, time with SpO_2 <90% and the apnea-hypopnea index) was markedly greater after the administration of chloral hydrate than before sedation. This clearly shows that chloral hydrate does not have the same effects on all newborns. As a consequence, we recorded values outside the normal limits only in individual newborns, while the average values remained within the normal range.

This is one of the few existing studies focusing on the effects of chloral hydrate sedation in the most vulnerable pediatric age group. We did not rely solely on monitoring the clinically discernible adverse events of sedation. Instead, we measured the impact of chloral hydrate on cardiorespiratory parameters in a prospective manner, which is a significant advantage of our study. To our knowledge, this is the first study that investigated the effects of chloral hydrate using polygraphy. In previous studies, vital signs were recorded at fixed intervals (every 5 minutes[3,4] or every 15-30 minutes[8,9]), whereas in our case, we used computer processing to analyze continuously measured parameters. Consequently, our findings do not support the results of a prior observational study by Treluyer et al.[8] in 20 children aged 2.13±1.43 years, in whom no statistically significant change in the vital functions was found after rectal administration of 75 mg/kg of chloral hydrate. Similar results were found in the study by Coskun et al.[30] in 360 patients aged 19 ± 4.5 months.

The major limitation of the present study is the difference in the duration of polygraphic recordings before and after the administration of chloral hydrate. For a more accurate comparison of these two periods, the recording time should be the same length for both parts of the study. Furthermore, the two parts of the study are not

entirely comparable due to the unequal external conditions during the recording and thus possible confounding factors. Future studies are therefore needed, together with a superior study protocol involving cardiorespiratory recording on two separate, consecutive days, of the same duration and at the same time of the day in each case, on the first day without sedation and on the second day after the administration of chloral hydrate. To directly distinguish between the effects of chloral hydrate and the influences caused by varying sleep stages, a full-channel polysomnography, rather than polygraphy, would be the more appropriate investigation method.

The small sample size is another limitation of our research, especially for the subgroup in which we measured $ETCO_2$. In addition, our study population was not homogeneous. Participating newborns differed in gestational age, antenatal history, Apgar score and clinical diagnoses. At the same time, great inter-individual variability could very well be the advantage of our research, since this sample is representative – it reflects the actual population in which we use chloral hydrate sedation in clinical practice.

To conclude, in a group of term newborns with normal cardiovascular and respiratory physiology, sedation with oral chloral hydrate at a dose of 40 mg/kg caused mild changes in cardiorespiratory parameters. Oxygenation and ventilation remained adequate in most cases, but the cardiorespiratory effects of chloral hydrate varied considerably among newborns. Our results could serve as a pilot for a larger study to determine the safety of chloral hydrate sedation in newborns.

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Original Article

Are general movements at 3-5 months correlated and compatible with the Bayley-III at 1,5-2 years age?

Aysu Kahraman[1], Ayşe Livanelioğlu[2], Özgün Kaya Kara[2], Murat Yurdakök[3]

[1]Department of Physiotherapy and Rehabilitation, Dr. Sami Ulus Maternity and Children's Health and Diseases Training and Research Hospital; [2]Department of Physiotherapy and Rehabilitation, Faculty of Health Sciences and [3]Neonatology Unit, Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Since early intervention is important in risky infants, it is also important to identify developmental problem as early as possible. There are various assessment methods for this. One of them is the General movements assessment (GMs), and the other one is the Bayley Scales of Infants and Toddler Developmental, third edition (Bayley-III). The present study aimed to compare the neurodevelopmental outcomes and Bayley-III scores at the age of 1.5-2 years with fidgety GMs. One hundred and twenty-six infants (57 females, 69 males) were assessed by the GMs at the corrected 3-5 months and also by the Bayley-III at the age of 1.5-2 years. According to the GMs, 21 infants exhibited the absence of fidgety movements, six infants exhibited abnormal fidgety movements, and 99 infants exhibited normal fidgety movements. According to the Bayley-III, 19 infants' motor scores, 13 infants' language scores, and 18 infants' cognitive scores were low (-2SD). Various neurodevelopmental problems were identified in 25.4% of the infants. As a result, although in the present study the Bayley-III underestimates the rates of motor impairment, it was found to be moderately compatible with the GMs at 3-5 months ($r= 0.4$, $p<0.001$). However, the GMs were better than the Bayley-III in predicting neurodevelopmental outcomes at the age of 1.5-2. Although the Bayley-III and GMs may be valuable tools for estimating the later outcomes of infants, care should be taken while interpreting their results.

Key words: fidgety movements, Bayley-3 infants and toddler developmental, neurodevelopmental outcome.

Because plasticity is rapid in the first two years, early diagnosis and treatment are essential in risky infants. Optimal treatment which is initiated at the early age for motor problems can reduce academic and psychosocial problems.[1] There are various assessment methods for the early identification or prediction of infant problems. One of these is the assessment of general movements (GMs), the other one is the Bayley-3 Infants and Toddler Developmental Scale (Bayley-III).[2,3]

GMs are gross movements that include all parts of the body.[2] They start during the 9th–10th postmenstrual week. These movements decrease in the fourth and fifth months after birth and are replaced by goal-directed motor behaviors.[4] The GM assessment is a good predictor for the identification of neurological impairments.[5] As a result of the developmental changes of the nervous system, GMs differ in the three periods of preterm, writhing, and fidgety movements (FM).[6] FM are seen around three to five months' post-term. FM are small movements in all directions with moderate speed and variable acceleration in the neck, trunk, and limbs.[7] While abnormal (AF), absent (F-), or sporadic FM indicate an increased risk of neurological dysfunction, normal FM (F+) have a high predictive value for normal development outcome.[8]

The Bayley-III is an assessment scale that is widely used to measure cognitive, language and motor conditions of infants. This scale

✉ Aysu Kahraman
aysum@hotmail.com

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assesses the development of infants and toddlers between 1 and 42 months of age.[9] It is a discriminatory tool that compares a child's performance with other children.[10]

The present study aimed to compare the neurodevelopmental outcomes and Bayley-III scores at the age of 1.5-2 with fidgety GMs.

Material and Methods

This study was carried out between January 2012 - January 2016 with 57 females and 69 males, 126 infants in total, who applied to Department of Physiotherapy and Rehabilitation, Faculty of Health Sciences, Hacettepe University, Ankara, Turkey.

Infants with major malformations of the brain or other organs, infants with a chromosomal deformity, and infants whose parents no longer volunteered to participate or bring their infant for regular follow-up were discontinued from the study. This study was designed as a prospective trial of premature infants. The permission of the Hacettepe University Ethics Committee (GO 14/66–30) was received and written informed consent was obtained from the guardian of each participant.

The infants included in the study were evaluated according to Prechtl's general movement assessment. Infants aged between 9 - 17 weeks post-term were recorded for 3-5 minute while they were lying on supine position, awake and active.[6] Evaluations of GMs were made by two experienced authors who had attended basic and advanced courses on GMs (basic: 2010, advanced: 2012). This was a blind study, the authors did not know anything about the infants' newborn period or previous problems during follow-up.

The Bayley-III was administered by another experienced author once for each participant at the corrected 18th or 24th months. The results were interpreted according to the composite score.

Statistical analysis

The data were analyzed using the IBM SPSS for Windows Version 16.0 software program. The Kolmogorov-Smirnov test was used to determine whether data had a normal distribution. Parametric tests were used for data with a normal distribution and non-parametric tests for data without a normal distribution. Mean, and standard deviation were calculated for numerical variables. Categorical variables were shown with numbers and percentages. The Kruskal-Wallis test was used to compare the data of more than two independent groups. On the other hand, the Mann-Whitney U test was used to compare the data of two independent groups. The Spearman correlation test was used to assess concordance between the data. Statistical results were interpreted at a 95% confidence interval with a $p < 0.05$ (two-tailed) significance level.

Results

The clinical and demographic characteristics of infants are presented in Table I.

According to the GMs analysis, in the 3-5-month period, 99 infants exhibited F+, six infants exhibited AF, and 21 infants exhibited F- movements. Of the infants, 94 with F+ had normal development, two had hypertonia, one had delayed motor development, and two had pervasive developmental disorder. One of the infants with AF had Cerebral Palsy (CP), one had hypertonia, one had hearing loss, two had

Table I. Demographic and clinical characteristics of infants.

	Infant (n=126)
Gestational age (Mean ± SD)	29.79 ± 2.69
Birth weight (Mean ± SD)	1350.68 ± 496.81
Jaundice (n)	71 (56.3%)
Respiratory distress syndrome (n)	72 (57.1%)
Pneumonia (n)	26 (20.6%)
Sepsis (n)	44 (34.9%)
Intraventricular hemorrhage (n)	90 (71.4%)
Bronchopulmonary dysplasia (n)	45 (35.7%)

Table II. Neurological outcome of infants.

	Infant (n=126)	GMs (n=126)
Normal neurological outcome	94 (74.6%)	94 F+
Cerebral Palsy	16 (12.7%)	15 F-, 1 AF
Hypotonia	1 (0.8%)	1 F-
Hypertonia	6 (4.8%)	2 F+, 1 AF, 3 F-
Motor development delay	2 (1.6%)	1 F+, 1F-
Walking impairment	1 (0.8%)	1 F-
Hear loss	1 (0.8%)	1 AF
Speech impairment	1 (0.8%)	1 AF
Pervasive developmental disorder	4 (3.1%)	2 F+, 2 AF

Table III. The comparison of Bayley-III Score and fidgety in infants.

	Bayley-III Motor (Mean ± SD)	Bayley-III Language (Mean ± SD)	Bayley-III Cognitive (Mean ± SD)
F+ (n=99)	97.21 ± 9.45	98.15 ± 8.85	98.66 ± 9.66
F- (n=21)	72.67 ± 23.15	81.87 ± 21.39	76.48 ± 21.65
AF (n=6)	86.17 ± 23.20	69 ± 4.9	76.17 ± 19.03

pervasive developmental disorder, and one had a speech impairment. On the other hand, 15 of the infants with F- had CP, one had hypotonia, three had hypertonia, one had walking impairment, and one had delayed motor development. The neurological outcomes of the infants are presented in Table II.

Since 63 of the infants could be followed up to 1.5 years old, and the remaining 63 up to 2 years old, the Bayley-III was applied at these ages. The Bayley-III motor scores were -2 SD below normal in 19 infants and four of these infants had normal development, 13 had CP, one had hypertonia, and one had hypotonia. The Bayley-III motor scores were +1 SD above normal in 6 infants, and one of these infants did not hear, one had hypertonia, and four exhibited normal development. The Bayley-III language scores were -2 SD below normal in 13 infants, and five of these infants had CP, one had a speech impairment, one had hypertonia, one did not hear, two had pervasive developmental delay, and three exhibited normal development. The Bayley-III language scores were +1 SD above normal in 7 infants, and one of them had pervasive developmental delay, one had hypertonia, one had CP, and four exhibited normal development. The Bayley-III cognitive scores were -2 SD below normal in 18 infants, and nine of them had CP, one had hypertonia, one did not hear, two had pervasive developmental delay, one had motor retardation, one had a speech impairment, and three exhibited normal development. The Bayley-III cognitive scores were +1 SD above normal in 6 infants, and one of these infants had CP, one had hypertonia, and four exhibited normal development.

While infants with F- had the lowest Bayley-III motor scores, infants with AF had the lowest Bayley-III language scores (Table III). Cognitive scores were found to be low in infants with both F- and AF. F+ infants' Bayley-III language and cognitive scores differed from those of F- and AF infants. However, when it is examined from the Bayley-III motor scores point of view, there is a difference between F+ and F-, but not between F+ and AF.

Our results show that the GMs analysis and Bayley III scores are moderately compatible ($r= 0.4$, $p< 0.001$). However, GMs were better than the Bayley III in predicting neurodevelopmental outcome at the age of 1.5-2 years.

Discussion

For risky infants is important to identify developmental problems as early as possible. The Bayley-III and GMs may be valuable tools for estimating the later outcomes of these infants.

The predictive value of AF is low. Infants with AF can show normal development or can have CP or minor neurological deficit.[11,12] However, certain recent studies have shown that AF can be related to fine motor dysfunctions or autism spectrum disorder.[13,14] Zappella et al.[14] have reported that abnormal GMs were observed more frequently in infants who were diagnosed with autism spectrum disorder later. The fact that infants with AF had the lowest Bayley-III language score and a low cognitive score in our study shows that these infants may have more sensory influences than the motor. Our results indicate that AF movements, even if they do not predict CP, are still consistent with a minor neurological impairment and behavioral, sensory, or cognitive developmental disabilities in the infants.

The presence of fidgety movements does not always show normal development.[15] In the present study, all infants with F+ did not have normal development at the age of 1.5-2 years. However, their Bayley-III scores were within normal limits. Furthermore, three infants with F- and mild CP had Bayley-III scores within normal limits. From this point of view, both tools do not have the ability to determine the developmental delay fully.

Fairbairn et al.[16] showed that the Bayley-III performance at one year of age was not very successful at predicting the performance at three years of age. Anderson et al.[17] reported that the Bayley-III had inflated scores and therefore was a poor predictor of cognitive and motor impairment. Spittle et al.[3] indicated that although the Bayley-III motor scale at the age of two years could predict motor developmental retardation at four years of age in infants who were born earlier than 30 gestational weeks, it underestimated the rates of motor impairment. Although similar results were obtained in our study, the Bayley is not a predictive tool, and it was developed as a discriminatory tool. Again, Peralta-Carcelen et al.[18] found out that children's performance in the Bayley-III varies with age. In our study, the Bayley-III assessment was performed at the age of 1.5-2. All of these are the limitations of the present study.

As a result, although the Bayley-III underestimate the rates of motor impairment in our study, it was found to be moderately compatible with the GMs analysis at 3-5 months of age. However, GMs were better than the Bayley III in predicting neurodevelopmental outcomes at the age of 1.5-2 years. The Bayley-III and GMs may be valuable tools for estimating the later outcomes of infants, but care should be taken when interpreting their results. The later outcome could be decided more accurately if a more holistic assessment is performed with clinical findings, the duration of follow-up and the correct timing. Since the environment and the family can affect the development of a child during the time past, it should be kept in mind that none of the tests can succeed one hundred percent in the prediction of the later outcome.

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Original Article

Assessment of motor development using the Alberta Infant Motor Scale in full-term infants

Büşra Kepenek-Varol[1], Zeynep Hoşbay[2], Selçuk Varol[3], Emel Torun[4]

[1]Department of Physiotherapy and Rehabilitation, Faculty of Health Sciences, Nuh Naci Yazgan University, Kayseri; [2]Department of Physiotherapy and Rehabilitation, Faculty of Health Sciences, Biruni University, İstanbul; [3]Department of Pediatrics, Yeşilhisar State Hospital, Kayseri; [4]Department of Pediatrics, Bezmialem Vakif University Medical Faculty, İstanbul, Turkey.

ABSTRACT

The Alberta Infant Motor Scale (AIMS) is a well-known, norm-referenced scale that evaluates the gross motor development of children from birth to 18 months. The aim of the study was to compare the Canadian norms with the AIMS scores of a Turkish sample of infants, and to investigate whether the current reference values of the AIMS are representative for Turkish full-term infants. The study was conducted with 411 Turkish infants of both sexes (195 girls and 216 boys), born with gestational age 38 weeks and older, weighing ≥2500 g at birth. Motor performance of all the cases at different ages were assessed with the AIMS which was used by a physiotherapist. The mean AIMS scores of Turkish infants were compared with the norm values of the original AIMS established in a Canadian sample of infants. The results showed no statistically significant differences between the AIMS scores of Turkish and Canadian infants during the first 18 months of life except at 0-<1 and 2-<3 months of age. The AIMS scores were significantly lower in Turkish infants than in Canadian infants at 0-<1 ($p=0.025$) and 2-<3 ($p=0.042$) months of age. In conclusion, the AIMS can be used in Turkish children to assess gross motor development, especially after 4 months of age. However, this paper was presented as a preliminary study to compare AIMS results between Turkish and Canadian infants, and further studies are needed to realize the Turkish validation of AIMS.

Key words: Alberta Infant Motor Scale, infants, motor development, motor skills.

Early detection of developmental delay in children, especially at 0-2 years of age, is important in terms of allowing early intervention. Motor development assessment is generally performed in the detection of delays and monitoring the achievement of new skills. Clinicians interested in early intervention such as doctors, physiotherapists, and occupational therapists are involved in assessing the motor status of infants, and usually use standardized neuromotor assessment instruments to help make decisions about a child's motor development.[1] Appropriate evaluations are necessary to identify motor developments and there are a number of tools available for physicians to use such as The General Movements Assessment,[2] Test of Infant Motor Performance,[3] The Bayley Scale of Infant and Toddler Development (especially the third edition, Bayley-III),[4] and the Alberta Infant Motor Scale (AIMS),[3,5,6] which are commonly used. The General Movement Assessment is an example method to assess infants up to 5-6 months and the predictive value of the assessment is high, especially between 2 and 4 months of age.[2] The Test of Infant Motor Performance is also a predictive tool to evaluate motor performance of infants under 4 months of age.[3] Bayley-III evaluates the gross motor development of infants and young children, and the fine motor skills, cognitive ability, language skills, and social-emotional behaviors up to 42 months of age.[4] The AIMS is a frequently used tool to evaluate the gross motor development of children in the early stage of life.[1,5]

✉ Büşra Kepenek-Varol
busrakepenek@gmail.com

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AIMS is a norm-referenced scale that evaluates the gross motor development of children from birth to 18 months. The scale was developed by Piper and Darrah[5,7] in Alberta, Canada, and reference values were established in a Canadian sample of infants. The scale allows the evaluation of gross motor function development of children, identifying children in need of early intervention, and monitoring treatment programs. The clinical application of AIMS is simple, the spontaneous movements of the child are evaluated by observation, and the test can be completed in a short time.[5,8]

AIMS has been used internationally as a clinical or research outcome measure for assessing gross motor abilities of term or preterm infants, although it is a Canadian norm-references measure.[9-13] The validity of the AIMS was obtained for infants in Spain,[12] a South African region,[14] Brazil,[15] Japan,[16] China,[17] and Taiwan.[18] Although AIMS is commonly used in various countries, the question remains as to whether current Canadian norm values represent the AIMS scores of infants with different cultural backgrounds.[11,19,20] In the literature, it has been reported that Brazilian children,[11] Dutch children,[21] and Flemish infants[22] showed differences in motor performance assessed by the AIMS compared with Canadian norm values, and it has been recommended that new reference values should be established for the AIMS of infants in other contries.[11,21,22] However, in another study performed by Syrengelas et al.[13], the motor performance and AIMS scores in full-term Greek children were similar to Canadian norms. In addition, Darrah et al.[20], who developed AIMS, demonstrated that AIMS continued to perform remarkably similarly to previous studies, the current normative values had remained valid for over a 20 years, and it might not be necessery to investigate ethnic or international differences. However, Saccani et al.[11] reported that the AIMS scores of Brazilian infants were lower than the scores of Canadian norms,[5] and the authors emphasized that to establish reference values for the AIMS of infants was important across cultures. AIMS has been widely used as a measure of clinical outcome and research worldwide,[9,10,13,18] including Turkey.[23,24] To our knowledge, no study has investigated AIMS reference values in Turkish infants. The aim of this study was to compare the Canadian norms with the AIMS scores of a Turkish sample of infants, and to investigate whether the current reference values of AIMS were representative for Turkish full-term infants during the first 18 months of age.

Material and Methods

Subjects

A total of 411 healthy full-term infants aged between 5 days and 18 months were assessed using AIMS. The study was conducted with Turkish infants of both sexes, born with gestational age 38 weeks and older, and weighing ≥2500 g at birth. The total sample consisted of 195 girls and 216 boys. The age and sex distribution per month of age is shown in Table I. The exclusion criteria consisted of infants with a history of pre-, peri- or post-natal problems associated with risk for developmental delay, congenital malformations, orthopedic or neurologic diseases, genetic syndromes, and status of the child that would not allow for assessment (such as crying, restlessness, acute or chronic disease). No infants were assessed more than once. Parents were informed about the aim of the study and informed consents

Table I. Demographic and clinical characteristics of infants.

Characteristics	Results
Gender, n	
Girls	195
Boys	216
Gestational age (week), mean ± SD	39.1 ± 1.08
Birth weight (g), mean ± SD	3301.4 ± 475.3
Type of birth, n	
Vaginal	187
Cesarean section	224

n = number of participants, SD = standard deviation

were received. The study was aproved by the Medical Ethics Committee of Bezmialem Vakıf University, Turkey (54022451-050.05.04-16/191).

Assessment tool and procedure

AIMS is a well-known, norm-referenced, discriminative tool used to evaluate gross motor development in early infancy. It can be performed by any health professional with a background in infant motor development and an understanding of the main components of movements. AIMS is a measure of gross motor development based on dynamic motor theory and neuromaturational theory. It was developed by Canadian physiotherapists, and the normative values were established using a cohort of 2202 Canadian infants.[5] The scale comprises 58 items that assess the spontaneous movements of infants in the prone (21 items), supine (9 items), sitting (12 items), and standing (16 items) positions. Items are defined that detail weight-bearing ability, postural alignment, and the control of antigravity muscles during observation of motor skills of the infants.[5-7] The least and most observed items in each position are defined as a 'window' of current motor development. AIMS is graded on a score sheet as observed items (one point) or not observed (zero point) within this window; a manual is available to provide more details on scoring movements. The total raw score is the sum of the scores for the four positions, which can range between 0 to 58; higher scores indicate better motor development. The total raw score is converted to an age-based percentile rank, varying from 5 to 90% for comparison norms from a sample of Canadian infants.[5] The validity and reliability of AIMS has been demonstrated.[6,12,18,25] AIMS has many advantages such as easy evaluation through observation, it is noninvasive, inexpensive, requires minimal space, it can be applied quickly (approximately 20-30 minutes), and does not require excessive handling of the child.[5,6]

The children were properly examined by an expert pediatrician before evaluating with AIMS, and then directed to the physiotherapist in accordance with the inclusion criteria. All 411 infants at different ages were assessed once using AIMS. AIMS was used by a physiotherapist who had more than 8 years' experience in pediatric physiotherapy (especially in evaluation and treatment of infants), and had used AIMS extensively. AIMS was applied in a quiet room with adequate space and toys required for the assessment, and adjustable room temperature. The child was underdressed or dressed lightly and observed in the evaluation room in the presence of the mother or father or both, and he/she was active and awake during the assessment. Toys were used to encourage and prompt some children to move to different positions. The AIMS assessment took approximately 25 minutes per infant, including waiting for the infant to adjust to the surroundings. At the end of the test, the total raw score was calculated and the percentile ranks were established. All assessments were performed by the same physiotherapist, and the scale was performed at each month of age.

Data analyses

Statistical analyses were performed using SPSS version 20 for Windows. The quantitative variables were presented as means with standard deviations. In this study, there were 18 groups according to the age of the children. The G*Power v3.1 program (Universitat Kiel, Germany)[26] was used to calculate the sample size on the basis of findings from a previous study,[13] which revealed that at least 20 children for each age group could provide a power of 80%. The mean AIMS scores, standard deviations, and percentiles were calculated for each group. The one-sample t-test was used to compare the mean AIMS scores of the study population with the normative values of Canadian infants[5] for each group. Statistical significance was accepted as $p<0.05$.

Results

The study consisted of 411 full-term infants whose gestational age ranged between 38

and 42 weeks. The demographic and clinical characteristics of infants are shown in Table I. Table II and Figure 1 provide information about the percentiles of Turkish full-term infants by age groups.

Table III presents the means and standard deviations of the AIMS scores of Turkish and Canadian infants, and a comparison of mean AIMS scores of Turkish infants and the original Canadian scores for 0 to 18 months according to age groups. In general, the original Canadian scores were higher compared with the scores of Turkish infants across several age groups. Statistically significant differences were observed between Turkish and Canadian infants only at 0-<1 (p=0.025) and 2-<3 (p=0.042) months of age, and there were no statistically significant differences for any other age groups.

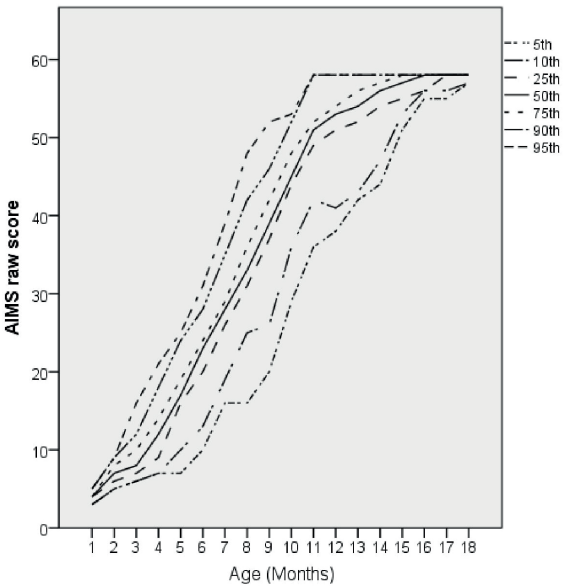

Fig. 1. Percentile ranks of AIMS-scores in Turkish infants.

Discussion

The main purpose of the present study was to provide the preliminary results of AIMS scores in a cohort of Turkish infants. The AIMS scores of the infants included in this study were compared with the original Canadian norms.[5] As a result, the AIMS scores of the Turkish and

Table II. The percentile ranks of AIMS scores of Turkish full-term infants.

Age (months)	n	Percentile ranks						
		5th	10th	25th	50th	75th	90th	95th
0-<1	21	3	3	4	4	4	5	5
1-<2	39	5	5	6	7	8	9	9
2-<3	32	6	6	7	8	10	12	16
3-<4	23	7	7	9	12	14	18	21
4-<5	20	7	10	16	17	19	24	25
5-<6	23	10	13	20	23	24	28	31
6-<7	20	16	19	26	28	29	35	39
7-<8	20	16	25	31	33	36	42	48
8-<9	25	20	26	37	39	42	46	52
9-<10	21	29	36	44	45	48	52	53
10-<11	22	36	42	49	51	52	58	58
11-<12	20	38	41	51	53	54	58	58
12-<13	21	42	43	52	54	56	58	58
13-<14	20	44	47	54	56	57	58	58
14-<15	22	51	53	55	57	58	58	58
15-<16	20	55	56	56	58	58	58	58
16-<17	22	55	56	58	58	58	58	58
17-<18	20	57	57	58	58	58	58	58

Table III. Comparison of the mean-AIMS scores of Turkish and Canadian infants.

Age (months)	Turkish infants				Canadian infants*				M_T-M_C	p
	n	girls/boys	M_T	SD	n	girls/boys	M_C	SD		
0-<1	21	11/10	4.1	0.76	22	9/23	4.5	1.37	-0.40	0.025
1-<2	39	15/24	6.97	1.38	56	29/27	7.3	1.96	-0.33	0.150
2-<3	32	16/16	8.78	2.72	118	58/60	9.8	2.42	-1.02	0.042
3-<4	23	12/11	11.96	3.74	90	45/45	12.6	3.29	-0.64	0.419
4-<5	20	7/13	17.15	4.1	122	53/69	17.9	4.15	-0.75	0.455
5-<6	23	13/10	22.04	4.92	189	109/80	23.2	4.75	-1.16	0.272
6-<7	20	4/16	27.45	5.24	225	106/119	28.3	5.50	-0.85	0.478
7-<8	20	13/7	33.05	6.54	222	102/120	32.3	6.85	0.75	0.614
8-<9	25	11/14	38.36	7.15	220	111/109	38.9	8.69	-0.54	0.709
9-<10	21	10/11	44.71	5.5	189	84/105	45.5	7.47	-0.79	0.521
10-<11	22	14/8	50.14	5.21	155	74/81	49.3	5.92	-0.84	0.460
11-<12	20	8/12	51.8	4.94	155	78/77	51.3	7.11	-0.50	0.656
12-<13	21	8/13	52.95	4.58	124	71/53	54.6	4.52	-1.65	0.115
13-<14	20	12/8	54.75	3.75	86	39/47	55.6	5.01	-0.85	0.324
14-<15	22	13/9	56.27	2.02	61	25/36	56.9	1.97	-0.63	0.162
15-<16	20	10/10	57.4	0.99	40	21/19	57.8	0.45	-0.40	0.088
16-<17	22	10/12	57.64	0.84	49	21/28	57.8	0.55	-0.16	0.376
17-<18	20	8/12	57.85	0.36	49	21/28	57.9	0.35	-0.05	0.549

M_T = mean in Turkish infants; M_C = mean in Canadian infants; n = number of participants; SD = standard deviation
*The mean AIMS-scores, n, and SD values of Canadian infant reported as in the AIMS manual (Reference 5: Piper MC, Darrah J. Motor Assessment of the Developing Infant. Philadelphia: WB Saunders, 1994: 205.)

Canadian infants were similar, except the first and third months of age. Canadian infants seem to have had earlier motor development in the first months than Turkish infants, and they showed higher scores compared with Turkish infants across several age groups, but there was a statistically significant difference only in the first month and third month of age. Especially after the 6th month, AIMS scores were quite similar. To our knowledge, AIMS has not yet been validated in a Turkish population, and the current study is presented as a preliminary study to determine the AIMS scores of Turkish full-term infants.

AIMS is a discriminative scale used to assess gross motor development of children from birth to 18 months of age and provides total raw scores. The scale is performed by observing the spontanous movements of a child in different positions; it is inexpensive, practical, does not require excessive handling of the child, and can be completed in a short-time.[5,9,6] Due to these reasons, AIMS has been widely used as a measure of clinical outcomes and research, both in preterm and term infants around the world.[9,10,13,18] Nevertheless, several studies indicated that AIMS normative data would be inadequate for children of different cultures.[11,15,22,27] One such study by Fleuren et al.[21] emphasized that new reference values of AIMS needed to be established for Dutch children, and the authors also suggested that all other European countries needed to determine the need for new reference values. Another study performed by Syrengelas et al.[13] indicated that Greek infants showed gross motor maturity similar to Canadian infants. In contrast, Darrah et al.[20], who developed AIMS, demonstrated that AIMS continued to perform remarkably similarly to previous studies. The authors asserted that the current normative values had remained valid for over 20 years and it might not be necessery to investigate ethnic

or international differences.[20] It is important to note that the sample of this research published in 2014, consisted of Canadian infants, and it is not possible to compare the sample directly with international populations. However, it is clear that the original scores of AIMS should be used as they have been in the past.[20] Conflictingly, in 2016, Saccani et al.[11] found that the AIMS scores of Brazilian and Canadian infants were different, and emphasized the need to create new reference values for AIMS scores of infants for different cultures. The authors also reported that the AIMS scores of Brazilian infants were significantly lower than the original AIMS scores in the first 3 months of life.[11] The results of another study performed by Syrengelas et al.[13] showed that Greek infants at 2-<3 months received significantly higher AIMS scores than Canadian infants, but there were no significant differences for the other age levels till 18 months, and the authors attributed these differences to possible variations in child-rearing and parental care.

Etnicity, child-rearing practices, and cultural differences could affect the gross motor development of infants.[28,29] In addition, maternal care including the baby's sleep and play position, the time spent with the child, and the ability to play with toys are also important for the development of the child.[11,30,31] De Kegel et al.[22] demonstrated that Flemish infants had lower AIMS scores than the Canadian norms, they also questioned the sleep and play positioning of infants, and postulated that the lower scores of Flemish infants were related to the sleep position and play time in different positions such as in the supine and prone position or in a sitting device. It is known that in some areas in Turkey, babies are swaddled in the early months and / or are not lain in the prone position. As a matter of fact, some babies who were brought to our clinic for a gross motor development assessment were swaddled. The sleep and play positions of the infants may be related to infant gross motor development, and 'tummy time' is an important position for developing the upper body strength in order to achieve movement against gravity.[32,33] Although the sleep positions of children and also the time they spend on their tummy during awake periods were not known in present study, many parents reported that they had difficulty keeping their infant in the prone position while awake. Therefore, cultural differences such as swaddling, parent's child-rearing practices or socio-economic factors may play a role in the gross motor abilities of infants. In addition, although there was no statistically significant difference between the AIMS scores of Turkish and Canadian infants at 2 months of age, Turkish infants showed a lower motor performance than Canadian infants in the same age range. It should be kept in mind that the AIMS scores of Turkish infants were lower than the original AIMS scores in all age groups until the first 5 months of life, and it remains to be investigated as to whether this difference was clinically important or if it was a random result. Hovewer, the gross motor milestones were found similar across five diverse countries,[34] and another study[35] found similar results across four countries including Turkey. It has also been reported in studies that environmental or/ and familial factors may adversely affect child development.[36,37] Saccani et al.[27] investigated gross motor development of Brazilian, Greek and Canadian infants assessed with AIMS, and reported that in the second year of life differences in the motor development were milder and at 15 months of age were similar in the three groups. Whether the gross motor development domains vary in healthy children across different countries and different factors such as ethnicity, cultural or socio-economic factors has not been established. Further research is needed to make clear whether the AIMS score may be affected by different social, cultural or ethnic factors. This differences in the scores of Turkish and Canadian infants might be due to the fact that there is less movement performance capacity in the first months of life and fewer items can be evaluated in the subcategories. AIMS provides fewer items to assess gross motor development of infants within the first month of life.

Another issue that should be considered is the opinions about the low sensitivity of AIMS in early life.[11,38] According to a systematic review, it may be better to use more detailed tests such as the Bayley test or Test of Infant Motor Performance (TIMP) for the first months of life.[38] Another study indicated that only a few items were suitable for assessing infants in the early months, and evaluations of gross motor development using AIMS from three to nine months of age were best.[39] In the present study, the differences of AIMS scores between Turkish and Canadian infants in the first months of life may be due to the lack of sensitivity of AIMS.

The main limitation of the present study is that the sample of the study may not reflect the general AIMS scores of Turkish infants because the study was conducted in only one city in Turkey. The fact remains, however, the city from which the data were provided is the most populous metropolis in the country, and receives immigration from every city in the country. When considered from this point of view, the data collected from Istanbul may reflect a large part of the country, but data collected from different areas of the country may be more reliable in order to reach safe conclusions. In addition, the power analysis for this study was calculated on the basis of findings from a previous study[13] with a 0.05 significance level, which found that 20 subjects for each age group could provide a power of 80%, and the study produced enough data to provide this power. Another limitation is that only one observer assessed all the infants, thus intra- and inter-observer consistency could not be evaluated. Finally, the effects of socioeconomic factors on motor development of infants were not investigated.

In conclusion, our findings suggest that AIMS can be used in Turkish children, especially after 4 months of age. However, this paper is presented as a preliminary study to compare AIMS results between Turkish and Canadian infants, and we strongly recommend that a validation study including cultural adaptations and data collected from different areas of the country should be performed in order to draw more reliable conclusions. AIMS can be used as a valuable, easy and functional assessment tool in the early identification of risky infants in maternity hospitals, routine health services or centers that privode early intervention in Turkey, as well as a basis for research studies. However, further studies are needed to determine if AIMS is valid for the greater Turkish infant population in different cities to assess gross motor development and determine developmental delays. In our opinion, one of the future goals should be to realize the Turkish validation of AIMS in a cohort of full-term and also preterm Turkish infants.

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Case Report

A novel homozygous nonsense mutation (p.Y78*) in *TMPRSS6* gene causing iron-refractory iron deficiency anemia (IRIDA) in two siblings

Seda Çakmaklı[1], Çiğdem Kaplan[2], Mehmet Uzunoğlu[2], Merve Büyükbayram[2], Emel Görgülü[2], Nurten Özkan Zarif[2], Ebru Yılmaz Keskin[3]

[1]*Department of Medical Genetics, Necip Fazıl City Hospital, Kahramanmaraş;* [3]*Division of Pediatric Hematology and Oncology,*
[2]*Department of Pediatrics, Süleyman Demirel University Faculty of Medicine, Isparta, Turkey.*

ABSTRACT

Iron-refractory iron deficiency anemia (IRIDA) is an inherited iron metabolism disorder caused by mutations in *TMPRSS6* gene encoding matriptase-2, which results in increased hepcidin synthesis. The hallmarks of the disease are hypochromic microcytic anemia, low transferrin saturation, slightly low or normal ferritin levels in contrast to classic iron deficiency anemia (IDA), inadequate response to oral iron, and only a partial response to parenteral iron. We report here a 6-year-old Syrian boy with unexplained microcytic anemia since one year of age. Genetic analysis of the *TMPRSS6* gene revealed a novel homozygous nonsense mutation in exon 3 (c.234C>G; p.Y78* or p.Tyr78*). In the presence of hypochromic microcytic anemia accompanied by atypical iron parameters not in accordance with classic IDA, and inadequate response to iron therapy, IRIDA should be remembered in the differential diagnosis.

Key words: iron deficiency anemia, hepcidin, *TMPRSS6*, matriptase-2.

Iron deficiency anemia (IDA) has constituted a serious public health problem for centuries. It develops most commonly due to inadequate dietary intake. In the presence of hypochromic microcytic anemia, IDA is the first underlying cause to be considered. However, some inherited conditions with variable clinical characteristics may also result in microcytic anemia by causing defective iron metabolism.

Iron-refractory IDA (IRIDA) described about a decade ago is one of the inherited iron metabolism disorders, and develops due to loss-of-function mutations in *TMPRSS6* gene.[1] As a result, affected individuals have inappropriately elevated hepcidin levels in contrast to classic IDA cases, whose serum hepcidin levels decrease markedly to promote intestinal iron absorption.[2,3] Remarkably, despite congenital, severe iron deficiency, affected cases display normal growth and intellectual development.[2]

We present here a child who was followed up for unexplained microcytic anemia since early childhood. Eventually, the case was diagnosed as IRIDA by genetic analysis which revealed a novel homozygous nonsense *TMPRSS6* mutation.

Case Report

A 6-year-old Syrian male patient born to first-degree cousin marriage was admitted due to microcytic anemia known since one year of age. He had history of inadequate response to oral iron therapy including the use of ferrous glycine sulfate ordered several times before by different physicians. Bone marrow aspiration studies had not identified the underlying cause, either. Although not proven, thalassemia intermedia had been thought in the differential diagnosis, and the case had been transfused twice.

✉ Ebru Yılmaz Keskin
ebruyilmaz81@hotmail.com

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Among the family members, the 10-year-old brother also had microcytic anemia, and the mother had history of IDA during pregnancy.

At the time of admission to our clinic, the patient was using 2.5 mg/kg/d iron (III)-hydroxide polymaltose complex for two weeks. Laboratory data of the patient and his available family members at the time of admission are presented in Table I. In addition, our case had negative tissue transglutaminase IgA and IgG antibodies examined for celiac disease accompanied by a normal serum IgA level, and his hemoglobin (Hb) electrophoresis examination results were within normal range, HbA0 level being 96.5%.

In the presence of microcytic anemia for many years which was unresponsive to iron therapy, low transferrin saturation accompanied by normal serum ferritin level in the absence of an infection or an inflammatory condition, IRIDA was thought as the possible diagnosis.

For the purpose of confirming the diagnosis, *TMPRSS6* gene sequence analysis of all coding exons and exon-intron boundaries was performed by next generation sequencing analysis (Miseq-Illumina Inc.). A novel homozygous mutation in exon 3 (c.234C>G; according to NM_153609.3) that causes a premature stop codon (p.Y78* or p.Tyr78*) was identified in the index case and his similarly affected sibling. In addition, the 15-year-old sister and the parents were found heterozygous for the same mutation. To validate the mutation in this family, we performed Sanger sequencing for the third exon of the *TMPRSS6* gene in all the members of the family. Results of the Sanger sequencing analysis confirmed the variant we detected by next generation sequencing analysis (Fig. 1).

The identified nonsense mutation was neither found in Exome Aggregation Consortium nor 1000 Genomes databases. Prediction of the functional effect of the mutation was done by using in silico analysis tool MutationTaster (http://www.mutationtaster.org/). MutationTaster prediction was disease-causing.[4] Variant was submitted to ClinVar; submission accession number is SCV000864243.1 (https://www.ncbi. nlm.nih.gov/clinvar).

The patient received intravenous iron therapy. Response to treatment is summarized in Table II. This case report was written after obtaining the parents' written informed consent.

Table I. Laboratory findings of the patient and his available family members at the time of admission.

	Proband	15-year-old sister	10-year-old brother
Hemoglobin (g/L)	7.2	13.7	8.9
Hematocrit (%)	26.2	40.4	29.7
Red blood cells (10^6 /μL)	5.25	5.35	5.91
MCV (fL)	49.8	75.6	50.2
MCH (pg)	13.8	25.6	15.0
MCHC (g/dl)	27.6	33.8	29.9
RDW (%)	21.5	16.2	21
White blood cells (10^3 /μL)	5.2	5.9	6.2
Platelets (10^3 /μL)	422	182	410
TSI (%)	6.7	23.2	6.2
Ferritin (ng/ml)	37	10	51
C-reactive protein	Negative	Negative	Negative
TMPRSS6 mutation	c.234C>G (p.Y78*) (p.Tyr78*) Homozygous	c.234C>G (p.Y78*) (p.Tyr78*) Heterozygous	c.234C>G (p.Y78*) (p.Tyr78*) Homozygous

MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; TSI: transferrin saturation index.

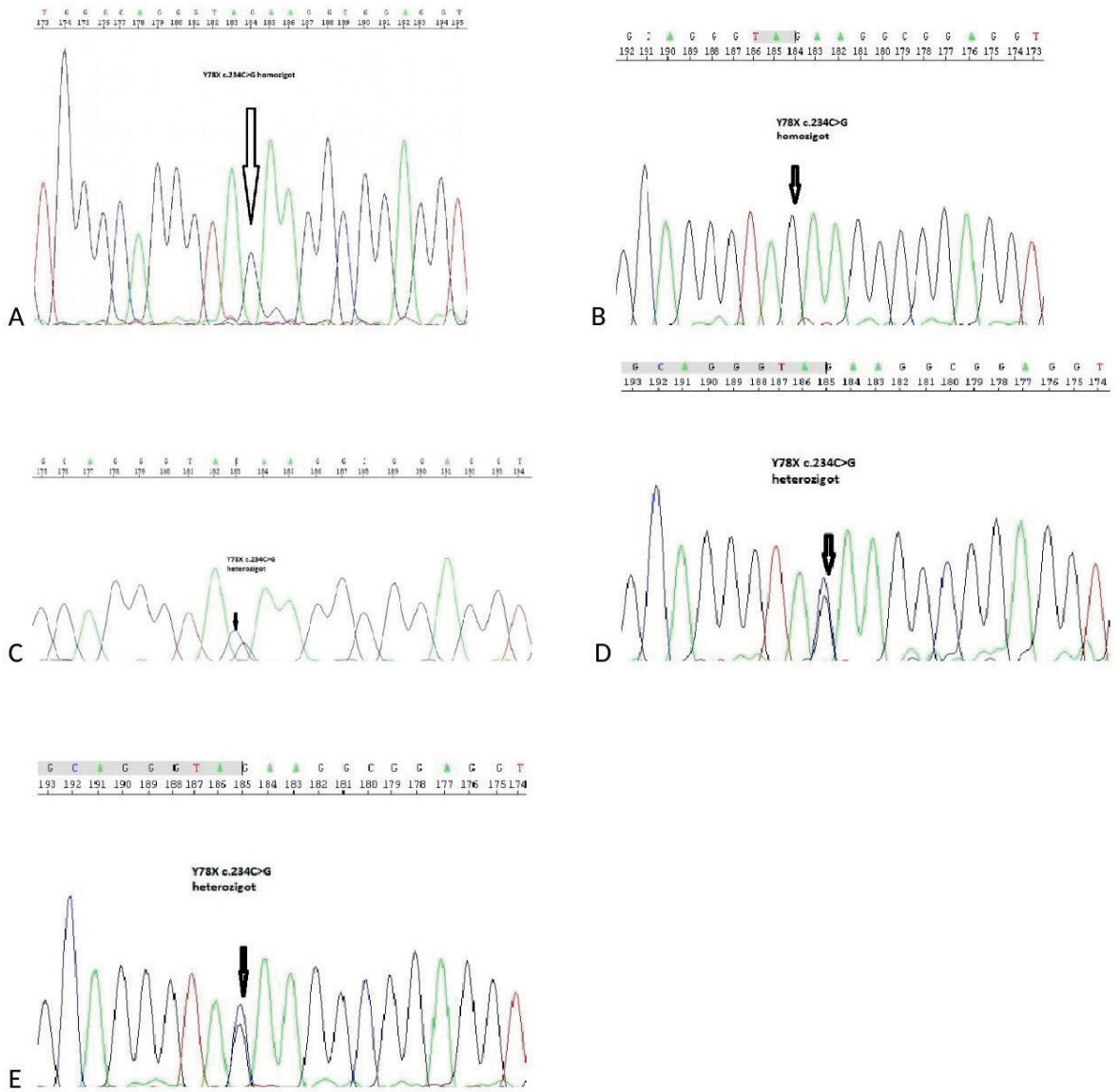

Fig. 1. Partial genome sequences of the *TMPRSS6* gene of (A) the patient (B) the patient's affected brother (C) the father (D) the mother (E) the patient's unaffected sister.

Discussion

In a child with with hypochromic microcytic anemia, acquired IDA is the main disorder to be considered, whereas for congenital microcytic hypochromic anemias, the differential diagnosis is dominated by the thalassemia syndromes. Rarer forms of congenital microcytic anemias characterized by certain defects in iron transport, iron uptake, and mitochondrial iron utilization should also be included in the differential diagnosis. Among these are IRIDA, divalent metal transporter 1 (DMT1) deficiency, congenital hypotransferrinemia, some hereditary forms of sideroblastic anemia, and aceruloplasminemia.[5] Although data regarding the prevalence of these rare congenital microcytic anemias are lacking, the recent increase in published IRIDA cases and affected families suggests that IRIDA may be the most common form.

IRIDA is an autosomal recessive disorder caused by mutations in the *TMPRSS6*

Table II. Laboratory findings of the proband before and after intravenous (IV) iron replacement.

	Before IV iron	One week after IV iron	One month after IV iron	Two months after IV iron	Three months after IV iron	Five months after IV iron
Hemoglobin (g/dl)	7.2	8.2	8.5	8.2	8.8	10.0
Hematocrit (%)	26.2	29.2	28.7	27.3	29.2	30.6
Red blood cells (10^6 /μL)	5.25	5.62	5.36	5.05	5.23	5.78
MCV (fL)	49.8	52.0	53.5	54.1	55.8	53.0
MCH (pg)	13.8	14.5	15.9	16.1	16.9	17.3
MCHC (g/dl)	27.6	27.9	29.7	29.9	30.3	32.6
RDW (%)	21.5	24.3	25.4	22.0	20.2	19.2
TSI (%)	6.7	n.a.	5.2	1.7	3.6	2.5
Ferritin (ng/ml)	37	258	236	218	196	206

MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red cell distribution width; TSI: transferrin saturation index; n.a.: not available.

gene which encodes matriptase-2, a type II transmembrane serine protease.[1-3] *TMPRSS6* gene is located on chromosome 22, contains 18 exons; all of the exons are coding. Matriptase-2 contains a large extracellular region harbouring several structural domains. It is involved in downregulation of hepcidin expression, the master regulator of iron metabolism in hepatocytes.

In a previously untreated individual with hypochromic microcytic anemia, clues for an IRIDA diagnosis from the initial assessment of iron status include two patterns: 1) the degree of microcytosis (MCV 45–65 fL range) relative to the anemia (Hb 6–8 g/dL range); and (2) a markedly low serum iron level and low transferrin saturation index (usually <5%) in the presence of a slightly low or even normal ferritin.[2,6] At his first admission to us, our case had a moderate degree of anemia (Hb level, 7.2 g/dL) accompanied by profound microcytosis (MCV level, 49.8 fL), and his transferrin saturation level was low (6.7%), while his ferritin level was within the normal range in the absence of a known infection or an inflammatory condition. In the presence of these findings, we strongly suspected IRIDA in our case.

Efficient intestinal iron absorption requires an acidic duodenal environment and a functioning duodenal epithelium. Common reasons for poor iron absorption include achlorhydria due to chronic proton pump inhibition and damage to the duodenum (eg, celiac sprue).[7] Our case had no history of any drug use, and in his laboratory evaluation, antibodies associated with celiac disease were found to be negative. Inadequate response to oral iron therapy in the absence of simultaneous drug use or a disorder such as an inflammatory bowel disease further supported the diagnosis of IRIDA in our case.

Ideally, the plasma or urinary hepcidin levels could be measured to easily distinguish true iron deficiency from IRIDA. In IRIDA, normal-to-elevated hepcidin levels are expected in contrast to classic IDA where hepcidin levels decrease to promote intestinal iron absorption.[2] However, although more than a decade has passed since the discovery of hepcidin, there is yet no hepcidin assay approved by the Food and Drug Administration available for clinical use. Once a hepcidin assay becomes available, it may serve as a useful aid in the diagnosis of IRIDA. Our patient's hepcidin level was not measured due to technical equipment restrictions although we expect it to be in normal or above the normal range.

In a case suspected to have IRIDA with certain laboratory and clinical data mentioned above, the diagnosis of IRIDA is very likely especially

if the iron deficiency truly appears to have its onset in infancy or childhood.[2] The only current diagnostic test for IRIDA is sequencing of the *TMPRSS6* gene. In our case, diagnosis of IRIDA was confirmed by mutational analysis.

Interestingly, in contrast to the usual form of IDA, IRIDA may not be associated with neuropsychological impairments as affected cases display normal growth and intellectual development.[2,8] The potential role of *TMPRSS6* in brain iron homeostasis was speculated, and it was hypothesized that inappropriately elevated hepcidin and normal to high ferritin levels compared with poor iron reserves may protect the brain in IRIDA.[8]

As expected in IRIDA cases, our patient displayed findings of defective iron utilization as evidenced by an elevation of ferritin levels after intravenous iron therapy with an only mild correction of his anemia. This observation may be explained by inappropriately elevated hepcidin levels in IRIDA patients. Hepcidin exerts its iron regulatory effects by binding to ferroportin which is the only known cellular iron exporter. It is expressed on the basolateral membrane of enterocytes, on the plasma membrane of macrophages and in hepatocytes. Since hepcidin binding leads to the internalization and degradation of ferroportin in lysosomes, dietary iron absorption and mobilization of iron from macrophage stores are decreased if hepcidin levels are elevated.[2] Therefore, the decrease in ferroportin expression through hepcidin not only explains the development of iron deficiency, but also unresponsiveness to oral iron in IRIDA cases.

In heterozygous carriers of a *TMPRSS6* mutation, iron deficiency was reported to develop under certain clinical conditions (such as in pregnancy), an observation inconsistent with recessive transmission.[9] Similarly, *TMPRSS6*-haploinsufficient mice were observed to be more susceptible to iron deficiency under conditions of iron restriction or an increased iron requirement.[10,11] Supporting these observations, the mother of our case had history of iron deficiency anemia during pregnancy which may be associated with her carrier status.

In few IRIDA cases followed-up until adulthood, increase in Hb levels to acceptable values was observed.[12] This was explained by the consequence of the greater availability of the limited amount of dietary iron for erythropoiesis in adults affected by IRIDA than in pediatric cases who need iron also for body growth.

Premature termination codons that are followed by an intron that is located more than 50–55 nucleotides downstream are generally expected to trigger Nonsense-mediated mRNA decay (NMD).[13] NMD is a mechanism that selectively eliminates mRNAs harboring premature termination codons.[14] We report here a C to G transversion of the fifth base of exon 3 which causes a premature stop codon (p.Y78*). We expect that this mutation in our patient leads to no protein being translated at all and haploinsufficiency through NMD mechanism.

Up to now, at least 45 mutations including missense, nonsense, frameshifts and splicing defects in the *TMPRSS6* gene leading to IRIDA phenotype have been reported in the literature.[2] To the best of our knowledge, only six nonsense mutations have been reported in *TMPRSS6* gene so far: p.Y355*, p.Y393*, p.S561*, p.R599*, p.Q405* and p.K752*.[15] The variation we detected in our patient (c.234C>G; according to NM_153609.3), is a nonsense mutation which has not been reported before.

De Falco et al.[16], stated that patients harboring two nonsense mutations present a more severe anemia and microcytosis compared with the patients carrying either 2 missense mutations or 1 missense and 1 nonsense mutation. Our patient's Hb level and mean corpuscular volume (MCV) of his erythrocytes at admission were 7.2 g/dL and 49.8 fL, respectively, while his 10-year old brother had an Hb of 8.9 g/dL and an MCV of 50.2 fL. Anemia of the two siblings may have been even more severe during their early

childhood, a time period when iron requirement for body growth is more marked.

In conclusion, we report a novel homozygous nonsense mutation in *TMPRSS6* gene in a 6-year-old Syrian boy and his sibling with unexplained microcytic anemia before. Through the timely genetic diagnosis of this disorder with a quite favorable prognosis, unnecessary (invasive) examinations can be avoided. Our results expand the mutation spectrum of the *TMPRSS6* gene contributing new data on genotype-phenotype correlations in IRIDA. Further studies are required to better understand the effect of *TMPRSS6* gene alterations on the phenotype and protein function.

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Case Report

A rare cause of epileptic encephalopathy: a beta-propeller protein associated neurodegeneration case with a new mutation and literature review

Nezir Özgün[1], Leyla Özer[2], Ahmet Yaramış[3]

[1]Department of Pediatrics, Division of Pediatric Neurology, Diyarbakir Children's Hospital, Diyarbakır; [2]Mikrogen Genetic Diagnostic Center Ankara; [3]Department of Pediatrics, Division of Pediatric Neurology, Diyarbakır Dicle University Faculty of Medicine, Diyarbakır, Turkey.

ABSTRACT

In this report, detailed clinical features of a female patient and a new mutation that was not previously identified in the WD repeat-containing protein 45 (WDR45) gene are presented in order to contribute to the information in the literature on the phenotype as well as genotype of Beta-Propeller Protein Associated Neurodegeneration. Whole Exome Sequencing (WES) analysis was done since etiology could not be determined. Our case was admitted to the hospital due to epilepsy, growth retardation and autism. Her family history was unremarkable except consanguineous marriage. She had tonic seizures twice at the age of 7 and 12 months and had continual seizures after 16 months. At the time, electroencephalography and brain MRI were performed twice were determined to be normal. Brain MRI Spectroscopy was also found to be normal at 35 months of age. Metabolic screening tests (acyl carnitine profile, urine organic acids, plasma amino acids, a very long chain fatty acid profile, etc.) were also normal. Genetic screening of the epilepsy panel for epileptic encephalopathies was negative. WES analysis revealed heterozygous previously unreported variant in intron 6 of the WDR45 gene, c.344+5G>A. In conclusion; Beta-Propeller Protein Associated Neurodegeneration should be considered as an option in the diagnosis of female patients with clinical findings of epilepsy, growth retardation and autism, with unspecified etiology.

Key words: beta-propeller protein associated neurodegeneration, WDR45 gene mutation, epileptic encephalopathy.

Beta-Propeller Protein Associated Neurodegeneration (BPAN), also known as Neurodegeneration with Brain Iron Accumulation type 5 (NBIA5) (OMIM # 300894) is an X-linked transient disease, mostly caused by de novo mutation in the WD repeat-containing protein 45 (WDR45) gene.[1-3] Mitochondrial abnormalities, autophagic defects, decrease in lysosomal functions, cellular iron accumulation and increase in oxidative stress can develop in the patient. The prevalence of NBIA group disorders is estimated to be less than 1/1.000.000.[4] The clinical course of the disease consists of two phases. The first phase begins in childhood and early-onset seizures, developmental retardation, cognitive impairment, speech disorder, motor dysfunctions such as ataxia, and behavioral disorders specific to autism spectrum can be seen. The second phase is subacute and begins in adolescence or early adulthood, presenting with neurological deterioration characterized by parkinsonism, dystonia and dementia.[5,6]

The diagnosis of suspected BPAN is based on clinical findings and hypo-intensity, suggestive of iron accumulation in brain magnetic resonance imaging (MRI).[7,8] The definitive diagnosis is established by means of molecular testing, identifying a heterozygous WDR45 pathogenic variant in females, and a hemizygous WDR45 pathogenic variant or deletion of WDR45 in males. Somatic mosaism has been reported rarely in girls.[1]

✉ Nezir Özgün
nezirozgun@hotmail.com

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There is no definite cure of BPAN. Supportive treatment is given for seizures and movement disorders.[9]

In this report, detailed clinical features of a female patient with non-syndromic epileptic encephalopathy, growth retardation, autism, and a new mutation that was not previously identified in the WDR45 gene are presented, in order to contribute to the information in the literature on the phenotype as well as genotype of the disease.

Case Report

Clinical History

A 40-month-old girl presented with epilepsy and growth retardation. After an eventless pregnancy, she was born at 37 weeks, weighted 3000 gram and without asphyxia. Developmental milestones: head control at 5 months, sitting without support at 11 months, walking at 21 months, first word at 25 months of age. Her parents had consanguineous marriage. She had two healthy brothers, 5 and 6 years old. No family history of any neurological disease was determined. She had the first seizure at the age of 7 months, when she had a fever. She was found to be staring at a fixed point and then having myoclonic jerks. She had a second seizure, similar to the first, at 12 months of age, again when she had a fever. Electroencephalogram (EEG) taken at that time and brain MRI taken to investigate growth retardation were normal. Febrile seizure was considered and that's why an antiepileptic drug was not prescribed. When the patient was 16 months old, phenobarbital (6 mg/kg/day, in 2 doses) was initiated upon having an attack of clonic contractions following fixed gaze in a fever-free period. She had absence, myoclonic and/or tonic seizures for 5-6 times per month despite phenobarbital. Then, pyridoxine (vitamin B6) was initiated. As it failed too, Sodium Valproate was given and continued for 2 months at increased doses (40 mg/kg/day (2 doses)). Afterwards, Sodium Valproate was discontinued and levetiracetam was started, due to the increase in seizures. With levetiracetam (60 mg/kg/day, 2 doses) and phenobarbital combination, her seizures were reduced to 2-3 times per month but increased again to 3-4 per month when she was 35 months of age. When the patient was 36 months old, phenobarbital was discontinued and clobazam (1 mg/kg/day, 2 doses) was started. Two months after starting clobazam, levetiracetam and clobazam combination stopped the seizures completely. The patient had already applied to the child psychiatry department due to speech delay, lack of communication skills, and stereotypic movements at 30 months of age and since then she was followed up with a diagnosis of autism. In the latest examination, her speech was consistent with 36 months of development although her calendar age was 52 months, and her ability to communicate and meet individual needs was consistent with 32 months (Denver Developmental Screening Test). She had gait ataxia, eye contact, moderate mental and speech delay and stereotyped movements. Currently she receives special education support and has had no seizure in 14 months. For this study, written informed consent was obtained from her parents after parents were fully informed.

Laboratory and Imaging

EEG and brain MRI taken when she was 13 months were reported as normal. Brain MRI Spectroscopy was also found to be normal at 35 months of age. Metabolic screening tests (acyl carnitine profile, urine organic acids, plasma amino acids, a very long chain fatty acid profile, etc.) were also normal. Genetic screening of the epilepsy panel for epileptic encephalopathies was negative. Since etiology could not be determined, Whole Exome Sequencing (WES) was requested. No pathological discharge was detected in EEGs repeated at 35 and 45 months of age.

The patient was confirmed as BPAN by means of WES and brain MRI taken repeatedly including T2 axial gradient sequence at 45 months of age. There was no signal change supporting iron accumulation in brain MRI (Fig. 1).

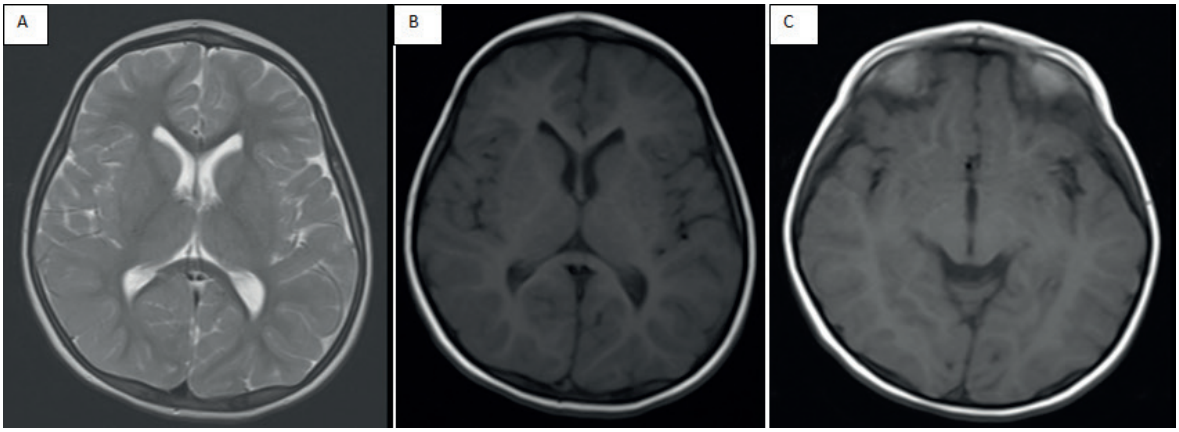

Fig. 1. Brain MRI at 45 months of age shows no evidence of iron deposition. A: T2 Axial Globus Pallidus section, B: T1 Axial Globus Pallidus section, C: T1 Axial Substanti Nigra section.

Genetic Analysis

Genomic DNA was isolated from peripheral blood samples using the QIAamp DNA Blood Mini kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. WES analysis revealed heterozygous previously unreported variant in intron 6 of the WDR45 gene, c.344+5G>A (g.48934299C>T) (Centogene, Germany). Detected variant was also analysed and confirmed by Sanger sequencing according to the manufacturer's protocols. Sanger sequence analysis image is shown in Figure 2. The amplicons were analyzed by direct sequencing with ABI 3500 (Life Technologies, Waltham, Massachusetts, USA). Analysis of sequence results was done by Mutation Surveyor Programme (SoftGenetics, USA).The mutation was considered as *de novo* because the genetic analyses of the parents were normal.

Discussion

BPAN is a rare disease with 68 cases reported in the literature so far.[10] Here, we present the case of a female patient presenting with clinic findings of epileptic encephalopathy with a previously unidentified mutation.

The development and common use of the techniques such as MRI, Next-Generation Sequencing (NGS) and WES, facilitated the early diagnosis of the disease and contributed to its awareness.[11] Although iron deposition in the brain has been often described in globus pallidus, it can be seen as hypointensity in areas such as cerebellum and substantia nigra on T2-weighted images, depending on the type of the disease.[7,8] As an additional and specific finding, hyperintense halo can be seen in T1-weighted images of substantia nigra and

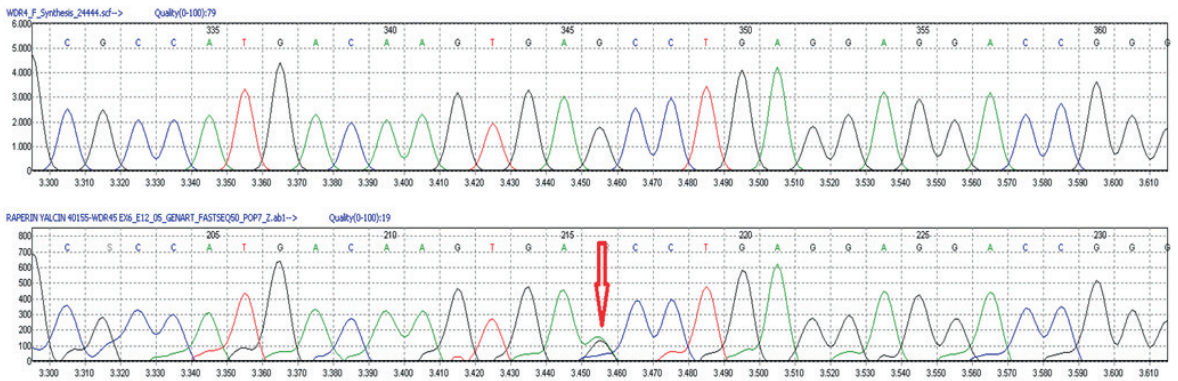

Fig. 2. Sanger sequence analysis of the detected mutation.

cerebral peduncle.[12] However, brain MR can be normal especially in early childhood, as it was in our patient. In some patients, significant signs may develop on brain MRI results in the late period when movement disturbances begin.[1,13] NGS and WES enabled early diagnosis without significant clinical findings and/or iron accumulation in basal ganglia.[1,14]

The clinical presentation of BPAN may show significant phenotypic variance. However, its main characteristic clinical features in childhood are usually early onset seizures with poor treatment response, speech, motor and mental delay. Initially febrile seizures are seen and then non-provocative, tonic, tonic-clonic, absence or myoclonic seizures.[8,15] Epilepsy is usually most severe in childhood and gets mild in later years. Multiple types of seizures can be seen in the same patient.[1] The clinic findings of our patient was consistent with epileptic encephalopathy[16], revealing itself with continuous epileptic activity and neurological and cognitive impairment. Her initial seizures were febrile, then afebrile, absence, tonic-clonic and myoclonic seizures. The decrease in seizures after clobazam may be due to the effect of the drug or the natural course of the disease, alleviating epilepsy at later ages. Another interesting feature in our patient was, despite her frequent seizures, epileptic discharge or ground rhythm irregularity was not detected on the interictal EEG, taken three times. Although the prevalence of NBIA group disorders having neurodegeneration is estimated to be less than one in a million[4], it is probably higher. In a series of 655 patients who had epileptic encephalopathy clinic findings and negative results for known pathogenic variant genes, Carvill et al.[17] screened the patients for the WDR45 gene using the NGS method and determined 7 BPAN patients, where all were females and four of them had previously unspecified mutations.

Morikawa et al.[18] reported 6 patients (2 females and 4 males), having a mutation in the WDR45 gene and a history of infantile spasm. Infantile spasms started before the age of one in 5 patients, and at 46 months of age in one. Only two patients had iron accumulation in the brain. Female patients had milder phenotypic features.[18]

Of the cases published in the literature to date, 85.9% are girls, 67.7% had epileptic seizures, that usually started in childhood, and 90.2% displayed no iron accumulation on brain MR.[10]

As far as we know, this is the first BPAN case in childhood age group, reported from Turkey. Upon mutation database search, the c.344+5G>A mutation detected in the WDR45 gene has not been previously reported. The mutation was considered de novo because of the normal genetic analysis of the parents. Our case contributes to the information in the literature, regarding phenotype with epilepsy, growth retardation and autism, comprising most of the symptoms seen in the first stage of the disease, and also regarding genotype with the new mutation.

In conclusion, BPAN should be considered as an option in the diagnosis of female patients having clinical findings of epilepsy, growth retardation and autism, with unspecified etiology.

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Case Report

Novel mutation and severe respiratory failure in congenital disorders of glycosylation Type Ix

Betül Kılıç[1], Nejmiye Akkuş[2]

Departments of [1]Pediatric Neurology and [2]Medical Genetics, University of Health Sciences, Kocaeli Derince Training and Research Hospital, Kocaeli, Turkey.

ABSTRACT

Congenital glycosylation disorders (CDG) are a group of rare hereditary metabolic diseases that result from abnormal protein and lipid glycosylation. Virtually all organ systems can be affected, and neurological involvement is particularly severe and disabling. More than 100 CDG types have been reported to date and those numbers are rapidly increasing. Each type is very rare, and the clinical characteristics of each subtype are difficult to determine. There are large numbers of biochemically unresolved cases defined as CDG-Ix. In this report, we present a 5-year-old boy who had dysmorphic features, hypotonia, developmental and mental delay, epileptic spasms, recurrent apnea and respiratory failure that led to the diagnosis of an unreported mutation of a rare form of CDG-Ix. This mutation in the STT3B gene affects the catalytic subunit of the oligosaccharyltransferase and the recipient substrate properties, which in part have the same functions in N-glycosylation. A novel homozygous mutation in the STT3B presence of c.38C>G that encodes p.S13W (p.Ser13Trp) was detected with next generation sequencing. The CDG clinical spectrum can be unusual, ranging from dysfunction of certain organs to severe multiple system disorders. Respiratory failure has rarely been reported in these cases. Increased types and numbers of patients constitute symptom variety. The identification of new genes and genotype-phenotype relationships may expand the family of CDG.

Key words: novel mutation, STT3B, congenital disorders of glycosylation Type Ix, respiratory failure.

Congenital disorders of glycosylation (CDG) comprise a group of inherited congenital metabolic disorders caused by protein or lipid glycosylation defects. To date, more than a hundred types of CDG have been defined although it is difficult to diagnose each subtype.[1,2]

In localization of intracellular molecular defects, CDG cases are categorized into two types: CDG I or II.[4-7] Two types of defects in protein N-glycosylation are related to a series of mixed pathways involving combining and processing proteins. These pathways occur in three cellular compartments: the Golgi apparatus, the endoplasmic reticulum and the cytosol. CDG-I is caused by defects in the enzymes that provide the synthesis and transfer of the oligosaccharide in the endoplasmic reticulum (ER). Pathologies leading to CDG-II are associated with different mechanisms: enzymes involved in the regulation of the N-glycan chain in the Golgi apparatus, sugar carriers and intracellular proteins such as the COG complex.[8]

After the latest revision to the terminology, the CDG type is detected according to the affected gene name or protein name.[4] However, the prevalence of this group of disorders is not exactly known. As long as the basic disorder remains unknown, diseases are called CDG-Ix.[9]

Patients with CDG-I present with mental and psychomotor delay, seizures, muscle hypotonia, neurological signs, ophthalmological anomalies, failure to thrive, blood coagulation defects, endocrine abnormalities, and volatile dysmorphic features.[8]

✉ Betül Kılıç
betulklc82@gmail.com

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Approximately 15% of all CDG families are subtyped as CDG-Ix owing to unidentified glycosylation defects.[11] These defects may include deficiencies in oligosaccharyltransferase (OST) activity or dolichol biosynthesis from mevalonate. These may include, for example, analysis of the dolichol biosynthetic pathway from mevalonate or measurement of OST activity.

In this article, we describe a 5-year-old patient with a rare unregistered respiratory failure who was diagnosed with CDG-Ix using new generation sequencing (NGS). We detected a homozygous mutation in *STT3B* and confirmed the presence of c.38C>G that encodes p.S13W (p.Ser13Trp) and were able to diagnose CDG-Ix. The patient had episodes of infantile spasm and showed symptoms of respiratory failure, which are very rarely reported in CDG Ix.

Case Report

A 5-year-old boy was the first-born child of healthy and consanguineous Turkish parents. Lower fetal movements and intrauterine growth retardation were reported during pregnancy. He was born at week 38 and weighed 2660 g (SD, −3.25) with a length of 47 cm (SD, −4 SD), Apgar score of 10/10, and occipitofrontal circumference of 34 cm (SD, −2). At three months of age, few psychomotor acquisitions, dysmorphic features, generalized hyporeflexia, and hypotonia were noticed. The fundus oculi exhibited minor pale papillae. An electroretinogram recorded few responses to stimulation. Evoked responses in the auditory brainstem were normal. He developed epileptic spasms at the age of 1 and received benzodiazepine treatment.

The patient first visited our hospital due to an infection-related phase of respiratory insufficiency at five years of age. He demonstrated dysmorphic features including hirsutism, narrow forehead, short palpebral fissure, hypertelorism, antimongoloid slanting eyes, and long eyelashes. Clinical findings included axial hypotonia, body adiposity, muscular weakness, and progressive developmental delay. Moreover, recurrent apnea was observed, for which long-term oxygen supplementation with cannulation was initiated followed by invasive ventilation via tracheostomy.

Laboratory investigation results were normal for peripheral blood count, serum cholesterol, lactate, amino acids, albumin, alkaline phosphatase, prothrombin time and activated partial thromboplastin time, thyroid stimulating hormone, thyroxine, and thyroxine-binding globulin levels. A temporal increase in renal failure and serum transaminases was also noted. Metabolic screening (amino acids, plasma amino acid, urinary organic acids, ammonia, biotinidase, phytanic acid, lactate, vitamin B12, blood pH, carnitine acyl carnitine levels, and long-chain fatty acids) revealed no abnormal findings.

Abdominal ultrasound and echocardiogram confirmed the absence of abnormalities in the liver and heart. However, renal ultrasound revealed bilateral, grade 1 pelviectasis. Electroencephalography examination revealed a burst suppression pattern. Brain magnetic resonance imaging showed a generalized reduction in myelination; moreover, the third and fourth ventricles were observed to be wide with gliotic changes around the lateral ventricle.

The high-performance liquid chromatography method was used for measurement of transferrin glycoforms in serum. Quantification of transferrin glycoforms was performed with selective absorbance at ~214 nm. All plasma transferrin levels (asialo-, monosialo-, disialo-, trisialo-, tetrasialo-, and pentasialotransferrin) were found to be normal (Fig. 1).

The patient's karyotype was 46, XY. In peripheral leukocytes, mitochondrial DNA and XY chromosome were in the normal order without any large deletion. Additionally, array comparative genomic hybridization revealed no abnormal findings.

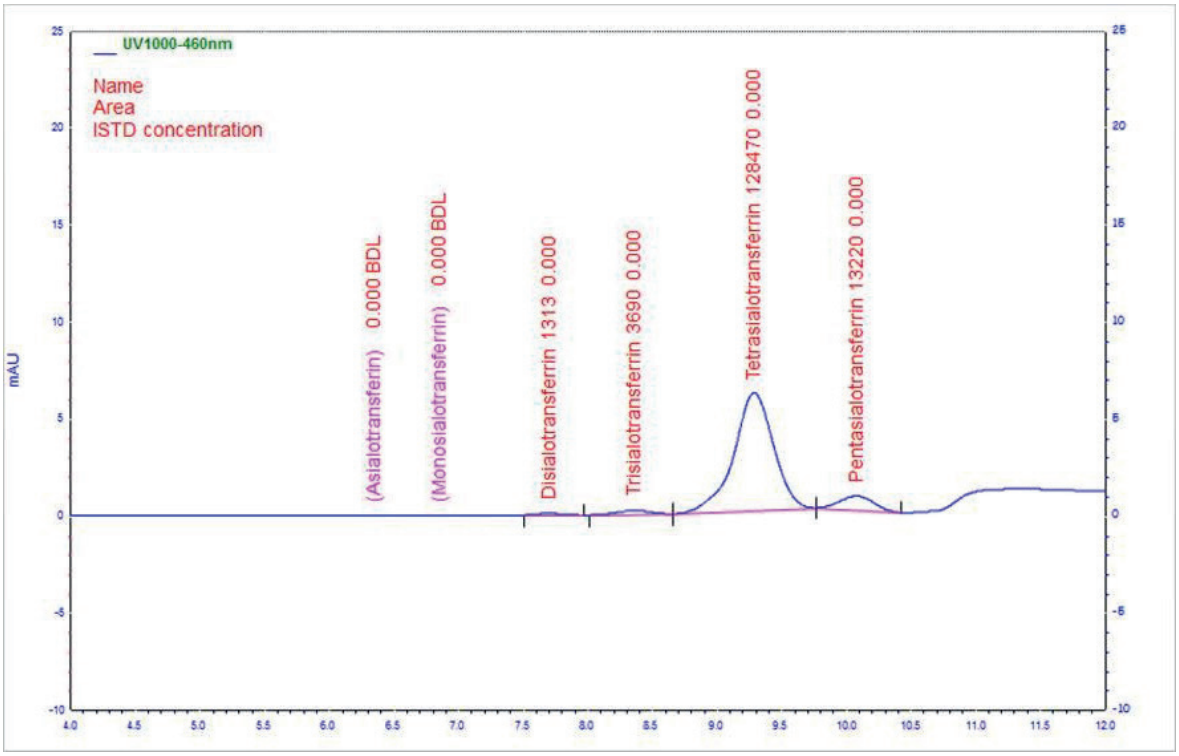

Fig. 1. Patient's plasma transferrin levels are in normal limits.

Genomic DNA was used to perform exome sequencing. Briefly, peripheral blood leukocytes were extracted and exomes were captured using TruSight One Panel (Illumina Inc., San Diego, CA, USA) that is capable of enriching a 12-Mb region spanning 4813 genes. The NextSeq platform (Illumina Inc.) was used for sequencing. Burrow-Wheeler Aligner (version 0.7.12, MEM algorithm) was used for aligning sequence reads with those of the reference hg19 genome.[3] Candidate variants were confirmed using custom-designed primers for Sanger sequencing.

Subsequently, NGS was used to diagnose CDG Ix by detecting a homozygous mutation in *STT3B* and with Sanger sequencing to confirm the presence of c. 38C>G that encodes p.S13W (p.Ser13Trp) (Fig. 2). Results of the genetic analysis revealed that the patient's parents were heterozygous for every mutated allele. Moreover, the patient was found to be homozygous for c.38C>G (NM_178862.1) that encodes protein p.Ser13Trp. This mutation is a new variation not found in the ClinVar database. However, the mutation found in SIFT, PolyPhen and MutationTaster databases is disease-specific. Written informed consent was obtained from the patient's parents for publication of the case.

Discussion

We report a patient with a formerly unlisted category of CDG arising from a mutation in *STT3B*. *STT3B* is a 22.8-Mb OST complex gene located on chromosome 3 (9,771,508–32,612,897).

OST catalyzes the transfer of the oligosaccharide from a lipid-linked oligosaccharide donor to the asparagine residue of glycosylation receptor sites or sequons in newly synthesized proteins. These enzymes are heteromeric complexes consisting of several membrane-related subunits, where *STT3* is a catalytic center.[12]

The genome contains two different *STT3* genes, *STT3A* and *STT3B*. Therefore, at least

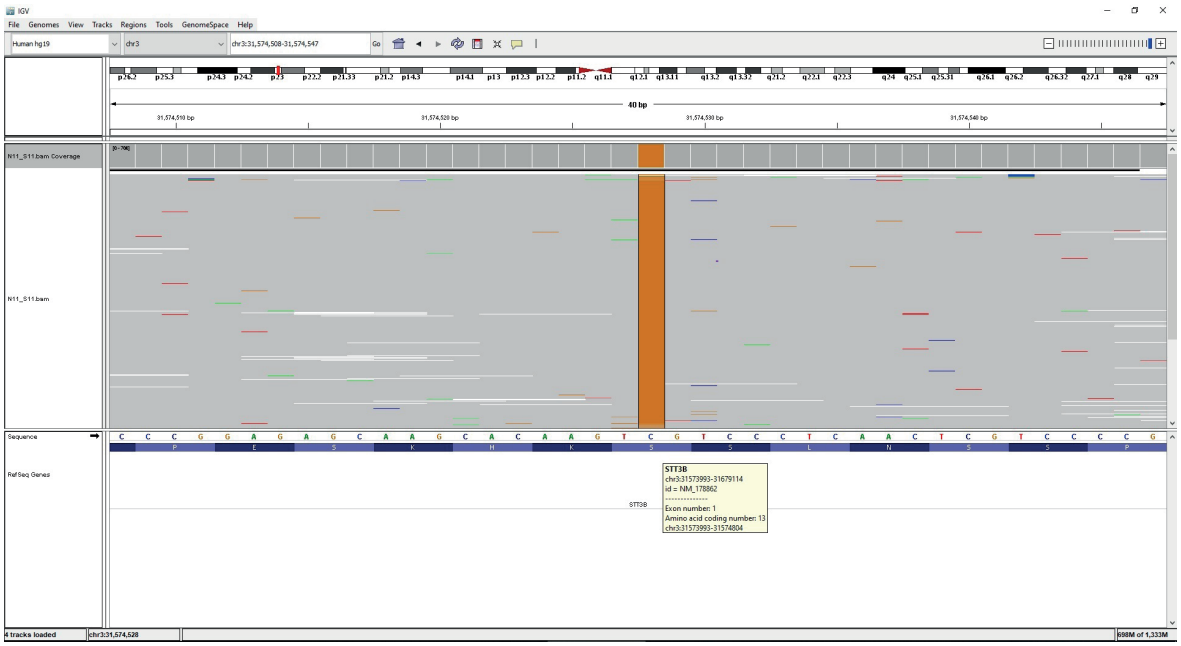

Fig. 2. Sanger sequencing showed the presence of c. 38C>G that encodes p.S13W (p.Ser13Trp).

two different OST isoforms are formed. The *STT3A* isoform primarily glycosylates substrate polypeptides cotranslationally, while the *STT3B* isoform provides cotranslational and post-translocational glycosylation of sequons that are skipped by the *STT3A* isoform.[13]

The oligosaccharides are transferred to N-linked glycoproteins with the *STT3B* isoform. It has also been reported that *STT3B*-dependent N-glycosylation plays a role in clearing misfolded proteins due to ER-related degradation.[14,15] The *STT3B* isoform is thought to have an important function in protein homeostasis in ER.

Measurement of transferrin glycoforms in serum is a laboratory method used to identify suspected CDG patients. However, since the protein is mainly glycosylated with the *STT3A* isoform of OST, potential CDG-*STT3B* cases cannot be determined by serum transferrin measurement.[16,17]

Almost all types of CDG manifest in infancy. As oligosaccharides in both glycolipids and glycoproteins have essential biological functions, defects in the synthesis of these molecules results in broad, multi-system clinical symptoms.[18] These symptoms may include one or more of the following: failure to thrive, developmental delay, hepatopathy, hypotonia/neurological abnormalities, hypoglycemia, protein-losing enteropathy, eye abnormalities, abnormal immunological findings, skin abnormalities, and skeletal findings.[19] For instance, an individual with *STT3B* mutation was reported to present with severe developmental delay, seizure disorder, microcephaly, failure to thrive and liver and genitourinary abnormalities.[6,17]

Most CDG types and multiple pathways manifest in an autosomal recessive manner. ALG13-, MGAT1-, SSR4-, and SLC35A2-CDG manifest in an X-linked manner. Homozygous mutations in genes located on chromosome 3p23 result in *STT3B*-CDG (CDG-Ix).[20]

Our patient had significant congenital and developmental abnormalities. The homozygous mutation (c.38C> G) in *STT3B* causes a significant reduction in *STT3B* protein and hypoglycosylation of *STT3B*-specific substrates. In our case, the parents were heterozygous for every mutated allele and healthy. Accordingly, it can be concluded that clinical phenotypes occur only when the *STT3B* level dramatically

decreases with the mutation in both alleles of the gene.

In this study, we present a patient diagnosed with CDG-Ix with a novel homozygous mutation in *STT3B* and a rare clinical presentation of recurrent apnea and respiratory failure. Respiratory failure has rarely been reported in patients with CDG-Ix. In the present case, there was recurrent apnea and respiratory failure, which necessitated tracheostomy.

Although curative treatment cannot be initiated in most cases, definitive diagnosis is necessary for palliative care. Defining the disorder type is crucial in diagnosing the disorder, instructing parents, and offering suitable genetic counseling.[6]

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Case Report

Rotavirus encephalopathy with concomitant acute cerebellitis: report of a case and review of the literature

Cem Paketçi[1], Pınar Edem[1], Derya Okur[1], Fatma Ceren Sarıoğlu[2], Handan Güleryüz[2], Erhan Bayram[1], Semra Hız Kurul[1], Uluç Yiş[1]

Divisions of [1]Pediatric Neurology, Department of Pediatrics, [2]Pediatric Radiology, Dokuz Eylül University Faculty of Medicine, İzmir, Turkey.

ABSTRACT

Rotavirus is a leading cause of gastroenteritis in children under 5 years of age. It is known that neurological manifestations like seizures, encephalopathy and encephalitis can rarely be seen due to rotavirus infections. Cerebellar involvement is extremely rare. We present an uncommon neurological manifestation of rotavirus infection in a 4-year-old Turkish child who presented with hypotonia, reduced consciousness and mutism. Magnetic resonance imaging revealed diffusion abnormalities in the splenium of corpus callosum and nucleus dentatus bilaterally. She was diagnosed with rotavirus cerebellitis. She improved well with dexamethasone and intravenous immunoglobulin but still has dysarthria and poor fine motor coordination.

Key words: central nervous system infection, encephalitis, encephalopathy, mutism, rotavirus.

Rotavirus is a common cause of gastroenteritis in children and may be complicated by central nervous system involvement. Afebrile seizures are common, but acute cerebellitis is a quite rare neurological complication. Cerebellar involvement has characteristic clinical and radiologic features.[1] There is no established treatment for this condition, but some cases have been treated with intravenous immunoglobulin (IVIG) or steroids. We present a case of rotavirus associated acute cerebellitis treated with dexamethasone with a relatively favorable outcome.

Case Report

A four-year-old previously healthy girl was admitted to hospital with complaints of diarrhea, vomiting and fever. Intravenous hydration was administered with the diagnosis of acute gastroenteritis and dehydration. She was discharged with prescription of metoclopramide. Three days after discharge, she was readmitted to our hospital with altered mental status and inability to speak. Her neurologic symptoms started three days after diarrhea on the day of admission to our hospital. There was no history of infectious disease in the last month. She was fully vaccinated excluding rotavirus vaccine, which is not a routine component of vaccine schedule in Turkey. No vaccine has been administered in the last three months.

Neurological examination revealed a deterioration in the level of consciousness with a Glasgow Coma Scale score of 10 (E2, V3, M5) and generalized muscle weakness. Muscle strength was 2/5 in upper and 3/5 in lower limbs symmetrically. Deep tendon reflexes were normal. She could not speak and could not walk. Her cranial nerve examination was unremarkable. Cerebellar tests could not be done due to consciousness disturbance. Remainder of the physical examination was normal.

Laboratory data, including serum glucose,

✉ Cem Paketçi
paketci@hotmail.com

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ammonia, lactate an electrolyte levels were normal except for an elevated C-reactive protein of 2.08 mg/dl (N: 0.2 – 0.5 mg/dl). Cranial computed tomography was normal.

Magnetic resonance imaging (MRI) of the brain showed a hyperintensity in the splenium of the corpus callosum (SCC) on T2-weighted and diffusion weighted images with corresponding diffusion restriction on Apparent Diffusion Coefficient (ADC) mapping (Fig. 1). Cerebrospinal fluid (CSF) examination revealed no cells, protein 51.6 mg/dl (N: <40 mg/dl) and glucose 81 mg/dl (N: 60 – 80 mg/dl) with simultaneous blood glucose of 113 mg/dl. Empirical treatment with intravenous ceftriaxone, vancomycin and acyclovir were initiated. Rotavirus antigen was positive in stool specimen 3 days after the onset of diarrhea, on the first day of onset of neurologic symptoms at the admission to our hospital. Tentative diagnosis was rotavirus-associated mild encephalopathy with a reversible splenial lesion (MERS).

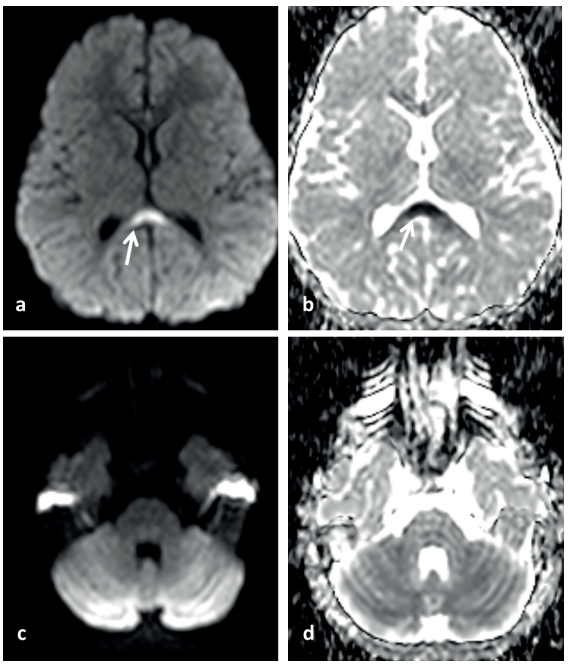

Fig. 1. Magnetic resonance (MR) imaging of patient on day-1. B 1000-weighted (a) and ADC (b) images showed that the diffusion restriction in the splenium of corpus callosum (arrows). B 1000-weighted (c) and ADC (d) images also showed no abnormalities in the cerebellum initially.

On day-2, she was still encephalopathic, mute and muscle strength were the same as admission. Electroencephalography was normal. Antimicrobial treatment was discontinued once CSF culture revealed no bacterial growth and PCR was negative for herpes simplex virus, enterovirus and adenovirus. Rotavirus could not be tested in CSF. Intravenous immunoglobulin was given for two days (1 g/kg/day). On day-3, despite the improvement in consciousness, she was still mute which is a well-known feature of acute cerebellitis. Hence a control brain MRI was requested on suspicion of acute cerebellitis. This showed reduced diffusion in dentate nuclei. There was also a slight signal deviation on the T2-weighted sequence in the same area. Previous lesion at SCC disappeared (Fig. 2). After a diagnosis of acute cerebellitis, dexamethasone (1 mg/kg/day) was administered at third day of admission and continued for 5 days followed by dose tapering of 5 days. From the 5th day of admission, our patient had improved consciousness, but she was still hypotonic and bedridden. Her movements were uncoordinated and unpredictable. Mutism became more prominent. She received physical therapy daily. After two weeks of illness, she was still mute, had head and neck control but could not sit without support.

Four months after onset, a follow-up brain MRI indicated marked cerebellar atrophy (Fig. 3). She was able to walk for a few steps with a wide-base gait and able to speak two or three word sentences. Her speech was still slow and dysarthric. She was able to walk but her fine motor coordination was poor. She still continues to improve, but the recovery is slow. An informed consent was received from the parents for publication.

Discussion

We report a case of rotavirus encephalopathy with concomitant acute cerebellitis. Although

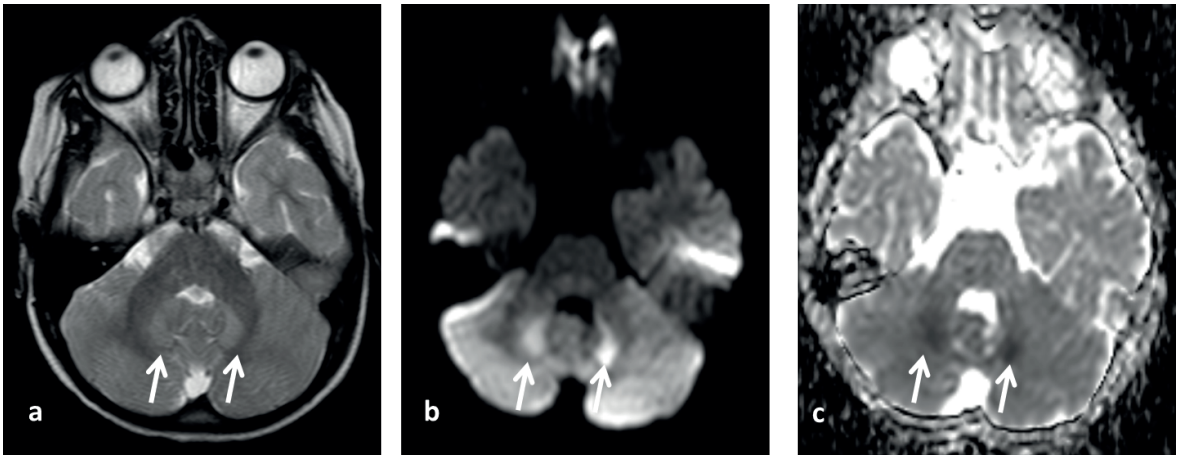

Fig. 2. Magnetic resonance (MR) imaging of patient on day-3. T2-weighted axial image (a) demonstrated that the high signal intensity in dentate nuclei (arrows). B 1000-weighted (b) and ADC (c) images also showed that the diffusion restriction in the same areas (arrows).

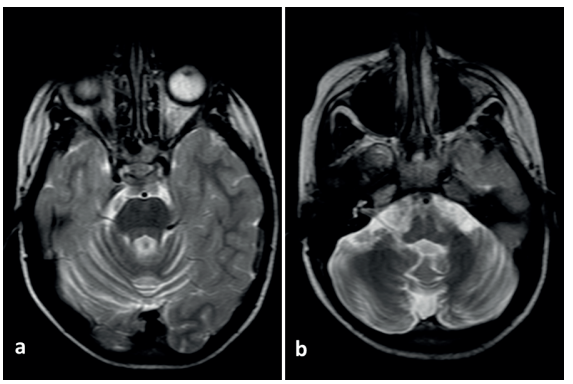

Fig. 3. Follow-up magnetic resonance (MR) imaging at four months. Diffuse cerebellar atrophy was seen in T2-weighted axial images (a-b).

rotavirus infection is common, neurological manifestations occur in 2%-5% of patients with rotavirus gastroenteritis.[2] The pathogenesis of neurological signs of rotavirus infection is not clearly understood. Direct invasion of central nervous system, high radical activity of nitric oxide metabolites due to the damage of enterocytes by toxin like proteins such as the non-structural protein 4 and dissemination of the virus by the enteric nervous system are possible mechanisms.[2,3]

Cerebellitis associated with rotavirus infection has been described previously. However, there are only few patients in the literature. To date 23 cases with cerebellar involvement due to rotavirus infection have been reported (Table I). The clinical features of rotavirus associated encephalopathy and cerebellitis can vary widely. Consciousness disturbance, mutism, ataxia and hypotonia are the most characteristic findings. Seizures and intentional tremor are also frequent. Mutism is the most important and distinct finding of acute cerebellitis associated with rotavirus infection.[1] Cerebellar mutism is generally a common complication of posterior fossa surgery in children. Non-surgical cerebellar mutism is rare, but can be seen after vascular events, trauma or inflammation.[4] Although the underlying mechanism is unclear, it is suggested to result from impaired coordination of the articulatory muscles due to the damage of cerebello-thalamo-cerebral pathway.[5] Cerebellar mutism was seen as an initial symptom in most patients with rotavirus cerebellitis, including our patient (19 of the 24 patients). Three patients suffered from dysarthtria. The mean duration of rotavirus associated cerebellar mutism is about a month as in our case.[1]

The typical MRI findings of rotavirus associated acute cerebellitis are a reversible splenial lesion in the early stages, abnormal signal intensity in the cerebellar white matter/nuclei and increased signal intensity in the cerebellar cortex respectively. The most likely finding in

Table I. Clinical and magnetic resonance imaging (MRI) findings of rotavirus cerebellitis.

Patient	Age (y) / Sex	Symptoms S	CD	CS	Other	Rotavirus (Stool / CSF)	EEG	MRI findings Initial	Follow-up	Treatment	Outcome
1 Nigrovic[15]	3/F	+	+	A, M	H	+/+	NA	CG/V	NA	Steroid (20 mg/kg/d, 5d)	A, moderate aphasia
2 Shiihara[2]	2/F	+	+	A, Dy, T	H	+/-	Occipital delta-teta wave	Normal	CA	MP (30mg/kg/d, 3d, twice)	Dy, T
3 Shiihara[2]	4/M	-	+	A, Dy T	H	+/-	Normal	CG/V	CA	MP (30 mg/kg/d, 3d)	Dy, T
4 Dickey[16]	3/F	-	+	A, Dy, M	H	+/+	Encephalopathy	CG/V	NA	Antibiotics, antiviral	Motor and slurred speech
5 Takanashi[1]	3/F	-	+	A, Dy, M		+/NA		CC*,CW/N*,CG/V	CA		A, Dy
6 Takanashi[1]	4/F	+	+	A, Dy, M		+/NA		CC*,CG/V	CA		Dy
7 Takanashi[1]	4/F	+	+	Dy, M		+/NA		CC*,CG/V	CA		A
8 Takanashi[1]	3/F	-	+	Dy, M		+/NA		CC*, CW/N*,CG/V*	CA		T, Dy
9 Takanashi[1]	2/M	-	+	Dy, M		NA/NA	Slow activity (8 of 11 patients)	CC*, CW/N*,CG/V	CA	CS (10 patients), IVIG (5 patients)	Dy
10 Takanashi[1]	3/F	+	+	Dy, M		+/NA		CC*, CW/N*,CG/V	CA		Dy
11 Takanashi[1]	2/F	-	+	Dy, M		+/NA		CW/N*,CG/V	CA		Dy
12 Takanashi[1]	3/M	-	+	Dy, M		+/NA		Normal	CA		Normal
13 Takanashi[1]	2/F	-	+	Dy, M		+/NA		CG/V*	CA		A, Mental retardation
14 Takanashi[1]	2/F	-	+	A, Vertigo		+/NA		Normal	Normal		Normal
15 Takanashi[1]	3/M	-	+	Dy, M		+/NA		CW/N*,CG/V	CA		Dy
16 Kubota[17]	1.5/M	+	+	NA	H	+/NA	Bilateral frontal delta activity	CW/N,CG/V*	CA	Fluid therapy	Autism
17 Kubota[17]	3/M	-	+	A, M, T	H	+/-	Normal	CW/N*,CG/V*	CA	MP (30mg/kg/d, 3d, 3 courses)	Dysmetria, slurred speech
18 Kubota[17]	2/F	+	+	A, M, T		+/NA	Bilateral frontal delta activity	CC*,CW/N*,CG/V*	CA	IVIG (1 g/kg/d, 1d) + Pulse MP (twice)	Slurred speech
19 Mori[7]	4/F	+	+	A, Dy	UMN signs (+)	+/-	Diffuse slow waves	CC*,CG/V*	Normal	Dx (0.4mg/kg/d, 4d)	Normal
20 Thompson[18]	4/F	+	+	A, M	H	+/+	Slow background activity, left frontal delta activity	CG/V	CA	Antibiotics, antiviral	A, Dy
21 Kato[19]	3/F	-	+	A, M, T	-	+/-	Normal	CW/N*,CG/V*	Normal	P (5mg/kg/d, 5d)	Normal
22 Engan[12]	4/F		+	A, M	H	+/-	Normal	CC*,CW/N*	NA	P (2mg/kg/d, 14d, tapered 30d)	Slurred speech, poor fine motor coordination
23 Bosetti[8]	2/F	-	+	M	Dystonia	+/NA	Diffuse slowing	CW/N	Normal	Dx, antibiotics, antiviral	Normal
24 Present case	4/F	-	+	A, M	H	+/NA	Normal	CC*, CW/N*	CA	IVIG (1 g/kg/d, 2d) + Dx (1 mg/kg/d, 5d, tapered 5d)	Dy, poor fine motor coordination

A: ataxia; CA: cerebellar atrophy, CC: corpus callosum, CD: consciousness disturbance, CS: cerebellar signs, CG/V cerebellar gray matter/vermis, CW/N: cerebellar white matter/nuclei, d: day; Dy: dysarthria; Dx: dexamethasone; EEG: electroencephalography, H, Hypotonia; M: mutism, NA: not available, S: seizure, T: tremor, MP: methylprednisolone, P: prednisolone, UMN: upper motor neuron
*: with reduced diffusion

the long term follow-up is cerebellar atrophy.[1] First MRI of our patient showed a T2-weighted hyperintensity and restricted diffusion in the SCC. Acute consciousness alteration after three days of fever, diarrhea and vomiting, demonstrates acute encephalopaty. Clinical findings and isolated MRI lesion with restricted diffusion in the SCC suggested rotavirus-associated MERS. But prominent mutism with hypotonia pointed cerebellar involvement. Second MRI on day 3 showed a T2-weighted hyperintensity with reduced diffusion in dentate nuclei. Restricted diffusion at SCC disappeared. These findings supported the diagnosis of acute cerebellitis and consistent with the literature.

Although somnolence, altered mental status and the loss of consciousness considered an encephalitis, lack of pleocytosis and negative culture results in the CSF suggested an encephalopathy rather than an encephalitis. Acute demyelinating encephalomyelitis (ADEM) often presents altered mental status, focal neurological signs and seizures which develop days to weeks after a viral infection or vaccination. Unlike our patient, mild CSF pleocytosis is expected and MRI lesions in ADEM, are typically asymmetrical, usually involve the white matter and resolve over months.[6]

Cerebellar atrophy is the most common finding on follow-up MRI (17 of the 24 patients). Three patients' follow-up MRI was not available/not done, four were normal. Speech disturbance is the most frequent long-term sequelae. All of the patients with speech disturbance as an outcome had cerebellar atrophy on follow-up MRI. Inversely there were no speech disturbance for four patients with cerebellar atrophy. Follow-up MRI of our patient at four months from onset showed cerebellar atrophy as in most patients.

No specific recommended treatment for rotavirus cerebellitis is available. Treatment with corticosteroids (methylprednisolone, prednisolone, dexamethasone) and IVIG has been used. Only two cases were treated with dexamethasone in the literature before and both showed complete recovery. One of these patients received dexamethasone therapy 0.4 mg/kg/day for 4 days starting from day 5.[7] The other patient was started on dexamethasone on day 2, the dose was unknown.[8] Both patients recovered completely without any sequelae. This patient was treated with IVIG and dexamethasone in an attempt to suppress an expected inflammatory process. She received infusions of 1g/kg IVIG for two days starting from day two and 1 mg/kg/g dexamethasone for five days starting from day three. Our patient has mild dysarthria and poor fine motor coordination after four months but she still continues to improve. Dexamethasone may be a good choice in the treatment of patients with acute cerebellitis and may be preferred due to its strong anti-inflammatory effects.

The impact of rotavirus vaccination on seizures and other neurological complications is still not clear and research results are contradictory.[9,10] Although the majority of studies are focused on seizures, it has been shown that the vaccination may be protective against severe complications of rotavirus.[11] Only one of the patients in the literature had information about vaccination who was not vaccinated like our patient.[12]

Severe complications of rotavirus infections are rare and pathogenesis remains unclear. Genotype variations of rotavirus, endotheliitis, cytokines such as interleukin 6 and 10 are emphasized.[13] Also cellular immunodeficiency is associated with severity of rotavirus infection.[14] In our patient, we did not identify the genotype of the virus. The patient may have an underlying immunodeficiency that we could not show with standard immunologic tests.

Acute cerebellitis associated with rotavirus has a distinct disease course with reduced consciousness and cerebellar mutism. Although rare, it should be considered especially in patients with a reversible splenial lesion because early and appropriate treatment may improve outcome. Dexamethasone treatment should be considered. Also rotavirus vaccination should be take into account to prevent severe complications.

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Case Report

Early-onset neonatal sepsis caused by *Neisseria meningitidis* serogroup B: case report and literature review of a 102-year period

Tayyib Mubashar[1], Paul Christopher Turner[2], Siba Prosad Paul[3]

[1]*Medical School, University of Exeter, Exeter; Departments of* [2]*Medical Microbiology and* [3]*Paediatric, Torbay Hospital, Torquay, United Kingdom.*

ABSTRACT

A 36-week-2-day-old male infant was admitted to the neonatal unit with respiratory distress, hypoglycaemia and suspected early onset neonatal sepsis for respiratory support, monitoring and intravenous antibiotics. His initial C-reactive protein was 12 mg/L, this increased to 66 mg/L at 24 hours. Blood cultures at 48 hours confirmed *Neisseria meningitidis* serogroup B. As the isolate was sensitive to benzylpenicillin the same antibiotic was continued for a total of 7 days. His mother remained asymptomatic but was monitored closely. Ciprofloxacin chemoprophylaxis was given to close family contacts. *Neisseria meningitidis* causing early onset neonatal sepsis is extremely rare and neonates may have minimal symptoms at presentation. A table reviewing all documented cases of early onset neonatal sepsis caused by *Neisseria meningitidis* over a 102-year time period is included. There is need for early identification and initiation of empirical antibiotic therapy pending confirmation and sensitivities.

Key words: neonatal sepsis, *Neisseria meningitidis*, invasive meningococcal disease, antibiotics, chemoprophylaxis.

Neonatal sepsis is defined as 'a systemic inflammatory response syndrome specifically presenting with abnormalities of temperature and/or leukocytosis in an infant from birth to 4 weeks of age as a result of proven infection'.[1] It can present as neonatal meningitis, septicaemia or a combination of both. Early onset neonatal sepsis (EOS) presents in the first 6 days of life with late onset neonatal sepsis presenting at 7–28 days.[1] A prospective multicenter surveillance study involving 12 English neonatal units over 3 years (2006-2008) with 124 confirmed isolates reported the most common organisms responsible for EOS were: Group B Streptococcus (GBS) (50%), *Escherichia coli* (18%), *Listeria monocytogenes* (6%), Streptococcus spp. (6%) and *Staphylococcus aureus* (5%).[2] The same study also reported the incidence of EOS at 0.9/1,000 live births.[2]

✉ Siba Prosad Paul
siba@doctors.org.uk

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Neisseria meningitidis causes invasive meningococcal disease (IMD). Although rare in developed countries, it remains one of the most feared infectious diseases. A data linkage project by Public Health England estimated the total burden of IMD in England to be 5,115 laboratory-confirmed cases over a five-year period (2007-2011) with group B meningococci responsible for 87.33% (n: 4,034) of hospitalised cases.[3] The same study reported that infants (<1 year-old) accounted for 1,115/4,619 (24.14%) of cases, although no specific further breakdown for neonates were given. *Neisseria meningitidis* as a causative organism in EOS is extremely rare.[4,5] This article describes a case of early onset neonatal sepsis caused by *Neisseria meningitidis*.

Case Report

A 36-week-2-day-old male was born following a normal vaginal delivery, with poor respiratory effort, tachycardia, pallor and hypotonia. The neonate was resuscitated via face mask

with a T-piece used to deliver intermittent positive pressure ventilation. As the neonate's spontaneous respiratory effort remained inadequate, he was started on positive end expiratory pressure of 5-8 cm water pressure and transferred to the neonatal unit. Initial neonatal blood gases demonstrated a respiratory acidosis (pH 7.11; $PaCO_2$ 12kPa).

On admission his temperature was 37.3˚C, pulse rate 140/min, respiratory rate 44/min, and blood pressure 59/39 mmHg. Blood glucose on admission was 1.1 mmol/L (19.8 mg/dl) and a 10% dextrose bolus of 2 ml/kg was administered. No maternal risk factors were identified. However, clinical indicators for possible EOS included: a) altered behaviour/ responsiveness b) tachycardia c) signs of respiratory distress syndrome (RDS) needing CPAP support d) prematurity e) hypoglycaemia.

Routine blood investigations and blood cultures were obtained, and the neonate was commenced on intravenous benzylpenicillin (50 mg/kg/dose twice daily) and gentamicin (5 mg/kg/dose 36 hourly). Chest X-ray demonstrated moderate RDS. C-reactive protein (CRP) was 12 mg/L increasing to 66 mg/L at 24 hours; a white blood cell count of $4.6x10^9$/L increasing to $13.2x10^9$/L. Blood gases improved as did blood glucose. He was weaned off CPAP at 24 hours.

CSF samples obtained at 31 hours of age showed no evidence of meningitis. Blood cultures at 48 hours identified *Neisseria meningitidis* serogroup B. Following a discussion with a medical microbiologist and as the neonate was improving clinically, penicillin was continued for a total of 7 days, and gentamicin was discontinued after the third dose. Antibiotic sensitivities confirmed that the organism was susceptible to penicillin [minimum inhibitory concentration (MIC) 0.06 mg/L] and cefotaxime (MIC 0.004 mg/L). His CRP had settled to 9 mg/L on day-5.

Close contact chemoprophylaxis with ciprofloxacin was prescribed for the parents and grandparents. The neonate was discharged at day-12 and reported as doing well at clinic follow-up few months later. Participation involved informed consent.

Discussion

The isolate from the neonate was *Neisseria meningitidis* group B type 1 (subtype NT/NT). IMD in neonates has been caused by all major serogroups of *Neisseria meningitidis* such as A, B, C, Y and W135.[4-8] However, literature reviews highlight the preponderance of *Neisseria meningitidis* serogroup B as the major causative agent in neonatal IMD.[4,6-9]

Koplick, in 1916, published the first case of neonatal meningococcal meningitis (NMM).[9] The exact incidence of *Neisseria meningitidis* as a causative agent for neonatal sepsis is not well known.[6] A review article reported 15/424 (3.5%) cases of confirmed neonatal bacterial meningitis in England and Wales that were due to *Neisseria meningitidis* over a 5-year period (1985-87, 1996-97).[10] A more recent study from France between 2001 and 2013 found that 23/831 (2.8%) cases of neonatal bacterial meningitis were caused by *Neisseria meningitidis*.[9]

A literature review conducted using the PubMed, Google Scholar, and Scirus databases revealed 21 published cases of EOS caused by *Neisseria meningitidis* over a 102-year period (1916 - 2018).[4,6,8,9,11] Table I highlights the clinico-pathological outcomes of 16 cases of EOS where adequate records were available. The 16 cases of EOS caused by *Neisseria meningitidis* were due to NMM (n: 7), septicaemia (n: 5) or a combination of both (n: 3). The mortality rate in neonates from EOS caused by *Neisseria meningitidis* was 40% (6/15) based on this literature review over the 102-year period.

A review of the literature by Kiray Baş et al.[4] identified significant risk factors for neonatal meningococcaemia which included: prematurity, maternal acute viral infections, functional asplenia, crowded environments, maternal smoking or neonates exposed to passive tobacco smoking. EOS cases caused

Table I. Clinico-pathological outcomes of early-onset neonatal sepsis caused by *Neisseria meningitidis* over a 102-year period (1916 - 2018).[4,6,8,9,11]

Author(s), year of publication	Day of onset	Clinical features in neonate	Maternal swab for *N. meningitidis*	Neonatal culture positive sample types	Sero-group Identified	Treatment	Outcomes
Koplick, 1916	3	-	N/A	CSF	N/A	Pre-antibiotic era	Survived, developed hydrocephalus
Carmona et al, 1953	2	-	N/A	CSF	N/A	Penicillin	Survived
Carmona et al, 1953	4	-	N/A	CSF	N/A	Penicillin	Survived
Kao et al, 1956	4	-	N/A	CSF	N/A	Penicillin, chloramphenicol, sulfadiazine	Died
Sunderland et al, 1972	1	Fever, irritability	Positive	CSF	C	Ampicillin, kanamycin	Died
Embree et al, 1987	1	-	N/A	CSF, blood	W135	Ampicillin, gentamicin	Survived
Chugh et al, 1988	3	Irritability	N/A	CSF	A	Penicillin, cefotaxime, gentamicin	Died
Bhutta et al, 1991	1	Hypotension, petechiae, prolonged resuscitation	N/A	Negative for neonate.	N/A	Penicillin, cefotaxime	Died Mother's blood and CSF cultures grew *N. meningitidis*
Ellis et al, 1992	3	Conjunctivitis	Positive	CSF, blood	C	Penicillin, cefotaxime	Survived
Ellis et al, 1992	3	Conjunctivitis	Positive	CSF, blood	W135	Penicillin, cefotaxime	Survived
Casanova et al, 2000	3	-	N/A	Blood	B	Penicillin	Survived
Lo et al, 2003	1	Petechiae	N/A	Blood	C	Ampicillin, gentamicin	Died
Shepard et al, 2003	5 cases (1 on day-0, 3 on day-1, 1 on day-6	N/A	N/A	N/A	Predominantly serogroup C	N/A	N/A
Kurlenda et al, 2010	1	Respiratory distress, convulsion	Positive	N/A	B	Penicillin, cefotaxime	Survived
Smith et al, 2015	5	Petechiae, unresponsive	N/A	Blood	β-lactamase negative *N. meningitidis*	N/A	Died
Bilal et al, 2016	1	N/A	N/A	CSF	N/A	N/A	N/A
Chacon-Cruz et al, 2017	3	Conjunctivitis, irritability, tachypnea, poor feeding	Positive	Blood	Y	Ceftriaxone	Survived
Our case	0	Respiratory distress, hypoglycaemia, hypotonia	N/A	Blood	B	Penicillin, Gentamicin	Survived

CSF: cerebrospinal fluid; N/A: not available.

by *Neisseria meningitidis* may have minimal symptoms as was the cases in 6/15 babies or may have minimal symptoms at presentation.[4] It is vital that based on risk factors, red flags and clinical suspicion for IMD, the neonate undergoes early investigations and empirical antibiotic therapy with intravenous benzylpenicillin and gentamicin is initiated. Depending on the response and clinical progress, the antibiotic regime may be changed to cefotaxime or another appropriate antibiotic based on the sensitivity profile. However, cases of meningococcal infection truly resistant to penicillin are extremely rare although the MIC as well as higher dosing need to be kept in mind.

In cases of IMD resulting in EOS, it is important to investigate genitourinary colonization of the mother and consider nasopharyngeal carriage among close contacts. Although the nasopharyngeal carriage rate is high in the community, IMD in neonates remains a rarity, possibly due to the protective effect of maternal antibodies passed on from mother to fetus *in-utero*.[4] However, prematurity and low birth weight could have an impact on this.[4] The availability of the immunizations in the UK against Meningococcal B and C as well as the quadrivalent vaccine could impact on the epidemiology of IMD in the future.

It is important to liaise with Public Heath England or similar national organizations and offer chemoprophylaxis to all close contacts including healthcare professionals who may have come into contact with the neonate's respiratory secretions.[4] The mother should also be monitored closely and if she becomes unwell or there is any suspicion of maternal sepsis, then blood cultures and appropriate antibiotic therapy e.g. intravenous ceftriaxone should be started promptly.

EOS due to IMD is extremely rare but can be life-threatening. Whilst rare, with symptom manifestation being atypical compared to older age groups, a raised index of suspicion should lead to prompt administration of appropriate intravenous antibiotics as well as taking blood/CSF cultures as this is likely to be associated with a better outcome. Further research is needed to facilitate a consensus, as currently there are no guidelines for empirical treatment of neonatal IMD.

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Case Report

Dropped head related lamin A/C associated congenital muscular dystrophy case; previously defined as emery-dreifuss muscular dystrophy

Hande Tekin[1], Sanem Yılmaz[2], Hasan Tekgül[2], Sarenur Gökben[2], Gül Aktan[2]

[1]Department of Pediatric Neurology, Çiğli Treatment Hospital, İzmir; [2]Division of Child Neurology, Department of Pediatrics, Ege University Faculty of Medicine, İzmir, Turkey.

ABSTRACT

Dropped head syndrome can be seen in many neuromuscular diseases. However, there are very few diseases in which neck extensors are weak among neuromuscular diseases. A 7 years old boy who had weakness of the neck extensor muscles, creatinine kinase elevation and dystrophy findings in biopsy followed up with the preliminary diagnosis of muscular dystrophy is presented. We detected p.N456K (c.1368C> A) heterozygote mutation by the gene sequencing in the Lamin A/C associated (LMNA) gene. This mutation was previously reported as Emery-Dreifuss muscular dystrophy.

Key words: congenital muscular dystrophy, LMNA, dropped head.

Congenital muscular dystrophy can be seen in clinically and genetically different forms. Most of the patients present with muscle weakness, hypotonia and delayed motor development within the first 2 years.[1] The diagnosis of a large number of identified genes, mutation-dependent clinic or even different clinics, even in the same mutation, can make diagnosis difficult. It is important to evaluate the mutations detected with clinical clues and their compliance with previously defined diseases. The cases that were defined as a variant form of a disease in the past as the diseases defined over time are now known with a new name and different clinical features.

LMNA-associated congenital muscular dystrophy was first described in 2005.[2] Transmission can be autosomal recessive, dominant or de nova. LMNA gene screening identified heterozygous de novo LMNA mutations in 15 patients by Quijano-Roy et al.[3]

It has two forms; milder form and severe form. Patients with the milder form can walk but cannot control their head because of neck extensor weakness. Severe form have poor movements, may need mechanic respiration and cannot stand.

Herein, we presented a case of LMNA-associated congenital muscular dystrophy, which previously detected a p.N456K (c.1368C> A) heterozygous mutation in the LMNA gene identified for Emery Dreifuss muscular dystrophy (EDMD).

Case Report

A 7-year-old male patient, who was born healthy from a family with a cousin marriage of second degree, applied with difficulty in walking and complaints of not being able to standing up without a support from his sitting position.

It was learned that the patient could sit at 6-7 months of age, began to take steps at 18 months, but could hold his head when he was

✉ Hande Tekin
gazetecihande@yahoo.com.tr

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36 months. At the current physical examination, neck extensors were weak, his head was falling occasionally, and weakness in other muscle groups was indistinct. Lordosis of the patient was increased, hypotonic and deep tendon reflexes were not obtained (Fig. 1). The mental capacity of the patient was normal according to his age.

Creatine kinase levels of the patient were 7-8-fold (1050-950-1208 mg/dl) higher than normal. Multiplex Ligation-dependent Probe Amplification (MLPA) genetic analysis was found negative for spinal muscular dystrophy (SMN 1-2) and Duchenne muscular dystrophy. Myopathic changes were present in proximal muscles in electromyography. Sensory and motor nerve conduction studies was normal. Polyphasic, short-duration, low-amplitude motor unit action potentials (MUAPs) was detected. Cranial magnetic resonance imaging was normal. Echocardiography was normal. 24-hour Holter monitoring was normal. Pulmonary function test was normal. Dystrophic changes like shape-size difference, degeneration and regeneration were observed in the biopsy of the patient's triceps muscle (Fig. 2). There was an increase in interstitial tissue detected by Gromi trichrome stain. Modified tricrom, NADH-TR, SDH and COX staining were normal. In Rust, dPas, Oil Red O and crystal violet staining, there were not material accumulation and amyloidosis. Fast myosin and type 2 / type 1 myofiber ratio was normal. Immunohistochemical evaluation showed that perinuclear emerin, sarcolemmal distrophin, spectrin, distroglikan, merosin, dysferlin, sarcoglycan, caveolin 3 and lamin A / C were stained normally. No inflammatory staining of the stains with CD45 and CD68 was detected (Fig. 2). With these findings, muscle biopsy had non-inflammatory primary myopathic and / or dystrophic disease findings. When the clinical and laboratory findings of the patient were evaluated, it was noteworthy that the patient was still suffering from inability to holding the head still, and LMNA and selenoprotein (SEPN1) gene sequencing was performed. A p.N456K (c.1368C> A) heterozygote mutation was detected in the LMNA gene. This mutation was not detected in the mother and father. It was defined as a disease-causing according to the silico analysis.

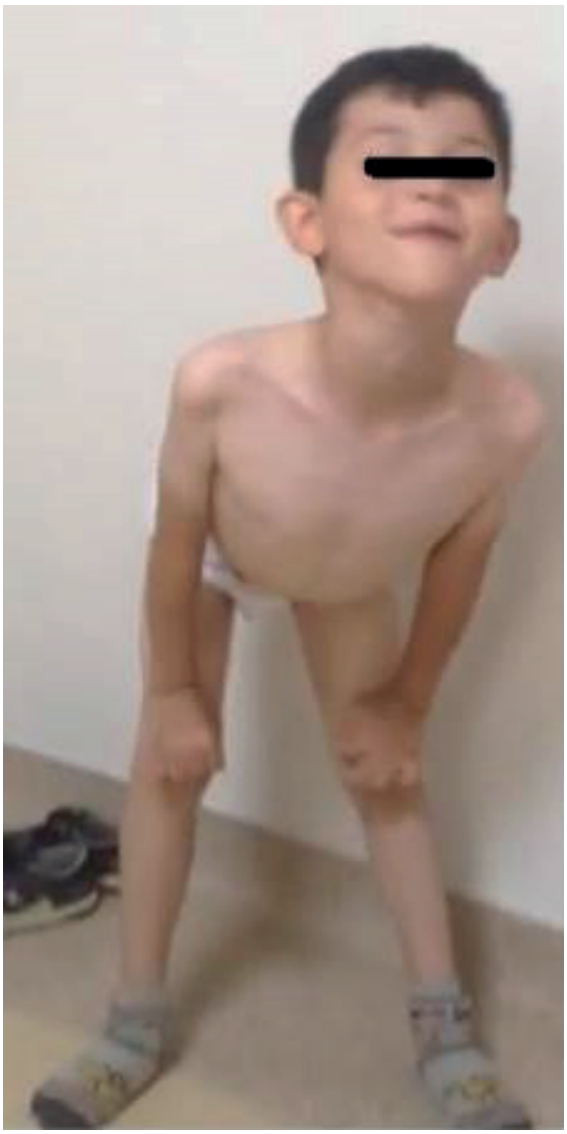

Fig. 1. Patient with weak neck flexors and Gower sign.

It was thought that the early onset of the disease, the lack of specific orientation of muscle biopsy, and the evaluation of patients in the literature suggest that the patient has LMNA-associated congenital muscular dystrophy (LMNA-CMD). An informed consent was received from parents for any publication.

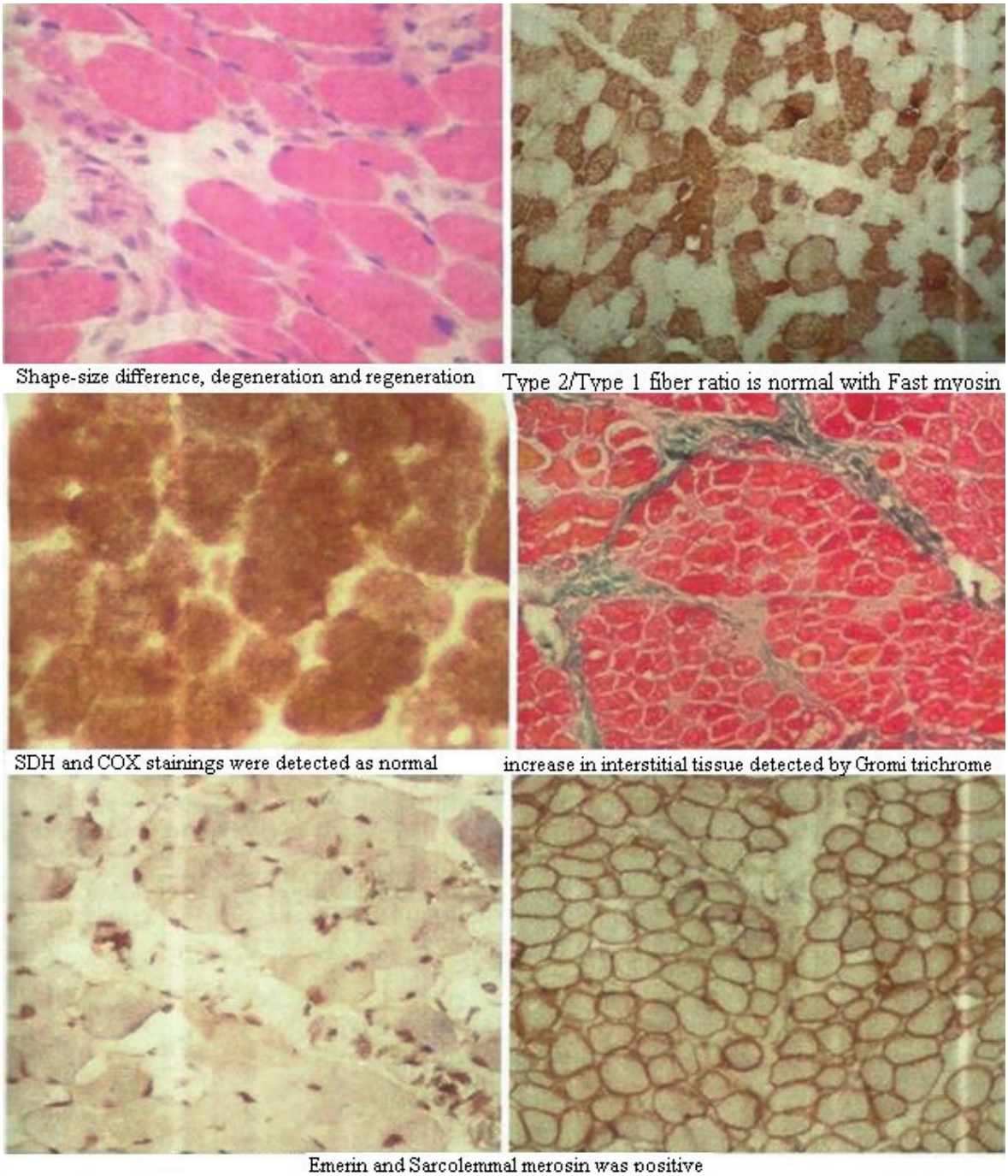

Fig. 2. Muscular biopsy findings.

Discussion

Weakness in neck extension is called 'dropped head syndrome' and this condition can be seen in many neuromuscular diseases. LMNA and SEPN1 mutations should be investigated in neuromuscular diseases where the weakest muscle group is neck extensors or the only finding is neck extensor weakness.[2]

Lamin A gene (LMNA) codes for type A laminates that constitute the nuclear envelope

proteins. Among the diseases associated with this gene are Autosomal Dominant and Autosomal Recessive Emery Dreifuss muscular dystrophy, limb girdle muscular dystrophy type 1b (LGMD1B), Cardiomyopathy with transmission defect, Charcot-Marie-Tooth type 2b and LMNA associated congenital muscular dystrophy.[4] LMNA mutation in patients presenting with dropped head was called LMNA-associated congenital muscular dystrophy by Quijano-Roy et al.[3] LMNA-associated congenital muscular dystrophy may be presented with early onset, especially if the patient has 'dropped head' due to weakness in the neck muscles, and moderate CK (creatinine kinase) elevation. There was widespread muscle weakness in our patient, but most obvious weakness was in the neck muscles and there were about 10 times higher CK values.

In these patients, biopsy findings support non-inflammatory primary myopathic and / or dystrophic disease findings but are not specific diagnostic features. In addition, type 1 fiber atrophy is more likely to occur in patient biopsies in the LMNA-CMD group.[3,5] In our patient, dystrophic changes were observed as expected in muscle biopsy, but there was no change in the ratio of type 2 / type 1 myofibrils to type 1 myofibril atrophy, which is usually seen or has a hint for diagnosis. One reason for this was that our patient had a slight weakness in other muscle groups other than the neck muscles. Patients with type 1 myofibril atrophy were more severe than our patients.[6,7] Lymphocytic inflammation was observed in biopsies of some patients with severe form. No inflammation was noted in both our and other dropped head patients.[3,8] Immunohistochemical stain for lamina A / C was found to be as normal. Most of the immunohistochemical staining in CMD-LMNA patients were normal and our patient also required genetic study.

Sanger sequencing of the LMNA gene coding exons and flanking introns from the genomic DNA p.N456K (c.1368C> A) heterozygote mutation was detected. Most of the LMNA de nova mutations can be normal variant. So parental genetic analysis or in silico analysis or functional studies should be evaluated. We proved that the mutation is de nova and disease-causing with in silico analysis. This mutation was preceded by a case of Emery Dreifuss muscular dystrophy, which had a moderate CK elevation (7-fold) and non-specific findings in progressive course at 2 years of age in 2000.[9] This patient with the same mutation appears to be presenting with severe involvement at an early age according to EDMD. EDMD is usually seen age 10 and is characterized by wasting and weakness of shoulders, upper arms and the calf muscles. LMNA-associated CMD (LMNA-CMD) muscle weakness becomes apparent in infancy or early childhood and can worsen quickly

Some clues in the differential diagnosis of LMNA-associated muscle diseases are prominent (Table I). We believe that this mutation, previously described, is LMNA-CMD when both our patient and the patient who is published in the literature are considered.

Similar cases were detected in the European and French Laminopathies / EDMD research networks and mutation identified by LMNA-CMD in 5 of 21 patients diagnosed as EDMD2 and LGMD Type 1 and lost walking ability before infancy or before 15 years of age.[3,10]

The patients were divided into two groups by Quijano-Roy et al.[3] severe and mild. Early onset motor weakness, lack of spontaneous movement, need for ventilator and severe ventricular tachycardia were classified as severe form, and none of the patients in this group were able to walk. The mild group consists of patients who can walk and have no other serious organ involvement. However, the most prominent feature of this group is that neck extensors are relatively weak compared to other muscle groups. Our patient has been in the mild group since birth, with no ventilator requirement, being able to walk and especially having weakness in the neck muscles. Cardiomyopathy and cardiac arrhythmias are reported frequently in these patients.[3,5] Our

Table I. Diferantial diagnosis of muscular dystrophy with LMNA mutation.

	EDMD TYPE 2	LGMD-TYPE 1B	Severe L-CMD	Mild L-CMD	Our Patient	Described EDMD by Bonne in 2000.
Inheritence	OD/OR	OD	OD/OR	OD/OR	De Nova	Sporadic
Age-Onset	Late Childhood-Adolescent	Childhood-Adolescent-Adult	Birth	Birth-infancy-early childhood	infancy	Age 2
Most Affected Muscle Group	Scapuloperoneal	Hip-Shoulder	Severe hypotonia, diffuse limb and axial muscle weakness, generalized amyotrophy	Neck extensor, peroneal, proximal lower extremite	Neck extensor, scapuloperoneal	Neck extensor- diffuse
CK Levels	N-mildly elevated	N-mildly elevated	2-10 times elevated	2-10 times elevated	7-8 times elevated	7 times elevated
Contracture	Elbow-ankle-neck	Hip-Shoulder	Ankles, fingers, wrists,rigid spine	Elbow, rigid spine		Rigid spine, elbow,ankle,wrist
Respiratory Support Requirement	No	No	Yes	No	No	No
Able to Walk	Yes	Yes/ elderly No	No	Yes	Yes (age 7)	No (age 16)
Cardiac Conduction Defect/ Cardiomyopathy	Usually/ Often	Usually/ Less frequently	Yes/Yes	Yes/ Less frequently	No/No	No / na
Histopathology	Variation in fiber size, increase in internal nuclei, increase in endomysial connective tissue, and necrotic fibers. Emerin is normally expressed.	Fiber size variation with small angulated fibers	Fiber size variation, increased endomysial conective tissue, necrosis and regeneration, lymphocytic inflammation type 1 atrophic fibers	Fiber size variation, increased endomysial conective tissue, necrosis and regeneration, type 1 atrophic fibers	Dystrophic changes like shape-size difference, degeneration and regeneration	Variation in fiber size, significant increase in internal nuclei, a mild increase in endomysial connective tissue and necrotic fibers. Type 1 fibers were predominant and often relatively atrophic.

patient's ECO and 24-hour electrocardiography examinations were normal.

Respiratory distress or failure occurs in the early-onset group at the first age, whereas in the dropped head group it is usually observed before the age of 8.[3] Our patient had no complaints and the respiratory function test was normal according to his age.

In some of the patients, white matter anomalies were also reported in cranial examinations.[6,11] No abnormal findings were found in the cranial imaging of our patient. Patients should be assessed for respiratory, cardiac, and cranial involvement.

We think that our patient and the patient who was previously referred to as EDMD are also LMNA-CMD.

Patients with symptoms of non-specific muscular dystrophy, especially those with weakness in the neck muscles, whose symptoms have begun at a young age, should be evaluated for LMNA-CMD prior to invasive procedures.

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Case Report

Peters Plus syndrome: a recognizable clinical entity

Gizem Ürel Demir[1], Naz Güleray Lafcı[2], Özlem Akgün Doğan[1], Pelin Özlem Şimşek Kiper[1], Gülen Eda Utine[1]

[1]Division of Pediatric Genetics, Department of Pediatrics and [2]Department of Medical Genetics, Hacettepe University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Peters plus syndrome is a rare genetic condition wherein multiple systemic involvement with distinctive facial features are manifested, whilst the hallmark is Peters anomaly, occuring from anterior segment dysgenesis. Homozygous variants in the B3GLCT gene were identified to underlie this disorder. We here report on a one-month-old female patient with typical features characteristic of Peters plus syndrome in whom a homozygous pathogenic mutation in the B3GLCT gene was detected.

Key words: Peters anomaly, Peters plus syndrome, B3GLCT.

Peters plus syndrome (PPS) (MIM 261540) is an autosomal recessive disorder characterized clinically by anterior chamber eye abnormalities and a constellation of multiple systemic defects.[1,2] Developmental delay, limb shortening with brachydactyly, typical craniofacial features, and cleft palate/lip are among the other common findings.[1,2] Although bilateral Peters anomaly is the most common ophtalmologic abnormality in PPS, unilateral involvement or a different anterior chamber defect may also be observed.[1] Homozygous mutations in the *B3GLCT* (MIM 610308) on chromosome 13q12.3 have been described in the etiology in PPS.[3] We describe a one-month-old female patient with manifestations of PPS which was confirmed by *B3GLCT* sequencing analysis.

Case Report

A one-month-old female infant with dysmorphic features and disproportionate short stature was referred to our department for further evaluation. She was born at 39 weeks of gestation to first-cousin parents with a birthweight of 2320 g (-2.39 SD) and length of 43 cm (-3.01 SD) by cesarean section. The pregnancy was remarkable with the history of intrauterine growth retardation and short limbs which were detected after 28 weeks of gestation. Prenatal ultrasonography did not demonstrate any other abnormalities including eye findings or craniofacial pathologies. On admission, physical examination revealed a body length of 47 cm (-2.67 SD), a weight of 2,870 g (-2.16 SD) and a head circumference of 37 cm (-0.08 SD) compatible with growth retardation. Disproportionate shortening of the limbs with an arm span of 40 cm and brachydactyly was noted as well. She had a large anterior fontanelle with prominent forehead, widow's peak, hypertelorism, deep set eyes, broad and low nasal bridge with a bulbous tip, long philtrum, thin upper lip, and retromicrognathia. (Fig. 1). Peters anomaly was detected on eye examination performed due to corneal opacity, nystagmus and microphthalmia. Her metabolic screening was normal. Echocardiography revealed secundum atrial septal defect, peripheral pulmonary stenosis and trivial mitral valve regurgitation. Renal ultrasonography was unremarkable. Considering the distinctive findings of eye and disproportionate short stature, the patient was

✉ Gizem Ürel Demir
gizemurel@gmail.com

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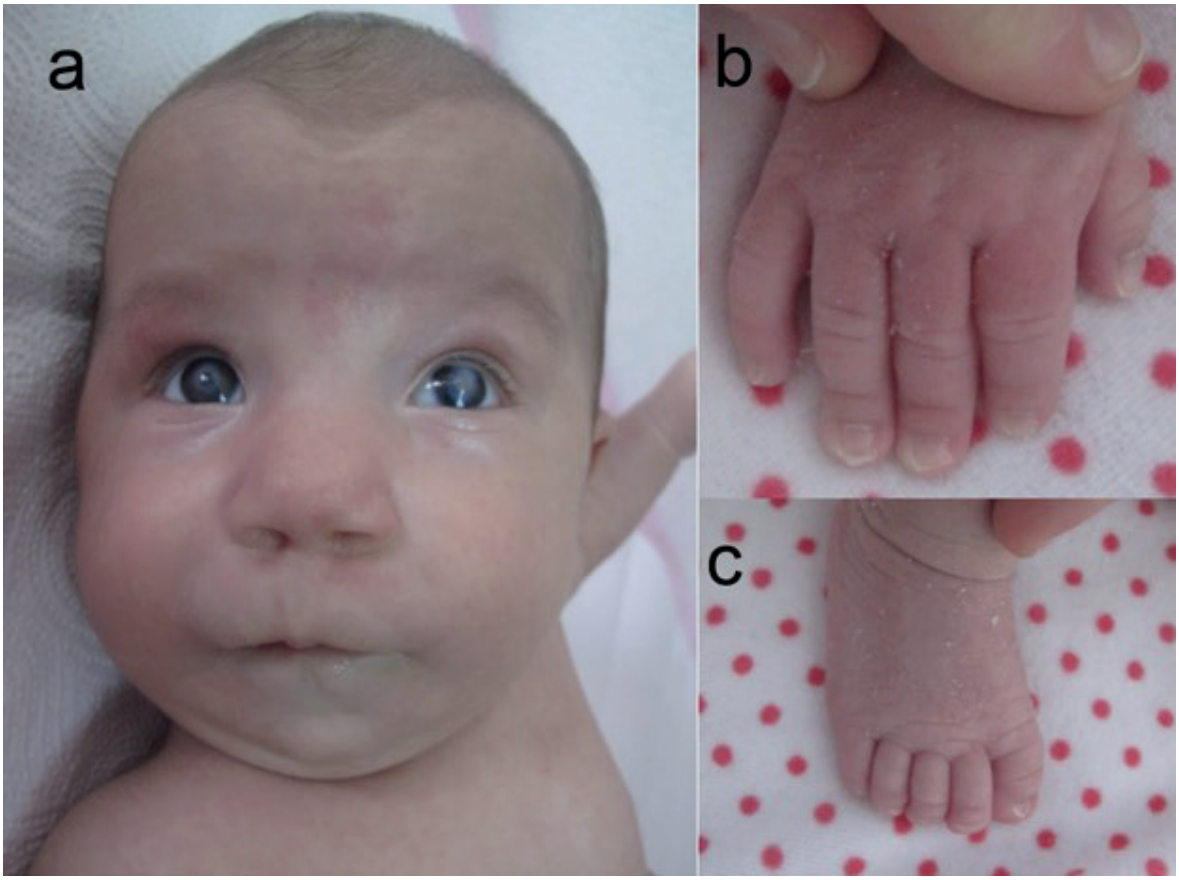

Fig. 1. Face (a), hand (b) and foot (c) appearance of the patient. Note bilateral corneal opacity, widow's peak, hypertelorism, low nasal bridge with a bulbous tip, long philtrum, thin upper lip, retromicrognathia (a), and brachydactyly (b,c).

clinically diagnosed with PPS. After informed consent was taken from the family, peripheral blood samples were obtained from the patient and her parents. *B3GLCT* sequence analysis was performed and a pathogenic homozygous splice site mutation (c.660+1 G>A) was detected in the patient which was heterozygous in the parents confirming the autosomal recessive inheritance (Fig. 2).

The patient had bilateral corneal transplantation in order to improve visual acuity. Her evaluation at 2 $^{5/12}$ years of age revealed body weight of 8500 g (-3.24 SD), height of 77 cm (-3.90) and head circumference of 46.5 cm (-0.94 SD). She achieved head and neck control at 6 months of age, sitting without support at 1 year of age, and walking at around 2 years of age. She was able to speak in sentences by 2 years of age. Denver developmental screening test was normal for her age.

Written informed consent to publish photographs was obtained from the family.

Discussion

In this clinical report, we have described a one-month-old female patient with characteristic features of Peters plus syndrome in whom a homozygous pathogenic mutation in *B3GLCT* was detected.

PPS is an autosomal recessive condition defined by ocular anomalies, short limbs with broad distal extremities, typical facial features, and variable systemic abnormalities.[1,2] In 1988, Saal et al.[4] reported two sisters with anterior

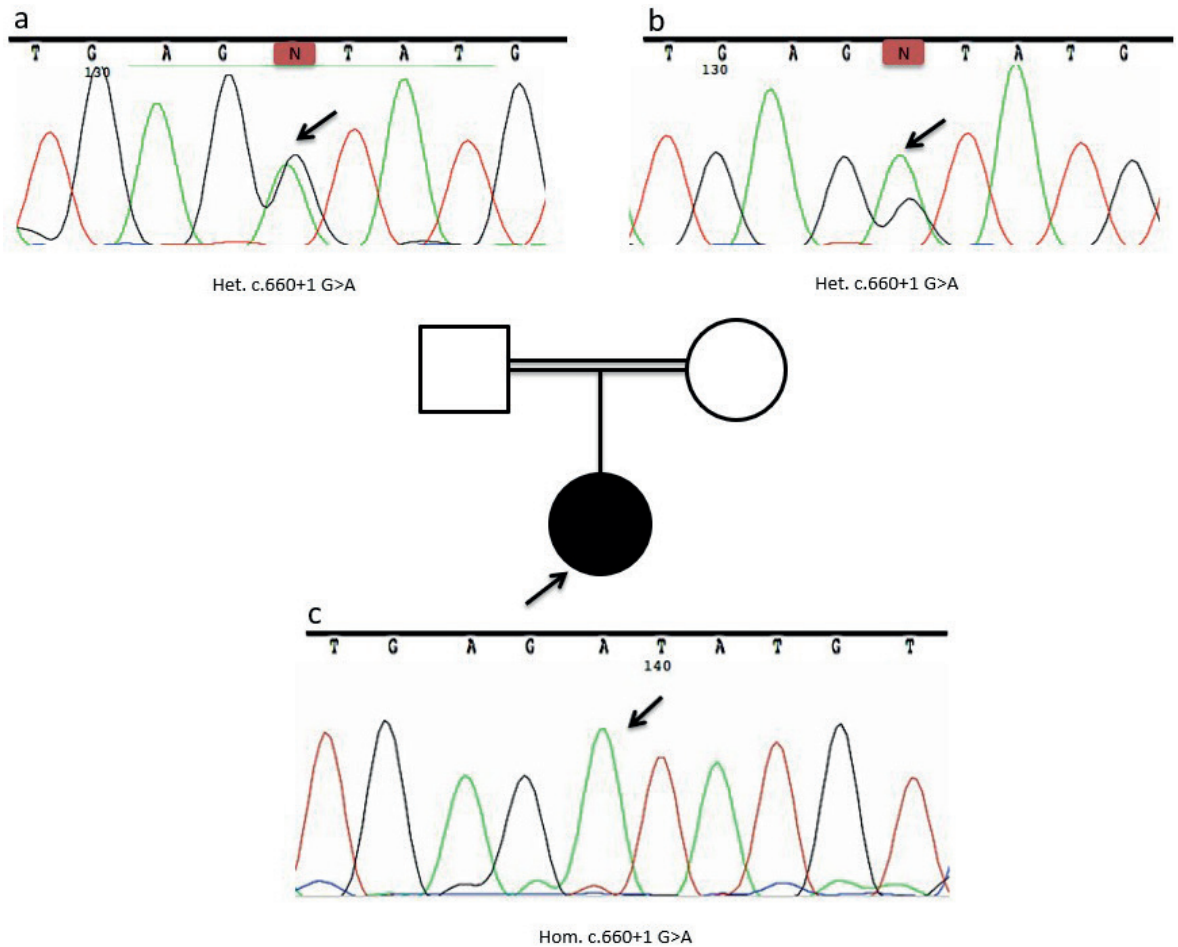

Fig. 2. Sequence analysis of the B3GLCT gene in the patient (homozygous) (c) and the parents (heterozygous) (a,b) shows a splice site mutation (c.660+1 G>A).

chamber anomalies of consanguineous parents suggesting autosomal recessive inheritance despite of resembling Robinow syndrome with the other associated features. Subsequently, Van Schooneveld et al.[5], in 1984, described 11 patients with similar phenotype and termed the entity as Peters plus syndrome (PPS).

Peters anomaly is the most common anterior chamber defect of the eye in PPS.[6-8] The most obvious feature of the Peters anomaly, which is a severe congenital anterior segment dysgenesis, is corneal opacity. This finding may be accompanied by various eye anomalies such as thinning or absence of corneal endothelium and Descemet's membrane, iridocorneal adhesions, and occasionally microphthalmia and cataracts.[6-8] Glaucoma is present in half of the patients at birth, and exacerbates the visual impairment.[1,9] Bilateral Peters anomaly was detected in the present patient as aforementioned and she had bilateral corneal transplantation whithin the first month of life in order to improve visual acuity and she is still under follow-up in terms of glaucoma.

Typical facial features of PPS include round face, a prominent forehead, hypertelorism, long philtrum, micrognathia, ear anomalies, Cupid's bow shaped upper lips, a thin vermillion border and a broad neck.[1] Rhizomelic shortening with broad hands and brachydactyly are the constant components of the syndrome while, other skeletal findings including thorax deformities, kyphoscoliosis, vertebral segmentation defects, short metatarsal and metacarpal bones,

clinodactyly of the fifth finger, syndactyly, and pes cavus can also be observed.[1,2,10] Growth retardation is prenatal onset and good response to growth hormone replacement therapy has been reported previously.[1,11] Growth retardation along with the facial and skeletal findings of the present patient were quite suggestive of PPS.

Individuals with PPS exhibit varying degrees of intellectual disability with or without structural brain abnormalities and epilepsy.[1,2,12,13] Microcephaly, macrocephaly, cleft palate/lip, and conductive hearing loss have also been reported among the other manifestations, however, these findings were all absent in our patient.[1,2,13,14] Defects affecting the central nervous system, including hydrocephaly, agenesis of the corpus callosum, and neural tube defects have also been described.[6,12,13,15,16] Congenital heart defects including atrial septal defect, ventricular septal defect, aortic stenosis, pulmonary stenosis, bicuspid pulmonary valve, hypoplastic left heart, absence of right pulmonary artery and vein have been reported as associated with PPS.[1,2,4,16,17] In this case, atrial septal defect and peripheral pulmonary stenosis were detected. Abnormalities involving the genitourinary system include hydronephrosis, vesicoureteral reflux, ectopic kidney, renal agenesis, renal and ureteral duplication, renal hypoplasia, oligomeganephronia, multicystic dysplastic kidney, and glomerulocystic kidneys, which were not present in our patient.[6,14,16]

Prenatal diagnosis of PPS is possible, however, it remains difficult due to nonspesific findings and limitations of ultrasound imaging. The prenatal ultrasound features defined in association with the syndrome are microcephalia, ventriculomegaly, agenesis of corpus callosum, microphthalmia, hyperechogenicity of the anterior chamber, cleft lip/palate, shortening of long bones, multicystic kidney, and dysmorphic facial characteristics of PPS.[18,19] PPS should be kept in mind in a fetus with typical ocular abnormalities, unusual facial appearance and shortness of long tubular bones, especially in the presence of a positive family history. In such instances, prenatal diagnosis might be an option for the couples. In the present case growth retardation and short limbs were detected prenatally, however, evidence was insufficient to establish the diagnosis of PPS because of the absence of the finding of scrutiny of eyes.

Based on all clinical findings, including anterior chamber defect of the eye, short stature with short limbs, and prominent facial features, PPS was suspected and the diagnosis was confirmed by molecular analysis of *B3GLCT*. *B3GLCT*, previously called *B3GALTL*, which is located on chromosome 13q12.3 was identified as the causative gene for PPS by Lesnik Oberstein et al.[3] in 2006. *B3GLCT* encodes a β1,3-glucosyltransferase which is involved in the glycosylation and functions by adding glucose to O-fucose attached to thrombospondin type 1 repeats (TSRs) by protein O-fucosyltransferase 2 (POFUT2).[20] Loss-of-function mutations in the *B3GLCT* cause degradation of glycosylation.[21] The splice site mutation in intron 8 [c.660+1G>A] is the most common mutation reported and also is present in our patient.[21] Unlike the other defects of enzymes that are involved in proteoglycan biosynthesis, *B3GLCT* defects are not related to joint dislocations, radioulnar synostosis, and generalized osteopenia. Peters anomaly, short stature and brachydactyly constitute the mandatory triad that is essential for the recognition of classic PPS.

In conclusion, patients with anterior chamber defects of the eye, particularly Peters anomaly, and other common systemic manifestations should prompt consideration of PPS. *B3GLCT* sequence analysis should be undertaken in order to confirm the clinical diagnosis for providing appropriate genetic counseling and options of prenatal diagnosis.

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Case Report

Grisel's syndrome presenting with neck pain: an atypical case

Emre Bilgin, Gökhan Çavuş, Vedat Açık, Yurdal Gezercan, Ali İhsan Ökten

Department of Neurosurgery, Adana City Training and Research Hospital, Adana, Turkey.

ABSTRACT

Grisel's syndrome is non-traumatic inflammatory subluxation of the atlantoaxial joints presenting clinically as torticollis, neck pain, and reduced neck mobility. Several pathogens have been implicated in its etiology. Early diagnosis and treatment are vital for Grisel's syndrome to avoid serious neurological complications. This study reports the case of a 6-year-old girl who complained of pain and curvature of the neck following an upper respiratory tract influenza infection. Notably, the patient's neck pain and curvature worsened toward the end of her normal day for a week. This pattern is rare, but it represents an important example of Grisel's syndrome as a condition that varies through the day.

Key words: atlantoaxial joints, torticollis, viral infection, pain.

Grisel's syndrome is non-traumatic inflammatory subluxation of the atlantoaxial joints; it presents clinically as torticollis and neck pain, and a decrease in neck mobility is observed, with surgical interventions showing hyperlaxity of the alar and transverse ligaments of atlantoaxial joints. The condition is diagnosed by clinical and neuroradiological examinations and can be cured completely via medical means. Several pathogens, including the Epstein-Barr virus, *Streptococcus pyogenes*, and *Staphylococcus aureus*, and Kawasaki disease have been implicated in its etiology. Early diagnosis and treatment are vital for patients with Grisel's syndrome as it can cause serious neurological complications if left untreated.

Here, we present the case of a 6-year old girl who complained of pain and curvature of the neck following an upper respiratory tract influenza infection. She had no history of trauma. For a week, the patient's neck pain and curvature had worsened toward the end of each day. This pattern is rare, but it represents an important example of Grisel's syndrome as a condition that varies through the day.

✉ Emre Bilgin
dremreblgn@gmail.com

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Case Report

A young 6-year-old girl was brought to the Neurosurgery outpatient clinic complaining of pain and curvature of the neck, which she had been experiencing since a week. Her parents explained that the girl's neck mobility was normal after waking up in the morning, with the curvature and pain in the neck area increasing progressively through the day (Fig. 1). The pain and limited neck mobility were associated with clinical torticollis.

The neck-related problems began following influenza-like symptoms, such as fever and nasal discharge, which started two weeks prior to presentation. Specific blood infection parameters included a sedimentation rate of 38 mm/h, a C-reactive protein level of 74.30 mg/L, and a white blood cell count of 12.6×10^3 cells/mm^3. Reproduction was not observed in any of the body fluid samples sent for culture. Under microscopy, the blood peripheral smear revealed atypical cells (Downey cells), which were considered to have developed secondary to the viral infection.

Fig. 1. The case's torticollis that intensified through the day (a-6:00 in the morning, b-12:00 midday, c-22:00 in the evening)

Three-dimensional (3D) reformatted cervical computed tomography (CT) revealed narrowing at the right anterior of the atlantoaxial joint range on cervical 1 (C1) and cervical 2 (C2), and image matching with subluxation was detected (Fig. 2). Because the patient did not experience torticollis in the early hours of the morning and there was only a small amount of pain at this time, a further 3D reformatted cervical CT scan was performed at 06:00 AM. This revealed that the atlantoaxial distance was normal and that the subluxation had been repaired (Fig. 3).

Superficial neck ultrasonography revealed multiple reactive, ovoid-shaped lymph nodes within the submandibular area on both cervical chains; one on the right was 23 mm long and one on the left was 17 mm long. Contrast-enhanced cervical magnetic resonance imaging (MRI) showed bilateral submandibular reactive lymph nodes and signal variations related to infection inflammation. These exhibited intense contrast and did not form distinct boundaries, and which hold the paravertebral tissues through C1 and C2 vertebrae toward inferior on nasopharynx left lateral wall, have been imaged (Fig. 4).

On the basis of these findings, Grisel's syndrome was diagnosed and the patient was administered an appropriate antibiotic treatment (ceftriaxone 2 × 50 mg/kg and clindamycin 3 × 40 mg/kg

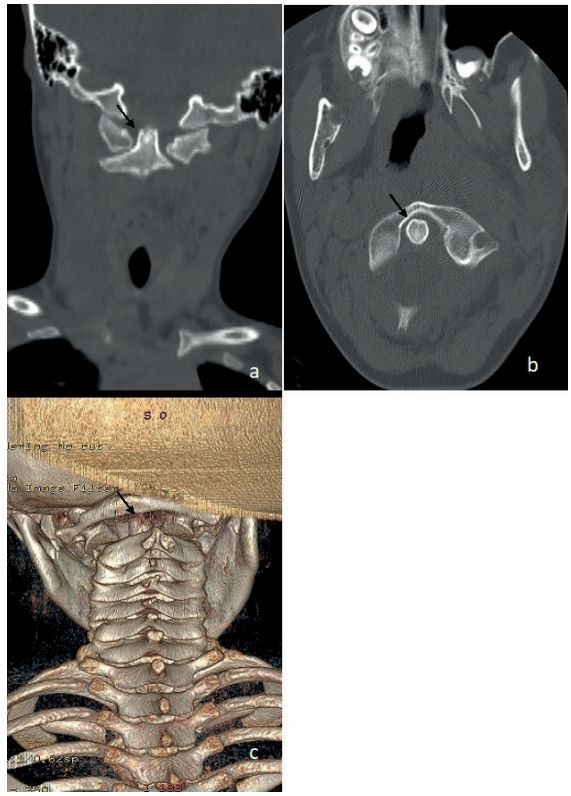

Fig. 2. Narrowing and image matching with subluxation at right anterior of atlanto-axial joint range in coronal (a) axial (b) section and reformat 3D cervical (c) CT images (black arrows)

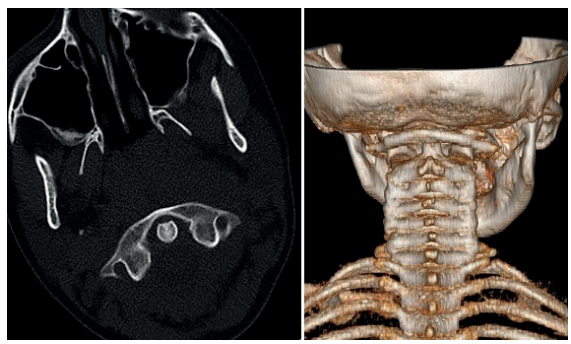

Fig. 3. It is observed that the atlanto-axial distance is normal and subluxation is non-existent in the axial section (a) and reformat 3D (b) cervical CT images.

intravenously, and ibuprofen syrup 4 × 20 mg/kg cups orally). Following two weeks of treatment, clinical, laboratory, and radiological findings showed the patient had recovered, and she was discharged home. During the two weeks after discharge, the patient was

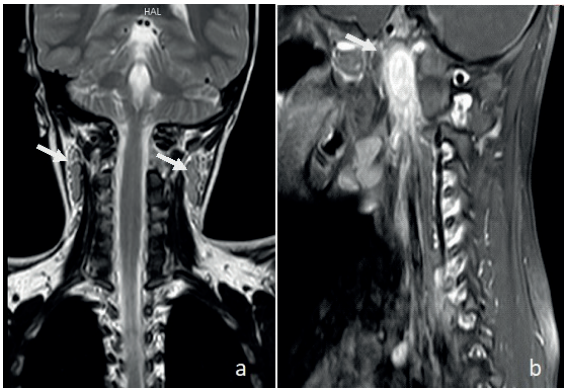

Fig. 4. Fusiform shaped lymph nodes with think cortexes on the bilateral submandibular area in contrast enhanced cervical MRI coronal (a) and sagittal (b) sections (white arrows), and soft tissue inflammation showing the substance retention in the parapharyngeal area, can be observed (white arrow.

mobilized with a cervical corset. Follow-up examinations at the outpatient clinic one month after discharge detected no pathology and there was no dislocation in the control CT (Fig. 5).

The child's parents provided written informed consent for the publication of this case report.

Discussion

Grisel's syndrome is characterized by rotator subluxation of the atlantoaxial junction of the first and second cervical spines (C1 and C2). It is non-traumatic and rare, appearing after an inflammatory process.[1-3] Grisel's syndrome has been reported after otorhinolaryngology infections,[4-6] or with osseous, ocular, ligamentous, psychiatric, and neurological disorders after head and neck surgery.[7] In addition, torticollis in childhood can be associated with infective agents.[8] The syndrome can be caused by various infectious agents, including *Streptococcus pyogenes*, *Bacteroides ureolyticus*,[9] *Mycobacterium tuberculosis*,[10] *Pseudomonas aeruginosa*, *Staphylococcus aureus*, methicillin-resistant *Staphylococcus epidermidis*, the Epstein–Barr virus, and Kawasaki disease.[11,12] In our case study, it was concluded that Grisel's syndrome developed secondary to the patient's recent upper respiratory tract viral infection.

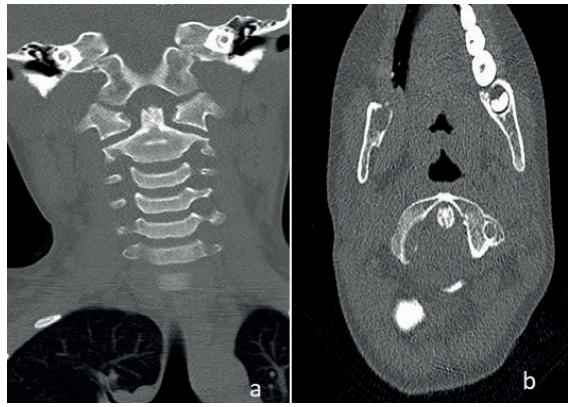

Fig. 5. It is observed that there are no subluxation and the ordinary atlanto-axial joint distance is normal in the cervical CT images in coronal (a) axial (b) sections.

No microorganisms could be reproduced in the blood or urine cultures, and there were atypical lymphocytes in the peripheral blood smear that supported the occurrence of such a viral infection.

Grisel's syndrome, which is generally accompanied by torticollis, presents with curvature of the neck, pain in the neck, and limited neck mobility.[13,14] The torticollis appears very soon after the start of a head and neck infection or following infection of the otorhinolaryngological tracts.[15] Unusually, the patient in our case study did not experience torticollis when she woke in the morning, but the torticollis intensified toward the evening. This pattern has been rarely seen in the literature. In addition, there have been no previous reports of radiology findings that show the absence of subluxation in the morning but its presence later in the day, accompanying the torticollis.

The early diagnosis of Grisel's syndrome is of vital importance. Serious neurological complications can occur if the condition goes unnoticed or is improperly treated. The condition is diagnosed radiologically using radiographs, CT, and MRI to show atlantoaxial subluxation. It is difficult to diagnose with radiographs alone.[16] The best modality for diagnosing atlantoaxial subluxation is CT, with 3D CT providing the optimal view of this pathology. MRI may show abnormalities in the soft tissue and

nervous tissue that are complementary to the radiological examination.[17] These examinations were utilized during the clinical management of the present case.

When Grisel's syndrome is diagnosed early, the primary treatment is conservative. Laboratory tests are required for the early diagnosis and to establish the etiology, and antibiotic treatment, bed rest, external fixation, anti-inflammatory drugs, and muscle relaxants are used against the active infectious agent; plasma exchange or immunoglobulin treatment must be started promptly in cases with a resistant form of infectious agent.[18] Treatment selection for the atlantoaxial joint subluxation depends on the severity of the injury. In non-serious injuries, physiotherapy and manipulation may be used; open surgery is indicated for serious subluxations.[19] According to the classification of subluxations of the atlantoaxial joint of Fielding and Hawkins[20] (Table I), types 1 and 2 represent the most common groups with no neurological deficiency, and types 3 and 4 are the groups that may lead to serious neurological deficiencies associated with spinal cord compression. The subluxations of the atlantoaxial joint were type 1 according to the Fielding classification in our case study.

Antibiotics, muscle relaxants, anti-inflammatory drugs, physiotherapy, and soft cervical corsets are recommended for the treatment of patients with type 1 subluxation. More-invasive methods, such as a halo vest, are included if neurological deficiency starts. In the most severe cases with repetitive subluxation, or in cases with difficult reduction, occiput (C0) C0–C1–C2 arthrodesis may be applied on the C1–C2 posterior arthrodesis line.[19] In the present case, we started treatment using antibiotics, muscle relaxants, and anti-inflammatory drugs, and then applied immobilization with a cervical corset.

Grisel's syndrome should be included in the differential diagnosis for malformation of the occipital, atlas, and axis bones that affect the craniocervical joint, and foramen magnum malformations. Atlantoaxial instability, infections, trauma, inflammation, malignancy, and Sandifer syndrome[21,22] should be ended as atlantoaxial instability like Grisel's syndrome.

In conclusion, Grisel's syndrome should be kept in mind for pediatric cases with neck pain and torticollis following a recent upper respiratory tract infection. Painful torticollis that shows a fatal course in the morning and intensifies

Table I. Fielding and hawking classification, reported in 1977.[20]

Type 1	Most common and is characterized by a simple rotation without anterior displacement of the atlas and the transverse ligament is undamaged	Antibiotics, muscle relaxant, massage therapy, and immobilization with a soft collar
Type 2	The rotatory subluxation is associated with anterior displacement of the atlas ≤ 5 mm and transverse ligament deficit	Reduction and cervical traction with a rigid collar
Type 3	Anterior displacement of the atlas is > 5 mm, both lateral atlantoaxial joints are subluxated anteriorly, the transverse ligament and the articular facets are damaged	Both type 3 and 4 subluxation are highly unstable lesions and, in most cases, associated with neurological symptom. It is necessary cervical traction with "halo vest" and, in the event of neurological symptoms, decompression and arthrodesis of C1 C2
Type 4	Quite rare, more frequent in adults with rheumatoid arthritis characterized by rotation and posterior dislocation of atlas	Both type 3 and 4 subluxation are highly unstable lesions and, in most cases, associated with neurological symptom. It is necessary cervical traction with "halo vest" and, in the event of neurological symptoms, decompression and arthrodesis of C1 C2

toward the evening may also indicate Grisel's syndrome. Progressive neurological deficit and permanent neck deformity can be prevented with appropriate and prompt medical treatment.

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Case Report

A rare presentation of adrenal adenoma in infancy: isolated Cushing's syndrome

Oğuzhan Şal[1], Gülçin Yeğen[2], Pelin Oğuzkurt[3]

[3]Department of Pediatric Surgery, [1]Koç University School of Medicine, İstanbul; [2]Department of Pathology, İstanbul University İstanbul Faculty of Medicine, İstanbul, Turkey.

ABSTRACT

Adrenocortical tumors are rare in children. Most of these tumors present with endocrinological manifestations, majority of which with virilizing features alone or in combination with over production of other adrenal hormones. However, it is uncommon of an adrenocortical tumor to present solely with Cushing's syndrome. In this paper we discuss the clinical presentation and management of a 5-month-old infant who had presented with Cushing's syndrome due to a functioning adrenocortical adenoma without androgen and mineralocorticoid excess, and made a brief review on the clinical and histopathological characteristics of adrenocortical tumors.

Key words: adrenocortical, adenoma, Cushing's syndrome, non-virilizing, wieneke.

Adrenocortical tumors (ACT) are among the rarest malignancies in children.[1] The annual worldwide incidence of childhood ACT is reported to be 0.3-0.38 per million children below the age of 15 years. The term ACT in children includes both benign adrenocortical adenomas (ACA) and malignant adrenocortical carcinomas (ACC), however histopathological classification of ACT is troublesome. Adrenocortical carcinomas are associated with recurrences and furthermore with death with a 5-year event free survival estimate of 46-54%.[2] Best prognostic factors for ACT are early detection, complete resection of the mass, and good constructed pre and post-operative steroid regimen.[3] It is already known that most of the ACT are endocrinologically active and usually present with virilization, precocious puberty with or without Cushing syndrome (CS) and rarely with isolated CS.[1,4]

The differentiation of malignant from benign tumor is difficult even with histopathological examination; therefore, final diagnosis relies on the evaluation of clinical manifestations, laboratory data, imaging, histopathological evaluation and clinical behavior of the tumor. Wieneke Scoring System is used to distinguish between malignant and benign adrenal tumors. In this paper, we report an infant who presented with isolated CS related to right adrenal adenoma according to Wieneke criteria.

Case Report

A 5-month-old female infant was admitted to the pediatric clinic due to progressive weight gain and facial swelling. She was born to non-consanguineous parents at the 38th week of gestation by C-section. Her birth weight was 3120 gr (25 percentile), birth height and head circumference were in 50 and between 25-50 percentiles, respectively. She was breastfed and later supplemented with formula feeding.

Her weight at presentation was 9 kg (>95th percentile). Vital signs including heart rate, blood pressure and respiratory rate were within normal limits for her age. In physical examination she had Cushingoid facies, buffalo hump, generalized excessive body fat, rashes in perianal and neck region. There were no signs of facial acne or virilization and she had

✉ Oğuzhan Şal
osal13@ku.edu.tr

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no further remarkable physical examination findings. In laboratory examinations, serum biochemical laboratory results were within normal ranges Na:147nmol/L (normal:135-150), K: 4.7mmol/L (normal:3.5-5.5), urea:13md/dL (normal:11-38), creatinine:0.4mg/dL (normal:0.17-0.42), whereas morning cortisol was 939.1 nmol/L (normal: 111-656 nmol/L); Adrenocorticotropic hormone (ACTH) was less than 5pg/mL (normal: 10.0-63.3 pg/mL). Abdominal ultrasound showed a hypoechoic, well-defined solid mass of 3.5x5 cm, in the right adrenal region. Abdominal magnetic resonance imaging (MRI) showed regular, contrast enhanced lesion of 38x40x45 mm (Fig. 1a) which compressed the inferior vena cava (Fig. 1b). A perioperative premedication with intravenous methylprednisolone was given to prevent adrenal insufficiency.

Right adrenalectomy was performed through a right subcostal incision. The cranial portion of the lesion had invaded the Glisson's capsule of the liver and also compressed the inferior vena cava medially. The mass was completely excised with the adherent liver parenchyma.

Gross pathological examination revealed a solid, yellowish tumor adjacent to the liver parenchyma. It was measured 5x4x4 cm and weighed 35 g. At microscopic examination encapsulated nodular lesion with trabecular-focally pseudoacinar pattern was seen (Fig. 2a). Tumor was consisted of eosinophilic cells with oval to round nucleus with fine chromatin. Although the tumor had expansive borders and was generally encapsulated, focally the capsule was wiped up and the tumor cells intermingled with the hepatocytes (Fig. 2b). There was no necrosis or vascular invasion. Four mitoses were observed at 20 HPF, but no atypical mitotic figures were observed. Immunohistochemically tumor cells were positive for melan-A (Fig. 2c), focally for inhibin alpha and were negative for cytokeratin, chromogranin A, synaptophysin, HepPar-1, glypican 3 and p53. Ki67 proliferation index was 10% (Fig 2d). Histopathological findings were compatible with adrenocortical adenoma according to Wieneke classification system (Table I) and a Stage 1 adrenocortical tumor according to pediatric adrenocortical tumor staging criteria.

The patient showed an uneventful recovery and discharged with oral hydrocortisone 5 days after the surgery. At 3 months follow-up, oral hydrocortisone was tapered and stopped

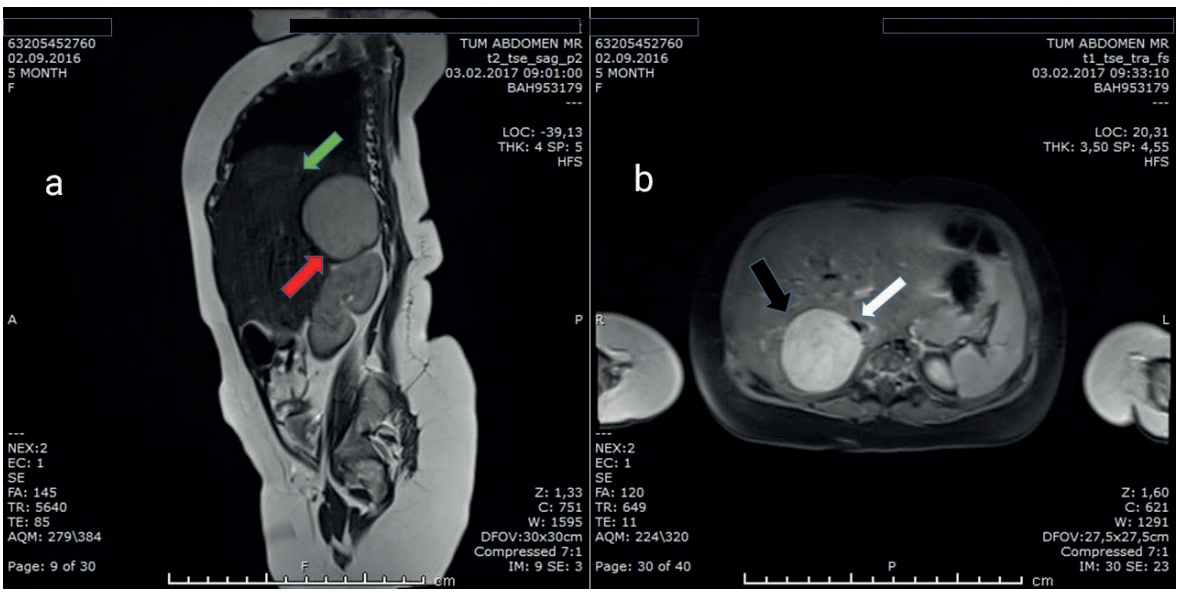

Fig. 1a. Right adrenal mass (shown with red arrow) and its close proximity to liver (shown with green arrow). **1b.** The mass (shown with black arrow) and its compression on inferior vena cava (shown with white arrow).

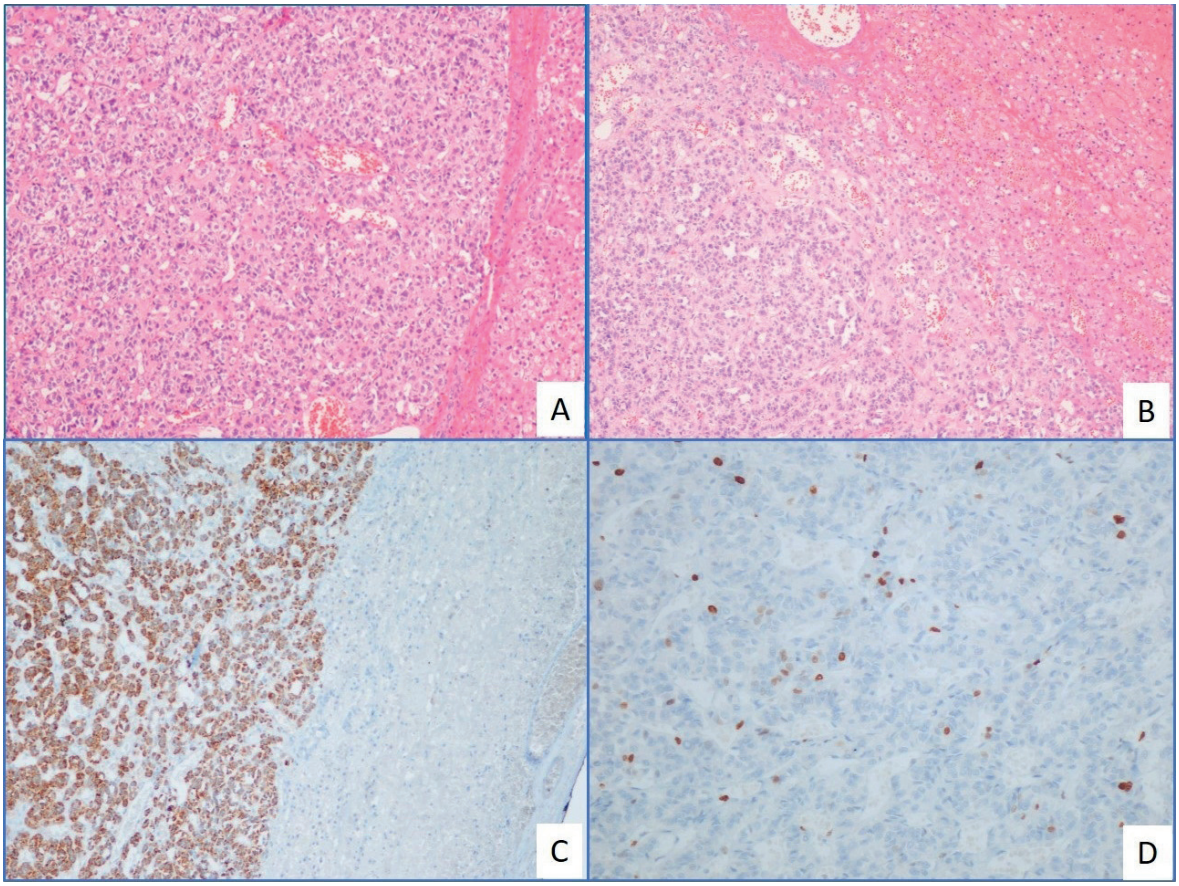

Fig. 2. Pathology sections of the tumor.

Table I. Wieneke criteria.

Wieneke criteria
Tumor weight >400 gr
Tumor size >10,5
Extension into periadrenal soft tissues and/or adjacent organs*
Invasion into vena cava
Venous invasion
Capsular invasion*
Presence of tumor necrosis
>15 mitoses per 20 HPF
Presence of atypical mitotic figures

(*Positive criteria present in our case)
Tumors showing ≤2 of features were classified as benign, ≥ 4 criteria as malignant, whereas tumors with 3 features were classified as indeterminate for malignancy.

through regular ACTH and cortisol monitoring (Table II). The cortisol and ACTH levels were 4.3μg/dl (normal range: 3.7-9.4 μg/dl) and 17pg/ml (normal range:10-60 pg/ml), respectively at the time of cessation of replacement treatment. The postoperative serum biochemistry results were normal. During the follow-up period, her Cushingoid appearance resolved at one-year of age (Fig 3a and b).

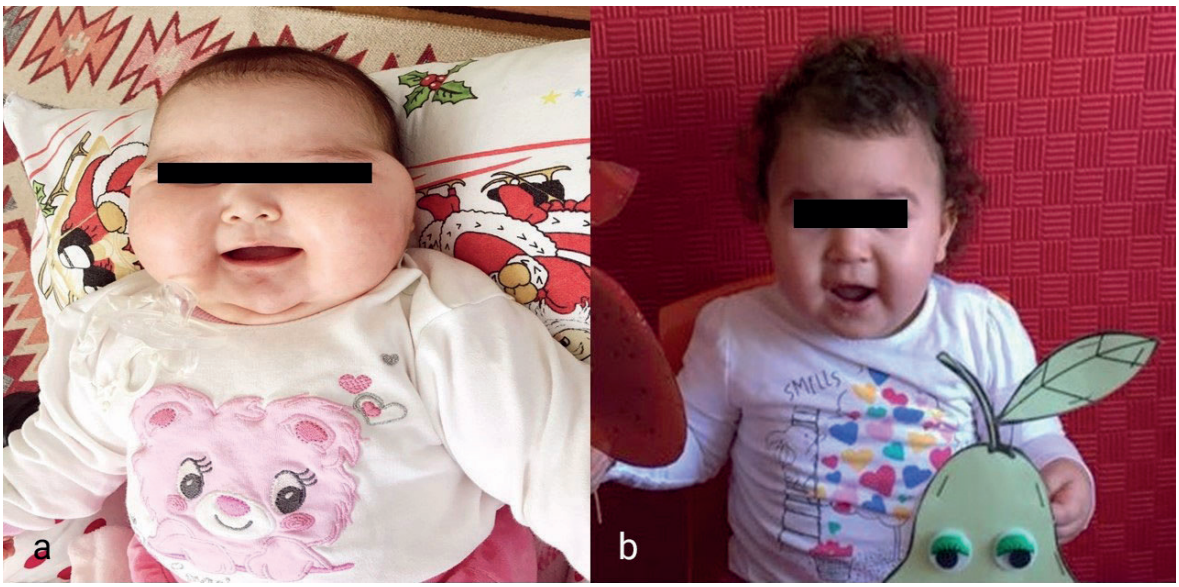

Fig. 3a and b. Cushingoid facies of the patient before and after the treatment.

Table II. ACTH and cortisol levels of the patient before and after surgery.

Date	ACTH	Cortisol
28.01.2017	<5.00 pg/mL (normal:10-63)	939,1 nmol/L(normal:111-656)
08.02.2017	Adrenalectomy was performed.	
15.02.2017	22,2 pg/mL (normal:10-63)	7,8 µg/dl (normal:3.7-19.4)
03.03.2017	28,1 pg/mL (normal:10-63)	4.0 µg/dl (normal:3.7-19.4)
05.04.2017	31,8 pg/mL (normal:10-63)	3.0 µg/dl (normal:3.7-19.4)
Cessation of replacement treatment		
02.05.2017	17 pg/mL (normal:10-63)	4,3 µg/dl (normal:3.7-19.4)
02.06.2017	24 pg/mL (normal:10-63)	8,7 µg/dl (normal:3.7-19.4)
16.08.2017	14 pg/mL (normal:10-63)	5,4 µg/dl (normal:3.7-19.4)

An informed consent to publish the case report including the photos was obtained from parents of the patient.

Discussion

In this report we presented a 5-month-old girl who showed discriminating clinical features of glucocorticoid excess, particularly excessive weight gain, Cushingoid facies, buffalo hump, and generalized increased body fat. She had biochemical evidence of excessive glucocorticoid production and ACTH suppression. CS in pediatric population usually manifests by slowed linear growth, extremely accelerated weight gain, facial puffiness, buffalo hump; rarely it presents itself by virilizing symptoms such as pubic and axillary hair development, and breast development.[5] CS can be ACTH-dependent or ACTH-independent. ACTH independent causes are primary adrenocortical hyperfunction, adrenocortical tumors (ACT), primary pigmented nodular adrenocortical disease, bilateral macronodular adrenal hyperplasia, and iatrogenic CS.[6]

The serum cortisol level of the presented patient was elevated whereas the ACTH level was severely suppressed, therefore an ACTH-independent cause of CS was suggested to be the etiology after excluding the iatrogenic CS. Oral and topical glucocorticoid therapies

in infants and young children with diaper dermatitis are the most common causes of iatrogenic CS, although other applications such as inhalation, ocular and nasal drops including glucocorticoids may also result in hypercortisolism.[7] In iatrogenic CS, all affected cases have low ACTH and cortisol levels. The presented patient did not have a history of exposure to exogenous glucocorticoids.

Adrenocortical tumors are rare in infancy and occur primarily in children between one to five years of age (60%), with a peak in incidence below 4 years of age.[7] To the best of our knowledge ACA and ACC presenting early in infancy with isolated CS are very rare and reported through a few case reports.[8-10]

In approximately 15-20% of CS cases, ACT are the etiology behind the disease. After clinical and laboratory assessment; preferred radiological approaches are ultrasound, computerized tomography (CT) or magnetic resonance imaging (MRI) ; in MRI fat–rich nature of ACA is suggested to be quite useful in distinguishing them from ACC .[11,12] Although CT has been the primary choice of imaging, MRI has superior traits such as better visualization of possible anatomical relationship between the mass and the adjacent anatomical structures.[11] FDG-PET scans are also quite useful in differentiation between ACC and ACA.[13] In our patient abdominal ultrasonography clearly defined hypoechoic right adrenal tumor, which was confirmed with MRI as a contrast enhanced lesion compressing the inferior vena cava. Imaging studies help in the surgical planning and the staging of the disease as well as predicting the resectability of the lesion such as tumor size, invasion to the adjacent structures and vascular invasion or thrombosis.[14]

Surgery is the most important procedure in the treatment of ACT. Endocrinological preparation of the patient prior to operation is vital in order to successfully avoid life threatening acute adrenal insufficiency due to the hypoplastic contralateral adrenal gland secondary to prolonged suppression of ACTH secretion from the pituitary.[15,16]

Adrenocortical tumors in children have a less aggressive clinical behavior when compared to their adult counterpart and furthermore application of histopathological criteria of adult ACT will result mostly in overdiagnosis of pediatric cases as carcinoma.[17-20] In childhood ACT, there is no single pathological feature that can predict the malignant behavior. Wieneke et.al proposed a set of macroscopic and microscopic criteria for histopathologic diagnosis of malignant ACT.[18] Nevertheless, the predictive value of the system is yet to be validated.[18] Besides the histopathologic criteria, a modified pediatric staging system based on tumor weight or volume and resectability was accepted to show prognostic significance.[22] In our case, adverse pathological features that had been mentioned in Wieneke Scoring System were focal capsule invasion with subsequent liver parenchymal invasion. The presented patient was diagnosed as ACA with young age at presentation, small tumor size and low tumor volume, and absence of tumor necrosis and atypical mitotic figures; however, focal invasion of liver parenchyma was considered as an important factor in favor of malignancy, so a close follow-up with endocrinological evaluation and ultrasound examination is recommended.

Adrenocortical tumors are rare in pediatric population. They rarely represent themselves with isolated CS; in this paper we presented a rare clinical picture of ACA; a 5-month-old infant with an isolated CS associated with a right ACA. Pathophysiology, diagnostic laboratory tests, imaging findings and treatment modalities are discussed. According to the Wieneke criteria this case was diagnosed as an adenoma. Distinction between ACA and ACC is quite challenging, therefore further pathological clarification between them is significant, creating necessity of ACT registry to obtain large data to have new guideline criteria.

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Case Report

Obstructive jaundice and severe pancreatitis due to the foramen of Winslow hernia with multiple anomalies

Neslihan Ekşi Bozbulut[1], İsmail Akdulum[2], Sinan Sarı[1], İbrahim Onur Özen[3], Yusuf Hakan Çavuşoğlu[3], Öznur Boyunağa[2], Aydın Dalgıç[4], Buket Dalgıç[1]

Departments of [1]Pediatric Gastroenterology, [2]Pediatric Radiology, [3]Pediatric Surgery and [4]General Surgery, Gazi University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Internal hernia through the foramen of Winslow is a very rare condition, especially in children. Here we report a 16-month-old girl who presented with obstructive jaundice and elevation of pancreatic enzymes and was ultimately diagnosed with internal hernia and malrotation by radiologic investigation and open approach surgery. To the best of our knowledge, obstructive jaundice with pancreatitis and other congenital abnormalities in children with the foramen of Winslow hernia have not been reported previously in the literature.

Key words: internal hernia, the foramen of Winslow, pancreatitis, obstructive jaundice.

Internal hernias are defined as protrusion of a viscus through a natural or secondary (postsurgical, traumatic, etc.) orifice of the abdominal cavity.[1] The foramen of Winslow (Foramen epiploicum) is a passage between the greater (general peritoneal space) and lesser sac (omental bursa), allowing communication between these two spaces.[1] Herniation of the intestines through this foramen constitutes only 8% of internal hernias. The rate of preoperative diagnosis is very low in these patients.[2] Typical patients are middle-aged, with a sudden onset of severe abdominal pain as a symptom of acute intestinal obstruction.[2,3] Rarely, patients present with jaundice because of the pressure of the herniated intestines on the common bile duct.[1] It is a very rare condition in children and it is difficult to suspect this clinical situation in this age group.[2-4] Accompanying pancreatitis has not been reported before in children. Herein, we report a case of an infant with multiple congenital anomalies who presented with pancreatitis and obstructive jaundice secondary to a foramen of Winslow hernia.

✉ Neslihan Ekşi Bozbulut
neslihaneksi@hotmail.com

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Case Report

A 16-month-old girl who was previously healthy was admitted to our hospital with a one-month history of restlessness, loss of appetite, abdominal pain, jaundice, and acholic stool. She had no pruritus. She had been hospitalized for 20 days in another clinic. Abdominal ultrasonography showed dilatation of intrahepatic bile ducts and ductus choledochus, and abnormal anatomic position of the head of the pancreas. For further examination, the patient was referred to our hospital.

On physical examination, her body weight was 7000 g (< 3p) and height was 73 cm (3-10 p). Her skin and sclera was icteric, spleen and liver were palpable 4 and 1 cm, respectively. She had also dysmorphic facial characteristics (broad nasal root, long eyelashes, prominent forehead) and bilateral clinodactyly.

Blood tests revealed elevated transaminases, serum total bilirubin, conjugated bilirubin, amylase, and lipase (Table I). Millimetric

Table I. Laboratory findings.

Laboratory results	Admission	Postoperative (5th day)	Postoperative (one month)
ALT (Range: 0-40 U/L)	71 U/L	68 U/L	19 U/L
AST (Range: 0-40 U/L)	152 U/L	124 U/L	42 U/L
GGT (Range: 0-50 U/L)	810 U/L	421 U/L	36 U/L
Total bilirubin (Range: 0.3-1.2 mg/dl)	15.3 mg/dl	7.3 mg/dl	0.94 mg/dl
Conjugated bilirubin (Range: 0-0.5 mg/dl)	13.3 mg/dl	6.3 mg/dl	0.15 mg/dl
Amylase (Range: 28-100 U/L)	1376 U/L	136 U/L	57 U/L
Lipase (Range: 0-67 U/L)	1813 U/L	107 U/L	30 U/L

ALT: Alanine transaminase, AST: Aspartate transaminase, GGT: Gamma-glutamyltransferase.

echogenicity in the gallbladder lumen, dilatation of the common bile duct and intrahepatic bile ducts, and right pelvic ectopic kidney were observed on abdominal ultrasonography. Secundum atrial septal defect (ASD) was detected by transthoracic echocardiographic assessment.

Magnetic resonance cholangiopancreatography (MRCP) revealed dilatation of choledochus, common hepatic, and the intrahepatic bile ducts; structural abnormality of the pancreas (the head and the uncinate process extending into the hepatic hilum and upward to the lesser curvature of the stomach); splenomegaly; and intestinal malrotation (Fig. 1). Duodenojejunal junction and jejunal loops were located on the right side of the midline at upper gastrointestinal series. Computer tomography (CT) of the abdomen revealed more detail about the anatomy of the pancreas and vascular structures. Many dilated venous collaterals were observed at the confluence of the main portal vein and the splenic vein, which also covered the head of the pancreas, and the right portal vein narrowed at confluence. The hepatic artery arose from the celiac trunks and ran inferiorly, crossing the superior mesenteric

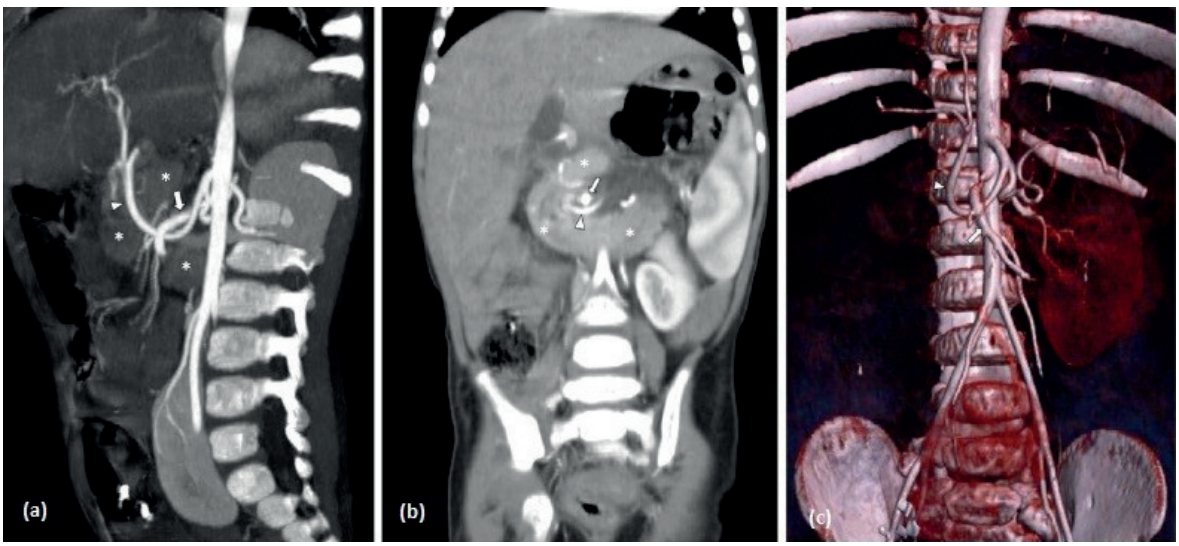

Fig. 1. Magnetic resonance cholangiopancreatography (MRCP) findings, sagittal oblique 5 mm maximum intensity projection (MIP) (a), 1 mm coronal multiplanar reformat (MPR) (b) and anterior view volume rendering technique (VRT) (c) images demonstrate superior mesenteric artery (SMA) (arrow) runs above hepatic artery (arrow head) and hepatic artery elongated. Pancreas (*) head and uncinate process shifted upwards and herniated through the foramen of Winslow. SMA proximal side is narrowed due to compression of herniated structures.

artery inferiorly, reaching the liver by traveling parallel to the splenic vein, while the head of the pancreas was turned incompletely around the hepatic artery and celiac trunk (Fig. 1); prominent Wirsung channel was also seen. Posterior arcus fusion defect of the vertebra was detected at the 5th lumbar and sacral vertebra on abdominal CT. Herniation of appendix, cecum, ascending colon, and a portion of the small intestine through the foramen of Winslow into the lesser sac, disrupted anatomic position of the pancreas (uncinate process pushing the stomach upwards), compression of the common bile duct by the duodenum, and malrotation were identified by open approach surgical exploration. Intestines were mobilized with traction behind the hepatoduodenal ligament. Position abnormality of the pancreas returned to normal after reduction of the intestines. External compression of the foramen of Winslow hernia on the biliary and pancreatic ducts caused obstructive jaundice and acute pancreatitis.

Her postoperative recovery was good with no complications. After surgery, her jaundice completely disappeared, and all laboratory abnormalities showed significant improvement (Table I). She was discharged from the hospital five days after the operation. One month after the surgery, laboratory results all returned to normal references. On the third month of her follow-up period, she had remained asymptomatic, abdominal ultrasonography revealed no abnormalities of the biliary tract and pancreas.

Informed consent of the subject was received from the family.

Discussion

The foramen of Winslow hernia accounts for 8% of internal hernias. It is seen much less frequently in children than in adults, and rare cases have been reported until now.[2-4] Etiological factors might be an enlarged foramen of Winslow, mobile ascending colon caused by failure of secondary fusion of the colon with the posterior abdominal wall, common mesentery for the whole intestine, or a mobile small intestine resulting from abnormal length of the mesentery.[1,4]

The foramen of Winslow hernia is difficult to diagnose both clinically and radiologically. The most common clinical presentation of foramen of Winslow hernia is intestinal obstruction. Most of the reported pediatric patients in the literature presented with clinical findings of acute intestinal obstruction.[2,4]

Differently from other pediatric cases, our patient did not have serious abdominal pain or vomiting that suggested intestinal obstruction. Because of the separation of the lesser sac from the anterior abdominal wall, clinical findings of intestinal obstruction, even intestinal necrosis, might be overlooked in these patients.[2] In some particular patients, obstructive jaundice was reported due to the direct compression of the bile ducts by the herniating viscus.[3,5] We observed obstructive jaundice and pancreatitis due to direct compression of the common bile duct because of the disrupted anatomic position of the pancreas and duodenum in our case. Similarly, an adult case was reported with obstructive jaundice and acute pancreatitis caused by herniation of the small bowel through the foramen of Winslow, like our case the common bile duct was compressed by the herniated bowel and believed that acute pancreatitis was due to this compression.[5]

The rate of preoperative diagnosis by clinical findings and laboratory abnormalities including imaging is very low, and most patients are diagnosed by laparotomy. "Narrowed portal vein" sign on CT imaging is considered to be a clue for the foramen of Winslow hernia, as seen in our case.[6] Intestinal rotation anomalies were present in all pediatric patients.[2-4] Our patient also has multiple congenital anomalies, atypical facial appearance, clinodactyly, right pelvic ectopic kidney, secundum ASD, and posterior arcus fusion defect of the vertebra, but we have not found a specific genetic reason for these congenital anomalies yet.

In conclusion, foramen of Winslow hernia is a very difficult entity to diagnose, especially in children. Although acute intestinal obstruction is the most common presentation, different signs and symptoms, such as obstructive jaundice and pancreatitis, can be observed in these patients without intestinal obstruction as a symptom. We need to be careful and heighten awareness of its identification, both clinically and radiologically, in patients presenting with these findings.

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Case Report

A rare cause of acute abdominal pain in a patient with Primary ciliary dyskinesia with situs inversus totalis

Kısmet Çıkı[1], Özlem Boybeyi Türer[2], Mina Hızal[3], Gökçen Dilşa Tuğcu[3], Nagehan Emiralioğlu[3], Ebru Yalçın[3], Deniz Doğru Ersöz[3], Nural Kiper[3], Uğur Özçelik[3]

Departments of [1]Pediatrics, [2]Pediatric Surgery and [3]Pediatric Pulmonology, Hacettepe University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

Primary ciliary dyskinesia (PCD) is a rare, genetic disease characterized by ciliary dysfunction. Patients may present with respiratory distress during neonatal period; chronic sinopulmonary disease, bronchiectasis, recurrent otitis media, sinusitis and infertility in later periods. About 50% of PCD patients have situs inversus totalis and 6-12% have situs ambiguous known as heterotaxy syndromes. Herein, we present a case of PCD and accompanying situs inversus who had acute abdominal pain and was diagnosed with torsion of one of the multiple spleens. Evaluation of acute abdominal pain in these patients has great importance since the internal organs are not at their typical locations.

Key words: Kartagener syndrome, primary ciliary dyskinesia, spleen torsion.

Primary ciliary dyskinesia (PCD) is a rare, genetic disease characterized by ciliary dysfunction. Patients may present with respiratory distress during neonatal period; chronic sinopulmonary disease, bronchiectasis, recurrent otitis media, sinusitis and infertility in later periods. About 50% of PCD patients have situs inversus totalis and 6-12% have situs ambiguous known as heterotaxy syndromes.[1,2] Situs inversus is a condition in which the arrangement of the internal organs is a mirror image of normal anatomy. In heterotaxy syndrome internal organs arrange non-mirror image, abnormal, and in mixed location. Cardiac, large vessel and lung anomalies, polysplenia and asplenia can be seen in situs ambiguous.

Therefore, evaluation of acute abdominal pain in these patients has great importance since the internal organs are not at their typical locations. Herein, we present a case of PCD and accompanying situs inversus who had acute abdominal pain and was diagnosed with torsion of one of the multiple spleens.

Case Report

A 15-year-old male patient was referred to our hospital for acute abdominal pain with the initial diagnosis of acute appendicitis. He has been followed at our hospital with the diagnosis of PCD for two years. He presented with an abdominal pain that was more prominent in lower right regions. There was no fever, vomiting, diarrhea, constipation, urinary frequency or dysuria. On physical examination he had tenderness on palpation, defense and rebound tenderness at the lower right. There was no swelling, tenderness and pain in testicular examination. There were rales in the lung bases. Leukocytosis was found in blood count with 72,5% neutrophil predominance, other laboratory tests were within normal limits. Oral feeding was discontinued because

✉ Kısmet Çıkı
dr.ksmet@hotmail.com

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of the acute abdomen. Intravenous fluid support was started. Abdominal ultrasonography was performed with the preliminary diagnosis of acute appendicitis. It was found that the liver was located on the left side, the spleen was located on the right side and there were six accessory spleens around the spleen. There was no vascularization in the largest accessory spleen (5x6x6 cm). An emergent surgery was performed since he had acute abdominal signs and it was suspected that there was torsion of one of the multiple spleens. Peroperatively it was seen that there were five accessory spleens and the largest one was swollen, edematous and rotated five times on itself (Fig. 1). Torsed spleen and all accessory spleens were excised. The original spleen and its ligaments were normal and not excised. Appendectomy was also performed since he had situs inversus totalis. On pathological examination, inflammation and congestion was seen in torsed accessory spleen. On the fourth day after the operation, the patient was discharged with cure. The patient is still under follow-up uneventfully.

Informed consent was received from the family.

Discussion

Primary ciliary dyskinesia is a genetic disorder characterized by motile silia dysfunction, impaired mucociliary clearance and recurrent respiratory infections. It is an autosomal recessive disorder but X-linked inheritance pattern has rarely been reported. It affects approximately 1 in 15,000-20,000 individuals.[3] The first PCD case who had bronchiectasis and situs inversus was reported by Siewart. Bronchiectasis, sinusitis and situs inversus triad was described by Kartagener in 1933 and it is known as Kartagener syndrome.[2] Respiratory distress is seen in the newborn; cough, nasal congestion, nasal polyp, chronic pansinusitis, recurrent middle ear infection, hearing loss, recurrent lower respiratory tract infection, and bronchiectasis are seen in the follow up. Males are 100% infertile, whereas fertility is decreased in females. Cystic kidney, cystic-cholestatic liver, skeletal deformities, hydrocephalus, developmental retardation, retinitis pigmentosa, blindness and deafness may also occur due to non-motile ciliary dysfunction. Clinical and diagnostic tests are both used for diagnosis. Genetic tests, cilia examination in electron microscopy, cilia motility study and nasal nitric oxide are diagnostic tests. Genetic studies and expert evaluation are recommended for clinical conditions of immotile silia dysfunction in patients with PCD.[4]

PCD and situs inversus are both seen as a result of ciliary dysfunction. Airway epithelium cilia defects cause PCD while nodal cilia, which play an important role during embryogenesis,

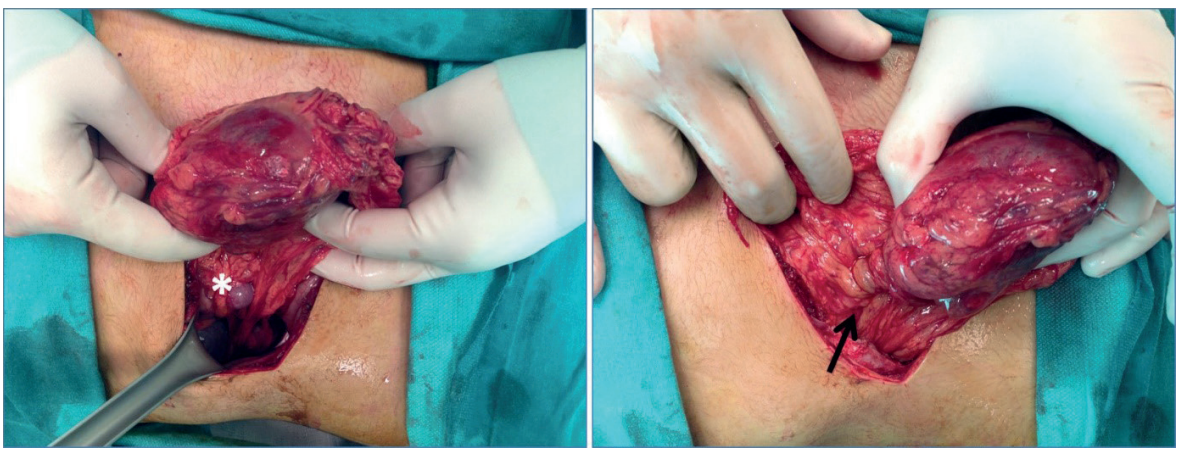

Fig. 1. The surgical view; the accessory spleens are seen in the left hand side (*) and the torsion of the largest accessory spleen is seen in the right hand side (torsed vascular pedicle signed with an arrow).

defects cause situs inversus.[5] Situs inversus is a condition in which the arrangement of the internal organs is a mirror image of normal anatomy. In situs ambiguous, internal organs arrange an abnormal, non-mirror image, and exist in mixed location. The prevalence of isolated situs inversus totalis is 1/8500; heterotaxy is 1/10 000. About 50% of PCD patients have situs inversus totalis and 6-12% have situs ambiguous known as heterotaxy syndromes. The true prevalence may be even higher, as many PCD patients do not routinely have investigations to define their abdominal laterality defects. Although the prevalence of an approximate heterotaxy in PCD patients is known, the PCD prevalence in heterotaxy syndrome is unknown.[6] Heterotaxy syndromes are divided into 2 groups according to whether it is polysplenia or asplenia. Heterotaxy syndromes with polysplenia are called left isomerism; heterotaxy syndromes with asplenia are called right isomerism. Left isomerism is characterized with partial pulmonary venous return anomalies, polysplenia, left superior vena cava, left inferior vena cava, left atrium, and bilateral two-lobe lungs while right isomerism is characterized with right ventricle, bilateral superior vena cava, total pulmonary venous return anomaly, pulmonary atresia, pulmonary stenosis, bilateral three lobed lungs and asplenia. Different locations of the organs, biliary atresia, choledocholithiasis, annular pancreas, short pancreas, pancreatitis due to short pancreas, malrotation, obstruction, volvulus, inferior vena cava and portal venous system anomalies are gastrointestinal complications of heterotaxis syndromes. Vascular anomalies may cause bleeding and thrombosis in abdominal surgeries.[7]

Location and number of spleens are variable in heterotaxy syndromes with polysplenia. Although they are usually asymptomatic, they can mimic lymphadenopathy or a tumor. It can also cause symptoms due to torsion, haemorrhage, spontaneous rupture or cyst formation.[8] The torsion of spleen at polysplenia is a very rare condition causing acute abdominal pain and has also been reported in a few reports in the literature. Patients present with pain, vomiting and nausea in the splenic torsion. It has been reported that the pain may mimic acute appendicitis.[9] Our patient's pain was more prominent in the right lower torso and we initially considered as acute appendicitis. Splenic torsion is rarely diagnosed preoperatively because it is an extremely rare entity. Imaging methods are used to diagnose and some cases need laporotomy for an exact diagnosis. The excision of torsed spleen is the prompt treatment.[9]

The coincidence of PCD with this rare condition is also very rare. There were some reported cases in literature of splenic torsion in heterotaxy syndromes with polysplenia and first case was reported by Ackerman in 1982.[10] Splenic torsion is reported in heterotaxy syndromes with polysplenia in the literature. There was no information as to whether PCD evaluation was performed in these cases. We could find only one case who had PCD and splenic torsion in the literature.[11]

Polysplenia should be kept in mind for patients who are followed up with PCD and situs inversus ('Kartagener Syndrome') and abdominal imaging should definitely be performed. Splenic torsion should be considered in the differential diagnosis of patients who are known to have polysplenia and admitted with the complaint of abdominal pain. Awareness of this entity is important in the exact diagnosis and prompt treatment of such cases.

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Case Report

Neonatal iliopsoas abscess presenting with transient cyanosis of a single extremity: a case report and review of the literature

Mustafa Şenol Akın[1], Damla Ağaçkıran[1], Emre Ünal[3], Özlem Özkale Yavuz[3], Şule Yiğit[2]

[2]Division of Neonatology, [1]Department of Pediatrics and [3]Department of Radiology, Hacettepe University Faculty of Medicine, Ankara, Turkey.

ABSTRACT

A newborn baby with an unusual complaint of transient left leg cyanosis during crying, who was diagnosed with a iliopsoas abscess is presented. Newborn cases diagnosed with an iliopsoas abscess in the English literature are summarized and differences in clinical presentations are discussed.

Key words: transient single extremity cyanosis, iliopsoas abscess, newborn.

The iliopsoas abscess is rare in children, while it is highly rare in the newborn period. To date, 24 cases with iliopsoas abscess occurring in the neonatal period have been reported in the literature (Table I). The etiological factors in most of the cases have not been identified. Birth trauma and local hemorrhage in the iliopsoas muscle have been accused as leading factors for abscess formation. The most common symptoms of iliopsoas abscess in newborns are pain during hip movement and swelling of thigh. We present here a case who presented with an unusual symptom; transient left leg cyanosis becoming evident during crying.

Case Report

The patient was a 3650gr female baby, born to a 29 years old mother at 37 weeks and 5 days of gestation with cesarean section. Pregnancy was uneventful. However, the baby was taken to the neonatal intensive care unit (NICU) on the first day of life due to sub-febrile fever. During NICU care antibiotic treatment was not given since acute phase reactants and blood culture were negative and fever did not persist.

The patient was discharged on the 5th day. After discharge the mother noticed that her baby had cyanosis on her left leg when she cried. On 13th day of life she was admitted to our hospital due to increase in cyanosis during crying and limitation of left hip movement. Physical examination revealed nothing except, slight limitation of left hip movement and slight swelling of thigh. The left thigh circumference was measured as 18 cm while the right thigh circumference was 17 cm. Pulse oxymeter showed no difference in oxygen saturation between right and left leg when the baby was peaceful. However left legs oxygen saturation decreased to 60% while baby was crying and cyanosis occurred on the left leg while the right leg saturation was still 100%. Cyanosis disappeared and left leg saturation increased when the baby calmed down. Treatment of vancomycin and amikacin were given considering septic arthritis.

Hemogram resulted as follows; hemoglobin 12.4 g / dl, white blood cell count 17,700 / mm^3, immature total ratio 0.31, platelet 688,000 /

✉ Mustafa Şenol Akın
mustafasenolakin@gmail.com

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mm^3, c-reactive protein 2,5 mg / dl, complete urinalysis was normal and blood culture was negative. Echocardiography was normal.

Doppler ultrasonography (US) showed normal arterial and venous blood flow of the lower extremity on both sides, but it was noticed that femoral artery and vein blood flow was interrupted temporarily on the left side when the baby cried. There was no thrombus or occlusion in the vascular structures. Superficial ultrasound revealed cutaneous and subcutaneous edema findings and a few small reactive lymph nodes on the left inguinal and femoral region suggesting infectious or inflammatory pathologies. Left hip joint was normal on US. Abdominal US showed asymmetrical thickness of left psoas muscle. For detail evaluation magnetic resonance imaging (MRI) was done without contrast. Abdominal MRI revealed multifocal abscess in iliopsoas muscle and soft tissue inflammation in the inguinal region (Fig. 1ab). We think that when intraabdominal pressure increased with crying, the left iliopsoas abscess compressed the iliac vasculature with mass effect and disturbed the blood flow and as a result, her left leg became cyanotic. The abscess was aspirated with a 19-gauge needle under US guidance by the interventional radiology department. Methicillin-resistant staphylococcus aureus was determined in the pus sent from the abscess. After the abscess was drainage, the patient's symptoms disappeared within the same day. The patient was discharged, after receiving vancomycin amikacin therapy for 4 weeks. Permission was obtained from the parents for publication of this case and informed consent was obtained from the family.

Discussion

The abscess of iliopsoas is rarely seen in the newborn period. We found 24 cases in the literature. These cases are summarized in Table I. Hospital admission usually occurs after the 2nd week of life, so it is thought that abscess formation occurs in the postnatal period. Our case applied on the 13th day of life. Six out of 24 cases in the literature had a fever and most of the time there is no symptom of fever as in our case. Of the 24 cases in the literature, 15 were male, the male / female ratio was 1.6, and if we include our case, the rate is 1.5. The most common symptom is pain and swelling on the lower extremity. In addition, inguinal region lymph nodes are palpable on the relevant side. Our case is the first case which was presented with cyanosis of a single leg while crying.

In very few cases, white blood cell count increase was determined. As in our case, there has been

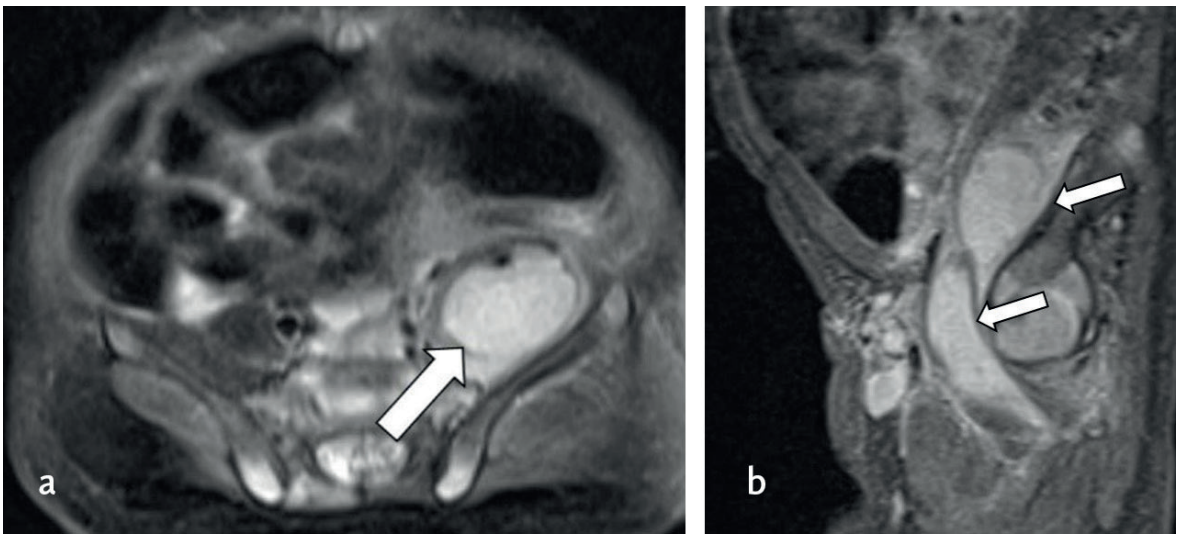

Fig. 1. Axial (a) and sagittal (b) T2-weighted images of MRI shows multiple abscess in iliopsoas muscle (arrows).

Table I. Twenty-four newborn psoas abscess cases in the literature.

Order	Author (publication date)	Gender	Age at presentation (day)	Fever	Swelling	Limitation of Movement/ pain	Location	Leukocyte count	CRP (mg/dl)	Treatment	Source of infection	Microorganism
1	Sedaghatian et al. (1978)[6]	female	15	+	-	-	right	10,000	absent	Ab and major operation	Unknown	SA
2	Zych et al. (1985)[6]	male	31	-	+	+	right	11,400	absent	Ab and major operation	Unknown	SA
3	Schut et al. (1988)[6]	female	21	-	+	-	right and left	26,000	absent	Ab and Major operation	Unknown	SA
4	Singer et al. (1993)[6]	female	15	-	+	+	left	31,400	absent	Ab and major operation	Unknown	SA
5	Edgar et al. (1993)[3]	male	21	-	+	-	right	8,700	absent	Ab and major operation	Catheter infection	SA
6	Edgar et al. (1993)[3]	male	17	-	+	+	right	18,600	absent	Ab therapy	Unknown	SA
7	Lucas et al. (1997)[6]	male	24	-	+	+	right	18,310	absent	Ab and major operation	Unknown	SA
8	Natsume et al. (1997)[6]	male	18	+	+	+	left	41,800	absent	USGPD, Ab therapy	Ureteral infection	MRSA
9	Andreou et al. (1997)[6]	female	18	-	-	-	right	-	absent	Ab and minor operation	Bacteriemia	KP
10	Prassopoulos et al. (1998)[6]	male	22	-	-	+	left	-	absent	Ab Therapy	Unknown	SA
11	Dib et al. (2000)[6]	female	21	-	+	+	left	22,000	4,3	USGPD, Ab therapy	Unknown	SA
12	Dib et al. (2000)[6]	female	14	-	+	+	left	20,100	2,3	USGPD, Ab therapy	Unknown	SA
13	Yano et al. (2004)[6]	female	11	-	+	+	right	20,900	5,01	Ab and minor operation	İntramusculer bleeding	SA
14	Okada et al. (2004)[6]	female	27	+	+	-	right	26,600	2,5	Ab and minor operation	Unknown	MRSA
15	Vastyan and MacKinnon (2006)[6]	male	28	-	+	+	right	34,200	absent	Ab and major operation	Unknown	SA
16	Okan et al. (2009)[6]	male	26	-	+	+	left	33,000	22	Ab and major operation	Pustular lesion	SA
17	Atsushi Horiuchi (2012)[6]	male	22	-	+	+	right	36,700	26,4	Ab and minor operation	Septic arthritis of pelvis	SP
18	Rakesh Mondal and Sumantra Sarkar (2012)[7]	male	26	+	-	+	not known	23,000	25	USGPD and 4 week ab. therapy	Unknown	MRSA
19	Karabayir N et al. (2012)[8]	male	12	+	+	+	right	26,900	31,3	USGPD and ab. therapy	Unknown and immunodeficiency	Not determined/ SA?
20	Ramnik Patel (2013)[3]	female	13	-	+	+	left	24,050	10,7	Open surgery and Ab. (2 weeks)	Unknown	Not determined
21	Minakshi Sham (2014)[4]	male	6	+	+	+	right	21,000	absent	Open surgery and Ab therapy but lost at post op 2nd day	Unknown	SA
22	Minakshi Sham (2014)[4]	male	21	-	+	+	right	12,000	absent	Open surgery and Ab. therapy	Unknown	SA
23	Al-Zaiem MM et al. (2014)[9]	Male	28	-	+	+	right	34,100	3,2	USGPD and Ab. Therapy then open surgery	Unknown	MRSA
24	Young-Mi Han (2015)[10]	male	20	-	+	-	left	21,330	3,34	Open surgery and Ab. therapy	Unknown	SA
25	Our case	female	13	-	+	+	left	17,700	2,5	USGPD and Ab. therapy	Unknown	MRSA

Ab: Antibiotic, USGPD: Ultrasound-guided percutaneous drainage, SA: *Staphylococcus aureus* MRSA: Methicillin-resistant *staphylococcus aureus*, KP: *Klebsiella Pneumoniae*, SP: *Streptococcus pneumoniae*

a slight increase in the acute phase reactors and an increase in white blood cell count has not been detected in most of the cases. Although US and computerized tomography (CT) have been used for diagnosis in most of the cases; MRI was used in our case after US evaluation. Compared to CT, advantages of MRI are no radiation exposure and high soft tissue resolution.

The first step of the treatment of abscess is drainage of the abscess. Usually antibiotic therapy alone is not sufficient for treatment. However, two cases in the literature were treated with only antibiotic therapy and their abscesses retracted with only antibiotic therapy.[1,2] In the literature, as in our case, US guided needle drainage was used at six cases and no additional open surgery was needed. There have been other treatment options ranging from a small cut made in the inguinal region to large open surgery. It should be debated which treatment method is better. However, it is suggested that the least invasive method should be tried first during the neonatal period and if it fails, more invasive methods should be performed.

Microorganism could be produced from blood cultures in a few cases while it is mostly produced from pus culture. Staphylococcus aureus was the most frequently isolated microorganism from iliopsoas abscess. The microorganisms were *Staph. Aureus* in 16 cases, methicillin-resistant Staphylococcus aureus (MRSA) in 5 cases with our case, *Klebsialla pneumonia* in one case, *Staph. Hominis* in one case and *Streptococcus pneumonia* in one case. In only one of these cases was no agent isolated. As in our case, the presence of elevated CRP, pain during hip movement and swelling of the thigh were the most common symptoms leading doctors to decide to start antibiotics. Septic arthritis was the most common preliminary diagnosis. It is recommended that antibiotic therapy should be given for 4 to 6 weeks, depending on the patient's clinic and doctor's assessment.

The etiological factors leading to abscess formation in the iliopsoas in neonates have not been identified. Birth trauma and possible intramuscular hemorrhage are accused reasons for abscess formation. Due to the weak immune system of newborns, a possible microorganism in the skin flora or gastrointestinal flora can reach the damaged region by hematologic route and may form an abscess there. Intramuscular injections for prophylactic treatments may cause the spread of skin or hospital flora. In our case, there may be healthcare-associated infection. MRSA detection and hospitalization history support this.

In the literature, one case was lost due to sepsis two days after open drainage of iliopsoas abscess but the prognosis of other patients with early diagnosis and abscess drainage is quite good.[3] None of the cases in the literature report sequelae or re-abscess formation occurring in the same region.

Transient local cyanosis is not a common finding in newborns. We found two cases with local cyanosis in the literature. One case's symptom was left leg cyanosis due to large bladder compression as a complication of circumcision.[4] The other case presented with upper left limb cyanosis and was diagnosed with raynaud phenomenon.[5] However in both cases cyanosis were not transient.

Our patient is the only case in the literature that presented with transient left leg cyanosis during crying and was discharged completely healthy after 4 weeks of amikacin and vancomycin treatment.

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Children with extreme hyperferritinemia are at risk of receiving more chemotherapy than necessary

Seung Beom Han, Soo-Young Lee

Department of Pediatrics, Bucheon St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea

Dear Editor,

We read with interest the article by Çakan et al.[1] and wish to add our own experience. The authors reported a 9-year-old boy with macrophage activation syndrome (MAS) secondary to systemic juvenile idiopathic arthritis (sJIA). We agree with their opinion that genetic testing for familial hemophagocytic lymphohistiocytosis (HLH) should be performed when a patient with MAS exhibits sustained hyperferritinemia. However, there is another important lesson when the patient exhibits extreme hyperferritinemia.

A 16-month-old girl was referred from a general hospital to our hospital with a 10-day fever. On physical examination, there were no abnormalities except for palpable 3cm liver and 5-cm spleen below the costal margin. Blood tests showed increased acute phase reactants and elevated liver enzymes. Considering the possibility of serious bacterial infection and sepsis, empiric antibiotics (cefotaxime 200 mg/kg/day) and intravenous immunoglobulin (400 mg/kg for 5 days) were administered.[2] However, her clinical and laboratory findings became aggravated and met the HLH diagnostic criteria (Table I).[3] She was transferred to pediatric hematology team and received 40 weeks of chemotherapy including etoposide (150 mg/m^2; i.e., the HLH-2004 protocol). She did not show any relapse of the disease during therapy and entered remission when she was 28 months old. Two months after terminating therapy, she visited the hospital again with fever, rash, and arthritis. Only then, was she eventually diagnosed with sJIA with the presenting manifestation of MAS. She now receives maintenance therapy for sJIA and has experienced no further relapse.

✉ Soo-Young Lee
sylee@catholic.ac.kr

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Table I. Laboratory findings of the patient.

Parameters	1st day	8th day	36th day	1 year later
Leukocytes, /mm^3	15,700	3,800	5,000	5,700
Hemoglobin, g/dl	11.6	6.4	11.2	13.6
Platelet, /mm^3	188,000	51,000	432,000	272,000
CRP, mg/L	184	56	0.4	0.2
ESR, mm/hour	56	28	12	4
AST, IU/L	199	1,006	22	25
ALT, IU/L	109	397	17	24
Ferritin, ng/ml	3,730	97,100	1,500	43
Triglyceride, mg/dl	-	288	107	87
Fibrinogen, mg/dl	-	139	197	280
LDH, U/L	-	2,131	823	392

ALT: alanine aminotransferase, AST: aspartate aminotransferase, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, LDH; lactate dehydrogenase.

Reviewing her medical records in detail, we noted that 40 weeks of chemotherapy including etoposide might not have been necessary for disease management. Children with MAS complicating sJIA can have good outcomes with short-term immunomodulators (i.e., the 8-week steroids and cyclosporine).[4] However, when children with unexplained MAS exhibit extreme hyperferritinemia (ferritin: 100,000 ng/ml),[1,5] they are at risk of receiving long-term potentially toxic chemotherapy because their overwhelming clinical manifestations usually meet the HLH diagnostic criteria.[6] MAS is a serious, life-threatening complication of childhood systemic inflammatory disorders.[1,3] Therefore, prompt initiation of adequate treatment is essential for the survival of affected children.[4-6] At the same time, careful monitoring of therapeutic response is also necessary to avoid overtreatment of MAS.

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Types of Articles:

Original Article: Original articles should report scientific findings within the pediatric field of interest. They must include a unstructured abstract of less than 300 words, to appear after the title page. The manuscript should be structured with the following headings: introduction, material and methods and Results. The introduction should put the study in context with the current literature and reflect the purpose of the study. The material and method should include the study methodology, the setting for the study (although affiliation must be covered), the subjects (number and type), the treatment or intervention, principal outcomes measured, and the type of statistical analysis. The Results section should include the outcome of the study and statistical significance, if appropriate. The Conclusion(s) states the significance of the results and limitations of the study.

Case Reports: Case reports should contain accounts of rare syndromes, new diagnostic tools and methods, new kinds of treatment and laboratory research with foreseeable practical application. Case Reports should consist of an unstructured abstract that summarizes the case(s), a brief introduction (recommended length, 1-2 paragraphs), a section that details patient presentation, initial diagnosis and outcome, as well as a discussion that includes a brief review of the relevant literature and describes how this case brings new understanding to the disease process.

Review Articles: Review articles should contain an objective account of different points of view and an internationally representative bibliography. Authors considering submission of review articles of medical progress are encouraged to consult with the Editor before submission.

Structured manuscript for systematic reviews are recommended. Headings should include: Unstructured abstract, Introduction, Data Sources, Study Selection, Data Extraction, Results, Limitations, and Conclusions. The general instructions regarding submission (including cover letter, title page requirements, journal style guidance, and conflict of interest statements) also apply to Review Articles.

Short communication: Short communications are opinion pieces consisting of a main point and supporting discussion. They may address general issues or controversies in the field of pediatrics. No abstract is needed but the general submission instructions (including cover letter, title page, journal style guidance, and conflict of interest statements) also apply to short communications.

Clinicopathological exercises: Authors can submit for consideration an clinicopathological exercises that is interesting, instructive, and visually attractive, along with a few lines of explanatory text and references No abstract, discussion or conclusion are required but the general submission instructions (including cover letter, title page, journal style guidance, and conflict of interest statements) also apply to clinicopathological exercises.

Letters to the Editor: Letters to the Editor should pertain to papers published in The Turkish Journal of Pediatrics within the past year. Provide a unique title for the Letter on the title page with complete contact information for the author(s). Double-space the text of the Letter. References, including reference to the pertinent article(s) in The Journal, should conform to style for manuscripts. No abstract is needed for a letter to editor. Letters to the Editor may be sent to the authors of the original article to which the Letter was written for review and/or Reply. If the Editors choose to publish the Reply, it will be published in the same issue as the Letter to the Editor.

Summary / Abstract and key words: An informative summary not exceeding 300 words must accompany each manuscript, Unexplained abbreviations and references should not be given. The abstract should state the objective (the purpose of the study and hypothesis) and summarize the rationale for the study. Following the material and methods it is critically important that all significant findings should be presented in the abstract. The abstracts should be unstructured.

The authors should list three to five key words or phrases taken from Index Medicus

Medical Subject Headings (http://www.nlm.nih.gov/mesh/MBrowser.html).

Abbreviations: Abbreviations must conform to accepted standards. Laboratory slang, clinical jargon and colloquialisms must be avoided. The full term for an abbreviation should precede its first use in the text. Generally, abbreviations should be limited to those defined in the AMA Manual of Style, current edition

Informed Consent: The Materials and Methods section should include a statement that the research was reviewed and approved by an institutional review board, and that participation involved informed consent. Please give information concerning where and when the study was approved, with the report number. The ethics committee report should also be uploaded to the online system.

Written consent from the family is required before a case report can be published. On submission, authors must attest that they have written consent from the family. Instances where there are extenuating circumstances in which family consent may be problematic will be handled on a case-by-case basis. A sentence stating that informed consent was received from the family should be added to the case section of the main document.

Conflicts of Interest: A conflict of interest is defined as any financial, personal, academically competitive or intellectual relationship between the author, referee or editor that involves article submission, referee evaluation, editorial decision and author-referee-editor correspondence and that could lead to a prejudiced and unfair action during the evaluation stage.

The authors must declare any financial relationship or conflict of interest areas that need to be known regarding the study. All financial contributions and sponsorships for the study and all financial relationships and conflict of interest areas should be included in the copyright transfer form that accompanies the article. The authors must explain the study sponsor's role in collecting, evaluating and analyzing the data, writing the article and submitting it for publication. Declaration of conflict of interest should be given at the end of the manuscript. If there is no conflict of interest, this section must include a "The authors declare no conflict of interest" statement at the end of the manuscript. All sources of funding should be mentioned in the manuscript.

The evaluation process for the article is not started if a conflict of interest statement is not present at the time the article is sent. If a conflict of interest becomes apparent after the article is published, the journal may make an official correction or withdraw the entire article.

The referees also need to declare all their interests that they feel may represent a potential conflict of interest. The referees must declare to the editor any conflicts of interest that may prejudice their evaluation of the article and must avoid such evaluation of certain articles if a potential prejudice is present.

The editors who will make the final decision on the articles cannot have any personal, professional or financial relationship with any of the authors of the articles they will decide on. The editors will also try to avoid any referees who have a possible conflict of interest, for example those working in the same department or facility as any of the authors.

Tables: Tables should be numbered with Roman numerals according to the order of mention in the text, each table must be typed double-spaced. A title for each table must be given. The first letter of each word should be capitalized. Tables should be self-explanatory and supplement, not duplicate the text. If a table or any data therein have been previously published, â footnote must give full credit to the original source. Tables should be added as a separate file.

Figures: Figures and illustrations should be of direct interest to the subject of the paper and numbered with Arabic numerals according to order of reference in the text. Art should be created / scanned and saved as either TIFF or JPEG format, submitted as a separate file, and not embedded in the text file. All lettering must be professional and in proportion to the drawing, graph, or photograph. A list of legends for the figures and illustrations must also be added as a separate file.

References: References to the literature should be limited to those cited by the author, arranged and numbered according to order they appear in the text; do not alphabetize. In text, tables, and legends, identify references with superscript Arabic numerals at the end of the sentence (after the full stop). List all authors up to 6; if more than 6, list the first 3 followed by "et al."

If reference to an author is made in the text the reference must be given after the name of the author rather than at the end of a sentence.

Example: In a study by Bier DM et al.23 the prevelance of…….

Names of journals are to be abbreviated in accordance with the style of Index Medicus.

References to journal articles:

Example: Kardelen F, Çeliker A, Özer S, et al. Sinus node dysfunction in children and adolescents: treatment by implantation of a permanent pacemaker in 26 patients. Turk J Pediatr 2002; 44: 312-316.

References to books:

Example: 2. Praat RTC, The Genetics of Neurological Disorders. London: Oxford University Press, 1967: 173-174.

References to chapters in books:

Example: 3, Kissane M. Development of the kidney and congenital malformations. In: heptinstall RH (ed). Pathology of the Kidney (2nd ed) Vol. 1. Boston: Little, Brown and Co, 1974: 69-109.

Acknowledgement: An "acknowledgement(s)" section may be added following these sections to thank those who helped the study or preparation of the article, if any. The acknowledgements are placed at the end of the article, before the references. This section contains statements of gratitude for personal, technical or material help, etc. If a grant has been received for the study this should also be given in the acknowledgments.

Submission of article: All manuscripts must be submitted on-line through the web site http://www.turkishjournalpediatrics.org. The corresponding author should upload the "Turkish Journal of Pediatrics Author Responsibility and Copyright Transfer Form" with the main manuscript. The copyright transfer form can be downloaded during the online submission process.

Announcements: Announcements of scheduled meetings, symposia, or postgraduate courses may be sent to the publisher for consideration at least 5 months in advance of the meeting date.

Footnote: If the study has been presented at a scientific meeting or is part of a thesis this can be added to the end of the manuscript as a footnote.

No manuscript will be considered for review until all authors have completed an uploaded the copyright form.

Checklist

- Please check your article as described below before sending it to the Editor.
- Covering letter
- Turkish Journal of Pediatrics Author Responsibility and Copyright Transfer Form.
- Title page
  - Title of article
  - Short running title
  - Name(s), academic degrees, and affiliations of author(s)
  - Name, address, and business and home telephone numbers of corresponding author
  - Word count and number of tables and figures
- Article
  - Original articles: Summary, Introduction, Material and Methods, Results, Discussion,
  - Case Reports: Summary, Introduction, Case Report, Discussion,
  - Key words
- Manuscript should be uploaded in a blinded manner; any affiliation must be covered.
- Declaration of informed consent in the material methods section
- Declaration of conflict of interest at the end of the manuscript.
- References on a separate page
- Tables should be uploaded separately
- Figures should be uploaded separately

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