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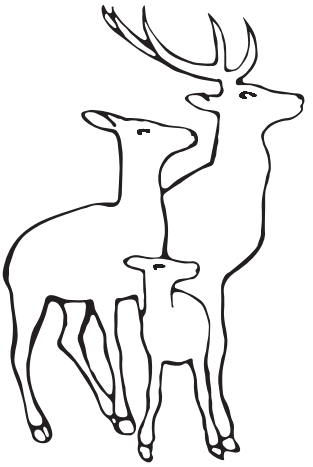

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Review

Zinc deficiency and febrile seizure: a systematic review and meta-analysis

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ABSTRACT

Background and objectives. Zinc has been reported to be low in children with febrile seizure compared to febrile cases without seizures, but results are inconsistent. A meta-analysis was performed to systematically evaluate the serum level of zinc in febrile children aged between 6-72 months with or without seizures.

Material and methods. A systematic search of databases was performed from January 2000 to January 2019. Studies comparing the serum level of zinc in febrile children with or without seizure were selected.

Results. The major outcome was serum level of zinc. Random effect model was used to calculate pooled standardized mean differences (SMD) with 95% confidence intervals (CIs). A total of 31 articles were included. Meta-analysis suggested that the serum level of zinc is lower in patients with febrile seizure versus febrile cases without seizure (SMD: -1.2, 95%CI= (-1.47, -0.93). In subgroup and sensitivity analysis no significant change was observed in pooled SMD. In meta-regression analysis sample size as a continuous variable had a significant influence on between-study variance (p= 0.02). According to cumulative analysis the difference of serum level of zinc in febrile children with or without seizure decreased with time.

Conclusion. This meta-result indicated a significant association of zinc deficiency with seizure in febrile children. It is suspected that decreased level of zinc may be involved in seizure occurrences and it may play a role in the pathogenesis of febrile seizure.

Key words: febrile seizure, children, zinc levels.

Seizure is one of the most common life-threatening events in childhood.[1] Febrile seizure (FS) has been defined as seizure in correlation with a febrile disease without the infection of the central nervous system (CNS) or acute imbalance of electrolytes in absence of previous afebrile seizures in children aged more than 1 month. The prevalence of FS is 3-5% worldwide.[2,3] FS could be simple or complex which is differed in frequency and duration.[4] Seizure is a result of abnormal and extreme discharge of cortical neurons in the CNS. Neuronal synapses are responsible for the composition of excitatory or inhibitory signals in the post-synaptic space as well as chipping in the basal making of neuronal networks in the brain.[5] Trace elements have several influences in the CNS. They are involved in Ion channels, synaptogenesis, membrane lipid peroxidation, etc. Over the past years, it was considered that trace elements may play a role in the pathogenesis of febrile seizure. It has been suggested that zinc as an essential trace element plays a role in the pathogenesis of seizures.[6] There are many studies comparing the serum level of zinc in febrile seizure. In some studies levels of zinc in febrile seizure have been reported to be lower when compared with febrile cases without seizures[7-9] but in some studies no significant difference was reported.[10-12]

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To the best of our knowledge there is only one systematic review and one meta-analysis evaluating the serum or CSF level of zinc in patients with FS.[2,13] They reviewed published papers until 2013 and had different inclusion criteria compared with the present study. In one systematic review[2] the control group included were febrile cases, afebrile seizure and healthy children. On the other hand, there are more than 15 published papers comparing the serum level of zinc in febrile children with or without seizures between the years 2013 and 2018 with different results.[7,8,14-22] Therefore, the present updated review was carried out to systematically evaluate relevant papers comparing the serum level of zinc in febrile patients with or without seizures until January 2019.

Material and Methods

Research strategy and study selection

Published papers were searched by 2 independent researchers (EB and FH) in relevant databases including Pubmed, Scopus, Cochrane library and web of science from January 1th, 2000 up to January 31th, 2019. Also e-publications ahead of print were searched in Pubmed. Disagreement between the two independent researchers was verified by the third researcher (AA). The reference lists of all verified studies were also checked to detect additional relevant articles. Searched key words were ["febrile seizure" OR "febrile convulsion"] AND [“level” OR “concentration” OR “profile”] AND [“zinc”] AND [“serum” OR “plasma”] and ["deficiency"]. The present systematic review was conducted according to the standard protocol for meta-analyses of observational studies. Inclusion criteria were original case-control, nested case-control and cross-sectional papers studying febrile children aged 6-72 month with or without seizures without any neurological disorders. Full-text of English articles and English abstract of non-English articles were included. Exclusion criteria were animal or laboratory studies, review studies, randomized clinical trials (RCT), non-controlled studies, healthy controls or control groups with epilepsy, afebrile seizure or any neurological complications or having received any pharmacological treatment. Articles which did not provide necessary data including patient's age, number of patients in each group and mean±SD of zinc level were excluded.

The following information was extracted from each article included: first author, year of publication, country of study, number of subjects in case and control groups, mean±SD of zinc level in both groups, significant difference between groups and method of zinc measurement. Studies were scored according to NOS (Newcastle-Ottawa Quality Assessment Scale Case-Control Studies).[23] Articles with at least 5 stars were considered as good quality and included in the meta-analysis. The present research was reviewed and approved by Mashhad University of Medical Sciences review board (code: 970646, ethic code: IR.MUMS.MEDICAL.REC.1398). Because the present study was a systematic review and did not relate to patients directly, it was not necessary to provide inform consent form.

Quantitative data synthesis and data analysis

The data were extracted and meta-analysis was used to pool them. Results were expressed as standardized mean differences (SMD) with 95% confidence intervals (95%CI). If a study only reported the median, range and/or inter-quartile range (IQR); mean and standard deviation (SD) were estimated, as described by Wan et al.[24] Heterogeneity was checked using I^2 statistics. Heterogeneity was considered significant if I^2 was more than 50%. In significant heterogeneity, data were analyzed using random effect modelling.[25] Sensitivity analysis was applied to the evaluation of the results constancy. In order to discover the probable sources of heterogeneity, subgroup analysis, based on study sample size, method of zinc measurement and match of demographic characteristics was performed. Meta-regression was also performed to further explore which variables contribute to

substantial heterogeneity. Cumulative analysis was also performed to detect the time-based changes in the magnitude of research findings.

Publication bias was also verified by visually check of Begg's funnel plot symmetry and Egger's regression test.[26] There is significant publication bias if P< 0.05. The data were analyzed using comprehensive Meta-analysis software version 2.2.064 (Biostat, Englewood,NJ, USA).

Results

Search and study selection

The primary literature search retrieved 558 articles. Some articles were removed because they were not original studies; some were excluded because of duplication. Some articles were excluded, because they were books, book section, review paper or animal studies. Some studies were excluded because they had no control group or they had a control group with different inclusion criteria. Finally, 31 articles were included in the present review.[7-12,14-22,27-42] All were case-control or cross sectional. The diagram of the study selection process is presented in Figure 1. Details of studies included are presented in Table I.

Serum level of zinc

Serum level of zinc was reported in 31 studies. Totally 3642 febrile children with or without seizures were included as FS and febrile group without seizure. In the FS group there were 1803 patients and in the febrile group without seizure there were 1839 patients. A significant lower serum level of zinc in febrile seizure versus febrile children without seizure was reported in 27 studies.[7-9,14-22,28-42] In 4 studies no significant difference between groups was reported.[10-12,27] Totally 31 studies were included in the meta-analysis.[7-12,14-22,27-42] Meta-analysis using random effect modelling, suggested that patients with FS had a significantly lower serum level of zinc in comparison with febrile patients without seizure (SMD: -1.2, 95% CI: (-1.47, -0.93), P value<0.001, I^2: 92.62%). Forest plot is shown in Figure 2.

Heterogeneity analysis

To assess the probable sources of heterogeneity in the results, subgroup, meta-regression and sensitivity analysis were carry out. In the subgroup analysis, the SMD of serum levels of zinc did not differ substantially according to sample size, method of zinc measurement or matching of demographic characteristics (Table II). Higher serum levels of zinc were observed in the febrile seizure patients compared with febrile cases without seizure across all subgroups. To additionally explore the potential sources of heterogeneity within the results; meta-regression analysis was performed by considering all the potential factors including sample size, method of zinc measurement and matching of demographic characteristics. According to the meta-regression analysis, sample size (p= 0.02) was found to be a significant contributing factor for

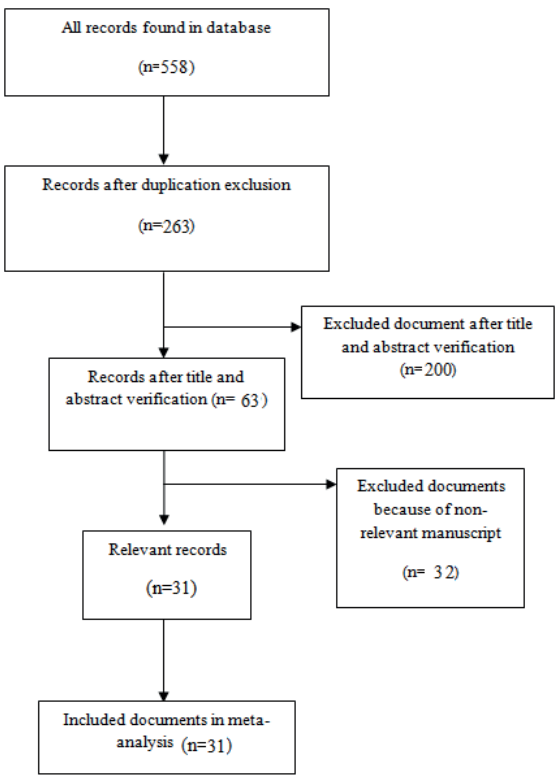

Fig. 1. Diagram of study selection.

Table I. Chracterstics of eligible studies included in pooled SMD analysis.

Author	Country	Age group (month)	Sample size (FS)	Sample size (febrile)	Serum level of zinc(FS)	Serum level of zinc (control)	Significant difference	Matched	Method of measurement	Study design
El-masry (2018)	Egypt	6-60	40	40	44.27 ± 26.5 (µg/dl)	69.9 ± 33.94 (µg/dl)	yes	yes	Other methods	Case-control
Hubaira (2018)	India	6-60	100	100	61.53 ± 15.87 (µg/dl)	71.90 ± 18.5 (µg/dl)	yes	yes	Colorimetric test kits	Case-control
Sampathkumar (2018)	India	6-72	100	200	59.17 ± 10.39 (µg/dl)	73.12 ± 15.15 (µg/dl)	yes	Not declared	Calorimetric method	Cross sectional
Maheshwari (2018)	Pakistan	6-36	60	60	62.82 ± 14.66 (µg/dl)	79.03 ± 24.17 (µg/dl)	yes	yes	Randox Elisa kit	Cross sectional
Pravin Soni (2017)	India	6-60	120	120	62.5 ± 3.43 (µg/dl)	72.14 ± 7.39 (µg/dl)	yes	Not declared	Colorimetric Test kits	Case-control
Nemichandra (2017)	India	6-60	82	82	8.93 ± 2.01 (µmol/l)	12.74 ± 3.47 (µmol/l)	yes	yes	Calorimetric method	Case-control
Khajeh (2016)	Iran	6-60	30	30	56.86 ± 52.78 (µg/dl)	96.14 ± 53.46 (µg/dl)	yes	yes	Spectrophotometry	Case-control
Shokrzadeh(2016)	Iran	6-72	92	93	0.43 ± 0.38 (mg/l)	0.66 ± 0.37 (mg/l)	yes	Not declared	Spectrophotometry	Case-control
Bonu (2016)	India	6-60	44	40	76.8 ± 24.4 (mg/l)	90.1 ± 14.6 (mg/l)	yes	yes	Spectrophotometry	Case-control
Namakin (2016)	Iran	6-60	48	48	80.5 ± 21.7	117.2 ± 35.5	yes	yes	Spectrophotometry	Case-control
Gatto (2015)	India	6-60	100	100	61.53 ± 15.87 (µg/dl)	71.90 ± 18.50 (µg/dl)	yes	Not declared	Colorimetric test kits	Case-control
Pannerselvam (2015)	India	6-60	50	50	68.4 ± 25 (µg/dl)	94.1 ± 36.8 (µg/dl)	yes	Not declared	Calorimetric method	Cross sectional
Sreenivasa (2015)	India	6-60	50	50	61.53 ± 16.87 (µg/dl)	50.49 ± 15.17 (µg/dl)	yes	Not declared	Colorimetric method	Cross sectional
Salah (2014)	Egypt	6-72	20	20	52.8 ± 24.4 (µg/dl)	56.1 ± 23.5 (µg/dl)	no	yes	Spectrophotometry	Cross sectional
S. Joshi (2014)	India	6-60	50	50	155.09 ± 17.57 (µg/dl)	132.04 ± 14.7 (µg/dl)	no	yes	Spectrophotometry	Case-control

mg: milligram, µg: microgram, dl: deciliter, l: liter, SMD: strandardized mean difference.

Table I. Continue.

Author	Country	Age group (month)	Sample size (FS)	Sample size (febrile)	Serum level of zinc(FS)	Serum level of zinc (control)	Significant difference	Matched	Method of measurement	Study design
Mohammad Aly (2014)	Egypt	6-36	40	40	53±29 (μg/dl)	95 ± 11 (μg/dl)	yes	yes	Colorimetric method	Case-control
Taherya (2013)	Iran	6-60	40	40	70 (μg/dl)	90 (μg/dl)	yes	Not declared	Spectrophotometry	Case-control
Salehiomran (2013)	Iran	6-72	50	50	0.704 ± 0.179 (mg/l)	0.585 ± 0.166 (mg/l)	yes	yes	Spectrophotometry	Case-control
Amouian (2013)	Iran	6-60	160	160	13.33 ± 2.66 (μg/dl)	13.69 ± 2.60 (μg/dl)	no	yes	Photometric method	Case-control
Iyshwarya (2013)	India	6-60	20	20	67.25 ± 4.97 (μg/dL)	50.49 ± 5.17 (μg/dl)	yes	Not declared	Spectrophotometry	Case-control
Kafadar (2012)	Turkey	6-72	45	23	107.12 ± 21.66 (μg/dl)	110.49 ± 35.03 (μg/dl)	no	Not declared	Spectrophotometry	Cross sectional
Okposio (2012)	Nigerian	6-60	90	90	90.3 ± 33.0 (μg/dl)	58.7 ± 25.4 (μg/dl)	yes	yes	Spectrophotometry	Case-control
Modarresi (2011)	Iran	9-60	30	30	130.54 ± 25.89 (μg/dl)	93.39 ± 73.88 (μg/dl)	yes	Not declared	Spectrophotometry	Cross sectional
Margaretha (2010)	indonesia	6-60	25	25	13.72 ± 0.45 (μmol/l)	8.83 ± 1.23 (μmol/l)	yes	Not declared	Not declared	Cross sectional
Heydarian (2010)	Iran	6-60	30	30	758.33 ± 80.29 (μg/L)	663.7 ± 107.6 (μg/L)	yes	Not declared	Spectrophotometry	Case-control
Amiri (2010)	Iran	6-60	30	30	107.87 ± 28.79 (μg/dl)	66.13 ± 18.97 (μg/dl)	yes	Not declared	Spectrophotometry	Case-control
Radhakrishnan Palliana (2010)	India	6-60	75	50	90.38 ± 6.88 (μg/dl)	81.84 ± 13.23 (μg/dl)	yes	Not declared	Spectrophotometry	Case-control
Ehsanipour (2009)	Iran	6-60	34	40	90.1 ± 14.6 (mg/l)	76.8 ± 24.4 (mg/l)	yes	yes	Spectrophotometry	Case-control
Talebian (2009)	Iran	6-72	60	60	116.280 ± 50.1 (mg/dl)	146 ± 56.6 (mg/dl)	yes	Not declared	Centerior kit	Case-control
Mullah (2008)	Bangladesh	5-60	50	30	749.33 ± 73.19 (μg/l)	464 ± 64.57 (μg/l)	yes	yes	Spectrophotometry	Cross sectional

mg: milligram, μg: microgram, dl: deciliter, l: liter, SMD: strandardized mean difference.

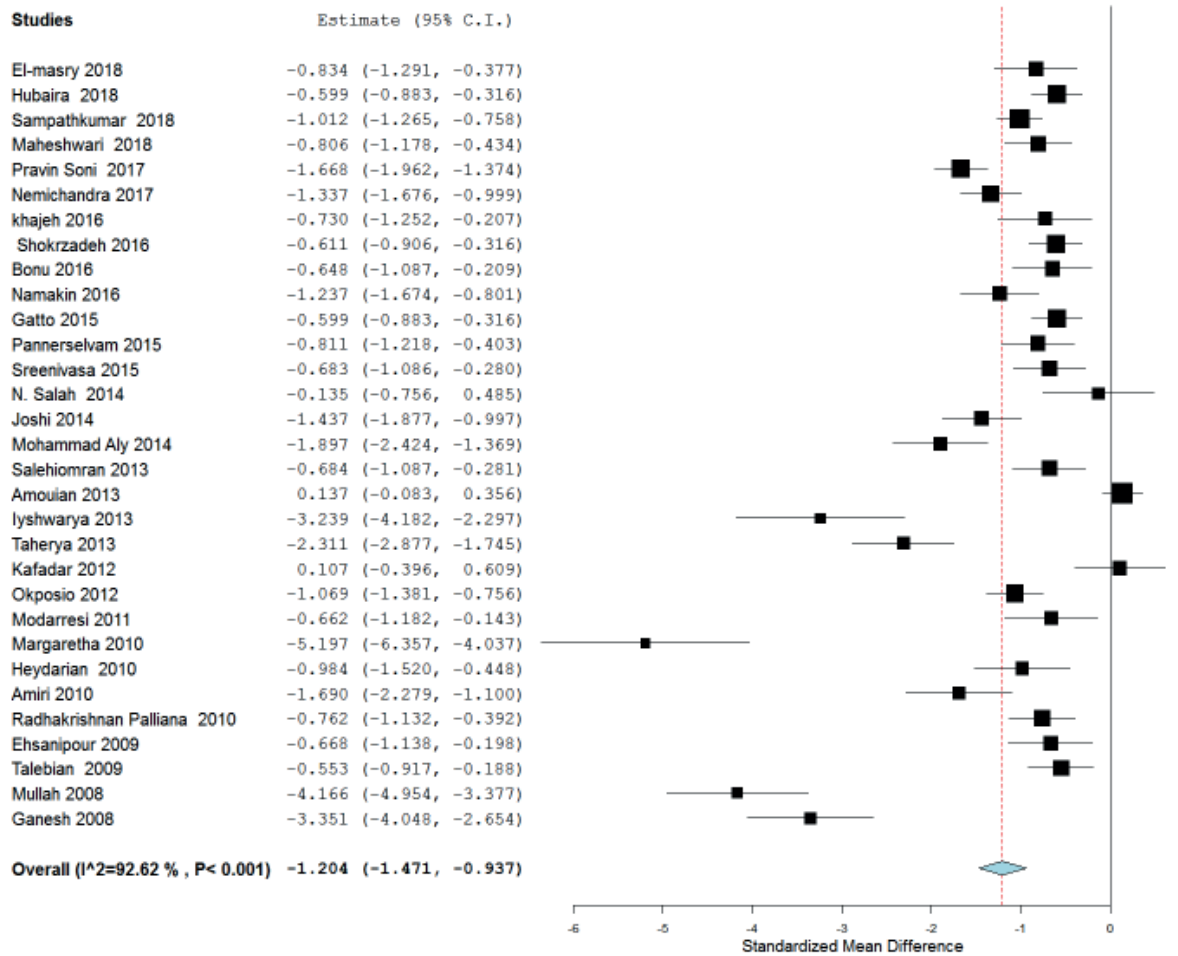

Fig. 2. The serum level of zinc in febrile children with or without seizure in pooled SMD.

Table II. Subgroup analysis of articles included according to sample size, method of zinc measurement and matched demographic characteristics.

Sub groups	No. of studies	Difference between FS and febrile group without seizure		Test of heterogeneity		
		SMD (95% CI)	p value	Model	I^2(%)	P value
Sample size						
Less than 100	16	-1.66(-2.21,-1.1)	<0.001	R	93.67	<0.001
100 or more	15	-0.82 (-1.08, -0.57)	<0.001	R	89.53	<0.001
Method of zinc measurement						
Spectrophotometry	18	-1.29(-1.67,-0.9)	<0.001	R	91.58	<0.001
Other methods	13	-1.08(-1.46, -0.7)	<0.001	R	93.08	<0.001
Demographic chracteristics matching						
Matched	16	-1.17(-1.57, -0.76)	<0.001	R	93.75	<0.001
Not declared	15	-1.23(-1.59, -0.87)	<0.001	R	91.38	<0.001

FS: febrile seizure, SMD: strandardized mean difference, CI: confidence interval, R: random.

between study variance for SMD analysis but not method of zinc measurement (p= 0.82) and matching of demographic characteristics (p= 0.58).

In the sensitivity analysis the effect of each study on the overall pooled SMD was assessed. According to sensitivity analysis no significant change in the direction of pooled SMD was observed, indicating the stability and robustness of the results. Forest plot is shown in Figure 3.

Cumulative analysis

In cumulative analysis studies were added according to date of publication and the results were summarized as each new study was added. According to cumulative analysis the difference of serum level of zinc in febrile children with or without seizures decreased over time. Forest plot is shown in Figure 4.

Publication bias

Begg's funnel plot and Egger's regression test are usually used to determine publication bias of the included studies. Egger's test revealed significant asymmetry (p value=0.0006). Funnel plot is shown in Figure 5.

Discussion

Presently, clinical evidence support the relationship between zinc deficiency and

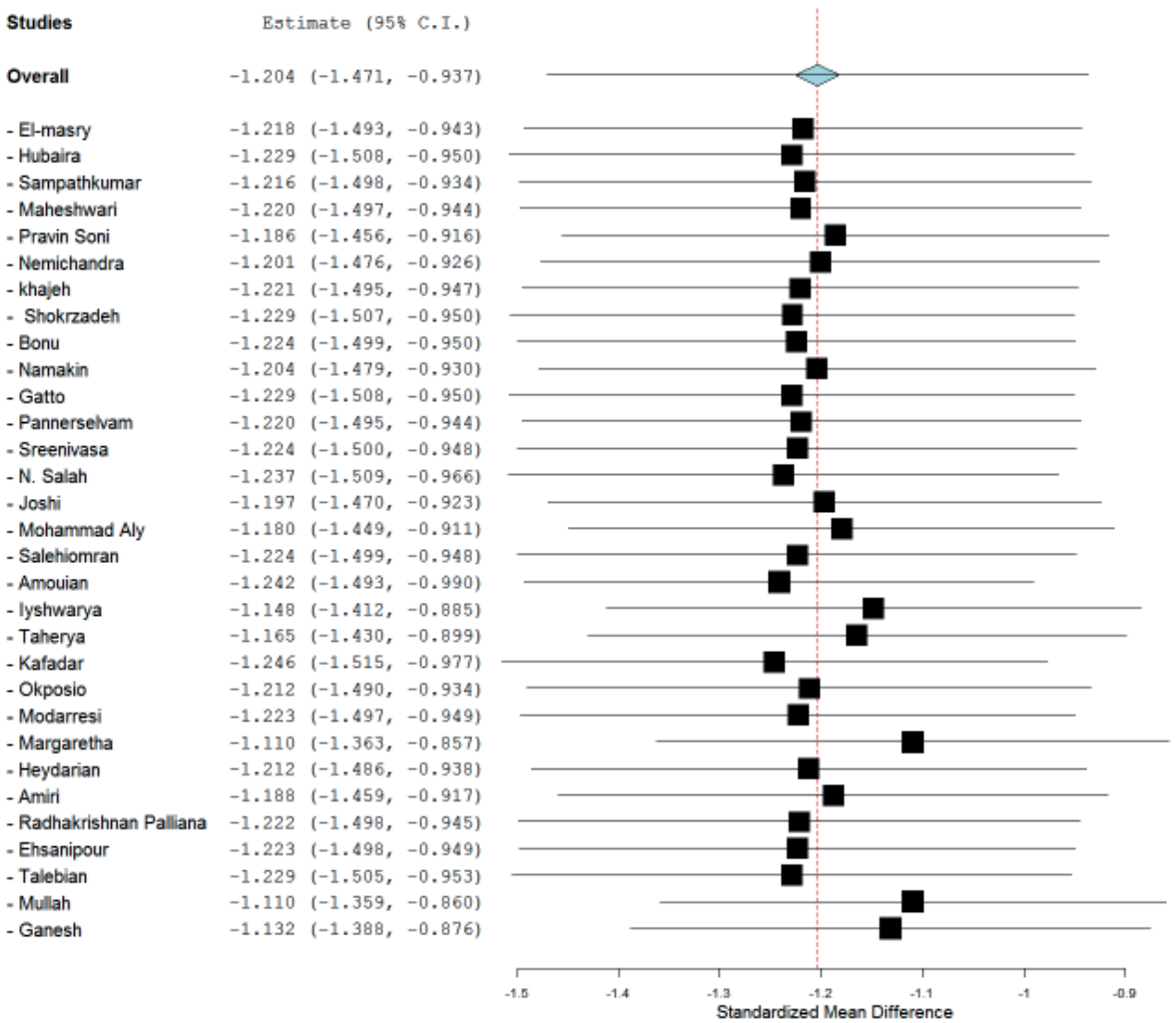

Fig. 3. Sensitivity analysis of included studies.

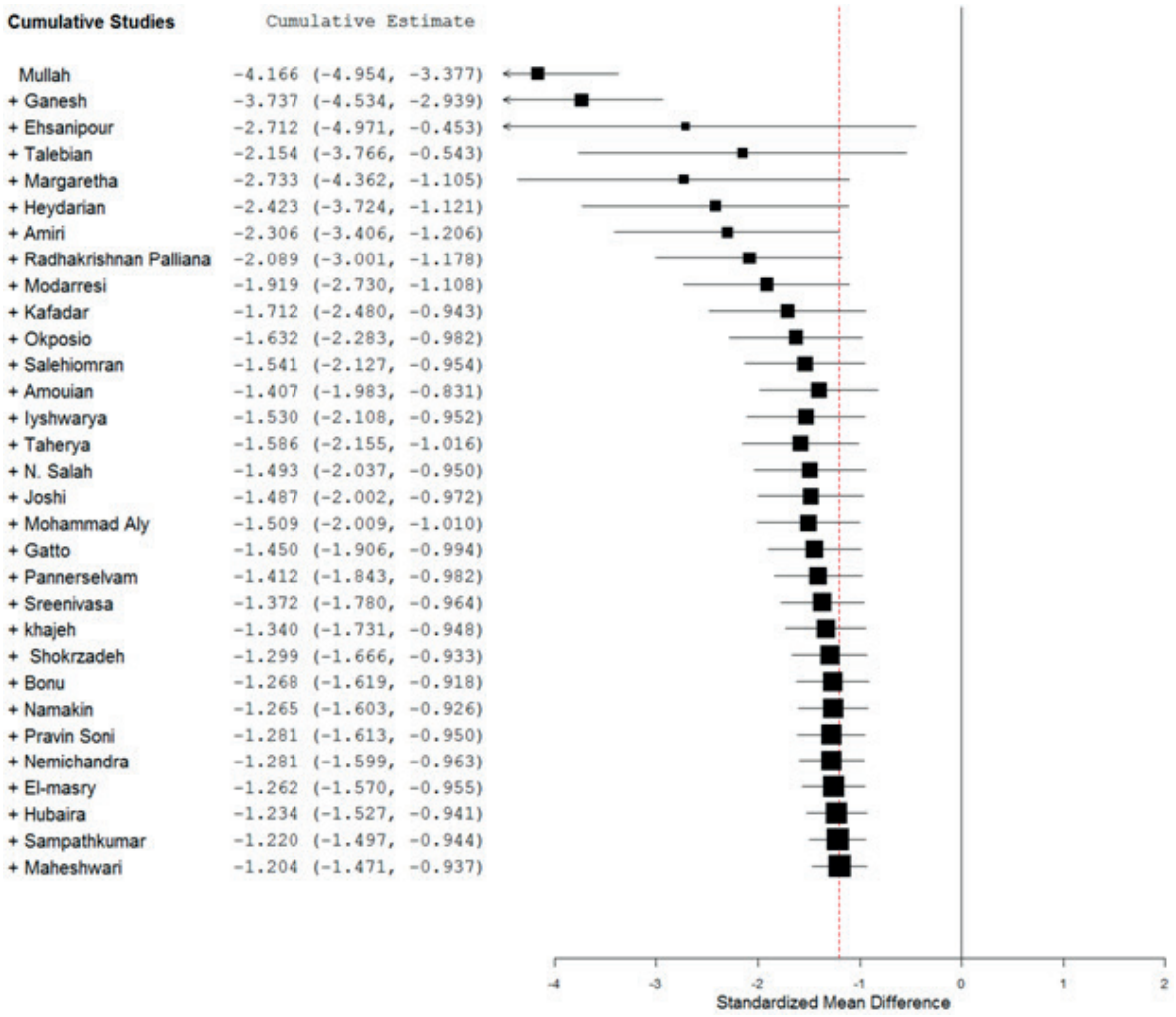

Fig. 4. Cumulative analysis of included studies.

seizure occurrence in febrile children. Therefore clinical data comparing the serum level of zinc in febrile children with or without seizures were systematically reviewed in the present study. According to a meta-analysis the serum level of zinc was significantly lower in FS children compared with febrile cases without seizure. In subgroup and sensitivity analysis no significant change was observed in pooled SMD. Sample size as continuous variable had a significant influence on between-study variance in the meta-regression analysis.

Daily intake of zinc is necessary because there is no storage system for zinc. Sources of zinc include whole grains, cheese, meat, shellfish and legumes.[43] Many people in developing countries suffer from zinc deficiency, including approximately 79% in south Asia.[44]

Zinc is one of the essential trace elements in the body, an especially high level is required in the human brain. It is involved in many biological activities, metabolism and differentiation of cells. It is also effective in normal functioning and development of brain cells.[45] Zinc has also been recognized as a cofactor in the synthesis and secretion of neurotransmitters.[46] Pyridoxal phosphate (PP) is an essential co-factor for the biosynthesis of neurotransmitters in brain neurons. Hippocampus with nearly 30 μg/g dry weight has the most amount of zinc in brain tissues. Zinc is essential for the synthesis of PP.[47] Imbalance of zinc leads to

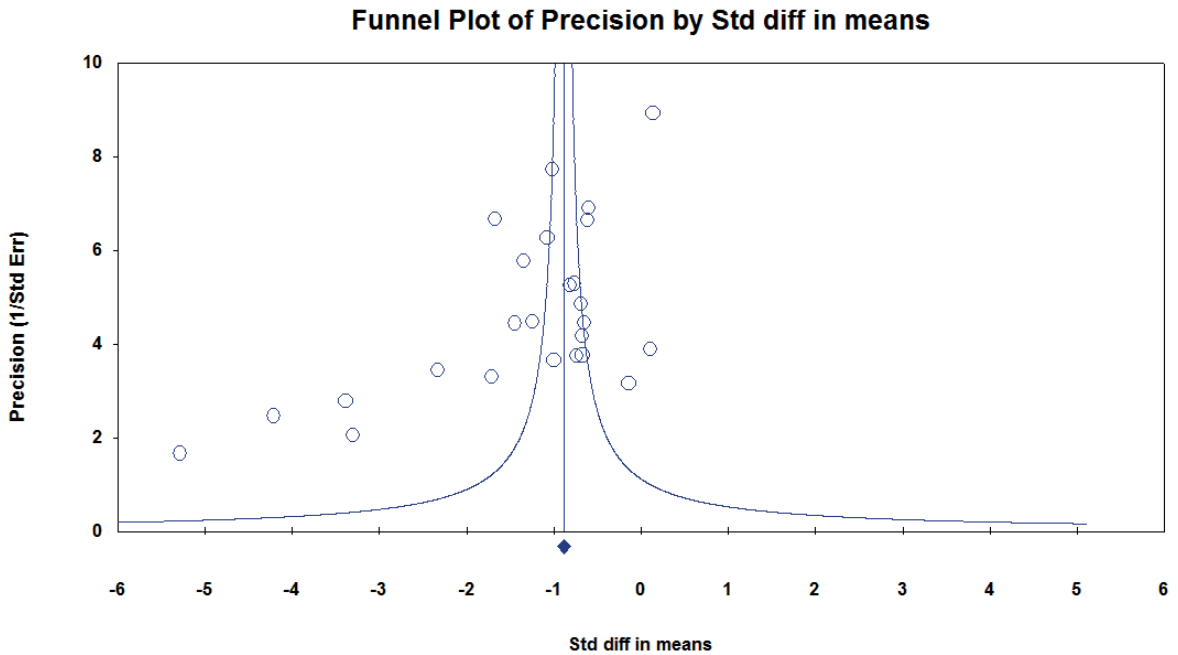

Fig. 5. The funnel plot of publication bias in pooled SMD analysis.

neurodegenerative disorders, oxidative stress, etc. Also the function of some proteins involved in seizures is affected by zinc imbalance. Some excitatory mechanisms can be suppressed by zinc. Zinc is a regulator of a rate-limiting enzyme, glutamic acid decarboxylase in the synthesis of gamma aminobutyric acid (GABA). Decrease of zinc leads to N-Mothy1-D-Asparate (NMDA) receptors activation, GABA inhibition and changes in calcium channel function. These changes could lead to seizure occurrence.[48-50] GABA brain level is reduced due to low serum level of zinc and as a result, threshold of seizure occurrence is reduced. Serum level of zinc can be affected by fever and acute phase response during infectious diseases. It is suggested that cytokines such as interleukin 6, interferon, tumor necrosis factor and interleukin 1 can reduce serum levels of zinc during infections.[51] So in cases with borderline serum level of zinc, infections may trigger hypozincemia.

As reviewed in the present study, the relationship between zinc and seizures was studied. To date, only one systematic review and one meta-analysis evaluating the serum or CSF levels of zinc in febrile seizures has been published, but the inclusion criteria were different.[2,13] Additionally, more than 15 papers have been published between 2013 and 2018 with different results. Therefore, we felt a new systematic review and meta-analysis was necessary.

Between studies heterogeneity was a common finding in the meta-analyses. In the present study, the meta-analysis showed significant heterogeneity in the pooled SMD analysis. Sensitivity analysis, subgroup analysis and meta-regression were used to discover the possible causes of between-study heterogeneity as well as heterogeneity decrement. The results of the present review were robust. According to sensitivity analysis any single study did not affect the estimated pooled SMD. Meta-regression confirmed that the sample size of the study had a significant effect on the pooled SMD and could be considered as a confounder but not the method of zinc measurement and group matching. Cumulative analysis showed a decreasing trend in the difference of serum levels of zinc in febrile children with or without seizures during the time. Despite dietary intake and supplementation reducing

micronutrient and nutrient deficiencies, remain a common problem in developing countries. Therefore, preventative zinc supplementation may outweigh any potentially adverse effects in areas where risk of zinc deficiency is high.

This meta-result indicated a significant association of zinc deficiency with seizures in febrile children. It is suspected that decreased level of zinc may be involved in seizure occurrence and it may play a role in the pathogenesis of febrile seizure.

The major limitations of the present study were: A) In spite of an attempt for a comprehensive search, it may be the case that some articles were missed. B) Despite subgroup analysis, significant heterogeneity was seen and could have influenced the results. C) Potential confounders were not verified in almost all of the studies so we were unable to analyze them. D) Publication bias may have affected the pooled results. E) Most included studies were case-controlled. It is well known that case-controlled studies have unavoidable limitations such as selection bias in case and control groups and many other confounders which may affect the pooled results.

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Original Article

Effect of long-term glucocorticoid therapy on bone mineral density of the patients with congenital adrenal hyperplasia

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ABSTRACT

Background and objectives. Congenital adrenal hyperplasia (CAH) is characterized by androgen excess which should be treated with life-long glucocorticoid therapy, thus can affect bone mineralization. We aimed to evaluate the bone mineral density (BMD) and determine the factors affecting bone mineralization in patients with CAH.

Method. This prospective case-control study was conducted in children, adolescents and young adults with classical 21-hydroxylase CAH, and age-, sex-, and pubertal stage matched healthy controls. $Lumbar_{1-4}$ BMD was determined by dual-energy X-ray absorptiometry. BMD z-score was calculated using national standards with respect to height age and was referred as "low BMD" if z-score < -1 SD. Univariate analyses were performed between low BMD and normal BMD groups, and multivariate logistic regression analysis was performed to assess the independent predictors of low BMD. Correlations of Body Mass Index (BMI)-z-score, average serum 17-hydroxyprogesterone level, duration of treatment, average and cumulative glucocorticoid doses with BMD z-score were evaluated with Spearman analyses.

Results. Each group included 37 cases. BMD z-score of patients with CAH [0.47 (-0.04 – 1.56)] was higher than control group [-0.43 (-0.82 –0.05)]; p= < 0.001. Number of patients with low BMD was similar in both groups; [CAH: 6(16.2%), control: 5(13.5%); p= 0.744]. BMI- z-score was higher in patients with CAH when compared to control group; p= < 0.001. BMI z-score was lower in low BMD group as comparison to normal BMD group; p= 0.041. Each 1.0 decrease in BMI z-score, risk of having low BMD was found to increase by 1.79 (%95 CI: 1.03-3.12, p= 0.040). BMI-z-score, average serum 17-hydroxyprogesterone level, duration of treatment, average and cumulative glucocorticoid doses were not found to be correlated with BMD z-score.

Conclusion. Long-term glucocorticoid therapy did not have negative effect on BMD of patients with CAH. Higher BMI z-score in patients with CAH may have a positive effect on preserving bone health. Precautions should be taken for increased risk of obesity.

Key words: congenital adrenal hyperplasia, bone mineral density, dual-energy X-ray absorptiometry scan, body mass index.

Congenital adrenal hyperplasia (CAH) is a disorder that is induced by deficiency of one of the enzymes needed for cortisol biosynthesis in adrenal cortex. 21-hydroxylase deficiency (21-OH) is the most common form, responsible for 90-95% of the cases. Treatment involves replacement of cortisol requirements; also, excessive androgen needs to be suppressed. The dose of glucocorticoid given to serve this purpose should be at a level to suppress excessive adrenal androgen production, without suppressing the hypothalamus-pituitary-adrenal axis entirely.[1]

Bone metabolism should be followed up in children with CAH as chronic glucocorticoid

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treatment may decrease bone mineralization by reducing skeletal growth factors, intestinal and renal calcium reabsorption, and increasing bone turnover. In addition, over suppression of androgens due to excessive treatment may also affect bone mineralization negatively.[2] Instead, insufficient treatment may result in elevation of androgens, thus, may have a protective effect against anticipated bone loss.[3]

Most of the studies on adult patients were shown to lead to decreased bone mineral density (BMD).[4,5] On the other hand, studies on children alone yielded conflicting results; some showed unchanged, others showed reduced or increased BMD using dual-energy X-ray absorptiometry (DEXA) in comparison to healthy children.[3,6-17] As those studies on children have variable results regarding the BMD in children with CAH, there is a need for further studies.

CAH is considered to be more common in Turkey than western countries due to a high frequency of consanguinity. A comprehensive review of patients with CAH in Turkey reported that 21-OH CAH accounts for 85.7% of the patients.[18] The aim of this study was to compare BMD in Turkish children, adolescents and young adults with classical 21-OH CAH to age, sex and pubertal stage matched healthy subjects, and determine the factors affecting the bone mineralization.

Material and Methods

This prospective case-control study was approved by Hacettepe University Ethics Committee (LUT 06/63 – 58) and funded by Hacettepe University Scientific Research Unit Grant (06-D07-101-005). Written informed consent was obtained from the parents of patients with CAH and the healthy controls for their participation in the study.

We included children older than three years, as well as adolescents and young adults with classical 21-OH CAH, who had been on glucocorticoid replacement for more than two years. Age, sex and pubertal stage matched healthy subjects composed the control group. None of the study subjects received calcium or vitamin D supplementation at the time of enrollment.

Diagnosis was made on clinical features (vomiting, dehydration, shock, failure to thrive, ambiguous genitalia in females, and hyperandrogenism in males) and elevated serum 17-hydroxypogesterone (17-OHP) > 300 nmol/L.[11] Patients were accepted as salt wasting 21-OH CAH if hyponatremia (serum sodium < 135 mmol/L), hyperkalemia (serum potassium > 5.5 mmol/L) and increased plasma renin activity was documented. They were considered as simple virilizing CAH if serum electrolytes and plasma renin activity were within normal limits at the time of initial diagnosis.

All patients with CAH and controls underwent thorough physical examination. We recorded chromosomal sex, pubertal stage, body weight to the nearest 0.1 kg, and height to the nearest 0.1 cm. The body mass index (BMI) was calculated using the formula; BMI=Weight(kg)/Height(m^2). Subjects were considered as overweight if a BMI percentile of 85-95 percentile with respect to national standards, and as obese if a BMI percentile greater than > 95 p with respect to national standards and BMI z-score was calculated.[19,20] Overweighed and obese subjects were analyzed together.

Average glucocorticoid dose was calculated as; sum of glucocorticoid replacement dose at each assessment divided by total number of assessments and presented as mg/m^2/day, hydrocortisone equivalents. Cumulative glucocorticoid dose was calculated as; average glucocorticoid dose x 365 x duration of treatment in years and presented as gr/m^2.[2,4] Glucocorticoid doses were converted to hydrocortisone equivalents as follows: 5 mg hydrocortisone=1 mg prednisolone. Average serum 17-OHP level (Radioimmune assay) was calculated as; sum of serum 17-OHP level at each assessment divided by total number of assessments and presented as mmol/L. Patients

were identified as poor control if mean serum 17-OHP > 60 nmol/L.[21]

The BMD of the patients and healthy subjects in the case and control groups were determined using DEXA, Hologic® QDR 4500A. The examination was performed in supine position BMD (gr/cm^2) and bone mineral content (BMC; gr) were determined from lumbar vertebrae (L_{1-4}). BMD z-score was calculated using national standards with respect to height age.[22] Included patients and healthy subjects were grouped according to BMD z-score, where BMD z-score higher than -1.0 was considered as "normal BMD", and those lower than -1.0 were considered as "low BMD".[23] Bone Mineral Apparent Density (BMAD; BMC/vertebral area$^{1.5}$; gr/cm^3) was calculated.[24]

Statistical analyses were performed using SPSS V15.0 for Windows (SPSS, Chicago, IL, USA). Categorical variables were presented as n (%), and were compared by Chi-square test, Fisher exact test or Yates correction, where appropriate. A p value of 0.05 was considered statistically significant. The distribution of numerical variables was investigated using visual and analytic methods and compared between patients and controls using Mann-Whitney U test. Results were presented as median (IQR; interquartile range). Univariate analyses were performed between patients and controls, and low BMD and normal BMD groups among both all subjects and only patients with CAH. Variables with p value < 0.10 in univariate analysis between low BMD and normal BMD groups were included in multivariate logistic regression analysis models (backward stepwise model) to assess the independent predictors of low BMD. Hosmer-Lemeshow goodness of fit statistics were used to assess the model fit. Spearman correlation analyses were performed between BMD z-score and BMI z-score, average serum 17-OHP level, average and cumulative glucocorticoid dose and duration of treatment in patients.

Results

A total number of 37 patients with classical 21-OH CAH (salt wasting; n= 26, simple virilizing; n= 11) were included in the study. Thirty-seven age, sex, and pubertal stage matched healthy subjects formed the control group. The age at the time of enrollment was 10.0 (7.5 – 15.5) years in patients with CAH and 10.5 (6.1 – 14.5) years in control group; p= 0.546. Each group included 15 (40.6%) male and 19 (51.4%) prepubertal subjects.

Median duration of follow-up in patients with CAH was 9.2 (IQR: 6.6 – 12.5) years. Twenty-two patients were diagnosed in the first year of life, of which 10 were diagnosed during the neonatal period. The median age of diagnosis of the remaining 15 patients diagnosed after the first year of life was 3.0 (IQR: 2.1 – 6.3) years. Patients were on steroid treatment for a median of 9.2 (6.4 – 12.5) years, and 11 out of 37 were on prednisolone treatment for 2 (1.0 - 3.6) years. With regards to gender assignment 20 patients with CAH (46,XX) were assigned female while, two patients with 46,XX were raised as male due to development of male gender identity. Clinical characteristics of patients with 21-OH CAH are shown in Table I. Fourteen patients were poor controlled.

BMD z-score of patients with CAH [0.47 (-0.04 – 1.56)] was higher than the control group [-0.43 (-0.82 – 0.05)]; p= < 0.001. There were similar number of patients with low BMD; [n (%); CAH: 6 (16.2%) vs control group:5 (13.5%); p= 0.744]. BMD, BMC, and BMAD of patients did not differ significantly from healthy controls (0.254, 0.701, 0.534 respectively). Height SDS of patients and controls were statistically similar; p= 0.234. However, BMI and BMI z-score were significantly higher in patients with CAH in comparison to controls. Likewise, there were more overweight/obese subjects in patients with CAH than in the control group; 54.1% vs 18.9%, p= 0.002. The data are summarized in Table II.

Table I. Clinical characteristics of patients with 21-hydroxylase congenital adrenal hyperplasia.

	n= 37
Age; years	10 (7.5 – 15.5)
Male; n (%)	15 (40.6)
Prepubertal status; n (%)	19 (51.4)
Salt wasting / simple virilizing n (%)	26 (70.1) / 11 (29.9)
Diagnosis within first year; n (%)	22 (59.5)
Bone age / chronological age at the time of diagnosis	1.63 (1.07 – 1.88)
Bone age / chronological age at the time of evaluation	1.06 (0.90 – 1.32)
Duration of treatment; years	9.2 (6.4 – 12.5)
Prednisolone treatment, n (%)	11 (29.7)
Average glucocorticoids dose, hydrocortisone equivalent; mg/m^2/day	15.2 (12.6 – 17.3)
Cumulative glucocorticoid dose, hydrocortisone equivalent; g/m^2	49.5 (28.8 – 69.4)
Average serum 17-hydroxyprogesterone level; nmol/L	44.8 (22.4 – 79.7)

Values are represented as n (%) or median (interquartile range) where appropriate.

Table II. Bone mineral densitometry and body mass index results in patients with congenital adrenal hyperplasia and control group.

	Patients with CAH (n= 37)	Control group (n= 37)	p-value
BMD z-score	0.47 (-0.04 – 1.56)	-0.43 (-0.82 – 0.05)	< 0.001
Low BMD, n (%)	6 (16.2)	5 (13.5)	0.744
BMD, gr/cm^2	0.640 (0.570 – 0.891)	0.621 (0.484 – 0.825)	0.254
BMC, gr	27.06 (19.64 – 47.65)	28.64 (15.11 – 48.50)	0.701
BMAD, gr/cm^3	0.206 (0.178 – 0.251)	0.197 (0.173 – 0.239)	0.534
Height SDS	-0.32 (-1.32 – 0.70)	0.14 (-0.46 – 0.74)	0.234
BMI, kg/m^2	20.7 (18.6 – 24.5)	17.7 (15.2 – 19.8)	< 0.001
BMI z-score	1.41 (0.91 – 1.95)	-0.12 (-0.48 – 0.92)	< 0.001
Overweight/Obesity, n (%)	20 (54.1)	7 (18.9)	0.002

Values are represented as n (%) or median (interquartile range) where appropriate.
BMD: Bone mineral density, BMC: Bone mineral content, BMAD: Bone mineral apparent density, BMI: Body mass index, CAH: Congenital adrenal hyperplasia, SDS: Standard deviation score.

Low BMD and normal BMD groups were compared according to age, BMI z-score, being CAH, male gender, prepubertal status and overweight/obesity as detailed in Table III. Of all comparisons, BMI z-score was found to be significantly lower in low BMD group as comparison to normal BMD group; p= 0.041. Multivariate logistic regression model including BMI z-score and male gender revealed that each 1.0 decrease in BMI z-score, risk of having low BMD increases by 1.79 (%95 CI: 1.03-3.12, p= 0.040).

Twenty-three patients with well controlled CAH [9.5 (6.8 – 14.9) years] and 23 control subjects [10.4 (6.0 – 12.3) years] were compared for BMD z-score, BMI z-score, and ratio of cases with low BMD. Each group included 8 (34.8%) male and 15 (65.2%) prepubertal subjects. Patients with well controlled CAH had higher BMD z-score [0.42 (-0.44 – 1.33) vs -0.60 (-0.89 – -0.08); p= 0.005], and BMI z-score [1.37 (1.05 – 2.16) vs -0.20 (-0.50 – 0.98); p= 0.003] as compared to control group. Four patients (17.4%) in CAH group and three subjects (13%) in control group

Table III. Clinical characteristics of included patients and healthy controls in low BMD and normal BMD groups.

	Low BMD (n= 11)	Normal BMD (n= 63)	p-value
BMD z-score	-1.36 (-1.55 – -1.29)	0.14 (-0.43 – 1.28)	<0.001
Age, years	7.5 (6.0 – 15.0)	11.3 (6.8 – 15.6)	0.335
BMI z-score	0.50 (-1.32 – 1.25)	0.95 (-0.13 – 1.72)	0.041
CAH, n (%)	6 (54.5)	31 (49.2)	0.744
Male, n (%)	2 (18.1)	31 (49.2)	0.056
Prepubertal status, n (%)	7 (63.6)	29 (46.0)	0.281
Overweight/Obese, n (%)	3 (27.3)	24 (38.1)	0.491

Values are represented as median (interquartile range) and n (%) where appropriate
BMD: Bone mineral density, BMI: Body mass index, CAH: Congenital adrenal hyperplasia.

Table IV. Correlation of body mass index z-score, 17-hydroxyprogesterone level, average glucocorticoid dose, cumulative glucocorticoid dose, and duration of treatment with bone mineral density z-score.

		BMI z-score	Average 17-OHP level; nmol/L	Average GC dose; mg/m^2/day	Cumulative GC dose, gr/m^2	Duration of treatment; years
BMD	r	0.193	0.117	0.296	0.229	0.194
z-score*	p	0.253	0.497	0.076	0.173	0.251

*Values are represented as r: correlation coefficient and p value; Spearman correlation analyses
BMD: Bone mineral density, BMI: Body mass index, GC: glucocorticoid, 17-OHP: 17-hydroxyprogesterone.

had low BMD; p= 0.500. Fourteen patients with poor controlled CAH [12.8 (8.0 – 16.0) years] and 14 control subjects [12.6 (6.4 – 16.0) years] were analyzed for same parameters. Each group included 7 male (50%) and 4 (28.6%) prepubertal subjects. BMD z- score [CAH: 0.64 (-0.04 – 1.94) vs Control: -0.26 (-0.71 – 0.72); p= 0.077] and BMI z-score [CAH: 0.64 (0.65 – 1.95) vs Control: -0.26 (-0.48 – 0.65); p= 0.001] were higher in patients with poor controlled CAH than control group. There were 2 subjects with low BMD in each group. BMD z-score and BMI z-score of patients with well controlled CAH and poor controlled CAH were similar; p= 0.546 and p= 0.841 respectively.

Among 37 patients with CAH, six patients had low BMD and 31 patients had normal BMD. Low BMD group included fewer male patients [n (%): 2 (33.3%) vs 16 (51.6%); p= 0.414], less overweight/obese patients [n (%): 2 (33.3%) vs 18 (58.1%) ; p= 0.266] as compared to normal BMD group, although not statistically significant. BMI z-score of CAH patients with low BMD was lower than control group but not statistically significant [Low BMD: 0.10 (-1.42 – 1.44) and Normal BMD: 1.44 (0.95 – 2.16); p= 0.113]. Ratio of prepubertal [n (%): 3 (50.0%) vs 16 (51.6%); p= 0.942] and poor controlled [n (%): 2 (33.3%) vs 12 (38.7%); p= 1.000] patients were similar in low and normal BMD groups. Salt wasting 21-OH CAH accounted for all patients in low BMD group and 65% of patients in normal BMD group; p= 0.080.

BMI z-score, average serum 17-OHP level, average glucocorticoid dose, cumulative glucocorticoid dose and duration of treatment were not found to be correlated with BMD z-score in patients with CAH as shown in Table IV.

Discussion

In this prospective study, we demonstrated that BMD z-score in patients with 21-OH CAH was increased in comparison to age, sex and puberty matched healthy controls. Previous studies

found that bone mineralization of children with CAH was either unchanged, reduced or increased when compared to healthy children.[3,6-17]

As reviewed in the clinical report of American Academy of Pediatrics about assessment of bone densitometry in children and adolescents, evaluation of results of DEXA is a debate and may require more than the calculation of z-score. Chronic illness may result in either delayed or advanced growth and pubertal development, which are factors that contribute to a low bone mass for age or gender. BMD, calculated by DEXA as BMC/cm^2, adjusts bone mineral content for the area, but not for the volume of bone. Bearing this in mind, if two people of different heights but similar ages have comparable volumetric bone density, the shorter person will be reported to have a lower BMD and BMD z-score than the taller one. Similarly, tall people have big and large vertebrae, thus BMD z-score will be higher than actual. Likewise, pubertal problems will cause alterations in bone size, geometry, and density that occur with sex-steroid exposure. Although controversy exists concerning the optimal method for adjusting variations in bone size, body composition, and maturity, BMD results are recommended to be adjusted for height or height age over age-, gender- specific z-score.[25] Besides, children with CAH often have accelerated puberty, whose outcome on bone mineralization cannot not be denied. Moreover, it is known that patients with CAH could be either taller or shorter than chronological age, while the bone age being either advanced or delayed. Ganesh et al.[15] reported that BMD and BMC results were well correlated with height for age of children with CAH. Bearing in mind that the presence of variations in the anthropometric measurements we evaluated BMD z-scores for height age as some studies in the literature.[13,15,16]

Increased risk of obesity in patients with CAH was emphasized in many studies and attributed to the glucocorticoid treatment to increase the fat mass rather than the increased serum androgens to increase the lean mass, the advanced bone age maturation and parental obesity.[9,19,26] As in line with those studies, we also found that there were approximately three times more obese subjects among patients with CAH than in control group. Moreover, our results showed that BMD was higher in patients with CAH when compared to the control group. This finding can be attributed to higher BMI z-score in patients as; each 1 decrease in standard deviation score of BMI was found to increase the risk of having low BMD by 1.79 supporting the previous studies.[9,13] The protection of higher BMI on bone density loss in adult patients with CAH was also emphasized in adult patients. The excess adipose tissue leading to increased conversion of estrogen from adrenal precursors and positive effect of a better nutritional condition on bone mineralization were said to be probable reasons to explain positive effect of increased BMI on BMD.[13,27] Moreover, mechanical loading on weight bearing bones may contribute to the higher BMD z-scores in patients with higher BMI z-score.[28]

Children with CAH require lifelong glucocorticoid treatment. Glucocorticoids give rise to deficient bone mineralization by direct suppression of osteoblastic activity, inhibiting calcium absorption from the gut, thereby leading to secondary hyperparathyroidism and increasing bone resorption by the osteoclasts, and inhibiting renal tubular calcium resorption.[2]

In our study, BMD z-score were found to be related neither with dose (mean or cumulative) nor the duration of the treatment as in line with the literature.[14] Similarly, currently used replacement doses of glucocorticoids was found not to have a major impact on bone in patients with CAH when bone mineralization was assessed by quantitative ultrasonometry.[29] Nevertheless, data about the effect of glucocorticoid treatment varies and many studies that relate osteopenia in patients with CAH to glucocorticoid use exist in the literature. Not only the dose but the duration of treatment was evaluated thoroughly and found to be

negatively correlated with bone mineralization in some studies.[9,13,15,17] Prednisolone treatment instead of hydrocortisone was shown to decrease bone mineralization.[5] Eleven patients in our study were on prednisolone treatment for 2.0 (1.0 - 3.6) years, which is quite a small percentage of total duration of treatment 9.2 (6.4 – 12.5) years. Thus, no detailed analyses were performed regarding steroid type.

Previous studies showed increased, decreased or unchanged bone mineralization in patients with poor controlled CAH as compared to patients with well controlled CAH.[6,9,21] In cases with poor controlled CAH, elevated sex steroids and decreased exposure to glucocorticoids may have positive effect on BMD. However, the ratio of patients with poor controlled CAH in low BMD and normal BMD groups were similar. In addition to this, patients with well controlled CAH and with poor controlled CAH had similar BMD z-scores, and serum 17-OHP levels were not correlated with BMD z-score.

Puberty is a significant stage of development which is known to affect bone mineralization. While Gussinye et al.[7] stated that prepubertal children with CAH had higher BMD z-score, Yilmaz et al.[30] showed that bone mineralization is affected positively during puberty. No difference in number of prepubertal subjects was observed between low BMD and normal BMD groups.

Patients with salt wasting 21-OH CAH were shown to have lower BMD when compared to late onset patients, but no variation between the forms of classical CAH was shown to exist with regards to bone mineralization.[12] We also noted similar percentage of patients with salt wasting 21-OH CAH in low BMD and normal BMD groups.

Small sample size, wide range in the age of subjects, inclusion patients on prednisolone treatment limit the results of our study to be generalized to all patients with CAH. Also current literature suggest the evidence of vitamin D deficiency and genetic factors in the bone mineralization in the patients, which we did not evaluated.[10,14,31] Despite the limitations of the current study, BMD z-score was not lower in patients with CAH, and this supports the idea that CAH itself does not affect bone mineralization negatively.

In conclusion, the results of our study revealed that an explicit negativity was not observed in bone mineralization of patients with 21-OH CAH patients followed up with appropriate treatment protocols. Moreover, higher BMI z-score may contribute to preservation of bone health in patients with CAH. In addition, it should be considered that the patients with CAH are at risk for obesity, and precautions are needed to be taken to this end.

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Original Article

Predictive value of an early amplitude-integrated electroencephalogram for short-term neurologic outcomes in preterm infants

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ABSTRACT

Background and objectives. The aim of this study was to compare serial scores of amplitude-integrated electroencephalography (aEEG) in preterm infants with favorable neurologic outcome compared with those with unfavorable neurologic outcome and to evaluate whether aEEG in the early days of life has predictive value for short-term neurologic outcome in preterm infants.

Methods. This prospective observational study included infants born at ≤32 weeks of gestational age and ≤1,500 g of birth weight. On the basis of brain ultrasonography findings, the infants were divided into two groups (favorable and unfavorable outcome group) at 36 weeks of corrected age or at discharge. aEEG was performed at 12-14 h (day-1), 46-48 h (day-2), 70-72 h (day-3), and 1 week (day-7) of life. The aEEG recordings were analyzed using the criteria described by Burdjalov et al.[23] and the serial scores of aEEG were compared between the two groups.

Results. Thirty five infants were enrolled and 18 infants and 17 infants were identified into both groups, respectively. Infants in the favorable outcome group showed high scores in almost all parameters and the score of all parameters increased over time. However, the scores of all components decreased in day-2 compared with those of day 1 in the unfavorable outcome group. The total score less than 3 of day-2 has predictive value of 70.6% of sensitivity and 72.2% of specificity for unfavorable outcome.

Conclusion. We found that aEEG is a useful predictor for short-term neurologic outcome in preterm infants.

Key words: amplitude-integrated electroencephalography; preterm infant; neurological outcomes; periventricular leukomalacia.

The survival rate of preterm infants has increased dramatically owing to the advances in neonatal intensive therapy.[1-3] However, this increased survival rate of preterm infants affects the morbidities such as intraventricular hemorrhage (IVH) and periventricular leukomalacia (PVL). The prevalence of brain damage and neurodevelopmental abnormalities will also increase as the survival rate increases.[4,5] Hence, monitoring of cerebral function has become important. However, it has not been generalized yet in neonatal intensive care units. Conventional electroencephalography (cEEG) has been the gold standard method for brain function monitoring for decades. Neonatal cEEG has been studied and correlated with neurodevelopmental outcomes in both full-term and preterm infants with intracranial lesions.[6-10] Because cEEG requires expertise in interpretation and is associated with some technical difficulties, it is difficult to routinely use in the neonatal intensive care

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units.[11] On the contrary, amplitude-integrated electroencephalography (aEEG) can be recorded from a single-channel EEG and is easy to interpret.[12] aEEG has become a bedside monitoring method in full-term asphyxiated infants and several studies have shown that an abnormal aEEG is predictive of the persistence of encephalopathy and impaired neurologic outcome when performed in the first few days of life.[13-16] Recently, several studies have reported a correlation of early aEEG parameters with short- and long-term neurological outcome in preterm infants.[17-22]

In the present study, we used aEEG scoring system described by Burdjalov et al.[23] to evaluate the relationship between scores of aEEG and unfavorable short-term neurologic outcome including IVH, PVL and death. We tried to compare serial scores of aEEG in the infants with favorable neurologic outcome compared with those with unfavorable neurologic outcome and to evaluate whether aEEG in the early days of life has predictive value of short-term neurologic outcome in preterm infants.

Material and Methods

A prospective observational study was conducted on preterm infants born between December 2016 and November 2017 in Pusan National University Hospital. Infants were included if they were born at ≤32 weeks of gestational age (GA) and weighed ≤1,500 g at birth. Infants were excluded if they had any of the following: 1) major congenital anomalies and/or chromosomal abnormality, 2) fetal hydrops, 3) metabolic disorders or central nervous system infection, or 4) death before 7days of life.

Data collection

The demographic characteristics were collected from the medical charts of the infants including the following parameters: GA, birth weight, sex, mode of delivery, 1 min and 5 min Apgar scores, initial arterial pH, respiratory distress syndrome, patent ductus arteriosus (defined as the need for ibuprofen medication or surgical ligation), and hospital stay duration.

Brain ultrasonography

Brain ultrasonography was performed during the first 3 days of life and then mostly once a week, depending on the clinical course of the patient, until discharge, transfer or death. A Vivid 7 Dimension ultrasound machine (GE Healthcare, Waukesha, WI, USA) with an 8.0-MHz transducer was used and the results were interpreted by 1 radiology specialist without knowing the history or the clinical status of the infants. Images were evaluated for germinal matrix-intraventricular hemorrhage according to the Papile[24] classification (grade I-IV) and PVL according to the de Vries[25] classification (grade I-IV). Short term neurologic outcome was assessed on the basis of ultrasonographic findings at 36 weeks of corrected age or at discharge. If there were different results on brain ultrasonography, the most recent was used. For the purpose of this study, Group A consisted of infants with favorable outcome which included normal brain lesions, IVH grade I or II, or PVL grade I or II. Group B consisted of infants who had unfavorable outcome including IVH grade III or IV, PVL grade III or IV, or death.

Amplitude-integrated electroencephalography

The aEEG recordings were performed in each infant four times: at 12-14 h, 46-48 h, 70-72 h, and 7 days after birth. At least three hours were recorded for each time points. We used a cerebral function monitor (Olympic CFM 6000, Natus Medical Incorporated, San Carlos, USA) with disc electrodes prepared with Elefix paste (Nihon Kohden Corporation, Tokyo, Japan) to achieve low impedance. Single channel monitoring with attached over bi-parietal areas was done according to the international 10-20 EEG system. The quality of the aEEG trace was monitored using a simultaneous continuous impedance trace, and aEEG tracings with an impedance of >20 kΩ were discarded. The aEEG tracings were interpreted according to a scoring system described by Burdjalov et al.[23] (Table I).

Table I. Cerebral function monitoring system reported by Burdjalov et al.[25]

Score	Continuity	Cycling	Amplitude of lower border	Bandwidth span and amplitude of lower border
0	Discontinuous	None	Severely depressed (< 3 μV)	Very depressed: low span (≤15 μV) and low voltage (5 μV)
1	Somewhat continuous	Waves first appear	Somewhat depressed (3-5 μV)	Very immature: high span (>20 μV) or moderate span (15-20 μV) and low voltage (5 μV)
2	Continuous	Not definite, somewhat cycling	Elevated (>5 μV)	Immature: high span (>20 μV) and high voltage (>5 μV)
3		Definite cycling, but interrupted		Maturing: moderate span (15-20 μV) and high voltage (>5 μV)
4		Definite cycling, non-interrupted		Mature: low span (<15 μV) and high voltage (>5 μV)
5		Regular and mature cycling		

Four components of the tracings were assessed: continuity (0-2), cycling (0-5), amplitude of the lower border (0-2), and bandwidth span and amplitude of lower border (0-4). These components were summated into a total score ranging from 0 to 13. The aEEG tracings were interpreted by two independent researchers, who were blind to other information.

Ethical approval for this study was granted by the institutional review board of Pusan National University Hospital (1603-006-064). Fully informed written consent was obtained from the parents of all infants.

Statistical analysis

Data were stored in a dedicated access database and verified for accuracy. Statistical analyses were performed with SPSS 22.0 (IBM Corp., Chicago, IL, USA) using raw scores. To investigate the agreement between the aEEG interpretations of the two examiners, we performed Bland-Altman analyses. The analyses presented here focused on the comparisons between two groups (Group A and Group B). Statistical comparison of the categorical variables was tested by Fisher's exact test. Continuous variables were tested by independent t-test or Wilcoxon rank sum test depending on whether the data conformed to the normality assumption. p-value <0.05 was regarded as statistically significant. Receiver operating characteristic and area under the curve (AUC) were generated to determine cutpoints and to calculate the sensitivity and specificity. Youden's index was used to determine the optimal cutpoints on the probability scale for best discriminating between Group A and Group B. Youden's index was defined as the optimal cutpoint that maximizes both sensitivity and specificity.

Results

During the study period, 43 preterm infants born at ≤32 weeks of GA and ≤1,500 g of birth weight were delivered at our hospital. Of them, 8 infants were excluded according to the exclusion criteria; congenital multiple anomaly (n= 1), fetal hydrops (n= 1), death before 7 days after birth (n= 4) or no parental consent (n=2). Of the 35 included infants, 18 infants and 17 infants were identified into the Group A and B, respectively (Fig. 1). The demographic and clinical characteristics of both groups are shown in Table II.

The mean GA and birth weight were 29.3 ± 2.2 weeks and 1139.5 ± 316.8 g, respectively in Group A. In Group B, the mean GA and birth weight was 28.0 ± 2.6 weeks and the mean

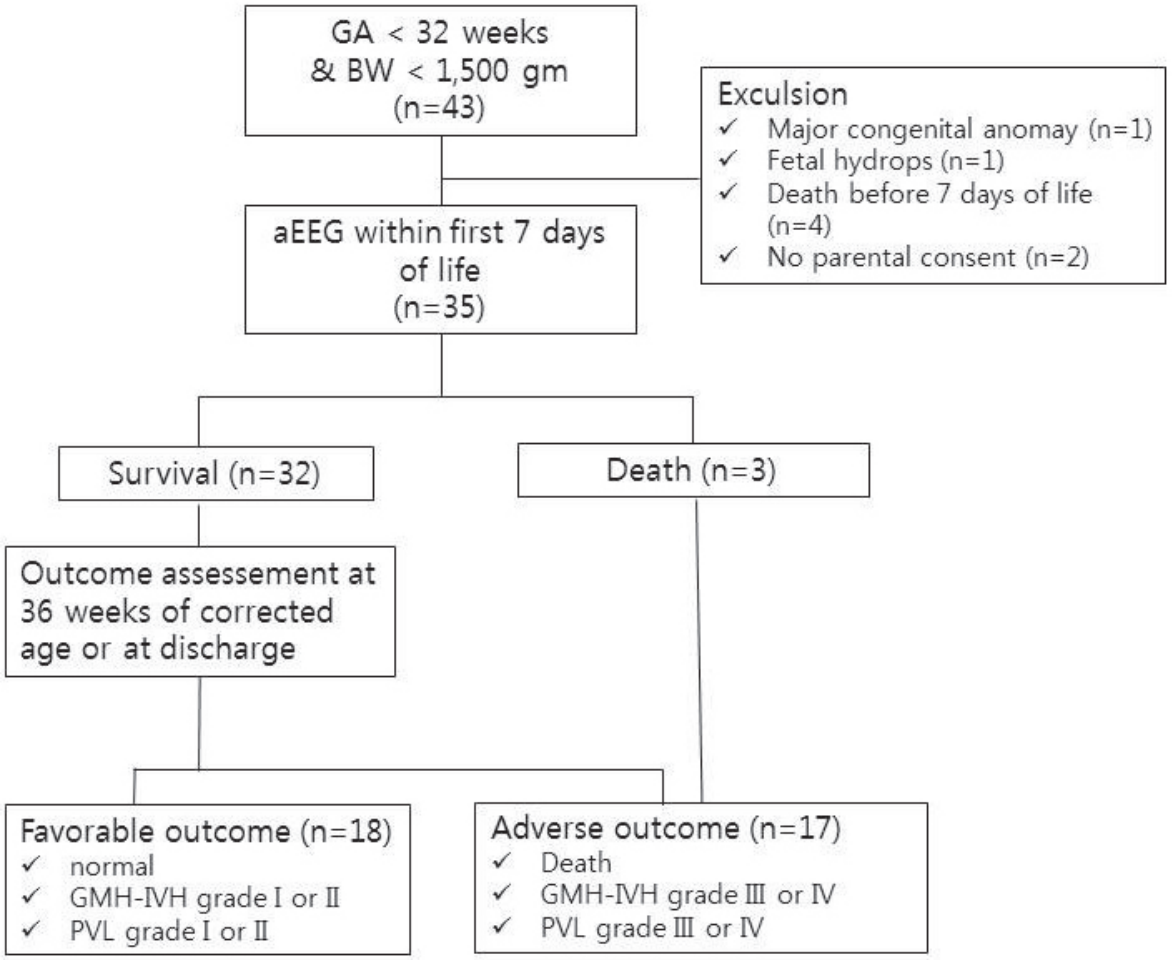

Fig. 1. Flowchart showing details of patients included and excluded.

BW: birth weight, aEEG: amplitude-integrated electroencephalography, GA: gestational age, GMH: germinal matrix hemorrhage, IVH: intraventricular hemorrhage, PVL: periventricular leukomalacia.

birth weight was 1023.0 ± 345.9 g (Table II). There was no significant difference between the two groups in demographic and clinical characteristics including in sex, delivery mode, initial arterial pH, incidence of patent ductus arteriosus, and hospital days. Initial systolic blood pressure and mean blood pressure were significantly lower in group B (p= 0.046 and p= 0.011 respectively) while incidence of respiratory distress syndrome was higher in group B (p= 0.025).

We performed the Bland-Altman analysis for total scores of Burdjalov score at each time points. The Bland-Altman plots show that most of the differences were within ± 1.96 SD, suggesting that the differences are not significant (Fig 2).

We found higher scores in all four component of each time point of the aEEG tracings in Group A than Group B and some of them had statistically significant differences. The mean score of continuity was significantly higher in group A (1.78) than that of group B (1.12) (p= 0.007). In case of cycling, patients in group A had significantly higher mean scores at day 2 and day 7 (p= 0.032 and p= 0.042, respectively). At the lower border, the only significant difference was at day-2, which was 1.17 and 0.94 in Group A and Group B, respectively (p= 0.020). At the band width, patients in Group A had

Table II. Comparison of demographic and clinical characteristics between the two groups.

Characteristics	Group A (n= 18)	Group B (n= 17)	p value
Male gender, n (%)	10 (55.6)	11 (64.7)	0.733
Gestational age, week	29.3 ± 2.2	28.0 ± 2.6	0.114
Birth weight, g	1139.5 ± 316.8	1023.0 ± 345.9	0.308
Cesarean section, n (%)	12 (66.7)	15 (88.2)	0.228
Apgar score at 1 min.§	5 (4-7)	4 (3-6)	0.095
Apgar score at 5 min.§	7 (5-8)	6 (4-7)	0.143
Initial arterial pH	7.4 ± 0.1	7.3 ± 0.1	0.191
Systolic blood pressure, mm Hg§	58 (38-80)	43 (32-87)	0.046*
Diastolic blood pressure, mm Hg§	29 (18-64)	21 (13-59)	0.064
Mean blood pressure, mm Hg§	38 (24-70)	27 (21-69)	0.011*
Ventilator support, n (%)	14 (77.8)	15 (88.2)	0.115
Respiratory distress syndrome, n (%)	7 (38.8)	12 (70.5)	0.025*
Patent ductus arteriosus, n (%)	5 (27.7)	10 (58.8)	0.061
Hospital stay duration, day§	58 (29-122)	68 (9-148)	0.801

Group A: infants with favorable outcome which included normal brain lesions, intraventricular hemorrhage (IVH) grade I or II, or periventricular leukomalacia (PVL) grade I or II.
Group B: infants who had unfavorable outcome including IVH grade III or IV, PVL grade III or IV, or death.
§ Results are presented as median (25th percentile - 75th percentile).
Two-sample test or Wilcoxon rank-sum test for continuous variables; Fisher's exact test for categorical variables.
* p value <0.05

higher mean scores but there is no significant difference. Total score was significantly higher at day-2 in group A (4.22 and 2.59, respectively).

All scores were increased as the postnatal days increased in Group A. However, there was a decrease in all four components of day-2 compared to day-1 in Group B, and then after day-3, there was increase in all four components like in Group A (Fig 3). Especially there was a statistically significant increase in cycling in Group A, while all components except lower border were decreased significantly in group B at the day-2 (Table III).

Receiver operator characteristics curves were created to provide the predictive value of the total Burdjalov score for unfavorable outcome in preterm infants (Fig 4). AUC was highest at the day-2. The cutpoints for the mean total Burdjalov score at day-2 was set at 3, as it presented the highest combined sensitivity and specificity, which were 70.6% and 72.2%, respectively (Table IV).

Discussion

The tool to predict neurodevelopmental outcome in preterm infants would be helpful for accurate parental counseling and early rehabilitation programs if needed. Although reports describing usefulness of aEEG in predicting outcome of preterm infants have been limited, several reports showed that possibility of aEEG in predicting outcome of preterm infants.[12,26,27] Chalak et al.[12] reported that low voltage discontinuous activity with burst suppression was an ominous finding predicting the occurrence of severe intracranial hemorrhage with 100% positive predictive value and specificity. Kidokoro et al.[26] reported that absent cycling on a EEG within 24 hour of age was associated with poor outcome in preterm infants.

Although there is still no consistent agreement in interpretation of recording in preterm infants, there are two common classifications and scoring systems of aEEG by Hellström-Hellström-Westas[28] and Burdjalov et al.[23] One

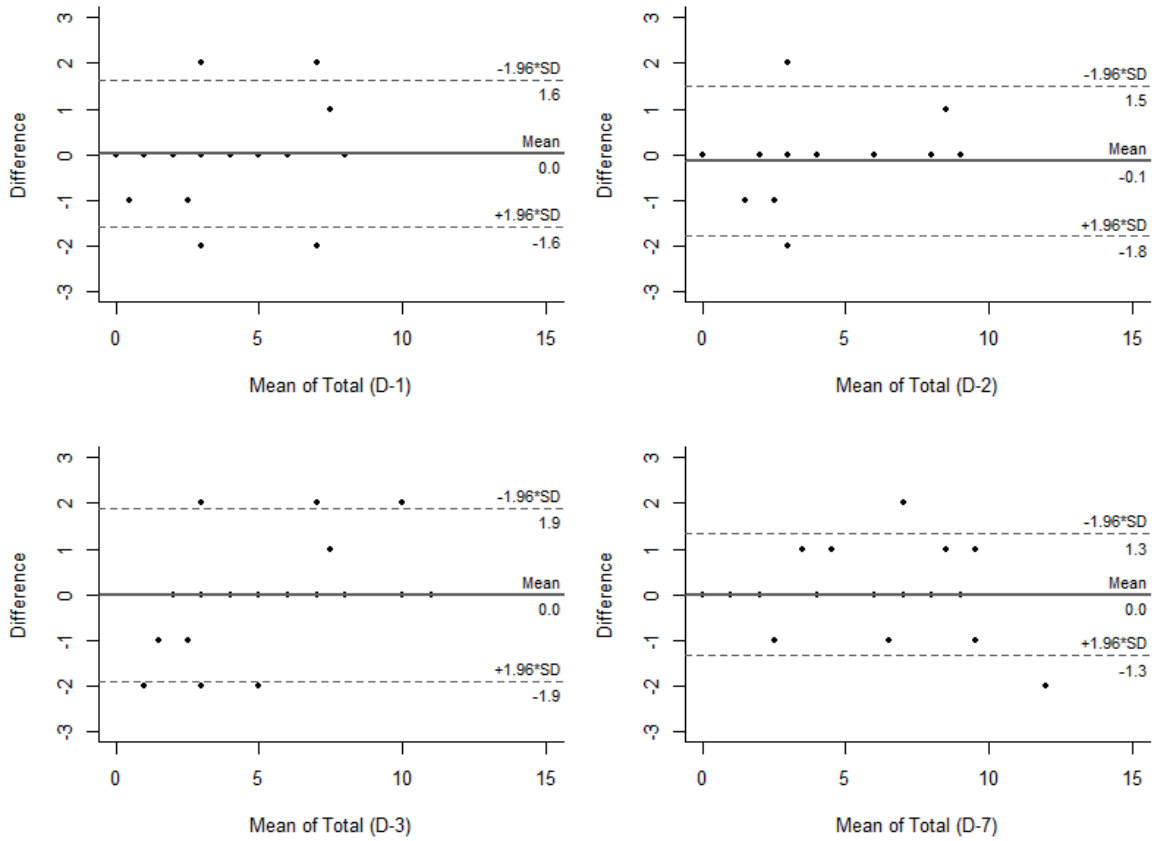

Fig. 2. Each graph shows the Bland-Altman plots and 95% confidence limits of the agreement between the 2 examiners for the total scores of aEEG from each time point. The mean of the examiners' scores is shown on the x-axis and differences are shown on the y-axis. The solid line indicates the mean differences between the examiners' scores, and the dashed lines indicate the 95% confidence intervals of these differences. (D: day)

main difference between the two classifications is that the Burdjalov score is primarily designed to describe the physiological maturation of electrocortical activity. On the other hand, Hellstrom-Westas score is designed to distinguish pathological and physiological patterns rather than to describe maturational changes over time.[29] We had evaluated the serial aEEGs during the first week of life with a scoring system described criteria by Burdjalov et al.[23] in our previous study.[30] At that study, we could confirm that the inter-rater agreement was high for most components of the tracing and that scoring system was objective and reproducible in repeated assessments of the aEEG tracing. Therefore, we adopted the criteria by Burdjalov et al.[23] again in this study. We performed to determine the extent of agreement between the aEEG interpretations of the two examiners using Bland-Altman analyses, and could confirm that most of the differences were within ± 1.96 SD, suggesting that the differences are not significant.

Several reports demonstrated that the maturation of the aEEG tracing depends on GA and postnatal age in preterm infant. In our previous study, we examined the serial development of aEEG during the first week of life using Burdjalov scoring system to evaluate preterm infants who had no abnormality of brain ultrasounds. We found that the greater the GA, the more mature the aEEG tracing is at birth. In addition, that study showed that progressive increases with advancing postnatal age in all four component scores and the total score regardless of GA even though in 24-26 weeks of GA. However, interestingly our

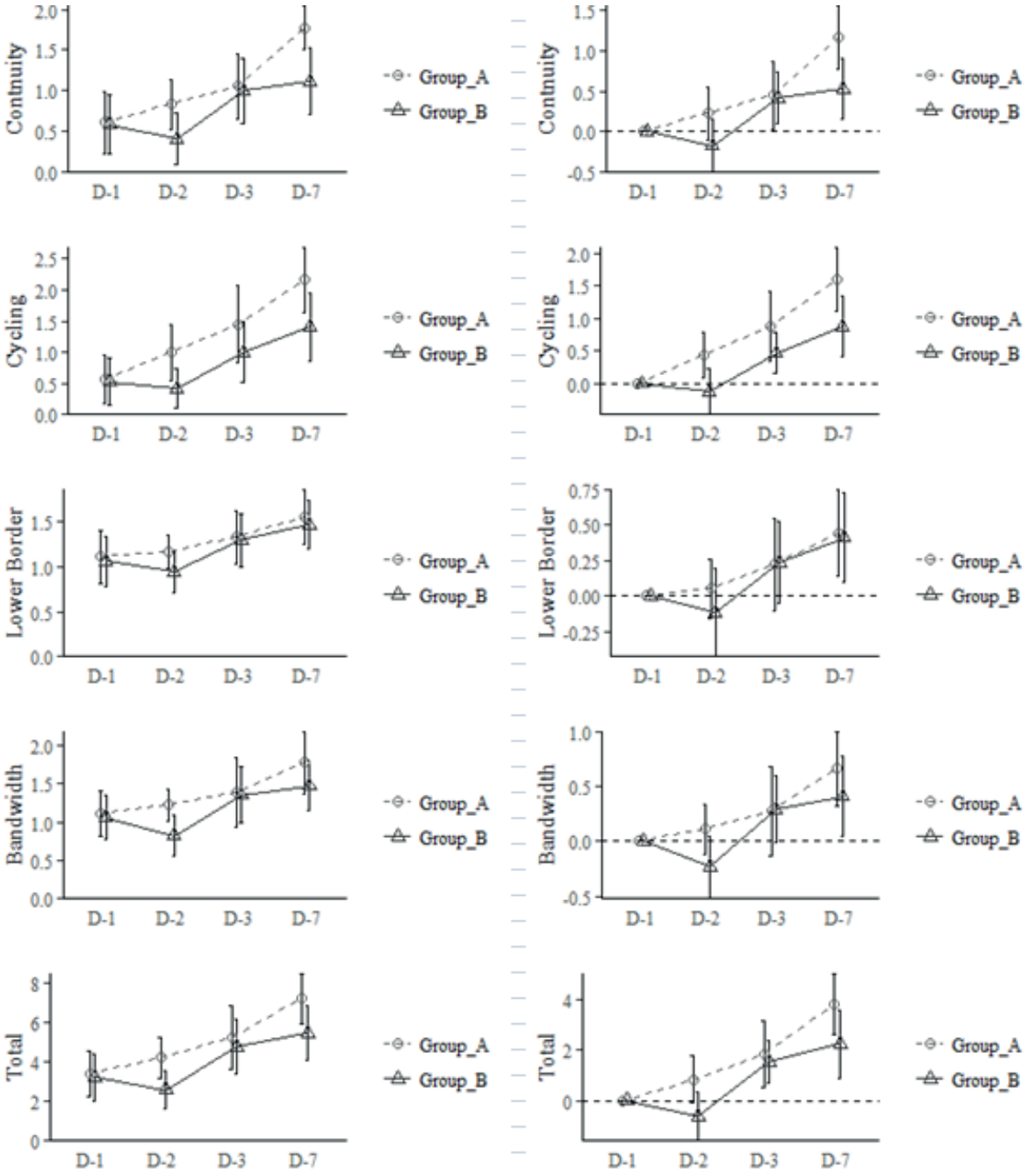

Fig. 3. Comparison of continuity, cycling, lower border, bandwidth and total score between the group A and the group B from each time points. The left side of the figure demonstrates the actual scores; the right side shows the differences from the first score. There was a decrease in all 4 components of day 2 compared with day 1 in group B. Then after day 3, there was an increase in all four components like in group A. (D: day)

results showed that scores of all components significantly decreased at day-2 compared with at day-1 in group B, while the scores were increased as postnatal age increase in Group A. In this study, GA and postnatal age did not differ between study groups. Therefore, those decreased score at day-2 in Group B might not be due to GA or postnatal age.

Table III. Scores of components of aEEG at each time point.

	Group A			
Components	Day 1	Day 2	Day 3	Day 7
Continuity	0.61 (0.78)	0.83 (0.62)	1.06 (0.80)	1.78 (0.55)
Cycling	0.56 (0.78)	1.00 (0.91)	1.44 (1.25)	2.17 (1.04)
Lower border	1.11 (0.58)	1.17 (0.38)	1.33 (0.59)	1.56 (0.62)
Bandwidth	1.11 (0.58)	1.22 (0.43)	1.39 (0.92)	1.78 (0.81)
Total	3.39 (2.33)	4.22 (2.13)	5.22 (3.26)	7.22 (2.53)
	Difference of mean from baseline (95% CI)			
Continuity	0 (reference)	0.22 (-0.10, 0.54)	0.44 (0.02, 0.87) †	1.17 (0.78, 1.56) †
Cycling	0 (reference)	0.44 (0.09, 0.79) †	0.89 (0.35, 1.43) †	1.61 (1.12, 2.10) †
Lower border	0 (reference)	0.06 (-0.15, 0.26)	0.22 (-0.10, 0.54)	0.44 (0.14, 0.75) †
Bandwidth	0 (reference)	0.11 (-0.12, 0.35)	0.28 (-0.13, 0.69)	0.67 (0.33, 1.01) †
Total	0 (reference)	0.83 (-0.10, 1.77)	1.83 (0.49, 3.18) †	3.83 (2.64, 5.03) †
	Group B			
	Day 1	Day 2	Day 3	Day 7
Continuity	0.59 (0.71)	0.41 (0.62)	1.00 (0.79)	1.12 (0.78) *
Cycling	0.53 (0.72)	0.41 (0.62) *	1.00 (0.94)	1.41 (1.06) *
Lower border	1.06 (0.56)	0.94 (0.43) *	1.29 (0.59)	1.47 (0.51)
Bandwidth	1.06 (0.56)	0.82 (0.53)	1.35 (0.70)	1.47 (0.62)
Total	3.24 (2.33)	2.59 (1.84) *	4.76 (2.68)	5.47 (2.74)
	Difference of mean from baseline (95% CI)			
Continuity	0 (reference)	-0.18 (-0.50, 0.15) †	0.41 (0.09, 0.73)	0.53 (0.16, 0.90) †
Cycling	0 (reference)	-0.12 (-0.48, 0.24) †	0.47 (0.15, 0.79)	0.88 (0.41, 1.36) †
Lower border	0 (reference)	-0.12 (-0.43, 0.19)	0.24 (-0.05, 0.52)	0.41 (0.09, 0.73)
Bandwidth	0 (reference)	-0.24 (-0.52, 0.05) †	0.29 (-0.01, 0.60)	0.41 (0.05, 0.78)
Total	0 (reference)	-0.65 (-1.61, 0.31) †	1.53 (0.67, 2.38)	2.24 (0.89, 3.58)

Group A: infants with favorable outcome which included normal brain lesions, intraventricular hemorrhage (IVH) grade I or II, or periventricular leukomalacia (PVL) grade I or II.
Group B: infants who had unfavorable outcome including IVH grade III or IV, PVL grade III or IV, or death.
Data are shown as mean (SD) except differences (95% CI)
* p value <0.05 compared with Group A at same time points (two-sample test).
† p value <0.05 compared with baseline (day-1) of the same parameter (two-sample test).

It is well known that the pathogenesis of the most common causes of disabling brain lesion such as IVH and PVL in preterm infants is likely to be associated with abnormalities of cerebral perfusion in the first days of life.[31-33] Kehrer et al.[33] performed quantitative measurement of cerebral blood flow volume using ultrasound flowmetry of the extracranial, brain feeding arteries in 32 preterm infants of 28-35 weeks of GA. They reported that the most pronounced increase in cerebral perfusion can be observed from the first to the second day of life in infants with normal brain and can be likely to represent a normal adaptive response of the cerebral circulation to postnatal conditions. Our results showed that scores of all components decreased significantly at day-2 compared with day-1 in Group B. The finding of this study is suggested that low score of aEEG might be due to failure of normal increase in cerebral perfusion during the first days of life, especially during the second day of life and therefore resulted in disabling brain lesions and adverse neurologic outcome sequentially. Interestingly, Bruns et al.[29] reported that there is a correlation between absence of cycling on the second day

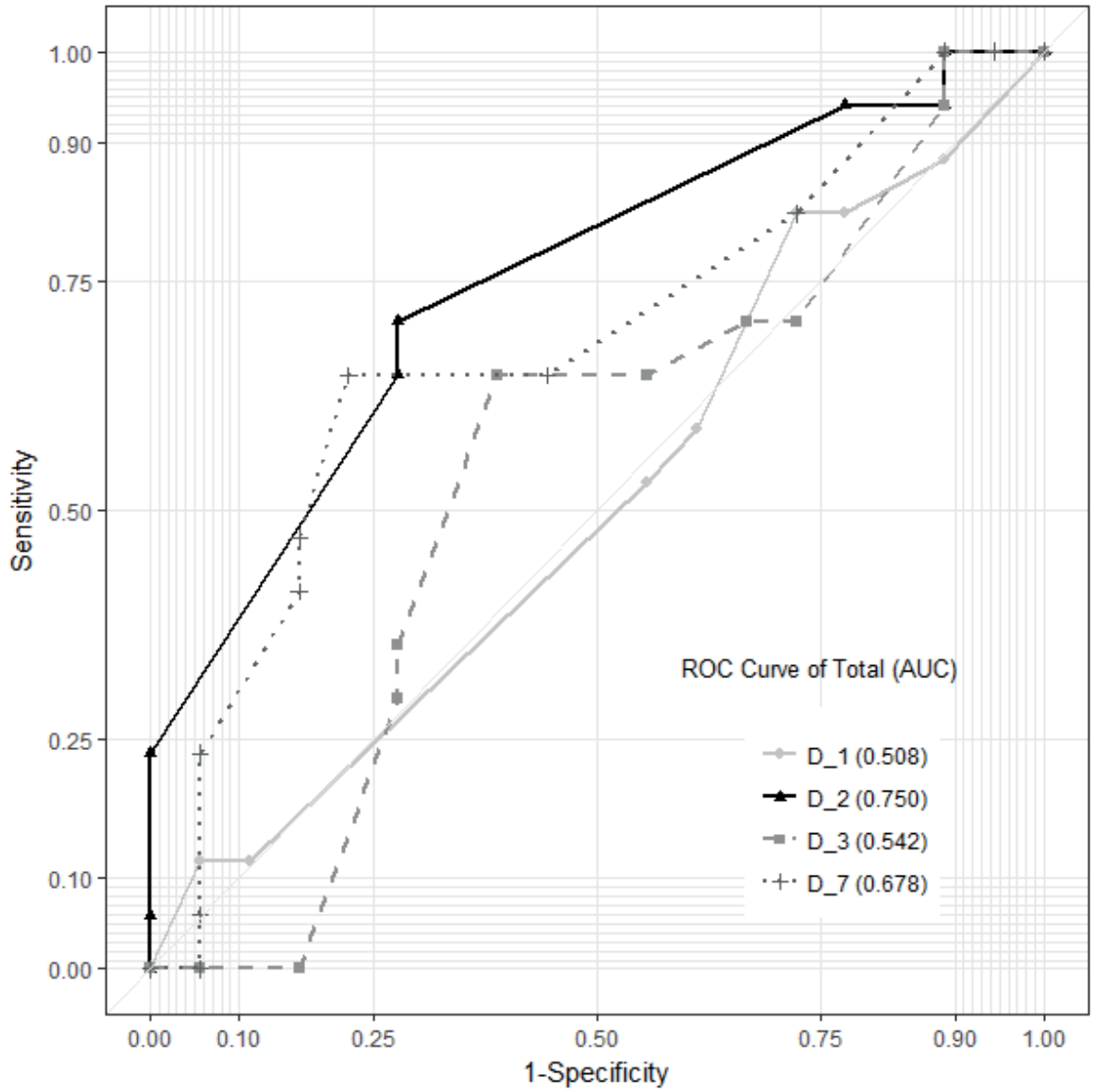

Fig. 4. Receiver operating characteristics curves calculated for total scores at each time point. The area under the curve is in brackets.

of life and the risk of death. In addition, Bowen et al.[34] measured aEEG activity in the first 48 hour of life in preterm infants and identified that there was no increased in EEG continuity or a EEG amplitude for 12 to 48 h in neonates who died or developed grades 3-4 IVH. Their results were in substantial agreement with our study and supported our results. However, we could not measure cerebral blood flow. Further study using aEEG and cerebral blood flow will be needed to understand this phenomenon.

We also tried to identify a numerical value to predict neurodevelopmental outcome in preterm infants using scoring system by Burdjalov et al.[23] We found that total score of day-2 has highest AUC and ≤3 of total score has predictive value of 70.6% of sensitivity and 72.2% of specificity for the unfavorable outcome. In fact, there may be NICU in which continuous aEEG monitoring is not possible due to lack of equipment. In that case, it might be helpful that unfavorable prognosis can be estimated if a

Table IV. Cutpoint, sensitivity and specificity of each time point in aEEG components.

Components	Time	AUC	Cutpoint§	Sensitivity	Specificity	p value
Continuity	D-1	0.500	1	88.2%	16.7%	NS
	D-2	0.683	0	64.7%	72.2%	0.025 *
	D-3	0.520	1	70.6%	33.3%	0.821
	D-7	0.742	1	64.7%	83.3%	0.009 *
Cycling	D-1	0.500	1	88.2%	16.7%	NS
	D-2	0.696	0	64.7%	72.2%	0.018 *
	D-3	0.598	1	70.6%	50.0%	0.249
	D-7	0.685	1	52.9%	77.8%	0.047 *
Lower border	D-1	0.523	1	82.4%	22.2%	NS
	D-2	0.603	0	11.8%	100.0%	0.276
	D-3	0.518	1	64.7%	38.9%	0.954
	D-7	0.556	1	52.9%	61.1%	0.716
Bandwidth	D-1	0.523	1	82.4%	22.2%	NS
	D-2	0.673	0	23.5%	100.0%	0.039 *
	D-3	0.515	1	64.7%	44.4%	0.926
	D-7	0.610	2	100.0%	16.7%	0.339
Total	D-1	0.508	4	82.4%	27.8%	NS
	D-2	0.750	3	70.6%	72.2%	0.004 *
	D-3	0.543	4	64.7%	61.1%	0.706
	D-7	0.678	6	64.7%	77.8%	0.074

§ Youden-index, D: day
* p value <0.05 compared from baseline in each parameters
AUC: area under the curve

score of ≤3 is obtained by conducting a second day of life.

This study had several limitations including small number of patients. It is impossible to know exactly when the aEEG changed because aEEG was not monitored continuously during the first seven days of life. Our study also overlooked some minor problems such as hypotension during the hospital days although we know that those events also may associate with adverse neurologic outcome. Further studies with larger numbers and continuously monitored were needed. Despite of these limitations, we demonstrated that preterm infants with lower scores of aEEG especially during the second day of life than the first day of life or those with total scores ≤3 during the second day of life are more likely to have short-term unfavorable outcome.

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Original Article

Immediate adverse reactions to intravenous immunoglobulin in primary immune deficiencies: a single center experience

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ABSTRACT

Background and objective. Adverse reactions related to intravenous immunoglobulin (IVIG) infusions vary from 1 to 81%, with an average of 20%. They may be classified as immediate; occurring during the infusion itself or delayed; occurring after the infusion has been ceased. In the present study, we aimed to evaluate the frequency of immediate adverse reactions due to IVIG infusions in primary immune deficiency (PID) patients.

Methods. The study population was composed of 109 patients. A total of 763 infusions were recorded for demographic data and adverse reactions.

Results. The participants included 32 girls (29%) and 77 boys (71%). The mean age was 11.8 ± 5.7 years (0.6-33.5 years). Early adverse events (AE) were recorded in 34 (4.5%) among 763 IVIG infusions including 30 mild (88.2%), 3 moderate (8.8%) and 1 severe (2.9%). The most common immediate adverse reactions were fever (29.4%) and headache (29.4%). The risk of AE was higher among primary antibody deficiency (PAD), compared to combined immunodeficiency (OR 2.61, 95%CI 1.061-6.475; p = 0.037).

Conclusions. Use of various intravenous immunoglobulin treatments should be considered with regard to side effect profiles observed. In our cohort, PID patient experienced mostly mild AE; PAD was associated with an increased risk of AE.

Key words: IVIG, adverse reaction, primary immunodeficiencies.

Intravenous immunoglobulin (IVIG) has been used for primary immune deficiencies (PID) since the 1980's.[1] It has been shown that IVIG treatment decrease the morbidity, mortality and the frequency of severe bacterial infections in X-linked agammaglobulinemia and common variable immune deficiency (CVID) patients.[2-6]

The frequency of adverse events seen in IVIG treatment varies between 1-81% with a mean value of 20%.[7-10] These are classified as early or late according to the time of occurrence of the reaction.[11] The most common early adverse events (AE) are fever, chills, headache, nausea, hypotension, myalgia, wheezing, back pain and rash.[11] Adverse reactions are reported to be related to product, patient or infusion characteristics. During the production process, ethanol precipitation or stabilizer addition may generate immunoglobulin aggregates resulting in AE.[7,8] Primary antibody deficient patients with very low levels of IgA are predisposed for reactions during IVIG treatment due to anti IgA antibodies which could be managed by the use of products containing trace amount of IgA.[12] Moreover, rapid rate especially at the initial phase of the infusion, presence of an acute infection, osmolality of the product, sodium

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and sugar contents and pH value may also be related to adverse reactions.[13] Since there are several concentrations and ingredients of IVIG products such as 5%, 10%.[14,15] To minimize rate-related adverse effects, infusions should be started slowly, at rates not above 0.01 ml/kg/minute. Infusion rate may be increased to 0.08 ml/kg/minute in the absence of any reactions.[15] In addition, the minimum duration of the infusion should be at least three hours.[12]

We hereby evaluated the early adverse events in our PID patient cohort during hospital-based IVIG administration and aimed to define risk factors associated with AE.

Material and Methods

A total of 109 PID patients receiving 763 IVIG infusions between the years of 2014-2016 were enrolled in the study. Diagnosis of PID was based on criteria of European Society for Immunodeficiency (ESID) and Pan-American Group for Immunodeficiency (PAGID) and classified by using the charts in International Union of Immunodeficiency Societies.[16-18] The study protocol was approved by the local Ethics Committee of Marmara University (IRB number: 09.2015.136) and a written informed consent for patients was obtained from either adult patients and parents of the children.

Demographic data and infusion details including current age, age at diagnosis, final diagnosis and age at first IVIG treatment, IVIG dose, the number of previous IVIG treatments, use of premedication, duration of infusion, serum IgG levels (mg/dl) prior to and after IVIG were recorded. Documentation of a patient's baseline and bi-annual virologic status, complete blood cell count, hepatic and renal function tests, and urinalysis were documented during IVIG therapy. A complete physical examination was performed before each infusion. Patients self-infusing IVIG as home therapy were excluded. Patients who had severe infection requiring hospital admission and had concomitant IVIG infusion were not enrolled into the study as well. The concentrations of the products were 5% and 10%. Patients received IVIG with a dose of 300-800 mg/kg (median: 500 mg/kg) every 3-4 weeks and the infusion rate was started at 0.01 ml/kg/minute (equaling 0.5 mg/kg/minute of 5% solution or 1 mg/kg/minute of 10% solution) and increased to 0.08 ml/kg/minute (4 or 8 mg/kg/ minute of 5% or 10% solution, respectively) in the absence of any reactions. All infusions were performed under the supervision of physician and nurse. Adverse reactions were recorded by the same physician (EN). The early AE of IVIG infusion were defined as mild including fever, chills, headache, rash, pruritus, urticaria, abdominal pain, myalgia, back pain, moderate as hypertension, wheezing, chest pain and severe as hypotension, anaphylaxis and impairment of consciousness.[11]

The infusion was suspended if any mild AE occurred and patients were treated accordingly. In case any moderate or severe AE developed, IVIG infusion was ceased, symptoms were treated accordingly and IVIG brand was switched to another one. Premedication was only given to patients who developed previous moderate to severe adverse reactions. Prophylaxis involved the use of single or several agents including: methylprednisolone (IV, 1 mg/kg/dose, maximum 40 mg given immediately prior to the infusion), antihistamine (IV or per oral, pheniramine maleate, 1 mg/kg/dose), paracetamol (per oral, 10 mg/kg/dose) given up to 1 h prior to the infusion.

Statistical analyses

Data was described as frequencies and medians with minimum-maximum values unless otherwise indicated. Continuous variables were analyzed by Independent Student's t-test and Mann-Whitney U tests as appropriate. Differences between the groups were assessed by chi-square analysis for categorical variables. All analyses were performed by the Statistical Package for the Social Sciences (SPSS) program (Version 16.0; SPSS Inc., Chicago, IL, USA) using default settings. Statistical significance level was set as $p<0.05$.

Results

A total of 109 patients (32 girls, 29.4%; 77 boys, 70.6%) with 763 IVIG infusions were included. The mean age was 11.8 ± 5.7 years (0.6-33.5 years). Distribution of age groups was as follows: <18 years (n = 829 75.2% and ≥18 years (n = 27) 24.8%. Demographic, clinical and laboratory features of patients is shown in Table I. PID cohort consisted of 65 (59.6%) combined immune deficiency (CID) and 44 (40.4%) primary antibody deficient (PAD) patients. The CID cohort consisted of CID with pending molecular diagnosis (n = 29, 26.6%), ataxia telangiectasia (n = 9, 8.2%), hyper IgE syndrome (n = 7, 6.4%), CD4 lymphopenia (n = 7, 6.4%), immunodeficiency / centromeric region instability / facial anomalies syndrome (ICF; n = 3, 2.7%), hyper IgM syndrome (n =2, 1.8%), DiGeorge syndrome (n = 2, 1.8%), Bloom syndrome (n = 2, 1.8%), MHC Class II deficiency (n = 1, 0.9%), Cernunnos syndrome (n = 1, 0.9%), Nijmegen breakage syndrome (n = 1, 0.9%), and Wiskott-Aldrich syndrome (WAS; n = 1, 0.9%). The PAD cohort consisted of CVID (n = 16, 14.6%), unclassified antibody deficiency (n = 16, 14.6%), Bruton disease (n = 4, 3.6%), IgG2 subclasses deficiency (n = 2, 1.8%), IgG2 subclasses deficiency with IgA deficiency (n = 1, 0.9%) , activation induced cytidine deaminase (AID) mutation (n = 1, 0.9%), CD55 deficiency (n = 1, 0.9%), CD21 deficiency (n = 1, 0.9%), CD19 deficiency (n = 1, 0.9%), phosphatidylinositol 3- kinase, catalytic, delta (PIK3CD), and p110 mutation (n = 1, (0.9%).

The early AE of IVIG infusion were defined as mild including fever, chills, headache, rash, pruritus, urticaria, abdominal pain, myalgia, back pain; moderate as hypertension, wheezing, chest pain; and severe as hypotension, anaphylaxis and impairment of consciousness.[11] The early AE were recorded in 34 (4.5%) infusions among 763 IVIG infusions including 30 mild (88.2%), 3 moderate (8.8%) and 1 severe (2.9%). The distribution of the AE is given in Figure 1. The recorded AE were 18 (6.3%) in 65 PAD patients with 290 infusions, while 16 (3.3%) in 44 combined immune deficiency patients among 473 infusions; having a PAD among PIDs was found to increase the AE risk with an OR of 2.61 (95%CI 1.061-6.475; p = 0.037). The distribution of AEs between two groups is presented at Table II.

The most common AE were fever (10/34, 29.4%) and headache (10/34, 29.4%). Mild AE were managed by antipyretics, antihistaminics and

Table I. Demographic, clinical and laboratory characteristics of patients (N = 109).

Features	Results
Age, year*	12 (0.6-33.5)
Gender (female/male), n (%)	32 (29.4%) / 77 (70.6%)
Age at onset, year*	1 (0.1-18)
Age at diagnosis, year *	1 (0.2-21)
Duration of follow-up, year*	2.5 (0.1-15)
Age at first IVIG administration, year*	6 (0.1-20)
IVIG dose, g/kg/dose*	0.5 (0.3-0.8)
Duration of infusion, hour*	4.5 (3-6.3)
Serum IgG level at diagnosis, mg/dl*	576 (6-1430)
Serum through IgG levels, mg/dl*	956 (464-2390)
Respiratory infections within the last month, n/N (%)	28/763 (3.7%)
Antibiotic use within the last month, n/N (%)	16/763 (2.1%)
Hospitalization within the last month, n/N (%)	1/763 (0.13%)
Time between IVIG infusion and infection occurrence, day*	20 (1-49)

*: results are presented as median (minimum-maximum)

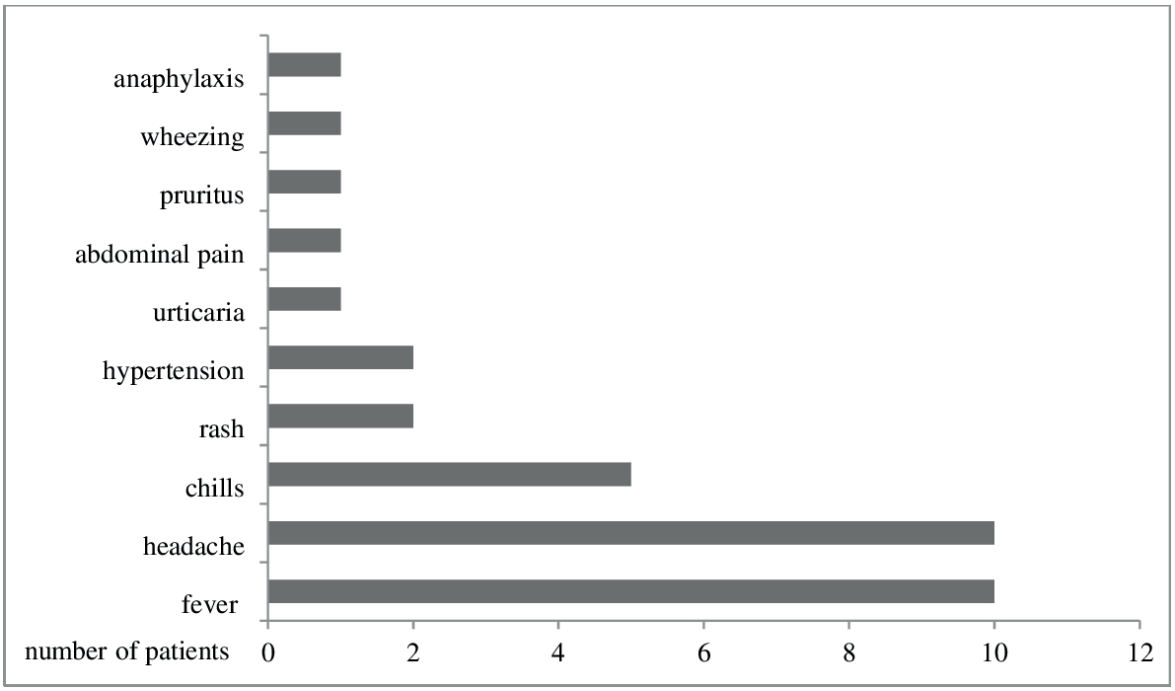

Fig. 1. Distribution of early adverse reactions to IVIG infusion.

Table II. Distribution of adverse events according to primary immune deficiency phenotype.

Adverse events	Primary antibody deficiency (N = 290 infusions in 65 patients)	Combined immune deficiency (N = 473 infusions in 44 patients)	p value
Mild	15 (5.2)	15 (3.2)	0.039
Fever, n (%)	3 (1.0)	7 (1.5)	>0.05
Headache, n (%)	10 (3.4)	-	<0.001
Chills, n (%)	1 (0.3)	4 (0.8)	>0.05
Urticaria, n (%)	1 (0.3)	-	>0.05
Pruritus, n (%)	-	1 (0.2)	>0.05
Abdominal pain, n (%)	-	1 (0.2)	>0.05
Rash, n (%)	-	2 (0.4)	>0.05
Moderate	3 (1.0)	-	>0.05
Wheezing, n (%)	1 (0.3)	-	>0.05
Hypertension, n (%)	2 (0.7)	-	>0.05
Severe	-	1 (0.2)	>0.05
Anaphylaxis, n (%)	-	1 (0.2)	>0.05
Total, n (%)	18 (6.2)	16 (3.4)	0.037

by decreasing the infusion rate. Moderate AE were documented in 3 patients and included increase in blood pressure (n = 2) and wheezing (n = 1); in these cases IVIG treatment was ceased; antihypertensive, short acting beta agonists and steroids were given. Only one patient experienced anaphylaxis and was treated with intramuscular epinephrine, antihistaminic and steroids.

Premedication was administered in 77 (10.1%) infusions in patients who developed adverse reactions during their previous infusions;

mild AE during the study period occurred in 6 (7.8%) of these infusions although they were receiving premedication. There was no significant difference between groups according to premedication use in the context of mild AE frequency, age at diagnosis, IVIG dose and infusion duration (Table III). The percentage of patients who received 5% IVIG among patients who required premedication was higher, compared to that of patients who did not require premedication (71/77, 92.2% vs. 531/686, 77.4%; OR 3.57, 95%CI 1.52-8.36; p = 0.002). Regarding various IVIG brands, number of adverse reactions for each brand were insufficient for accurate statistical analyses (Table IV).

The majority of patients (78.9%) were on IVIG products of 5% concentration. Nine AE were recorded in 161 infusions with IVIG products of 10% concentration (5.6%); 25 AE were recorded in 602 infusions with IVIG products of 5% concentration (4.2%; p>0.05).

Discussion

In this study, we evaluated 763 infusions in 109 patients with PID. The overall frequency of AE was 4.5% in which majority was mild reactions with a higher AE frequency in PAD group. Adverse reaction frequency was not found to be related to IVIG dose, duration and concentration. IVIG infusions with 5%

Table III. Comparison of patients according to use of premedication.

Features	Premedication		p value
	Yes (N = 77)	No (N = 686)	
Adverse events, n (%)	6 (7.8)	28 (4.1)	>0.05
Age at diagnosis, year	2 (0.08- 18)	0.66 (0.08-18)	>0.05
IVIG dose, g/kg/dose	0.57 ± 0.94	0.50 ± 0.84	>0.05
IVIG infusion duration, hours	5.2 ± 0.5	4.9 ± 0.6	>0.05
10% IVIG concentration, n (%)	6 (7.8)	155 (22.6)	0.002
5% IVIG concentration, n (%)	71 (92.2)	531 (77.4)	

IVIG: intravenous immunoglobulin
Premedication was administered in 77 infusions in patients who developed adverse reactions during their previous infusions.

Table IV. Adverse events associated with various IVIG brands and concentrations.

Adverse events	Brands (concentration)							Total
	Ig vena (5%)	Tegeline (5%)	Nanogam (5%)	Octagam (5%)	Phlebogamma (5%)	Kiovig (10%)	Gamunex-c (10%)	
Number of infusions, n	116	161	88	150	87	115	46	763
Fever, n (%)	1 (0.86)	1 (0.62)	3 (3.41)	1 (0.66)	2 (2.30)	2 (1.74)	-	10 (1.31)
Headache, n (%)	-	-	-	4 (2.66)	2 (2.30)	4 (3.48)	-	10 (1.31)
Chills, n (%)	-	1 (0.62)	1 (1.14)	3 (2.00)	-	-	-	5 (0.65)
Rash, n (%)	-	-	-	1 (0.66)	-	1 (0.87)	-	2 (0.26)
Hypertension, n (%)	-	-	-	1 (0.66)	1 (1.15)	-	-	2 (0.26)
Abdominal pain	-	-	-	-	-	1 (0.87)	-	1 (0.13)
Wheezing, n (%)	-	-	-	1 (0.66)	-	-	-	1 (0.13)
Itching, n (%)	-	-	-	1 (0.66)	-	-	-	1 (0.13)
Urticaria, n (%)	-	1 (0.62)	-	-	-	-	-	1 (0.13)
Anaphylaxis	-	-	-	-	-	1 (0.87)	-	1 (0.13)
Total events, n (%)	1 (0.86)	3 (1.86)	4 (4.55)	12 (8.00)	5 (5.75)	9 (7.83)	-	34 (4.46)

concentration was found to be frequent among infusions with premedication use.

The mean frequency of AE seen during IVIG treatment was reported to be 20% changing from 1 to 81%.[7-10] Galli et al.[19] reported 40% of AE seen in PID children. In addition, Dashti-Khavidaki et al.[20] documented 216 AE (7.2%) in 3,004 infusions for 13 years. In our cohort, the overall frequency of AE was 4.5% in which the majority was mild reactions with a higher AE frequency in PAD group. This data was compatible with the higher rate of AE reported for CVID group by Dashti-Khavidaki et al.[20] This entity was reported to be related to the generation of anti IgG and anti IgA antibodies in this group of patients.[21,22]

The recorded mild AE in our cohort consisted of mostly fever, headache and chills which were similar to data reported at previous studies.[20,23] The symptoms were managed with a decrease in infusion rate, antihistaminics, low dose steroids and antipyretics. Although not well clarified, the most reasonable cause of the fever was postulated to be an immune complex driven reaction.[24] The headache seen during IVIG infusion was asserted to be related to aseptic meningitis which can be controlled with antihistaminics, anti-inflammatory drugs and with decreased infusion rates.[25] It was also reported that higher doses and concentrations may increase headache ratios.[25,26] In our cohort, headache was not found to be related to infusion rate, dose or concentration.

In a study showing that frequency of infusions associated with AE was lower with the 5% concentration; the type, seriousness, and severity of AE detected were similar for both 5% and 10% concentrations of same brand.[27] Headache and fever were reported as most common AE in 10% concentrations of IVIG.[27,28] In our study we observed that headache and fever were most common AE for 10% concentration, whereas no differences were detected between IVIG concentrations. In another cohort, Souayah et al.[29] showed that premedication decreased AE seen in the home infusion of IVIG in patients with neuroimmunologic disorders. In our cohort, 77 infusions were given with premedication in patients who had a history of adverse events; adverse events was not observed in 71 of them (92.2%).

Kaba et al.[23] compared the rate of adverse events between various IVIG solutions which showed no difference. Our patients received 7 different brands of IVIG with various rates of adverse reactions. These data agree with a prior study data finding that preparations are not equally tolerated even with similar concentration.[30] If patients persistently develop adverse events following administration with a particular IVIG product, switching to another immunoglobulin product may result with fewer AE and safer infusions.[30]

Expression of a mutation of novel gain-of-function splice variant of the FcRIIa receptor in patients with CVID is reported to be associated with pro-inflammatory signaling toward IgG, which then induces recurrent anaphylactic reactions to IVIG.[31] We documented severe AE as anaphylaxis in only one patient. Among previous reports, no severe AE were recorded in 16,223 applications,[32,33] whereas one study reported severe AE in 3 patients with 2 of them evaluated as anaphylaxis.[20]

Adverse events are particularly likely in a patient who has not been given IVIG previously. A survey by the Immune Deficiency Foundation (IDF) displayed that as many as 34% of adverse reactions occurred during the first infusion of an IVIG product[34] with another study noting 7%.[20] It was also reported that switching among different brands increase the risk of AE.[35] Similar to the IDF's report, AE detected were noted in earlier infusions but not at first infusion among our cohort (data not shown). The main reasons for initial high reaction rates that reduce with subsequent doses of the same product are unknown. Therefore, the first infusion recommended to be given slowly at a dose of 0.5 to 1.0 mg/kg/min.[36] In addition, in infected patients, high rates of adverse reactions are related to the pattern of antigen–antibody

complexes, and rates can be reduced if the patient is apyrexial or receiving antibiotics.[37,38]

Our study has shown that adverse events during hospital based IVIG infusions are infrequent and that IVIG preparations and concentrations are equally tolerated. Use of various intravenous immunoglobulin treatments should be considered with regard to side effect profiles observed. In our cohort, PID patient experienced mild AE in the presence of PAD disorder and demanded antihistaminic premedication for 5% IVIG infusions. Therefore, identification of risk factors, use of adjunctive therapies for adverse events and trained medical supervision are measures to provide safe use of this medication.

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Original Article

Retrospective evaluation of childhood paraphenylenediamine intoxication due to black henna

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ABSTRACT

Background and objectives. Paraphenylenediamine (PPD) is a toxic substance in henna. Oral intake of this substance causes severe systemic toxicity. To the best of our knowledge there are no studies in the literature conducted only on children exposed to henna intoxication.

Methods. Twenty-three patients aged between 1 and 17 who referred to Nyala-Sudan Turkey Training and Research Hospital between May 2015 and June 2018 were evaluated retrospectively in terms of demographic, clinic and laboratory characteristics.

Results. Four (17.39%) patients were male and 19 (82.61%) were female. Average age of patients was 10.95 ± 3.2. Most of the referrals to the hospital following PPD intoxication occurred in the first 16 hours. All of the patients between 10 and 17 had taken henna for suicidal purposes. All patients had vomiting and agitation. The most common symptoms apart from these were gastrointestinal symptoms, tachycardia, tachypnea and dyspnea. Twelve (52.17%) patients had elevated liver function tests and 3 (13.04%) had developed renal failure. None of the patients had neurological complications. Two (8.70%) patients developed a need for tracheostomy. Average hospitalization period of patients was 8.5 days. Two patients died. One was in 1-5 age group and died due to renal complications, while the other was in 6-10 age group and died due to hepatic failure.

Conclusion. PPD intoxication is a life-threatening situation even in low doses. For this reason, even asymptomatic cases should undergo physical examination and should be followed closely in terms of respiratory tract obstruction. Ensuring hydration and diuresis in the early period, steroid and adrenalin therapy for prophylaxis in terms of respiratory tract obstruction are important and tracheostomy should not be abstained in necessary cases. It should not be forgotten that symptomatic treatment for organ systems and dialysis will decrease mortality and morbidity.

Key words: childhood, paraphenylenediamine, henna, intoxication.

Paraphenylenediamine (PPD) is a toxic substance found in henna which is used as traditional dye to give hair a dark color. Accidental or suicidal intake of henna causes severe systemic toxicity.[1] The primary substance giving rise to this situation is PPD.[2] Toxicity caused by henna is very common in Sudan. Globally, suicidal deaths have been found to increase 60% in the last 50 years.[2] Large-scaled studies have shown that PPD is responsible for 35% of suicidal deaths in Sudan.[3] The high rate of suicide with henna can be due to the fact that it is cheap and easily accessible or owing to previous suicide experiences.

Clinical course is various in toxications with PPD. However, there is no antidote and the intoxication may lead to mortality unless early intervention is initiated.[4] Although the lethal dose has been reported as 10 gram after oral intake and enteral absorption of PPD, it has also been reported lethal even in very small doses in some cases.[3] Suicidal henna intoxication generally occurs due to oral intake. The main part of the treatment consists of supportive treatment and it is an emergent medical

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condition. Symptomatic treatments include providing airway patency, endotracheal intubation, and tracheostomy if necessary, gastric lavage, providing fluid electrolyte balance and hemodialysis in case of renal failure.[2,4,5] To the best of our knowleadge there are no studies in literature evaluating exposure to henna intoxication including the pediatric age group only. The aim of this study was to evaluate the effects of henna intoxication in children.

Material and Methods

The present study protocol was approved by Nyala Sudan- Turkey Education and Research Hospital Ethics Commitee's (NSTH.03/903.07.03-09).

This study was a retrospective cohort study and files of 23 patients aged between 1 and 17 years who were referred to the Nyala-Sudan Turkey Training and Research Hospital between May 2015 and June 2018 were reviewed retrospectively. The patients were divided to three groups according to age as group 1; 1-5 years, group 2; 6-10, and group 3; 11-17 years. Patients' gender, marital status, pregnancy, reason for intoxication (suicidal/accidental/homicidal) were questioned. The time of referral to hospital was grouped as 0-8 hours, 9-16 hours, and 17 hours and more. Laboratory parameters; complete blood count, serum biochemistry (urea, creatinine, aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin, direct bilirubin), serum electrolytes (sodium, potassium, chlorine, calcium), coagulation parameters; prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT), , blood gas (pH, partial carbondioxide pressure, bicarbonate, anion gap) and total urine test were evaluated based on laboratory reference values of the hospital. Electrocardiogram (ECG) records were obtained from all patients. The patients were questioned for psychiatric diagnoses, known before referral for intoxication and evaluated in terms of mortality and morbidity. Complications were grouped as cardiovascular, respiratory, gastrointestinal, neurological, and renal. The methods of treatment for complications were grouped as medical treatment (adrenalin, steroid, hydration and diuresis), surgical treatment, and dialysis. All statistical analysis was conducted with the SPSS (Statistical Package for Social Sciences) for Windows version 19.0 release (IBM, Chicago, IL). Frequency and averages were calculated by using descriptive statistic tests.

Results

Of the 23 patients, 4 (17.39%) were male, 19 (82.61%) were female. There were 2 (8.70%) patients in 1-5 years age group 1, 8 (34.78%) in 6-10 years age group 2, and 13 (56.52%) in 11-17 years age group 3. Average age was 10.95±3.20 years. Three (23.07%) patients in group 3 were married, while 10 (76.92%) were single. One patient was pregnant. Average hospitalization period was 8.48 ± 1.12 days. In 13 (56.52%) of the patients' hospitalization time was more than 9 days. Demographic, epidemiological and clinical data of the patients are given in Table I. The patients mostly referred to the hospital within 16 hours following PPD intoxication (Table I). Only 4 had referred to the hospital in 17 hours and more. Average period of referral was 8.78 ± 1.9 hours. All the patients in group 3 had ingested henna for suicidal purposes, while the others had ingested accidentally. The way of henna ingested was oral in all patients. None of the patients had previous psychiatric diagnosis. It was found that symptoms of henna toxication started 3 hours or longer in 47.83% of the patients after ingestion, and 76.92% of these patients were in group 3. The symptoms started with in the first hour of intoxication in all the patients of the group 1, and 75% of the group 2. Vomiting and agitation were observed in all patients. The other most common symptoms were gastrointestinal symptoms, tachycardia, tachypnea and dyspnea. All children in group 1 had tachycardia, tachypnea and dyspnea. Angioedema was not observed in group 1 but it was found in 7 (30.43%) patients in other

Table I. Demographic, epidemiological and clinical data of the patients.

	Patients (n:23)		Group 1 (n:2)		Group 2 (n:8)		Group 1 (n:13)	
	Number (n)	Percentage (%)	Number (n)	Percentage (%)	Number (n)	Percentage (%)	Number (n)	Percentage (%)
Sex								
Male	4	17.39	1	50	3	37.50	0	0
Female	19	82.61	1	50	5	62.50	13	100
Marital status								
Married	3	13.04	0	0	0	0	3	23.07
Single	20	86.96	2	100	8	100	10	76.92
Pregnancy								
Yes	1	5.26	0	0	0	0	1	8.33
No	22	94.74	2	100	8	100	12	91.67
Referral time								
1-8 hours	13	56.52	1	50	6	75	6	46.15
9-16 hours	6	26.08	1	50	2	25	3	23.08
17 hours and more	4	17.4	0	0	0	0	4	30.77
Type of intoxication								
Suicidal	13	56.52	0	0	0	0	13	100
Accidental	10	43.48	2	100	8	100	0	0
Way of intake								
Oral	23	100	2	100	8	100	13	100
Other	0	0	0	0	0	0	0	0
Symptom onset time								
First 1 hours	9	39.13	2	100	6	75	1	7.69
1-3 hours	3	13.04	0	0	1	12.50	2	15.39
4 hours and more	11	47.83	0	0	1	12.50	10	76.92
Symptoms								
Gastrointestinal	17	73.91	1	50	6	75	10	76.92
Tachycardia	16	69.57	2	100	6	75	8	61.53
Tachypnea	15	65.22	2	100	5	62.50	8	61.53
Vomitting	23	100	2	100	8	100	13	100
Angioedema	7	30.43	0	0	3	37.50	4	30.76
Wooden tongue	2	8.70	0	0	0	0	2	15.38
Facial edema	5	21.74	1	50	1	12.50	3	23.07
Dyspnea	14	60.87	2	100	6	75	6	46.15
Agitation	23	100	2	100	8	100	13	100

*n: Number of patients

age groups. Wooden tongue was found only in 2 patients in group 3. Laboratory data of the patients was given in Table II. Twelve (52.17%) patients had elevated liver function tests, and in 3 of them (13.04%), the values were twice that of the normal level. The blood urea and creatinine levels were high in 7 (30.43%) patients. Metabolic acidosis nonresponsive to treatment and renal failure requiring dialysis developed in 3 (13.04%) of the 7 patients. Serum electrolyte imbalance (imbalance in at least one of the serum sodium, potassium, chlorine, calcium values) was observed in 5 (21.73%) patients. In terms of complete blood count

Table II. Laboratory test results of the patients.

Laboratory tests	Patients (n:23)	
	Number (n)	Percentage (%)
Serum biochemistry		
Elevated transaminases	12	52.17
Elevated urea/creatinine	7	30.43
Electrolyte imbalance	5	21.73
Coagulation		
Elevated INR**	9	39.13
Complete blood counting		
Leucocytosis	6	26.08
Eosinophilia	8	34.78
Thrombocytopenia	3	13.04
Urine analysis		
Proteinuria, hematuria, glucosuria	6	26.08

*n: Number of patients
**INR: International normalized ratio

parameters, leukocytosis was observed in 6 (26.08%) patients, eosinophilia was found in 8 (34.78%) patients, and thrombocytopenia was observed in 3 (13.04%) patients. Nine (39.13%) patients had elevated international normalized ratio (INR) values. Proteinuria, glucosuria and hematuria were observed in urine tests of 6 (26.08%) patients. ECG of 3 (13.04%) patients showed prolonged PR interval. Table III demonstrates treatment methods, complications and mortality of the patients. Seven (30.43%) patients had renal, 7 (30.43%) patients had respiratory, 3 (13.04%) patients had cardiovascular, and 3 (13.04%) patients had hepatic complications. None of the patients had neurological complications. All patients received a nasogastric catheter and gastric lavage was performed. Methylprednisolone was given to all patients, as was intravenous (IV) hydration and furosemide treatment to increase diuresis. Four (17.39%) patients received intramuscular (IM) adrenalin, 10 (43.47%) patients received symptomatic treatment for cardiopulmonary symptoms. Tracheostomy was performed on 2 (8.70%) patients. All patients were followed in the intensive care unit on the first three days. One patient (4.34%) in group 1 had died due to

Table III. Data of treatment options, complications and mortality of the patients.

Treatment	Patients (n:23)	
	Number (n)	Percentage (%)
Medical		
Gastric lavage	23	100
Methylprednisolone	23	100
Hydration	23	100
Furosemide	23	100
Adrenalin	4	17.39
Symptomatic treatment for cardiovasculary symptoms	10	43.47
Surgery	-	-
Tracheostomy	2	8.70
Dialysis	3	13.04
Complications		
Renal	7	30.43
Respiratory	7	30.43
Cardiovasculary	3	13.04
Hepatic	3	13.04
Neurologic	0	0
Mortality		
Renal failure	1	4.35
Hepatic failure	1	4.35
Total	2	8.70

*n: Number of patients

the renal complications, and one patient (4.34%) in group 2 died due to the hepatic failure.

Discussion

Paraphenylenediamine (PPD) is the main toxic substance in henna which is used for dying hair, hands and nails in Sudan.[1,2] PPD is an oxidative chemical; intoxications with this substance can cause hypersensitivity reactions and systemic side effects.[6-8] While there are studies about toxications with several substances in different geographies in Africa, suicidal PPD intoxications have been reported in Eastern African countries such as Sudan.[8]

Similar to the previous literature, most cases, especially in older children were suicidal. In Sudan, high rate of suicide was associated

with difficulty of life conditions, stress, low socioeconomic level, strict cultural rules, and psychological problems.[3] It has been reported that single people were frequently exposed to PPD intoxication, and attempt for suicide when compared with the married ones.[3] One of our patients was exposed to PPD intoxication in pregnancy. With early and effective treatment, neither the baby nor the mother had any complications. There is insufficient data about PPD intoxication in pregnancy in the literature.

The average hospital referral time was 8.78 ± 1.9 hours. In PPD intoxication, symptoms generally occur within the first hours, and may result in mortality and morbidity. Hospital referral times of the patients were relatively late in our study. Late referral time was considered to be associated with low socio-economic and education level, and transportation difficulties to emergency departments of hospitals.

In some patients, tracheostomy may be required due to obstruction in upper airway. The need for tracheostomy has been reported in higher rates for both adults and children in literature.[9-11] The patients who applied to our hospital after 17 hours or more due to intake of PPD was for 17% (4 patients). Two of the four patients applied to the hospital between 17 and 24 hours, and they did not need tracheostomy. The other two patients were admitted to our hospital after 48 hours, with severe respiratory obstruction findings and they underwent tracheostomy. In our study, the low number of patients with tracheostomy was considered to be due to a few number of patients admitted after 24 hours. This situation gives rise to thought that early admission and effective treatment may be related to decreased tracheostomy need.

In a study of 200 patients from Sudan, it was found that PPD intoxication peaked between 14 and 27 years of age and was generally taken for suicidal purposes.[3] In our study, PPD toxicity was observed more frequent in group 3. Similar to the other studies, in our study PPD was mostly taken orally. Other studies have shown that after high amount of henna intake for suicidal purposes, symptoms started generally within one hour.[3] Whereas symptoms started later (three or more hours) in our study. In Africa, especially in undeveloped countries such as Sudan, difficult economic conditions and low education levels have unfavorable effects to life styles of people. For this reason, many people try to overcome from their many medical conditions by themselves without hospital admission. In association with their religious beliefs, which is extremely fatalistic, African people have high tolerans levels when compared with western people, who usually prefer refer to seek medical care immediately. Older patients try to tolerate their complaints such as pain due to their beliefs as a result of their culture, and express these only when they become unbearable. In the younger age group (group 1 and 2), symptoms started in the first hour, similar to literature. Although there are no studies in the literature covering patients only in pediatric age group, an adult study including pediatric patients demostrated that accidental intake was more frequent in the pediatric age group.[9] The behaviour of recognizing objects by taking them into mouth especially within the first 3 years of age plays the main role in accidental PPD intake. There were also studies reporting that suicide rate was higher in puberty.[12,13] While the rate of suicidal PPD intake was 56.52% patients in our study, the rate of accidental intake was 43.48%.

Most frequent problems following PPD intoxication were reported as upper respiratory tract problems, hypersensitivity reactions, rhabdomyolysis resulting in renal failure, cardiac disorders presenting with dysrhythmia and liver disease presenting with elevated liver transaminases. Similar to the other studies, in our study, respiratory diseases were the most common complication which was followed by renal, cardiovascular, and lung complications.[3,8,9] In 82% of patients exposed to PPD, chocolate brown dark urine was reported due to hemolysis and rhabdomyolysis. While there are studies in literature reporting dialysis need following oliguria in adult patients

exposed to PPD, 3 (13.04%) of the patients received dialysis in our study.[9] In De Groot's study[14], it was reported that allergic contact dermatitis with the use of hair dyes including PPD or related chemicals could develop and such reactions could require hospitalization especially in children. Since cross-reactivity can develop for other hair dyes, textile paints, local anesthetics and rubber chemicals in most of those sensitized to PPD, contact with these materials should also be avoided.[14] It was reported that due to increased use of temporary black henna tattoos and inadequacy in the legal control of henna tattooing practice, an increase in PPD sensitization cases would be inevitable in the future and thus black henna tattooing should be controlled by health authorities.[14] However, it was remarkable in our study that PPD toxicity induced skin symptoms following oral intake were not seen in any of our patients.

The main steps of the treatment following PPD intoxication are providing continuation of airway, breathing, and circulation, gastric lavage following by nasogastric tube placement, hydration and providing forced diuresis to decrease the intensity of toxic substance in the plasma, close monitorization and symptomatic treatment in the intensive care unit for possible complications.[9] Ten (43.47%) patients received treatment for cardiovascular symptoms, 4 (17.39%) patients received adrenalin for severe angioedema not responding to methylprednisolone, 2 (8.7%) patients were applied tracheostomy for establishment of a more stable airway, and 3 (13.04%) patients received dialysis. Antihistamines and steroids are commonly used in the management of airway edema because of the possibility of a hypersensitivity reaction to PPD but there is no clinical evidence to support this type of treatment.[15] Also, in our study severe angioedema did not respond to steroids and we had to use adrenaline for treatment. In case of repiratory system complications, tracheostomy is a life saving procedure.[16]

In 4 studies including all age groups mortality rates were found as 33.30%, 11.90%, 9.50%, and 3.50%.[3,17-19] These lower rates were explained with early referral to hospital and detoxification treatment given to patients.[3,17-19] In a study by Elgamel et al.[3], mortality occured due to respiratory obstruction in 71.40% of the patients, and renal failure in 28.60% of the patients additionally mortality rate was found higher in the pediatric age group. In our study, mortality occured in two (8.70%) patients because of renal and hepatic failure. We explained low mortality rates with relative small number of our cases, providing airway safety promptly, effective diuresis and hydration, appropriate symptomatic treatment and close monitorization in the intensive care unit and early performing of dialysis and tracheostomy. In intoxications with PPD, mortality rates are found higher in the pediatric age group. These high rates can be explained by low tolerability of intoxication in childhood owing to inadequate metabolism and remove of the toxins.

In conclusion, to the best of our knowleage our study is the first in the literature including the pediatric age group only. PPD intoxication is a life-threatening situation even in low doses. Even in patients who are asymptomatic during referral patients should be carefully monitored for airway obstruction, renal, hepatic and cardiovascular complications. In the treatment, early hydration and forced diuresis is important. To prevent respiratory tract obstruction, IV steroids and when necessary IM adrenalin treatment can be applied for prophylaxis. Tracheostomy should not be avoided in necessary cases. Symptomatic treatments for organ failure and especially dialysis in case of renal failure can decrease mortality and morbidity.

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Original Article

Is there any relationship between initial hematological parameters and severity of scorpion envenomation?

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ABSTRACT

Background and objectives. Most cases of severe scorpion envenomation occur in children and are associated with significant morbidity and mortality. Excessive systemic inflammatory response, which leads to multiple organ involvement, is an emerging challenge during severe envenomation. The aim of this study was to investigate if there was any relationship between initial hematological parameters and severe envenomation in pediatric patients presenting with scorpion envenomation.

Method. This study was performed retrospectively, at the pediatric emergency unit and pediatric intensive care unit of the Çukurova University Medical School in Turkey. Two hundred and fifty-seven cases with scorpion envenomation, and a control group consisting of one hundred and fifteen healthy children were included in the study.

Results. White blood cell, neutrophil, lymphocyte, platelet, neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR) and PDW values of patients were higher than the controls ($p<0.05$). Mean NLR was 3.8 ± 4.7 in patients. Patients were analyzed with the help of the decision tree model, and it was seen that in patients who had applied to hospital in less than an hour after the scorpion sting, 87.5% of the patients whose NLR value was between the 0.519-1.969 interval (below 2.1 which we found as the cut-off value) did not need to be hospitalized in the intensive care unit, 54.1% of the patients whose NLR value was higher than 1.969 needed to be hospitalized at the intensive care unit.

Conclusions. Severe envenomation is associated with mortality and morbidity in children. Our findings showed that NLR seems to be a useful tool in predicting severe envenomation.

Key words: children, scorpion, severe envenomation, neutrophil/lymphocyte ratio.

Scorpion envenomation has been a medical problem for all continents (except for Antarctica) for centuries. It is more common in Central and South America, North Africa, the Middle East and South Asia.[1] There are 1753 types of scorpions known worldwide and 23 types in Turkey. The properties of the scorpion, such as type, age, size, and nutritional status, determine the severity of the envenomation. Also, the number of stings, depth of venom injection, location of stings in proximity to the head and neck, and age and health status of the victim determine the severity of envenomation.[2]

Depending on the scorpion, 66 to 90 percent of stings have signs and symptoms limited to local pain, paresthesias, and skin changes without systemic effects.[3] Systemic effects become apparent in 10 to 33 percent of patients. After envenomation, symptoms may begin immediately and typically progress to maximum severity within 5 hours. The clinical effects of stings are characterized

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by autonomic, cardiovascular, neurological and gastrointestinal effects. All of these are responsible for severe envenomation. Cardiovascular effects are atrial tachycardia, ventricular extrasystoles, T-wave inversion, ST-T wave changes, bundle-branch block. Catecholamine-induced myocarditis and myocardial ischemia results in pulmonary edema and cardiogenic shock.[4-6] Neurological effects: systemic envenomation is characterized by neuromuscular abnormalities resulting from effects on the somatic and cranial nerves. These include local pain and paresthesias, unexplained agitation or inconsolable crying, dysphagia, drooling, abnormal eye movements with blurred vision, slurred speech, tongue fasciculations, restlessness, fasciculations, shaking and jerking of the extremities, alternating opisthotonos and tetanic forward flexion of the body.[7] Gastrointestinal effects are vomiting, abdominal pain and diarrhea. Also, acute pancreatitis has been reported.[5,8]

Cardiovascular toxic effects and acute pulmonary edema are the most important complications of scorpion stings and the most frequent cause of death in the first 24 hours after the sting. Most cases of severe envenomation occur in children and are associated with morbidity and mortality.[9] Although there is no accurate data worldwide, a 20% mortality rate is reported in untreated infants and 10% in untreated school-aged children.[10]

Many studies have been conducted recently with the help of hematological parameters, especially, the ratio between the absolute number of neutrophils and the number of lymphocytes has been considered as a potential new biomarker predicting the worse clinical course of cancer, infectious diseases, cardiovascular diseases, end-stage renal disease, immunologic diseases, and schizophrenia. The platelet/lymphocyte ratio is also used to determine inflammation.[11-17]

As it is known, pro-inflammatory and anti-inflammatory cytokines and mediators are excreted in scorpion stings. The balance between the pro-inflammatory and anti-inflammatory mediators, in other words, the excessive systemic inflammatory response of the host determines the severity of the envenomation.[18] Therefore, the aim of this study was to investigate if there was any relationship between initial mean platelet volume (MPV), platelet distribution width (PDW), neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR) and severe envenomation in pediatric patients presenting with a scorpion sting.

Material and Methods

This study was performed retrospectively (between May 3, 2007, and December 31, 2018), at the pediatric emergency unit and pediatric intensive care unit of Çukurova University Medical School in Turkey. Two hundred and fifty-seven cases consisting of patients aged 18 years or younger who were treated with scorpion stings, and a control group consisting of one hundred and fifteen healthy children were included in the study. Children with infectious or inflammatory diseases and chronic disorders were excluded from this study's control group.

The study protocol was approved by the local institutional ethics committee (04.01.2019/84) and was performed in accordance with the Helsinki Declaration.

Age, gender, weight, time and date of presentation, place of first referral, types of scorpions, location, time and number of stings, region of residence (urban or rural), clinical signs and symptoms, administration of antivenom, number of antivenoms, treatment, hospitalization, length of hospital stay, and results were recorded for all patients. The patients were treated with antivenom against the Androctonus Crassicauda produced by Refik Saydam Hygiene Center Presidency.

Hematological parameters, which were obtained easily and economically through complete blood counts, to investigate the relation between the severity of the disease and prognosis were: White blood cells (WBC),

neutrophil, lymphocyte, platalate count (PLT), MPV, PDW, NLR and PLR were calculated from the complete blood count at admission.

Myocarditis was determined as follows: one finding of cardiac insufficiencies, such as tachycardia, murmur, gallop rhythm, or muffled heart sounds, and at least one of the following: change on ECG, altered cardiac function at Echocardiogram (ECHO), or an increase in troponin levels.[19-20] ECHO examinations were performed by a pediatric cardiologist.

According to Abroug's previous classification, our patients were categorized based on clinical findings.[21] According to this, the patients with local signs only were classified as stage I, the patients with local signs extending to the proximal side and/or mild systemic signs were classified as stage II, and the patients with life-threatening systemic signs were classified as stage III.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics Version 20.0 statistical software package. Categorical variables were expressed as numbers and percentages, whereas continuous variables were summarized as mean and standard deviation and as median and minimum-maximum where appropriate. For comparison of more than two groups, Oneway ANOVA or Kruskal Wallis test was used depending on whether the statistical hypotheses were fulfilled or not. To evaluate the correlations between measurements, the Pearson Correlation Coefficient or Spearman Rank Correlation Coefficient was used depending on whether the statistical hypotheses were fulfilled or not. A receiver operator characteristic (ROC) curve analysis was performed in order to identify the optimal cut-off point. Decision tree analysis with 10 fold cross-validation was applied to predict the probability of PICU stay. The statistical level of significance for all tests was considered to be 0.05.[22]

Results

We enrolled 257 patients (145 male), and 115 controls (72 male) in the study. There were no differences in age and gender between the patient and control groups. WBC, neutrophil, lymphocyte, platelet, NLR, PLR and PDW values of the patients were higher than the control group's ($p < 0.05$). Demographic and hematologic parameters of patients and control groups are shown in Table I.

According to Abroug's classification, patients were divided into three stages. There was a difference only for NLR value which from stage 1 to 3, the NLR was increasing and it was statistically significant ($p < 0.001$) but there was no difference for PLR, MPV, and PDW between the stages 1, 2 and 3 ($p > 0.05$).

There was a statistically significant difference for NLR and PDW values when the patients were separated according to rural or urban areas. The NLR and PLR values of the patients who were admitted to the hospital more than 1 hour after a scorpion sting were significantly higher (respectively $p < 0.001$, $p < 0.001$). Scorpion species were classified as yellow, black and unknown. PDW was higher in unknown species of scorpion stings ($p < 0.001$). Patients were evaluated in terms of the number of stings and the location of stings but there were no differences for NLR, PLR, MPV, and PDW ($p > 0.05$). Patients who were stung by scorpions at night had higher PLR values ($p = 0.029$). Patients who were given antivenom and who had myocarditis had higher NLR values (respectively $p < 0.001$, $p < 0.001$). The NLR and PLR values of the patients who were admitted to the pediatric intensive care unit (PICU) were significantly higher (respectively $p < 0.001$, $p = 0.020$), and PDW values were lower ($p = 0.004$). A comparison of epidemiological and clinical characteristics with hematological parameters in patients are shown in Table II.

Table I. Demographic, and hematologic parameters of patients and controls.

	Patient (n= 257) mean±SD median (min-max)	Control (n= 115) mean±SD median (min-max)	p
Age (Month)	7.9 ± 5.1	7.8 ± 5.2	0.565
	7 (0.2-19)	6.9 (0.2-0.9)	
Sex			
Male	145	72	0.951
Female	112	43	
WBC (10^3 μL)	13.9 ± 8.1	8.8 ± 2.5	<0.001
	11.6 (2.1-46.4)	8.2 (4.5-16.9)	
Neutrophil (10^3 μL)	8.2 ± 5.8	4.1 ± 1.7	<0.001
	6.3 (0.2-31)	3.8 (1.3-9.4)	
Lymphocyte (10^3 μL)	4.4 ± 4.4	3.7 ± 1.9	0.030
	3.1 (0.4-25.4)	3.1 (1.1-11.8)	
Platelete (10^3 μL)	345.9 ± 126.7	313.1 ± 70.1	0.002
	320 (110-779)	326 (164-490)	
NLR	3.8 ± 4.7	1.4 ± 0.9	<0.001
	1.9 (0.04-35.5)	1.3 (0.2-6.1)	
PLR	137.6 ± 109.7	99.8 ± 39.3	<0.001
	96.9 (14.7-559.7)	98.0 (21.3-212.6)	
MPV (fl)	8.0 ± 1.4	8.2 ± 1.3	0.189
	7.7 (5.7-14.9)	7.9 (6.2-13.1)	
PDW (fl)	21.1 ± 13.4	16.0 ± 4.1	<0.001
	16.4 (7.7-74)	16.3 (8.2-53.7)	

WBC: white blood cell, NLR: neutrophil/lymphocyte ratio lymphocyte, PLR: platelet/lymphocyte ratio, MPV: mean platelet volume, PDW: platelet distribution width.

Mean NLR was 3.8 ± 4.7 and was median: 1.9 (minimum: 0.04- maximum: 35.5) in patients (n= 257). One hundred and thirty-five patients (135/250) were admitted to the PICU. ROC curve analysis revealed that, in predicting PICU stay, the area under the curve for NLR was 0.643 (95% CI 0.575-0.711), p<0.001 (Fig. 1). The optimal cut-off point for NLR was obtained as 2.1 with 65.4% sensitivity and 70.5% specificity. There was a poor correlation between the length of PICU stay and NLR (p <0.001, r= 0.225). There was no association between length of PICU stay and PLR, MPV, PDW (p >0.05 for all).

Myocarditis was seen in 36 of the patients (36/135) admitted to the PICU. ROC curve analysis revealed that, in predicting myocarditis, the area under the curve for NLR was 0.671 (95% CI 0.560-0.782), p <0.001 (Fig. 2). The optimal cut-off point for NLR was obtained as 2.5 with 69.4% sensitivity and 64.2% specificity.

The decision tree analysis with 10 fold cross-validation was applied. The decision model generated from the dataset which is shown in Figure 3. All patients were divided into five subgroups (five nodes) from root node (PICU, non PICU) to time of admission (1 hour, more than 1 hour) and finally to NLR (≤0.519, 0.519-1.969, ≥1.969). The probability of PICU stays varied from 12.5% to 75.2%. For example, 71.8% of the patients who were admitted within the

Table II. A comparison of epidemiological and clinical characteristics with hematological parameters in patients.

	NLR mean±SD median (min-max)	PLR mean±SD median (min-max)	MPV (fl) mean±SD median (min-max)	PDW (fl) mean±SD median (min-max)
District	4.5 ± 5.5	144.3 ± 116.2	8.1 ± 1.3	15.8 ± 5.0
Urban (n=135)	2.4 (0.1-35.5)	100 (14.7-559.7)	8.0 (5.7-11.9)	16.2 (7.7-46.6)
Rural (n=122)	2.9 ± 3.1	130.2 ± 102.1	7.9 ± 1.4	25.7 ± 16.4
	1.5 (0.04-21.1)	93.7 (26.8-491.5)	7.5 (5.7-14.9)	16.8 (8.9-74)
p	0.003	0.303	0.374	<0.001
Time to admission	2.5 ± 3.2	107.8 ± 83.2	8.1 ± 1.4	20.4 ± 13.2
1 Hour (n=124)	1.3 (0.1-21.1)	83.4 (27.3-469.6)	7.7 (5.7-14.9)	16.3 (7.7-74.0)
> 1 Hour (n=133)	4.9 ± 5.4	165.5 ± 123.7	7.9 ± 1.4	22.1 ± 13.7
	3.1 (0.04-35.5)	134.8 (14.7-559.7)	7.7 (5.7-11.9)	16.6 (9.2-69.0)
p	<0.001	<0.001	0.485	0.370
Type of scorpion	3.5 ± 3.8	133.1 ± 105.9	7.9 ± 1.4	25.2 ± 16.5
Unknown (n=127)	1.9 (0.1-26)	93.5 (14.7-559.7)	7.5 (5.7-14.9)	16.9 (7.7-74)
Black (n=14)	3.0 ± 3.3	107.6 ± 88.5	8.4 ± 1.4	15.3 ± 2.0
	1.8 (0.4-11.6)	82.2 (32.8-368.3)	8.3 (5.9-10.9)	16.3 (11.1-16.7)
Yellow (n=116)	4.1 ± 5.4	146.0 ± 115.8	8.1 ± 1.3	17.6 ± 8.5
	2.0 (0.04-35.5)	103.5 (16.7-535.0)	7.9 (5.8-12.1)	16.2 (9.0-51.1)
p	0.265	0.368	0.226	<0.001
Time of sting	3.4 ± 4.4	126.0 ± 95.0	8.0 ± 1.4	20.7 ± 12.7
Day (n=160)	1.8 (0.04-35.5)	97.1 (14.7-535.0)	7.7 (5.7-14.9)	16.5 (9.0-69.0)
Night (n=97)	4.3 ± 4.9	156.8 ± 128.8	8.0 ± 1.2	21.9 ± 14.5
	2.7 (0.1-26.7)	96.6 (16.7-559.7)	8 (5.8-10.9)	16.4 (7.7-74)
p	0.136	0.029	0.999	0.055
Administration of antivenom	2.5 ± 2.7	120.6 ± 96.6	7.9 ± 1.4	22.6 ± 15.1
No (n=104)	1.5 (0.1-12)	89.6 (18.4-491.5)	7.6 (5.7-14.9)	16.7 (9-74)
Yes (n=153)	4.6 ± 5.4	149.7 ± 116.7	8.1 ± 1.3	20.0 ± 12.0
	2.6 (0.04-35.5)	113.3 (14.7-559.7)	7.9 (5.8-12.1)	16.3 (7.7-65.4)
p	<0.001	0.056	0.662	0.359
PICU stay	2.6 ± 3.8	119.7 ± 90.6	8.1 ± 1.4	19.0 ± 11.7
No (n=122)	1.2 (0.3-35.5)	84.5 (36.2-535.0)	7.7 (5.9-14.9)	16.8 (9.0-74.0)
Yes (n=135)	4.8 ± 5.0	155.3 ± 123.3	7.8 ± 1.2	24.1 ± 14.9
	2.9 (0.04-26.7)	104.8 (14.7-559.7)	7.5 (5.8-11.9)	16.7 (8.9-69.0)
p	<0.001	0.020	0.067	0.004
Myocarditis	3.3 ± 4.2	134.0 ± 108.3	8.0 ± 1.4	21.0 ± 13.3
No (n=221)	1.8 (0.1-35.5)	93.6 (16.7-559.7)	7.7 (5.7-14.9)	16.4 (7.7-74)
Yes (n=36)	6.5 ± 5.9	159.5 ± 117.4	8.2 ± 1.1	21.8 ± 14.3
	5.2 (0.04-26)	147.1 (14.7-486.0)	8.3 (6.3-10.2)	16.3 (10.2-65.4)
p	<0.001	0.197	0.395	0.767

NLR: neutrophil/lymphocyte ratio lymphocyte, PLR: platelet/lymphocyte ratio, MPV: mean platelet volume, PDW: platelet distribution width, PICU: pediatric intensive care unit.

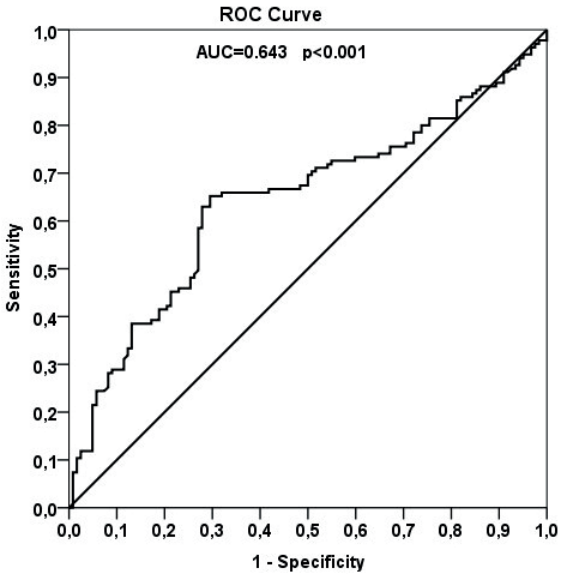


Fig. 1. ROC curve analysis of NLR predicts pediatric intensive care unit stay. Area under curve (AUC): 0.643.

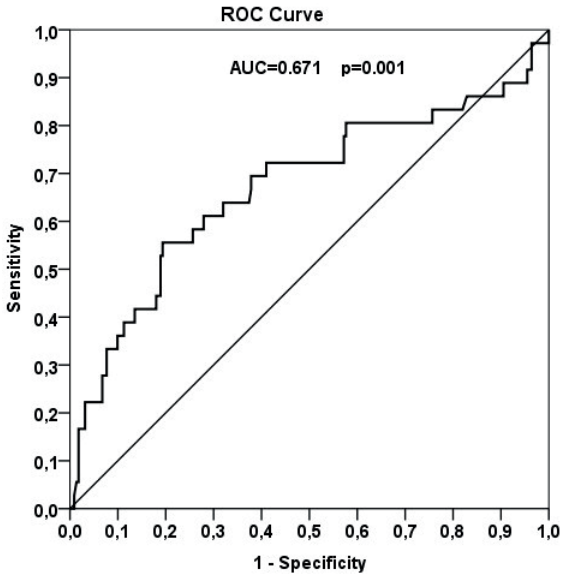
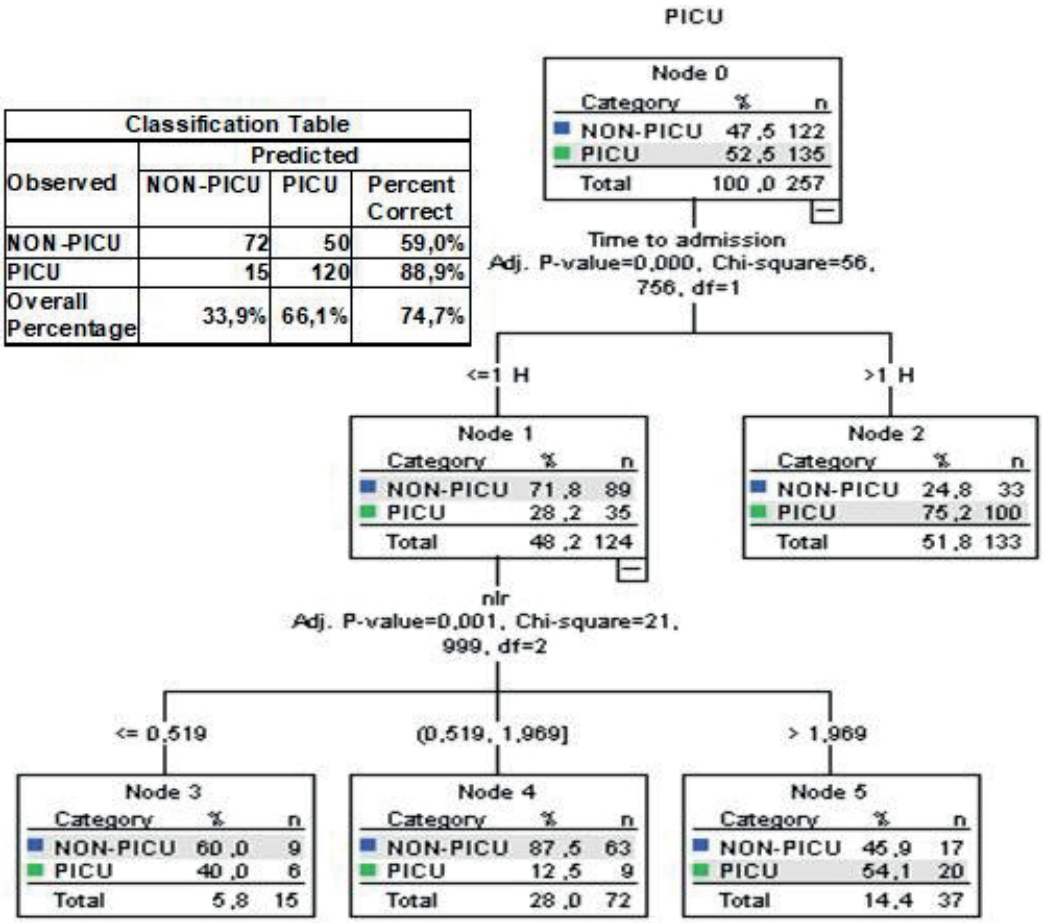


Fig. 2. ROC curve analysis of NLR predicts myocarditis. Area under curve (AUC): 0.671.



Classification Table

Observed	Predicted		
	NON-PICU	PICU	Percent Correct
NON-PICU	72	50	59,0%
PICU	15	120	88,9%
Overall Percentage	33,9%	66,1%	74,7%

Fig. 3. The decision tree model.

first hour of stinging did not require intensive care. Whereas, 75.2% of the patients who were admitted after more than one hour required intensive care stay. Additionally, 87.5% of the patients who applied within the first hour and whose NLR values were in the range of 0.519-1.969 did not require intensive care stay. (88.9% sensitivity, 59% specificity, positive predictive value 70.59%, negative predictive value 82.76%, accuracy 74.71%)

Discussion

Pro-inflammatory and anti-inflammatory cytokines and mediators are excreted in scorpion stings. The balance between the pro-inflammatory and anti-inflammatory mediators, in other words, the excessive systemic inflammatory response of the host determines the severity of the envenomation.[18] In addition, the properties of the scorpion, number of stings, depth of venom injection, location of stings, age and health status of the victim determine the severity of the envenomation. Severe envenomation is associated with morbidity and mortality. Therefore, it is the reason for hospitalization and increased costs. When the literature was reviewed, no study was found which investigated the relation between initial hematological parameters and the disease severity in scorpion envenomations. The correlation between platelet indices and scorpion envenomation was assessed only in one study.[23] To the best of our knowledge, our study is the first to investigate the role of initial hematological parameters in the severity of scorpion envenomation in children. When we compared our patients with healthy controls, WBC, neutrophil, lymphocyte, platelet count, PDW, NLR and PLR ratios were statistically significantly higher, whereas the MPV values of our patients were lower than the controls. However, this situation was not statistically significant.

In previous studies, leucocytosis has been reported in patients who were admitted due to scorpion stings.[18,24,25] In our study, it was determined that the WBC values of the patients were significantly higher than the control groups'.

Various studies have been published in which the MPV value in inflammatory diseases have been investigated. It has been reported in these studies (chronic urticaria, rheumatoid arthritis, chronic hepatitis B, myocardial infarction, an acute attack of FMF) that the MPV value is high in association with the severity of inflammation.[26-30] However, it has also been determined that MPV is in lower values in cystic fibrosis acute exacerbation.[31] In our study, the MPV values of our patients were found to be lower however, this was not statistically significant.

PDW value, which is one of the hematological parameters, has been stated to be associated with inflammation in many diseases: acute coronary syndrome, heart failure, sickle-cell disease, tuberculosis, bacteremia, chronic urticaria.[26,32-34] In our study, the PDW values of our patients who were admitted due to scorpion stinging, were found to be statistically significantly higher than the control group.

As an indicator of the inflammatory period, PLR has been the subject of many studies recently. The association between the inflammatory period indicators and disease have been investigated even in psychiatric diseases.[30,35-37] The PLR value was statistically significantly higher in our patients as well.

NLR which is prominent due to rapidity, easy detection and cost-effectiveness is being reported with increasing frequency as a reliable biomarker of systemic inflammation.[38-40] Djordjevic et al.[16] have investigated the relationship between hematological parameters and prognosis in critically ill patients with bacteremia, and have reported that high MPV and high NLR are very good independent predictors in predicting lethal outcome. It has been reported that NLR is a useful tool for clinical severity and prognosis in inflammatory and malignant diseases.[41-43] The mean NLR in our patients was 3.8 ± 4.7 and statistically

significantly higher than the control group. This suggested that it was related to severe inflammation post scorpion stinging.

In patients who applied from urban areas the NLR was found to be higher, whereas in scorpion stinging which happened at night the PLR values were found to be higher, and in patients who applied to the hospital later than an hour post scorpion stinging both the NLR and PLR values were significantly higher and this suggested increasingly inflammation response. When considered according to scorpion types there was no difference between the scorpion types for NLR, PLR, and MPV, however, the PDW value was higher in stings with unknown scorpion types. When the patients were divided according to Abroug's calcification, NLR significantly increased from stage 1 to stage 3 and this supported the idea that when envenomation severity increased the NLR increased. Additionally, the fact that the NLR value was significantly higher in patients who were given antivenoms, patients who needed intensive care and patients who had myocarditis was considered as related to the severity of the disease.

In the prediction of admission of patients to intensive care due to scorpion stings, initial NLR was a useful parameter. The optimal cut-off point for NLR was obtained as 2.1 with 65.4% sensitivity and 70.5% specificity. When the literature is reviewed, various publications about NLR cut-off value in children can be found. The NLR cut-off value has been reported as 1.97 in predicting sepsis, as 2.86 in gastrointestinal hemorrhage in Henoch-Schönlein purpura, like 1.4 to assess the inflammation in rheumatoid arthritis and as 2.51 in Kawasaki disease.[15,44-46] However, in the literature, there is no cut-off value for the prediction of severity in children who applied with scorpion stings. In a retrospective study that evaluated adult patients who applied with snake bites, it was reported that the patients with a high NLR value were found to have a longer hospital stay.[47] Nevertheless, we have determined a weak correlation between the intensive care unit stay duration and NLR. We have determined that NLR can be used in predicting myocarditis which is another picture that indicates the severity of scorpion envenomation. The optimal cut-off point for NLR was obtained as 2.5 with 69.4% sensitivity and 64.2% specificity for myocarditis.

When 257 patients, who were admitted to the hospital due to scorpion stings, were analyzed with the help of the decision tree model it was seen that 71.8% of the patients who were admitted to the emergency department within the first hour did not need to be hospitalized at the intensive care unit. On the other hand, 75.2% of the patients who were admitted to the hospital later than an hour after the scorpion sting needed to be hospitalized in the intensive care unit. In patients who had applied to the hospital in less than an hour after the scorpion sting 87.5% of the patients whose NLR value was between the 0.519-1.969 interval (below 2.1 which we found as the cut-off value) did not need to be hospitalized in the intensive care unit, 54.1% of the patients whose NLR value was higher than 1.969 needed to be hospitalized in the intensive care unit. Interestingly, six (40%) out of the 15 patients whose NLR value was lower than 0.519 and who had applied to the hospital within the first hour after the scorpion sting also needed to be hospitalized in the intensive care unit. When these six children whose NLR values were low were examined, it was found that they were all under 1 year old and their lymphocyte and neutrophil counts were (7.7-25.3) and (2.6-8.0) respectively. As it is known, there is lymphocyte dominance in children under 1-year-old and this age group in under risk for severe envenomation after scorpion stings. The fact that these six children had lymphocytosis, and were younger than one-year-old explains why they needed to be hospitalized at in intensive care units despite having low NLR values.

The major limitations of our study were being retrospective, being the only center and including pediatric patients with restricted racial properties.

In conclusion, systemic inflammation occurs after scorpion stings and the degree of inflammation determines severe envenomation. After scorpion stings, severe envenomation risk increases especially in pediatric patients.[48] This situation is associated with mortality and morbidity. High NLR, which will be calculated from the complete blood count at the admission of the patient to the hospital, can predict severe envenomation. High NLR in scorpion stings may be helpful during the decision stage. High NLR in scorpion stings may be a helpful tool in deciding when the patient should be transported to a more advanced center than the sub-urban areas where there is limited access to severe envenomation treatment and especially patient care resources. However, we believe that more studies are needed about NLR, which is quite popular lately, and other hematological parameters.

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Original Article

Role of kallistatin in pediatric patients with pulmonary arterial hypertension

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ABSTRACT

Background and objectives. Kallistatin, a serine proteinase inhibitor, exerts its effect by vascular repair, angiogenesis inhibition, strong vasodilation, inhibition of vascular endothelial growth factor (VEGF), anti-inflammation, and anti-apoptosis. We hypothesized as to whether it has a protective role in pulmonary arterial hypertension (PAH).

Methods. The study included 5 subgroups (78 patients; 44 male): Eisenmenger syndrome (n=16), PAH with left to right shunt (n=20), idiopathic PAH (n=7), patients with left to right shunt without PAH (n=19), and patients with innocent heart murmur (n=16). Physical examination, chest radiography, electrocardiography, and transthoracic echocardiography (TTE) were performed for each patient. PAH diagnosis was confirmed by catheterization. Serum kallistatin, tumor necrosis factor alpha (TNF-α), Interleukin-10 (IL-10) and N-terminal pro b-type natriuretic peptide (NT-proBNP) levels were studied for each patient.

Results. The lowest median kallistatin value was found in Eisenmenger syndrome: 1.19 (0.87-3.30) μg/ml. The highest value belonged to control group with innocent murmur: 2.89 (1.19-5.66) μg/ml. Serum levels of kallistatin were significantly lower in patients with PAH ($p<0.05$). TNF-α values were increased and IL-10 values were decreased in pulmonary hypertension. However; no correlation was found between kallistatin levels and cytokines.

Conclusions. Kallistatin may have a protective effect in pulmonary arterial hypertension by repairing vascular damage, inhibition of angiogenesis, strong vasodilator effect, inhibiting VEGF, and anti-inflammatory mechanism of action. To our knowledge, our study is the first one that shows the role of kallistatin in pulmonary hypertension. Kallistatin may represent a promising novel therapeutic approach for pulmonary hypertension in the near future.

Key words: kallistatin, pulmonary hypertension, TNFα, IL-10.

Pulmonary hypertension is a progressive obliterative vasculopathy. Underlying mechanisms are very complex; there is not a single but group of events. The one that takes the main role seems to be endothelial dysfunction. Other events are changes in function of platelet and ion channels, calcium homeostasis, inflammation, angiogenesis etc.[1] Pulmonary arterial hypertension (PAH) is a subgroup of pulmonary hypertension that has idiopathic, genetic, and comorbid etiologies (e.g., connective tissue disorders, HIV infection, schistosomiasis, etc.).[2] It is characterized by occlusion of pulmonary arterioles that results the increase in the pulmonary vascular resistance, right ventricular hypertrophy, and finally right sided heart failure.

In recent years, there is an increased understanding of the pathophysiology of

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pulmonary hypertension which is thought to be helpful in finding the novel PAH-specific therapies.

Kallistatin is a serine proteinase inhibitor originally known as a tissue kallikrein inhibitor. The main ways of action of it are inhibiting inflammation, oxidative stress, apoptosis, and angiogenesis.[3,4] Herein; by this study, we aimed to discuss the protective role of kallistatin in pulmonary arterial hypertension. Perhaps in near future it may represent a promising novel therapeutic approach for pulmonary hypertension.

Material and Methods

The study was performed in our university between December 2015- January 2017. The study was approved by the Institutional Ethics Committee (06.02.2015; 2015/799).

The study included a total of 78 patients. The study population was classified under 5 groups. Group 1: Eisenmenger syndrome, Group 2: PAH patients with left to right shunt, Group 3: idiopathic PAH, Group 4: patients with left to right shunt but without PAH, Group 5 (controls): patients with innocent murmur.

The following data for each patient were obtained: age, weight, sex, and non-cardiac medical problems. Underlying cardiac pathology was recorded except for groups 3 and 5. Physical examination, chest radiography, electrocardiography, and transthoracic echocardiography (TTE) were done for each patient. Parents were informed about the study and written consent was taken from each of them before being included in the study. In addition, pulmonary hypertensive ones (Group 1, 2, 3) were also informed about cardiac catheterization and its complications; a written consent was also taken for the procedure from the parents.

Cardiac catheterization

Angiography was performed in pulmonary hypertensive patients. Following heparinization and a dose of intravenous antibiotic, hemodynamic and angiographic evaluations were performed. The procedure included measurements of pressure (systolic and diastolic and/or mean) and oxygen saturation in the superior vena cava, inferior vena cava, right atrium, pulmonary artery, right ventricle (RV), and the aorta. Pressure recordings were obtained by using fluid-filled catheter systems, and these systems were calibrated to zero at the midaxillary line.

The diagnosis of each patient was determined according to current guidelines[5] (mean pulmonary artery pressure-PAP ≥25 mm Hg, pulmonary artery wedge pressure <15 mm Hg).

According to the clinical classification by Simonneau et al.[6]; Eisenmenger's syndrome includes all intra- and extra-cardiac defects as well as systemic-to-pulmonary shunts.

Idiopathic PAH is defined in the guidelines as PAH with no underlying disease known to be associated.

Serum kallistatin, cytokine, and NT-proBNP levels

Blood samples for kallistatin was taken by the tubes centrifuged at 4000 rpm for 10 min at 4 1C. The serum was kept at -80°C as frozen until required. Kallistatin levels were determined by ELISA (R&D Systems, Inc. Minneapolis, USA) as previously described.[7]

Concentrations of TNF-α and IL-10 in plasma were measured in picograms per milliliter (pg/ml) by commercially available enzyme-linked immunosorbent assay kits (Cayman Chemical, Ann Arbor, MI, USA).

Five ml blood samples were drawn from both patient and control groups for N terminal pro brain natriuretic peptide (NT-proBNP). Blood samples were centrifuged at +4°C and 1500 rpm for 5 minutes. Plasma part of the upper phase was taken in to another tube for NT-proBNP calculation. Samples were preserved at -80°C till the study date. Later on, they were

studied with ELISA method using Biomedica NT-proBNP N-terminal pro-BNP commercial kits (NT- ProBNP enzyme immuno assay kit Biomedica, Bratislava, Slovakia) and Elecsys® 1010 aoutoanalyzer (Roche Diagnostics, Basel, Switzerland). Results were expresses as fmol/ml (1 fmol/ml = 16.1 pg/ml).

Statistical analysis

The Shapiro–Wilk test was used to analyze the distribution of the data. If the data presented with a normal distribution, analysis of variance (ANOVA) followed by the Tukey's test was used to evaluate the results. Comparisons of means were performed with Student's t-test. Comparisons of medians were performed with the Mann–Whitney U-test. Correlations were calculated with Pearson product moment or Spearman rank order, as determined by the normalcy of data distribution. Differences were considered significant at $p < 0.05$.

Results

Totally 78 patients [44 male with a mean age of 12.1 ± 4.3 (range: 3-18 years), 34 female with a mean age of 12.0 ± 4.1 (range: 4-17 years)] were included in the study. The first group had 16 patients with Eisenmenger syndrome. Underlying congenital heart diseases of this group were ventricular septal defect (n= 13) and patent ductus arterious (n= 13). The second group had 20 pulmonary hypertensive patients with left to right shunt. Group 3 included 7 patients with idiopathic pulmonary hypertension. Group 4 had 19 patients with left to right shunt but without pulmonary arterial hypertension. The last group, Group 5 included the 16 patients with innocent heart murmur.

In Group 1, 6 patients used endothelin receptor antagonist (bosentan/macitentan) treatment as a monothereapy. The rest of the patients in group 1 had combination therapy (bosentan/macitentan+ phosphodiesterase-5 inhibitor sildenafil, bosentan/macitentan+ PGI2 analog (ilioprost), bosentan/macitentan+ ilioprost+ sildenafil). Bosentan was switched to macitentan due to drug side effects in 5 patients. In group 3, vasoreactivity was negative in all patients. Two patients received only endothelin receptor antagonist (macitentan). The rest of the 5 patients received combination therapy (endothelin receptor antagonist+ sildenafil or endothelin receptor antagonist+ sildenafil+ilioprost).

Median serum kallistatin levels were summarized for each group in Table I. The lowest value was found in Group 1 (Eisenmenger syndrome); the highest value belonged to control group with innocent murmur. Serum levels of kallistatin were significantly lower ($p < 0.05$) in pulmonary hypertensive patients (Group 1, 2, 3). However; no statistically significant difference was detected between those 3 groups. Negative correlation was detected between mean pulmonary arterial pressure and serum kallistatin levels (rho: - 0.303; p= 0.009).

Mean value of TNF-α of pulmonary hypertensive group was 10.5 ± 4.3 pg/ml, TNF-α of control group was 9.7 ± 2.4 pg/ml. There was no statistically significant difference between these two groups. Mean value of IL-10 of pulmonary hypertension group was 14.6±2.82 pg/ml and

Table I. Median serum kallistatin and NT-proBNP levels according to groups.

Groups	Kallistatin (μg/ ml)*	NT ProBNP levels (pg/ml)*
Group 1: Eisenmenger syndrome (n=16)	1.19 (0.87-3.30)	123 (20-3,586)
Group 2: Left to right shunt with PAH (n=20)	1.79 (1.13-3.60)	657 (20-4,840)
Group 3: Idiopathic PAH (n=7)	1.51 (1.07-2.02)	510 (125-2,470)
Group 4: Left to right shunt without PAH (n=19)	2.52 (0.87-3.21)	57 (20-185)
Group 5: Control patients (n=16)	2.89 (1.19-5.66)	20 (20-51)

*Data are presented as median (minimum - maximum)
PAH: pulmonary arterial hypertension.

control group was 15 ± 4 pg/ml. In addition; no correlation was found between kallistatin levels and cytokines TNF-α and IL-10.

Median NT-proBNP levels for groups are summarized in Table I. There was a statistically significant difference between the Eisenmenger group and the control group ($p < 0.05$). However; there was no significant differences between pulmonary hypertensive groups such as Groups 1, 2, and 3. Moreover, there was no statistically significant difference between the control group and the left to right shunt group without PAH. NT-proBNP levels of pulmonary hypertensive groups were significantly higher than the patients without pulmonary hypertension ($p < 0.05$).

Bosentan treatment was started in 39 patients (92.8%), but in 10 (23.8%) patients, bosentan was switched to macitentan due to drug side effects or shortening in 6 minutes walking distance. Bosentan, sildenafil and iloprost combined therapy was started in one of the patients for poor clinical status. In one patient sildenafil and in another patient single macitentan treatment was started. In follow up, 29 patients (69%) received only bosentan. Of the 42 patients, 9 (21.4%) received combined drug therapy consisting of endothelin receptor blocker, phosphodiesterase-5 inhibitor or PGI2 analog. In patients who received bosentan, liver toxicity was seen in 1 patient and thrombocytopenia in 4 patients. Macitentan was used in 12 patients aged between 14 and 22 years who received single or combined treatment. The duration of macitentan use was 16.5 months in these patients.

Discussion

Pulmonary arterial hypertension is a big burden for the healthcare system. Morbidity is high and prognosis is poor. There is no ideal treatment that can reverse or prevent the disease process. Treatment given for symptomatic relief is long term and expensive which means a huge financial load. Also most pulmonary hypertensive patients should have sedentary life that means the loss of manpower. Further understanding the pathogenesis may lead us to take steps in the development of new treatment strategies. If the quality of life of such patients improves, they may participate in work life more effectively and contribute to country economics.

Kallistatin is an endogenous protein; a member of the serine proteinase inhibitors. In previous studies it was shown that kallistatin has a role in vascular repair, angiogenesis inhibition, strong vasodilatation, VEGF inhibition, anti-inflammation and anti-apoptosis. The most conspicuous point is that all of these events have a role in the pathogenesis of pulmonary hypertension and we hypothesized that kallistatin may have a protective role in pulmonary hypertension. In order to support this hypothesis, we could not find any study in literature concerning the role of kallistatin in pulmonary hypertension. Therefore, we discuss other studies about kallistatin that might be a clue for preventing pulmonary hypertension. The main event in the pathogenesis of pulmonary arterial hypertension is endothelial dysfunction and it has been reported that kallistatin had a role in vascular repair by promoting mobility, viability, and function of endothelial progenitor cells.[8,9]

Vascular smooth muscle proliferation is also known to be an important mechanism in pulmonary hypertension and kallistatin was found to have role in the inhibition of angiogenesis in hepatocellular carcinoma[10], which also promotes apoptosis and autophagy of breast cancer cell.[11] The other mechanism of pulmonary hypertension is increased intravascular thrombosis and vasodilator effect of kallistatin was proven previously by Chao et al.[12] Vascular endothelial growth factor is known to be increased in pulmonary arterial hypertension. In breast and hepatocellular cancer kallistatin was demonstrated to inhibit VEGF secretion.[13]

Inflammation is a major feature of pulmonary arterial hypertension. Immune processes are altered; there is usually a failure to resolve inflammation. As a result of inflammation circulating levels of certain cytokines and chemokines are abnormally elevated that lead to vascular remodeling in pulmonary arterial hypertension.

In our study we investigated the role of TNF-α and IL-10 in pulmonary arterial hypertension and its relation with kallistatin. We have found that TNF-α increased and IL-10 level decreased in our study; but there was no statistically significant difference which could be related to the small number of patients. Decrement in IL-10 level could be explained as consumption of anti-inflammatory cytokines during the inflammatory process (pulmonary hypertension). In fact, kallistatin levels were found to be significantly low in pulmonary hypertension; no correlation was found between cytokine and kallistatin levels.

Soon et al.[14] described that TNF-α and IL-10 levels were significantly raised in the PAH group as a whole compared with healthy control subjects. They suggested that increased levels of anti-inflammatory cytokines such as IL-10 might be related to compensatory mechanisms also another important detail was high IL-10 found in patients who were prescribed prostanoids.

The anti-inflammatory role of kallistatin was studied in various organ systems like kidney, liver, heart and lungs. Its protective effects were shown in salt-induced renal injury, inflammation, and fibrosis; carbon tetrachloride-induced liver fibrosis; and cardiac remodeling after chronic myocardial infarction.[15-18] Furthermore, arthritis in a rat model was shown to be inhibited by adenovirus-mediated human kallistatin gene therapy.[19]

Reduced kallistatin levels are strongly associated with increased lung inflammation and mortality of sepsis-related ARDS in hospitalized patients by Lin et al.[20]. They came to the conclusion that kallistatin ameliorates lipopolysaccharide-induced lung injury and inflammation which could be supportive data for our hypothesis.

Consequently, kallistatin may have a protective effect in pulmonary arterial hypertension by repairing vascular damage, inhibition of angiogenesis, strong vasodilator effect, inhibiting VEGF, anti-inflammatory mechanism of action.

The main restriction of the study is that our study is the only one in the literature therefore we do not have a chance to compare it with others. The number of patients is limited. Low levels of kallistatin may be related to either a decreased production or increase in consumption.

It would be better if we had the chance to compare kallistatin levels of patients before and after starting anti-pulmonary hypertensive drugs to evaluate the effectiveness of the therapy.

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Original Article

New guidelines for diagnosis of rheumatic fever; do they apply to all populations?

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ABSTRACT

Background and objectives. To evaluate the efficacy of recently updated Jones criteria for diagnosis of rheumatic fever in high incidence populations like Egypt.

Methods. Clinical data of 891 Egyptian patients with rheumatic fever, aged 5-15 years in a highly specialized rheumatic fever clinic were reviewed retrospectively from March 2014 to March 2016. Discriminant analysis was used to detect the most effective predictors for diagnosis of rheumatic fever in our patients incorporating echocardiographic criteria. We compared our results to the most recent update by the American Heart Association.

Results. The most effective predictors of rheumatic fever included arthritis, carditis, chorea, aortic regurgitation, grades of mitral regurgitation ≥10mm length and velocity ≥2.5 m/s, thick anterior mitral valve leaflets, elevated acute phase reactants, positive family history and prolonged PR interval. Our predictors showed a high sensitivity of 93%, a specificity of 62% and an overall prediction accuracy of 81.4%.

Conclusion. We concluded that strict application of updated Jones criteria may lead to under diagnosis of rheumatic fever in highly endemic countries. We recommend further studies to examine the sensitivity of the most recent update of Jones criteria on other highly endemic populations.

Key words: rheumatic fever, Egypt, subclinical carditis, limiting arthralgia, prediction model.

Egypt has a high prevalence of rheumatic fever; seen in 5.1[1,2] per 1,000 school children. Prompt diagnosis of acute rheumatic fever is important for initiating treatment.[2,3] Jones Criteria were defined by Dr. T.D. Jones to diagnose acute rheumatic fever[4] and have been periodically updated. The 1992 update was the most widely used.[5] Modifications aimed at improving specificity were conducted, however at the expense of sensitivity; hence it has not been sensitive enough to pick up disease in high incidence populations.[5,6] The World Health Organization developed criteria for the diagnosis of primary and recurrent episodes of rheumatic fever and based on this modified Jones also introduced minimal echocardiographic criteria diagnosing pathological regurgitation, however subclinical carditis hasn't yet been included in Jones criteria.[7] The Australian guidelines modified criteria for diagnosis of acute rheumatic fever and proposed additional criteria for high-risk groups[8,9] The Australian guidelines applied the World Health Federation criteria for diagnosing rheumatic heart disease by echocardiography.[10] Furthermore, the American Heart Association introduced an updated revision of Jones criteria to meet current advances.[11] In this study we reviewed the criteria for diagnosis of acute rheumatic fever in our patients. We compared our results with the most recent update by the American Heart Association[11] to emphasize the application of this update on our Egyptian

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children aiming at improving the diagnosis of rheumatic fever and avoiding the impending burden of rheumatic heart disease as a result of under diagnosis of this serious disease in highly endemic areas like Egypt.

Material and Methods

This is a retrospective study of 5-15 year-old Egyptian patients diagnosed with rheumatic fever, from March 2014 to March 2016. Children were enrolled if they didn't have comorbidities that may affect the heart, joints or brain. We adhered strictly to the updated Jones criteria (positive two major or one major and two minor manifestations plus evidence of recent streptococcal infection). Proven rheumatic fever patients completely fulfilled these criteria while probable rheumatic fever patients were short by either one major or one minor manifestation. We aimed to examine and emphasize the significance of addition of the new diagnostic criteria of rheumatic fever described in the recent guidelines on our children especially subclinical carditis. Also to observe the echocardiographic diagnostic criteria for pathological regurgitation in proved rheumatic fever Egyptian patients and to compare our results with both World Health Organization and World Health Federation criteria.

A data collection sheet was filled, including history taking, Baseline examination and related laboratory investigations. Screening for positive Jones criteria and new debatable criteria namely aseptic monoarthritis, limiting polyarthralgia, and subclinical carditis were done. Aseptic monoarthritis refers to swelling, redness and hotness involving one big joint with limitation of movement, elevated acute phase reactants and dramatic improvement on salicylates treatment.

Limiting polyarthralgia refers to limiting and/or fleeting arthralgia without signs of inflammation (no swelling, redness nor hotness) involving big joints with elevated acute phase reactants and dramatic improvement on salicylates treatment.

Echocardiography was performed for detection of valvular dysfunction[7,9,10] and rheumatic morphological changes.[10] Trivial regurgitation was classified according to jet length into jets <10 mm, ≥10 mm and ≥20 mm and according to jet velocity into jets <2.5 m/sec, ≥2.5 m/sec and >3 m/sec.[7,9,10] Regurgitant lengths more than trivial were divided into mild, moderate and severe. Mild regurgitation has a small jet, with a vena contracta width ~3mm, moderate regurgitation has an intermediate jet with a vena contracta width 3-6.9 mm while a severe mitral regurgitation has a large jet with vena contracta width ≥7mm. The continuous wave signal of the jet is faint or incomplete in case of mild regurgitation, dense in moderate and denser in severe regurgitation. The more severe the aortic regurgitation, the less the pressure half time (500 ms in mild reaching 200 ms in severe) with diastolic flow reversal in descending aorta in severe aortic regurgitation.[12-14]

Subclinical carditis refers to significant regurgitation of mitral or aortic valve (≥10 mm in length and ≥2.5 m/sec velocity) ± rheumatic valve morphological changes despite the absence of any auscultated murmur.[7]

The research was reviewed and approved by an institutional review board and participation involved informed consent. The study was approved on 12th of November 2017 by Cairo University Committee and reviewed and approved on 30th of January 2018 by the Scientific Committee in Cairo University Children Hospitals and finally approved by Faculty of Medicine, Cairo University Committee on 25th of February 2018; Report number: 164367.

Statistical methods

All data were gathered, statistically analyzed and tabulated. The presence of individual signs and symptoms were compared between proven and probable rheumatic fever using chi-square tests for binomial variables which were presented as numbers and percentages. Continuous variables were assessed using t-test analysis and were presented as mean ± standard deviation.

The statistically significant clinical variables (p value≤0.05) were used as independent predictors of proven rheumatic fever utilizing discriminant analysis technique. This multivariate statistical method derives a prediction equation as a linear combination of the independent variables that will discriminate best between groups in the dependent variable (proven and probable rheumatic fever). Functions at group centroid indicate the average discriminant score for subjects in the two groups. Patients are located in the prediction equation according to their discriminant score (unstandardized canonical discriminant coefficients) then diagnosed as probable or proven rheumatic fever accordingly. Stepwise techniques were next done to detect the best predictors of rheumatic fever. Eigen value, canonical correlation, Wilks' lambda, p value, sensitivity and specificity were calculated for the whole model. The bigger the Eigen value and the smaller the Wilks' lambda, the stronger the discriminating power between proved and probable rheumatic fever.

We used receiver operating characteristic (ROC) curves and area under ROC curve (AUC) to visually and statistically assess sensitivity, specificity and overall performance of our prediction model. Statistical analyses were performed on SPSS 21 statistical software (Statistical Package for Social Science).

Results

A total of 891 children aged 5-15 years were diagnosed with rheumatic fever during the period of study, 53.4% (476) were females, with a mean age of 9 ± 2.5 years; 62.5% (557) were diagnosed as proven rheumatic fever while 37.5% (334) were diagnosed as probable rheumatic fever. The most common presentations were, arthritis followed by subclinical carditis and carditis (Table I). The most common associations between major criteria in our patients were between arthritis and carditis (14.6%), followed by arthritis and subclinical carditis (11.4%), carditis and limiting arthralgia (5.8%), subclinical carditis and chorea (3.1%) and carditis and chorea (2.9%). Four patients had carditis and subcutaneous nodules, two patients had arthritis and erythema marginatum, only one patient had arthritis and subcutaneous nodules.

Echocardiographic study of our patients detected mitral regurgitation in 509 patients.

Table I. Clinical features of the study group.

Features	Total (N=891)	Proven RF (N=557)	Probable RF (N=334)	P value
Arthritis	532 (59.7%)	398 (71.5%)	134 (40.1%)	0.0001*
Carditis	271 (30.4%)	247 (44.3%)	24 (7.2%)	0.0001*
Chorea	64 (7.2%)	64 (11.5%)	0 (0%)	0.0001*
Subcutaneous nodules	4 (0.4%)	4 (0.70%)	0 (0%)	0.06
Erythema marginatum	3 (0.3%)	2 (0.36%)	1 (0.3%)	0.684
Subclinical carditis	299 (33.6%)	114 (20.5%)	185 (55.4%)	0.0001*
Limited arthralgia	170 (19.1%)	64 (20.5%)	106 (31.7%)	0.0001*
Fever	441 (49.5%)	317 (56.9%)	124 (37.1%)	0.0001*
Arthralgia	343 (38.5%)	201 (36.1%)	142 (42.5%)	0.033*
Prolonged PR interval	18 (2%)	18 (3.2%)	0 (0%)	0.0001*
Elevated ESR, CRP	346 (38.8%)	269 (48.3 %)	77 (23.0%)	0.0001*
Elevated ASO	395 (44.3%)	270 (48.5 %)	125 (37.4%)	0.0001*
Positive family history	163 (18.3%)	86 (15.4 %)	77 (23.0%)	0.003*

*: p value <0.05
ASO: antistreptolysin-O, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, RF: rheumatic fever.

Table II. Grades of mitral regurgitation in patients with mitral regurgitation.

Grades of mitral regurgitation	Total (N=509)	Proven RF (N=325)	Probable RF (N=184)
Trivial MR: 7-9 mm	7 (1.4%)	-	7 (3.8%)
Trivial MR: 10-20 mm	263 (51.7%)	116 (35.7%)	147 (79.9%)
Mild MR ≥20 mm	32 (6.3%)	15 (4.6%)	17 (9.2%)
Moderate MR	136 (26.7%)	124 (38.2%)	12 (6.5%)
Severe MR	71 (13.9%)	70 (21.5%)	1 (0.5%)

MR: mitral regurgitation, RF: rheumatic fever.
*The frequency of patients with MR ≥10 mm was higher in proven rheumatic fever (325/325; 100%), compared to probable rheumatic fever (177/184; 96%; p= 0.001).
Patients are classified into mild, moderate and severe MR according to color flow MR jet, vena contracta width, continuous wave signal of jet and flow convergence zone.

The frequency of patients with grades of mitral regurgitation of jet length ≥10 mm was higher in proven rheumatic fever (325/325; 100%), compared to probable rheumatic fever (177/184; 96%; p= 0.001, Table II). Rheumatic morphological changes in the mitral valve was detected in 23.9% (213) of our patients (Fig. 1); of those 39.9% (85) had one morphological change while 60.1% (128) had two or more morphological changes. Morphological features included thick mitral valve leaflets, thick subvalvular apparatus, lack of systolic coaptation of mitral valve, restricted posterior mitral valve leaflet, and mitral valve prolapse (Fig. 1). The frequency of two or more morphological changes was in proven rheumatic fever (104/156; 66.7%), compared to probable rheumatic fever (24/57; 42.1%, p= 0.001). Twelve patients had rheumatic mitral valve morphological changes without any functional regurgitation.

Aortic regurgitation was detected in 258 patients. The frequency of patients with grades of aortic regurgitation jet length ≥10 mm was higher in proven rheumatic fever (203/204; 99.5%), compared to probable rheumatic fever (49/54; 90.7%, p= 0.001, Table III). Thick aortic cusps were detected in 3.9% (35) of our patients;

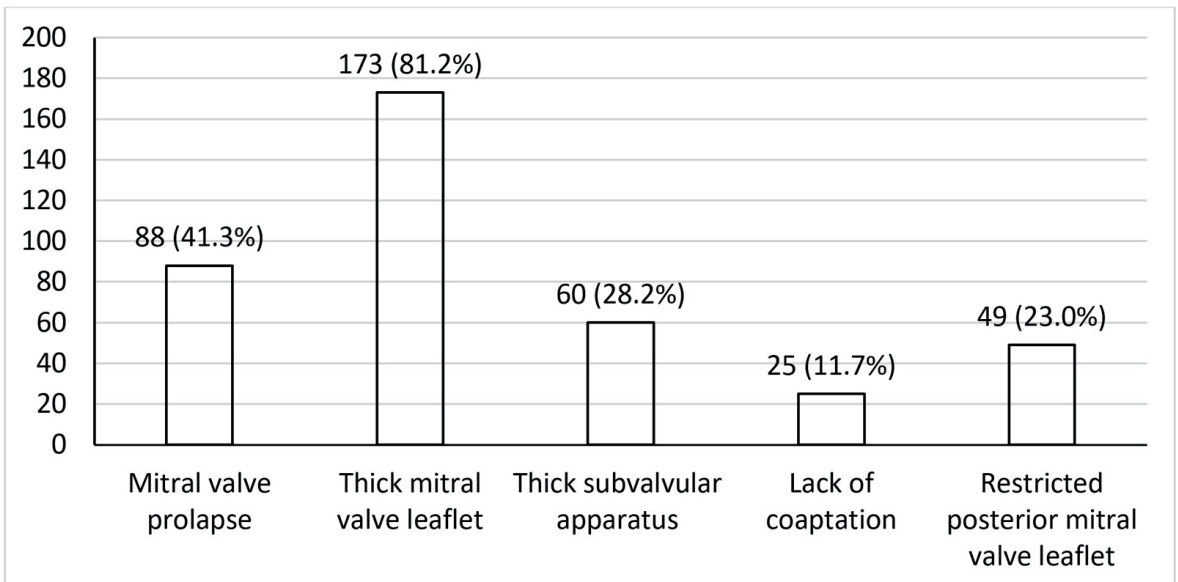

Fig. 1. Distribution of rheumatic morphological changes involving mitral valve; morphological changes were detected in 213 patients.

Table III. Grades of aortic regurgitation in patients with aortic regurgitation.

Grades of aortic regurgitation	Total (N=258)	Proven RF (N=204)	Probable RF (N=54)
Trivial AR (7-9 mm length)	6 (2.3%)	1 (0.5%)	5 (9.3%)
Trivial AR (10-20 mm length)	119 (45.9%)	79 (38.7%)	40 (74.1%)
Mild AR (>20 mm length)	52 (20.1%)	48 (23.5%)	4 (7.4%)
Moderate AR	57 (22.0%)	51 (25%)	6 (11.1%)
Severe AR	25 (9.7%)	25 (12.3%)	0 (0%)

AR: aortic regurgitation, RF: rheumatic fever.
*The frequency of patients with AR ≥10 mm was higher in proven rheumatic fever (203/204; 99.5%), compared to probable rheumatic fever (49/54; 90.7%; p= 0.001).
Patients are classified mild, moderate and severe AR according to color flow AR jet width, vena contracta width, continuous wave signal of jet, pressure half time, and diastolic flow reversal.

4.7% (26/557) in proven rheumatic fever and 2.7% (9/334) in probable rheumatic fever with (p= 0.097). Thick aortic cusps were detected in six patients without any functional aortic regurgitation. Double valve affection (mitral and aortic regurgitation) was detected in 24% (214) of our patients.

In our patients, 33.6% (299) had subclinical carditis; 38.1% (114) had proven rheumatic fever while 61.9% (185) had probable rheumatic fever (p= 0.0001). In patients with subclinical carditis, 42.8% (128) had associated other rheumatic fever major criteria where 34.4% had arthritis, 33.4% had limiting polyarthralgia, 9.4% had chorea and 0.6 % had erythema marginatum.

Trivial mitral regurgitation (jet length 10-20 mm) was detected in 29.5 % (263) of patients, 44% (116) had proven rheumatic fever (p= 0.0001). In patients with trivial mitral regurgitation (jet length 10-20 mm), 31.6% (83) had associated arthritis; 80.2% had proven rheumatic fever (p= 0.0001); 9.5% (25) had associated chorea, and all had proven rheumatic fever (p= 0.0001).

Trivial aortic regurgitation (jet length 10-20 mm) was detected in 13.4% (119) of our patients; 66.4% (79) had proven rheumatic fever (p= 0.0001). In patients with trivial aortic regurgitation (jet length 10-20 mm), 41.2% (49) had associated arthritis; 93.9% of these patients had proven rheumatic fever (p= 0.0001); 10.9 %

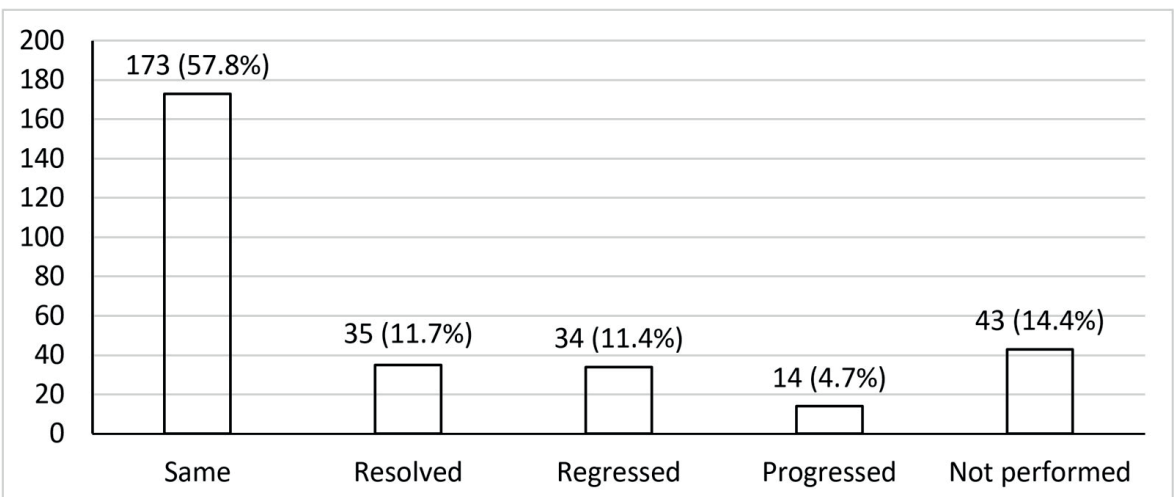

Fig. 2. Echocardiographic follow-up in patients with subclinical carditis; Follow-up echocardiography was scheduled after 1-year for 299 patients with subclinical carditis. In two patients, mitral regurgitation became clinically auscultated.

(13) had associated chorea, and all had proven rheumatic fever (p= 0.003).

Follow up echocardiography was scheduled after 1 year for all patients with subclinical carditis (Fig. 2). The follow up was missed in only 14.4% (n: 43) of them and in two patients, mitral regurgitation became clinically auscultated. All patients showed good compliance to secondary prophylaxis of long acting penicillin, except five patients who showed persistent lesions by follow up.

We used discriminant analysis to detect the most effective predictors of rheumatic fever in our children where significant variables by univariate analysis (p value ≤0.05) have been used as independent predictors for diagnosis of rheumatic fever to construct the prediction model (Table IV). The final prediction model after step wise approach included 9 variables: arthritis, carditis, chorea, aortic regurgitation, grades of mitral regurgitation ≥10 mm length & velocity ≥2.5 m/s, thick anterior mitral valve leaflets, elevated erythrocyte sedimentation rate and C- reactive protein, positive family history, and prolonged PR interval (Table V). The final prediction model had an Eigen value 0.783, canonical correlation 0.663, Wilks' lambda 0.561, p value 0.0001, sensitivity 93% and specificity 62% with overall prediction accuracy of 81.4%.

ROC curve of final model was done with AUC 0.889 (Fig. 3).

Discriminant analysis was also used to detect the best echocardiographic predictors of proven rheumatic fever where significant echocardiographic criteria by univariate analysis (p ≤0.05) have been used as independent predictors for diagnosis of rheumatic fever to construct echocardiographic criteria prediction model (Table VI). The final echocardiographic prediction model after stepwise approach included 4 variables namely: grades of mitral regurgitation ≥10 mm length & velocity ≥2.5 m/s, grades of aortic regurgitation ≥10 mm length & velocity ≥2.5 m/s, ≥2 rheumatic mitral morphological changes, thick mitral valve leaflets. The final echocardiographic criteria prediction model had an Eigen value 0.1, canonical correlation 0.301, Wilks' lambda 0.909, and p value 0 .0001. The model had a sensitivity of 98.6%, specificity 7.2% and an overall prediction accuracy of 64.3% as it correctly classified 549 out of 557 patients with proven rheumatic fever, 24 out of 334 patients with probable rheumatic fever however, 310 patients were recommended to be classified as proven rheumatic fever by our model though being diagnosed as probable rheumatic fever according to the updated Jones criteria.

Discussion

Our study showed a predominance of females with rheumatic fever (53.4%) this was similar to other studies.[15,16] In contrast to a study where rheumatic fever was more in males (62.5%).[17] Age of our patients (5-15 years) was similar to other studies.[18] However rheumatic fever in ages <5 years or >15 years has been reported by some studies.[19,20]

Similar to what have been reported by other studies;[21,22] arthritis, carditis and chorea were the most common major manifestations encountered in our patients. Mitral regurgitation was the most common valvular lesion followed by aortic regurgitation which is similar to other studies.[23] Although mitral stenosis is uncommon before 10 years of age,[24] 1.7% of our patients had mitral stenosis and 1.5% had both mitral regurgitation and stenosis. However, aortic stenosis wasn't detected in our patients which adds to the evidence that rheumatic heart disease is an uncommon cause of aortic stenosis.[25]

Several studies, as in ours, have documented the prevalence of subclinical carditis and its association with other major criteria.[26-29] Most of these studies, as in our study, used World Health Organization criteria in diagnosing a pathological regurgitation.[26,29-37]

Table IV. The performance of predictors in the diagnosis of rheumatic fever.

Predictors of rheumatic fever	Standardized canonical discriminant function coefficients*	P-value	Sensitivity (%)	Specificity (%)	AUC
Arthritis	0.672	0.0001	74.8	55.7	0.657
Carditis	0.434	0.0001	91.1	49.8	0.684
Chorea	0.495	0.0001	93.8	40	0.549
Subclinical carditis	-0.175	0.0001	38.1	25.2	0.462
Limiting arthralgia	-0.172	0.0001	37.6	31.6	0.465
Elevated ESR, CRP	0.264	0.0001	77.7	47.2	0.626
Fever	0.090	0.0001	71.9	46.7	0.599
Arthralgia	0.136	0.033	58.6	35.0	0.468
Positive family history	-0.092	0.003	52.8	35.3	0.554
Prolonged PR interval	0.098	0.0001	100	38.3	0.516
Elevated ASO	0.078	0.0001	68.4	42.1	0.555
Mitral regurgitation	0.091	0.022	59.0	33.5	0.540
Aortic regurgitation	0.193	0.0001	79.1	44.2	0.602
Grades of MR ≥10 mm length & ≥2.5 m/s velocity	0.175	0.0001	64.7	40.3	0.606
Grades of AR ≥10mm length & ≥2.5 m/s velocity	- 0.076	0.0001	80.2	44.5	0.615
Rheumatic morphological features of mitral valve ≥2	-0.183	0.001	81.6	40.6	0.561
Mitral stenosis	0.040	0.006	100	38.1	0.513
Thick MV leaflets	0.294	0.0001	81.5	42.1	0.579
Thick subvalvular apparatus	0.047	0.013	78.3	38.6	0.513
Lack of systolic coaptation	0.042	0.0001	100	38.6	0.522
Restricted PMVL	0.016	0.003	81.6	38.6	0.512

AR: aortic regurgitation, ASO: antistreptolysin-O, AUC: area under the curve, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, MR: mitral regurgitation, MV: mitral valve, PMVL: posterior mitral valve leaflets.
*The standardized canonical discriminant coefficients can be used to rank the importance of each variable in the prediction model. A high standardized discriminant function coefficient means that the groups differ a lot on that variable.

A meta-analysis was done to study the prevalence and outcome of subclinical carditis in acute rheumatic fever including more than 1700 rheumatic fever cases in studies done during 1996 through March 2005.[38] Of 63 articles, 23 articles only documented the prevalence of subclinical carditis in their study population.[38] World Health Organization criteria were used completely in 12 studies and incompletely in five studies; the remaining six studies did not specify criteria used.[38] In this meta-analysis, age range of patients was similar to our study.[38] The prevalence of subclinical carditis ranged from 0% in one study[39] to 53% in 23 studies.[38] The weighted pooled prevalence of subclinical carditis in acute rheumatic fever in this meta-analysis was 16.8%. Eleven studies only attempted to follow-up their patients.[38] Follow up revealed progressive valve dysfunction in some studies[29,40,41] improvement and even resolution in other studies.[37,42,43] Others found new cases of subclinical carditis, mostly diagnosed within the first year of follow-up.[44] One study reported recurrent subclinical carditis after 4 years of initial resolution.[28] The weighted pooled prevalence of persistence or deterioration (3–23 months) after acute rheumatic fever diagnosis was 44.7%. Of

Table V. Final prediction model of rheumatic fever.

Best predictors of rheumatic fever	Standardized canonical discriminant function coefficients*	AUC
Arthritis	0.621	0.657
Carditis	0.435	0.684
Chorea	0.314	0.549
Aortic regurgitation	0.196	0.602
Grades of MR ≥10 mm length & velocity ≥2.5 m/s	0.177	0.606
Elevated ESR, CRP	0.273	0.626
Prolonged PR interval	-0.117	0.516
Thick mitral valve leaflets	0.293	0.579
Positive family history	-0.425	0.554

AUC: area under the curve, CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, MR: mitral regurgitation.
*The standardized canonical discriminant coefficients can be used to rank the importance of each variable in the prediction model. A high standardized discriminant function coefficient means that the groups differ a lot on that variable.

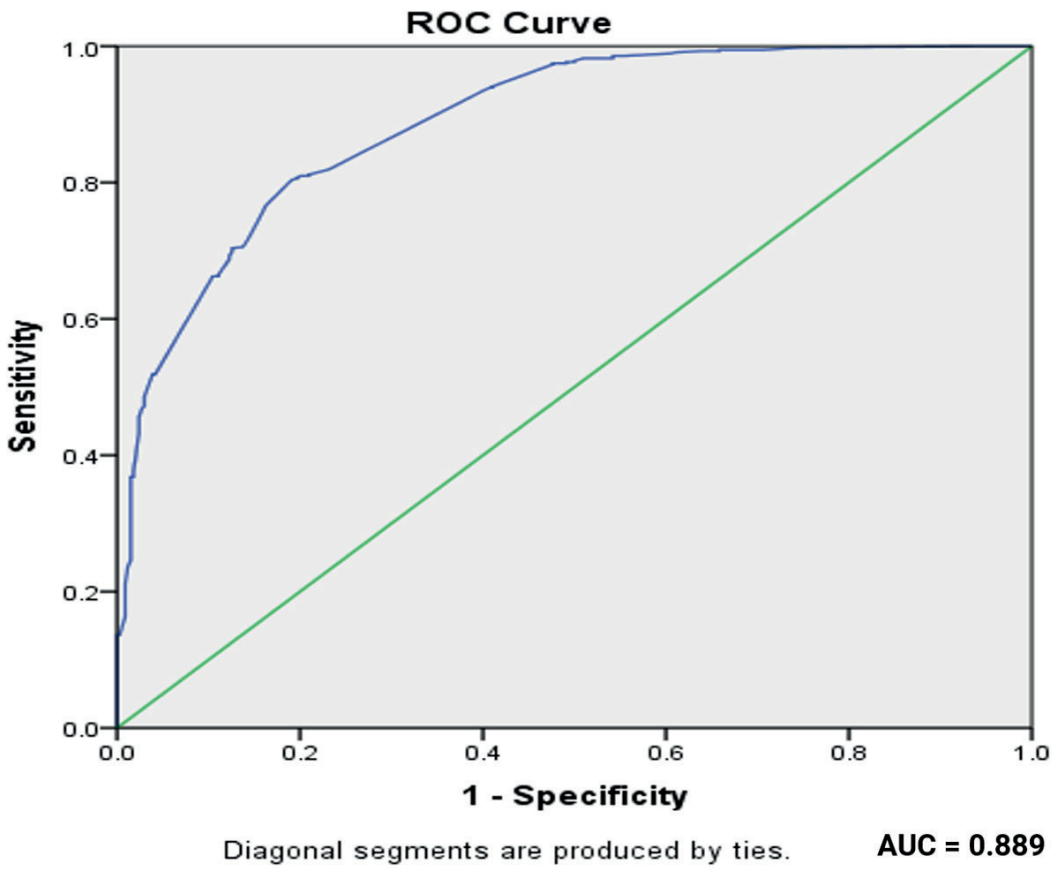

Fig. 3. Receiver operating characteristic (ROC)* curve of rheumatic fever prediction model. ROC curves were done to visually and statistically assess the sensitivity, specificity, and overall performance of predictors of proved rheumatic fever. The area under the ROC curve of the whole prediction model was significantly high: 0.889.

*ROC curve, is a graphical plot of the sensitivity vs. (1 - specificity) for a binary classifier system as its discrimination threshold is varied. The area under the ROC curve (AUC) corresponds to the probability that a physician using the prediction model will correctly classify a pair of patients with and without disease.

Table VI. The contribution of significant echocardiographic criteria in the prediction model.

Significant echocardiographic criteria	Standardized canonical discriminant function coefficients
Grades of mitral regurgitation ≥10mm length & velocity ≥2.5m/s	0.330
Grades of aortic regurgitation ≥10mm length & velocity ≥2.5m/s	0.544
Rheumatic mitral morphological changes (2 or more)	-0.836
Mitral stenosis	0.202
Mitral valve prolapse	0.148
Thick mitral valve leaflets	0.803
Thick subvalvular apparatus	0.025
Lack of systolic coaptation	0.153
Restricted posterior mitral valve leaflets	0.142

The standardized canonical discriminant coefficients can be used to rank the importance of each variable in the prediction model. A high standardized discriminant function coefficient means that the groups (proven and probable rheumatic fever) differ a lot on that variable.

patients followed for (>23 months), two thirds had persistence and one third had resolution.[38]

The number of patients followed up in these studies was very small.[38] Unlike our study, most studies didn't detail which patients received secondary prophylaxis and few studies reported whether valvular regurgitation remained subclinical or became clinical at any stage.[38] The predictors of improvement, persistence or deterioration of subclinical carditis were unknown. However, this improvement or deterioration emphasizes the significance of early diagnosis, early prescription of secondary prophylaxis and close follow up for patients with subclinical carditis.

Vijaya's echocardiographic criteria were evolved using the common echocardiographic features detected in 492 patients with isolated manifestations of acute rheumatic fever such as arthritis or chorea[45] and its efficacy in an Indian population was tested in a prospective double blinded study including 333 patients and showed sensitivity of 81% and specificity of 93%.[32] This study also used World Health Organization criteria but unless valvular regurgitation was associated with rheumatic morphological features with an echo score of ≥6, it was not taken as pathological. Vijaya's echocardiographic criteria avoided over diagnosis, however, morphological changes are often minimal in acute carditis, so we believe that if patients with isolated pathological regurgitation were not considered rheumatic or even probable rheumatic fever, many patients would be missed.

Our study strongly agrees with the recommendations of World Health Federation, Australian guidelines and the new American Heart Association updated revision of Jones Criteria[9-11] that subclinical carditis should be added as a major criterion in the diagnosis of rheumatic fever in high risk populations like Egypt. The set of morphological changes that indicate the diagnosis of rheumatic heart disease as well as the recommended two echocardiographic categories by World Health Federation were strongly applicable on our patients. On the other hand, we still believe that we should rely upon the World Health Organization criteria[7] in diagnosing a pathological regurgitation. Trivial pansystolic, mosaic mitral regurgitation of jet length 10-20 mm and velocity ≥2.5 m/sec should not be taken lightly nor considered physiological as stated by World Health Federation, Australian guidelines and approved later by American Heart Association.[9-11] This was very much proven by our study.

In our patients, 19.1% (170) had limiting polyarthralgia; 58.8% of them had associated

subclinical carditis, 30.6% had carditis, while 8.2% had chorea. If limiting polyarthralgia was taken lightly many patients with subclinical carditis would not have been diagnosed. Similarly, in an Indian study 70% of their patients had polyarthralgia; subclinical carditis was detected in 46.9% of them.[45] We believe that limiting, fleeting polyarthralgia involving big joints that was associated with elevated erythrocyte sedimentation rate and showed dramatic response to salicylates should be added as a major criterion in the diagnosis of rheumatic fever especially in high risk populations as recommended by Australian guidelines and American Heart Association[8,9,11] however we suggest that non limiting, non-fleeting polyarthralgia should still be considered a minor criterion as it didn't show a high significance for diagnosis of rheumatic fever in our patients.

In 127 patients with acute rheumatic fever presentation, 7 patients (5.5%) had monoarthritis; two patients had arthritis in one knee while five patients had arthritis in one ankle. Five patients had associated carditis, two had subclinical carditis, three had mitral regurgitation, while four patients had aortic regurgitation. Elevated erythrocyte sedimentation rate, C-reactive protein and antistreptolysin O titer were reported in all 7 patients. Monoarthritis resolved dramatically after a trial of salicylate treatment in all 7 patients. Our study emphasizes the significance of monoarthritis as stated by many studies[31,46,47] and we also strongly suggest its addition as a major criterion in diagnosis of rheumatic fever especially in high risk populations as stated by Australian guidelines[9] and American Heart Association.[11]

Erythema marginatum was reported in 3 patients only (0.3%), this low incidence was similarly reported by other studies.[45,48] Two patients had associated carditis, one had subclinical carditis and one had arthritis which indicates that this criterion shouldn't be considered irrelevant as described by other studies.[45] Besides, low incidence might be due the evanescent nature of these lesions, so it could be missed even by expert clinicians especially in dark-skinned patients.

The incidence of subcutaneous nodules in our patients was very low too; reported in 4 patients only (0.4%). One patient had associated arthritis, one patient had chorea and all four patients had severe carditis with a p value 0.002. Subcutaneous nodules were more frequently reported in patients with severe carditis by many other studies too.[5,45]

Our study as well as many other studies pointed to the importance of positive family history reported in 18.3% of our patients with a p value 0.003 in diagnosis of rheumatic fever. We believe, as proven by other researches, that genetic susceptibility factors among patients with acute rheumatic fever might point to a totally new set of diagnostic tools.[3,49,50]

We concluded that echocardiography should be performed in all patients with suspected rheumatic fever and subclinical carditis should be considered a diagnostic major criterion. We recommend adopting World Health Organization criteria when diagnosing a pathological regurgitation. Regurgitation or stenosis should definitely be considered rheumatic by echocardiography if associated with rheumatic morphological features. Patients with isolated rheumatic morphological changes or with isolated pathological regurgitation or stenosis should be diagnosed probable rheumatic fever. Limiting, fleeting polyarthralgia and aseptic monoarthritis showing dramatic response to salicylates should be considered as diagnostic major criteria in high risk populations. Positive family history showed a high significance in diagnosis of our rheumatic fever patients so future genetic studies are mandatory. Highly suspicious patients despite not fulfilling Jones criteria should be diagnosed probable rheumatic fever, prescribed long acting penicillin and followed up yearly to revise diagnosis. We compared our results to the most recent update by the

American Heart Association. We concluded that strict application of updated Jones criteria may lead to under diagnosis of rheumatic fever in highly endemic countries. We recommend further studies to examine the sensitivity of the most recent update of Jones criteria on other highly endemic populations.

Unfortunately, there is no single laboratory test that definitely establishes the diagnosis of acute rheumatic fever which makes the diagnosis difficult and mainly based on clinical criteria. We adhered strictly to the updated jones criteria in diagnosis of proven rheumatic fever patients. However highly suspicious patients despite not completely fulfilling the criteria were diagnosed as probable rheumatic fever, prescribed secondary prophylaxis and followed up yearly for possible change of diagnosis.

Retrospective studies are known to carry risk of inconsistent methodology; however, we were following a standard protocol which minimized any possible risk of bias.

We only conducted 1 follow up echocardiographic study after 1 year for patients with subclinical carditis and 14.4% missed their follow up despite being contacted. A prospective long-term follow up is needed in the future to demonstrate its potential significance.

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Original Article

Evaluating the effects of different doses of ursodeoxycholic acid on neonatal jaundice

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ABSTRACT

Background. Icterus tends to be one of the most prevalent causes of neonatal hospitalization. The present study aimed to evaluate the effects of the different doses of ursodeoxycholic acid (UDCA) on neonatal jaundice.

Method. In this study, 120 newborns who were hospitalized for phototherapy were randomly assigned. Group A received phototherapy and UDCA 5 mg/kg/dose every 12 hours orally, group B patients were treated with phototherapy and UDCA 7.5 mg/kg/dose every 12 hours orally. Group C received phototherapy with a placebo. All patients were evaluated for bilirubin levels, the duration of phototherapy, and the length of hospital stay.

Results. The mean bilirubin level at hospital admission was 19.88 ± 2.33 mg/dl in group A, 19.33 ± 2.51 mg/dl in group B, and 19.76 ± 2.64 mg/dl in group C (p= 0.58). The groups receiving phototherapy with UDCA showed a significant decrease in the bilirubin level. Bilirubin level decreased to 10.04 ± 1.11mg/dl in group A, 8.82 ± 1.11 mg/dl in group B, and 12.04 ± 2.05 mg/dl in group C (p= 0.000). Furthermore, the mean duration of phototherapy, as well as the average length of hospital stay, were significantly lower in group B as compared to the other groups (p= 0.000).

Conclusion. The findings of this study indicated that the administration of UDCA in addition to phototherapy could effectively decrease the length of hospital stay and bilirubin levels in neonatal hyperbilirubinemia. However, further studies with a larger sample size are required before one can recommend the routine use of UDCA for the treatment of neonatal jaundice.

Key words: bilirubin, hospital stay, neonatal jaundice, ursobil, ursodeoxycholic acid.

Hyperbilirubinemia tends to be one of the most prevalent health issues in neonates. Its prevalence has been reported in half of the term and the majority of the preterm neonates.[1] Phototherapy is usually the mainstay for the treatment of indirect (unconjugated) hyperbilirubinemia. It reduces the plasma levels of unconjugated bilirubin, preventing kernicterus and decreasing the need for exchange transfusion.[2] However, the use of this method is accompanied by several short or long term side effects. the short-term effects include interference in the maternal-infant relationship, dehydration, diarrhea, maculopapular rash, patent ductus arteriosus, and high hospital costs.[2-4] Several studies have been published to correlate phototherapy with the long-term side effects such as neoplasm, nevi, café au lait spots, and allergic diseases such as asthma, rhinitis, conjunctivitis, and the immune and inflammatory response.[2]

So far, various medications such as activated charcoal, d-penicillamine, phenobarbital, metalloporphyrin, clofibrate, and bile salts have been used for the treatment of indirect hyperbilirubinemia.[5-8] The use of phenobarbital has been observed to reduce indirect hyperbilirubinemia and the duration of phototherapy; while, phenobarbital was shown to lead to some side effects in patients such as drowsiness, reduced rate of breastfeeding, and neurological disorders.[8]

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Recently, ursodeoxycholic acid (UDCA) has been proposed to be used in the treatment of neonatal jaundice. Dietary bile salt administration induces a large, persistent decrease in plasma unconjugated bilirubin (UCB) concentrations in Gunn rats.[9] UDCA enhances UCB turnover by increasing its fecal disposal. These results support the application of oral bile salt treatment in patients with unconjugated hyperbilirubinemia.[9] It has already been widely used in the treatment of cholestatic hepatic diseases, gallstones, and primary biliary cholangitis and has resulted in a decrease in indirect bilirubin levels and the severity of jaundice.[10] UDCA has been reported to play its role through anti-inflammatory, anti-apoptosis, and immunomodulatory effects. It might also control the changes in bile acid levels. Besides, UDCA increases the secretion of bile acids from the liver, improves cell signaling, and preserves the integrity of mitochondria.[10]

Some studies have already evaluated the effects and dosing of UDCA in neonatal hyperbilirubinemia. For instance, Honar et al.[11] suggested that using UDCA (10 mg/kg) along with phototherapy leads to a significant decrease in the indirect bilirubin level in neonatal jaundice. However, the information regarding its exact dosing in the treatment of neonatal jaundice is lacking.[12-15] Considering the side effects of phototherapy[2] and the effectiveness of UDCA on neonatal jaundice, this study was conducted to evaluate the effects of different doses of UDCA on decreasing neonatal hyperbilirubinemia and their length of hospital stay.

Material and Methods

The present study is a clinical trial, trying to evaluate the effects of UDCA in patients who were hospitalized with neonatal jaundice in the Pediatric Hospital of Tabriz, Iran, from January to December 2017. The inclusion criteria were having a birth weight of higher than 2500 g, gestational age of over 35 weeks, total bilirubin level of 14-25 mg/dl, and a direct bilirubin level of lower than 2 mg/dl. The policy for treatment of neonatal hyperbilirubinemia in our center is based on AAP guidelines.[3] The neonates who had Rh or ABO incompatibility (with positive direct coombs test), G6PD deficiency, direct hyperbilirubinemia, sepsis, Crigler-Najjar syndrome, thyroid disorders, hepatic diseases, and diabetic mothers were excluded from the study. We exclude G6PD deficiency, and thyroid disorders by routine lab tests. Crigler-Najjar Syndrome was ruled out by unresponsive hyperbilirubinemia to phototherapy in follow up.

This study was approved by the Ethics Committee for Clinical Trials of Tabriz University of Medical Sciences and was registered in IRCT with the registration code of IRCT201701313915N18. Informed written consent was obtained from parents.

All of the neonates who had inclusion criteria and admitted only for the treatment of indirect hyperbilirubinemia were enrolled in this study based on AAP recommendations. The included neonates were randomly allocated into three groups, each consisting of 40 neonates. The patients were assigned to the groups randomly using a random number table. The patients in group A received intensive phototherapy (LED lamps) and UDCA was administered 10 mg/kg in two divided doses (5 mg/kg/dose). Those in group B received phototherapy along with 15 mg/kg of UDCA in two divided doses (7.5 mg/kg/dose). Group C was the control group and the patients received phototherapy and the placebo. The termination criteria for phototherapy were decrease in total bilirubin concentration less than 50% exchange threshold or bilirubin less than 10 mg/dl. Throughout the study, the responsible physicians were kept blind to the grouping of the patients. Total and indirect bilirubin levels were measured by 1-1.5 ml blood at the 6, 12, 24, and 48 hours after the admission and phototherapy. Changes in the bowel habits (i.e., diarrhea or constipation), and other complications such as vomiting, food intolerance, and nutritional disorders were compared in three groups. In this study, the

primary outcome was the reduction of bilirubin level, and the secondary outcome was the length of stay in the hospital and the duration of phototherapy.

The demographic characteristics of the infants as well as bilirubin levels were recorded in a checklist. The gathered data were then fed into SPSS Software, Version 16 and the statistical analyses were performed by ANOVA and Chi-square tests. A p-value of less than 0.05 was considered significant in this study.

Results

This study was conducted on 120 neonates suffering from neonatal jaundice. The patients were allocated in 3 groups: group A (receiving phototherapy and UDCA 5 mg/kg/dose every 12 hours orally), group B (receiving phototherapy and UDCA 7.5 mg/kg/dose every 12 hours orally), and group C (just receiving phototherapy and placebo). Twenty-two patients (55%) in group A, 24 patients (60%) in group B, and 22 patients (55%) in group C were boys, and the rest were girls (p= 0.66). The three groups were not significantly different regarding the mean age (p= 0.26), gestational age at the time of delivery (p= 0.49), and body weight at the time of admission (p= 0.66) and discharge from the hospital (p= 0.06). However, the weight gain during hospitalization was observed to be significantly higher in the control group as compared to the other groups (p= 0.001) (Table I).

The direct bilirubin level was found to be lower than 2 mg/dl in all of the neonates. The indirect bilirubin level was also monitored at the time of admission and discharge from the hospital, as well as 6, 12, 24, and 48 hours after hospitalization. The results showed significant differences among the three groups, six hours after hospitalization. The bilirubin levels in groups A and B were significantly lower than that of the patients in group C, at 6 hours after hospitalization (Table II). The comparison of bilirubin levels revealed that the patients receiving UDCA had lower levels as compared to the patients in the control group, at all measured times, except for upon the admission (Fig. 1).

Table I. General findings of the study groups.

	5-mg group (A) N=40	7.5-mg group (B) N=40	Control (C) N=40	p-value
Age (days)	5.91 ± 2.49	5.1 ± 2.47	5.91 ± 2.49	0.26
Gestational age (weeks)	37.75 ± 4.93	38.1 ± 1.05	38.55 ± 1.01	0.49
Admission weight (gr)	3175.41 ± 561.53	2961.71 ± 440.03	3192.91 ± 431.02	0.08
Discharge weight (gr)	3200.69 ± 557.59	3008.29 ± 445.03	3269.17 ± 708.38	0.06
Weight changes during admission (gr)	25.27	42.46	76.36	0.00

Table II. Average bilirubin levels in study groups at different times.

	5-mg group (A) N=40	7.5-mg group (B) N=40	Control (C) N=40	p-value
Indirect bilirubin at admission (mg/dl)	19.88 ± 2.33	19.33 ± 2.51	19.76 ± 2.64	0.58
Direct bilirubin at admission (mg/dl)	0.51 ± 0.11	0.91 ± 0.42	0.48 ± 0.12	0.39
Indirect bilirubin at 6th hour(mg/dl)	16.82 ± 2.0	15.78 ± 2.44	17.2 ± 2.51	0.01
Indirect bilirubin at 12th hour(mg/dl)	13.24 ± 2.16	12.07 ± 2.15	14.66 ± 2.55	0.000
Indirect bilirubin at 24th hour(mg/dl)	10.04 ± 1.11	8.82 ± 1.11	12.04 ± 2.05	0.000
Indirect bilirubin at 48th hour(mg/dl)	7.96 ± 1.75	6.21 ± 1.06	10.17 ± 1.96	0.000
Indirect bilirubin at discharge (mg/dl)	4.75 ± 1.07	4.74 ± 0.63	7.88 ± 2.11	0.000

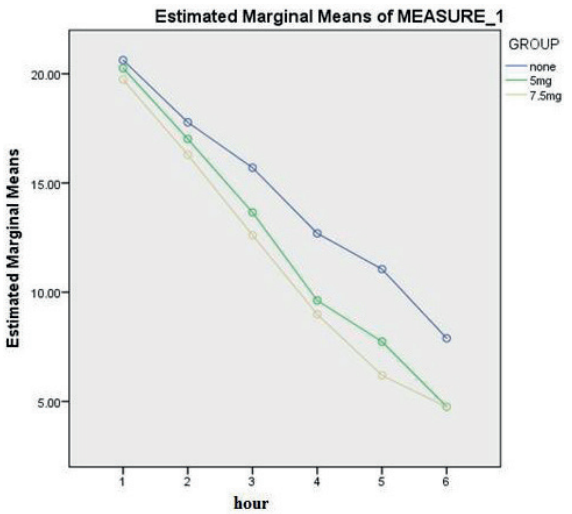

Fig. 1. The bilirubin levels in the three groups.

Furthermore, among the two groups receiving UDCA, the reduction of bilirubin was higher in the group of patients for whom the higher doses of medication were administered (Table III). The mean length of hospital stay was 29.47 ± 16.80 hours for the patients in group A, 21.35 ± 8.12 hours for the patients in group B, and 45.97 ± 18.01 hours for the patients in group C (p= 0.000). In all studied patients, the predominant source of nutrition was breast milk (p= 0.43) (Table IV). There were no cases of vomiting, food intolerance, or constipation in studied patients. Diarrhea was not seen in patients who received Ursobil significantly more than the placebo group.

Discussion

Ursodeoxycholic acid (UDCA) is widely used in the treatment of chronic cholestatic liver diseases. Recently, some studies have attempted to investigate the effect of this medication on infants with indirect hyperbilirubinemia. Based on the results of the present study higher doses of UDCA are more effective in decreasing the indirect bilirubin levels.

In a study conducted by Honar et al.[11] the total bilirubin level of the infants 12, 24, and 48 hours after hospitalization was reported to be 12, 10, and 9.8 mg/dl in the UDCA group and

Table III. Comparison of indirect bilirubin levels in different groups.

	5-mg group (A) N=40	7.5-mg group (B) N=40	Control (C) N=40	p-value
Indirect bilirubin at admission (mg/dl)	19.88 ± 2.33	-	19.76 ± 2.64	0.83
	-	19.33 ± 2.51	19.76 ± 2.64	0.45
	19.88 ± 2.33	19.33 ± 2.51	-	0.30
Indirect bilirubin at 6th hour(mg/dl)	16.82 ± 2.0	-	17.2 ± 2.51	0.89
	-	15.78 ± 2.44	17.2 ± 2.51	0.04
	16.82 ± 2.0	15.78 ± 2.44	-	0.04
Indirect bilirubin at 12th hour(mg/dl)	13.24 ± 2.16	-	14.66 ± 2.55	0.009
	-	12.07 ± 2.15	14.66 ± 2.55	0.000
	13.24 ± 2.16	12.07 ± 2.15	-	0.01
Indirect bilirubin at 24th hour(mg/dl)	10.04 ± 1.11	-	12.04 ± 2.05	0.000
	-	8.82 ± 1.11	12.04 ± 2.05	0.000
	10.04 ± 1.11	8.82 ± 1.11	-	0.001
Indirect bilirubin at 48th hour(mg/dl)	7.96 ± 1.75	-	10.17 ± 1.96	0.000
	-	6.21 ± 1.06	10.17 ± 1.96	0.000
	7.96 ± 1.75	6.21 ± 1.06	-	0.000
Indirect bilirubin at discharge (mg/dl)	4.75 ± 1.07	-	7.88 ± 2.11	0.000
	-	4.74 ± 0.63	7.88 ± 2.11	0.000
	4.75 ± 1.07	4.74 ± 0.63	-	0.97

Table IV. Hospital stay duration and type of nutrition in three groups.

	Variable	Control		5mg group		7.5 mg group		pvalue
		Frequency	percent	Frequency	percent	Frequency	percent	
Neonatal duration of hospital stay	<1 day	7	17.5	17	42.5	29	72.5	0.000
	2 day	10	25	20	50	10	25	
	3 day	21	52.5	1	2.5	1	2.5	
	>3day	2	5	2	5	0	0	
Type of feeding	Breast milk	28	70	34	85	30	75	0.43
	Breast milk + formula	9	22.5	3	7.5	6	15	
	Formula only	3	7.5	3	7.5	4	10	

14.4, 12.5, and 10.1 mg/dl in the control group, respectively (p= 0/05). Hassan et al.[16] reported in their study that the mean indirect bilirubin levels of the infants 12, 24, and 36 hours after hospitalization were 11.7, 8.8, and 7.6 mg/dl in the UDCA group and 14.6, 13.2, and 10.2 mg/dl in the control group, respectively. They observed that the reduction of bilirubin levels in the patients receiving UDCA was significantly higher than that of the patients in the control group (p= 0.00). In Jafari et al.'s[17] study, the mean indirect bilirubin level in the infants was measured. They illustrated that the reduction of bilirubin levels in the UDCA -receiving groups was significantly higher when compared to the control group. They also reported that the most effective dose of this medication for bilirubin reduction is 10 mg/kg/day. In another study, conducted by George et al.[18] the administration of UDCA reduced serum bilirubin level in 4 of the five infants suffering from cholestasis.

On the other hand, Maldonado et al.[19] investigated neonatal icterus in their study and found that UDCA is more effective than phenobarbital in reducing bilirubin levels. However, in Rina et al.[20] study, the use of UDCA failed to bring about significant changes in hepatic parameters over seven days. In their study, the reduction of total bilirubin level was reported to be 2.2 mg/dl in the patients receiving UDCA and 1.7 mg/dl in the patients of the control group (p= 0.80). Altogether, a review of the previous studies showed that the use of UDCA has led to a decrease in the infants bilirubin level. It should be noted that this medication has been administered along with phototherapy and the additional administration of UDCA has enhanced the therapeutic effects of phototherapy. Therefore, the findings of the present study are consistent with those of other studies.

In the study by Honar et al.[11], the average length of hospital stay was reported to be 15.5 hours for the group of patients receiving UDCA and 44.6 hours for those in the control group. They found that there was a significant association between the duration of stay in the hospital and the administration of UDCA (p= 0.00). In another study, Hassan et al.[16] reported the average length of stay in the hospital to be 23.2 hours for the neonates receiving UDCA and 41.1 hours for those in the control group (p= 0.00). Jafari et al.[17] also reported similar findings. However, in Lewis et al.[22] study, the length of hospital stay for the infants receiving UDCA was found to be similar to that of the infants receiving phenobarbital. In other words, in their study, the administration of Ursobil failed to depict significant changes in the infants length of stay in the hospital (p= 0.45). The findings of the present study are similar to Honar's[11] and Hassan's[16] studies. In our study, as in other studies, the administration of UDCA in patients led to a significant decrease in hospital stay and phototherapy duration. However, although the findings of our study and many other studies indicated the effectiveness of administering UDCA along with phototherapy, further studies with a larger sample size are required before routine administration of UDCA for neonatal

hyperbilirubinemia. The strengths of this study were the assessment of two different doses of UDCA and the investigation of a large number of patients. Its limitations, on the other hand, were the lack of long-term follow-up and the absence of multicenter trials. It is recommended future studies with a large number of patients, long term follow up of patients and also in neonates with severe hyperbilirubinemia near the exchange transfusion threshold before routine use of UDCA.

The findings of this study indicated that the administration of UDCA in addition to phototherapy, on infants suffering from neonatal jaundice might lead to the shorter length of hospital stay and rapid decrease in the unconjugated bilirubin concentrations and limited brain exposure to its neurotoxic effects.

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Original Article

Effect of gestational diabetes on the vitamin D levels in the neonates: a case control study

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ABSTRACT

Background and objectives. The present study was conducted to determine the effect of maternal gestational diabetes on the Vitamin D levels of the mother and their newborns and to compare it with healthy mother-infant pairs.

Methods. The study design was a Case Control study. It was conducted at the antenatal unit of Obstetrics and Neonatal unit of Pediatrics department of a tertiary care hospital in costal Karnataka. Consecutive sample of otherwise healthy pregnant women presenting with Gestational Diabetes Mellitus (GDM) and their healthy term neonates were taken as study group. The weight matched healthy mothers and their healthy term neonates were taken as controls. The blood samples of the mothers, at term and the cord blood samples of the neonates were collected for estimating the Vitamin D levels. Vitamin D levels in the cases and controls were the primary outcome measures.

Results. The mean value of Vitamin D levels in the GDM mothers was 10.74 ng/ml and in the mothers forming the control group was 23.53ng/ml (p value <0.001). The mean value of Vitamin D levels in GDM babies was 8.47ng/ml and was 19.51ng/ml in the control (p value <0.001).

Conclusion. Comparison of Vitamin D levels of mothers and infants of both groups showed a positive correlation. GDM seems to exacerbate the Vitamin D deficiency in the mothers and their neonates.

Key words: infants of diabetic mothers, gestational diabetes, deficiency.

Vitamin D is the most common nutritional deficiency and a public health issue worldwide, with the number of people affected by vitamin D deficiency or insufficiency reaching nearly 1 billion.[1,2] Various adverse fetal outcomes have been linked with Vitamin D deficiency during pregnancy including low birth weight, neonatal hypocalcaemia, abortions, impaired development and rickets.

Some previous studies have linked decreased Vitamin D levels with onset of GDM, so it was postulated that an infant of a diabetic mother may also have decreased levels of Vitamin D when compared to healthy mothers.

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Material and Methods

An institutional ethical and scientific committee approval for the research and the informed consent was taken before starting the study (Ref. INST.EC/EC/092/2015-16; dated 16/11/2015). An informed consent was taken before enrolling any of the mother-infant pair. The maternal sampling was done along with routine investigations and cord blood was taken from neonates to prevent unnecessary invasive pricks.

Mothers diagnosed with GDM were enrolled in the study. Any mother, who was on any drug affecting Vitamin D levels, or had history of thyroid disease, metabolic bone diseases or pre gestational type 1 or type 2 diabetes mellitus were excluded. Any neonates who were preterm or had low birth weight or multiple gestation were also excluded from the study.

Thirty consecutive, live born, healthy infants born to mothers with GDM were enrolled into study. A complete data sheet was prepared for both the mother and infant, including their anthropometric measurements and laboratory values including HbA1c and vitamin D levels. Thirty healthy age, weight and gestational age matched controls were enrolled as the control group.

The 75gm Glucose Tolerance Test was done to diagnose GDM. according to Carpenter and Couston guidelines.[3] Any mother with overt diabetes mellitus were excluded from the study.

The blood samples of mothers in both the cases and controls groups were collected at 37 weeks of gestation. After delivery, the cord blood sample of their newborns were collected. Measurement of the Vitamin D level was done using ELISA, via a commercially available kit.

For this study, we followed the US Endocrine Society guidelines for the vitamin D levels.[4] We took vitamin D levels less than 20ng/ml as deficiency, whereas, 21-29ng/ml was taken as insufficiency, >30ng/ml was considered sufficiency and >150ng/ml was considered toxicity. As the Endocrine society guidelines have been standardized for all ages and hold true for neonates as well, and the data was collected only for comparative analysis, for the purpose of this study, we used the same criteria for Vitamin D insufficiency and deficiency for both mothers and their neonates. Vitamin D levels of mothers and infants born to GDM mothers were compared to normal controls.

Statistical analysis

Descriptive statistics of the data collected were calculated and presented with suitable tables and diagrams. Quantitative variables such as age of mother, Vitamin D levels in mother and baby, period of gestation of baby, baby length, head circumference and weight were expressed in mean and standard deviation. Qualitative variables such as Vitamin D deficiency states in mother and baby, HbA1C levels was expressed in terms of percentage. Chi-square test was used for determination of age, weight and Body mass Index (BMI) of mother and birth weight of baby and association of HbA1C, maternal age with Vitamin D deficiency. Independent t test was used to determine difference in mean length of baby. After finding normality with Shapirowilk test, Mann-Whitney U test was used for comparison of Vitamin D levels in GDM and healthy mothers and Vitamin D levels in GDM babies and healthy babies. A p value of <0.05 was taken as significant.

Statistical analyses were performed using statistical software.

Results

Anthropometric data were taken and analysed for both cases and control groups (Table I). There was no statistically significant difference between the mothers of the cases or control groups on the basis of body weight or BMI. A significant difference was found in the ages (30 ± 4.3 years and 27.6 ± 4.5 years for cases and controls respectively; p value 0.034), as one mother in control group had age less than 20 years.

Among the neonates, no statistically significant difference was found on the basis of mean gestational age, birth weight, length or head circumference in either group. Majority of mothers in our cases (14; 46.67%) were managed with oral hypoglycemics, 12 (40%) were managed by diet modification alone while only 4 (13.33%) required insulin. Out of 30 mothers in the case group, 16 (53.3%) had HbA1c levels ≤6%.

Vitamin D levels for the cases and controls are given in Table II. All (100%; n= 30) of the GDM mothers and infants born to them had Vitamin D deficiency (serum values <20ng/ml). In control group of mothers, 13(43.33%) were deficient, 14(46.66%) were insufficient and only 3(10%) had normal Vitamin D levels. Among their healthy infants (n= 30), 20(66.67%) were deficient, 8(26.67%) were insufficient, while only 2(6.67%) had normal levels of Vitamin D.

Table I. Mean anthropometric measurements of mothers and infants.

	Cases	Controls	p value
Mean age of mother (years)	30.0 ± 4.3	27.6 ± 4.5	0.034
Mean weight (kg) of mother	67.3 ± 8.44	64.1 ± 8.61	0.151
Mean body mass index (kg/m^2) of mother	27.4 ± 3.19	26.5 ± 3.54	0.328
Mean gestational age(days)	268.9 ± 6.70	268.9 ± 9.07	0.987
Mean birth weight of neonate (kg)	3.1 ± 0.36	3.2 ± 0.41	0.343
Mean length(cms) of neonate	49.2 ± 1.3	48.9 ± 0.99	0.378
Mean head circumference of neonate (cms)	34.0 ± 0.83	33.9 ± 0.86	0.432

Table II. Comparison of vitamin D levels in GDM mothers and their newborns with healthy controls.

Mothers	Median [IQR] (ng/ml)	p value	Mann Whitney U
Cases	10.5[8.17-14.32]	0.000	9.00
Controls	21.9[20.03-28.42]		
Neonates	Median [IQR] (ng/ml)	p value	Mann Whitney U
Cases	8.9[6.73-10.80]	0.000	14.50
Controls	17.9[15.15-21.80]		

Lowest value of Vitamin D level in the mothers was 2.21 ng/ml and in the neonates, the level was 4.31 ng/ml.

However, the median values of the Vitamin D levels of the two groups had a very significant difference. In the mothers, the median value among cases was 10.5 (8.17-14.32) ng/ml, which was significantly lower than the median value 21.9 (20.03-28.42) ng/ml in controls. In the neonates, the median value in the case group was 8.9 (6.73-10.8) ng/ml as compared to 17.9 (15.15-21.80)ng/ml in the control group. The p value for both groups was 0.000 which was highly significant.

The individual mother-neonate pair showed a moderate to high level of positive correlation in their serum Vitamin D levels in both the cases and control (Fig. 1). However, this correlation appears more in cases than that of controls. Hence, it can be inferred that there was a significant positive correlation, i.e. with a decrease in Vitamin D levels of mothers a corresponding decrease in Vitamin D levels of their neonates can be expected. We did not get any positive correlation between maternal HbA1C levels and their neonate's vitamin D levels.

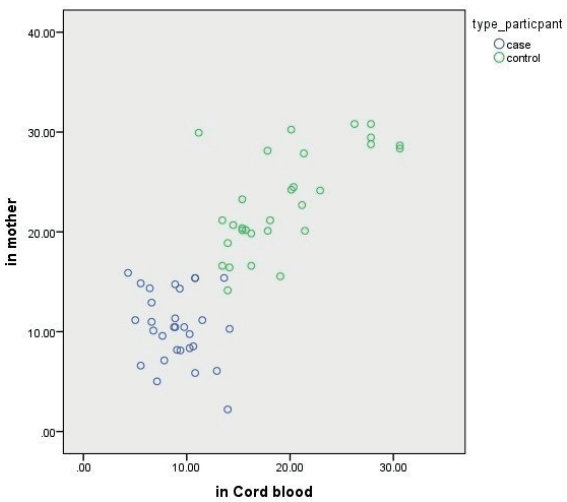

Fig. 1. The distribution scatter plot of the vitamin D levels in the mother with vitamin D levels in the infant. Pearson's correlation was carried out and coefficient was found to be 0.79.

Discussion

In our study, we did not find any statistically significant difference between gestational age and anthropometry among the neonates of GDM and healthy controls. We believe the tight glycemic control achieved by the GDM mothers to be the reason behind this finding in our study. All the GDM mothers and their babies had

Vitamin D deficiency. In our study, although both cases and controls had a high number of participants with vitamin D deficiency, the mean value of Vitamin D levels in the GDM mothers was significantly lower than that of the controls. Similarly, the mean value of Vitamin D levels in GDM babies was significantly lower when compared to the controls. Individual mother-neonate pairs showed a high positive correlation, signifying the neonatal vitamin D levels to be directly influenced by the maternal levels. No correlation was found between the glycemic control of the mother and the neonatal vitamin D levels.

There have been many studies about Vitamin D deficiency among pregnant women and their cord blood, but no specific comparative studies between GDM and normal mothers has been published to our knowledge. Kumar et al.[5], studied 106 mother and cord blood samples for Vitamin D levels. They found majority of the mothers (70.7%) and neonates (83.01%) to have hypovitaminosis D. More neonates born to both mothers with hypovitaminosis D (93.3%) had low vitamin D levels than those born to mothers having normal Vitamin D (61.3%). Like our study, they also found a significant correlation between maternal and cord blood Vitamin D levels.

A similar study was done by Aly et al.[6], evaluating Vitamin D levels in 92 pregnant women at the end of the 3rd trimester and their newborns. Compared to pregnant women with adequate vitamin D levels, significantly higher number of women deficient in Vitamin D had infants with Vitamin D deficiency.Another study conducted by Rajoria et al.[7], on 250 pregnant patients and their newborns showed results similar to ours. Their maternal and cord blood samples also showed significant positive correlation (r= 0.90, p value <0.001).

A systemic review and meta-analysis noted that Vitamin D insufficiency was associated with high risk of GDM.[8] A previous meta-analysis done by Poel et al.[9] in 2012 also found a highly significant difference between the vitamin D levels in pregnant women with GDM compared to those without. The Vitamin D levels of diabetic pregnant women was significantly lower with a p value being 0.018. Similar results were also demonstrated by Soheilykhah et al.[10] in their case control study comparing GDM, Impaired Glucose Tolerant (IGT) women and normal women by their Vitamin D status. They found a 2.66 fold increased risk of Vitamin D deficiency in GDM group when compared to control. These all supported our results where all GDM mothers had Vitamin D deficiency and a mean Vitamin D levels significantly lower than the controls.

Triunfo et al.[11] in their paper have described lower circulating Vitamin D levels as a potential cause for development of gestational diabetes. They discussed the effect of Vitamin D on insulin sensitivity of tissues, genetic variations or polymorphisms of Vitamin D receptors and Vitamin D being an anti-inflammatory agent as potential causes for association of Vitamin D deficiency with GDM. These factors may explain the very significant difference found in the serum Vitamin D levels of pregnant women with and without GDM in our study.

We could not find any previously published articles about the Vitamin D deficiency in infants born to GDM mothers, despite extensive literature search. Our study shows that GDM is not only associated with significant Vitamin D deficiency in the mothers but also their neonates.

In a study done by Naik et al.[12], cord blood Vitamin D levels were estimated in 50 term healthy neonates. Majority of babies had values between 5 to 15ng/ml. This study showed that most of the neonates are born with deficient Vitamin D levels, even in tropical climates. Our study showed similar results where only two babies out of total 60 (3.33%), had normal vitamin D levels.

Our study was limited by the small sample size. Moreover, interventional studies are needed

to evaluate the effect of maternal vitamin D supplementation on the neonatal levels.

The strong correlation between Vitamin D levels in mothers and their newborn indicate that adequate Vitamin D intake for pregnant women should be emphasized through maternal supplementation which might achieve the double effect of preventing Vitamin D deficiency in both mothers and children. GDM in mothers also seems to exacerbate the Vitamin D deficiency in their neonates. Hence, we conclude that all mothers should be recommended Vitamin D prophylaxis during pregnancy, but it should be specifically recommended for pregnant women with GDM.

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Original Article

Emotional support for parents with premature children admitted to a neonatal intensive care unit: a qualitative phenomenological study

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ABSTRACT

Background and objectives. Parents who have a premature child in neonatal intensive care units (NICUs) are in a stressful situation. The aim of this paper is to analyze the emotional support received by parents with premature children admitted to NICUs.

Methods. A phenomenological qualitative study with an explanatory and interpretative approach was employed.

Results. The findings are: 1) The experience and emotions of a premature delivery; showing sadness, guilt and despair, stress, anxiety, and uncertainty over the future of their child. 2) The emotional support received by the father/mother of the partner; discussion of how their partner is cared for, as well as the care given to the premature child and other children in the family; the stress that this causes them on not being able visit all at once. 3) The emotional support offered by the health professionals (doctors, nurses, etc.); parents indicate that they have received very strong support from the nurses, but also that they were not always asked about their feelings when in the NICU. 4) The informal emotional support of relatives and parents in the NICU. After talking with other *support mothers*, the mothers then felt less guilty.

Conclusion. As regards premature birth, the mothers showed feelings of sadness and guilt, asked themselves where they had failed and what they had done wrong.

Key words: premature infant, low birth weight, emotional support, NICU, qualitative research.

Premature birth is that which occurs before week 37 of gestation or before 259 days counting from the first day of the last menstruation[1], or as other authors indicate, before the organs are sufficiently mature to permit normal post-natal survival.[2] Premature birth is a crisis process for mothers. In 2000, Caplan et al.[3] found feelings of anticipated grief in these mothers as well as a sense of failure associated with the birth of the baby before full term.

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Stressful situations described in the literature to which parents may be subjected are diverse. The birth of a premature child can lead to a strong imbalance of roles, which may subsequently give rise to a situational crisis.[4-6] When a baby is admitted to a Neonatal Intensive Care Unit (NICU), this can be a very stressful moment for the parents, for whom the main concern is the diagnosis and prognosis of their child.[6] Additional to this is the fear of not being able to be with the baby, as well as the disconcerting factor of the advanced technology and the general environment in the NICU itself.[4] In these units, health professionals focus their attention on the care of the patient, in many

cases ignoring the other family members.[7]

It should be remembered that the parents can be on an emotional roller-coaster, where the birth of the baby is presented as something surprising and unexpected, for which they were not prepared.[8]

Other stressors can be added to the experience of premature birth, such as the separation from contacts and friendships, loss of intimacy present in the home, in addition to conflict between couples that can end up in separation or divorce.[9]

In view of all this, emotional support is as a key factor in promoting an adaptive approach for mothers and fathers when faced with the birth of a premature child. On the other hand, the complex interactions among issues such as prematurity and paternity, the stressful nature of the environment, and personal circumstances give rise to a wide range of emotional responses.[10]

Several sources of emotional support to parents are described, among them, that of the nurses of the unit. In this respect, the majority of parents in the study by O'Brien et al.[6], reported having received a high level of support from the nurses in the Irish NICU. However, emotional support scored lowest in the functional support provided by the neonatal nursing staff. An important part of nurses' responsibility is to provide support to parents. After observing their child's condition, parents often start seeking hope and feel anxious when encouraging comments from nurses and doctors are not forthcoming; in this context, a sense of desperation is one of the most stressful factors reported by most parents, since any glimmer of hope provides them with the emotional strength to continue the struggle.[5]

The family-centered care of the Newborn Individualized Developmental Care and Assessment Program (NIDCAP) has in current times changed its aims towards ensuring a quality paradigm focusing on the means of promoting family well-being and on parents' participation from the birth of the child.[8] Family-centered care in the NICU entails the active participation of both parents in the daily care of the premature newborn.[8,11] Its basic principles are dignity and respect, and sharing information[11] This philosophy of care should therefore facilitate the establishment of individualized patient attention, meaning that the mother and father feel more secure, reducing anxiety by establishing a therapeutic relationship with the nursing team.[12]

Another source of emotional support is from other parents with premature infants admitted to the unit. These parents-friends (peers) are an aid for promoting an adaptive approach on the part of the parents. Mothers from a culture other than the prevailing one are often geographically distant from their own parents and members of their extended family. However, even when social support was available in their own language, mothers often experienced a feeling of failure, isolation, and a lack of understanding on the part of family and friends to whom they normally feel close. Instead of this usual support network, such mothers tend to talk with fathers-mothers-friends who have already had similar experiences to their own.[10]

We aimed to analyze the emotional support received by the parents with premature children admitted into the Neonatal Intensive Care Unit (NICU).

Material and Methods

Design

We present a phenomenological qualitative study having an explanatory and interpretative approach. It aims to determine the sources of emotional support for parents of premature infants and the importance that these parents give to such sources It facilitates the study of the phenomena in the natural context in which they are produced and to understand the corresponding social reality by sharing and interpreting the importance of the individuals involved.[13] The study sees the individual as integrated within the environment; the

individual is the only source of information for responding to the question posed. In the study that concerns us here, this individual is the mother and father of the premature baby.[14]

Participating population

The participants were the mothers and fathers of premature infants admitted into the NICU.

Inclusion criteria:

- Fathers/ mothers of infants admitted into the NICU.
- Speakers of Spanish or Catalan (or else understand one or both).

Exclusion criteria:

- Parents with cognitive or behavioral disorders
- Mothers admitted for causes other than childbirth
- Denial of consent to record their voice during the interview.

The sample was theoretical, non-probabilistic, intentional and reasonable. Sample units were chosen on the basis of the representation of discourse variability. Individuals capable of providing a good response to the study questions were looked for, as well as the possibility that they were familiar with the study phenomena. To this end, a decision tree was drawn up providing those profiles that a priori would be explored (Fig.1).

It was additionally considered that the sample would be accumulative and sequential, that is to say, the sampling ended with the saturation of data, as well as flexible, circular and reflexive, meaning that the sampling decision could lead to the discovery of new aspects that would need to be studied, or in order to ascertain whether distinct informants were more suitable.

The sampling was performed through a recruiter, who was provided with the characteristics required of the informant.

Data collection

Data were collected between April 2016 and February 2018. Data collection was carried out by nurses working in the NICU who are also researchers in this study, collecting data from informants whose children were not awarded to these professional´s care collaborators working in the NICU, collecting data from the informants whose children were not awarded to these workers' care.

The interview used was in depth, semi-structured and individual, with a script of the

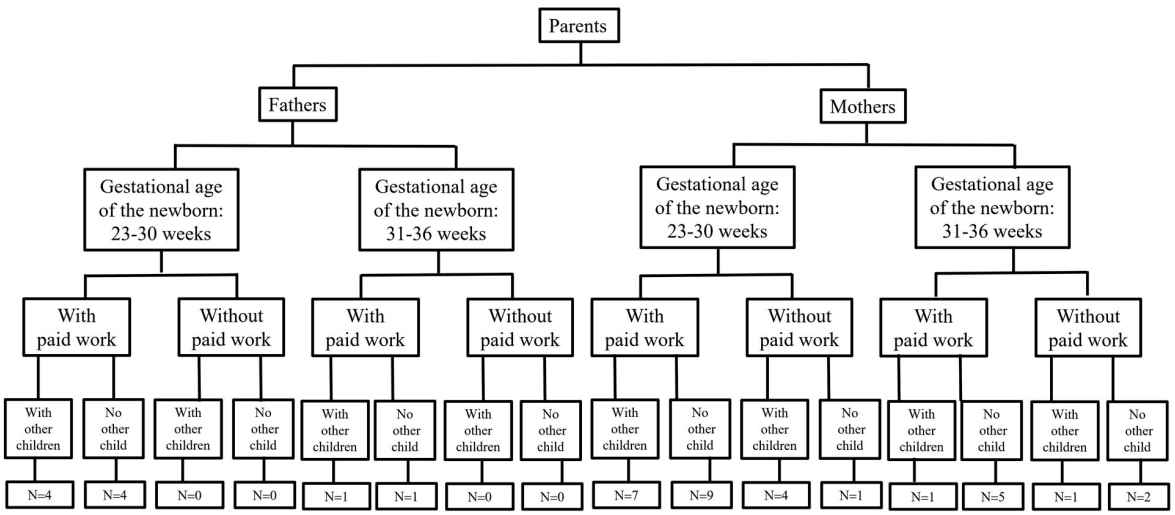

Fig. 1. Decision tree showing the profiles examined and the number of participants in each subgroup.

basic aspects to explore, open, and focused on the aims of the study. A pilot interview was previously carried out with a mother, the results of which have not been included in this study. The interview script evolved as the interviews were carried out and as the data analysis from these interviews was undertaken, thus shaping the content of all subsequent interviews. They were carried out in a setting that was private, had good lighting conditions and was free from noise and other interruptions. The nursing office of the NICU was made available for this purpose. The interview lasted about 60 minutes. The investigator had a notebook in which his/her observations and impressions during the interview were recorded.

There was a total number of 40 interviews, of which 10 were with men and 30 with women. The profile of men without paid work was not left pending further research as it was not possible to find fathers that fulfilled these characteristics.

The data collection was deemed to be terminated when there was saturation of information, that is to say, when the interviews with new parents did not provide data different to that already collected.

Data analysis

Data analysis was performed at the same time as collection. The interviews were recorded in audio and were transcribed literally, after which they were returned to the participants for them to read, confirm or rectify anything that did not reflect their experiences.

Following receipt of the source documents, these were protected by removing all elements that could identify the participants and other individuals that appeared in the conversation, and by assigning fictitious names to the participants (maintaining gender).

Subsequently, the transcriptions were analyzed, first, by reading the text repeatedly so that the investigator became familiar with the conversation, preparing pre-analytical intuitions and coding the findings that appeared in the interviews that were then grouped into categories and families. Once finalized, an explanatory framework was created, and the findings of the analysis were contrasted with the original data.

As an aid to analysis the Atlas Ti 6.2 computer program was used.

With the aim of increasing the validity and consistency of the results obtained, a triangulation was performed by the investigators and specialists. The data were analyzed independently by two investigators not connected to the NICU in order to subsequently reach a consensus on the findings. The findings were then presented to specialists for their validation (Table I).

Ethical and legal aspects

Permission to conduct the study was requested from the Neonatology Unit. The study was approved by the Ethics Committee of the Vall d'Hebron Research Institute, Barcelona. Spain. PR(AMI) 342/2015.

An informative document was written for the participating parents. Informed consent was requested prior to the data collection, and are available to those who require them. The transcriptions were stored electronically and password protected; access to these documents was exclusive to the research team.

Participants were informed of the relevant data that had been obtained during the course of the study. This information has been communicated to those that expressed a wished to have it; in the case of preferring not to be informed, this decision was respected.

Results

The field work was carried out in the Neonatal Intensive Care Unit (NICU) of the Hospital Universitario Vall d'Hebron, Barcelona, Spain, which is a third-level, high-technology hospital.[15] The Neonatal Unit has 25 critical

Table I. Guba Rigor Criteria based on Pla M, 1999 (reference: 14).

Criteria	Description	Procedures
Credibility	True value of an investigation to the extent that it may be credible	Data collection technique is explicit
		Extensive and intensive observations are made
		The data are triangulated by investigators and experts
		Feedback is obtained from the informants
		The collections of data and the analysis are concomitant
		The data are presented with examples (verbatim)
Transferability	Degree in which the discoveries of the investigation can be applied to other subjects or contexts	Profiles to explore are prepared in order to ensure the homogeneity and heterogeneity of the sample
		The sample is described (see Table I, sociodemographic aspects)
Dependency or consistency	Stability of the data	The status and the role of the investigators are described
		The physical, social and interpersonal context of the informants are described
		Information collection techniques are described
Confirmability	Makes reference to the neutrality	Particular registers are collected, textual transcriptions and direct quotes are made
		The suppositions are checked with the participants
		The information is collected by direct recording
		The position of the investigator is explained

beds, 20 semi-critical beds, and 24 basic-care beds. There were 970 admissions in this unit throughout 2017. This NICU is an open unit, allowing 24/7 access to the parents of the babies admitted. Parents of diverse nationalities and ethnic groups, as well as different academic profiles, were interviewed. The characteristics of the participants are shown in Table II. An elevated mean age was observed in both the fathers (38.8 years) and in the mothers (35.05 years).

The present study shows distinct categories and families: 1) the experience and emotions of a premature delivery; 2) the emotional support received by the father/mother of the spouse in the event of a premature delivery, and that offered by their spouse/partner; 3) the emotional support offered by the health professionals (doctors, nurses, etc.); 4) the informal emotional support offered by relatives and other fathers/mothers in the NICU undergoing the same situation. The emerging category 'emotional distance' appears throughout this study. The informed consent was signed both by the informant and the researcher before recording the interview.

The experience and emotions of a premature delivery

Regarding the event of a premature birth, participants experienced feelings of sadness and guilt (parents of the newborn), as well as disappointment and anxiety for not having achieved a full-term pregnancy (mothers).

Cristina (aged 37), explains her experiences and opinions in the following way:

"She was very small; just when you start to see something more of the pregnancy… I've got a bigger tummy and then suddenly… it's not there. Other mums say: "I just can't wait any longer for this baby! I'm so heavy, everything's a real pain"…I didn't have the time to feel like this…it's like when you have something and then they take it away from you. It's taken for granted that it should be super-nice and super-happy, but this is more like a wake,

Table II. Sociodemographic data of the participating parents.

Mothers (n=30)	Fathers (n=10)
Mean age: 35.05 years	Mean age: 38.8 years
20-29 years, n=5	20-29 years, n=1
30-39 years, n=17	30-39 years, n=5
40-49 years, n=8	40-49 years, n=3
50-59 years, n=0	50-59 years, n=1
Nationality	Nationality
Spanish, n=24	Spanish, n=9
Romanian, n=2	Ecuadorean, n=1
Pakistani, n=1	
Ecuadorean, n=1	
Moroccan, n=1	
Uruguayan, n=1	
Ethnicity	Ethnicity
Caucasian, n=25	Caucasian, n=9
Muslim, n=2	South American, n=1
South American, n=2	
Gypsy, n=1	
Births	Births
Twins, n=8	Twins, n=2
Single, n=22	Single, n=8
Level of studies	Level of studies
Primary, n=5	Primary, n=1
Secondary, n=11	Secondary, n=5
University, n=14	University, n= 4
Duration of gestation	Duration of gestation
23- 30 weeks, n=21	23- 30 weeks, n=8
31-36 weeks, n=9	31-36 weeks, n=2
Work situation	Work situation
Active paid work, n=22	Active paid work, n=10
Without paid work, n=8	Without paid work, n=0
Number of children	Number of children
Have 1 child, n=17	Have 1 child, n=5
Have more than 1 child, n=13	Have more than 1 child, n=5

or a feeling of sadness, of guilt…I wasn't up to the demands of the delivery, or the birth of a child at full-term… It doesn't even have a name, my poor little thing"

Emma (aged 43) explains her experience of the premature delivery of a twin pregnancy:

"I'd already thought about prematurity… I said, well, just hang on until week 29 or 30, to the end of December or the beginning of January. I was expecting to have two premature babies but nothing so extreme, week 25. It was a real shock to be operated on for this… 'Save my children', that was the only thing that interested me, I wasn't worried that that my blood pressure was high, nothing bothered me". Just that fear, the fear that they wouldn't survive".

In the account given by some of the participants, because an explanation for the premature

delivery is not available, this lack is attributed to God's will.

Rafaela (aged 36) explains her experience of pregnancy by insemination and her premature delivery in the following way:

"God knows why it has happened, why it had to be born this way. Of course, you end up saying; "if it had been two or three months more inside me, this would not have happened. It's… well, you've gone and left your heart there. I thought that when the artificial insemination was carried out, I might have had a miscarriage in the first few months, but this didn't happen. What actually happened never ever came into my mind before; it was only later that I learned about artificial insemination being one of the causes of pre-eclampsia".

Maria (aged 30) explains her pregnancy and her daughter's premature birth. Other cultures also reveal the same feelings of guilt by the mother for not having made things better for the baby during pregnancy, and because of the premature delivery itself.

"Really, I just don't know what happened, I think that it was my fault, I was very stressed in the last 3-4 months because of things at home, family things… One day I wanted to quit work, nobody understood me. I told them I wanted to quit work and they told me, OK, do what you want, but in reality they wanted me to continue working. In the previous pregnancy I did not look after myself, I didn't know how to, but in this one, I looked after myself very well, my husband has always made lunch, dinner, for me, he gave me fruit, because my husband knew that the other child was born with low weight, and wanted the daughter to born safely (...). I know my husband says that it's not my fault; but I know that it's all down to me, because I didn't do things right, I worked, did work around the house, I put on weight…I called my husband and told him to come quickly, I was very scared: she's very small, I don't know what's going to happen, I don't know if she'll make it, don't know what's going to happen. I was crying, praying, I was in surgery for 3-4 hours. I just don't know what is going to happen…"

Oriol (aged 29), talks about his partner's pregnancy and his daughter's premature birth:

"You're worried about what might happen, the first 4-5 hours are very important, their first seconds, how to react… well, yes, the NICU is quite impressive, because it's a unit that, I mean, listen, things are more serious in here and especially it's like how my wife said, I can't do anything. You feel impotent on not being to do anything. It's all new, I don't know how to react. We are first-time parents, we have to take it all in…at first it was a shock, not severe, very severe, very sudden and very bad…We have to move forward".

Gloria (aged 42) explains her pregnancy and the premature delivery of her twin daughters, as well as her feelings of guilt.

"I felt guilty, it might have been my age that influenced thing, but here, there's a lot of young people in the same situation as us, so if it happens to you, it just happens and that's that…"

Emotional distance

In our study, some mothers and fathers explain situations that we identify as emotional distance. Emotional distance can be defined, in the case that concerns us here, as a distant view taken with regard to the current problem, that is to say, after the birth of the premature baby and its admission into the NICU. This emotional distance leads to a situation in which the parents do not feel as if they were the parents of their newborn child and, consequently, delegate the care and attention of their baby to professionals, showing ambivalent feelings about the new situation.

Cristina (aged 37) explains the emotional distance she felt towards her daughter.

"You feel any connection, but it's your baby...yes, I see her more as a person that you need to bring up, I don't have this baby feeling, all cuddles and baby-talk… I see her more like just a person".

Gloria (aged 42), explains the emotional distance with her daughters.

"There is like a kind of, like a…an emptiness inside, no? There is like a kind of distance. Whereas you say it's mine, of course…that as long as you do not touch it and let a few days or hours pass by, well… The connection, that these are my own daughters, really is present, of course. But I'm not sure what to think. Can they survive, so small? That's what I think… it's what was in my head at that time".

Rafaela (aged 36), explains her emotional roller-coaster.

"No, I believe that hormonally it is also a roller coaster… the emotions and all the moods. You are making normal progress. At first you cannot stop crying, later on talking, you remember and you are still emotional…"

Formal emotional support received by the parents from the professionals (nurses, doctors and psychologists)

Neonatal nurses are expected to show competence and a willingness to involve mothers and fathers as soon as possible in the care of their baby, and also to be open to developing a relationship of confidence with the parents.

Abel (aged 38) describes the help received from the nurses.

"They [the nurses] didn't ask me about my emotions or feelings. I think my wife needed help, because I gave her support. I helped her to cry, because all of this was stored up inside and yes, she did need a professional to help her."

Yolanda (aged 34) describes the help she received from the nurses in the Unit.

"And later, where I got a lot of support, it was with the nurses. Sometimes I was a little… I don't know, they help you, they explain things to you… they even explain things to you later on, when you're at home, what will happen… for example, me with breastfeeding… they really have helped a lot. They gave me real encouragement, above all. So that I don't get obsessed with things".

Rebeca (aged 34) comments the following on the psychological support that she received.

"Well, the psychologist is there… but for me, it was better talking to the mums than with the psychologist. M. is a really good person; she came specially to look for me so we could talk about things…"

Gerardo (aged 33) describes the psychological support that he received.

"Professional psychological support? No, professional no, because you know what you have to come to terms with anyway. You just have to get your head around it, you've drawn the short straw… when things are fine, then… you just tell it like an anecdote and that's it. I didn't need a psychologist. I talked it over with whoever I needed to, my parents, my sister and that's that. You know, I just don't need to explain it to anybody else".

Clara (aged 28) recalls the psychological support that she received

"On the other hand… the psychologist did come round. But, well, if you don't really believe in psychologists… we were on the floor above where the baby was admitted, there were a few very bad days and she came to see me twice, the next time I went down to see her. I never saw her again; she came and told me that I seemed fine."

Carlos (aged 37) describes the help received from the nurses.

"They offered us psychological support several times. And finally, one day when I saw my missus in a bit of a state and that, I said "if you want, come and talk to her sometime. Let's see if you can help us with anything". And, well, she came along one day to talk to us, but from my point of view, I don't really think it helped me much. Because what I'm going through I have go through anyway. And to talk about it, well, I'll talk to someone that I know and trust and I'll tell them everything I would have told her anyway".

Informal emotional support offered / received by their partner / husband

Due to the distressing situation experienced by the fathers in NICU, the health care systems have changed their objectives towards the quality of care paradigm, promoting well-being centered on the family, and in the continued and maintained participation by the fathers. The benefits that are observed from this active participation by the fathers in the care are: reduction in the stay, improved well-being of the fathers, and an improved neuro-behavioral development of the baby. However, the concerns expressed by the fathers are about the well-being of their partner and their baby.[8]

Luis (aged 31), discusses the pregnancy and premature delivery of his son, and how he cared for his wife

"You have to try, poor thing [his wife], to be as affectionate as possible at that moment and explain to her that, look, everything's fine, it's okay, you know with the tubes and all that. I've seen him and he looks just great, he's a little fighter. And anyway, at that moment you couldn't really tell her even if there was something wrong, you just can't give her any bad news, poor thing (…). So, well, you just try to cheer her up".

Emotional support received by first-time parents from other parents whose child is in the same NICU.

The participants' conversation mentions the help received by parents from other support parents whose own children have also been admitted into the NICU. The place used for the exchange of experiences was "the milk room", where the extraction of milk is carried out. This was a meeting point where both positive and negative experiences were exchanged.

Rebeca (aged 34), describes the help she received from other support mothers with infants admitted to the NICU.

"It's far easier for me to talk to the other mums than to the psychologist". "The milk room is an interesting place.... I had the chance to speak to other mums there (...), of course, you always meet the same mums there. You end up explaining your worries and you can let off steam. Or maybe you meet other mums who tell what's happened to them and that's just as good. They cheer you up, they tell you what's easier and what works really well…"

Josefa (aged 43), describes her own experience of this help.

"Well, it's really good and you get to know people in the NICU. For example, in the milk room, there's lots of different experiences. Some people speak more positively than others. Other times they start talking and all you get are only problems, pain and misery, and you so end up saying that your milk has run out…. For example, people who have already experienced this situation have a bit of perspective and can explain it. Lots of mums are alone, because they're from other places, probably the husband can't be there with them because he's working. We give each other emotional support, but if we needed any other kind of help, we would ask for it. On the trips home and back again, my husband and I talked about how we were both managing".

Clara (aged 28), talks about the help received by other support mothers with infants admitted in the NICU.

"It's just like being at the hairdresser's when you're in the milk room. People are always asking you things like 'how are you doing?'; 'How many weeks ago was your baby born?'; 'How much does he weigh?'; 'How are you coping with all the stuff?'; 'How long have you been here?'. You hear about everything in the milk room, about benefits, how they work them out, things you have to hand into get them, all of that, and that's a big plus (…). It's been really useful for me, yes… you share these moments, you say, if there's anything here, there's a bit of humanity, and that's not easy to find in other situations. You connect, you feel supported and comforted. You feel like you're not the only one going through all of this. And later, you get help from the mums who have more time and then you help the new mums"

Diana (aged 32) considers the help that she received through social networks;

"There is a group of mothers called "Vall d'Hebron prematures", on Facebook. I gave it a like. It has been good for me because you end up reading other cases. And the other day I read an article by one of the girls and I could really identify with that. You know? I also had my child here. I've been helped here by other mums and dads who've got through all of this…".

José (aged 51), talks about the help received from other support mothers/fathers.

"D. [another father] told me that he was in the Vall d'Hebron prematures group on Facebook; I took part in this group, too. I have been in contact with parents who had a premature child, even with one of my friends, who I knew has two 11-year-old twins of 11 years and one of them was monitored at home, what a drag…"

However, some parents comment on the fact that they were unaware of the social network support groups for parents with premature infants.

Raquel (aged 41) discusses not knowing about the social network groups, adding that the support received from other mothers with infants in the Unit was sufficient.

"I haven't been to any support group, and I didn't know about the Vall d'Hebron prematures group on Facebook, I did not read any of the comments from other parents (…). But I really liked the milk room, it is the place where most of the mums go to. I got to talk to other mums about my children, and they talked to me about theirs. The milk room was really useful for me, basically because you talk to other mums about their situation, and sometimes you say, well, things aren't so bad for me. And we also talk about our personal situations and things".

Discussion

The mothers in this study showed feelings of guilt and sadness, searching for the causes that triggered premature delivery, in addition to a sense of fear regarding the prognosis and survival of their baby. In his text on crisis intervention, Roberts[15] highlights the prior conditions for crisis: the most important precipitating factor is a stressful or dangerous event. However, two other conditions are also required: (a) the subject's perception that the stressful event will lead to considerable unease; and (b) the subject's inability to resolve the disruption through the coping methods being used to that end.[10,15] Other authors report a profound change in social roles and expectations. In the case that concerns us, the mother finds herself with a baby in a critical state, instead of being able to take up the expected role of a mother with her full-term baby. This experience can lead to post-traumatic stress disorders[10,16]

When mothers do not receive enough support from the staff that looks after their child, they turn to God. We agree with the observation by Heidari[5] which states that parents are searching for hope and hoping to talk with the doctors and nurses. When these needs are not satisfied, they resort to God and to prayer to cope with the stress.

According to Borrero[17], a suitable *management* of these feelings should be carried out by the nurse responsible, a nurse trained in family relationships. Care is required in such circumstances that is based on listening to experiences, information, in accordance with a guide on participation, and directed towards stabilizing the role of the mother as soon as possible so as to reduce stress.

On the other hand, we must take into account that in the NICU, nurses have responsibilities that go further than caring for the newborn child, such as the commitment to the parents, especially the mother, to accompany them in the first visits to the NICU, inform on the condition of the baby, answer questions, give emotional support, encourage the visit and the contact, participate in the nursing care and inform about the procedures and treatments performed.[12]

The findings of this present study, as regards the feelings expressed by mothers on premature delivery, are in agreement with that reported by Frello et al.[12] On the admission of their

child to an NICU, mothers feel a sense of guilt, disappointment and anxiety, as well as the need for emotional support at this difficult time.

As reported by Shin and White-Traut[18], negative feelings on the health of the baby and a lack of confidence in the medical team lead to a higher level of stress than the simple fact of having a baby in the NICU. Our study's findings differ from those of Heidari[5] and Shin et al.[18], since we observed few signs of any lack of confidence in the doctors and nurses in the department; stress in our study is identified more with uncertainty about the future and about the baby's development and progress and is also linked to a lack of knowledge regarding the causes of the premature delivery. In view of all this, we infer that frequent and open communication by the nursing staff, based on empathy, active listening and the handling of topics on which parents show concern, could contribute to a reduction in the anxiety felt by these parents and to facilitate a more relaxed experience of their child's hospitalization.

In 2017, Heidari[5] showed that when the parents become anxious due to having a baby in the NICU, they look for alternative ways to improve their child's health and its chances of survival. This action in itself helps the parents to cope better and to gain a little control over the situation. For many parents, constant praying may offer them a sense of calm, hope, and acceptance. These findings can help to improve both clinical performance and quality of care through a focus on considering the parents' emotional state and through enabling nurses to communicate with the parents in keeping with their behavior and spiritual needs.

In our study, the fathers stated that they were not always asked about their emotions and feelings during their stay in the NICU. According to Frello et al.[12], nurses may forget that fathers need support and emotional guidance, thereby undermining the interpersonal relationships that are seen as one of the challenge in the movement towards humanizing care. Health professionals are concerned with perfecting their technical abilities, in some cases neglecting the care of individuals. A failure to recognize the importance of the mother and to include her needs in nursing care is seen as indifference.

Parents subjected to the stressful situation of a premature birth can show emotional distance and also reveal themselves to be, in effect, on an emotional roller-coaster, that is to say, they are characterized by marked emotional ups and downs. The results of this study coincide with the findings of Ionio et al.[19] who suggested that, during the mother-child interaction, mothers of premature infants are more distant than are mothers of full-term infants. They also find it easier to withdraw from interacting with their baby. On the other hand, the fathers of pre-term babies are more prone to resilience than stress. Likewise, those mothers showing stress, negative feelings (anxiety, depression, and anger), as well as post-traumatic symptoms, showed a maternal behavior of distancing themselves from the infant during their interactions. Ionio et al.[19] found that the perception of a dysfunctional mother-child interaction from the point of view of the mothers could have an influence on greater distancing during the interaction. This shows that if the mother does not perceive the child as appropriate to her expectations and to the interactions, the infant does not reinforce the mother's own sense of being mother and probably may be more distance in the interaction with her baby.[19]

O'Brien et al.[6] pointed out the time of admission of the premature infant into the NICU as a stressful time and full of anxiety for the parents. They pointed out the concerns for the diagnosis and the highly technological environment and worries about a change in their functions as parents. We have not found authors describing the sense of separation felt by the parents from their hospitalized baby and the feeling, also described by the parents in the present study, on a lack of bonding with the baby possibly determined by the circumstances of birth and restrictions on contact with their child and any decision-taking involved.

The informants identified the attention received from the nurses as 'very good'; however, in some of the conversations, deficiencies in communication were noted, as well as in the psychological care given to fathers. As indicated by certain authors, it is essential that the NICU team welcomes parents and establishes effective therapeutic communication with them, avoiding the use of technical terms distinct from maternal reality, which only serve to mark professionals as withholders of knowledge.[12]

Our study also enquires into the emotional support provided by the psychologist assigned to the NICU. The conversations reveal a rejection of the help provided by this healthcare professional, this being associated with the social connotations of mental illness that such professional support involves.

There is evidence in the literature that nurses provide most of the communication on emotional support for the parents in NICUs, and that this type of support is appreciated by parents.[20]

Of the studies that consider the relationship established between nurses and mothers with babies admitted to the NICU, it is possible to perceive that there is margin for improvement, despite the effort recognized.[10,12]

The information and observations provided by the informants in this study was in agreement with that expressed by certain authors. Ardal et al.[10], showed that mothers felt less guilty, less anxious, and had more confidence, after talking with their support mother-friend. The support parents-friends reduced the sense of isolation felt in relation to the birth; similarly, the conscientious use of the support mother-friend experience itself, in response to the concerns of the first-time mother, seemed to have a profound impact on this situation.

As well as that which has previously been mentioned, other sources of emotional support were identified, such as social networks and *WhatsApp* groups. In this case, support is derived from the discovery that one's own story is not unique, which will then lead to a better acceptance of the situation.

The findings of the present study show the importance of support among peers, that is to say, among parents going through a similar situation. This support becomes key in overcoming the problem, the distress and the pressure of having a newborn child who has been admitted into an NICU. This coincides with the views reported by Ardal et al.[10], which highlights the importance of mutual support for vulnerable parents.

In conclusion, in the context of a premature birth, mothers expressed feelings of sadness and guilt, asking themselves where they had failed and what they did wrong. Mothers and fathers are both afraid that their child, with a low birth weight and related immaturity problems due to prematurity (sometimes extreme), may not survive which is sometimes extreme.

In light of this stressful situation, parents undergo an emotional roller coaster and have ambivalent feelings about this new situation. As a defense mechanism, they adopt emotional distance, delegating the care of their baby to the health professionals so as to process the situation being faced, and for which they were not prepared. Consequently, parents require emotional support that may come from the health professionals themselves, but may also be in the shape of informal support.

In the ambit of professional-emotional support, parents identify the nurses as a key part, since they accompany the parents, inform them, and instruct them in childcare. Nurses must therefore take an interest in the process being undergone, attempting to give support and help to parents according to their expressed needs.

Another source of professional-emotional is that provided by the NICU psychologist, which was shown to be factor that was not determining, almost certainly due to the social connotations that psychological treatment entails.

As regards non-professional support, parents identified as very beneficial the exchange of experiences with other mothers and fathers whose children had also been admitted into the Unit, as well as support groups on social networks, and the use of WhatsApp groups in which they can give voice to their worries and compare their experiences with other parents who are possibly going through a similar experience, or whose child has overcome prematurity.

The nurse needs to know about these sources of support and should guide families towards them, in order for them to understand their emotions when facing the situation, they are experiencing with their children.

Among the limitations there is the difficulty of the father taking part in the interview, as studies have shown the difficulty of combining work, caring for the hospitalized wife, for other children and for the premature child; for all of these reasons, there are still profiles in this study that require fuller exploration.

Other limitations are those common to qualitative methodology as regards the generalization of data. For this reason, this qualitative-research study cannot be extrapolated to all communities.

Finally, an additional limitation was that the informants did not provide all the information that was actually available to them, due to the fact that the researchers were from the service area in which their child had been admitted; they therefore retained whatever information that might be conflictive in such cases.

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Original Article

Congenital myotonia: a review of twenty cases and a new splice-site mutation in the CLCN1 gene

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ABSTRACT

Background and objectives. Congenital Myotonia (CM) is a disease caused by mutations in the skeletal muscle chloride channel gene (CLCN1). Mutations can be transmitted as autosomal dominant (Thomsen's disease) or recessive (Becker's disease). CM is more common in men and Becker myotonia may be 10 times more common than Thomsen myotonia. Genotypic and phenotypic characteristics of CM may vary according to geographical region and ethnicity.

Method. In this study, we present the genotypic and phenotypic characteristics of 20 Turkish CM patients all diagnosed by molecular genetic testing.

The clinical and laboratory features of the patients with mutation in CLCN1 gene were retrospectively analyzed.

Results. Eleven of the patients were female. c.1064+1G>A splice-site change, p.Arg338X (c.1012 C>T) stop codon, p.Gly190Ser (c.568_569delinsTC) missense mutations were detected. Eight of the 20 patients were found to be compatible with Becker type and 12 with Thomsen type, based on mode of inheritance, neurological examination findings and genetic test results.

Conclusion. The c.1064+1G>A splice-site change mutation, defined for the first time in this study, expands the spectrum of mutations in the CLCN1 gene. Thomsen type and female gender were observed to be more frequent in this series of patients from Turkey.

Key words: Congenital myotonia, Thomsen disease, Becker disease, CLCN1 gene.

Congenital Myotonia (CM) is a disease caused by mutations in the skeletal muscle chloride channel gene (CLCN1).[1] The sarcolemmal chloride conductance is reduced due to the defect in the CLCN1 gene encoding the ClC-1 chloride channel, resulting in both clinically and also electrically delayed relaxation of the hyperpolarized muscle. Affected patients describe muscle stiffness after a strong contraction.[2-4] The mutation in the CLCN1 gene is divided into two subgroups according to mode of inheritance: autosomal dominant (Thomsen disease, OMIM#160800) and autosomal recessive (Becker disease, OMIM#255700).[5] Genotype-phenotype correlations are variable and various intermediate forms have been described between Thomsen and Becker phenotypes.[6] Thomsen and Becker forms can be distinguished by time of onset, mode of inheritance, and clinical findings. Clinical and genetic characteristics of these two forms are summarized in Table I.[4,5,7-13]

CM was reported to be more common in men.[7,14] The incidence was initially estimated as 1:23.000 for the Thomsen type and 1:50.000 for the Becker type[15]; in subsequent studies, however, Becker type was reported to be more common.[16] The prevalence of CM was estimated to be 1:10.000

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Table I. Clinical and genetic characteristics of Thomsen and Becker myotonia.

	Thomsen	Becker
Gene and gene location	CLCN1 / 7q35	CLCN1 / 7q35
Inheritance type	Autosomal Dominant	Autosomal Recessive
Onset age	At any age	Early Childhood
Male/Female Ratio	1.3	2.0
Myotonia Severity	From asymptomatic to severe	Usually severe
Muscle Hypertrophy	Rare, light and generalized	More frequent and mostly in the lower extremities
Episodic weakness	None	Rare
Permanent weakness	None	Rare
Increased findings over the years	Usually does not increase	Usually increases

in Northern Scandinavia[17,18], while 1-10: 100,000 worldwide.[19]

Genotypic and phenotypic characteristics of CM may vary according to geographical region and ethnicity.[6,7,14-18] There are limited number of studies on CM in Turkey, which are mostly related to neurophysiological characteristics of the disease and not diagnosed by molecular genetic testing at all cases.[20-22] There are no studies reporting genotypic and phenotypic characteristics of patients.

In this study we present the genotypic and phenotypic characteristics of 20 CM patients from three Turkish families, all diagnosed by molecular genetic testing. We determined three different mutations and one of these is a novel mutation identified for the first time in the CLCN1 gene.

Material and Methods

Analysis of mutations in CLCN1 gene was performed in 5 index cases from three families who applied to the pediatric neurology outpatient clinic of our hospital with the symptoms suggesting nondystrophic CM, in the light of clinical examination and laboratory findings. Upon mutation-positive test results, family screening was performed. Mutational analysis in the CLCN1 gene was performed in 24 individuals from three families, including parents, children and one aunt in the first family (Fig. 1). The clinical and laboratory features of the patients with mutation were retrospectively analyzed. The mode of inheritance was determined according to the neurological examination findings and genetic results. Consent form was obtained after providing a detailed information from all patients or their legal guardians. The study was conducted in accordance with the rules of the Helsinki Declaration of Ethics. We received ethics committee approval-Diyarbakır Gazi Yaşargil Training and Research Hospital (Number: 356/25.10.2019).

Myotonia Assessment

Neurological examination was performed to search for myotonic findings, particularly in the tongue, jaw, eyelid and limbs. Handgrip myotonia, being unable to open the eyes after a strong eyelid closure and percussion myotonia were examined. The semi-quantitative scale of myotonic impairment[15] was used to grade the severity of myotonia. Readily noticeable myotonia hindering daily life activities was defined as severe myotonia (+++). The case is defined as moderate myotonia (++) if some minor troubles in daily life can be noticed and reported by the individuals, but it is called as mild myotonia (+) in case it has no noticable negative effects on daily life but can be diagnosed only by examination.

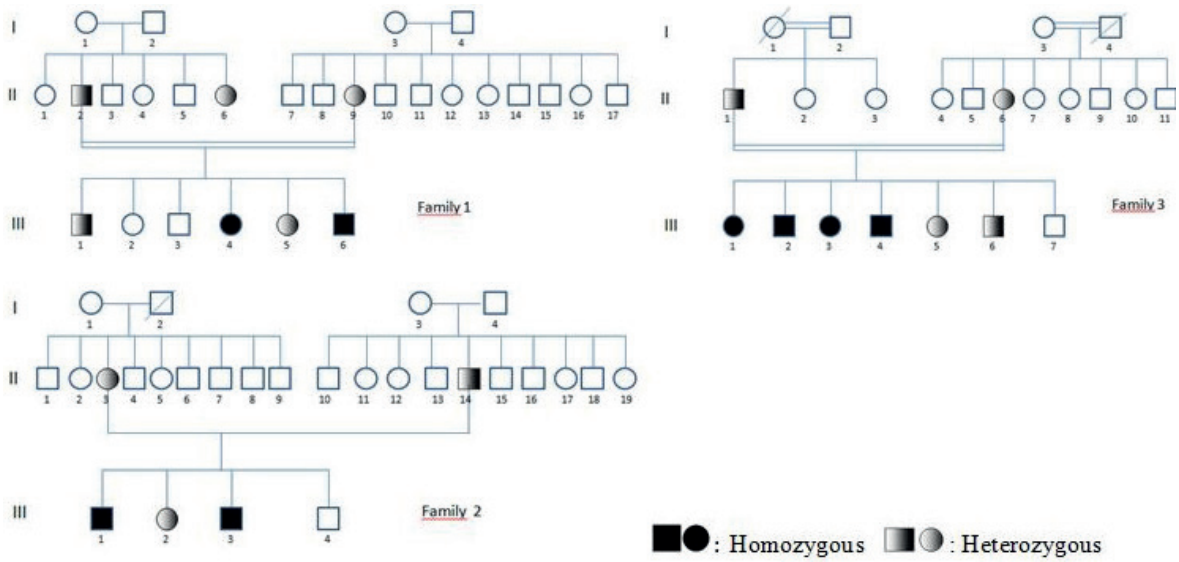

Fig. 1. Pedigree of families.

Genetic Diagnosis

Exons 1-23 of the CLCN1 gene were amplified by *polymerase chain reaction* (PCR) and then DNA sequencing was performed. DNA isolation from peripheral blood samples was performed using saline precipitation method. Exons of the CLCN1 gene obtained from isolated DNAs were amplified by PCR. Amplification was confirmed by agarose gel electrophoresis. Illumina's Nextera XT Library Prep Kit was used for making sequencing libraries from the amplified exon regions for next generation sequencing. Purification was then performed using AMPure XP kit (Beckman Coulter) and DNA libraries were loaded onto Illumina Miseq System using MiSeq v2 Reagent kits. The BAM files provided by Miseq instrument were viewed and analyzed by IGV program. Cases with mutations were confirmed by Sanger sequencing.

Results

General Clinical Characteristics

Mutations were detected in 20 cases. Eleven of the patients (55%) were female. The parents had first-degree kinship relation in the first and third families. Difficulty in opening hands, worsening in cold weather and palliating after repetitive contractions, difficulty in motility and fatigue were common complaints of the patients who were diagnosed to have myotonia in neurological examination. Developmental phases such as sitting, crawling and walking were normal in all patients with mutation. Complaints and clinical findings of all patients with myotonia began within the first 3 years. All patients, either heterozygous or homozygous, were able to perform daily life activities independently without any support. Homozygous mutation was detected in each index patient. Muscle hypertrophy was determined only in patients with homozygous transmission in addition to myotonia, in their neurological examination. Mild hypertrophy was determined in the members of the first and second families especially localized in the lower extremities, while generalized and marked muscular hypertrophy (Hercules' appearance) in all family members with homozygous mutations in the third family (Fig. 2). Electromyographic examination (EMG) was done only in one person, the index case, from each family, considering that it is a painful procedure. Motor unit potentials were normal but myotonic discharges were observed. Clinical findings remained unchanged in the first and second families while increased in the third family over the years.

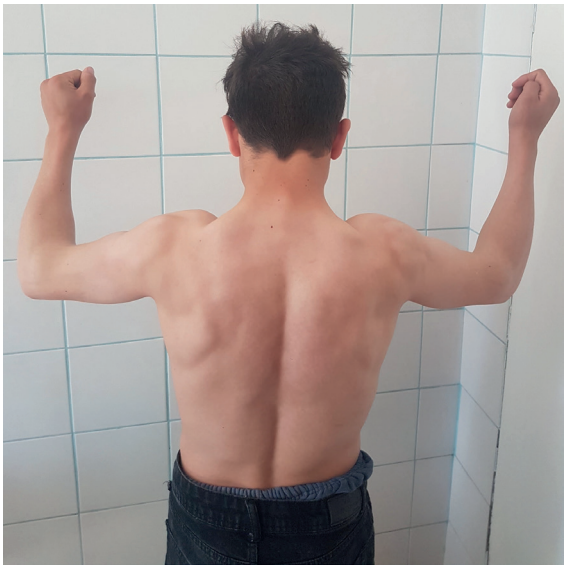

Fig. 2. Example of generalized muscle hypertrophy and Hercules' appearance in the patients with homozygous p.Gly190Ser mutation.

Demographic, clinical, genetic, and laboratory characteristics of patients with mutation and the grade that the patient was affected by myotonia are summarized in Table II. Eight (40%) of the 20 patients were found to be compatible with Becker type and 12 (60%) with Thomsen type, based on mode of inheritance, neurological examination findings and genetic test results.

Genetic Test Results

In the first family, c.1064+1G>A splice-site change mutation was detected in intron 9 of the CLCN1 gene. The mutation was confirmed by Sanger sequencing. Sanger sequencing image of the detected mutation is shown in Figure 3. The mutation was homozygous in two index siblings and heterozygous in all other family members.

In the second family, p.Arg338X (c.1012 C>T) stop codon mutation was detected in exon 9 of the CLCN1 gene. The mutation was homozygous in index cases and heterozygous in sisters and parents. No mutation was detected in six-year-old brother.

In the third family, p.Gly190Ser (c.568_569delinsTC) missense mutation was detected in the exon 5 of the CLCN1 gene. The mutation was homozygous in the four oldest children and heterozygous in the other family members. No mutation was detected in the two-year-old brother.

Treatment

Treatment was not needed in the patients with the Thomsen variant because myotonia was mild and causing no difficulties in daily life. Treatment was initiated for the patients in all three families diagnosed as severe myotonia based on the semi-quantitative scale of myotonic impairment and suffering difficulties in daily life activities such as walking, going up the stairs and writing. Primarily carbamazepine was initially given to the patients of the first and second families, while directly mexiletine was prescribed to the patients of the third family. Mexiletine was initiated when carbamazepine failed to resolve the complaints significantly, even at increased doses of 20 mg/kg/day. In all patients with severe myotonia according to the

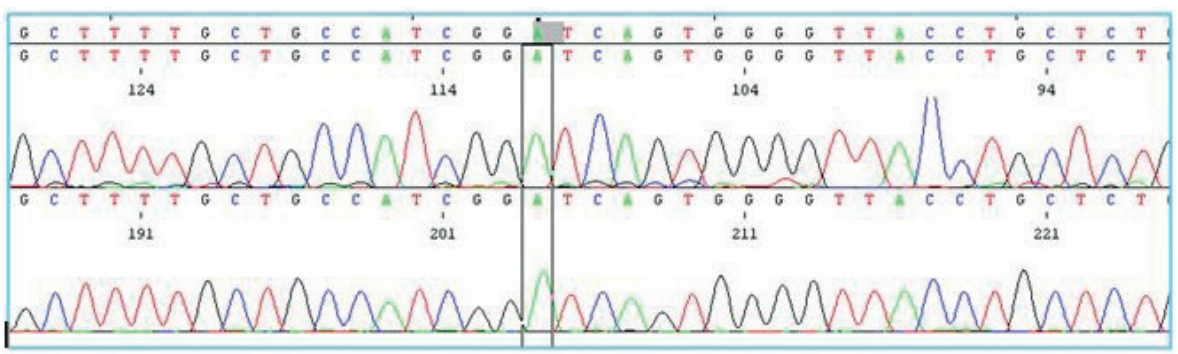

Fig. 3. Sanger sequencing image of c.1064+1G>A splice-site change mutation.

Table II. Demographic, clinical, genetic, and laboratory characteristics of patients with mutation.

Patient ID	Age Gender	CLNC1 Gene Mutations	Inheritance types	Myotonia Severity	Location of myotoni	Triggers / Comforter	Muscle Hypertrophy	Muscle Pain	CK (N:29-138) (U/L)	EMG
P1/II/2	41,M	c.1064 +1G>A Heterozygous	OD Thomsen	None	-	-	None	-	137	Nd
P1/II/6	32,F	c.1064 +1G>A Heterozygous	OD Thomsen	Moderate	LE, UE, Eyelid	Cold, Rest / Warm up	None	+	285	Nd
P1/II/9	35,F	c.1064 +1G>A Heterozygous	OD Thomsen	Mild	LE, UE	-	None	-	142	Nd
P1/III/1	18,M	c.1064 +1G>A Heterozygous	OD Thomsen	Mild	LE, UE	-	None	-	98	Nd
P1/III/4 *	11,F	c.1064 +1G>A Homozygous	OR Becker	Severe	LE, UE, Jaw, Eyelid	Cold, Stress, Hunger; Rest / Warm-up	Mild	+	341	Myopathic changes (-) Myotonic discharges (+)
P1/III/5	6,F	c.1064 +1G>A Heterozygous	OD Thomsen	None	-	-	None	-	122	Nd
P1/III/6 *	3,M	c.1064 +1G>A Homozygous	OR Becker	Severe	LE, UE, Jaw, Eyelid	Cold, Rest / Warm-up	Mild	+	273	Nd
P2/II/3	38,F	p.Arg338X (c.1012 C>T) Heterozygous	OD Thomsen	Moderate	LE, UE	Cold, Rest / Warm up	None	-	125	Nd
P2/II/14	38,M	p.Arg338X(c.1012 C>T) Heterozygous	OD Thomsen	Moderate	LE, UE	Cold, Rest / Warm up	None	-	48	Nd

M: Male, F: Female , OD: Autosomal Dominant, OR: Autosomal Recessive, Myotonia severity: Severe: Pronounced myotonia, myotonia is a handicap in daily work; Moderate: Myotonia have some negative effects on daily life, Mild: No symptoms, but obvious myotonia at examination, LE: Lower extremity, UE: Upper extremity, Nd: Not done CK: creatine kinase

Table II. Continue.

Patient ID	Age Gender	CLNC1 Gene Mutations	Inheritance types	Myotonia Severity	Location of myotoni	Triggers / Comforter	Muscle Hypertrophy	Muscle Pain	CK (N:29-138) (U/L)	EMG
P2/III/1 *	15,M	p.Arg338X (c.1012 C>T)	OR	Severe	LE, UE, Jaw, Eyelid	Cold, Rest / Warm-up	Mild	+	418	Myopathic changes
		Homozygous	Becker							(-) Myotonic discharges (+)
P2/III/2	14,F	p.Arg338X (c.1012 C>T)	OD	None	-		None	-	145	Nd
		Heterozygous	Thomsen			-				
P2/III/3 *	11,M	p.Arg338X (c.1012 C>T)	OR	Severe	LE, UE, Jaw, Eyelid		Mild	+	389	Nd
		Homozygous	Becker			Cold, Rest / Warm-up				
P3/II/1	43, M	p.Gly190Ser	OD	None	-		None	-	103	Nd
		(c.568_569 delinsTC)	Thomsen			-				
		Heterozygous								
P3/II/6	37, F	p.Gly190Ser	OD	Mild	LE, UE		Mild	-	388	Nd
		(c.568_569 delinsTC)	Thomsen			Cold, Rest / Warm-up				
		Heterozygous								
P3/III/1	17, F	p.Gly190Ser	OR	Severe	LE, UE, Jaw, Eyelid, Tongue		Severe	+	502	Nd
		(c.568_569 delinsTC)	Becker			Cold, Rest, Stress / Warm-up				
		Homozygous								
P3/III/2*	15, M	p.Gly190Ser	OR	Severe	LE, UE, Jaw, Eyelid, Tongue		Severe	+	478	Myopathic changes
		(c.568_569 delinsTC)	Becker			Cold, Rest, Stress / Warm-up				(-) Myotonic discharges (+)
		Homozygous								

M: Male, F: Female , OD: Autosomal Dominant, OR: Autosomal Recessive, Myotonia severity: Severe: Pronounced myotonia, myotonia is a handicap in daily work; Moderate: Myotonia have some negative effects on daily life, Mild: No symptoms, but obvious myotonia at examination, LE: Lower extremity, UE: Upper extremity, Nd: Not done CK: creatine kinase

Table II. Continue.

Patient ID	Age Gender	CLNC1 Gene Mutations	Inheritance types	Myotonia Severity	Location of myotoni	Triggers / Comforter	Muscle Hypertrophy	Muscle Pain	CK (N:29-138) (U/L)	EMG
P3/III/3	14, F	p.Gly190Ser	OR	Severe	LE, UE, Jaw, Eyelid, Tongue		Severe	+	590	Nd
		(c.568_569 delinsTC)	Becker			Cold, Rest, Stress / Warm-up				
		Homozygous								
P3/III/4	13, M	p.Gly190Ser	OR	Severe	LE, UE, Jaw, Eyelid, Tongue		Moderate	+	612	Nd
		(c.568_569 delinsTC)	Becker			Cold, Rest, Stress / Warm-up				
		Homozygous								
P3/III/5	11, F	p.Gly190Ser	OD	Moderate	LE, UE		Mild	-	314	Nd
		(c.568_569 delinsTC)	Thomsen			Cold, Rest / Warm-up				
		Heterozygous								
P3/III/6	6, M	p.Gly190Ser	OD	Moderate	LE, UE		Mild	-	209	Nd
		(c.568_569 delinsTC)	Thomsen			Cold, Rest / Warm-up				
		Heterozygous								

M: Male, F: Female , OD: Autosomal Dominant, OR: Autosomal Recessive, Myotonia severity: Severe: Pronounced myotonia, myotonia is a handicap in daily work; Moderate: Myotonia have some negative effects on daily life, Mild: No symptoms, but obvious myotonia at examination, LE: Lower extremity, UE: Upper extremity, Nd: Not done CK: creatine kinase

semi-quantitative scale of myotonic impairment and given mexiletine (2x150 mg), the severity of myotonia regressed to moderate and on some of the days to mild.

Discussion

In this study, phenotypic and genotypic characteristics of Turkey's series of CM cases are presented, mostly consisting of pediatric patients and verified by molecular genetic testing.

The first mutations in CLCN1, both recessive and dominant, have been reported in the early 1990s[1], and more than 200 pathogenic mutations were identified so far.[23] Myotonia-inducing mutations are spread throughout the entire sequence of the channel protein and are mostly seen as deletion, insertion, duplication, frame shift, stop codon, 'missense' or 'splice-site' mutations.[11,23] In CM, the same mutation may show both homozygous and heterozygous transition. Heterozygous mutations are usually asymptomatic, but marked myotonia may occur without symptoms of weakness and myopathy. There may be significant phenotypic differences even among family members carrying the same mutation.[4,24-26] This phenotypic difference may be due to variable expression, incomplete penetrance, the effect of mutant alleles on wild type channel proteins, allele expression, gene dose effect and specific variability of channel dysfunction.[27,28] Clinical and electrophysiological findings may be sufficient to establish the diagnosis, but do not allow any distinction between recessive and dominant forms. It is difficult to evaluate heredity and genotype-phenotype correlation without examining the patient and pedigree, even if they have the same mutation. Only the father and six years old girl were asymptomatic while the other 4 members of the family with heterozygous transition of c.1064+1G>A mutation, that we identified the first time, had mild to moderate myotonia. Severe myotonia and mild muscle hypertrophy in the lower extremities were determined in those patients with homozygous transition. Clinical findings were generally consistent with the literature. These results allow us to expand the spectrum of CLCN1 alleles as well as verify that the c.1064+1G>A splice-site change mutation is both heterozygous and homozygous dominant mutation with full penetration.

The p.Arg338X (c.1012 C>T) stop codon mutation detected in the second family and the p.Gly190Ser missense mutation detected in the third family were previously identified in the literature.[29,30] Also these mutations displayed both homozygous and heterozygous transition. Disease onset age, heat sensitivity, severity of myotonia and distribution characteristics of our patients with p.Arg338X (c.1012 C>T) stop codon mutation were similar to the clinical features of the patient described in detail by Ulzi et al.[29] In our patients however, those with homozygous mutation had mild muscle hypertrophy, while their patient had no hypertrophy but hypotrophy. This difference may be related to the p.Gly190Ser mutation besides the p.Arg338X mutation of the patient they identified, or to some other factors.

The p.Gly190Ser missense mutation detected in the third family was first described by Shalata et al.[30] in 12 members of an Arab family. Although the father had a heterozygous mutation, he had had no symptoms or complaints, and he even had successful results in athletics competitions during his military service. Generalized muscle hypertrophy and Hercules' appearance were determined in the patients with homozygous p.Gly190Ser mutation, unlike mild muscle hypertrophy observed in the lower extremities of the other two mutations of our cases, in the homozygous variants. The phenotype of our both heterozygous and homozygous patients was quite similar to the patient series reported by Shalata et al.[30]

CM is the most common hereditary skeletal muscle canalopathy.[13,15] Becker myotonia may be 10 times more common than Thomsen myotonia.[6] There are very few reports published on Thomsen myotonia. In a French-Canadian

cohort of 50 patients, 27 patients were reported as Becker type and 9 patients as Thomsen type.[31] Another study conducted with 142 families from West Germany, reported that 73% of the cases were Becker type while 19% were Thomsen type.[25] In our series of 20 patients, clinical and genetic transmission compatible with Thomsen myotonia was determined in 12 (60%) and with Becker myotonia in 8 (40%) patients. In a study from England with patients that were genetically proven to have skeletal muscle canalopathy, 99 patients had Thomsen and 69 had Becker variants and two types were observed in similar rates.[13] Again, 86.1% of the cases reported from China were reported to be Thomsen variants.[32] The findings in our series as well as in the other studies suggest that the incidence of the two types vary in accordance with ethnicity and regions. CM was reported to be seen nearly twice in males than in females[7,14], but female (55%) and male (45%) rates were almost equal in our patient series.

Electromyography (EMG), by displaying myotonic discharges, is useful in demonstrating myotonic disorder and in distinguishing CM from paramyotonia congenita or periodic paralysis, but not in distinguishing between dominant and recessive variants of CM.[16,33] Sequence analysis of CLCN1 detects more than 95% of CM pathogenic variants of both autosomal recessive and autosomal dominant forms. Deletion/duplication analysis in the CLCN1 gene detects the remaining 1-5% mutations.[9] EMG was performed only in 3 patients, because clinical findings of our patients were consistent with nondystrophic CM. The other patients underwent only genetic examination. It may be in favor of patients' comfort to perform directly genetic examination, if the clinic view of the patient is compatible with nondystrophic CM.

Mexiletin, a derivative of lidocaine, is the most commonly used treatment option with proven efficacy in randomized controlled trials.[34-36] Besides Mexiletine, various sodium channel blockers such as phenytoin and carbamazepine can be used. Besides these agents, lithium, tocainide, and trimeprazine have also been tested.[37] In two recent studies, ranolazine[38] and lamotrigine[39] were suggested to be used in nondystrophic CM. Lamotrigine was reported to be considered as a cheaper and effective first choice because of the high cost and side effects of Mexiletin.[39] We could not obtain an adequate clinical response despite prescribing up to 20 mg/kg/day doses of carbamazepine, although Lyons et al.[40] noted a dramatic response to low-dose carbamazepine in patients with Becker variant. Based on the semiquantitative scale of myotonic impairment, a significant decrease was determined in the severity of myotonia in all patients that started mexiletine after stopping carbamazepine.

In conclusion, the c.1064+1G>A splice-site change mutation, defined for the first time in this study, expands the spectrum of mutations in the CLCN1 gene and contributes to genotype-phenotype correlation. Although Becker type and the male gender were reported to be more common in the literature so far, Thomsen type and female gender were observed to be more frequent in this series of patients from Turkey, which was also confirmed genetically.

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Original Article

Management of pediatric cardiac transplantation candidates with pulmonary hypertension and high pulmonary vascular resistance

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ABSTRACT

Background and objectives. Right ventricular failure is an important cause of mortality and morbidity after orthotopic heart transplantation (OHT). The right ventricle of the donor may fail to accommodate to the high pulmonary vascular resistance (PVR) of the recipient. Pulmonary hypertension (PH) due to chronic heart failure with PVRi > 4 Wood units.m^2, transpulmonary gradient > 15 mmHg adversely affect the outcome of OHT. In this study we aimed to evaluate management strategies in our pediatric cardiac transplantation candidates with PH and high PVR prior to OHT.

Method. Twenty-six cardiac transplantation candidates (age: 10.2 ± 4.6, 1-17 years) underwent cardiac catheterization for the determination of PVR and pulmonary arterial pressure. They were admitted to the hospital and received 1-3 days of intravenous (IV) vasodilator therapy; 0.5-3 μg/kg/min nitroglyserin and/or 0.5-3 μg/kg/min nitroprusside, 5-15 μg/kg/min dobutamin and/or dopamin to keep systolic blood pressure above 80 mmHg.

Results. Thirteen patients had dilated cardiomyopathy (CMP), 11 had restrictive CMP, one had hypertrophic CMP and one had congenital heart disease (CHD). Nineteen of the 26 patients underwent OHT.

Mean pulmonary arterial pressure of the patients ranged between 11 and 82 mmHg (30.4 ± 16 mmHg) and PVRi between 0.41-21.4 Wood units.m^2 (5.3 ± 5.7). Nine patients had PVRi above 4 Wood units.m^2. Six of these patients had IV treatment for longer than three days and some received specific anti-PH treatment. Eventually they underwent a pulmonary vasoreactivity test with IV iloprost and six had PVRi <4 Wood units.m^2. Five of them underwent OHT.

Conclusion. Cardiac transplantation candidates with PH and high PVR should be evaluated after conditioning with vasodilator and inotropic treatment. Specific treatment for PH and vasoreactivity testing may help selected patients reenter the transplantation list.

Key words: cardiac transplantation, pulmonary hypertension, pulmonary vascular resistance, right ventricular failure.

Advanced heart failure patients considered for heart transplantation frequently have Pulmonary hypertension (PH). Patients with restrictive and dilated cardiomyopathy (CMP), who have chronic heart failure with elevated filling pressures are especially under risk. Severe PH is considered a contraindication for heart transplantation as PH leads to right ventricular dysfunction. Right ventricular failure is an important cause of mortality and morbidity after orthotopic heart transplantation (OHT).[1-3]

The right ventricle of the donor may fail to accommodate the high PVR of the recipient.

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Preoperative pulmonary artery pressure and PVR were found to affect mortality after heart transplantation.[2-7] Pulmonary hypertension due to chronic heart failure with PVRi > 4 Wood units.m^2, transpulmonary gradient > 15 mmHg adversely affect the outcome of OHT. Thus patients are evaluated with cardiac catheterization before consideration for cardiac transplantation. Several drugs are used before and/or during cardiac catheterization to decrease pulmonary arterial pressure and pulmonary vascular resistance (PVR) and to test the reversibility of high PVR.[3-5,8-11]

We aimed to evaluate management strategies in our pediatric cardiac transplantation candidates with PH and high PVR prior to OHT.

Material and Methods

Twenty-six cardiac transplantation candidates (age: 10.2 ± 4.6, range 1-17 years) underwent cardiac catheterization for determination of PVR and pulmonary arterial pressure. They were admitted to the hospital and received 1-3 days of IV vasodilator therapy; 0.5-3 μg/kg/min nitroglyserin and/or 0.5-3 μg/kg/min nitroprusside, 5-15 μg/kg/min dobutamin and/or dopamin to keep systolic blood pressure above 80 mmHg, in addition to their usual drugs such as furocemide, spironolactone, angiotensin converting enzyme inhibitors and beta blockers. All patients who had PH and high PVRi underwent a pulmonary vasoreactivity test with IV iloprost. Patients with PH and PVRi greater than 4 Wood units.m^2 after vasoreactivity test received additional therapy including IV vasodilator and inotropic treatment for longer than three days. Additionally, five patients received specific anti-PH therapy. This study was approved by the ethics committee of our university (28/05/2019-KA19/199).

Results

Thirteen patients had dilated CMP, 11 had restrictive CMP, one had hypertrophic CMP and one had severe left ventricular failure years after a Rastelli operation for transposition of great arteries, ventricular septal defect and pulmonary stenosis. Nineteen of the 26 patients underwent OHT. Mean pulmonary arterial pressure of the patients ranged between 11 and 82 mmHg (30.4 ± 16 mmHg), PVRi between 0.41-21.4 Wood units.m^2 (5.3 ± 5.7). Nine patients had PVRi above 4 Wood units.m^2. Six of these patients were reevaluated after receiving IV treatment for longer than three days. Additionally, five patients received specific anti-PH drugs before reevaluation. Patient 1 received sildenafil for 7 days besides intravenous inotropic and vasodilator treatment of 10 days. Patient 4 received bosentan for one month. Patient 7 received bosentan for one week which was stopped due to elevation of liver enzyme levels. She received inhaled iloprost for four months and was admitted to the hospital 7 days before cardiac catheterization and was put on dobutamin and nitroglyserin. IV iloprost was started 24 hours before catheterization. Patient 8 received inhaled iloprost for six months and bosentan for five months, and was admitted to the hospital three days before cardiac catheterization and was put on dobutamin and nitroglyserin. Patient 9 was put on bosentan for two months, and was admitted to the hospital 10 days before cardiac catheterization and was put on dobutamin, nitroglyserin, and nitroprusside. He also received IV iloprost for 24 hours before catheterization.

Eventually all underwent pulmonary vasoreactivity test with IV iloprost. Six patients had PVRi <4 Wood units.m^2. Five of these underwent OHT. The four-year-old patient (Patient 3) with restrictive CMP had a PVRi of 8.8 Wood units.m^2. After OHT PVRi was 5.27 Wood units.m^2. Despite bosentan and inhaled iloprost treatment he died due to right heart failure three months after transplantation. The course of the other five patients was uneventful. The hemodynamic variables and clinical course of these 9 patients are summarized in Table I.

Informed consent was obtained from all individual participants included in the study.

Table I. Hemodynamic variables and clinical course of 9 patients with high PVRI.

Patient	Age year	Cardiac diagnosis	MPAPp mmHg	PVRIp Wood U.m^2	MPAPv mmHg	PVRIv Wood U.m^2	Management for PH	MPAPa mmHg	PVRIa Wood U.m^2	Result
1	14.5	DCMP	59.0	21.4			0.5-3 µg/kg/min NG and 0.5-3 µg/kg/min nitroprusside 10 days, 10 µg/kg/min dobutamin 9 days, 25 mg sildenafil four times/day for 7 days	52	12.08	
							Vasoreactivity test with iloprost	44.0	3.94	STX
2	5.5	DCMP	37.0	4.9	24.0	1.1				Died before TX
3	4.0	RCMP	36.0	14.6			0.5-3 µg/kg/min NG and 0.5-3 µg/kg/min nitroprusside 8 days, 10 µg/kg/min dobutamin 8 days	37.0	8.8	Died 3 months after TX with RV failure
4	11.0	RCMP	35.0	13.9	35.0	12.5	Bosentan 62.5 mg twice daily for 1 month, 0.5-3 µg/kg/min NG, 10 µg/kg/min dobutamin for 7 days before the second cardiac catheterization	24.0	3.39	STX
5	13.5	RCMP	25.0	6.1	25.0	4.30				STX
6	11.0	DCMP	40.0	6.2	35.0	1.16				STX
7	3.0	RCMP	42.0	7.8			Inhaled iloprost 4 months, Bosentan was stopped due to side effects, 7 days dobutamin and NG, 1 day IV iloprost	25	0.8	STX
8	3.5	RCMP	47	8.3		4.3	Inhaled iloprost six months. Bosentan five months, 3 days dobutamin, NG	49	10.1	
							Vasoreactivity test with iloprost		4.2	In TX list
9	15	RCMP	82	18.6			Bosentan for 2 months, 10 days dobutamin, NG, 1 day IV iloprost	96	9.3	
							Vasoreactivity test with iloprost	64	2.8	Not in TX list

DCMP: dilated cardiomyopathy, MPAPa: mean pulmonary artery pressure after antiPH treatment, MPAPp: mean pulmonary arter pressure before vasoreactivity test, MPAPv: mean pulmonary artery pressure after vasoreactivity test, NG: nitroglyserin, PH: pulmonary hypertension, PVRIa: pulmonary vascular resistance after antiPH treatment, PVRIp: pulmonary vascular resistance before vasoreactivity test, PVRIv: pulmonary vascular resistance after vasoreactivity test, RCMP: restrictive cardiomyopathy, RV: right ventricle, STX: successful transplantation, TX: transplantation.

Discussion

Pulmonary hypertension and right ventricular failure is an important cause of mortality and morbidity after OHT.[1-7] Elevated PVRi is generally considered to be a contraindication for heart transplantation in most centers.[2,3,11] In some studies, it has been reported that high PVRi should not be a contraindication and this is not related to mortality for OHT.[12,13] Chiu et al.[12] reported that survival was similar in both unmatched and propensity-matched analyses of groups of patients using either a threshold of PVRI ≥6 or PVRI ≥9 WUx m^2. The evolving management of right ventricular dysfunction following OHT, use of mechanical circulatory support and targeted therapy for pulmonary hypertension may allow survival of transplanted patients with high PVRI. In spite of favorable results, concerns about adverse outcome of pre-transplantation pulmonary hypertension continues. Costard-Jackle et al.[4] reported a 3.3% mortality rate due to PH or right ventricular failure in 301 patients which was responsible for 26% of deaths within 90 days after OHT. A pediatric study reported by Addonizio et al.[14] correlated outcomes of pediatric patients with various levels of PVRi. Pulmonary vascular resistance index above 6 Wood units.m^2 was related with poor survival due to right ventricular failure. Gajarski et al.[15] compared their results in pediatric heart transplant recipients with a mean PVRi of 11.5 ± 3.5 Wood units.m^2 with those having mean PVRi of 2.3 ± 0.4 Wood units.m^2. After prostaglandin E_1 infusion as a vasodilator, PVRi decreased to 3.9 ± 0.9 Wood units.m^2 in those with high PVRi. They concluded that the reactivity of the pulmonary vascular bed rather than the absolute measure of the PVR correlated with outcome as the mortality was similar in two groups.

Patients with chronic heart failure may have PH resulting from several reasons. Increased left ventricular end-diastolic pressure causing increased left atrial and increased pulmonary capillary pressures eventually leads to high pulmonary arterial pressure. Pulmonary hypertension due to heart failure was classically thought to develop as a passive consequence of high filling pressures of the left ventricle.[16-19] However pulmonary pressures and PVR may remain high after heart transplantation for a varying time and may decrease gradually.[7,11] This suggests the occurrence of structural changes in pulmonary vessels. Delgado et al.[20] demonstrated an increase in the medial thickness of the muscular pulmonary arteries of patients dying early after OHT. In patients with congestive heart failure persistent elevation of left ventricular end-diastolic pressure leads to passive pulmonary venous congestion and reactive pulmonary vasoconstriction.[16,17,21] Persistent elevation of pulmonary capillary pressure due to increased left ventricular filling pressure may result in histological changes in the pulmonary vasculature.[16-19,22] At the beginning PVR is reversible with pulmonary vasodilators until prolonged pulmonary venous congestion causes remodelling of the pulmonary arterial wall with abnormalities of the elastic fibers, intimal fibrosis and medial hypertrophy. At this time PH may be defined as fixed as it is resistant to pulmonary vasodilators. Ortiz et al.[23] have investigated the evolution of right heart pressures in an adult series in the first year after heart transplantation with respect to background cardiac disease. The right heart pressures showed an important decrease in the first days after heart transplantation with stabilization by the third month but without returning to normal. However, the PVRi in this series was less than 4 Wood units.m^2. We applied intravenous drugs for deloading and for decreasing afterload to optimize the hemodynamics before evaluating the PVR. This conditioning is important as pulmonary edema and systemic vasoconstriction may adversely affect pulmonary pressure and cardiac index. Several reports in pediatric and adult patients have stated that intensive treatment with inotropes, vasodilators and in some cases left ventricular assist device can lower PVR and allow for transplantation.[3,11,24,25] Mahajan et al.[9] reported a series of 21 adult patients with dilated CMP with persistent moderate to severe PH despite intravenous medical therapy. All

patients received 1 to 3 days of IV cardiac drug therapy including vasodilators, inotropes and diuretics with the goal of optimizing hemodynamics with maximally tolerated doses of IV vasodilators. Twenty-one patients who had persistent moderate to severe PH underwent testing with 100% oxygen and inhaled nitric oxide. Nitric oxide caused a more significant decrease in pulmonary arterial pressure and PVRi than oxygen. Nine of the patients had PVR decreased below 4 Wood units and were included in the transplantation list.

Several drugs have been used for testing the reversibility of high PVR. Sablotzki et al.[26] investigated hemodynamic effects of inhaled aerosolized iloprost and inhaled nitric oxide in heart transplant candidates with elevated PVR. They showed that inhaled iloprost induced pulmonary vasodilation greater than the effects of 10 and 30 ppm nitric oxide and they recommended iloprost as a routine screening drug for vascular reactivity in heart transplantation candidates. We used intravenous iloprost for this purpose. Adenosine was also successfully used by Haywood et al.[8] for the reversal of pulmonary vasoconstriction in biventricular failure and was found to be superior to nitroprusside. In another study however adenosine was less effective with more side effects than inhaled nitric oxide in a group of patients with pulmonary arterial hypertension.[27]

Despite cautious treatment with vasodilators, inotropes and diuretics to optimize hemodynamics and to decrease pulmonary edema before evaluation of pulmonary arterial pressure and PVR some patients still have high PVR not allowing transplantation. Specific treatment for PH can further help to relist patients with high PVR.[3,5,11,28,29] Kao et al.[28] reported a 14 year-old patient with dilated CMP who was previously operated for aortic coarctation and ventricular septal defect in infancy. The patient had a PVRi of 27 Wood units.m^2. After two months of continuous prostacyclin infusion of up to 14 ng/kg/min repeat cardiac catheterization revealed PVRi of 3.7 Wood units.m^2. She underwent heart transplantation one month later with extracorporeal membrane oxygenation support for the first four days. Perez-Villa et al.[29] reported one of the initial experiences with bosentan, an oral endothelin-receptor antagonist to overcome high pulmonary artery pressure and PVR in 7 patients who were considered ineligible for heart transplantation. After six weeks of bosentan therapy five of them had a PVR <2.5 Wood units and underwent successful heart transplantation.

The use of a left ventricular assist device (LVAD) has been proposed as an effective treatment for reducing PVRi in potential heart transplant candidate's refractory to medical vasodilator therapies.[30,31] In current studies after LVAD implantation, the patients experienced a profound decrease in PVRi on follow-up cardiac catheterization. The resulting dramatic improvement in PVRi in a relatively short period of time allowed for successful OHT.[25,32] In addition, the use of ventricular support devices help to avoid sudden loss of OHT candidates waiting for transplant. In our study, we showed that the patients with high pulmonary artery pressure and PVRi could be included in the waiting list after treatment with vasodilators, inotropes and diuretics. It was also possible to detect reversible PVR after vasodilator, inotropic and diuretic treatment. These therapies have been shown to reduce the incidence of right heart failure and potentially reduce the morbidity and mortality of post-transplantation right heart failure in single institution reports.[3,33,34]

Several studies showed that the current treatments after OHT decrease the contribution of PVRI elevation on right ventricular failure and early mortality.[13,35] Improved management of PH and right ventricular dysfunction may have changed the relationship between PVR and post-transplant mortality.

In this study, the lack of comparative analysis due to the small number of patients was a limitation. Non-standardization of specific anti-PH treatment was considered as a disadvantage.

In conclusion cardiac transplantation candidates with PH and high PVR should be evaluated after conditioning with vasodilator and inotropic treatment. Specific treatment for PH and vasoreactivity testing may help selected patients reenter the transplantation list.

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Original Article

Corneal endothelial morphology and anterior segment parameters in children with type 1 diabetes mellitus

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ABSTRACT

Background and objectives. To compare the corneal endothelial morphology and anterior segment parameters in type 1 diabetes mellitus children (T1DM) and healthy controls.

Methods. This cross-sectional prospective study included 56 patients with T1DM and 50 eyes of 50 age-matched controls. Endothelial morphology was analyzed with EM-3000 specular microscopy, and anterior parameters were analyzed with Sirius Scheimpflug topography. Endothelial cell density (ECD), coefficient of variation (CV) of cell area, central corneal thickness (CCT), anterior chamber depth (ACD), iridocorneal angle (ICA), K1 and K2, pupillary diameter (PD), horizontal visible iris diameter (HVID), duration of T1DM, and HbA1c levels were noted.

Results. The mean age of the T1DM group was 14.3 ± 3.2 years, compared to 13.2 ± 3.7 years in the healthy group ($p = 0.140$). The mean duration of diabetes mellitus was 4.5 ± 3.5 years. The mean HbA1c was 9.5 ± 1.9% (minimum 6%, maximum 14%). The mean values of CCT were 556 ± 30 μm and 536 ± 36 μm in T1DM and healthy groups, respectively ($p = 0.003$). The mean values of ACD were 3.69 ± 0.31 mm and 3.83 ± 0.27 mm in T1DM and healthy groups, respectively ($p = 0.02$). The mean values of PD were 4.29 ± 1.2 mm and 5.17 ± 1.36 mm in T1DM and healthy groups, respectively ($p = 0.001$). There was no statistically significant difference between groups in terms of ECD, CV, ICA, K1, K2, and HVID ($p > 0.05$).

Conclusion. Type 1 diabetes mellitus children have thicker corneas, shallower anterior chamber depth, and smaller pupillary diameter than healthy subjects.

Key words: anterior segment parameters, corneal topography, endothelial morphology, type 1 diabetes mellitus.

Type 1 diabetes mellitus (T1DM) is the most common metabolic disease in childhood. It is a chronic illness characterized by high blood glucose levels due to an inability to produce insulin. This disease affects 500,000 patients globally.[1] The prevalence of T1DM in Turkey is 75/100000.[2] T1DM is a systemic disease that can affect all organ systems.[3-5] Diabetic retinopathy is the most common complication of T1DM.[6] But it can also affect the anterior segment of the patients.[7-13] Some studies have demonstrated that patients with T1DM had greater central corneal thickness (CCT) than non-diabetic subjects.[11] Anterior chamber depth (ACD) was found to be shallower in DM patients due to thickening of lens because of hyperglycemia.[7-9] T1DM can also cause sympathetic autonomic neuropathy. T1DM can affect response and the duration time of the sympathomimetics.[13] Because of these effects on sympathomimetics, pupillary diameter was found to be smaller in diabetic patients.[13] The main outcomes of these cited studies have addressed crystalline lens and anterior chamber depth; however, morphologic and functional changes in cornea and anterior segment parameters have been studied less frequently in diabetic children. This

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study aimed to compare corneal endothelial morphology and anterior segment parameters in diabetic children and healthy controls by corneal topography and noncontact specular microscopy.

Material and Methods

The eyes of 56 patients with T1DM and the eyes of 50 age-sex matched healthy subjects were examined in this study. T1DM patients were referred to us through pediatric endocrinology clinics between June 2018 and July 2018. Ethical Committee approval for the study was obtained (protocol number 09.2018.120). Written informed consent was obtained from all the parents of the children.

All participants underwent a total ophthalmic examination. Best-corrected visual acuity, slit-lamp examination, intraocular pressure measurements with pneumotonometer, fundus examination, and refraction measurements with autokeratorefractometer were performed. Refraction measurements and fundus examination were performed after cycloplegia. The duration of DM, age, gender, and Hemoglobin A1c (HbA1c) levels were recorded for diabetic children. Patients with any of the following criteria were excluded from the study: > 18 years old, contact lens users, previous ocular trauma, history of ocular surgery, ocular inflammation, refractive errors > ± 1.00 (spherical or cylindrical), corneal disease, and cataracts. Measurements of endothelial morphology, such as endothelial cell density (ECD) and coefficient of variation (CV) of cell area were examined by noncontact specular microscopy using an EM-3000 Specular Microscope (CBD/Tomey, Phoenix, AZ, USA). Anterior segment parameters, such as CCT, ACD, iridocorneal angle (ICA), sim K1 and K2, pupillary diameter (PD), and horizontal visible iris diameter (HVID) were examined by Sirius Scheimpflug topography (Costruzione Strumenti Oftalmici, Florence, Italy). The Siruis topography examination was performed 2 days after cycloplegia by the same person. Because of the correlation between right and left eye, only the right eyes of the participants were analyzed. HbA1c levels were obtained from pediatric clinic on the same day.

Statistical analyses were performed using the SPSS software version 21. Descriptive analyses were presented using means and standard deviations for normally distributed variables. An assessment of normality was done using the Kolmogorov-Smirnov test. The independent t, Man-Whitney U, Chi-squared, and Pearson correlation tests were used for analyses. A p-value of less than 0.05 was considered to show a statistically significant result.

Results

In the T1DM group, the female/male ratio was 32/24, while it was 28/22 in the healthy group (p = 0.454). The mean age was 14.3 ± 3.2 years in the T1DM group and 13.2 ± 3.7 years in the healthy group (p = 0.140). The mean duration of diabetes mellitus was 4.5 ± 3.5 years. The mean HbA1c was 9.5 ± 1.9% (minimum 6%, maximum 14%).

In terms of corneal endothelial morphology, the mean ECD values were 2975 ± 248 cells/mm^2 and 3012 ± 257 cells/mm^2 in the T1DM and healthy groups, respectively. There was no statistically significant difference between groups (p = 0.458). The mean CV values were 0.36 ± 0.06 and 0.35 ± 0.08 in the T1DM and healthy groups, respectively. Regarding CV, there was no statistically significant difference (p = 0.608). Regarding topographic anterior segment parameters, the mean values of CCT were 556 ± 30 μm and 536 ± 36 μm in T1DM and healthy groups, respectively (p = 0.003) (Fig. 1). The mean values of ACD were 3.69 ± 0.31 mm and 3.83 ± 0.27 mm in T1DM and healthy groups, respectively (p = 0.02) (Fig. 2). The mean values of ICA were 44.1 ± 6.6 and 45.5 ± 7.3 in T1DM and healthy groups, respectively (p = 0.297). The mean values of HVID were 12.15 ± 0.53 mm and 12.14 ± 0.44 mm in the T1DM and healthy groups, respectively (p = 0.914). In the T1DM

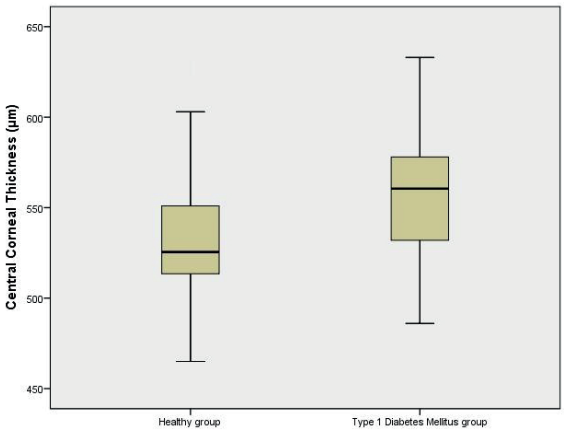

Fig. 1. Central corneal thickness values in groups.

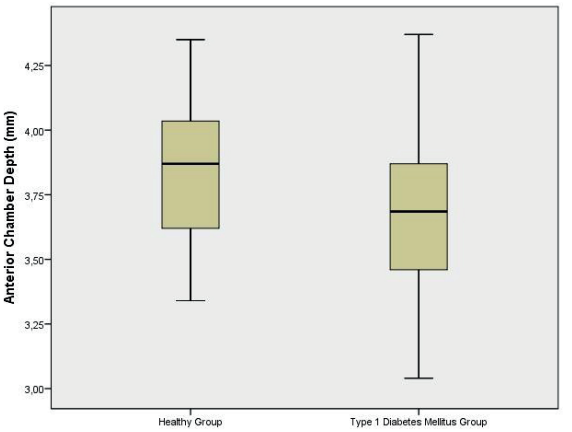

Fig. 2. Anterior chamber depth in groups.

group, the mean values of K1 and K2 were 42.75 ± 1.41 mm 42.37 ± 1.5 mm, respectively, and these values were 43.69 ± 1.64 mm and 44.03 ± 1.59 mm in the healthy group (p = 0.191 and p = 0.634, respectively). The mean PD values were 4.29 ± 1.2 mm and 5.17 ± 1.36 mm in the T1DM and healthy groups, respectively (p = 0.001) (Fig. 3).

A significant positive correlation was detected when comparing the duration of diabetes and CCT (r = 0.277 and p = 0.038). This correlation is presented in Figure 4. No statistically significant correlations were found between HbA1c levels and PD, CCT, and ACD. No statistically significant correlations were found between the duration of DM, PD, and ACD. In T1DM groups, females had higher CVs than males (mean CV in females, 0.38 ± 0.06; mean CV in males, 0.32 ± 0.04; p < 0.001).

Discussion

The Diabetic Control and Complications Trial (DCCT) has reported a lower risk for microvascular complications of T1DM.[14] Beside microvascular complications, T1DM can also affect anterior segment parameters. Multiple studies have reported that T1DM affects the lens.[1,7,15] In this study, we report that T1DM also affects corneal and anterior segment parameters.

Anbar et al[16]. found that ECD and CV values were lower in T1DM patients than the healthy controls, but they did not find any correlation

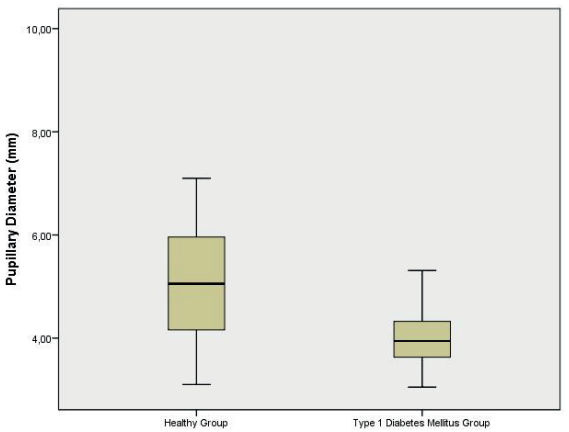

Fig. 3. Pupillary diameter in groups.

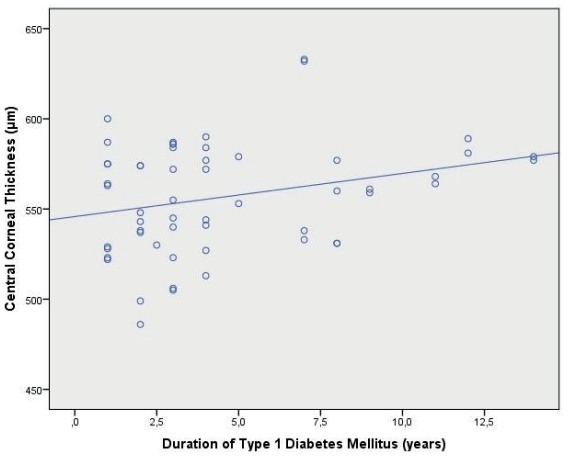

Fig. 4. Correlation between CCT and duration of type 1 DM.

between ECD with the following variables: the age of the patients, gender, HbA1C level, Body mass index and hemoglobin level. They only found a correlation with duration of T1DM and ECD. The duration of T1DM was also identified as a risk factor for changes in the polymegathism and pleomorphism in their study. These authors have established an increase in polymegathism and decrease in pleomorphism in T1DM children.[16] Unlike Anbar et al.[16] we found no difference in terms of endothelial parameters.

According to this study, there was no difference in endothelial parameters when comparing the T1DM and healthy groups. The same results were obtained by Larsson et al.[17] Their study included 49 patients with T1DM and 60 patients with T2DM, and their outcomes concluded that type 1 and type 2 diabetes patients did not differ from their controls in ECD. Also, Larsson et al.[17] noticed a significant decrease in endothelial cell hexagonality and abnormalities in endothelial cell morphologic characteristics in T1DM patients when compared to their controls.

As in this study, Ozdamar et al.[18] also found that CCT values were higher in diabetic patients than the healthy group. These authors compared one hundred diabetic patients with one hundred-forty-five control subjects. In diabetic patients, the mean CCT value was 564 ± 30 μm, compared to 558 ± 35 μm in the healthy controls. Although they compared adult diabetes patients, the results were the same as the current study. In another study with diabetic children, Urban et al.[19] compared 123 eyes of T1DM children with 124 eyes of a control group. These authors reported that T1DM children had a CCT value of 550 ± 30 μm, while control subjects had a value of 530 ± 33 μm. Tiutiuca et al.[20] found that T1DM children had a CCT value of 541 ± 30 in their right eye, and control subjects had a CCT value of 528 ± 33. These studies supported our results. Additionally, there is a positive correlation between T1DM duration and CCT in our study. Busted et al.[21] also reported that thicker CCT and lower ECD were correlated with duration of DM. Lee et al.[22] found a correlation between duration of DM and thicker CCT; these authors also reported that there was no correlation between duration of DM and ECD. Although there is a pathogenic hypothesis for this, such as corneal endothelial pump dysfunction and swelling cornea, any strong associations have not been established. The reasons for these contracting findings must be investigated in pathological studies.

Wiemer et al.[7] investigated the effects of type 1 and type 2 DM on the cornea with scheimplug topography. Subjects were investigated for asphericity of anterior and posterior corneal surfaces and corneal power. The authors did not find a significant difference between diabetic subjects and healthy subjects. Uzel et al.[15] also did not detect any difference in K1 or K2 when comparing the T1DM and healthy groups. In this current study, we also did not find any statistically significant difference in K1 and K2.

Impaired glucose metabolism can cause swelling of the lens. Decreased anterior chamber may occur due to metabolic swelling of the lens.[15] Multiple studies have investigated and found shallower anterior chambers in T1DM patients compared to a healthy group.[10,15] As in those studies, our T1DM patients had significantly shallower ACD than our healthy group. However, there was no correlation between T1DM duration and ACD, as also reported by Uzel et al.[15] These authors also identify a thicker lens as the reason for the decreased ACD in T1DM patients.

T1DM can also cause sympathetic autonomic neuropathy. Thus, T1DM can affect response and the duration time of the sympathomimetics.[13,15] Studies have reported smaller PD in T1DM patients relative to healthy groups (e.g., like Lei et al.[13]). These authors also report that T1DM patients with diabetic retinopathy had a smaller PD. It In the current study we could not compare the effect of the diabetic retinopathy, because none of our patients had developed it. Uzel et al.[15] reported that this smaller PD also negatively correlated with HBA1c levels, indicating a relation between pupil size and poor diabetes control.[15] In this study, there was

no correlation between HBA1c, PD, CCT, and ACD. We also could not detect any correlation between T1DM duration and PD.

In the T1DM group, females had a higher CV than males, but the other parameters showed no sex differences. The study by Saw et al.[10] included 1453 healthy children between 7 and 9 years old. Males were found to have a longer axial length, flatter corneal curvature radius, deeper ACD, and vitreous chamber than the females. They also reported a longer axial length and a deeper vitreous chamber in taller children.[10] Therefore, different outcomes of gender might also be related to body height differences.

Consequently, T1DM was found to affect anterior segment parameters. Diabetic children have thicker cornea, lower ACD, and smaller PD. Although the duration of DM affects CCT, it does not affect PD or ACD. We recommend that these factors should be taken into consideration during the examination of patients with T1DM.

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Case Report

Two cases of Vici syndrome presenting with corpus callosum agenesis, albinism, and severe developmental delay

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ABSTRACT

Background. Vici syndrome is a rare autosomal recessive disease with phenotypically heterogeneous presentation. Characteristic features of the disease are oculocutaneous albinism, corpus callosum agenesis, cataract, cardiomyopathy, and immunodeficiency.

Case. Here we report two Turkish patients with Vici syndrome. One of these patients had a novel mutation in *EPG5* and presented with idiopathic thrombocytopenic purpura (ITP) and maculopapular rashes similar to Stevens–Johnson syndrome, which has been previously reported in only a few cases in the literature.

Conclusion. Vici syndrome presents with a typical phenotype which may facilitate diagnosis for infants with multisystemic disorders. ITP and maculopapular rashes might be added to the spectrum of findings of patients with Vici syndrome.

Key words: Vici syndrome, autophagy, oculocutaneous albinism, EPG5 mutation.

Vici syndrome is a rare multisystemic autosomal recessive disease characterized by corpus callosum agenesis, oculocutaneous hypopigmentation, cataract, cardiomyopathy, severe developmental delay, combined immunodeficiency, and variable multisystemic features. It was first reported by Dionisi-Vici in two siblings in 1988.[1-3] It occurs as a result of biallelic loss-of-function mutations in the *EPG5* gene on chromosome 18q12.3, which encodes autophagy regulator ectopic P granular protein 5 (*EPG5*). It is a neurodevelopmental disorder of the autophagy pathway.[4,5] Impaired autophagy results in multisystemic defects affecting organs such as the heart, brain, and immune system.[6]

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Here we describe two patients who displayed the clinical features of Vici syndrome and carried homozygous mutations in the *EPG5* gene. One of these patients had a novel homozygous mutation in the *EPG5* gene and presented with idiopathic thrombocytopenic purpura (ITP) and maculopapular rashes similar to Stevens–Johnson syndrome, which has been previously reported in only a few cases in the literature.

Case Report

Case 1

Case 1 was a male infant born as the third child of healthy, consanguineous (first cousins) Turkish parents. The patient's brother had died an hour after birth due to respiratory insufficiency. He was born by normal vaginal delivery with meconium-stained amniotic fluid at gestational age of 36 weeks with a birth weight of 2.8 kg and normal head circumference.

The patient developed acute respiratory failure after birth and was monitored in the neonatal intensive care unit with mechanical ventilation for 20 days. He was hospitalized for 4 months due to recurrent respiratory tract infections. Corpus callosum agenesis was suspected in trans-fontanel ultrasound and confirmed by magnetic resonance imaging (MRI) of the brain. In echocardiography, biventricular hypertrophy, mild mitral insufficiency, and cardiomyopathy were detected. The patient also had feeding difficulties. Tracheal aspiration was noticed during deglutition studies. He had recurrent aspiration pneumonia, and a gastronomy tube was needed after 4 months.

Laboratory investigations performed upon suspicion of metabolic disease were normal, including serum lactate, pyruvate, carnitine, acylcarnitines, very-long-chain fatty acid levels, and urine organic acids. Plasma and urine amino acid levels were also normal, along with sweat chloride test, serological tests for cytomegalovirus, rubella, toxoplasma, and herpes simplex virus, and chromosome analysis (46, XY). At the age of 2 months, complete blood count revealed a low platelet count (15,000/ mm^3). ITP was suspected, and bone marrow biopsy findings were consistent with ITP. After two infusions of intravenous immunoglobulin (IVIG), his platelet count increased.

The patient was admitted to our hospital at the age of 5 months with bronchopneumonia requiring intubation and mechanical ventilation. His body weight, length, and head circumference were below the third percentile for sex and age. Hypopigmented skin and hair, micrognathia, high-arched palate, low-set ears, broad nasal bridge, almond-shaped eyes, and truncal hypotonia were observed on physical examination. He had no social smile or object tracking. In ophthalmologic examination, bilateral anterior subcapsular cataract was noted, while the retina was normal.

Muscles enzymes were elevated (CPK: 604 U/L, AST: 195 U/L, and LDH: 2504 U/L). He also had elevated levels of ALT (126 U/L) and GGT (112 U/L). Electroencephalogram revealed no epileptic activity, and there were no abnormal findings on abdominal ultrasonography or electromyography. The parents did not consent to a muscle biopsy. Immunological investigations revealed low IgA and CD19 levels. ITP recurred at 6 months of age, and again platelet count increased after administration of IVIG. He also developed diffuse maculopapular rash similar to Stevens–Johnson syndrome at 6 months of age without accompanying fever or other signs of viral infection. Diagnostic tests to determine the etiology of the rash did not yield positive results. Skin biopsy was performed because the rashes persisted for more than two weeks and revealed nondiagnostic inflammatory cells.

The patient's respiratory functions progressively declined and he died at 3 years of age due to pulmonary infection. Autopsy could not be performed.

Because the patient showed the typical features of Vici syndrome, a molecular study of the *EPG5* gene was performed and homozygous c.2653delA (p.Thr885fs) mutation was detected in exon 14 (Fig.1). The variant was not found on publicly available databases of human genetic variations.

Case 2

Case 2 was another male infant born as the second child of healthy, consanguineous (first cousins) Turkish parents. He was born by normal vaginal delivery at 37 weeks' gestation

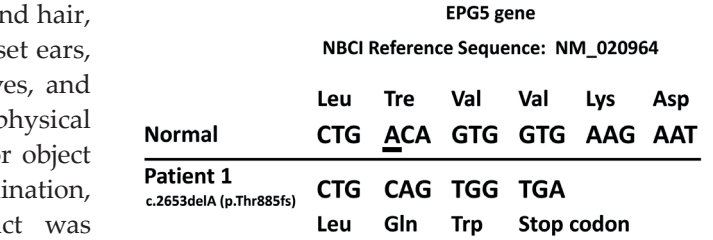

Fig. 1. Schematic presentation of *EPG5* mutation in Patient 1. c.2653delA (p.Thr885fs) mutation results in the occurrence of a premature stop codon (TGA) leading to cessation of synthesis of the protein encoded by the *EPG5* gene.

with a birth weight of 3.0 kg and normal head circumference. He presented at 2 weeks of age with poor feeding and failure to thrive. He was hospitalized with pneumonia at 2 months of age.

He was admitted to our hospital at the age of 5 months with poor weight gain and frequent upper respiratory tract infections. His family history included a sister who had died at the age of 4 months due to similar complaints. Physical examination revealed hypopigmented skin and hair, micrognathia, low-set ears, and truncal hypotonia. He was unable to hold his head up, could not track objects, and lacked social smile. Complete blood count, liver and kidney function tests, and metabolic analyses were normal.

On ophthalmological examination, macular reflexes were absent and peripapillary atrophy was detected. Echocardiography demonstrated hypertrophic cardiomyopathy and left ventricular hypertrophy. Cranial MRI revealed corpus callosum agenesis and delayed myelination. Lymphocyte subgroups and immunoglobulin levels were normal in immunological investigations.

The patient had difficulty feeding. Aspiration was observed during deglutition studies and a nasogastric feeding tube was placed. There were no abnormal findings in abdominal ultrasound and he had no history of seizure.

Because his clinical presentation was consistent with Vici syndrome, a molecular study of the *EPG5* gene was performed and revealed a homozygous c.7447C>T (p.Arg2483*) mutation. The patient was discharged from the hospital after his pneumonia resolved. He is now 24 months of age and still under follow-up.

Informed consent was received from the families for publication of cases.

Discussion

The incidence of Vici syndrome is unknown. To date, approximately 80 cases have been reported in the literature.[7] In addition to its principal features, other nonspecific characteristics have also been reported, including severe developmental delay, acquired microcephaly, and progressive growth failure, which can support the diagnosis.[8,9]

Vici syndrome occurs as a result of biallelic mutations of the *EPG5* gene.[10,11] In our patients, genetic testing showed c.2653delA (p.Thr885fs) and c.7447C>T (p.Arg2483*) mutations in *EPG5*, confirming the diagnosis of Vici syndrome. This is the first report of homozygous c.2653delA (p.Thr885fs) mutation in exon 14 in the literature.

The two patients described in this report both had postnatal growth retardation, oculocutaneous hypopigmentation, agenesis of corpus callosum, cardiomyopathy, significant hypotonia, and recurrent respiratory tract infections, all of which support a diagnosis of Vici syndrome.

Developmental delay is common in patients with Vici syndrome.[6] Our patients also had severe developmental delay. Affected children can acquire social smile and some degree of head control, but these were absent in both of our patients.[6] In contrast, although two-thirds of patients have refractory seizures, this was not observed in our cases.

Although head circumference is generally normal at birth, microcephaly develops progressively within the first year of life in Vici syndrome.[6] Both of our patients had acquired microcephaly despite normal head circumference at birth. In addition to corpus callosum agenesis, cranial imaging may demonstrate pontine hypoplasia, delayed myelination, and reduced white matter.[9,12] However, no additional findings aside from agenesis of corpus callosum were detected in cranial imaging studies of our patients.

Oculocutaneous hypopigmentation is among the principal features of Vici syndrome.[13] Maculopapular rashes similar to Stevens–Johnson syndrome were also reported

in 6 children, though the cause has not been identified.[9] One of our patients had maculopapular rashes and we were unable to determine the cause based on laboratory investigations and skin biopsy. Considering the multisystem involvement of Vici syndrome, there are numerous mechanisms that may be responsible for these rashes. The underlying pathophysiology of this phenomenon may be elucidated in the future as more cases are reported and evaluated in light of emerging evidence.

In this article we present a case of Vici syndrome with attacks of ITP in addition to the classic features of the disease. To our knowledge, ITP has been previously reported in only one pair of siblings with Vici syndrome.[14] One of these siblings had a single ITP attack, while the other had recurrent attacks. Although immune dysfunction is often described in patients with Vici syndrome, autoimmune syndromes such as ITP are not yet recognized as a feature of the disease. Defective autophagy affects many organs and may also trigger an autoimmune mechanism by unknown pathways. In children, ITP is frequently triggered by an infectious illness. This finding may explain by the recurrent infections as well. Further case reports and investigational studies are needed to understand the association between ITP and autophagy disorders.

Bilateral cataract is known as a main finding of Vici syndrome. However, in a study including 50 patients, the prevalence of cataract was approximately 75%.[12] Similarly, only one of our patients had cataract. The ophthalmological features of Vici syndrome were described by Filloux et al.[13] as optic nerve hypoplasia, visual defect, nystagmus, and fundus hypopigmentation. The second patient in this study exhibited peripapillary atrophy. Ophthalmologic evaluation has essential for these children, not only to detect cataract but also to identify other ophthalmologic pathologies.

Combined immunodeficiency is common in Vici syndrome, with highly variable severity.[15] Although one of the patients in this report had normal immunologic evaluation, the other had some degree of hypogammaglobulinemia and low CD19 level. However, both of our patients had frequent respiratory tract infections. There are many contributing factors in recurrent respiratory tract infections in Vici syndrome. Although patients seem to have a normal immune status, they can present with recurrent infections starting early in life. Investigation of these other factors is important. It should be kept in mind that these patients may have swallowing disorders, as in our cases, particularly due to the underlying hypotonia.

Vici syndrome is a progressive disease with poor prognosis. Median survival time is approximately 24 months, and treatment consists of supportive therapeutic interventions. The main causes of death in these patients are recurrent infections due to immune dysfunction and cardiomyopathy.[7] A better understanding of the underlying pathophysiology might facilitate the development of targeted treatments in the future.

In conclusion, Vici syndrome presents with a typical phenotype characterized by corpus callosum agenesis, cataract, hypopigmentation, cardiomyopathy, immunodeficiency, developmental delay, and acquired microcephaly. Keeping this in mind may facilitate diagnosis for infants with multisystemic disorders. We report a case of Vici syndrome with ITP and maculopapular rashes resembling Stevens–Johnson syndrome, which has been reported in only a few cases to date. ITP and maculopapular rashes might be added to the spectrum of findings of patients with Vici syndrome.

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Case Report

The remarkable response to ponatinib therapy in a child with blastic phase of chronic myeloid leukemia

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ABSTRACT

Background. Chronic myeloid leukemia (CML) rarely occurs in children and adolescents, which shows more aggressive features like high risk of more advanced disease at the time of diagnosis. Suboptimal response to tyrosine kinase inhibitors (TKIs), adverse events, or advanced disease may impede the treatment.

Case. Herein we present a nine-year-old chronic phase CML case. He had no major molecular response (MMR) to imatinib, which was switched to dasatinib. MMR was ensured for 24 months, yet he developed a lymphoid blastic phase under dasatinib. He obtained a remarkable response to ponatinib when administered in parallel to multiagent induction chemotherapy.

Conclusion. Ponatinib therapy is effective and promising as a bridge to hematopoietic stem cell transplantation in children. Although more studies are necessary to determine indications, dose, efficacy, and safety data.

Key words: chronic myeloid leukemia, blastic phase, ponatinib.

Chronic myeloid leukemia (CML) accounts for 2-3% of leukemias in children and adolescents, which is less common than adults, and the disease shows more aggressive features like higher white blood cell counts (WBC), larger spleen size, and a high risk of more advanced disease at the time of diagnosis in children and adolescents.[1,2] Treatment with tyrosine kinase inhibitors (TKIs) has improved outcomes and changed first-line therapy from hematopoietic stem cell transplantation (HSCT) to TKIs in children. However, there is still a risk of growth disturbance due to TKI, which is a unique side effect in pediatric patients.[3] The second-generation (2G) TKIs such as dasatinib, nilotinib, and third-generation (3G) TKI, such as ponatinib, have expanded the treatment options that defers from HSCT.[2] However, to date ponatinib has not been approved for children, and there is very limited data on dose, safety, and efficacy in children. Herein, we present a child with lymphoid blastic phase (BP) CML who had a remarkable response to ponatinib therapy when administered in parallel to acute lymphoblastic leukemia (ALL) induction treatment.

Case Report

A nine-year-old, previously healthy boy presented with fatigue, loss of weight, and bone pain. He was found to have splenomegaly, WBC of 200 x 10^9/L, and 100% t(9;22) translocation by fluorescence in situ hybridization (FISH) analysis in Azerbaijan. Chronic phase CML (CML-CP) was diagnosed, and imatinib was initiated with a 300 mg/m^2 dose daily. After a five-month follow-up in Azerbaijan, he presented to our center. At admission, he had complete cytogenetic response, except the BCR/ABL1 transcript level was 0.19 international scale (IS) by polymerase chain reaction (PCR) assay. No matched family donors were present. During follow-up, he developed severe bone pain resistant to analgesics and severe neutropenia due to imatinib. At 12 months, he

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had no major molecular response (MMR) (BCR/ABL1 0.29 IS), and imatinib was replaced with dasatinib (60 mg/m^2 per day). He achieved MMR at the 6th month of dasatinib therapy (BCR/ABL1, 0.09 IS). Nevertheless, the BCR-ABL1 transcript level was 0.04, 0.21, and 0.69 IS at 18, 21, and 24 months respectively, under dasatinib. Unfortunately, at 27 months under dasatinib, he presented with neutropenic fever, and a bone marrow aspirate revealed ALL with precursor B cell phenotype. The patient had no central nervous system or testicular involvement. Bone marrow FISH examination demonstrated t(9p;22q) and the patient was started Berlin-Frankfurt-Münster-based (BFM) induction chemotherapy combined with ponatinib (15 mg daily, 13 mg/m^2/daily), as approved by Ministry of Health of Turkey and consent of family for this use without pediatric indication was obtained. Ponatinib started on day 15 of induction chemotherapy. Karyotyping at the time of progress to CML-BP lymphoid did not show additional chromosomal aberrations. The gene sequencing ABL kinase domain including threonine-to-isoleucine mutation at position 315 (T315I) showed no mutation at the time of the blast crisis. The blasts in the peripheral blood were disappeared in the first week of therapy. Bone marrow aspirate on day 15 showed 11% blasts, and BCR/ABL1, 25.74 IS. He achieved hematological remission, and with a BCR/ABL1, 0.25 IS on day 33 of induction chemotherapy along with ponatinib. Complete molecular response (CMR) with a quantification limit of 0.0063% IS under the ponatinib and multiagent chemotherapy was achieved by the 3rd month of treatment initiation. No occurrences of adverse events including QT prolongation, hypertension, and arterial occlusion, were observed. Additionally, the patient had not experienced hepatotoxicity or prolonged neutropenia in comparison with observed data from the BFM ALL trial. He underwent HSCT from a haploidentical mother (5/10 matched) after he gained CMR. At the last follow-up, he is alive with CMR. The consent of the parents of the patient is included in the hospital clinical documents.

Discussion

Recently, 2G-TKIs dasatinib and nilotinib were approved for children, along with imatinib, which was approved in 2003. Up-front TKI choice depends on efficacy, safety, availability, administration, cost, and restrictions of the insurance system.[2] The limited data showed that target responses were met early, and deep molecular responses achieved with 2G-TKI, though at 18 months, are similar for imatinib.[2,4] The presented case demonstrated a suboptimal response to imatinib, and he experienced toxicity requiring TKI change. He sustained remission with dasatinib initially but progressed to the lymphoblastic leukemic phase subsequently. Mutations of the BCR/ABL1 kinase domain were excluded, and leukemic progression occurred despite good adherence to the drug. If failure to 2G-TKIs is observed, allogeneic HSCT is recommended.[2] The prognosis of blast crisis of CML-CP while on TKI therapy is poorer than de-novo CML-BP.[2,5] Millot et al.[5] reported overall survival (OS) rate to be 74% at 60 months in 17 children for patients diagnosed in CML-BP, whereas an OS rate of 41% at 60 months in 21 children receiving imatinib for CML-CP progressed to predominant lymphoid blastic phase. Our patient presented with a blastic crisis under 2G-TKI, so we preferred ponatinib as a 3G-TKI, nilotinib therapy was ignored. Ponatinib therapy with BFM based induction chemotherapy was initiated as a bridge to HSCT.

There is limited experience with ponatinib in children, and a safe dose has not determined.[6-9] Nickel et al.[6] described an adolescent patient who lost response to imatinib due to T315I mutation. The patient was started on ponatinib at 45 mg daily and achieved a CMR after four months of therapy. They reduced the dose to 15 mg daily to avoid toxicity. They concluded that ponatinib provided excellent disease control, but the patient had a decline in height velocity.[6] Yamamoto et al.[7] described a child with relapsed Philadelphia chromosome-positive ALL treated with ponatinib before HSCT. The patient received ponatinib monotherapy at

15 mg (16.8 mg/m^2 per day) and achieved a complete remission at day 20 after the initiation of ponatinib without any severe adverse event.[7] Millot et al.[8] presented ponatinib experience in childhood Philadelphia positive leukemias in 14 patients, at a dose range of 15-41 mg/m^2, median 27 mg/m^2, and a median duration of 2.5 months. They concluded that ponatinib might be an additional treatment option for children with Ph+ leukemias.[8] Rossoff et al.[9] reported 9 CML patients that were started on ponatinib with the median dose of 20 (range, 9–26) mg/m^2. They concluded that ponatinib was well tolerated, with no grade 4 or 5 adverse events.[9]

In the presented case, 15 mg/daily ponatinib, and induction chemotherapy for ALL achieved a remarkable response without any severe toxicity. Remission can be expected with conventional chemotherapy. However, ponatinib ensured CMR at the 3rd month of therapy which was accepted as the "add-on" benefit of ponatinib. Ponatinib therapy is effective and promising as a bridge to HSCT in children. However, the indications, dose, efficacy, and safety data are required.

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Case Report

Recurrent debilitating calf pain associated with fasciitis in Familial Mediterranean fever and response to canacinumab

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ABSTRACT

Background. Myalgia is one of the presenting symptoms of Familial Mediterranean Fever (FMF), which is reported at a rate of 27-39.6%. Recurrent fasciitis in those cases are extremely rare. We aimed to present a case with FMF having radiologically proven fasciitis.

Case. An 11-year-old male patient with a diagnosis of FMF and M694V homozygote mutation, using colchicine regularly at a dose of 2 mg/day (0.08 mg/kg/day) for 4 years; was admitted to the hospital with severe pain and tenderness on the right calf. There were no accompanying symptoms like fever or abdominal pain. He described three similar episodes with pain and tenderness in left and right calves, which were not accompanied by fever in the last 6 months. The erythrocyte sedimentation rate and C-reactive protein levels were high and serum creatinine kinase was normal. The day after initiation of non-steroidal anti-inflammatory drug (NSAID), his complaints regressed. However, after a week, he again had a severe calf pain. Lower extremity arterial and venous doppler ultrasonography was normal. Increased peripheral signal intensity and fasciitis around the soleus muscle was defined in MRI. With NSAIDs, myalgia disappeared in a few days and acute phase reactants decreased within a week. In the follow up, canakinumab was prescribed due to febrile attacks as frequent as once a month and calf pain observed almost weekly. Thereafter, both febrile attacks and recurrent debilitating calf pain were completely ceased.

Conclusion. There are three cases in the literature with fasciitis related myalgia. With this case, we wanted to emphasize fasciitis as a cause of FMF associated myalgia on MRI. In such cases MRI may be helpful to demonstrate fascial involvement.

Key words: Familial Mediaterranean Fever, fasciitis.

Familial Mediterranean Fever (FMF) is the most common monogenic auto-inflammatory disease in the world due to autosomal recessive mutation in MEFV gene located on the short arm of chromosome 16. Myalgia is defined as pain and/or tenderness in the extremities in the absence of joint swelling or signs of underlying osteomyelitis.[1] It is a well-known clinical manifestation of FMF, but it is not as common as arthralgia, fever, abdominal or chest pain. When directed questions are used, myalgia may be detected in up to 20-25% of the patients with FMF.[1,2]

There are four known patterns of myalgia associated with FMF: Spontaneous myalgia, exercise–induced myalgia, protracted febrile myalgia and colchicine-induced neuromyopathy.[1-4] Not all the patients with myalgia are evaluated with MRI or muscle biopsy. However, in some cases, fasciitis may be the underlying cause when evaluated radiographically. Furthermore, recurrent fasciitis in cases with FMF is extremely rarely

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described. Herein, we aimed to present a child with FMF who was radiologically diagnosed with fasciitis and reviewed the literature for previously reported cases with FMF and fasciitis.

Case Report

An 11-year-old male patient with a diagnosis of FMF and M694V homozygous mutation, using colchicine regularly at a dose of 2 mg/day (0.08 mg/kg/day) for 4 years was admitted with a debilitating pain and tenderness on the right calf leading him to use wheel chair. He denied any accompanying symptom like fever or abdominal pain. He described three similar episodes of pain and tenderness in his both calves, which were not accompanied by fever in the last 6 months. Tenderness was assessed with palpation on the right calf on physical examination. The laboratory findings were as follows; erythrocyte sedimentation rate (ESR): 44 mm/h (N: 0-20), C-reactive protein (CRP): 48.5 mg/L (N: 0-5), leukocytes: 9500 U/L (N: 4500-15000) (22.6% lymphocyte, 69.4% leukocyte), creatinine 0.6 mg/dl (N 0.5-1.2), sodium 139 mmol/L (N 134-150), potassium: 3.74 mmol/L (N: 3.5-5.5), calcium: 9.8 mg/dl (N: 8.8-10.8), phosphorus: 4.8 mg/dl (N: 4-7), magnesium: 2.5 mg/dl, total protein: 8.2 g/dl (N: 5.7-8.0), albumin: 4 g/dl (N: 3.5-5.2), creatinine kinase: 150 mg/dl (N: 0-171), lactate dehydrogenase: 207 U/L (N: 110-295), AST: 32 U/L (N:0-50), ALT: 20 U/L (N:0-50).

The day after initiation of non-steroidal anti-inflammatory drug (NSAID), his complaints regressed. In the control of acute phase reactants, CRP decreased to 11.7 mg/L (N: 0-5) and ESR decreased to 31 mm/h. However, after a week, he had a calf pain again. There were no pathological findings in lower extremity arterial or venous doppler ultrasonography. A thin fluid accumulation and a slight increase in peripheral signal intensity were detected in the soleus muscle in magnetic resonance imaging (MR) and was compatible with fasciitis (Figs. 1 and 2). Right lower extremity electromyography for muscle involvement was normal. The patient continued on non-steroidal anti-inflammatory therapy and colchicine dosage (2 mg/day) was not changed. Myalgia disappeared within a week. In the follow-up period, the patient had attacks of abdominal pain, fever, arthritis at least once a month and leg pain in both calves as frequent as once a week. As he had been accepted as resistant to colchicine, canakinumab was instituted. Since then, he neither had isolated

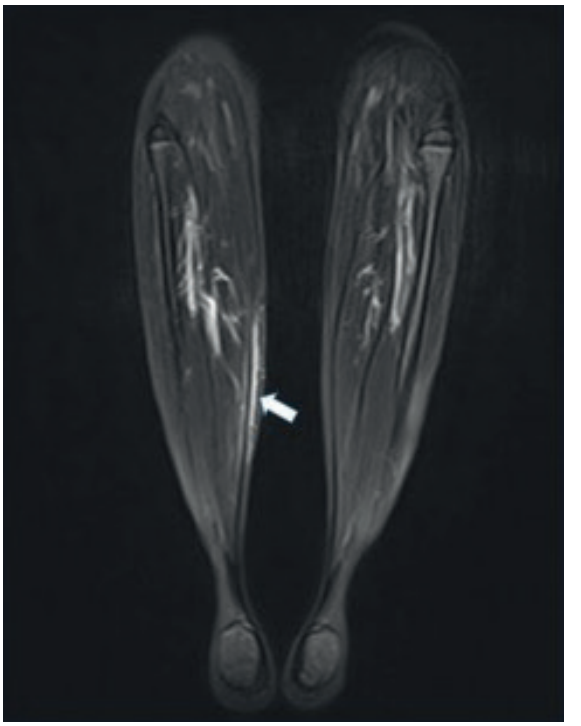

Fig. 1. A thin fluid accumulation along with the fascia of the soleus and a slight increase in peripheral signal intensity in the coronal sections of magnetic resonance imaging (MRI) (white arrow).

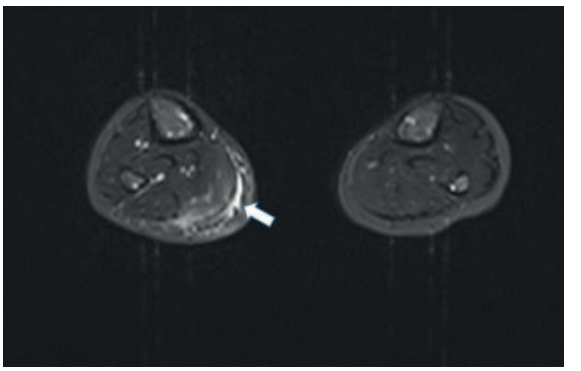

Fig. 2. The same findings in the tansverse sections of MRI (white arrow).

leg pain due to fasciitis nor any febrile attacks of FMF. An informed consent was received from the family.

Discussion

Fasciitis in autoimmune or auto inflammatory diseases have been reported in several cases. It is mostly seen in patients with tumor necrosis factor receptor-associated periodic syndrome (TRAPS).[5,6] In addition, patients with psoriatic arthritis, polyarteritis nodosa, eosinophilic fasciitis and inflammatory myopathy with abundant macrophages may have fasciitis.[4,7] In FMF, fasciitis has rarely been reported in a few cases, although myalgia is a well-known clinical component.

Abnormal muscular patterns in FMF are spontaneous myalgia, exercise-induced myalgia, protracted febrile myalgia syndrome (PFMS) and colchicine-induced neuromyopathy.[2,4] Spontaneous myalgia is defined as febrile myalgia not related to exercise or any other precipitating factor, which lasts for about 8 hours to 1 day. Fever is low grade and pain is mild to moderate. Exertional myalgia is the most common form, which lasts from hours to a few days, subsides with rest and occurs without fever or elevated levels of acute phase reactants.[1-4] The most severe form is PFMS, which is usually symmetrical lasting more than 5 days with normal muscle enzyme levels and elevated levels of inflammation markers (ESR 80 mm/h, CRP ≥5 mg/dl) in an FMF patient with fever (≥38°C) and at least M694V mutation in one allele. Muscle biopsy and electromyography (EMG) findings are usually normal. Response to NSAIDs or spontaneous recovery is unlikely, whereas a dramatic response to steroids is typical.[8] Colchicine-induced myopathy is characterized by proximal muscle weakness mostly without pain in the presence of peripheral neuropathy, elevated muscle enzymes leading to rhabdomyolysis, and myopathic changes and axonal polyneuropathy on EMG. Lysosomal vacuolar changes without inflammatory cell infiltration are observed in muscle cells.[9]

Patients diagnosed with FMF and recurrent calf pain have been reported[10], however, not all the patients with myalgia are evaluated with MRI or muscle biopsy on every occasion. There have been 3 previously reported cases with fasciitis and FMF (Table I).

Kotevoglu et al.[12] reported a 13-year-old girl with FMF presenting with fever and muscle pain in her right leg and knee. The patient had two similar episodes of muscle pain and maintained complete recovery after steroid treatment. MRI revealed non-specific edema of the subcutaneous fat tissue and increased signal intensity of the intermuscular septa and the distal lateral part of the medial gastrocnemius muscle. In addition, biopsy of the cutaneous and subcutaneous tissue with the fascia of the muscle revealed inflammatory infiltration with leukocytes, lymphocytes and eosinophils consistent with fasciitis. Although the patient maintained complete recovery after steroid treatment on the previous two episodes, she did not need steroids for the last attack as her symptoms resolved spontaneously. Her medical history was remarkable for attacks of abdominal pain and fever. She was found to have MEFV gene mutations and was diagnosed with FMF. She began to receive colchicine thereafter and suffered no attacks during the following two years. Muscle pain in her legs was thought to be associated with PFMS.

Fujikawa et al.[4] reported a 22-year-old Japanese man with fever and prolonged severe myalgia of the upper and lower extremities lasting for 3 weeks accompanied by elevated CRP and normal CK levels, suggestive of PFMS. Thickening of the fascia was observed on MRI. He responded quickly to prednisolone. Upon his medical history of recurrent fever, arthralgia, erythema, headaches, chest pain starting at 10 years of age and family history including a sister diagnosed with FMF, colchicine was instituted with the diagnosis of FMF. Genetic analysis revealed mutations in MEFV gene.

Umeda et al.[13] reported fasciitis in a 64-year-old Japanese woman. She had recurrent fever

Table I. Characteristics of patients with FMF and fasciitis.

	Case 1 (Ref no 11)	Case 3 (Ref no 4)	Case 2 (Ref no 12)	Our case
Age	13	22	64	11
Gender	F	M	F	M
Nationality	Turkish	Japanese	Japanese	Turkish
Fever	(+)	(+)	(+)	(-)
CRP/ESR	↑	↑	↑	↑
CPK	N	N	N	N
Localization of myalgia	Right calf	Upper and lower extremities	Left thigh	Left and right calves
Muscle biopsy	Leukocyte, lymphocyte and eosinophil infiltration	ND	Neutophilic infiltration both into the fascia and vacular endothelium of the fascia	ND
MRI findings	Diffuse edema of the subcutaneous fat tissue, increased signal intensity of the intermuscular septa and distal lateral part of the medial gastrocnemius muscle	Thickening of the fascia	High intensity in femoral muscle and fascia	Fasciitis around, and a slight increase in the signal intensity of the soleus muscle
Treatment for myalgia	Steroids / none	Steroids (dramatic response)	Unresponive to steroids Tocilizumab	NSAID
Recurrence	Under colchicine: None	None	Under colchicine: Yes Under tocilizumab: None	Under colchicine: Yes Under canakinumab: None
Number of myalgia attacks	3	First attack	Several undiagnosed attacks for the last 7 years	4
MEFV gene mutation	M694V/V726A	E148Q/ E148Q P369S-R408Q	E148Q/-	M694V/M694V

F: female, M: male, ND: not done, NSAID: non sterodid antiinflamatuar drug.

and myalgia attacks for the last 7 years in her medical history without a specific diagnosis. She also had localized pain on her left thigh at admission. She had normal levels of CK and high levels of CRP as seen in PFMS. MRI of the lower limbs obtained several times for limb pain revealed high intensity of muscles and fascia appearing in different sites at each attack. Muscle biopsy obtained from quadriceps muscle revealed neutrophilic infiltration into the fascia and vascular endothelium of the fascia compatible with fasciitis. She responded well to colchicine and was diagnosed with FMF using the Tel-Hashomer criteria. Although this case has similarities with the previous one, myalgia has been considered as "spontaneous myalgia". The patient responded well to tocilizumab treatment in the follow up.

The first case was thought to have PFMS. However, it is usually seen symmetrically in extremities and is rarely controlled without steroids in the early period. In addition, muscle biopsies are usually normal in PFMS.[8] The patient had a more localized pain in the right calf and fasciitis was established in the muscle

biopsy. The findings of the second case with fasciitis was compatible with PFMS. The third case with a localized pain on left thigh was considered as spontaneous myalgia, however, she had fever and high levels of CRP, which are not expected in spontaneous myalgia. Our case also had a localized pain on calves at different periods and myalgia episodes lasted only for a few days and resolved without steroid prescription. None of the cases had a history of exercise and colchicine induced myalgia was excluded due to normal CK levels. It seems possible to gather all these patients with myalgia and FMF under one title, as FMF associated fasciitis. In addition, as the findings of patient two were compatible with PFMS, looking for fasciitis with MRI in further cases in the acute phase would elucidate whether it is characteristic for PFMS as seen in TRAPS.

FMF attacks more frequent than one per month despite the maximum tolerable doses of colchicine has been frequently used to define colchicine resistance.[14] In such patients, anti-IL-1 biologic agents are the first treatment of choice.[14] Anakinra and canakinumab are the available anti-IL-1 agents in our country. We preferred canakinumab in our case and he had a complete response to febrile attacks and debilitating myalgia.

In conclusion, severe recurrent myalgia especially in calves or thighs, along with high levels of acute phase reactants and normal levels of CK in patients with FMF may emphasize a distinct form of myalgia marked by fasciitis, which does not require steroids. In such cases, MRI may be helpful and NSAIDs may be initially preferred before using steroids. Canakinumab may be useful for recurrent severe myalgia as well as frequent FMF attacks due to inadequate response to colchicine.

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Case Report

A mysterious case with abdominal pain and syndrome of inappropriate anti-diuretic hormone secretion

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ABSTRACT

Background. Acute intermittent porphyria (AIP) is a rare, hereditary, metabolic disease caused by a defect in heme biosynthesis. Hormonal changes may trigger porphyria attacks.

Case. Here we present a 17 -year- old adolescent refugee mother who applied to the pediatric emergency department with the complaint of diffuse abdominal pain at puerperium. The patient was hypertensive, and had convulsions after admission. Hyponatremia (serum sodium; 121 meq/L) was detected, and syndrome of inappropriate anti-diuretic hormone secretion (SIADH) was found to be the cause of hyponatremia which responded well to fluid restriction. Infectious, autoimmune and toxicologic laboratory work-up did not reveal any specific pathologies. Despite prompt utilization of analgesic treatment, the patient continued to have unbearable abdominal pain. The preference of prone position to relieve the pain and the family history of a mother who had died with similar symptoms, led us to the diagnosis of AIP. Genetic analysis showed a heterozygous mutation in hydroxymethylbilane synthase (HMBS) gene (c160+6T>A) which confirmed our diagnosis.

Conclusion. Acute porphyrias should be considered in differential diagnosis of abdominal pain, especially when there are accompanying symptoms like hyponatremia, seizures, mental changes and hypertension.

Key words: abdominal pain, acute intermittent porphyria, SIADH.

Acute porphyias are rare, metabolic diseases caused by the mutations of the genes involved in heme biosynthesis.[1] Acute intermittent porphyria (AIP) is inherited in an autosomal dominant fashion and is the most common acute porphyria type in Europe.[2] Certain drugs including oral contraceptives, hormonal changes, low carbohydrate intake are known precipitating factors for acute attacks. Vomiting and constipation accompanying abdominal pain may mimic acute abdomen in children.[3] Electrolyte imbalances, especially hyponatremia due to syndrome of inappropriate anti-diuretic hormone secretion (SIADH), may be seen during an attack.

Here, we present an adolescent refugee mother who was diagnosed with AIP. The porphyria attack manifested with abdominal pain, SIADH and hypertension at puerperium period.

Case Report

A seventeen -year- old Syrian refugee female patient applied to the pediatric emergency department with the complaints of vomiting and abdominal pain. The patient had given birth to her second child 35 days ago and she had constipation for more than a week. On physical examination, she was pale and looked like she was in pain. She had diffuse tenderness

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in her abdomen. Her blood pressure was 150/90 mmhg; the patient also had a tonic-clonic seizure when she was under observation.

In laboratory evaluation her serum sodium level was 121 meq/L; hypertonic saline infusion was administered due to the seizure. Her serum potassium level was 4.4 meq/L, chloride level was 83 meq/L, serum urea was 58 mg/dl, serum creatinine was 0.86 mg/dl and uric acid level was 7 mg/dl. Serum glucose level and complete blood count were normal. In urinary dipstick test microscopic hematuria and positive urobilinogen were detected. Abdominal ultrasonography did not reveal any specific pathologies.

The patient was hospitalized for further investigation. Oral amlodipine and enalapril treatments were started in a stepwise manner for hypertension. Cranial imaging was normal. Hypophyseal hormone levels and pituitary magnetic resonance imaging showed normal findings, therefore Sheehan syndrome due to post-partum hemorrhage was excluded. Blood osmolarity, urinary osmolarity were 278 mOsm/kg and 489 mOsm/kg, respectively. Urinary sodium concentration was >40 mEq/L and the patient was euvolemic. Volume restriction and salt replacement were performed with the diagnosis of SIADH. Despite normalization of serum sodium level, her severe abdominal pain persisted.

Amylase and lipase levels which were in normal range on admission (98 U/L and 4 U/L, respectively), rose on the tenth day of hospitalization(155U/Land41U/L,respectively). Repeated ultrasonographic evaluation was consistent with pancreatitis. However, magnetic resonance cholangiopancreatography was found to be normal except gallbladder sludge. Intravenous sulperazone treatment was started. During her follow-up, a decrease was observed in levels of amylase and lipase. Her abdominal pain had subsided as well. Due to the difficulties in communication and probably because of the postpartum depression, the follow-up was getting harder. We learned that the patient's mother had died at a young age with similar symptoms. During the second week of her hospitalization she had some psychiatric behaviors such as auditory hallucinations and she attempted suicide attempt by cutting her wrists with a sharp metal. The patient was immediately consulted to a pediatric psychiatrist. Escitalopram and risperidone treatments were started. Postpartum depression and hyponatremia (though her sodium level was 135 mEq/L at that time) was thought to be the cause of the psychiatric symptoms.

Her abdominal pain was increasing in intensity during her follow-up; paracetamol, nonsteroidal anti-inflammatory drugs and finally oral tramadol were used to relieve the pain. Toxicologic investigation for serum mercury, lead and cadmium levels were in normal range. Anti-nuclear antibody, anti-dsDNA and anti-neutrophil cytoplasmic antibody tests were normal. Clinical findings and laboratory investigations did not reveal any infectious etiologies.

Vomiting accompanying abdominal pain led us to stop giving anything peroral finally. Gastrointestinal endoscopy was performed to exclude gastric pathologies, it did not show any abnormalities. Oral ondansetron treatment partially decreased vomiting attacks. She did not tolerate oral nutrition; therefore total parenteral nutrition was started.

During an abdominal crisis the patient usually preferred prone position. She also had a family history of a mother dying at a young age with similar symptoms, suggesting a genetic disease. She had constipation and vomiting. The urine color of the patient was not red, but she had positive urobilinogen test in several urine analyses. Acute porphyria was the preliminary diagnosis.

The Watson-Schwartz test which is the screening test for acute porphyria was positive. To confirm the diagnosis serum and urine porphyrin levels were ordered. Urine total porphyrin level was 418 nmol/L (<35), erythrocyte protoporohyrin

was 0.6 μmol/L (0-1.4), total plasma porphyrin was 26.1 nmol/L (0-10). Genetic analysis showed a heterozygous mutation in HMBS gene (c160+6T>A).

Intravenous fluid containing 12.5% dextrose was administered. Her abdominal pain subsided. After a few days, 3 mg/kg/day intravenous hemin was administered for two days. The patient's abdominal pain relieved. Before discharge, the patient's serum sodium was 139 meq/L, creatinine was 0.97 mg/dl. Her blood pressure was normal, amlodipine and enalapril treatments were discontinued. Her psychiatric symptoms subsided totally.

Informed consent was received from the family.

Discussion

Acute Intermittent Porphyria is an autosomal dominant rare metabolic disease with an estimated prevalence of one in 75.000 in Europe.[1] Hydroxymethylbilane synthase (HMBS) mutations cause disorder in heme biosynthesis. Onset of symptoms may be delayed until adulthood. The most common presentation in children is abdominal pain which may mimic an acute abdomen and it may be associated with vomiting and constipation.[3] Electrolyte imbalances especially hyponatremia due to SIADH are seen. Gastric and renal losses may contribute to the development of hyponatremia. Our patient was a post-partum refugee girl, who had severe abdominal pain together with SIADH induced hyponatremia.

Hypertension is a common finding in patients manifested with AIP. In a population-based retrospective study, hypertension prevalence was shown to be 56% in AIP patients and porphyrin precursors were speculated to have a cytotoxic and/or vasospastic effect on renal arteries.[4] Autonomic dysfunction/ neuropathy contributes to the development of hypertension.[5] Our patient was hypertensive on admission, anti-hypertensive medication was started.

Although many of the patients remain asymptomatic, the attacks of AIP might be precipitated by several factors including drugs, alcohol, low calorie intake, hormonal changes in women.[6] A relation with the onset of porphyrias with birth and menstrual cycles were defined in the literature. Increased estrogen and progesterone levels may trigger acute porphyria attacks by increasing the porphyrin precursors via inducing the first enzyme in heme biosynthesis.[7] In a retrospective study of Andersson et al.[8], they showed that oral contraceptives caused attack in 24% of AIP patients. Our patient was healthy until the age of 17 and she had a two -year- old daughter. Her complaints started after the birth of her second child.

In a report from Belgium, a 22 -year- old female patient was admitted to the intensive care unit with severe hyponatremia and abdominal pain. Imaging of the abdomen was normal, she also had SIADH. The patient was in a premenstrual phase and she was using oral contraceptives. She was diagnosed with AIP.[9]

In AIP neurologic and psychiatric symptoms may be seen. The mechanism of neurological issues are not clear. However, the symptoms may result from increased neurotoxic porphyrin precursors. On admission, our patient had a seizure which probably resulted from hyponatremia. Without an interpreter's help we could not communicate with her. That caused difficulties in understanding the developing psychological issues, and finally she made a suicide attempt. She was started on escitalopram and risperidone treatment. In a case report from China, they presented a patient with SIADH who was then diagnosed with AIP, it was mentioned that the patient's mother had committed a suicide due to pain symptoms when her child was five years old.[10]

Singh et al.[11] reported a 35 -year- old male patient, who applied to hospital with complaints of altered mental status and vomiting. The patient had severe hyponatremia due to SIADH. Despite improvement in sodium level,

the patient had severe tonic-clonic seizures. Hemin was not available; the patient's attack was treated with hemodialysis.

A reddish color may develop in urine samples of acute porphyria patients due to the excess porphyrin precursors, especially when the urine is exposed to light. An abnormal color change was not noticed in our patient's urine, but urobilinogen was positive in her urinary analyses. The diagnosis was clinically made with the pattern of abdominal pain (she usually preferred the prone position) and togetherness of SIADH. SIADH development in acute porphyria may be associated with a vascular damage to hypothalamus due to high levels of circulating δ-aminolevulinic acid (ALA) and porphobilinogen (PBG). Also, persistent nausea and vomiting may contribute to SIADH.[9]

High levels of ALA, PBG and porphyrin are diagnostic. Treatment consists of intravenous high dextrose fluids and hemin. Our patient's abdominal pain partially subsided with dextrose infusion. After hemin was obtained and administered, there was a remarkable improvement in her pain and mood. Genetic analysis showed a heterozygous mutation in HMBS gene (c160+6T>A).

In conclusion, acute porphyrias must be considered in differential diagnosis of abdominal pain, especially when there are accompanying symptoms like hyponatremia, seizures, mental changes and hypertension. Early diagnosis and specific treatment with hemin are important to prevent complications.

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Case Report

Variant ataxia-telangiectasia in a child presenting with laryngeal dystonia

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ABSTRACT

Background. Dystonia is a common hyperkinetic movement disorder in children; however, making an early and definitive diagnosis of dystonia can sometimes be challenging for clinicians.

Case. Herein, we report a case of a 16 years-old girl presenting with laryngeal dystonia due to compound heterozygosity of a known pathogenic and a novel variant in the ATM gene. Serum alpha-fetoprotein level was elevated. Serum IgG, IgA, IgM and IgE levels were within normal range. Treatment with L-DOPA had no benefit. Her symptoms were dramatically improved by localized botulinum toxin injections.

Conclusion. Mutations in the ATM gene show a wide phenotypic spectrum from severe classical early-onset ataxia–telangiectasia (A-T) to late-onset milder variant A-T. Our findings highlight the importance of recognizing laryngeal dystonia as one of the clinical signs of A-T.

Key words: ataxia–telangiectasia, ATM gene, dysarthria, dystonia.

Dystonia is one of the most common hyperkinetic pediatric movement disorders. It is defined as involuntary, twisting movements and abnormal posture, caused by sustained or intermittent muscle contractions.[1] Clinical features of dystonia may be non-specific or atypical and can be diagnosed several years after the first symptoms manifest.[2] Dystonia prevents the acquisition of normal motor skills during critical periods of development in children and has negative impacts on activities of daily living and quality of life.[3]

Primary dystonia consists of a genetic heterogeneous group of disorders with onset of symptoms during childhood or adolescence. Advances in genetic techniques in the past years have widened the etiological spectrum of primary dystonia and other neurological conditions that can present as primary dystonia such as ataxia telangiectasia (A-T) and spinocerebellar ataxia type 3.[4-6]

The typical phenotype is childhood onset of focal dystonia, mainly in the lower limbs and more likely that can spread to multiple body parts over time.[7] Herein, we report a case of a 16 years-old girl presenting with laryngeal dystonia due to compound heterozygosity of a known pathogenic and a novel variant in the ATM gene.

Case Report

A 16-years-old female patient admitted with complaints of fatigability and dysarthria. She was the third child of non-consanguineous parents with no relevant medical familial history. She was born full term after a normal pregnancy. Her neuromotor development was normal until the age of 10 years when

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she developed fatigability and dysarthria. On physical examination, her weight, height, and head circumference were normal for her age. Neurologic examination was normal, except for dysarthria and facial weakness. There were no organomegaly and dysmorphic features.

Complete blood count, serum electrolytes, renal function, liver function, thyroid function tests and creatinine kinase level were normal. Cranial and cervical spine magnetic resonance imaging was normal. Myasthenia was suspected and further investigations were performed. Electromyography with repetitive nerve stimulation was normal. Acetylcholine receptor antibodies and MuSK antibodies were negative. Analysis of the CHRNE gene did not show a mutation.

For further genetic investigation of possible neurometabolic disease, TruSight Inherited Disease Sequencing Panel (Illumina Inc.) was used. She was found compound heterozygous for ATM variant (Fig. 1). The variant c.8147T>C (p.Val2716Ala) has been described[7], whereas nonsense variant c.7424T>G (p.Leu2475Ter) has not been described so far, but is predicted in silico to be pathogenic. The unaffected parents were heterozygous for the variants. Sanger sequencing in parents showed the biallelic origin of the two variants, with the mother harboring the c.8147T>C (p.Val2716Ala) variant, and the father harboring the c.7424T>G (p.Leu2475Ter) variant (Fig. 1).

Serum alpha-fetoprotein level was 65.32 ng/ml (reference range: 0–5.8). She had no clinical or laboratory signs of malignancy and abdominal ultrasound showed no abnormality. Serum IgG, IgA, IgM and IgE levels were within normal range. There was no history of recurrent sinopulmonary infections. At the age of eighteen, she developed oromandibular dystonia. Subsequently, treatment with L-DOPA was started at 4 mg/kg/day for a week and then the dose was increased to 8 mg/kg/day. Treatment with L-DOPA had no benefit. Oromandibular dystonia was dramatically improved by localized botulinum toxin injections into the genioglossus, mentalis, corrugator supercilii muscles with injection doses of 5 units and into the lateral pterygoid muscles with injection doses of 10 units. A written informed consent was obtained from the parents of the patient.

Discussion

We identified a variant form of A-T presented with laryngeal dystonia. A-T, due to mutations in the ATM gene, is a recessively inherited multisystem disorder. It is characterized by oculocutaneous telangiectasia, progressive cerebellar ataxia, choreoathetosis, recurrent sinopulmonary infections and an increased risk for cancer.[8,9]

Mutations in the ATM gene show a wide phenotypic spectrum from severe classical early-onset A-T to late-onset milder variant A-T. Clinical severity generally depends on the presence of ATM protein and kinase activity.[10] In variant form of A-T, movement disorders appear to dominate the clinical presentation. Atypical clinical manifestations including late-onset focal or generalized dystonia, resting tremor and myoclonus have been described.[11-13]

To date, the pathophysiology of dystonia in variant A-T has not been well understood. Eilam et al.[14] showed selective loss of dopaminergic nigrostriatal neurons in ATM-deficient mice and suggested that ATM deficiency could severely affect dopaminergic neurons in the central nervous system. Koepp et al.[15] showed a decreased tracer uptake in the striatum bilaterally in a 6-year-old girl with A-T and severe progressive dystonia.

The ATM gene plays a central role in DNA damage responses and patients with A-T demonstrate increased sensitivity to ionizing radiation. Even though cases of variant A-T have much milder neurological manifestations than in classic A-T, patients still have increased risk of malignancies.[12] It is important to provide counseling and monitoring for neoplasms and to take measures in order to lessen accumulating DNA damage from radiologic exposure and chemotherapeutic agents.

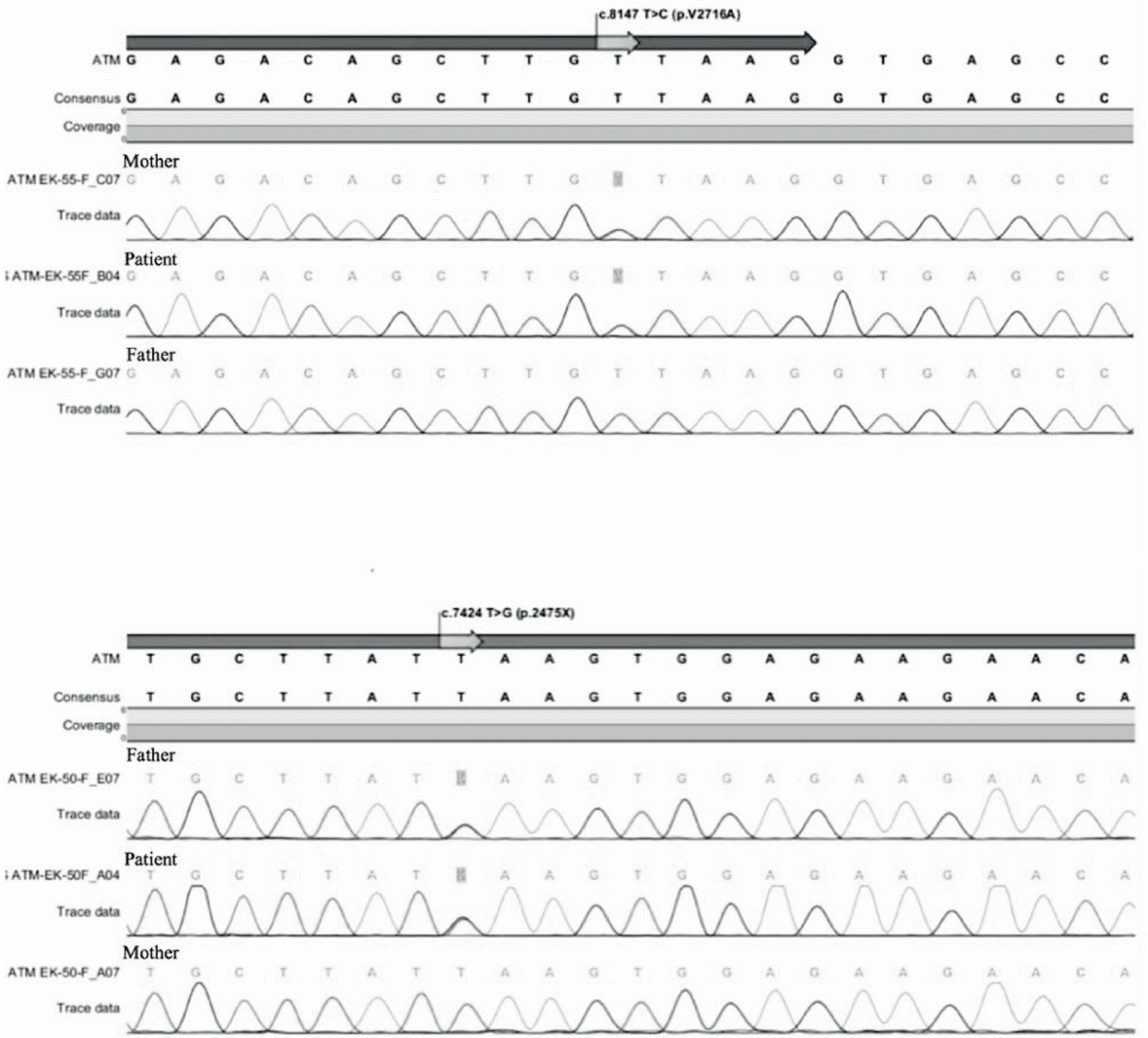

Fig. 1. Sanger sequencing in patient showed compound heterozygous variants, with the mother harboring the c.8147T>C (p.Val2716Ala) variant, and the father harboring the c.7424T>G (p.Leu2475Ter) variant.

In patients with dystonia due to ATM mutations, treatment is challenging and efficacy is typically incomplete. Botulinum toxin, levodopa, trihexyphenidyl, baclofen and clonazepam are considered the treatment of choice.[12,16] There are no specific studies for the treatment of movement disorders in A-T. In most cases, treatment is symptomatic and designed to reduce symptoms and improve the quality of life. Consequently, the optimal management of patients remains unclear. We observed dramatic improvement with localized botulinum toxin injections in our patient.

Clinicians should be aware of variant A-T when investigating dystonia with unknown etiology. Elevated serum alpha-fetoprotein level can be a low-cost useful screening tool. It is important to recognize variant form of A-T, as patients can avoid radiation exposures unnecessarily, as well as symptoms can improve with treatment. Therefore, A-T should be considered in a case of laryngeal dystonia, even without ataxia or telangiectasia.

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Case Report

A new location for pediatric immunoglobulin G4 related disease: the biceps muscle

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ABSTRACT

Background. Immunoglobulin G4 (IgG4)-related disease (IgG4-RD) is a systemic disorder of unknown etiology characterized by elevated serum IgG4 and tissue infiltration of IgG4-positive plasma cells. The disease was described in the pancreas, aorta, thyroid, salivary glands, periorbital tissues, kidneys, pericardium and lymph nodes.

Case. Here in, we report a first pediatric case report of IgG4-related disease who presented with a mass in skeletal muscle i.e., biceps muscle.

Conclusion. To the best of our knowledge, the involvement in skeletal muscle has previously not been reported in children.

Key words: immunoglobulin G4, immunoglobulin G4-related disease, biceps muscle.

Immunoglobulin G4 (IgG4)-related disease (IgG4-RD) is a systemic disorder of unknown etiology characterized by elevated serum IgG4 and tissue infiltration of IgG4-positive plasma cells.[1] IgG4 positive plasma cell infiltrations were first recognized in the pancreas. Until 2003, these abnormalities were not viewed as a distinct condition.[2-5] Later on, the abnormalities related to the disease were described in other organs and tissues like aorta, thyroid, salivary glands, periorbital tissues, kidneys, pericardium and lymph nodes.[2,3] But the involvement in skeletal muscle has not been reported.

To the best of our knowledge, this is the first pediatric case report of IgG4-related disease who presented with a mass in skeletal muscle i.e., biceps muscle.

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Case Report

A 14-year-old girl presented with swelling in the upper arm. Her physical examination indicated an approximately 6x7 cm swelling in the right upper arm. Other system examinations were unremarkable. She had no other complaints. On admission, blood laboratory tests revealed that white blood cell count was 23 500/mm^3, hemoglobin 8,7 g/dl, platelets 878 000/mm^3, erythrocyte sedimentation rate was 130 mm/h, C-reactive protein 124,6 mg/dl (normal <20 mg/dl). Liver and renal function tests, muscle enzymes, C3 and C4 were normal. Anti-nuclear antibody and anti-neutrophil cytoplasmic antibodies were negative. Serum IgG4 concentration was 606 mg/dl (normal <135 mg/dl). Lung x-ray and abdominal ultrasonography were reported as normal. Contrast-enhanced magnetic resonance imaging of the right upper arm revealed a well-defined 62x48x50-mm contrast-retained solid mass with septa in the biceps muscle in T1–weighted series (Fig. 1). Bone marrow aspiration was performed for possible malignancy exclusion, which was

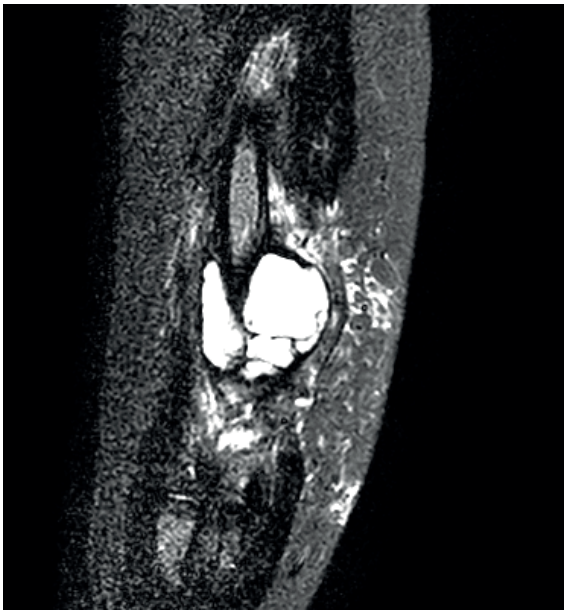

Fig. 1. Magnetic resonance imaging of right biceps muscle showed a well defined 62x48x50-mm solid mass.

unremarkable. Then the mass was surgically dissected. The result biopsy of the mass showed intensive lymphoplasmocytic infiltration, enriched with IgG4-positive plasma cells (with an IgG4/IgG ratio >40%), storiform fibrosis, and obliterative phlebitis (Fig. 2). There was no evidence of malignancy and granulomatous inflammation. In view of this, a diagnosis of 'IgG4-related disease' was rendered in this case. She was treated with prednisolone 2 milligram (mg) per kilogram (kg) per day per oral for a month. Then prednisolone therapy was slowly tapered. The mass shrunk to the level of being unpalpable. Acute phase reactants became within normal range. After follow up of 4 months, when prednisolone was tapered, her symptoms re-activated and the mass regrew. Then we started mycophenolate mofetil (MMF) 1200 mg per square meters per day as an additional therapy to prednisolone but she responded poorly to the three-month MMF treatment. At follow up, three months later, rituximab was added to treatment and we observed a significant decrease the in the mass

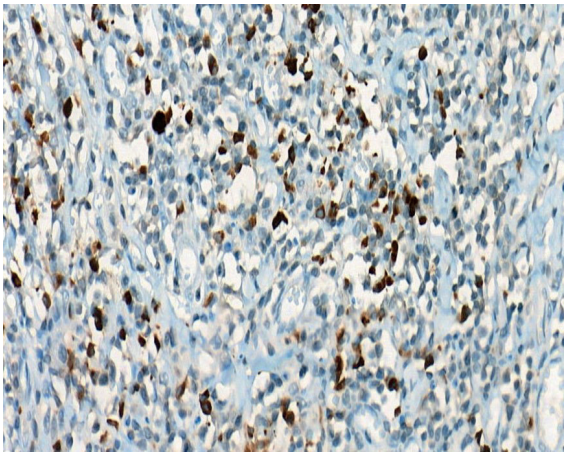

Fig. 2. IgG4 stain positive revealed an increased IgG4 plasma cell count.

volume (down to 2x2 cm) and prednisolone was stopped. She was successfully treated with rituximab for four months with resolution of symptoms.

Informed consent was received from the family.

Discussion

IgG4-RD is rare autoimmune disease condition. The exact etiology of IgG4-RD is unknown. Patients with IgG4-RD often present with tumor-like swelling. The three diagnostic criteria of Umehara are applicable to its diagnosis.[1] A diagnosis of probable IgG4-RD requires the presence of a tumor-like swelling (clinical/radiological examination showing characteristic diffuse or localized swelling or masses in single or multiple organs) and one of the other criteria. The other criteria: Hematological examination showing elevated serum IgG4 concentrations (> 135 mg/dl) and histopathological examination showing marked lymphocyte and plasmocyte infiltration, storiform fibrosis, and infiltration of IgG4+ plasma cells with a ratio of IgG4+/IgG+ plasma cells ≥ 40%, and a total of ≥ 10 IgG4+ plasma cells/ high power field (HPF). The presence of all three criteria indicates definite IgG4-RD.[6,7] The majority of patients have high serumIgG4

levels. But 30% of patients have normal serum IgG4 although typical histopathological and immunohistochemical findings.[6] It should be noted that IgG4+ plasma cells may also be seen in diseases such as Castleman disease, eosinophilic granulomatosis with polyangiitis, sarcoidosis, inflammatory bowel disease and lymphoma but in these diseases, storiform fibrosis and obliterative phlebitis are not seen. This is especially important in the differential diagnosis. IgG4-RD can affect many organs and systems, commonly pancreas and other retroperitoneal organs. Various organ involvements have been described such as salivary glands, periorbital tissues, lymph nodes, biliary tree, kidneys, lungs, meninges, prostate, thyroid, pericardium, and skin.[7,8] It is required for the differential diagnosis of many diseases in the diagnosis process. It is important to distinguish the disease from a variety of disorders, especially including neoplastic and inflammatory conditions.

Glucocorticoids are the most widely recommended agent at the first line of therapy. Usually the response is good. But the frequency of relapse is high with 25 to 50 %.[9,10] Besides, immunosuppressive therapy such as azathioprine or MMF are often require in many cases. In cases either recurrent or resistant disease, rituximab or bortezomib treatment is also recommended.[11,12]

In conclusion, IgG4-RD is rare disease. The biceps muscle is a clinically rare region for IgG4-RD. To the best of our knowledge this is the first pediatric case of IgG4-RD with upper arm muscle involvement. This case suggests that IgG4-RD can be consistent across a wide range of organ systems including skeletal muscles. If a patient presents with an tumoral formation in any organ and pathology cannot demonstrate malignancy, IgG4-RD should be considered.

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Case Report

Low function of natural killer cells in treated classic Menkes disease

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ABSTRACT

Background. Menkes disease (MD) is a rare lethal X-linked, multisystem disorder of copper metabolism resulting from mutations in the ATP7A gene. Features such as Ehlers- Danlos syndrome, trichopoliodystrophy, urologic and skeletal changes have been reported. We present a case of classic MD treated with copper infusions who suffered from persistent natural killer (NK) cell dysfunction.

Case. A 2-year-old, Caucasian male child presented at 8-month-old of age with persistent hypotonia, kinky hair and developmental regression. Diagnosis of MD was based on low serum levels of copper [5 mg/dl (18-37)] and ceruloplasmin [18 ug/dl (75-153)] and gene-targeted deletion/duplication analysis performed by the reference laboratory. Brain MRI showed mild hypoplasia of the cerebellar vermis and vascular tortuosity typical of MD. Copper chloride treatment was immediately initiated. The child became more alert with excellent eye contact and purposeful movements. The child was hospitalized for recurrent respiratory infections, each time caused by enterovirus as confirmed by multiplex polymerase chain reaction (PCR). Extensive immunologic studies were negative, except for a severe NK cell dysfunction on multiple occasions (0.6 NK lytic Units; N >2.6).

Conclusion. We postulate that NK cell dysfunction in a classic MD can be explained by the deficient incorporation of copper in the endoplasmic reticulum resulting in an abnormal Fenton chemistry within phagosomes.

Key words: Menkes disease, copper, natural killer cell, recurrent infection.

Classic Menkes disease (MD) is a rare lethal X-linked multisystem disorder of copper metabolism caused by mutations in the ATP7A gene and leading to low serum copper and ceruloplasmin levels. The ATP7A gene encodes a transmembrane copper-transporting P-type ATPase (MNK) localized to the trans-Golgi network (TGN). MNK protein is essential for systemic copper absorption and provides copper to secretory cuproenzymes traversing the TGN.[1] Parenteral copper administration is successful in normalizing copper and ceruloplasmin levels. It also improves the muscle tone and motor activity and most importantly prevents seizures as long as some MNK activity is preserved.[2] However, the primary clinical manifestations such as peculiar kinky hair (trichopoliodystrophy), hypopigmentation, connective tissue disturbances (Ehler Danlos syndrome) are frequently not improved by parenteral copper supplementation.[3-5]

Susceptibility of children with MD to recurrent infection is well documented and thought to be due to hematological consequences of copper deficiency and defective immune functioning due to copper deficiency. We present a male child with classic MD, who manifested with recurrent viral infections due to a proven natural killer (NK) cell dysfunction, which occurred despite continued parenteral copper supplementation. To our knowledge, such a feature has never been reported earlier in patients with MD.

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Case Report

A 3-year-old, Caucasian male child presented at 8-month-old of age with irritability, hypotonia, kinky hair and developmental regression. He was diagnosed with MD based on low serum levels of copper {5 mg/dl (18-37)} and ceruloplasmin {18 ug/dl (75-153)} and Pilli Torti on light microscopy. Gene-targeted deletion/duplication analysis performed by the reference laboratory (Dr. Stephen Kaler Laboratory) revealed a novel deletion of 363 bases involving the 3'end of ATP7A exon 12 (encoding the final 4 amino acids) plus a segment of intron 12 (c.2617_2626+342del)(p.Leu873_Gly876del). Brain MRI showed mild hypoplasia of the cerebellar vermis and vascular tortuosity typical of MD. Parenteral copper chloride treatment was immediately initiated. Following the initiation of copper treatment, the child became less irritable and more alert with excellent eye contact and purposeful movements. Peculiar hair, hypopigmentation, and connective tissue disturbances failed to improve.

The patient also required several in-patient admissions since the initiation of copper treatment. Recurrent viral upper respiratory tract infections were the main reasons for most of these admissions, each time caused by enterovirus as confirmed by multiplex polymerase chain reaction (PCR) testing on the nasopharyngeal secretions. In addition, he was also admitted for multiple episodes of fever of unknown origin. Extensive immunologic studies including serum immunoglobulin levels were negative.

On two occasions, peripheral blood was obtained to measure plasma copper levels and serum ceruloplasmin levels. Both times, NK quantification and measurements of Natural Killer (NK) activity were performed.[6] The first time at 13 months, plasma copper level was borderline low (64 mcg/dl; N: 75-153) while serum ceruloplasmin level was normal (20 mg/dl; N: 18-37). The second time at 23 months, both copper (76 mcg/dl; N: 75-153) and ceruloplasmin (19 mg/dl; N: 18-37) were normal. On both occasions, flow cytometry showed that the percentage of circulating CD16+CD56+ NK cells was normal (3%; N: 3-16) and NK cell function was decreased (0.6 NK lytic Units; N >2.6) (Table I). This finding of NK cell dysfunction has not been reported previously in MD. For reporting this case, informed consent was obtained from the mother of the patient.

Discussion

MD is a progressive disorder with varying severity of the clinical course. Severe forms of MD usually result in early childhood death. Either intravenous copper sulfate administration or subcutaneous administration of copper histidine, if initiated early, can successfully modify the disease progression and improve long-term clinical outcomes.[2] Though copper supplementation, intracellular cytoplasmic, nuclear and mitochondrial

Table I. Serial NK cell activity, plasma copper and ceruloplasmin levels.

Variables	Age 13 months	Age 23 months	Reference range
Plasma copper (mcg/dl)	64	76	75-153
Serum ceruloplasmin (mg/dl)	20	19	18-37
CD16+ CD56+ NK cells (%)	3	3	3-16
50:1 E:T Ratio (% lysis)	7	7	≥ 20
25:1 E:T Ratio (% lysis)	3	3	≥ 10
12.5:1 E:T Ratio (% lysis)	2	2	≥ 5
6.25:1 E:T Ratio (% lysis)	1	1	≥ 1
NK Lytic units	0.6	0.6	> 2.6

NK: natural killer cell, E: T ratio: Effector-to-target ratio.

copper levels are improved, but still, the trans face of the Golgi complex with secretory functions remain deficient in copper. Thus, functions of cuproenzymes in cytoplasm [Superoxide Dismutase 1(SOD), laccase], nucleus (metal-regulatory transcription factor 1) and mitochondria (cytochrome oxidase, SOD1/2) improve, while secretory enzymes (dopamine beta-hydroxylase, tyrosinase, peptidyl-alpha amidating enzyme, diamine oxidase, monoamine oxidase, lysyl oxidase, and SOD3) remain deficient[7], which could explain the persistent clinical manifestation despite normalization of copper and ceruloplasmin levels through parenteral copper supplementation.

Susceptibility to infections, including pulmonary and urinary tract infections and septicemia, has been reported in MD. Underlying systemic copper deficiency in MD and resultant neutropenia and humoral immunodeficiency are most probably the reason for increased susceptibility to infections in untreated patients.[7,8] Our index case was hospitalized several times after normalization of copper and ceruloplasmin levels. Most of these admissions included respiratory tract infections. Further evaluations confirmed rhino-enterovirus through PCR as the cause of these respiratory infections. Extensive immunologic studies were negative, except for a severe NK cell dysfunction (0.6 NK lytic Units; N >2.6). This finding of NK cell dysfunction has been unreported previously in human or animal models of MD. Previously, animal studies have shown that ATP7A copper transporter is required for macrophage-mediated killing of infectious organisms by enabling the transport of copper from Golgi complex to cytoplasmic vesicles.[9] It has also been postulated that phagosomal copper catalyzes the production of hydroxyl radicals from hydrogen peroxide via Fenton-like chemistry. We speculate that NK cell dysfunction in classic MD can be explained by the deficient incorporation of copper in trans face of the Golgi complex leading to a reduced Fenton chemistry within phagosomes.[10] NK cell dysfunction in classic MD has never been reported. We speculate that reduced NK cell function was the underlying mechanism for susceptibility to recurrent infections in the index case. We consider that NK cell dysfunction in classic MD can be explained by the deficient incorporation of copper in trans face of the Golgi complex.[10] Therefore, evaluation of NK cell function should be considered in patients with classic MD.

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Case Report

Pyloroduodenal intussusception due to diffuse juvenile polyposis in a 3 year-old child: case report

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ABSTRACT

Background. Pyloroduodenal intussusception (PDI) due to gastric and pyloric polyps is very rare and has not been reported previously in children.

Case. A 3 year-old boy was admitted with non-bilious vomiting and abdominal distention. Abdominal X-ray showed gastric air-fluid level and ultrasonography showed 5 cm intussusception at right upper quadrant. Upper gastrointestinal study showed gastric outlet obstruction. Multiple polyps at stomach and pylorus were detected in endoscopy. The explorative laparotomy revealed polyps originating from pylorus passing to duodenum and causing PDI. The polyps were excised to reduce the intussusception via duodenotomy.

Conclusion. PDI and pyloric polyps should be kept in mind in cases with radiological examinations revealing gastric outlet obstruction.

Key words: intussusceptions, pylorus, gastric outlet obstruction, polyps.

Juvenile polyps (JP) are epithelial or submucosal growths that protrude into the lumen of the bowel. They may cause bleeding, abdominal pain and intussusception. JP account 80% of all gastrointestinal polyps and the proximal colon is the most common localization.[1] Children with diffuse juvenile polyposis are usually 6 months to 5 years of age and present with rectal bleeding, prolapsus, protein-losing enteropathy, intussusception and malnutrition.[2] Polyps extrude into the bowel lumen may be propelled distally by peristalsis and may lead to intussusception. Gastrointestinal (GI) polyps account for 4% of all intussusceptions as a leading point in children.[3] Pyloroduodenal intussusception (PDI) due to gastrointestinal tumors was reported in adults and has not been reported in children previously.

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The case was presented at National Congress of Pediatric Surgery Associations in 25-28 October 2017, Edirne, Turkey.

Intussusception can be idiopathic (without leading point), due to a leading point or can occur postoperatively. The incidence of intussusception caused by a leading point in an infant or a child ranges from 1.5% to 12%.[4] Intestinal polyps are the most common cause of a leading point after Meckel's diverticulum. When classified into anatomic types, ileocolic intussusception is the most common (85%). PDI is not reported and/or classified as 'others'. We aimed to present the first case of PDI in a 3 year-old children to discuss the clinical features and treatment options for this rare type of gastric outlet obstruction.

Case Report

The informed consent prior to the inclusion of the case was obtained from the family of the patient. A 3-year-old boy with allogeneic hematopoietic stem cell transplantation due to severe combined immune deficiency (SCID) and adenosine-deaminase deficiency was admitted with non-bilious vomiting and abdominal distention. In his past medical history, he underwent both upper and lower

GI endoscopy because of bloody stool and was diagnosed with diffuse juvenile polyposis. He had protein-losing enteropathy and received intravenous immunoglobulin G, albumin and enzyme replacement treatment.

At admission, his vital signs were within normal limits. Complete blood count findings showed hemoglobin 9.8 g/dl, neutrophils $8x10^3$ mm^3, and platelet count was $95x10^3$ mm. Liver and renal function tests were normal. The physical examination revealed epigastric distention and a palpable mass at epigastrium. Ultrasonography of abdomen showed 5 cm intussusception at right upper quadrant (Fig. 1). Upper GI study confirmed gastric outlet obstruction (Fig. 1). The patient was diagnosed with PDI and surgical treatment was suggested to reduce intussusception. Before explorative laparotomy, upper GI endoscopy was performed under anesthesia and multiple polyps originating from the stomach and pylorus were detected. The pyloric orifice was displaced and obstructed with polyps (Fig. 2). It was not possible to pass the endoscope through pylorus and examine the duodenum because of protruding polyps into the duodenum. Surgical exploration via right transverse incision revealed polyps originating from pylorus passing to duodenum and causing PDI (Fig. 3). After transverse duodenotomy, polyps were excised and intussusception was reduced (Fig. 3). Since the gastrointestinal passage was obtained after polyp excision, there was no need for gastroduodenostomy. The postoperative period was uneventful and the histopathological evaluation of excised polyps confirmed diffuse juvenile polyposis of childhood. After the surgical treatment, the patient was followed-up for two years free of symptoms but died due to acute pulmonary thromboembolism at 5 years-of age.

Discussion

Diffuse JP of infancy present with multiple polyps in the entire GI tract in which stomach, distal colon and rectum are the most common localizations. Gastric polyposis protruding to pylorus and duodenum is seldom rare and reported in adults as a cause of gastroduodenal intussusceptions.[5] Herein, we report the first pediatric case of PDI due to diffuse JP.

Gastroduodenal or pyloroduodenal polyps may be part of polyposis syndrome or due to prolonged use of proton pump inhibitors.[6] Different from polyps, gastrointestinal tumors

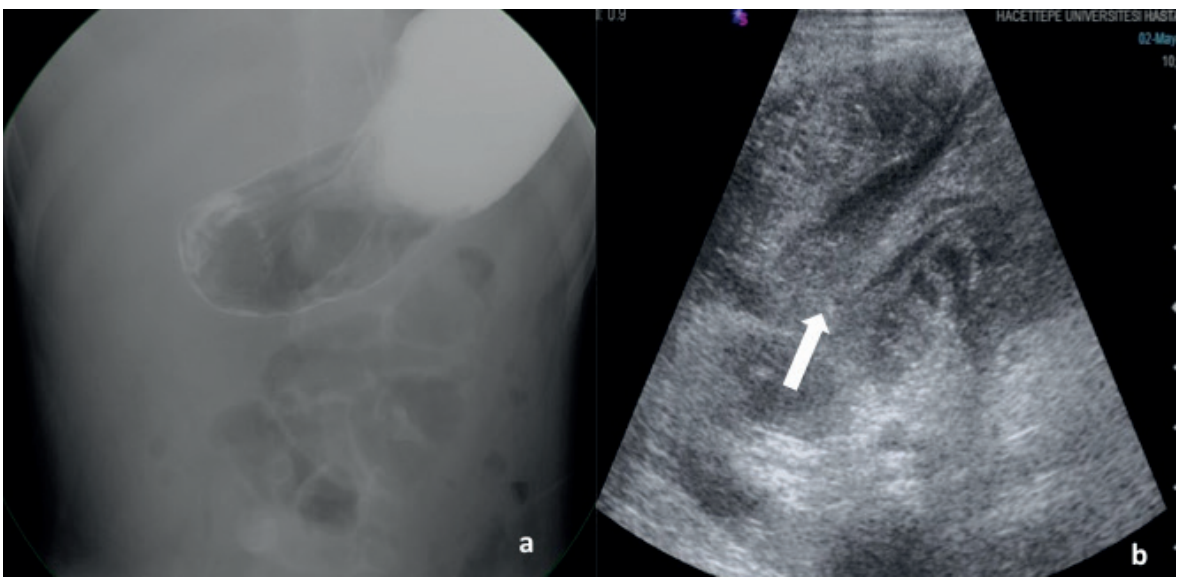

Fig. 1. Upper GI contrast graph showed gastric outlet obstruction (a). The ultrasonography showed target sign (arrow) and confirmed the diagnosis of PDI (b).

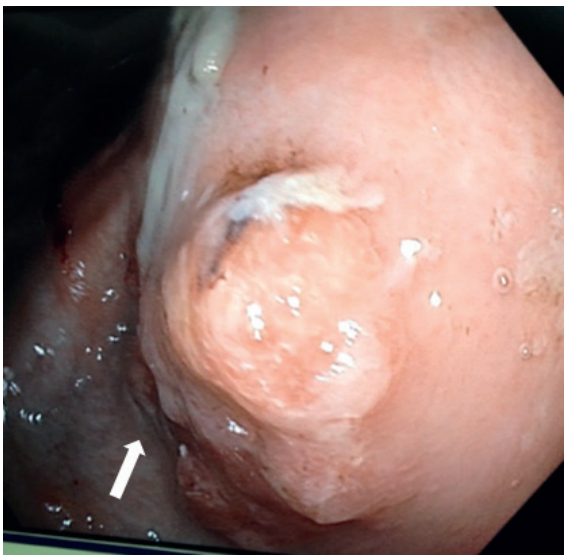

Fig. 2. The upper GI endoscopy showed gastric polyps. The pyloric orifice (arrow) was displaced and obstructed by polyps.

are a more common cause of gastroduodenal intussusception in adults and accounts for 10% of all adulthood intussusceptions.[7] PDI is extremely rare because gastric cardia, pylorus and duodenum are much more secure and less mobile than the small and large bowel. Despite other intussusceptions, no idiopathic occurrence was reported for PDI and almost all of the cases had a leading point including tumors and polyps. Similar to adult cases, diffuse JP involving the pylorus was the cause of PDI in our patient. Although, no causative relation has been defined between adenosine deamianse deficiency and gastrointestinal polyposis, intercellular deposition of toxins due to elevated adenosine and 2'-deoxyadenosine can be suggested as a cause for diffuse JP in our patient.

PDI and gastroduodenal intussusceptions presents with non-bilious vomiting and gastric outlet obstruction.[6] In addition, obstruction of pancreatic exocrine enzymes and bile due to displacement of surrounding tissues can be seen. The onset of symptoms is usually variable and a majority of patient's present acutely.[7] Episodic abdominal pain can be seen if the history is longstanding. Also, spontaneous reduction and recurrence of intussusception presents with cramped abdominal pain.

The diagnosis of PDI can be obtained by ultrasonography and typical target sign on the involved site reveals intussusception. Although contrast enema is both diagnostic and therapeutic in ileocolic and colonic intussusceptions, PDI requires upper GI contrast studies for diagnosis. The endoscopy of upper GI can be done not only for diagnosis but also for sampling of polyps for histopathological evaluations. In the current case, we confirmed the gastric outlet obstruction by contrast GI study. Additionally, it was not possible to pass the endoscope into the duodenum and the gastric outlet was fully obstructed by pyloric polyps. Computed tomography (CT) was used

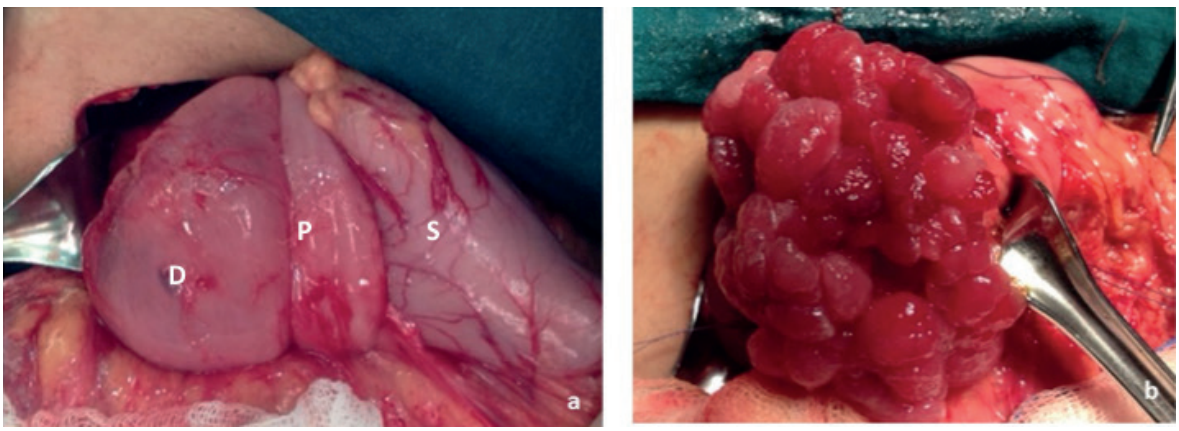

Fig. 3. The gross appearance of PDI (a). The surgical reduction of PDI and excision of polyps (b). (D: duodenum, P: pylorus, S: stomach).

as the most reliable tool for diagnosis of PDI in adults.[7] It is valid if tumor is suggested as a leading point. However, other radiologic interventions are adequate for diagnosis.

In the treatment of PDI, manual reduction of intussusception and surgical excision of polyps is recommended. In diffuse JP, multiple polyps obstructing the gastric outlet can be excised by duodenotomy or gastrotomy. We preferred to excised the polyps by incising the duodenum and exclude the other obstructing polyps in the distal duodenal segments. After excising all pyloric polyps, gastroduodenal passage was controlled and obstruction in the passage was eliminated. Gastroduodenostomy can be reserved for the patients, in case of inadequate polyp excision or if a clear gastroduodenal passage is not provided.

The outcome of our patient was eventful two years after the surgical treatment and follow-up endoscopies reveal no novel polyps in the stomach and pylorus. However, the patient died of pulmonary thromboembolism as a complication of SCID and adenosine deaminase deficiency.

In conclusion, PDI should be kept in mind as a cause of gastric outlet obstruction in patients who are admitted with non-bilious vomiting and abdominal distention. Infants with diffuse JP especially involving gastric antrum and pylorus may cause PDI and gastric outlet obstruction. Although surgical excision of the polyps is adequate in the treatment, gastroduodenostomy should be kept in mind if total excision of polyps is not possible.

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Case Report

Iatrogenic nasal synechiae in a premature newborn

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ABSTRACT

Background. Nasal obstruction may cause short- and long-term problems such as respiratory distress, cyanosis, apnea, difficulty during feeding, and failure to thrive during the newborn period; since newborns are obligatory nasal breathers. Compression effect of the nasal cannulas and prongs used during respiratory support and nasal aspiration applications to clear the airways of secretions may result in nasal synechiae and acquired iatrogenic nasal obstruction.

Case. In this case report, we present a premature newborn with nasal synechiae secondary to long-term nasal continuous positive airway pressure (nasal CPAP) applications and routine upper airway nursing care.

Conclusion. Severe nasal damage may occur in premature newborns receiving prolonged nasal CPAP support. To prevent this upper airway care should be conducted as gently as possible in premature newborns.

Key words: neonatology, nasal obstruction.

Newborn babies are obligatory nasal breathers. Short- and long-term problems such as respiratory distress, cyanosis, apnea, difficulty during feeding, and failure to thrive may be seen in patients with nasal obstruction.[1-3] Etiology of nasal obstruction in neonates involve congenital and acquired causes. Congenital anomalies include nasal agenesis, choanal stenosis/atresia, craniofacial anomalies, septal deviations and congenital masses. Acquired causes include obstructions due to birth traumas, infectious rhinitis, scar formation due to compression effect of nasal cannulas and prongs used during respiratory support, and nasal synechiae secondary to nasal aspirations to clear secretions.[1-5] In this case report, a premature newborn with nasal synechiae secondary to long-term nasal continuous positive airway pressure (nasal CPAP) applications and routine upper airway nursing care is presented.

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Case Report

A female neonate weighing 1160 grams (10–50p) was born via emergent cesarean section as the second baby from a twin-gestation of 30+5 weeks to a 40-year-old primipara mother because of clinically confirmed acute appendicitis in the mother and documented decelerations upon non-stress test examination. The baby was intubated and admitted to the newborn intensive care unit.

Prenatal history of the mother revealed primary hypertension, type 2 diabetes mellitus regulated with insulin therapy, Familial Mediterranean Fever regulated with colchicine therapy, sickle cell anemia trait, gluten-induced enteropathy and multinodular goiter. Fetal DNA analysis and antenatal ultrasonographic examinations were normal. There was no consanguinity between mother and father.

Starting from first days of life, thin nasal suction catheters did not progress easily through both nasal passages. Because bilateral choanal patency was revealed immediately after birth, findings were evaluated as an acquired stenosis, and nasal examination was planned.

Three doses of intratracheal surfactant therapy were introduced within the first 48 hours of life. Grade 3 intraventricular hemorrhage was detected during routine cranial ultrasonography on the 3rd day of life. Surgical ligation was performed due to hemodynamically significant and pharmacologically non-reactive patent ductus arteriosus on 25th day of life. Intubated mechanical ventilation was introduced for a total of 27 days. Extubation was successful on 27th day of life, and respiratory support was continued as nasal CPAP for a total of 35 days. This support was interchanged with nasal masks and bi-nasal prongs. On 35th and 58th days of life, two courses of dexamethasone therapy (0.5 mg/kg/day, 3 days) were given for bronchopulmonary dysplasia. Vitamin A treatment (5000 IU/dose, 3 doses/week, orally) was given starting from 35th day of life for a total of 3 weeks. Appropriate continuous moistening of the airway with humidifiers was introduced throughout the respiratory support at all times. Secretions were effectively cleared from the upper airways whenever needed with 6-Fr nasal suction catheters, with a negative pressure less than 80 mm Hg.

Due to lack of separation from Nasal CPAP, oxygen dependency (FiO2 50%) during feeding sessions, and increasingly difficult progression of nasal suction catheters during nasal aspirations, the patient was evaluated by otorhinolaryngology team on 60th day of life. Flexible fiberoptic nasal endoscopy revealed bilateral intensive nasal synechiae that occluded bilateral nasal passages almost totally. (Fig. 1) To prevent potential subsequent scars and synechiae, no endoscopic surgical intervention was performed. Palliative interventions to relieve the symptoms were introduced to the patient, such as serum physiologic nasal drops and intranasal steroids. Nasal aspirations were discontinued.

On 67th day of life, the patient was spontaneously separated from Nasal CPAP but continued to receive free oxygen. Gastrostomy procedure was planned for the patient, who had unsuccessful oral feeding sessions due

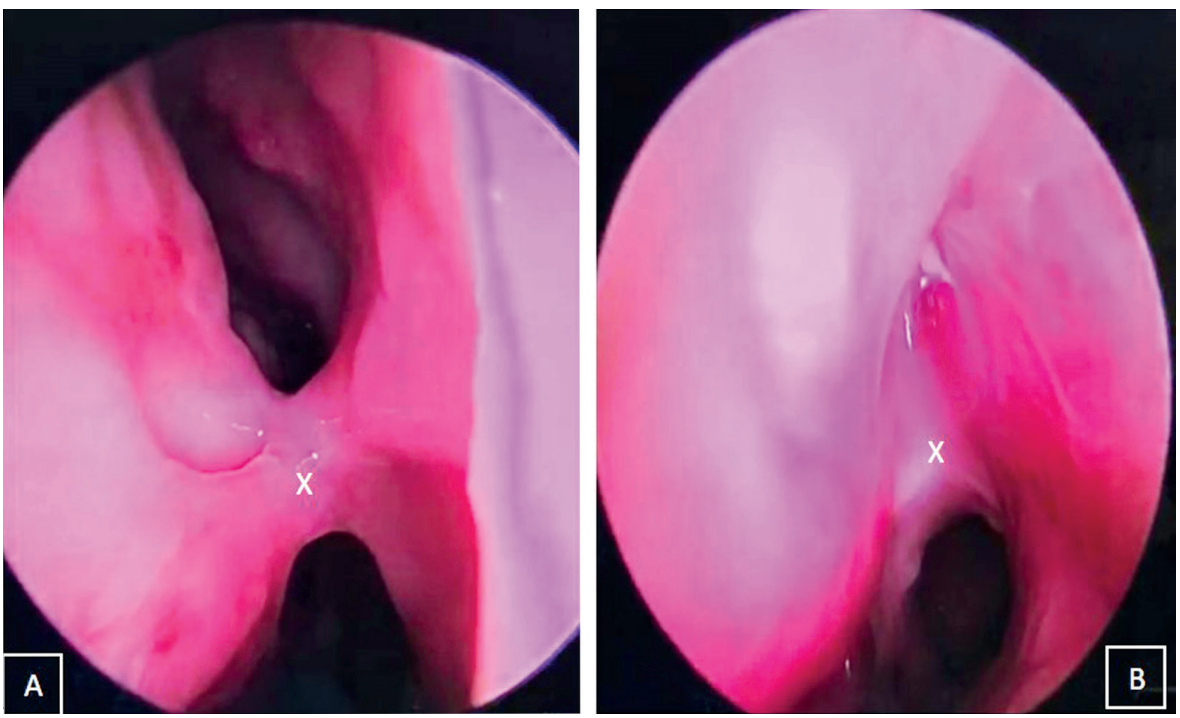

Fig. 1. Nasal cavity examination under Fiberoptic Flexible Bronchoscopy. A. Right nasal cavity. B. Left nasal cavity. X: Areas of synechiae.

to nasal passage narrowing and could only be fed with the help of orogastric feeding catheters. During the 20 days of pre-operative period, the patient received only free oxygen, bilateral nasal passages began to relieve, oxygen dependency gradually decreased and ultimately disappeared. Oral feeding was successful on 90th day of life. Reevaluation with the help of flexible fiberoptic nasal endoscopy revealed that nasal passage at the right choana was open and much improved. Patient was discharged on 99th day of life with outpatient follow-up planning. Informed consent has been obtained from the mother of the baby.

Discussion

Nasal obstructions may lead to short- and long-term morbidities such as respiratory distress, cyanosis, apnea, difficulty during feeding, and failure to thrive in newborns. Narrowing of nasal passages due to acquired causes is usually a manifestation of iatrogenic nasal trauma caused by nasal aspirations and results in progressively increasing respiratory distress and unsuccessful weaning from respiratory support.[1-5]

At present, the preferred method of non-invasive respiratory support in newborns is nasal CPAP application. To minimize pressure leakage, well-sitting interfaces are used. These interfaces include triangular mask-shaped soft interfaces that are closed on the nose and bi-nasal soft short prongs in the form of short tubes that fit on both nostrils. Interchanging use of these interfaces is recommended to minimize microtraumas.[1]

Regular cleaning of upper airways from secretions to keep passages open is especially needed in premature babies because their airways are relatively narrower and have a high resistance to air flow, which results in considerable decrease in delivery of nasal CPAP pressure to lungs when airways are obstructed by secretions. Because nasal aspirations itself may result in iatrogenic tissue damage, it should not be performed when patient has no need, should preferably be performed as gently as possible and should be performed with a negative pressure less than 80 mmHg.[1]

In the literature, wide spectrum of complications due to Nasal CPAP and upper airway aspirations were reported.[4,5] One study reported that nasal synechiae developed as a complication of nasal CPAP treatment in two (82%) of 82 premature newborns. In one of the babies' synechiae were bilateral, and was sent home with nasal oxygen at discharge. Because of nasal oxygen dependency, balloon dilatation was performed on the nasal cavity, but synechiae continued to be present at one-year follow-up. Synechiae were unilateral in the other infant, who had no respiratory problems and no feeding difficulties and thus needed no surgical intervention.[4]

In another study, which included 91 premature newborn babies who all received nasal CPAP, intranasal ulcerations were detected in six (3.3%) nostrils, granulations in nasal cavity were present in three (1.6%) nostrils, four nostrils (2.2%) had stenosis of the vestibular region.[5]

In our case, the nasal passages, which were already narrow, became progressively and near-completely obstructed secondary to microtrauma caused by nasal aspirations and nasogastric feeding catheters. Respiratory distress and feeding difficulty lasted long enough to suggest a gastrostomy procedure. Enlargement of nasal passages with advancement of postnatal age and discontinuation of nasal aspirations resulted in relief of nasal passages and unilateral re-opening of nasal airway. Cessation of respiratory distress made it possible to feed the infant orally. Because nasal synechiae are secondary to inflammatory response caused by microtraumas in nasal passages, as in the case presented here, aim should be to eliminate main causes of microtraumas as well as to relieve the symptoms of patient palliatively, rather than directly intervening with surgical procedures.

In conclusion, severe nasal damage may occur in premature newborns who receive nasal

CPAP support for long periods of time. Ongoing respiratory distress and unsuccessful weaning from respiratory support negatively affect oral feeding and weight gain, which results in prolonged hospitalization. Upper airway care should be done as gently as possible in premature newborns. Upper airway examinations should be done whenever needed to diagnose potential obstructions and synechiae.

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Case Report

Choroid plexus papilloma in extragonadal teratoma with predominantly neurogenic elements: a case report and review of the literature

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ABSTRACT

Background. Teratoma is a germ cell tumor that develops gonadal or extragonadal. Benign or malign somatic tumors can develop in teratoma. Choroid plexus papilloma is a benign, grade I intraventricular neoplasm that occur mostly in children. Choroid plexus papilloma in a teratoma is not often seen.

Case. We present the fifth case of a choroid plexus papilloma in a teratoma in the English literature. It was extragonadal and localized on the right side of the neck. It included only neuroglial tissue.

Conclusion. It is important to separate a teratoma with normal choroid plexus from a teratoma with choroid plexus tumor. Pathologists need to be aware of this entity in the distinction from other papillary neoplasms that may be primary or metastatic.

Key words: choroid plexus papilloma, teratoma, neuroglial tissue, extragonadal, newborn.

Teratoma is a germ cell neoplasm which has any combination of ectodermal, mesodermal and endodermal elements. The frequency of teratoma is 1 in 4,000 live births and 1-2% of the cases seen in the head and neck region.[1] Approximately 75% of this tumor group are mature teratomas, and about 12% of the cases are malignant and lethal.[2] Head and neck teratomas are most commonly seen at the cervical region, oropharynx is the second most common location. Congenital cervical teratoma is approximately 3% of the teratomas seen in childhood/infancy.[3] Since head and neck teratomas frequently presents with respiratory distress, surgeons should be aware of the clinical presentation and pathology of the teratoma.

Choroid plexus papillomas (CPP) are benign and slow-growing tumors derived from choroid plexus epithelium.[4] They are predominantly seen in children, and in the lateral ventricle, while they are seen in the fourth ventricle in adult. Many different localizations of the tumor have been documented.[5] The incidence of CPP is 0.3-0.6% of all brain tumors; 2-4% of the cases occur under the age of 15, and 10-20% of the cases occur within the first year of life.Teratoma is an unusual location for CPP. Four cases of CPP located in teratoma have been publishedin the literature so far.[6] All these cases were found in ovarian teratoma. Unlike the other cases, our case was at the cervical region.

Up to 90% of childhood teratomas contain derivatives from all three embryonic germ layers. Though teratomas develop from the three germ cell layers, some reports showed that they may arise from a single germ cell. The present teratoma included totally mature neuroglial tissue.

Case Report

A female neonate born with cesarean section at 39 weeks of age weighting 3350 grams had a mass

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on her neck at birth. The voluminous cervical mass was detected prenatally. It was followed up by a gynecologist until birth. She was operated on two weeks after birth. On postnatal magnetic resonance imaging (MRI) a 9 cm diameter mass with cystic and solid components was observed on the right side of the neck. It extended from the parapharyngeal region to the subcutaneous soft tissue. Teratoma, rhabdomyosarcoma and neuroblastoma were considered in the differential diagnosis. Tumor markers were within normal level. The lesion was removed by protecting vital organs and skin. There was no complication during the postoperative period. Macroscopically; the mass had cystic and solid areas. Microscopically; a CPP was found in the cyst. The diameter of the lesion was 0,6 cm. It was arranged of papillary structures with fibrovascular cores. Papillary structures showed complexity and there were acinar structures in some areas (Fig. 1). Epithelium of the CPP showed variably pseudostratification, surface epithelium of it was flat, and nuclei were oval and elongated (Fig. 2). Necrosis, mitosis and solid pattern was not observed. The other areas consisted of mature neuroglial tissue entirely (Fig. 3). Neuroglial tissue was hypercellular in some areas, but non-neoplastic. Immunohistochemical study showed that neuroglial tissue was positive for Alpha Thalassemia/Mental Retardation Syndrome X-Linked (ATRX), Glial fibrillary acidic protein (GFAP), Oligodendrocyte Transcription Factor 2 (OLIG2), and negative for The p53 gene (P53) and Isocitrate dehydrogenase 1 (IDH1). Antigen KI-67 (Ki67) index was very low.

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

Discussion

The teratoma of the head and neck region can shows unpredictable behavior and dramatic clinical presentation. Because of the potential for rapid growth of teratomas and as a result airway compression, the treatment of cervical and nasopharyngeal teratomas is immediate excision. Cervical teratoma is not common. In the literature 217 cases of cervical teratoma have been reported until 2009, and 90% of the cases were diagnosed in the pediatric population.[7]

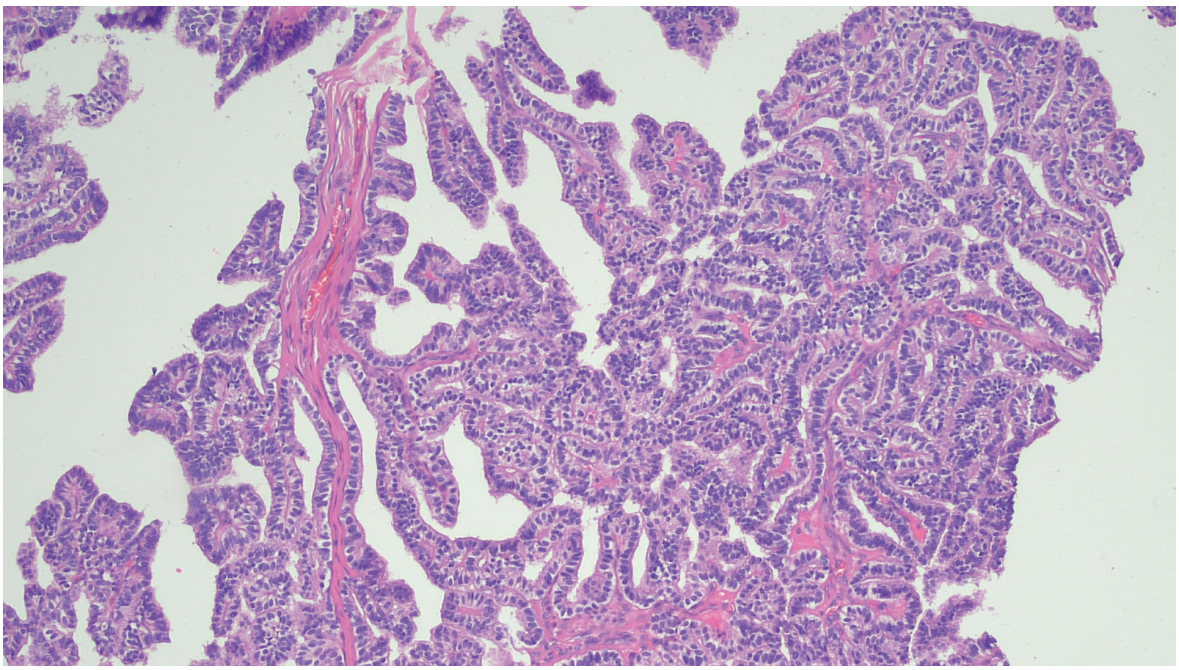

Fig. 1. HE X 200: Choroid plexus papilloma; papillary structures with fibrovascular cores and acinar structures in some areas.

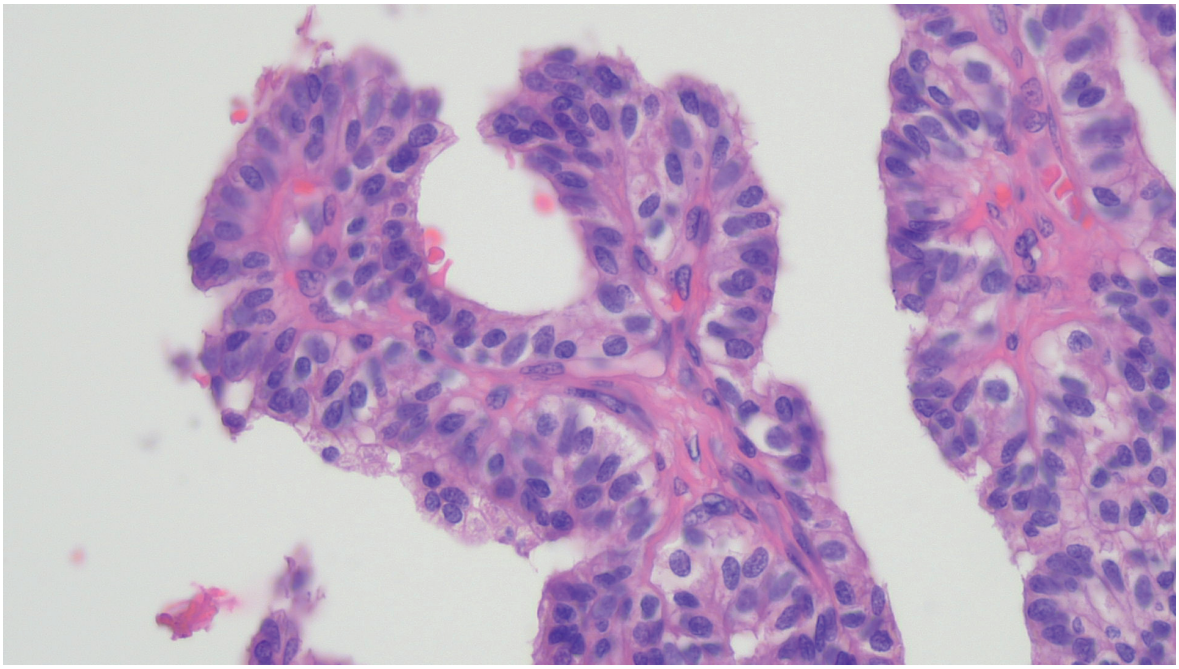

Fig. 2. HE X 400: Choroid plexus papilloma; variable pseudostratification of epithelium, flat surface epithelium, oval and elongated nuclei.

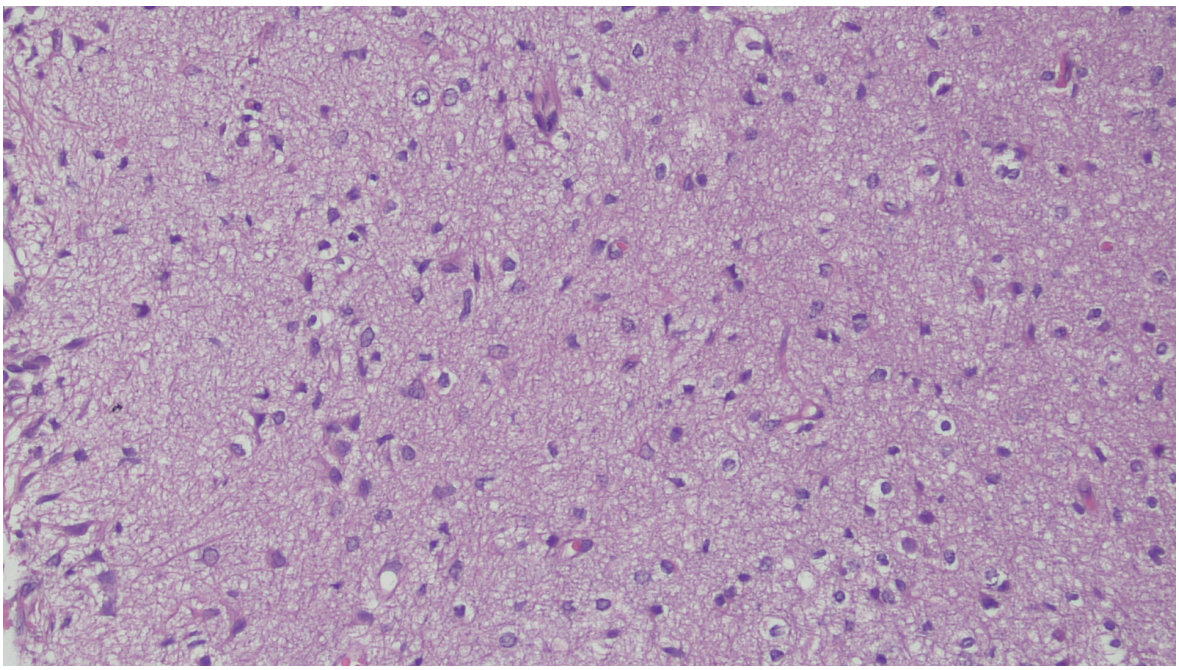

Fig. 3. HE X 200: Neuroglial tissues in different regions of teratoma (hypercellular and hypocellular areas).

The present case included CPP in teratoma of the head and neck region.

Choroid plexus in teratoma is commonly seen. It is important to separate a teratoma with choroid plexus from a teratoma with choroid plexus tumor. CPP contains more complex papillary architecture than non-neoplastic choroid plexus, and epithelium is flat in comparison with normal cobblestone-like surface. Only

four CPP in teratoma cases have been reported so far (Table I). In the present case, the diameter of the papilloma was very small, and was only visible at microscopic examination. In our case there was a delicate fibrovascular stalk covered by a single layer of cuboidal epithelial cells, arranged in a papillary configuration with fingerlike projections of tissue in the teratoma. The nucleus of the epithelium was slightly elongated.

Monodermal teratoma which includes only neuroglial tissue is uncommon in the literature. Akbulut et al.[8] described an ovarian teratoma that mainly consists of neurogenic elements including glial tissue, cuboidal cells lining the lumen of the cyst resembling ventricular ependyma, melanotic cells, ganglion cells, peripheral nerve and choroid plexus. Sharma et al.[9] reported a teratoma case that was predominantly composed of neurogenic tissue in the retroperitoneal region. Williams et al.[10] presented a monodermal teratoma which was named neurogenic ovarian cyst. It included neuroglial tissue with astrocytes, occasional Rosenthal fibers, oligodendrocytes, ependymal cells, scattered neurons with an overlying arachnoid layer and cerebellar tissue components. Our case consisted of only neuroglial tissue which included astrocyte, oligodendrocyte and choroid plexus. But it was not a monodermal teratoma because of the presence of choroid plexus. The choroid plexus has dual embryonic origin, with the choroid plexus epithelium originating from the ectoderm and the central stroma from the mesoderm.[11]

Our case was unique with its neuroglial component that includes CPP. CPP should be considered in the differential diagnosis with other papillary neoplasms which could be primary or metastatic such as serous, clear cell or endometroid gynecologic tumours, mesothelioma, papillary thyroid carcinoma, and lung adenocarcinoma.[12]

Immunohistochemically, choroid plexus epithelium-derived tumors react with pancytokeratin and epithelial membrane antigen (EMA), sometimes with vimentin, S-100 protein and synaptophysin. Focal GFAP reaction can be seen in epidermal differentiated choroid plexus tumor. But none of them are used in the differentiation of primary choroid plexus tumor from metastasis.

Two specific markers of choroid plexus tumor have been recently described. Kir7.1, a potassium inwardly-rectifying channel family member that may play a role in the transepithelial transport

Table I. Cases of choroid plexus papilloma in teratoma.

Case and reference	Year	Age	Localization	Diagnosis	Teratomatous elements	Size	Treatment
I[14]	2006	14	Ovary	CPP	Lungparenchyma, neuropil	ND	ND
II[15]	2011	26	Ovary	ACPP	Skin withadnexal structures, neuroglialelements, adiposetissue	9 × 7 × 7 cm	Cystectomy
III[16]	2013	32	Ovary	CPP	Skeletalmuscle, Adiposetissue	8 × 3 cm	Oophorectomy
IV[6]	2015	23	Ovary	CPP	Bone, cartilage, skin withadnexal structures, respiratorytype epithelium, ependym	22.5 × 20.5 cm	Salpingo-oophorectomy
Present case	2018	Newborn	Neck	CPP	Neuroglialelements	10 x 9,5 x 8,5	Resection

ACPP: atypical choroid plexus papilloma, CPP: choroid plexus papilloma, ND: not described.

of potassium. Stanniocalcin-1, a glycoprotein normally expressed in human choroid plexus that might participate in the regulation of cerebrospinal fluid calcium levels. Both normal choroid plexus and choroid plexus neoplasm react for these two immunohistochemical markers.[13] Therefore, Kir7.1 and Stanniocalcin-1 are sensitive and specific diagnostic markers for choroid plexus tumor. They are used in the differentiation of metastasis from choroid plexus tumor.

A choroid plexus papilloma located in a teratoma at neck region was presented. Pathologists need to be aware of this entity in the distinction from other papillary neoplasms that may be primary or metastatic.

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Case Report

Eczema herpeticum emerging during atopic dermatitis in infancy

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ABSTRACT

Background. Eczema herpeticum (EH) is an acute disseminated viral infection that develops in the presence of an existing skin lesion, often on the ground of atopic dermatitis (AD). Morbidity and mortality of EH can be minimized by starting antiviral therapy at the earliest time in diagnosis.

Case. Herein we report five infants diagnosed with EH in the course of AD treatment. All patients had early onset, moderate to severe AD and needed intermittent topical corticosteroid (TCS) therapy. In physical examination, newly formed, TCS-resistant vesiculo-papular skin lesions were recognised on the present dermatitis. The presence of AD with food allergy and moderate to severe eosinophilia were other prominent findings.

Conclusion. All patients were misdiagnosed as AD exacerbation. Therefore, EH should be considered in the differential diagnosis of AD exacerbation especially in the infants with moderate to severe AD.

Key words: atopic dermatitis, eczema herpeticum, Kaposi's varicelliform eruption, Herpes Simplex virus.

Atopic dermatitis (AD) is the most common inflammatory skin disease in childhood. The worldwide prevalence of AD has been reported to be 8-20%. It is characterized by chronic relapsing itchy lesions with different distribution according to age.[1,2] Eczema herpeticum (EH) (Kaposi's varicelliform eruption) is an acute disseminated viral infection, usually *Herpes Simplex* virus (HSV) type 1, that develops in the presence of an existing skin lesion, often on the ground of AD. Eczema herpeticum was first described in children and occurs with a higher prevalence in childhood however it can occur at any age.[3] Pathogenesis of EH is not fully understood but deterioration of skin barrier integrity and imbalance of the immune system are accused. Diagnosis is based on clinical findings. Early initiation of anti-viral therapy can prevent significant morbidity and mortality.[4]

Herein we report five infants diagnosed with EH in the course of AD treatment. A wide spectrum of clinical manifestations has been observed in the patients. We highlighted the clinical features, associations and therapeutic options in the context of the literature.

Case Report

The medical files of 1355 pediatric patients with AD between January 1, 2017 to December 31, 2017 at the pediatric allergy clinic in a tertiary centre (Allergy and Immunology Department of Health Sciences University Dr. Sami Ulus Maternity and Children's Health Training and Research Hospital Turkey) were reviewed retrospectively. Five of 1355 patients (% 0.36) were found to have EH during follow-up. The patients were treated by a multidisciplinary

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approach including departments of pediatric allergy immunology, infectious disease and dermatology. The characteristics of the cases are summarized in Table I.

All of the patients had early onset and moderate to severe AD and needed intermittent topical corticosteroid (TCS) therapy. TCS had been applied on the acute eczematous lesions within the past month in all patients. Despite repetitive TCS requirement, due to age limitation, topical calcineurin inhibitors had not been used. The most frequently affected area of the skin was the head region especially cheeks, followed by extremities and trunk (Fig. 1). The diagnosis of EH was made clinically by detection of active vesiculo-papules on the existing persistent eczematous lesions of the skin. The vesiculopustular lesions of EH recovered within in 5-15 days after onset of accurate treatment.

The laboratory findings are summarized in Table II. There was no significant increase in C-reactive protein (range=0-3 mg/L) or erythrocyte sedimentation rate (rate=0-20 mm/hr) although the white blood cells of the patients were high at diagnosis (mean 18.540 ± 6247/ μl). Serum *Herpes Simplex Virus* (HSV)-1 and HSV-2 IgM/IgG serology was negative in all patients. Due to refractory fever and irritability, lumbar puncture was performed in case 4 which revealed positive HSV type-1 by using Polymerase chain reaction (PCR) in the cerebrospinal fluid. The immunologic profiles of the patients were within normal limits despite one patient exhibited transient hypogammaglobulinemia of infancy. Atopy was evaluated by skin prick tests and specific IgE measurements. Standardized allergen extracts (Stallergens, Antony, France) were used for skin prick tests. Specific IgE serum levels to food allergens were performed on enzyme immunoassay system (Immulite Siemens, Germany). Food allergy was confirmed by the food challenge test in 4 patients in the follow-

Table I. The characteristics of the patients.

Case	Sex	Age (month)	Age (month) onset of AD/ diagnosis of EH	Distribution and characteristics of skin lesions	Fever	Seborrheic dermatitis	AD severity SCORAD index during EH/ currently
1	M	19	2/9	Grouped vesiculo-pustules on the eczematous skin of cheeks, left eyebrow and extremities, with secondary impetiginization	-	Over the scalp	60 - 57.2
2	F	22	1/9	Crusted grouped vesiculo-pustules on the eczematous skin of the cheeks, chin and forehead, eczematous plaques on the neck and trunk	-	-	40 - 30
3	F	25	1.5/4	Grouped vesiculo-pustules on the eczematous skin of cheeks with ulceration and secondary impetiginization, grouped vesicles on the eczematous skin of hand and eczematous plaques on the trunk	-	Over the scalp	66.7 - 0
4	M	24	2/4	Grouped vesiculo-pustules and blisters on the eczematous skin of the cheeks, chin, ears, neck, extensor aspects of the knees and elbows with secondary impetiginization	+	Behind the ear	85.7 - 40.7
5	F	17	4/4	Grouped vesicles on the cheeks, eyelid and eyebrow with secondary impetiginization	-	-	47.4 - 20

M: male, F: female, AD: atopic dermatitis, ED: eczema herpeticum.

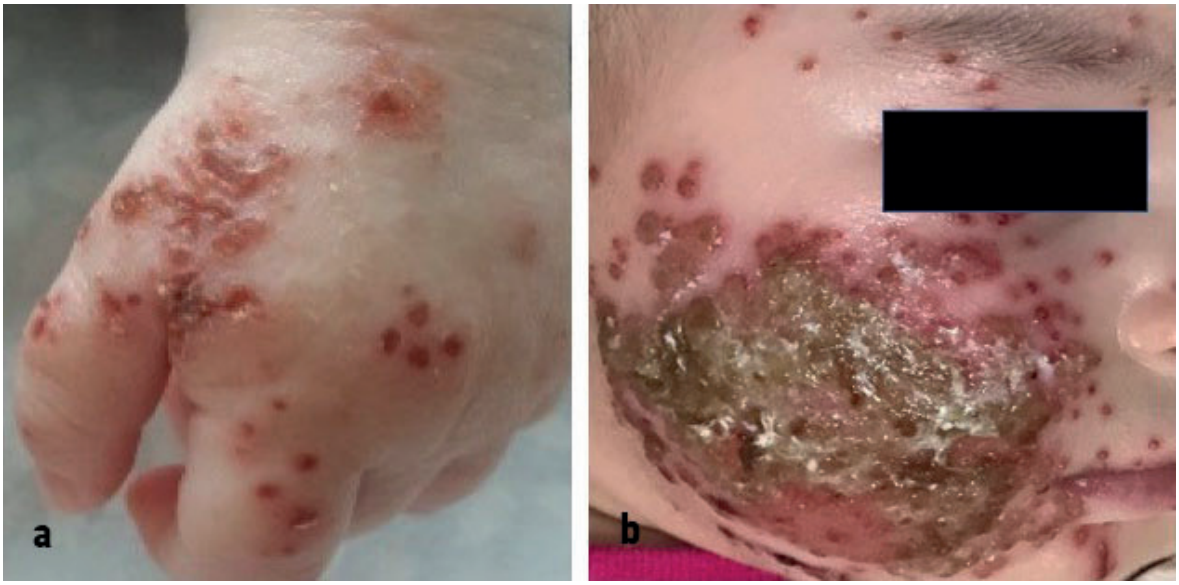

Fig. 1a. Grouped vesiculo-papules on the eczematous skin of hand. **1b.** Grouped vesiculo-papules on the eczematous skin of cheeks, eyelid and eyebrow with ulceration and secondary impetiginisation.

up period. Two patients are still on food elimination diet.

Four of the patients were hospitalized and median length of hospital stay was 9 days (IQR 5 to 21 days). Parenteral acyclovir and systemic antibiotherapy were administered to four of the patients. In one case, antimicrobial therapy was not administered because the lesions were crusted and there were no active vesicles at the time of diagnosis. The treatments applied before and after the diagnosis of EH are summarized in Table III.

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

Discussion

Atopic dermatitis is a chronic inflammatory skin disease characterized by intense pruritus with frequent relapsing courses with a higher incidence of certain cutaneous infections.[5]

Eczema herpeticum is one of the cutaneous infections in association with AD. The incidence of EH has not been well defined but it is known to be with a mortality of 1-9 %. Most commonly HSV type 1 and also HSV type 2, *Coxcakie Virus A16*, *Vaccinia Virus*, *Varicella Zoster Virus* are the contributing infectious etiologies that affect patients with EH.[4,6] The pathogenesis of EH is not completely understood. In a study it was reported that faster replication of HSV was observed in damaged skin than in normal skin.[7] High levels of serum IgE, serum eosinophil counts, thymus and activation regulated chemokine have also been associated with a higher risk of developing EH in patients with AD.[8]

Eczema herpeticum is a clinical diagnosis and characterized by grouping vesiculo-pustules followed by crusted, hemorrhagic, punched-out ulcers on the skin affected by a pre-existing dermatosis.[4] Secondary bacterial infection, mostly due to *S. aureus*, often occurs because of the inflammatory and extensive nature of the process.[9] In all of our patients, characteristic vesiculo-pustules were observed on the pre-existing dermatitis. Secondary impetiginization was common on the affected area which made the clinic difficult to diagnose accurately. In the patients with pre-existing AD with EH, underlying viral etiology may be underrecognized and misdiagnosed as AD

Table II. The laboratory findings of the patients.

Case	Skin prick test positivity	ESR (mm/hr) CRP (mg/L)	White blood cell (μL)	Absolute eosinophil count, eosinophil %	Absolute neutrophil count neutrophil%	Absolute lymphocyte count lymphocyte%	Total IgE (IU/ml)	Specific IgE Cow's milk/ Casein (Ku/L)	Specific IgE Hen's egg (Ku/L)
1	Cow's milk	8	13.600	4530	1632	6256	618	1.26	57.1
	Hen's egg	<3		33	12	46		1.35	
	Lentil Chickpeas								
	Potato								
2	Cow's milk	NP	13.800	900	4140	6762	24	-	-
	Hen's egg			8	30	49			
3	Hen's egg	3	28.400	4700	4800	16600	<18	-	-
		NP		16	17	58			
4	Cow's milk	NP	20.900	3200	2508	8778	743	12.3	33.6
	Hen's egg	<3		15	12	42			
	Wheat Potato								
5	Hen's egg	NP	16.000	300	5600	7200	58.9	-	7.28
		31		2	35	45			

*NP, Not Performed

*CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate.

Table III. The treatments applied before and after the diagnosis of Eczema herpeticum.

Case	Duration of TCS* usage before EH (week)**	Treatment of EH				
		Parenteral acyclovir	Parenteral antibiotic	TCS+	Topical antibiotic	Anti-histamine
1	8	+	+	+	+	+
2	20	-	-	+	+	-
3	6	+	+	+	-	-
4	4	+	+	+	-	+
5	1	+	+	+	+	-

*TCS: Topical corticosteroid.
**TCS treatment was applied intermittently, not all days of the week
+Topical corticosteroid was not applied in the active disease period
ED: Eczema herpeticum

exacerbation or as impetiginization. The upper body is the most common site of infection, with a predilection for the head and neck.[10] The most frequently affected area in all of our patients was the head, followed by extremities and trunk involvement.

Eczema herpeticum is associated with a flu-like syndrome, fever, malaise, lymphadenopathy and may be complicated by keratoconjunctivitis, meningitis and encephalitis.[11] We observed fever and restlessness in one case but did not observe central nervous system involvement findings in any of the patients.

Eczema herpeticum occurs most commonly in association with AD. However, it can also be seen in the course of other skin diseases that disrupt epidermal integrity such as psoriasis, pemphigus vulgaris, pemphigus foliaceus, pityriasis rubra pilaris, contact dermatitis (irritant and allergic), seborrheic dermatitis, cutaneous T-cell lymphoma.[12]

Our cases had early onset moderate to severe AD. Three cases were accompanied by seborrheic dermatitis on the head. Beck et al.[8] reported that patients who had AD with a history of EH (ADEH) had more severe AD, an earlier onset of skin disease and a higher prevalence of associated allergic diseases such as food allergy or asthma. In the study clinical features of ADEH+ versus ADEH− subjects were compared. ADEH+ patients were more commonly sensitized to many common allergens and higher serum total IgE and absolute eosinophil count was detected. Similarly, in our cases high levels of eosinophil count and total IgE was observed and all patients were sensitized to food allergens. Eosinophilia is a predictable finding in cases of AD with food allergy. However, the presence of moderate to severe eosinophilia in our patients is noteworthy. Three of our patients were found to have hypereosinophilia (> 1500/mm^3). One of the patients with hypereosinophilia improved clinically within six months and had no more eosinophilia on peripheral blood smear, while the other two patients continued with recurrent severe AD exacerbations and high eosinophil counts. Neither AD nor food allergy would be enough to explain the resistant and long-standing eosinophilia in these patients. We anticipate that an undefined immunological and/or genetic mechanism may lead to this predisposition to high allergy load and EH.

The diagnosis of our patients was made clinically by a pediatric allergist and a dermatologist. None of the patients had positive HSV serology which was taken from the peripheral blood sample at the active disease period. After active period HSV serology was not re-evaluated and this might have led to false negativity in serological evaluation. But in one of the patients HSV PCR positivity was detected from the sample taken from the cerebrospinal fluid. Supporting the clinical diagnosis; Tzank test has the highest sensitivity when compared to PCR, electron

microscopy, immunofluorescence, viral culture and serological tests.[13] For our patients Tzanck test was not performed. The low sensitivity of supporting laboratory tests in the diagnosis of EH further heightens the importance of clinical diagnosis. Clinical suspicion is essential and initiation of antiviral treatment should not be postponed.

Morbidity and mortality of EH can be minimized by starting antiviral therapy at the earliest time in diagnosis. The main treatment is acyclovir. Systemic antivirals and hospitalization are recommended for severe disease and immunocompromised patients. Secondary bacterial infection (mostly due to *Staphylococcus aureus*, *streptococcus pyogenes* and *pseudomonas*) which can hide the underlying viral pathogenesis and sepsis are the most important complications, and therefore systemic antibiotherapy should be added when necessary.[9] Parenteral acyclovir and systemic antibacterial therapy were given in patients except one whose skin lesions were all crusted and recovering at the time of the diagnosis. During the six months follow up of our patients, recurrence of EH was not observed.

Despite the high prevalence of AD in childhood, EH is a rare but severe and potentially fatal condition.[14] The eruption of EH in the patients with AD is usually misdiagnosed as an exacerbation of AD and causes delay in diagnosis with the use of unnecessary long-term potent local steroids. For all that delay in diagnosis leads to increased complications such as herpetic keratitis, viremia, sepsis and death.[9]

In patients with early onset, moderate to severe AD, when newly formed, local steroid-resistant vesiculopapular puched out lesions are recognised on the present dermatitis lesion, EH should be considered in the differential diagnosis. Infants with food allergy who have moderate to severe eosinophilia constitute a prominent risk group. Careful history taken with the awareness of the risk factors and characteristic cutaneous findings pointing EH will be helpful for the accurate diagnosis. The laboratory evaluation is not diagnostic but helpful for supporting the clinic. Early diagnosis and antiviral treatment on time can prevent major complications and death.

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Case Report

Congenital esophageal diverticulum in a very low birth weight infant: case report and review of literature

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ABSTRACT

Background. A diverticulum is an outpouching of a tubular organ that is classified as congenital and acquired according to the involved layers of the gastrointestinal wall. Congenital true diverticulum has been very rarely seen in neonatal period and it is very difficult to diagnose it especially in premature infants.

Case. A male infant was born with birth weight of 1000 g at 28th gestational week, was hospitalized for prematurity and respiratory distress. During follow up intermittent CO_2 retention was observed in blood gases. On the 17th day of hospitalization, esophageal dilatation was detected on X-ray and barium swallowed esophagram showed a saccular pouch on the distal esophagus. The patient was operated on 26th day of life and pathological specimen revealed true diverticulum of esophagus. The patient died due to respiratory failure and septic shock during hospitalization.

Conclusion. To the best of our knowledge this case is the smallest and youngest preterm infant diagnosed with congenital esophageal diverticulum. Prolonged and intermittent CO_2 retention such as in our case can be an atypical symptom of congenital diverticulum and it should be suspected in the differential diagnosis. Congenital esophageal diverticulum may be also seen in extremely preterm infants and can present with unusual symptoms.

Key words: congenital, esophageal diverticulum, newborn, premature.

A diverticulum is an outpouching of a tubular organ. Esophageal diverticulum is classified according to the location such as upper (pharyngoesophageal, Killion-Jamiesson or Zenker), middle or lower (epiphrenic). Besides anatomical location, esophageal diverticulum has also been described as pulsion or traction type according to the pathogenesis. Traction diverticula are true diverticula seen in mid-esophagus, associated with inflammation in mediastinum leading an external pressure on the esophagus. Pulsion diverticula are also classified into two groups; one is Zenker diverticule and the other is epiphrenic that both occur due to increased pressure on the upper and lower esophageal sphincter. Esophageal diverticula are also classified as; congenital (containing all layers of intestinal wall) and acquired (occurring in mucosal and submucosal layers via a defect in the muscular wall as herniation) like Zenker diverticulum.[1,2]

Congenital true diverticulum has very rarely been reported in newborns. Brintall and Kredelbagh[3] described two newborns with posterior hypopharyngeal congenital diverticule. None of them survived. These diverticula are usually small and asymptomatic; therefore, it is really difficult to diagnose especially in newborns. To the best of our knowledge here we reported the smallest and youngest infant diagnosed with congenital diverticulum.

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Case Report

A male infant was born by cesarean section at 28 weeks' gestation to a 22-year-old mother with Apgar 5 and 7 at 1 and 5 minutes. The birth weight was 1000 g (10-50th centile), birth length was 36 cm (50th centile) and head circumference was 25 cm (10-50th centile). The patient was hospitalized and monitored. Physical examination revealed respiratory distress and prematurity findings. No other congenital anomalies were observed with clinical and radiological findings. Early rescue surfactant was administered. He was given ampicillin and gentamicin therapies for sepsis. He could not be extubated during the first days of life due to the intermittent CO_2 retention that was not was compatible with the chest X-ray findings. Although chest radiograms did not reveal reticulogranular image and ventilator parameters were upgraded, there was no dramatical change in CO_2 levels during this period. This condition continued approximately once or twice a day. Ventilatory support status and blood gases changes are summarized in Table I. On the postnatal 17th day, a dilatation at esophagus was seen on chest radiogram (Fig. 1) and barium swallowed esophagram showed a saccular pouch on middle-distal esophagus (Fig. 2). Computed tomography of thorax illustrated a hypodense lesion with cystic characteristics with 30x14x15 mm in diameters forcing lung posteriorly in paraesophageal zone in the left hemithorax. After consultation with pediatric surgeons, esophagogastroduodenoscopy was performed in the operation room under general anesthesia and no evidence of diverticulum was detected. In addition to the persistence of unexplained intermittent CO_2 retention and persisting dilatation in the mid-esophageal region, the infant was operated on 26th postnatal day of life. After left thoracotomy at 5th intercostal area, grayish-like color mass with 3x1.5x 2 cm diameter was visualized between aorta and vertebra in posterior mediastinum left to distal esophagus. The mass was resected and no continuation or opening to any other part of esophagus or mediastinum was observed. The esophagus was primarily repaired and closed. Pathological evaluation of the mass revealed a true diverticulum containing all layers of esophagus. However, after the operation, the patient died on postnatal 34th day due to severe respiratory failure and septic shock. Informed consent was obtained from the parents.

Discussion

Herein, we described the smallest and youngest preterm infant diagnosed with congenital esophageal diverticulum. The clinical presentation of the case revealed unexplained intermittent respiratory acidosis and respiratory distress that resolved and reoccurred spontaneously. Most of the

Table I. Ventilatory support status (synchronized intermittent mandatory ventilation / continuous positive airway pressure / high frequency oscillation ventilation) and blood gases (pH / pCO_2) alterations of the patient before and after the interventions during the hospital stay.

Date of Blood Gases	pH	pCO_2 (mmHg)	Ventilatory support status
1st day (Before surfactant therapy)	7.22	64	SIMV mode
1st day (After surfactant therapy)	7.36	42	SIMV mode
3rd day	7.25	59	SIMV mode
7th day (Before extubation)	7.40	38	SIMV mode
7th day (After extubation)	7.21	92	Nasal CPAP mode
17th day	7.18	104	HFOV mode
26th day	7.26	59	SIMV mode
34th day	6.92	142	HFOV mode

pH: power of hydrogen, pCO_2: partial pressure of carbon dioxide, SIMV: synchronized intermittent mandatory ventilation, CPAP: continuous positive airway pressure, HFOV: high frequency oscillation ventilation.

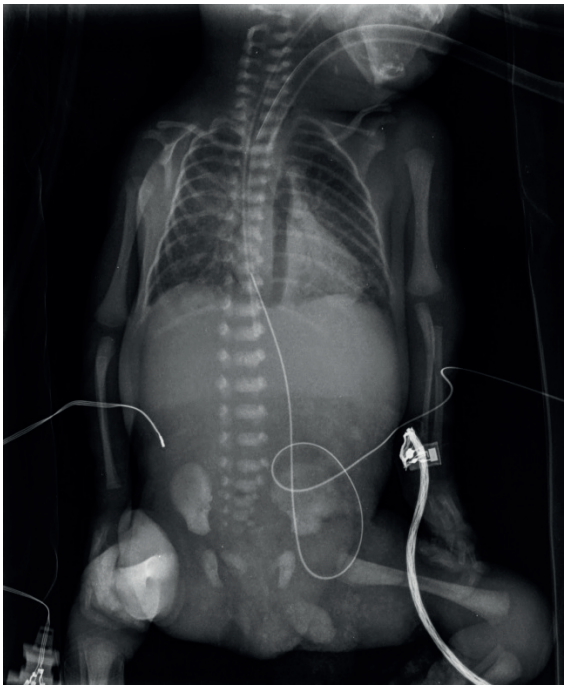

Fig. 1. Dilatation at esophagus seen on chest radiogram.

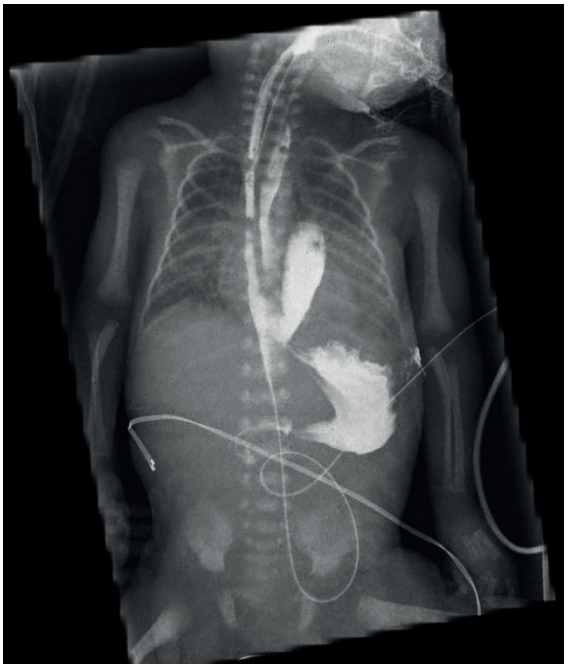

Fig. 2. Saccular pouch on middle-distal esophagus in barium swallowed esophagram.

esophageal diverticula are acquired and seen in middle-aged group, it has been very rarely been reported in infants.[4]

Holderman[5] first described a 5-day old infant misdiagnosed with congenital diverticulum in 1927, it was thought to be a class 3 tracheoesophageal fistula. Rush and Stingily[6] reported a newborn with supraclavicular mass causing respiratory obstruction. The autopsy revealed a large congenital cricopharyngeal diverticulum. Also, diverticulum in a newborn was reported by O'Bannon[7] on autopsy including a pouch anteriorly and inferiorly proximal to distal esophagus. In an extended review of literature, Poncher and Milles[8] could not find any examples of congenital esophagus diverticulum in 1933. In a more recent paper, an 8-day old term infant with stridor and poor feeding was diagnosed with upper esophageal diverticulum.[9] However, to our best of knowledge, no preterm infant had a diagnosis of esophageal diverticulum. Therefore, our case represents the smallest preterm infant diagnosed with congenital esophageal diverticulum in the literature.

Development of congenital diverticulum was suggested based on esophageal motor dysfunction by D'Abreu[10] in 1949 and it was manometrically confirmed by Cross et al.[11] in 1961. Kaye[12] reported esophageal motor dysfunction in 12 patients diagnosed with diverticule in 1974. Further theories supporting the development of diverticula were suggested by Ishigami et al.[13] in 1965. Our case had unexplained intermittent respiratory acidosis that did not response to ventilatory therapy and was not compatible with radiological findings. Therefore, motor dysfunction, impaired relaxation and delayed passage in esophagus might have caused increased pressure on the esophagus.[12] The weakness of the muscular layer of esophagus and pouch formation might have resulted in increased pressure against the lungs leading to worsening of pulmonary function. The blood gases might be found normal possibly when the size of the pouch and associated pressure against lungs decreased.

Clinical features are broad for esophageal diverticula. It can be asymptomatic but dysphagia is a common finding. When

symptoms occur, they are likely to be caused by associated underlying motility disorder. Other clinical features associated with esophageal diverticulum include feeding problems, weight loss, regurgitation, stridor, belching, bleeding, and cough. In a recent case report; esophageal diveticulum with bronchoesophageal fistula was diagnosed in case of unexplained cough or recurrent pneumonia.[14] In our case, intermittently increasing CO_2 levels were detected as the main and unusual symptom. We suggest that dilatation of esophagus and sac formation may have led to increased pressure on lungs. We also think that it resolved spontaneously when the pressure decreased.

According to adult American Gastroenterological Association guidelines, barium swallowed esophagogram is the gold standard diagnostic method.[15] On barium swallowed esophagogram, diverticulum will be illustrated as a distended, barium filled sac above the diaphragm. We showed the same similar findings in our patient. There was dilated, pouch-like formation seen in the mid-esophageal area. Endoscopy and bronchoscopy may also be useful for both confirming and assessing the degree of esophageal inflammation and obstruction. Carcinomas or other diseases should also be excluded by endoscopy. Although we performed an endoscopy, we could not diagnose the diverticulum. Endoscopy may be dangerous due to increased risk of perforation with misdirection of the scope into the diverticula and is not recommended in case of large diverticula because of incomplete emptying of pouch remnants.[9] In our case, thorax CT showed the diverticulum as a hypodense lesion with cystic characteristics, therefore we suggest to use of other diagnostic tools such as CT in suspected cases. This CT finding can be interrupted with esophageal duplication cyst. But approximately 90 percent of esophageal cysts do not communicate with the esophageal lumen.[16] In our case the lesion originated from the esophageal lumen. We could precisely differentiate this lesion from duplication cyst by pathological evaluation.

The optimal management of these cases are early and prompt surgery. Surgical approach for treatment of diverticulum is mainly myotomy, supported by various studies in which 80-100% of patients had good outcomes.[17] Left-sided thoracotomy is mostly preferred to visualize the esophagus. Our case underwent a left thoracotomy and after the resection of the sac, all esophageal layers were closed anatomically. Although the surgery was successful, the patient died due to problems of prematurity and neonatal sepsis. The prognosis varies according to several factors including the presentation, gestational age, and associated abnormalities. However, the prognosis may be poorer in preterm infants such as in our case. Therefore, we recommend prompt surgery in appropriate conditions after the diagnosis.

The strength of this case can be suggested as the youngest and smallest preterm infant in the literature to be diagnosed and operated on very promptly. Although symptoms were non-specific our findings may represent a clue for both neonatologists and pediatric surgeons. The weak point of this paper is that the problems may have been associated with prematurity and also there was no genetic data about this congenital anomaly.

Esophageal diverticula are frequently acquired and seen in the middle-aged group, it has very rarely been reported in infants and even rarer in premature infants. Clinicians should keep in mind and be aware of this condition in the case of prolonged intubation and extubation failure with prolonged CO_2 retention that could not be related to any other situation.

Herein, we reported an unusual case of a true congenital esophageal diverticulum in a very low birth weight premature infant. Although congenital esophageal diverticulum is rare in neonates, the clinical findings may vary from respiratory problems to feeding problems. The main mechanism responsible from variable symptoms may be increased esophageal pressure. In addition to barium graphics, other diagnostic tools such as endoscopy or thorax

CT should be performed in suspected cases. Prolonged and intermittent CO_2 retention such as in our case can be an atypical symptom of congenital diverticulum. In conclusion, we suggest that congenital esophageal diverticulum should be kept in mind in the differential diagnosis of infants with respiratory and feeding problems even in preterm babies.

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